Doctoral Programme in

Agrifood and Environmental Sciences

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Epidemiological studies on primary inoculum of *Venturia inaequalis* and *V. asperata* for apple scab management

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ABSTRACT

Apple scab, caused by the ascomycete *Venturia inaequalis*, is the most important disease of apple, causing great losses worldwide. Its management is largely based on fungicide treatments, especially targeting ascosporic infections. To reduce the pesticide input, sanitation practices against overwintering inoculum, decision support systems for an optimal timing of treatments, and resistant cultivars can be used. However, reducing pesticide pressure increases the risk of emerging new and/or secondary pathogens. This is the case of *V. asperata* that was isolated on cultivars resistant to *V. inaequalis* and already reported in several regions of Italy and France. It causes atypical scab-spots on fruit and leaves. Limited information is available on the epidemiology of *V. asperata*. The specific objectives of the thesis were: i) to set up a method of reducing the overwintering inoculum of apple scab by irrigating overwintering leaves on the ground in dry days, ii) to understand the aerial dispersal of ascospores with the under-canopy irrigation, and iii) to identify the conditions of maturation of pseudothecia and ascospore discharge of *V. asperata*. The effect of irrigation was evaluated comparing irrigated and non-irrigated leaf litter. Irrigation in dry days induced the release of a noticeable amount of ascospores of *V. inaequalis* under conditions unsuitable for infection, thus reducing the inoculum during rainy days. In mean of all trials, the ascosporic inoculum was reduced by approx. 50% in irrigated plots. In addition, irrigation caused an earlier depletion of the ascospore supply. Field trials were carried out to evaluate the efficacy of irrigation in reducing scab in untreated and fungicide-treated plots. By the end of the primary season, the overall incidence of infected leaves and fruit in untreated and fungicide-treated plots had decreased by more than 50%. To understand the aerial dispersal of ascospores of *V. inaequalis* with under-canopy irrigation, rotating-arm spore traps were placed at heights, ranging from 0.3 m to 3.0 m above the ground. Irrigation was carried out above overwintered apple leaves infected with scab in two different situations, i.e., in a wind-protected enclosure and under real orchard conditions. Ascospores were found to disperse above the irrigated layer, with more spores detected above the sprinklers than below. Based on these results, since ascospores could settle on susceptible apple tissues, it is essential to ensure a rain-free period of at least 24 hours post-irrigation to prevent scab infections. The primary infection season of *V. asperata* was studied by comparing the development of pseudothecia and ascospore discharge to *V. inaequalis* in overwintered leaf litters. *Venturia asperata* showed a delay in pseudothecial maturation and emptying in relation to degree days accumulation, compared to *V. inaequalis.* The time of spore release for *V. asperata* was postponed compared to *V. inaequalis*. The delayed spore ejection and pseudothecial development of *V. asperata,* in comparison with *V. inaequalis*, could partly explain the late appearance of symptoms during the growing season in the orchards. In conclusion, targeted irrigation could represent a sustainable and easily applicable method to reduce the incidence of apple scab, and consequently to reduce the number of treatments or increasing their efficacy. Further research on epidemiology of *V. asperata* is necessary to find the optimal weather conditions for ascospore discharge and for primary and secondary infections, as well as its latency period. Future breeding programs for apple should also take into consideration resistance against *V. asperata.*

INTRODUCTION

Apple scab: economic importance and impact of fungicide treatments

Apple scab, caused by the ascomycete *Venturia inaequalis* (Cke.) Wint., is the most important fungal disease of apple (*Malus* × *domestica* Borkh.) worldwide. Symptoms occur on leaves, fruit, petioles, sepals, pedicels and young shoots (MacHardy, 1996), but the main economic impact stems from the scab-like lesions that may deform the fruits. *Venturia inaequalis* is a hemibiotrophic fungus that overwinters mainly in infected leaves on the ground, where the development of pseudothecia takes place (MacHardy, 1996). The pseudothecia progressively mature and develop asci and ascospores during late winter and spring. Mature ascospores are released in spring when pseudothecia have been moistened by rain or heavy dew, and they are dispersed by wind. Ascospores, settled on susceptible apple tissues, start primary infections when conditions of leaf wetness duration and temperature are met (Mills, 1944; MacHardy and Gadoury, 1989; Stensvand et al., 1997). Conidia developed in scab lesions are responsible for the secondary dissemination and infections that may occur repeatedly during the entire growing season.

Apple scab is a major threat in most apple growing regions, and especially in temperate regions with cool-moist weather in spring and frequent rains in summer (MacHardy, 1996; MacHardy et al., 2001). In these regions, the losses directly result from infection on fruit and indirectly from leaf infections that may cause a premature defoliation. Such defoliation reduces plant growth and consequently the yield, and it increases the susceptibility to winter frost (Biggs and Stensvand, 2014; Roberts and Pierce, 1935). Under favourable conditions to the disease, the entire apple production may become unmarketable if proper control measures are not applied (Holb, 2006; Roberts and Pierce, 1935). A single scab lesion is sufficient to make fruit unmarketable, and therefore even a low incidence of scab means significant losses to the growers (Carisse and Dewdney, 2002).

Italy is the third largest apple producer in Europe, with about 55.000 hectares, of which 80% are located in northern Italy (almost half of these are produced in the region of Trentino-Alto Adige; Caponero, 2023). In the recent years, apple production in northern Italy had a significant renewal of cultivars, with the introduction of several new both scab-resistant and scab-susceptible cultivars. Resistant cultivars, although still a minor fraction of the total surface of the apple area in northern Italy, are increasing over the years, reaching more than 1.900 ha (Gregori et al., 2022). In northern Italy, spring is normally characterized by scattered rain showers and thunderstorms alternating with dry and clear-sky days (Laiti et al., 2014, 2018). Climatic conditions showed overall an increase of average temperatures in the last few years, with mild winters and hot summers, and an increase in extreme weather events (Caponero, 2023). These conditions cause both an anticipation in the primary infection season of *V. inaequalis,* with the first release of ascospores at the end of February in the Po Valley, and in an earlier onset of the apple growing season (Bugiani and Bariselli, 2024; Caponero, 2023).

Apple cultivars are vegetatively propagated, and therefore there is a high genetic uniformity of apple orchards worldwide, with few cultivars/clones prevailing. Most of the existing apple varieties are susceptible to apple scab, and intense chemical control programs are often needed, with associated costs and environmental pollution (Gessler and Pertot, 2012; MacHardy et al., 2001). Indeed, management of apple scab is largely based on fungicide treatments, targeting especially against primary-ascosporic infections (Aylor, 1998; MacHardy, 1996). For example, fungicides represent the greater proportion of pesticides applied to apples, and against apple scab they represent 72% to 80% of the total treatments applied in orchards (Sauphanor et al., 2009).

The number of fungicides applied per season depends on weather conditions, cultivar susceptibility, growing regions and management approaches. In integrated pest management (IPM) programs in northern Italy, on average 9-12 fungicide treatments are applied against apple scab during the primary season. In organic orchards, the number is 10–15 applications, because organic fungicides are commonly less persistent and effective than synthetic fungicides (Bilanci Fitosanitari 2020-2021 and 2022-2023; Branz A., Bugiani R., personal communications). In northern Italy the control strategy against *V. inaequalis* is mainly based on preventive fungicide treatments (e.g., captan, dithianon, copper compounds), applied as close as possible to infection conditions. A system for reliable weather forecasting is therefore crucial to optimise fungicide applications. If an unexpected rain occurs or the preventive fungicide has been washed off, some active substances (e.g., fluazinam, dodine, lime sulphur) can stop the infection even if, during rainfall, ascospores settled on susceptible tissue and germinated. If severe infections occur due to heavy, prolonged and repeated rainfalls and high ascospore spore release, fungicides with curative mechanism of action (e.g. difenoconazole, pyrimethanil, cyprodinil) can be applied (Andreatti et al., 2023; Bugiani and Bariselli, 2024; Caponero, 2023). In order to avoid breakdown of resistance and the development of virulent strains of *V. inaequalis*, scab-resistant cultivars are usually managed with a reduced spray schedule, applying fungicides to cover the most severe infections (Barchetti et al., 2023).

In the past, many growers have perceived synthetic fungicides as the sole effective and inexpensive control measure against apple scab (Carisse and Dewdney, 2002). However, this belief is slowly changing due to the increasing cost of fungicides, the reduced efficacy due to selection of fungicide resistant strains of *V. inaequalis*, the negative impact on human health and the environment, and the severe restrictions for pesticide residue levels on fruit imposed by regulations, and consequently by wholesalers and retailers (Antal et al., 2024; Didelot et al., 2016; Philion et al., 2023). Moreover, environmental and health regulation has resulted in severe restrictions or prohibition in the use of widely employed fungicides (Philion et al., 2023). In northern Italy, to date, apple scab is effectively controlled with several fungicide applications during the primary season for ascospore release, but the reduction of admitted active substances and climate change are making its control more difficult. The integration of new technologies and agronomic control measures with fungicide treatments will be essential to ensure sustainable and economically viable apple production (Caponero, 2023).

Management of apple scab

Much research on apple scab management has been focused at reducing overwintering inoculum with agronomic practices, finding the optimal timing for the treatments, replacing chemicals with biofungicides, and developing resistant cultivars.

Orchard sanitation practices aim to reduce ascosporic inoculum of *V. inaequalis* through mechanical removal of fallen leaves, such as leaf shredding, burning, or burying leaves in the soil (Sutton et al., 2000; Vincent et al., 2004). For example, treatments of urea (5% urea solution) before leaf fall or on the leaves on the ground accelerate the decomposition of leaf litter and may reduce the primary inoculum from 50 to 96% (Burchill, 1968; Burchill et al., 1965; Sutton et al., 2000). Cultural practices, such as a regular and balanced pruning, increasing air circulation can reduce leaf wetness duration and improve spray coverage (Carisse and Dewdney, 2002; Biggs and Stensvand, 2014). Nevertheless, a substantial reduction of ascosporic inoculum by sanitation and cultural practices is not sufficient to reduce scab infections under favorable disease conditions and without applying other control measures (Biggs and Stensvand, 2014). These practices are moreover time consuming and challenging for the growers.

Warning systems based on weather data, weather forecasting and decision support systems (DSSs), are widely used to alert growers on potential infection risks (Rossi et al., 2007; Trapman and Polfliet, 1997). The integration of different models (e.g., Mills infection periods with different modifications, and ascospore maturation) and forecasting tools in web-based DSSs, provide real-time advice in apple scab management, but the predicted risk of infection depends both on the accuracy of the model and weather forecast (Garofalo et al., 2016; Okoro et al., 2024). These models, based on weather data, have overall a good accuracy in predicting apple scab infections, even if discrepancies were observed sometimes between ascospore maturation and the outcomes of some forecasting models (Garofalo et al., 2016; Okoro et al., 2024). Moreover, if conditions for scab infections are frequent and close to each other, the efficacy of DSSs to optimize fungicide treatments is limited.

Several fungal isolates, yeasts, and bacteria demonstrated biocontrol activity under laboratory and greenhouse conditions against the biotrophic and saprophytic phases of *V. inaequalis*; however, they were insufficient to reduce apple scab at a satisfactory level in commercial orchards (Okoro et al., 2024; Shuttleworth, 2021). A promising solution for biocontrol against apple scab may be post-harvest applications, before leaf fall, to promote leaf decay in the orchard, thus reducing the overwintering inoculum (Okoro et al., 2024; Shuttleworth, 2021). For example, beneficial fungi such as *Athelia bombacina*, *Trichoderma* spp. and *Microsphaeropsis* sp., can reduce apple scab inoculum by decomposing the leaf litter and thus interfere with development of pseudothecia (Carisse et al., 2000; Okoro et al., 2024). Technological challenges, such as formulations to ensure long-term viability, storage conditions and reduction of production costs, need to be solved for a large-scale use of beneficial microorganisms in the field, as the disease control is often variable, and due to their low persistency, the number of applications is usually higher that chemical fungicides (MacHardy et al., 2001; Okoro et al., 2024).

The use of scab resistant cultivars is an effective alternative to reduce chemical treatments (Brun et al., 2008; Patocchi et al., 2020). The strategy to develop scab resistant cultivars implies the incorporation of *Rvi* resistance genes. The main resistance gene used in apple breeding programmes has been the *Rvi6* (=*Vf*) gene, and it has been deployed in several cultivars in commercial apple production (Caffier et al., 2012; Didelot et al., 2016; Gessler and Pertot, 2012). An important drawback of monogenic resistance is that it may be overcome, and therefore it is not a durable long-term solution. In fact, breakdown of resistance provided by the *Rvi6* gene has been observed in Europe since the 1990's. Virulent strains of *V. inaequalis* have become dominant in several European countries (Gessler and Pertot, 2012; Parisi et al., 1993, 2004) and later they were reported in other countries around the world (Papp et al., 2020). Therefore, to achieve durable resistance in apple, pyramiding multiple scab resistance genes in a single cultivar is necessary (Patocchi et al., 2020). Destroying leaf litter in winter and the application of a minimal fungicide spray schedule are recommended to the growers to prevent the development of virulent strains of *V. inaequalis* in apple resistant orchards (Caffier et al., 2012).

All the approaches described above have some practical, technological or economical limitations, and none of them are sufficiently effective to manage apple scab alone. For these reasons it will be necessary to find new and innovative solutions to integrate into current apple scab management practices. Rain is necessary for ascospore discharge from overwintered leaves on the ground and even low amounts of water (0.2 mm) are sufficient to wet the leaves and to induce a significant release of ascospores (Brook, 1966; Hirst and Stedman, 1962; Rossi et al., 2001). Therefore, sprinkler irrigation of overwintered apple leaves on the ground during periods of dry weather and before forecasted rainfall could promote the release of ascospores if conditions for infection are not met. Previous trials carried out in Denmark (Korsgaard, 2012, 2014, 2016) and France (Libourel, 2006) by using different irrigation methods and timings, showed that irrigation released ascospores of *V. inaequalis*, but its efficacy on spore release and on leaf and fruit infection was variable, and often insignificant. In this context, the development of a targeted irrigation system to release ascospores of *V. inaequalis* could represent a sustainable, effective and cheap technique to reduce the disease incidence and therefore reduce the number of fungicide applications and increase their efficacy.

Scab resistant cultivars and new-emerging diseases

Changes in the pathogen, the host or the environment can lead to new disease appearances in crops. In particular, the introduction of alien pathogens or hosts in a certain area, climate change and changes in agricultural practices can lead to the emergence of pre-existing pathogens as major diseases or can provide conditions for introduced pathogens to emerge (Anderson et al., 2004). Regarding the apple tree, a reduction in fungicide applications in scab-resistant cultivars may promote the development and outbreak of other diseases, including fruit rots, sooty blotch, and more recently Marssonina blotch (Boutry et al., 2023; Ellis et al., 1998).

An example of the occurrence of a new pathogen was observed since 2007 in southern France, where atypical scab-like symptoms appeared on fruits of apple cultivars ‘Ariane’, ‘Goldrush’ and ‘Prima’ carrying the resistance gene *Rvi6*. Morphological and molecular analysis identified the causal agent of the scab-like symptoms to be the ascomycete *Venturia asperata* Samuels & Sivan. (Caffier et al., 2012). *Venturia asperata* was first described by Samuels & Sivanesan (1975) in New Zealand and later in Canada by Cortlett (1985), and in both locations the fungus was reported as a saprotroph on overwintered apple leaves on the ground. In 2012, *V. asperata* was recorded for the first time in Italy (Emilia-Romagna Region), as atypical scab spots on fruits of the cultivar CIVG198/Modì®, carrying the *Rvi6* gene (Turan et al., 2019). Later, *V. asperata* was reported in the province of Trento (Italy) in 2018 (Gualandri et al., 2021; Prodorutti et al., 2023), and in the nearby province of South-Tyrol in 2019 (Öttl, 2021), on cultivars carrying the *Rvi6* gene in both provinces. In 2018, *V. asperata* was reported in China (Zhou et al., 2021).

Limited information is available on the epidemiology of *V. asperata*. Caffier et al. (2012) reported from a one-season trial comparing the release of ascospores of *V. asperata* with that of *V. inaequalis*. Pathogenicity trials under controlled environmental conditions (optimal for *V. inaequalis*) showed a weaker development of *V. asperata* on leaves compared to *V. inaequalis*, and efforts to reproduce symptoms on fruits by artificial inoculation almost all failed. These results may suggest that optimal conditions for infection of *V. asperata* differ from those of *V. inaequalis* (Caffier et al., 2012). Since *V. asperata* is spreading in key apple-growing regions across Europe on cultivars carrying the *Rvi6* gene, there is a need to study the epidemiology of this new emerging pathogen of apple. Improving knowledge on the epidemiology of *V. asperata* holds practical implications for management of apple scab, because it may influence the timing and the type of fungicide treatments, as well as the timing for other management practices (e.g, targeted irrigation for ascospore release). Future breeding programs and selection of new scab-resistant cultivars should consider developing durable resistance also for *V. asperata*, besides *V. inaequalis.*

AIM OF THE THESIS AND SPECIFIC OBJECTIVES

The overall aim of the thesis is the development of a sustainable and effective method for management of primary infections of *V. inaequalis* and improve knowledge on the epidemiology of *V. asperata* in northern Italy, which may become problematic in scab-resistant cultivars.

The specific objectives are:

- to implement a method of reducing primary inoculum of *V. inaequalis* by irrigating overwintering leaves on the ground before forecasted rainfall;
- to understand if ascospores of *V. inaequalis* released by targeted irrigation may disperse in the plant canopy, thus potentially inciting infection if an unexpected rain occurs shortly after the irrigation;
- to understand when primary inoculum (maturation of pseudothecia and ascospore discharge) of *V. asperata* occurs in northern Italy, in order to assess the risk of outbreak of this pathogen and find the right timing for fungicide treatments or for application of sprinkler irrigation.

The first objective was achieved by comparing ascospore discharge and pseudothecial maturation of *V. inaequalis* in irrigated apple leaf litter compared with non-irrigated leaf litter. Field trials were carried out to evaluate the efficacy of sprinkler irrigation on apple scab incidence. Irrigated and non-irrigated plots were either treated with different fungicide control strategies or not treated. This topic corresponds to the chapter "Irrigation targeted to provoke ejection of ascospores of *Venturia inaequalis* shortens the season for ascospore release and results in less apple scab". The paper was published in the journal "Plant Disease" in May 2024 (https://doi.org/10.1094/PDIS-07-23-1245-RE).

The aerial dispersal of ascospores of *V. ianequalis* was studied carrying out under-canopy irrigation over infected leaf litter, in two different conditions: in a wind-protected enclosure and in a wind-exposed apple orchard. Ascospores were captured with rotating-arm spore traps placed at heights ranging from 0.3 m to 3.0 m above the ground. This objective has been covered in the chapter "Aerial dispersal of *Venturia inaequalis* ascospores with under-canopy sprinkler irrigation for apple scab management". This paper has been published online in the "European Journal of Plant Pathology" in September 2024 (https://doi.org/10.1007/s10658-024-02949-3).

The study of *V. asperata* was carried out by checking the development of pseudothecia and ascospore discharge of *V. asperata* in comparison with *V. inaequalis* in infected leaf litters, in relation to rainfall and degree-day accumulation. The impact of daylight on ascospore discharge of *V. asperata* ascospore also studied. The third objective was covered in the chapter "Pseudothecium development and ascospore discharge in *Venturia asperata* and *V. inaequalis*: relation to environmental triggers". The paper was published in the journal "Phytopathologia Mediterranea" in December 2024 (https://doi.org/10.36253/phyto-15739).

IRRIGATION TARGETED TO PROVOKE EJECTION OF ASCOSPORES OF *VENTURIA INAEQUALIS* SHORTENS THE SEASON FOR ASCOSPORE RELEASE AND RESULTS IN LESS APPLE SCAB

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Abstract

Trials were carried out in apple orchards of Emilia-Romagna and Trentino-Alto Adige in northern Italy to investigate the effects of sprinkler irrigation on possible reduction in inoculum and subsequent disease pressure of *Venturia inaequalis*, the ascomycete causing apple scab. In spring, volumetric spore traps were placed above apple leaf litter containing pseudothecia with ascospores of the fungus.

Pseudothecia matured more rapidly in irrigated plots, and 95% of the total number of spores trapped in a season was reached on average 164 degree days (base temperature 0°C) earlier in irrigated compared to non-irrigated plots. On average for seven location/year combinations, more than 50% of the ascospores were trapped following irrigations carried out for two hours on sunny days before a forecasted rainfall. Subsequently, a much lower number of spores were trapped on rainy days following irrigation. Field trials with scab susceptible apple cultivars were carried out in the two regions to evaluate the efficacy of sprinkler irrigation on disease. Irrigated and non-irrigated plots were either treated with different fungicide control strategies or not treated. Irrigation significantly reduced the incidence of apple scab at both sites, and the overall number of infected leaves and fruit was reduced by more than 50%. Mid-day sprinkler irrigation can significantly reduce the inoculum pressure of *V. inaequalis* in apple orchards. This may be a sustainable management strategy, especially in areas with extended dry periods.

Keywords: apple, ascospore pattern, disease control, efficacy, sustainability

Introduction

Apple scab, caused by the ascomycete *Venturia inaequalis* (Cke.) Wint., is the most important fungal disease on apple (*Malus* × *domestica* Borkh.) worldwide. *Venturia inaequalis* is a hemibiotrophic fungus that overwinters in infected leaves on the ground where the development of its fruiting bodies (pseudothecia) takes place. During late winter and spring, the pseudothecia mature and develop asci and ascospores (Biggs and Stensvand 2014). In spring, when rain or heavy dew occur, pseudothecia release ascospores, which are disseminated by wind and start primary infections when conditions of sufficient leaf wetness duration and temperature are met (Mills 1944; MacHardy 1996; Stensvand et al. 1997, Philion et al. 2020).

The first ascospores in a growing season are usually mature and ready to be released around bud break of the apple tree, although ascospores can be mature ahead of bud break in rainy years and after bud break in dry years (Philion et al. 2012), which has also been shown for *V. pyrina* in pear (Rossi et al. 2009). The maturation and release of spores continues for 6-10 weeks, usually with a peak between the stages of pink bud and full bloom (MacHardy 1996). The peak of ascospore ejection roughly coincides with the flush of growth of the apple tree, maximizing opportunities for primary infection (MacHardy 1996). Subsequently, scab lesions with conidia develop on leaves and fruit and can cause secondary infections.

Wetting of leaves by rain is necessary for significant ascospore discharge (Moore 1958; Brook 1966; Rossi et al. 2001). Although ascospores of *V. inaequalis* can be released during periods of dew formation (Stensvand et al. 1998), dew rarely triggers substantial ascospore releases (Hirst and Stedman 1962; Brook 1969a), and dew periods are usually not sufficiently long to cause infections (Stensvand et al. 1998). The impact of raindrops on leaves during rain events induces ascospore release, thanks to periodic dampened vibrations on leaf surfaces (Alt and Kollar 2010). Rain of 0.2 mm or greater is sufficient to cause a measurable spore discharge (Brook 1966; Hirst and Stedman 1962). Most ascospores of *V. inaequalis* are released during daylight hours (Brook 1969b; Brook 1975; MacHardy and Gadoury 1986; Gadoury et al. 1998; Rossi et al. 2001), and the peak of ascospore release is usually the middle of the day (MacHardy and Gadoury 1986).

Temperature, in addition to influencing ascospore maturation (James and Sutton 1982), is an important factor affecting ascospore release. The rate of ascospore discharge is slow below 10°C (Hirst and Stedman 1962; MacHardy and Gadoury 1986; Gadoury et al. 1994; Stensvand et al. 1997). Above 10°C there is discrepancy among studies: some reported that above 10°C spore release is steady (Seem et al. 1979); others indicate a significant effect of increasing temperatures on spore release at

10-22°C (Brook 1976) or showed that temperature influences the rate of spore discharge over a range of 5-20°C (Rossi et al. 2003).

The time interval and the pattern of ascospore release of *V. inaequalis* during and following rain are somewhat unclear. The beginning of ascospore discharge is reported from a few minutes to one or more hours after leaves are wet by rain (Hirst and Stedman 1962; Stensvand et al. 1997; Rossi et al. 2003).

Management of apple scab is largely based on fungicide programs, especially against primary (ascosporic) infections (MacHardy 1996). Protectant (syn. preventive) and curative fungicides can be used, but the disease is commonly effectively controlled with multisite protectant fungicides (e.g., captan, dithianon, mancozeb, metiram, and copper compounds). Curative fungicides (e.g., difenoconazole and pyrimethanil) are used for post-infection applications, and they are often mixed with protectants to increase efficacy and reduce the risk of fungicide resistance (MacHardy 1996; Biggs and Stensvand 2014). Warning systems based on weather forecasting and decision support systems are used to alert growers of potential risk of infections (Trapman and Polfliet 1997; Rossi et al. 2007; Garofalo 2019).

Sanitation practices help reduce apple scab inoculum, thus increasing the efficacy of fungicide applications. Application of urea before leaf fall or to leaves on the ground promotes degradation of leaf litter and reduces the ascosporic inoculum from 50 to 96% (Burchill 1968; Burchill et al. 1965; Sutton et al. 2000). Removing or shredding apple leaf litter may also significantly reduce the ascosporic inoculum (Sutton et al. 2000; Vincent et al. 2004). Beneficial fungi, such as *Athelia bombacina*, *Trichoderma* spp., and *Microsphaeropsis* sp. can reduce apple scab inoculum by decomposing leaf litter or acting directly as antagonists of *V. inaequalis* (Carisse et al. 2000), but technological challenges need to be solved for a large-scale use of beneficial microorganisms in the field (MacHardy et al. 2001).

Irrigation of overwintering leaves on the ground during periods of dry weather may be a technique to reduce the ascosporic inoculum by stimulating the release of ascospores in periods with minimal risk of infection. Timed irrigations could promote the release of mature ascospores when conditions for infection are not met and therefore reduce the number of spores discharged on subsequent rainy days when infections could be incited. Dry spells have occurred with greater frequency in the recent years in many countries, as in 2022 in Italy. Such climatic changes could enhance the efficacy of this technique and promote its use in apple growing areas.

Field trials carried out in Denmark (Korsgaard 2012; 2014; 2016) indicated that artificial irrigation releases *V. inaequalis* ascospores; however, numbers were low compared to the total seasonal supply of spores. The effect of irrigation on leaf and fruit infection was variable, and the efficacy in reducing scab infections was often insignificant. Increasing the amount of water applied, irrigating the entire orchard floor, and an earlier start of treatments in spring, were proposed as possible improvements of the technique (Korsgaard 2014; 2016).

Possible benefits of overhead sprinkler irrigation to control apple scab were studied in France in a six-year field trial (Libourel 2006), and it was concluded that at least five hours of irrigation would be required to exhaust the spore potential. Martínez et al. (2021) in Uruguay also studied the effect of spray irrigation on ascospore discharge of *V. inaequalis*. Daytime and nighttime irrigations were carried out on four successive days, and considerable spore numbers were trapped during the first two to three consecutive days of irrigation. However, the effect of irrigation on the seasonal ascospore pattern and disease development was not reported.

The aim of this study was to implement a method of reducing primary inoculum of *V. inaequalis* by irrigating overwintering leaves on the ground before forecasted rainfall. The specific objectives were to: i) investigate if sprinkler irrigation can stimulate the release of ascospores of *V. inaequalis* enough to substantially reduce the inoculum from infected leaf litter overwintered on the ground; ii) assess the effect of sprinkler irrigation on the seasonal pattern of spore release; iii) verify if sprinkler irrigation can reduce the incidence of apple scab on leaves and fruit and increase the efficacy of a standard plant protection strategy.

Materials and methods

Sprinkler irrigation, development of pseudothecia and ascospore trapping

Irrigation trials were carried out in spring during the time of ascospore release of *V. inaequalis*, in open fields in Italy, in San Michele all'Adige (SM, coordinates 46.190015, 11.134925) in the Trentino-Alto Adige (TAA) Region from 2017 to 2021 and in Bologna (BO, coordinates 44.524286, 11.349701) in the Emilia-Romagna (ER) Region, in 2017 and 2018. Just before leaf fall of the year preceding the irrigation trial (between 25 October and 5 November according to the year; i.e., in 2016 to 2020 in SM and in 2016 and 2017 in BO), apple leaves with scab symptoms were collected from untreated trees (cv. Golden Delicious for SM and cv. Gala for BO) in selected plots of commercial orchards (in Borgo Valsugana, TAA and in Malborghetto di Boara, ER), where no fungicides had been applied during the entire growing season. Leaves were immediately placed on the ground in two plots in each location (plot sizes were 3 × 2 m in SM and 2 × 1 m in BO), at least 100 m apart from each other. The grass was removed by a light soil tillage (5 cm), and a layer of leaves was placed on the soil above a white non-woven fabric of permeable polypropylene (Ortoclima, Tenax s.p.a., Lecco, Italy) that prevented earthworms from degrading the leaves. Each plot was then covered with a wire mesh (1 × 1 cm) to keep the leaves in place. The leaves (approximately 150/m^2) were placed with the main infected surface (adaxial or abaxial) upwards in a single layer of leaves (not overlapping).

In the following spring (first and second week of March in BO and SM, respectively), a volumetric spore-trap (Lanzoni VPPS 2000, Lanzoni s.r.l., Bologna, Italy in BO and Myco-trap, Paul Illi Mech. Werkstatt, Wädenswil, Switzerland in SM) was placed above each leaf litter plot. In each location, a micro-sprinkler irrigation system (model 01 KR, Ecorain Irrigation Systems s.r.l., Rubano, Italy) was placed above one of the leaf litter plots in each orchard, while the other one was not irrigated. To homogeneously wet the entire litter surface, four sprinklers were placed 30 cm above the leaf litter plots in SM, while in BO three sprinklers were placed 2 m above the plots. Each sprinkler had a water volume of 35 L/h, equivalent to 2 and 4 mm/h in BO and SM, respectively.

From the second half of March until the end of May, corresponding to the expected length of the ascospore release period in the two regions, the leaf litter was irrigated before forecasted rainfall by Meteotrentino in SM (https://www.meteotrentino.it) and ARPA Emilia-Romagna in BO (https://www.arpae.it/it/temi-ambientali/meteo/previsioni-meteo). Irrigation was carried out if i) rain of any intensity was forecasted in the next 24-48 h, ii) no rain had occurred in the previous two (or more) days, and iii) the weather was dry and sunny on the day of irrigation. Irrigation was applied for two hours in the middle of the day, from 11 am to 1 pm (Central European Time, CET).

From 2018 to 2021, during the primary season for ascospore maturation and release in spring, the development of pseudothecia of *V. inaequalis* was evaluated weekly in SM. Fifty pseudothecia from ten leaves collected from both irrigated and non-irrigated leaf litter were observed under a light microscope, starting the first week of March. Leaves containing pseudothecia were soaked in water for ca. 10 minutes, then the pseudothecia were harvested, crushed on glass microscope slides and classified according to the stage of development as described by James and Sutton (1982). Pseudothecia were then divided in three main groups (immature, mature, and empty) defined as follows. The immature group included stages from pseudothecial initials to pseudothecia with most of the asci containing not yet pigmented (immature) spores. In the mature group, most asci contained mature ascospores (septate and pigmented). Pseudothecia were defined as empty if most of the asci were empty or aborted.

Ascospores were counted on rainy days (i.e., ≥ 0.2 mm rain occurring during that day) and on irrigation days. If a day without rain was preceded and followed by a rainy day, or was preceded by an irrigation day, spores were also counted. A microscope tape was mounted on a 7-day rotating clock cylinder in each trap. Spores were recorded as daily numbers. The microscope tapes were cut in pieces representing single days, placed on glass slides and counted with a light microscope. Spore counting on the tape was carried out according to Mandrioli (2000), by assessing along three parallel horizontal lines of the slides. The daily average number of the counted ascospores (average of the three horizontal lines of the slide) for each trap was calculated and used to tabulate total spore production and proportion of the total number of spores trapped during the primary season. On irrigation days, the beginning and the end of spore discharge was also assessed. The percentage of the total seasonal number of spores ejected by irrigations was calculated. The relative daily difference of ejected spores between irrigated and non-irrigated plots was calculated as: (spores in irrigated plots - spores in non-irrigated plots) / total seasonal spores in non-irrigated plots.

Weather data were recorded during all trials by weather stations (model TMF 500, Nesa s.r.l., Vidor, Treviso, Italy) located at the experimental sites. Summation of degree days (DD, base 0°C) from budbreak to the last day of spore ejection and from the first to the last day of spore ejection was calculated.

Effect of under-canopy irrigation on apple scab

From 2020 to 2022, the efficacy of under-canopy targeted irrigation on development of scab was assessed in an apple orchard in TAA, located in Borgo Valsugana (BV; coordinates 46.045564, 11.470860). The orchard was planted in 1999, with cv. Golden Delicious grafted on rootstock M9 and an inter-row distance of 3.5 m and 1.0 m distance of plants in the row.

The under-canopy irrigation system was composed of micro-sprinklers set up along the rows. The sprinklers (dynamic micro-sprinkler model 01 KR, Ecorain Irrigation Systems s.r.l., Rubano, Italy), each providing 35 L/h were placed 30 cm above the ground at a distance of 3 m along the rows. The irrigation covered the entire surface of the plot, with an intensity of approx. 3 mm/h. Irrigations were performed with the same decision scheme as described above.

Apple scab infections in irrigated (Irr) and non-irrigated (Non-irr) plots, treated with two fungicide control strategies were compared: full fungicide schedule with preventive and curative treatments (Prev+Cur), and a reduced fungicide schedule with only curative treatments (Cur). Irrigated and non-irrigated untreated control (Untr) plots were also included.

Irrigations and fungicide treatments in the three control strategies started around 10-15 April, just before bloom and continued until mid-May, in order to cover the stage of high risk of ascospore infections and to maximize the efficacy of irrigation on spore release in the period of the highest spore maturation.

Preventive treatments were carried out one to two days before a forecasted rainfall (https://www.meteotrentino.it) and when the RIMpro model (Le Berre 2023; Trapman and Polfliet 1997) gave a risk of infection. Curative fungicides were applied only after the estimated main infection events, i.e., prolonged rain and leaf wetness and severe infections indicated by RIMpro. At the beginning of the growing season, before the experiment started, one or two applications with a copper fungicide were carried out in all plots to control the first possible apple scab infections. After the end of the trial (late-May to early-June), preventive fungicides were applied in all plots following the conventional control strategy used in that area for secondary infections of apple scab (Andreatti et al. 2021). All fungicide treatments were applied with a towed atomizer (Dieter Waibl, Merano, Italy) equipped with anti-drift nozzles. The spray volume of the fungicide applications was 500 l/ha, and the dose of the products per hectare is shown in Supplementary Table S1.

Timed irrigation was done on six, seven and five instances in 2020, 2021 and 2022, respectively. The irrigations started on April 10 to 15 and ended on May 9 to 22 in the three seasons. Seven preventive and three curative, eight preventive and six curative, and five preventive and three curative fungicide treatments were applied during the trial period in 2020, 2021 and 2022, respectively (Supplementary Table S1). On the irrigation days, the preventive fungicide in the Prev+Cur plots was applied in the morning (at 9 am), before the irrigation. Assessments were carried out on June 15, 18 and 23 on shoots and on June 23, 18 and 23 on fruit in 2020, 2021 and 2022, respectively.

Effect of overhead irrigation on apple scab

Trials to understand if irrigation over the tree canopies can reduce the incidence of primary infections of apple scab were carried out in ER, in Malborghetto di Boara (MB; coordinates 44.858318, 11.655405) from 2017 to 2019. The orchard was planted in 2005, with cv. Gala grafted on rootstock M9, on 3.3 m inter-row and 1.2 m in-row planting distance.

Overhead irrigation was carried out by using the sprinkler irrigation system (irrigator model K15, Kofler s.r.l., Lagundo, Italy) already installed in the orchard and used by the grower for irrigation and frost protection. The sprinklers (rate flow 14 l/min) were placed 4 m above ground with a distance of 20 × 16.5 m, providing a relatively even distribution of 2 mm/h. Irrigations were performed with the same decision scheme as explained above.

Apple scab infections in irrigated (Irr) and non-irrigated (Non-irr) plots, untreated (Untr) or treated with a full fungicide control strategy (Prev+Cur) were compared. The control strategy in fungicide treated plots was set up by using the active ingredients commonly used in integrated pest management (IPM) in ER (Supplementary Table S2). Furthermore, the timing of fungicide treatments followed the conventional control strategy used in ER for the primary and secondary (conidial) infections of apple scab, meaning that the treatment decision was based on weather forecast (https://www.arpae.it/it/temi-ambientali/meteo/previsioni-meteo) and the infection risk obtained from the A-scab model (Rossi et al., 2007). Fungicides were applied with a towed atomizer (SAE Turbomatic, Ferrara, Italy).

Seven irrigations were carried out in 2017 and 2018 and ten irrigations in 2019. The irrigations started in early March and ended in late April. The number of fungicide treatments applied during the growing season were 15 in 2017 (nine preventive and six preventive+curative), 17 in 2018 (eleven preventive and six preventive+curative) and 13 in 2019 (six preventive and seven preventive+curative) (Supplementary Table S2). Assessments on leaves and fruit were carried out on 5/20/2017, 5/10/2018 and 5/8/2019.

Experimental design, assessments and data analysis

The *tidyverse* packages (Wickham et al. 2019) of the R language (version 4.2.2; R Core team 2023) were used to handle data and generate plots. Packages and functions used to fit individual models are listed below.

The cumulative proportion of empty pseudothecia was modeled as a function of accumulated DD since budbreak using binomial regression (generalized linear model, glm). Different link functions were compared using the Akaike Information Criterion (AIC). The effect of irrigation treatment, year, and interactions with accumulated DD were assessed using likelihood ratio tests (LRTs).

Time to ejection (recorded as DD, base 0°C) curves between Irr and Non-irr plots was studied with survival analysis. The nonparametric log-rank test (Peto et al. 1977) and variants (e.g., the Peto & Peto modification of the Gehan-Breslow-Wilcoxon test) were used to compare the Kaplan-Meier survival curves using the "survdiff" function of the survival package of R. The effect size of the difference in survival time (time to ejection) could not be analyzed with continuous time Cox regression since the proportional hazard (PH) assumption was not met. The accelerated failure time

(AFT) and discrete time interval Cox regression models were used as alternatives. Calculations of AFT were done with the “survreg” function of the survival package of R. Discrete Interval data were calculated with the “dataLong” function of the “discSurv” library. Logistic regression for the interval Cox regression was performed with glm using the Gompertz link (also called complementary log-log), and the hazard ratio was reported.

Both field experiments (BV and MB) were carried out in a randomized block design with four replicates, with plots of 28 and 12 plants in BV and MB, respectively. To assess apple scab infections that had developed during the primary ascospore release period, a disease assessment was carried out in mid-June and mid-May, in BV and MB, respectively, which was at the end of the ascospore infection season in the two locations.

In BV, 40 shoots/replicate for each treatment (20 vegetative and 20 bourse shoots) were randomly assessed for the presence of apple scab. For each shoot, the number of infected leaves and the total number of leaves was counted. In MB, 400 leaves/replicate were randomly assessed for the presence of apple scab, and the number of infected leaves was counted at the plot scale. A total of 250 and 50 fruit per replicate for each treatment were assessed by counting the number of infected fruit, in BV and MB, respectively.

The proportion of infected leaves per shoot (in BV), the proportion of infected leaves (in MB) and of infected fruit (in BV and MB) per plot (incidence) was modelled using logistic regression. Leaf scab incidence at the individual shoot scale in BV was modelled using “glmer” of the “lme4” package of R. The effect of fungicide treatments (Prev+Cur, Cur, Untr), irrigation, shoot type (vegetative, bourse), year and their interactions were modelled as fixed effects. Shoots within plots and plots within blocks were used as random effects. The shoot and plot terms in the random effects were used to account for the correlation between leaves within the same shoot and shoots within plots. Random effects were maintained in model selection based on AIC. Similarly, the effect of treatment, irrigation, year, and their interactions on leaf scab incidence in MB and fruit scab incidence in BV and MB were modelled using glm.

In all cases, different models were compared using likelihood ratios (LRT) with ANOVA tests for which the chi-square statistics (χ^2), difference in the number of parameters for the two models compared (degrees of freedom), and associated P-values were reported. Odds ratio (OR) and the associated z statistic was reported for individual effects of the selected models. Marginal means of leaf and fruit scab models of both sites were computed with "emmeans" to account for interactions.

The efficacy of irrigation in reducing scab infections on leaves and fruit between irrigated and non-irrigated treatments was calculated with the Abbott's formula (Abbott, 1925).

Results

Effect of sprinkler irrigation on development of pseudothecia

Over the four years of the trial, there was a different trend on pseudothecia development in irrigated and non-irrigated leaf litter. An overall delay in pseudothecia maturation and emptying in relation to DD accumulation was observed in non-irrigated plots (Fig. 1).

The first pseudothecia with mature ascospores were detected in early March in 2020 and 2021 and in late March in 2018 and 2019, while the first empty pseudothecia were observed in early April (April 9-11) in 2018, 2020 and 2021, and in late April in 2019. In all four years of the trial, the time of 95% empty pseudothecia was later in the non-irrigated compared to the irrigated plots (Table 1), on average 84 DD.

Although Probit and Logistic regression gave similar results, probit overall gave a better fit on rate of emptying pseudothecia (Fig. 1). Overall, the effect of DD accumulation was different between Irr and Non-irr treatments (LRT deviance = 13.6, df = 1, P < 0.001), but the effect of DD accumulation on the proportion of empty pseudothecia was similar for the different years (LRT deviance = 7, df =

3, P = 0.07). There was no Treatment × Year interaction and no triple interaction (DD × Treatment × Year) (P > 0.2 for both).

The probit regression coefficients gave a change in the z-score (or probit index) for a one-unit change in the predictor. The z-score for the effect of each DD accumulation was slightly smaller for Non-irr plots (0.006) compared to Irr plots (0.007).

Table 1. Summation of degree days (DD, base 0°C) from budbreak to 95% of the pseudothecia in irrigated or non-irrigated plots were empty in San Michele all'Adige.

Year	**DD 95% Irrigated (Σ)**	**DD 95% Non Irrigated (Σ)**
2018	753	814
2019	1046	1159
2020	944	1038
2021	785	851

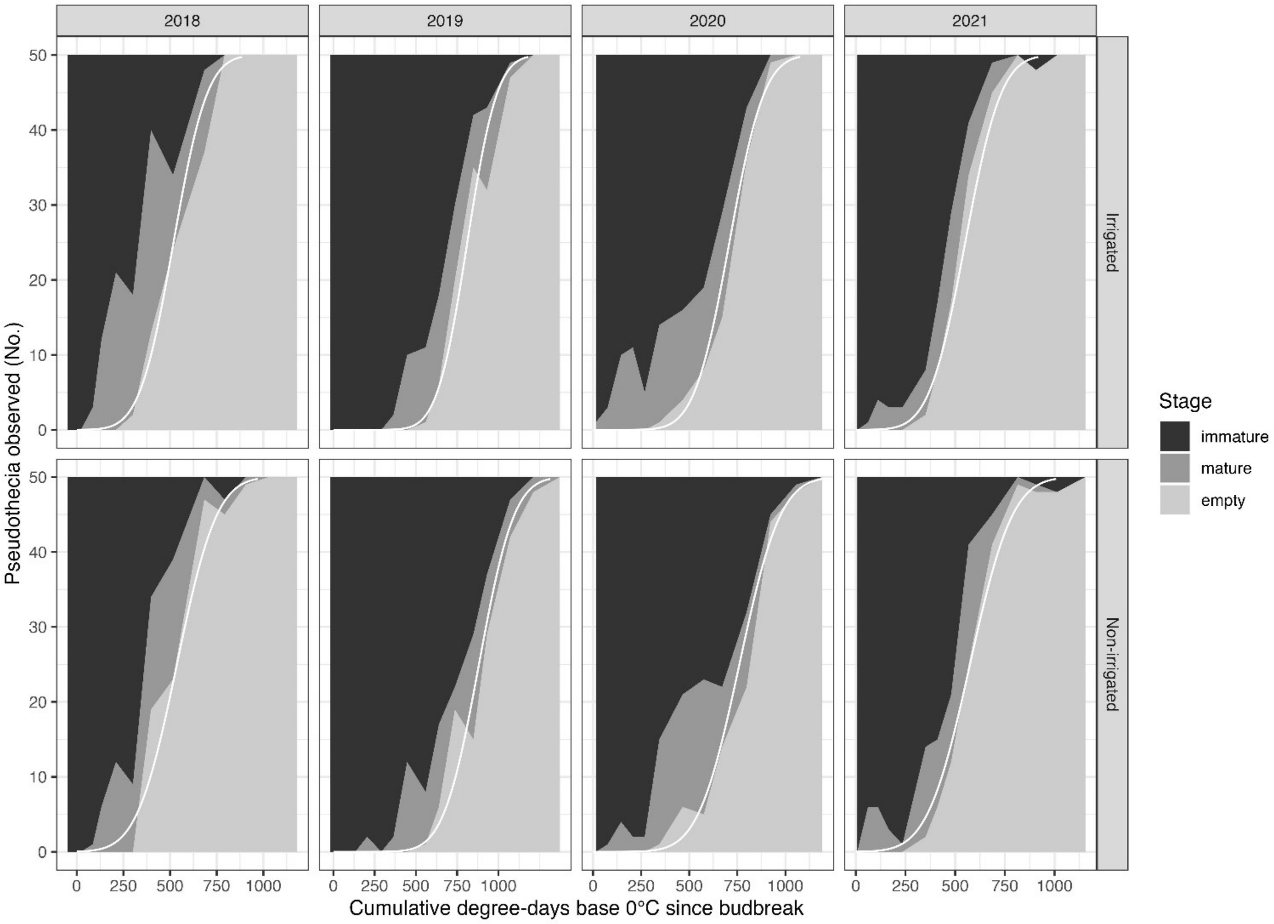

Fig. 1. Immature, mature and empty pseudothecia during the season for ascospore release of *Venturia inaequalis* in irrigated and non-irrigated leaf litter in relation to degree-day (base 0°C) accumulation in San Michele all'Adige. Weekly assessments of 50 pseudothecia per treatment were evaluated from 2018 to 2021. Pseudothecia emptying was modeled using probit, represented by the white line.

Effect of sprinkler irrigation on ascospore release

In the years of the study, ascospores were released from March 11-22 to May 6-14 in BO and from March 14-April 2 to May 24-June 8 in SM. Irrigations were carried out from 21 March to 12 May and 21 March to 30 May in BO and SM, respectively, and ranged between 5 and 11 per season (Fig. 2 A, Table 2). In the period in which ascospores were released, the total amount of rain was lower than 100 mm and higher than 200 mm in BO and SM, respectively (Table 2).

A noticeable release of ascospores of *V. inaequalis* was observed following irrigations in the Irr plots, while no release or very few spores were usually detected in the Non-irr plots. In a few cases (one each in BO 2017, SM 2017, and SM 2018; four times in SM 2019 and two times in SM 2020) spores were trapped in Non-irr plots during irrigation days (Fig. 2 C). On the contrary, when rainy days followed irrigations, a much lower number of spores was normally found in the Irr plots compared to the Non-irr ones (Fig. 2 B, C, D).

Irrigations were associated with a faster depletion of the ascospore potential (total amount of ascospores produced from leaf litter in the ascospore release season). The time of 95% spore trapping was on average reached 164 DD earlier in the Irr plots than in the Non-irr ones (Fig. 2 E, Table 2). Except for SM in 2019, when there was no difference in number of rainy days in Irr and Non-irr plots, the number of rainy days at time of 95% ascospore accumulation was 1 to 9 days less in Irr plots than in the Non-irr plots (Table 2).

The total number of ascospores trapped per season varied among the years, and a lower number of spores was trapped in BO compared to SM. However, on average considering all years and locations, there was only a difference of 24 spores (approx. 1% of seasonal total) between the irrigated and non-irrigated plots (Table 2). Considering each irrigation event, the spores were trapped within a few minutes after irrigation started and until 2-3 h after it had ended (data not shown). The yearly percentage of spores trapped during the rainy days following an irrigation was reduced by 10.7 to 81.1% (mean 54.4%) in the Irr compared to the Non-irr plots (Table 2).

For both sites and all years, irrigation resulted in a quicker depletion of the ascosporic supply. The log-rank statistic and variants all had P-values below 0.001, except for SM in 2019 (log-rank statistic = 8.1, P = 0.004) and BO 2018 (Gehan-Wilcoxon = -2.5, P = 0.01, Peto & Peto = 6.4, P = 0.01). The AFT model with a Weibull distribution best fit the data. The ratio of survival times in SM in 2019 was close to 1, indicating equal survival time. The hazard ratio from the discrete-time Cox regression analysis gave similar results.

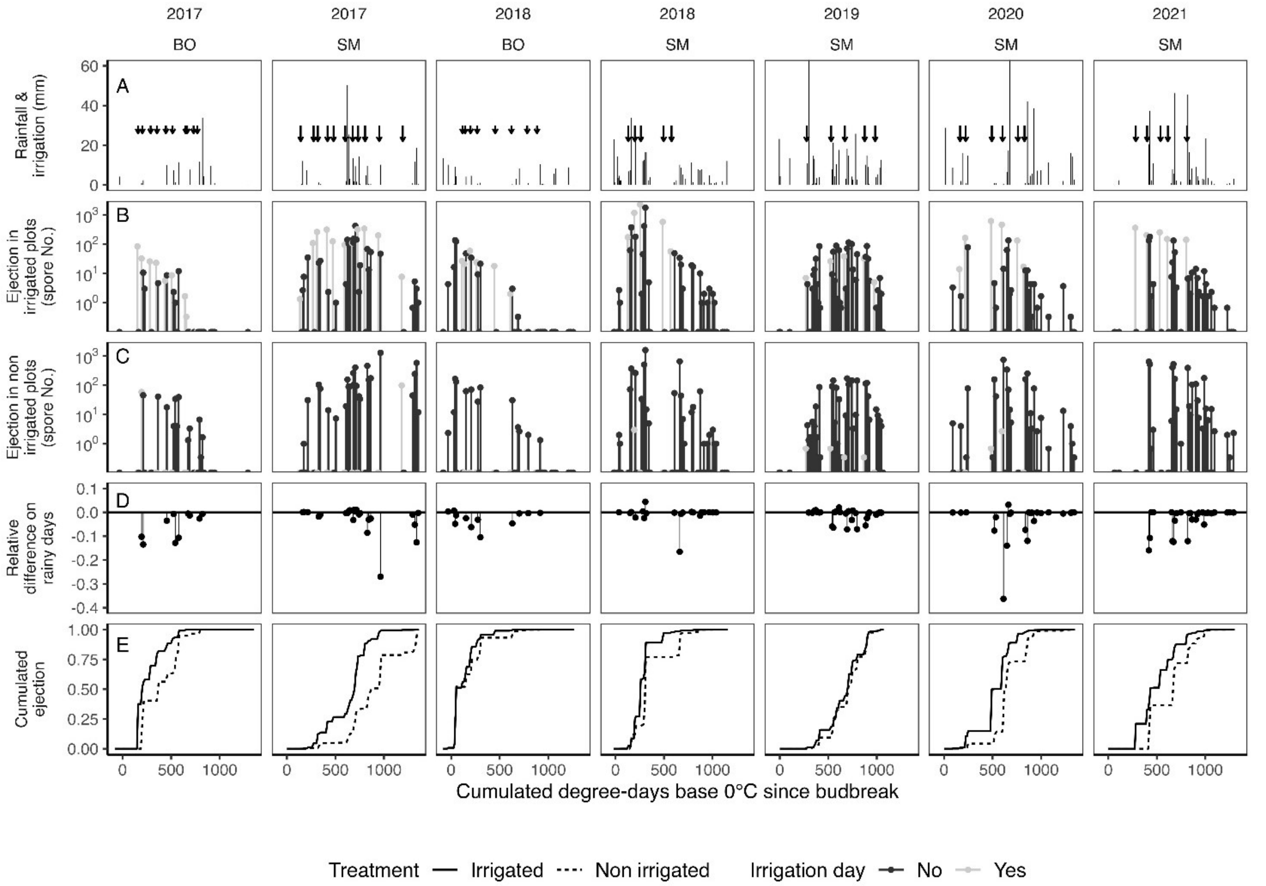

Fig. 2. Degree day accumulation (base temperature 0°C) since budbreak, in two locations over two (Bologna, BO) and five (San Michele all'Adige, SM) years, in relation to the following. A, daily rainfall (bars) and days of irrigation (arrows). Irrigation was four and eight mm over two hours per event in BO and SM, respectively; B and C, *Venturia inaequalis* ascospores released per day in irrigated (B) and non-irrigated plots (C), in rain days (black bars) and irrigation days (grey bars); D, relative difference of spores released in rain days in irrigated and non-irrigated plots (negative differences mean a higher number of spores in non-irrigated plots); E, proportion of cumulated seasonal trapping of ascospores in irrigated (continuous line) and non-irrigated (dashed line) plots.

Table 2. Trapping of ascospores of *Venturia inaequalis* in Bologna (BO, two years) and San Michele all'Adige (SM, five years) during irrigation and rain. Data refer to the period from time of budbreak to 95% of the season's spores being trapped.

Year	**Site**	**Number of irrigations**	**Total rain (mm)**	**Total spore number in irrigated plots**	**Total spore number in non-irrigated plots**	**DD in irrigated plots[a]**	**DD in non-irrigated plots[a]**	**Rainy days in irrigated plots[b]**	**Rainy days in non-irrigated plots[b]**	**Number of spores released by irrigation[c]**	**Percentage of spores released by irrigation[d]**
2017	BO	10	87.1	223.7	257.3	431.2	497.4	6	7	181.3	81.1
2017	SM	11	200.8	3407.7	4577.5	831.6	1181.8	21	23	1881.7	55.2
2018	BO	8	57.9	547.7	596.0	352.5	692.6	9	10	150.7	27.5
2018	SM	5	220.8	7255.7	3745.0	475.0	642.8	14	17	4251.0	58.6
2019	SM	5	332.2	1012.4	1400.3	654.4	654.4	26	26	108.7	10.7
2020	SM	6	293.0	1755.0	1999.0	684.1	858.8	13	19	1406.7	80.2
2021	SM	5	264.2	1719.3	3178.7	542.9	592.1	10	19	1108.0	64.4
Average				**2274.5**	**2250.5**	**567.4**	**731.4**	**14.1**	**17.3**	**1298.3**	**54.0**

[a] Summation of degree days (DD, base 0°C).

[b] Number of days with ≥ 0.2 mm rain.

[c] Total number of spores released by irrigations in the irrigated plots, counted as sum of spores trapped in irrigation days.

[d] Percentage of spores released by irrigations in the irrigated plots.

Effect of under-canopy irrigation on apple scab

The probability of a leaf showing scab symptoms was influenced by the fungicide treatment, irrigation, shoot type, and year. Since Blocks did not improve the model based on AIC, random effects were simplified to Shoots within Plots.

There were strong interactions for Fungicide × Year (LRT = 20, df = 4, $P < 0.001$), Irrigation × Year (LRT = 6.4, df = 2, P = 0.04), and Shoot Type × Year (LRT = 12, df = 2, P = 0.002). Fungicide and irrigation had an additive effect, but there was no overall interaction when years were pooled (LRT = 0.5, df = 2, P = 0.8). Similarly, fungicide (LRT = 2.3, df = 2, P = 0.3) and irrigation (LRT = 0.8, df = 1, P = 0.37) had the same effect on both bourse and vegetative shoots, and no interaction was observed for pooled years. However, the joint impact of fungicide and irrigation varied per year (Fungicide × Irrigation × Year, LRT = 21, df = 4, $P < 0.001$). Similarly, the triple interaction Irrigation × Shoot Type × Year was also significant (LRT = 12, df = 2, P = 0.002). The higher-level interaction did not improve the model (LRT = 8, df = 4, P = 0.08).

At the end of the season for ascospore release, apple leaf scab in the Untr Non-irr plots was lowest in 2021 and similar in 2020 and 2022 (Fig. 3). The Prev+Cur strategy was the most effective in all years, although the effect was lower in 2020 than in the other two years. The Cur strategy was equally effective in 2020 and 2022 and less effective in 2021. Irrigation in control plots was equally effective in 2020 and 2021, but less effective in 2022 (OR = 8.7, z = 4.8, $P < 0.001$). Scab on the vegetative shoots was higher than on bourse shoots for all three years, but differences between the two shoot types were more evident in 2022 (OR = 1.6, z = 4.3, $P < 0.001$) (Fig. 3).

Because of interactions, marginal means were used to simplify the interpretation of results. For all years and for both Irr vs. Non-irr, both Prev+Cur and Cur fungicides alone reduced scab compared to the Untr (no fungicide) control. The lowest efficacy observed (OR = 2.8, z = 3.2, P = 0.0013) was for Cur fungicides in Non-irr plots in 2021.

The best efficacy observed (OR = 168, z = 14, P < 0.0001), was for Prev+Cur in irrigated plots in 2022. In all years, Prev+Cur fungicides were better than only Cur (shoot types pooled). The smallest difference observed between these two treatments (OR = 2.2, z = 2.3, P = 0.02) was for Irr plots in 2020.

Similarly, for all years and for all fungicide strategies including the untreated control, Irr resulted in less leaf scab than Non-irr. The smallest OR observed (OR = 1.9, z = 2, P = 0.04) was for plots untreated with fungicides in 2022. Difference between Non-irr and Irr reached OR = 16 (z = 8.5, P < 0.0001) in plots treated with Prev+Cur in 2021.

For both 2020 (OR = 1.8, z = 1.8, P = 0.07) and 2021 (OR = 0.7, z= -1.3, P = 0.2), the reduced fungicide schedule in combination with irrigation (Cur Irr) resulted in a similar scab level as the full fungicide schedule in absence of irrigation (Prev+Cur Non-irr). Similarly, the reduced fungicide strategy in non-irrigated plots (Cur Non-irr) resulted in the same scab level as irrigated plots without fungicides (Untr Irr) in 2020 (OR = 0.7, z = -1.1, P = 0.28).

On average for the three years, the efficacy in reducing leaf scab of irrigation in non-fungicide treated plots (Untr Irr *vs*. Untr Non-irr) was 45%. Similarly, the reduction in leaf scab of Irr *vs*. Non-irr for Prev+Cur and Cur was 73% and 68%, respectively.

The incidence of fruit scab was influenced by year, irrigation, and fungicide treatments. In BV, Blocks did not improve the fruit scab model and were removed. Similarly, the Fungicide x Irrigation x Year interaction was non-significant (NS; LRT = 4, df = 4, P = 0.35).

Similar to foliar scab, efficacy of fungicide (LRT = 62, df = 4, P < 0.0001) and irrigation (LRT = 50, df = 2, P < 0.0001) varied with year. Contrary to foliar scab, fungicide efficacy on fruit varied depending if the plots were irrigated or not (LRT = 10, df = 2, P = 0.005).

Fruit scab in Untr Non-irr plots was highest in 2020 (Fig. 4). The Prev+Cur strategy had the best efficacy in all years, but it was weaker in 2021 (OR = 11, z = 3, P = 0.003) and 2022 (OR = 9, z = 3,

$P = 0.005$) than in 2020. The Cur strategy for fruit scab was more effective in 2020 than in 2021 ($OR = 6$, $z = 7$, $P < 0.001$) or 2022 ($OR = 2.7$, $z = 4$, $P < 0.001$). Irrigation was effective in all years, but its effect on fruit scab was greater in 2020 than in 2021 ($OR = 4$, $z = 6$, $P < 0.001$) or 2022 ($OR = 3.2$, $z = 5$, $P < 0.001$) (Fig. 4).

In fungicide treated plots, scab reduction on fruit from irrigation was more efficient than the simple additive effect. The effect was slightly larger for Prev+Cur ($OR = 0.3$, $z = -2$, $P = 0.05$) than for Cur ($OR = 0.6$, $z = -2.4$, $P = 0.01$).

Overall, averaging the year effect, Prev+Cur ($OR = 8.5$, $z = 3.4$, $P < 0.001$) and Cur ($OR = 4.3$, $z = 6.7$, $P < 0.001$) had a better effect on fruit scab in Irr plots vs. Non-irr plots, compared to the no fungicide control ($OR = 2.4$, $z = 9.6$, $P < 0.001$). The efficacy of Cur vs. Prev+Cur was weaker in Non-irr plots ($OR = 6.4$, $z = 6$, $P < 0.001$) than Irr plots ($OR = 13$, $z = 4$, $P < 0.001$). Cur Irr and Prev+Cur Non-irr showed a similar effect on fruit scab ($OR = 1.5$, $z = 1.1$, $P = 0.26$).

On average for the three years, the efficacy in reducing fruit scab of irrigation in non-fungicide treated plots (Untr Irr *vs*. Untr Non-irr) was 48%. Efficacy of irrigation in Prev+Cur and Cur plots was 59% and 71%, respectively.

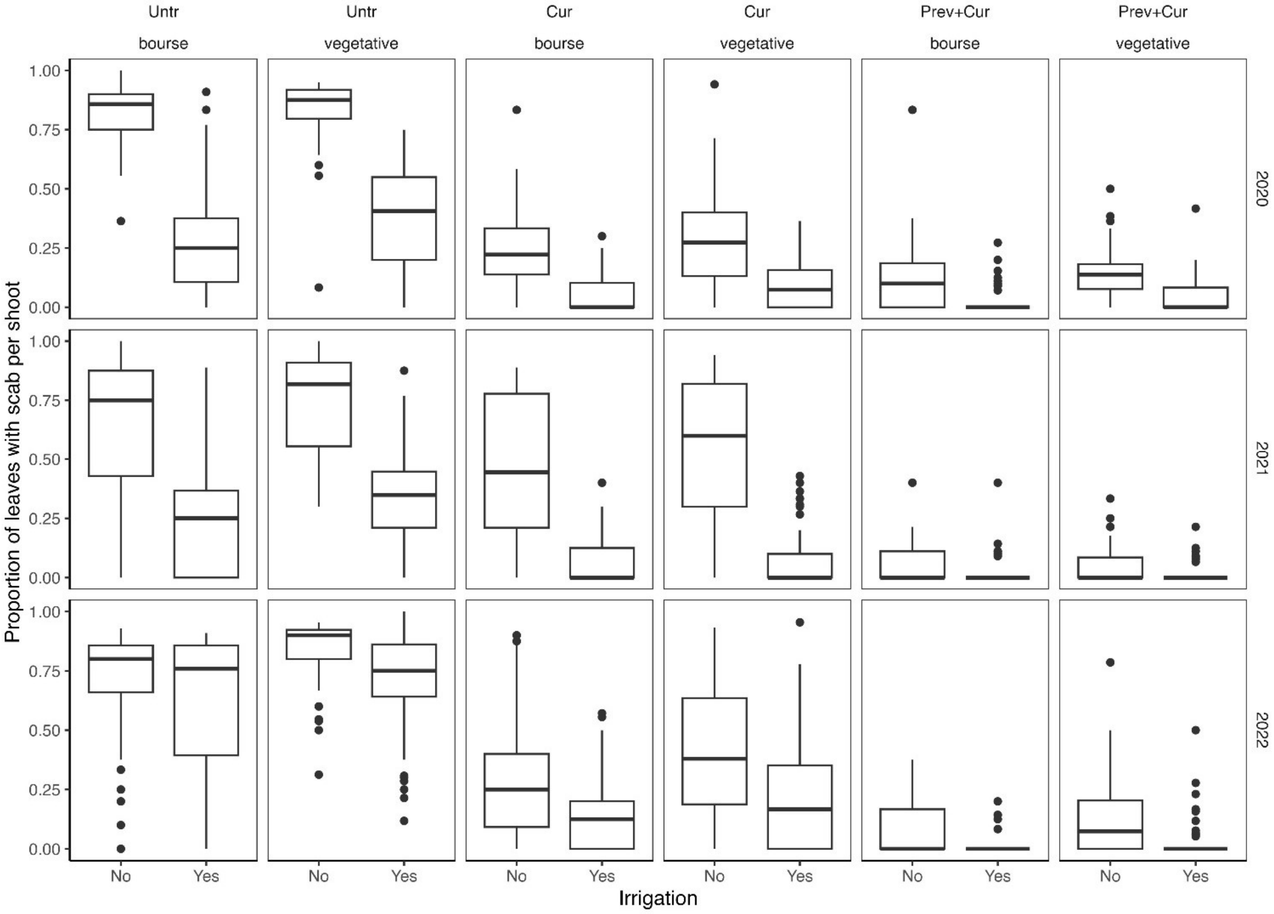

Fig. 3. Proportion of leaves with scab per bourse shoot or vegetative shoot assessed in mid to late June for curative (Cur), preventive+curative (Prev+Cur) fungicide treatments and untreated control (Untr), in non-irrigated (No) and irrigated (Yes) plots. Results of a three-year trial (2020 – 2022) in Borgo Valsugana, Trentino-Alto Adige Region.

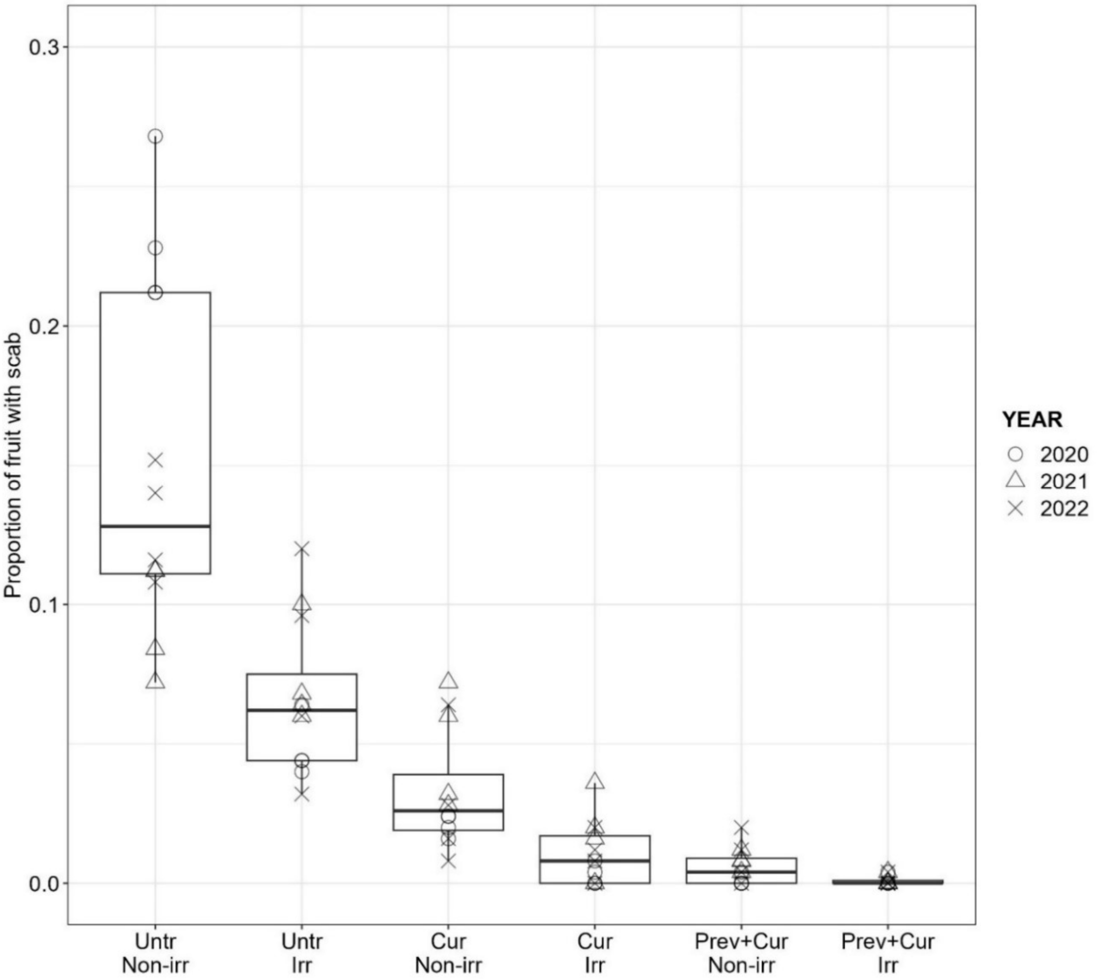

Fig. 4. Proportion of fruit with scab for the untreated control (Untr), curative (Cur) and preventive + curative (Prev+Cur) fungicide treatments in non-irrigated (Non-irr) and irrigated (Irr) plots. Different symbols show the fruit incidences of the three-year (2020-2022) trials carried out in Borgo Valsugana, Trentino-Alto Adige Region.

Effect of overhead irrigation on apple scab

The average scab incidence on leaves in Untr Non-irr plots ranged from 22 to 27% in the three-year trial. Incidence in Untr Irr plots was lower in 2018 compared to 2017 and 2019 (Fig. 5A). No apple scab infection on leaves was detected in fungicide treated plots in 2018 and 2019, and a very low incidence (0.13%) was observed in 2017 in Prev+Cur Non-irr plots (Fig. 5A). Irrigation in Untr plots reduced the percentage of scabbed leaves, and a higher efficacy (87%) was observed in 2018 (Fig. 5A).

Fungicide and irrigation had independent effect on leaf scab (no interaction) (LRT = 1.2, df = 1, P = 0.27), and fungicide efficacy was similar across years (LRT = 4, df = 2, P = 0.14). However, the efficacy of irrigation varied with year (LRT = 61, df = 1, P < 0.001). In 2018, irrigation worked better than in 2017 (OR = 0.28, z = 7, P < 0.001), whereas scab reduction due to irrigation in 2019 was similar to 2017 (OR = 0.8, z = 1.5, P = 0.13). Pooling the effect of fungicides, the OR values of scab incidence between non irrigated and irrigated plots were 2.8 (2017), 9.8 (2018) and 3.5 (2019).

Fruit showed a higher incidence of infection in 2018 in Untr Non-irr plots (average of 20%) (Fig. 5B). Only in 2017 apple scab was detected in the Prev+Cur treatments (1% incidence in Prev+Cur Non-irr; Fig. 5B).

Because fruit scab levels were low and caused fitted probabilities to be numerically equal to 0, logistic regression models for fruit scab in MB were modified and the reduced-bias estimates were computed using the brglm2 R package. Similar to leaf scab, fungicide and irrigation had independent action on fruit scab (LRT = 0, df = 1, P = 1), and fungicide had similar effect each year (LRT = 0.3, df = 2, P = 0.87). Again, irrigation likely had a varying effect between years (LRT = 19, df = 2, P < 0.001).

Irrigation reduced fruit scab all years, but irrigation in 2018 (OR = 0.023, z = 2.6, P = 0.01) was more efficient than in 2017. Irrigation in 2019 and 2017 had the same magnitude of effect (OR = 0.68, z = 0.7, P = 0.5). Overall, the average efficacy (2017 to 2019) of irrigation in reducing apple scab in Untr plots was 69% for leaves and 73% for fruit.

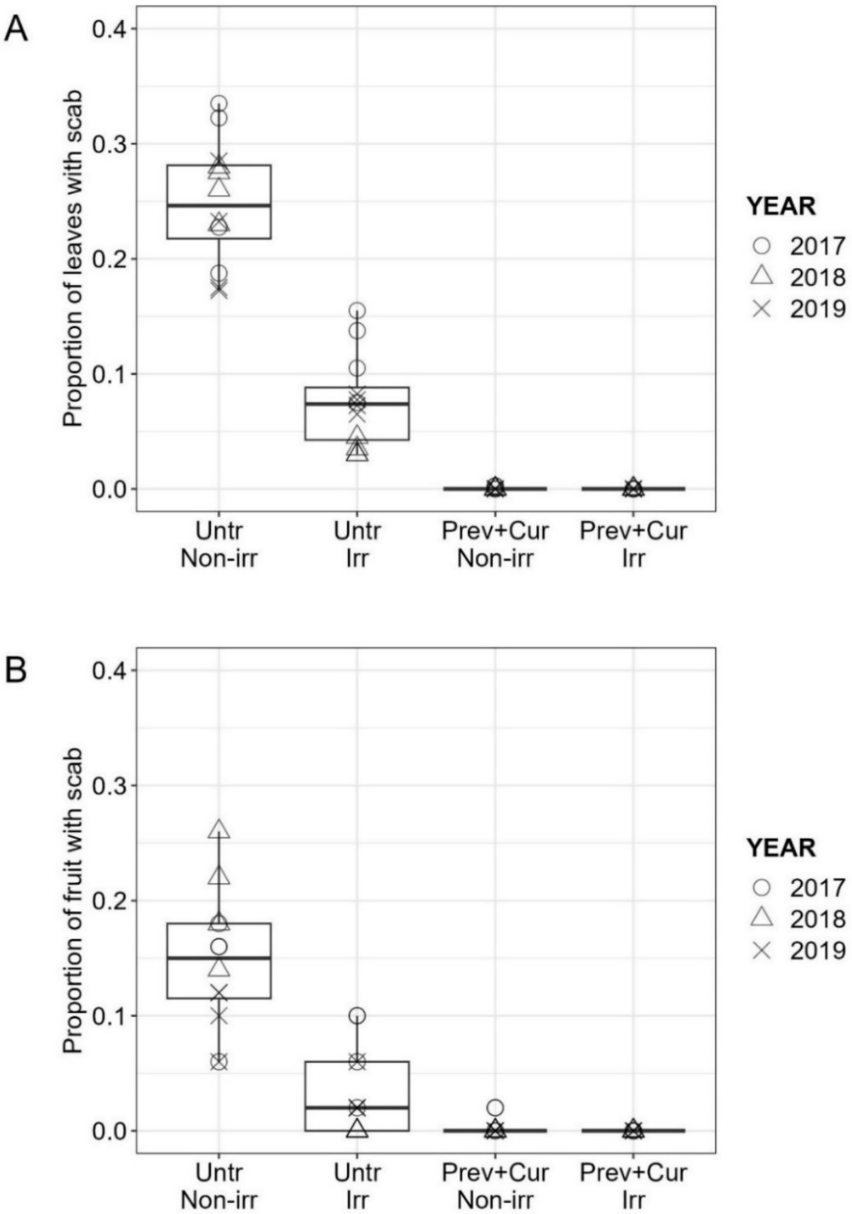

Fig. 5. Proportion of leaves (A) and fruit (B) with scab for fungicide (Prev+Cur) and no fungicide (Untr) treatments, in non-irrigated (Non-irr) and irrigated (Irr) plots. Different symbols show the leaf or fruit incidence in trials over three years (2017-2019) in Malborghetto di Boara, Emilia-Romagna Region.

Discussion

The present study, carried out for several years (2017 to 2022) in two important apple producing regions in Italy, demonstrates that irrigation performed ahead of forecasted rain may release a substantial amount of *V. inaequalis* ascospores on the day of irrigation and fewer spores in rainy days following irrigation. Furthermore, irrigation causes an earlier depletion of the ascospore supply in the leaf litter compared to no irrigation. In field trials, irrigation reduced the incidence of apple scab on leaves and fruit by more than 50% on average. Efficacy of irrigation in reducing scab was observed both in fungicide treated and untreated plots and by using both under-canopy and overhead irrigation systems. We are not aware of any other studies where the effect of irrigation on ascospore release of *V. inaequalis* and subsequent scab development has been studied to this extent.

Due to the sunny and dry weather at time of irrigation, the leaf litter dried in a few hours (commonly within two hours; Prodorutti, data not shown), and spore discharge ceased. Irrigation triggered spore release on the day of irrigation, and no or very few spores were found the day after irrigation if rain did not occur. Brief 2 h irrigations carried out around noon and using a low irrigation rate, seemed to be efficient in releasing a high number of ascospores. Our findings are consistent with MacHardy and Gadoury (1986) who assessed the peak of ascospore release in the middle of the day, and with Aylor and Sutton (1992) who stated that low rates of rainfall were able to release a significant amount of spores.

The efficacy of irrigation on spore discharge was influenced by seasonal weather conditions. For example, in SM in 2019, the low percentage (11%) of ejected spores during irrigation events could be due both to the high number of rainy days and high total precipitation during the season for ascospore release.

Irrigation caused pseudothecia to empty faster, and this effect was consistent during the four-year trial. It was also consistent with the faster depletion of the ascospore supply in irrigated plots in both locations.

The use of targeted irrigation is probably best suited for areas where ascospore release coincides with extended dry periods. In such conditions, irrigation would induce the discharge of high numbers of spores that had matured since the previous rain. Conversely, in climates where rain is more frequent and abundant, irrigation is likely less effective in reducing inoculum.

In most of the cases in this study, weather forecasts were correct, and the irrigations were carried out 24-48 h before rain occurred. Therefore, spores were ejected under conditions not suitable for infection. If rain events had occurred closer to irrigation, the planned spore ejection could have increased the risk of infections, because spores landing on plants could still be viable if rain occurred. Irrigation should therefore be carefully timed according to weather forecasts. The few cases when spores were trapped in Non-irr plots during irrigation days can be explained by the fact that in BO in 2017 and SM in 2017, a light rain (0.9 mm and <0.2 mm/below the detection threshold of the rain gauge, respectively) which was not forecasted, occurred in the evening of the irrigation day, and in SM in 2018, 2019 and 2020 heavy dew occurred during nights when a few ascospores were captured in the Non-irr plots.

In our trials, the efficacy of irrigation resulted in high numbers of spores released. We also evaluated the effect of irrigation on spore release, on pseudothecial development and on apple scab during the ascospore release season. In a Danish study, Korsgaard (2014, 2016) reported from several trials using irrigation as a strategy against apple scab. Irrigations lasting 5-10 minutes two times a day with one hour interval, using sprinklers with a flow rate of 90 - 160 l/h and providing 0.2-1.4 mm of water per irrigation, resulted in a low number of spores trapped. Irrigations of a few minutes with a high flow rate with sprinklers released spores, but this may not have been sufficient to ensure spore ejection over a period long enough to deplete the supply of mature spores. Additionally, water droplets in a high volume of water may have removed a significant number of spores from the air and thus reduced the number trapped. By irrigating for a longer time with lower flow rate sprinklers (35 l/h), as in the present study, spore ejection and the number of airborne spores was higher. Furthermore, in the study by Korsgaard (2014, 2016), the wet area following sprinkling did not cover the whole orchard area, and thus the potential ascospore producing area following a rain was larger than that following irrigation. This could have affected the total spore trapping.

In an investigation in France, it was stated that at least 5 h of irrigation would be necessary to exhaust the supply of mature spores (Libourel 2006). The author also concluded that to be effective, the irrigation must be carried out as close as possible to the forecasted rain and must be followed by at least 8 h dry plant tissue to ensure that the minimum time of wetting for scab infection is not reached. In our study, spores started to be released within a few minutes after the beginning of irrigation and continued for 2-3 h after irrigation had stopped, releasing a large amount of the season's spores. The thickness of the leaf litter may influence spore release. A layer of leaf litter of up to 20 cm, as used by Libourel (2006), may have required a longer wetting period to release spores as compared to the present study and the one by Korsgaard (2016), where only one layer of leaves was used. Moreover, we demonstrated that it is not necessary to irrigate as close as possible to the forecasted rain to reduce the spore potential. The method described by Libourel (2006) could increase the risk of scab infections if the plants remain wet after overhead irrigation, and the dry period between irrigation and rain is not sufficiently long.

In Uruguay, daytime and nighttime irrigations of apple leaf litter for up to four successive days were evaluated (Martinez et al., 2021). The percentage of ascospores released during the first day of irrigation varied between 32 and 77%, with an average of 53%, and the spore numbers typically decreased with successive irrigation days. It was concluded that at least three successive daily irrigations should be carried out before a forecasted rainy day to be effective (Martinez et al., 2021). However, this strategy seems difficult to apply in practice, since at least 3-4 days of dry weather are necessary before a rain. Furthermore, this method risks irrigating close to a rain event, potentially increasing survivability of previously ejected spores.

Our field trials were carried out by using either an under-canopy irrigation system (in BV) especially prepared for the study, or with an overhead irrigation system (in MB) already installed in the orchard and used conventionally for irrigation and frost protection. We could therefore confirm the efficacy of sprinkler irrigation in two different ways that could be easily applied in commercial orchards, and we demonstrated that both systems substantially reduced apple scab infections under field conditions. In the under-canopy trials, released spores, even if they had landed on plants, would not have been exposed to wet conditions necessary for infections to occur. In the overhead irrigation trials, irrigation of two hours in the middle of the day was not sufficiently long enough for infections to occur. The plants dried fast (in ca. two hours in sunny days), and treatments took place at least 24 h prior to the following rain.

Under-canopy irrigation reduced the incidence of scabbed leaves in each control strategy (Untr, Cur, Prev+Cur) and for both shoot types, even if a lower efficacy was observed in Untr Irr plots in 2022, compared to 2020 and 2021. No Fungicide x Irrigation interaction was observed on leaves in BV, meaning that the irrigation effect was the same for any control strategy, and there was no synergy between fungicide and irrigation. Fungicide and irrigation acted independently on leaf scab incidence and therefore seem to be complementary: in all cases irrigation improved the efficacy of fungicides. Overall, there was no significant difference in scabbed leaves between Cur Irr and Prev+Cur Non-irr treatments. This clearly indicates that irrigation could simplify the fungicide strategy, by reducing the number of treatments. The 2022 season was very dry in March and April, and rain and ascospore infections occurred late, starting from the end of April. The beginning of June (after the ascospores were released) was also rainy, and the last irrigation was carried out on 12 May, just prior to a severe infection forecasted by RIMpro. These climatic conditions could explain the lower efficacy of irrigation in Untr control plots in 2022.

On fruit in BV, irrigation was effective in all years and in each control strategy. Contrary to the leaf treatments, fungicide and irrigation had a significant interaction. Fungicide treatments (Prev+Cur and Cur) were more effective in Irr plots, and especially the Cur treatment showed the best effect in combination with irrigation. Scab in Untr Non-irr plots was highest in 2020, and the effect of irrigation in reducing infections was greater that year. Overall, treatment differences were larger in 2020. Similar to the leaves, no significant difference on scabbed fruit was observed between Cur Irr and Prev+Cur Non-irr treatments.

Due to the full fungicide schedule adopted in the trials in MB, treated plots showed no scab infection or a very low incidence of scab on either leaves or fruit at the end of the ascospore release season. Fungicide and irrigation had independent effects on apple scab (no interaction). In Untr plots, irrigation resulted in a significant disease reduction, and the best efficacy was observed in 2018 both on leaves and fruit, although there was a higher incidence assessed that year compared to 2017 and 2019.

In our study, we used irrigation in the period when mature ascospores were present, starting after bud break, with the aim to release matured spores before rain and therefore reduce infections. However, this work opens the possibility of using targeted irrigation in late winter, before bud break, to promote the maturation of pseudothecia and ascospores. Stensvand et al. (2006) demonstrated that weather conditions prior to bud break can affect the onset of spore release and that frequent rain in the month before bud break increased the proportion of mature spores at bud break. Roubal & Nicot (2016) stated that the duration of pseudothecial maturation was influenced by winter rain and an earlier onset of the ascospore release period was observed when there are many rainy days. Therefore, starting irrigation ahead of bud break could be considered as a method to accelerate ascospore maturation in order to reduce the period during which high ejection and fast growth occurs. To demonstrate an effect of irrigations ahead of apple bud break, further trials are needed.

In the present experiments, sprinkler irrigation took place with low water intensities for two hours; however, this method is applicable only if water supply is not a limiting factor in spring. In conclusion, targeted irrigation of ascospores of *V. inaequalis* could represent a sustainable and easily applicable method to reduce the incidence of apple scab. Irrigation should be integrated with fungicide control strategies, to reduce the number of treatments or increase their efficacy.

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Supplementary material

Supplementary Table S1. Fungicide applications carried out from 2020 to 2022 in the field trial in Borgo Valsugana in Trentino-Alto Adige Region.

Date	Product	Active ingredient	Dose of product/ha	Treatment
2020-03-20	Poltiglia Disperss	Copper (Bordeaux mixture)	1500 g	all treatments
2020-03-27	Poltiglia Disperss	Copper (Bordeaux mixture)	1500 g	all treatments
2020-04-10	Delan 70 WG	Dithianon	750 g	Prev+Cur
2020-04-18	Delan 70 WG	Dithianon	750 g	Prev+Cur
2020-04-25	Delan 70 WG	Dithianon	750 g	Prev+Cur
2020-04-28	Delan 70 WG	Dithianon	750 g	Prev+Cur
	Score 10 WG	Difenoconazole	550 g	Prev+Cur, Cur
2020-05-02	Delan 70 WG	Dithianon	750 g	Prev+Cur
	Difenzone	Difenoconazole	225 ml	Prev+Cur, Cur
2020-05-09	Delan 70 WG	Dithianon	750 g	Prev+Cur
2020-05-13	Delan 70 WG	Dithianon	750 g	Prev+Cur
	Difenzone	Difenoconazole	225 ml	Prev+Cur, Cur
2021-03-10	Poltiglia Disperss	Copper (Bordeaux mixture)	2250 g	all treatments
2021-04-10	Delan 70 WG	Dithianon	750 g	Prev+Cur
2021-04-14	Pyrus 400 SC	Pyrimethanil	1000 ml	Prev+Cur, Cur
2021-04-19	Delan 70 WG	Dithianon	750 g	Prev+Cur
2021-04-24	Delan 70 WG	Dithianon	750 g	Prev+Cur
2021-04-28	Delan 70 WG	Dithianon	750 g	Prev+Cur
	Pyrus 400 SC	Pyrimethanil	1000 ml	Prev+Cur, Cur
2021-05-01	Pyrus 400 SC	Pyrimethanil	1000 ml	Prev+Cur, Cur
2021-05-06	Delan 70 WG	Dithianon	750 g	Prev+Cur
2021-05-09	Delan 70 WG	Dithianon	750 g	Prev+Cur
2021-05-13	Difenzone	Difenoconazole	225 ml	Prev+Cur, Cur
2021-05-22	Delan 70 WG	Dithianon	750 g	Prev+Cur
2021-05-26	Difenzone	Difenoconazole	225 ml	Prev+Cur, Cur
2021-06-05	Delan 70 WG	Dithianon	750 g	Prev+Cur

	Score 10 WG	Difenoconazole	550 g	Prev+Cur, Cur
2022-03-29	Poltiglia Disperss	Copper (Bordeaux mixture)	2250 g	all treatments
2022-04-15	Delan 70 WG	Dithianon	750 g	Prev+Cur
2022-04-21	Delan 70 WG	Dithianon	750 g	Prev+Cur
2022-04-23	Pyrus 400 SC	Pyrimethanil	1000 ml	Prev+Cur, Cur
2022-04-29	Delan 70 WG	Dithianon	750 g	Prev+Cur
2022-05-02	Difcor	Difenoconazole	225 ml	Prev+Cur, Cur
2022-05-03	Delan 70 WG	Dithianon	750 g	Prev+Cur
2022-05-07	Difcor	Difenoconazole	225 ml	Prev+Cur, Cur
2022-05-12	Delan 70 WG	Dithianon	750 g	Prev+Cur

Supplementary Table S2. Fungicide applications carried out from 2017 to 2019 in the field trial in Malborghetto di Boara in Emilia-Romagna Region. All applications refer to the fungicide treatment (Preventive+Curative strategy).

Date	**Product**	**Active ingredient**	**Dose of product/ha**
2017-03-03	Cupravit 35 WG	Copper oxychloride	5000 g
2017-03-10	Polyram DF	Methiram	1500 g
	Oxycur	Copper oxychloride	2000 g
2017-03-16	Polyram DF	Methiram	2600 g
2017-03-21	Polyram DF	Methiram	1000 g
	Penncozeb DG	Mancozeb	2100 g
2017-03-24	Delan 70 WG	Dithianon	1500 g
	Chorus	Cyprodinil	500 g
2017-03-27	Delan 70 WG	Dithianon	1500 g
2017-04-01	Delan 70 WG	Dithianon	1500 g
	Chorus	Cyprodinil	500 g
2017-04-04	Delan 70 WG	Dithianon	700 g
	Fontelis	Penthiopyrad	900 ml
2017-04-10	Delan 70 WG	Dithianon	1500 g
	Chorus	Cyprodinil	500 g
2017-04-14	Fontelis	Penthiopyrad	900 ml
	Delan 70 WG	Dithianon	750 g
2017-04-18	Delan 70 WG	Dithianon	1500 g
	Score 10 WG	Difenoconazole	550 g
2017-04-24	Banjo	Fluazinam	1000 ml
2017-04-28	Banjo	Fluazinam	1000 ml
	Score 10 WG	Difenoconazole	600 g
2017-05-03	Merpan 80 WDG	Captan	2000 g
2017-05-07	Banjo	Fluazinam	1000 ml
	Score 10 WG	Difenoconazole	600 g
2018-03-14	Cupravit 35 WG	Copper oxychloride	4000 g
2018-03-23	Cupravit 35 WG	Copper oxychloride	2000 g
	Polyram DF	Methiram	2000 g
2018-03-28	Penncozeb DG	Mancozeb	2100 g
	Poltiglia Disperss	Copper (Bordeaux mixture)	3000 g
2018-03-30	Vision Plus	Dithianon+Pyrimethanil	1200 ml
	Delan 70 WG	Dithianon	500 g
2018-04-03	Vision Plus	Dithianon+Pyrimethanil	1200 ml
	Delan 70 WG	Dithianon	500 g
2018-04-05	Vision Plus	Dithianon+Pyrimethanil	1200 ml
	Delan 70 WG	Dithianon	500 g
2018-04-08	Sercadis	Fluxapyroxad	300 ml

	Delan PRO	Dithianon+Potassium phosphonates	2500 ml
2018-04-13	Sercadis	Fluxapyroxad	300 ml
	Delan PRO	Dithianon+Potassium phosphonates	2500 ml
	Score 10 WG	Difenoconazole	600 g
2018-04-19	Delan 70 WG	Dithianon	750 g
	Chorus	Cyprodinil	500 g
2018-04-27	Delan 70 WG	Dithianon	750 g
	Chorus	Cyprodinil	500 g
2018-04-30	Sercadis	Fluxapyroxad	300 ml
	Delan 70 WG	Dithianon	750 g
2018-05-04	Nando Maxi	Fluazinam	1500 ml
	Score 10 WG	Difenoconazole	600 g
2018-05-09	Merpan 80 WDG	Captan	2000 g
2018-05-11	Nando Maxi	Fluazinam	1500 ml
2018-05-18	Merpan 80 WDG	Captan	2000 g
2018-05-25	Merpan 80 WDG	Captan	2000 g
	Score 10 WG	Difenoconazole	600 g
2018-06-01	Merpan 80 WDG	Captan	2000 g
	Score 10 WG	Difenoconazole	600 g
2019-03-25	Vision Plus	Dithianon+Pyrimethanil	1200 ml
	Delan 70 WG	Dithianon	500 g
	Century Pro	Potassium phosphonates	1900 ml
2019-03-29	Vision Plus	Dithianon+Pyrimethanil	1200 m
2019-04-02	Fontelis	Penthiopyrad	900 ml
	Delan 70 WG	Dithianon	750 g
2019-04-06	Delan PRO	Dithianon+Potassium phosphonates	2500 ml
	Score 10 WG	Difenoconazole	600 g
2019-04-08	Fontelis	Penthiopyrad	900 ml
	Delan 70 WG	Dithianon	750 g
2019-04-12	Delan PRO	Dithianon+Potassium phosphonates	2500 ml
	Score 10 WG	Difenoconazole	600 g
2019-04-19	Sercadis	Fluxapyroxad	300 ml
	Delan 70 WG	Dithianon	750 g
	Century Pro	Potassium phosphonates	1900 ml
2019-04-24	Nando Maxi	Fluazinam	1500 ml
	Century Pro	Potassium phosphonates	1900 ml
	Score 10 WG	Difenoconazole	600 g
2019-04-30	Sercadis	Fluxapyroxad	300 ml
	Delan 70 WG	Dithianon	500 g
	Score 10 WG	Difenoconazole	600 g
2019-05-04	Merpan 80 WDG	Captan	2000 g
2019-05-06	Nando Maxi	Fluazinam	1500 ml
	Score 10 WG	Difenoconazole	600 g
2019-05-10	Merpan 80 WDG	Captan	2000 g

	Score 10 WG	Difenoconazole	600 g
2019-05-14	Merpan 80 WDG	Captan	2000 g
	Score 10 WG	Difenoconazole	750 g

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 infections, 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 hours 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 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 and Stensvand, 2014). Mature ascospores are released in spring when pseudothecia have been moistened by rain or heavy dew, and then they 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 (Mills, 1944; MacHardy, 1996; Stensvand et al., 1997, Philion et al., 2020). 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 and Anagnostakis, 1991). Ascospores are mostly released in daylight hours (Brook, 1969;

Brook, 1975; MacHardy and Gadoury, 1986; Gadoury et al., 1998; Rossi et al., 2001), and the peak of the release commonly occurs in the middle of the day (MacHardy and Gadoury, 1986).

The average dimensions of ascospores of *V. inaequalis* are 6 ×13 µm (Aylor and Kyiomoto, 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 and 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 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.).

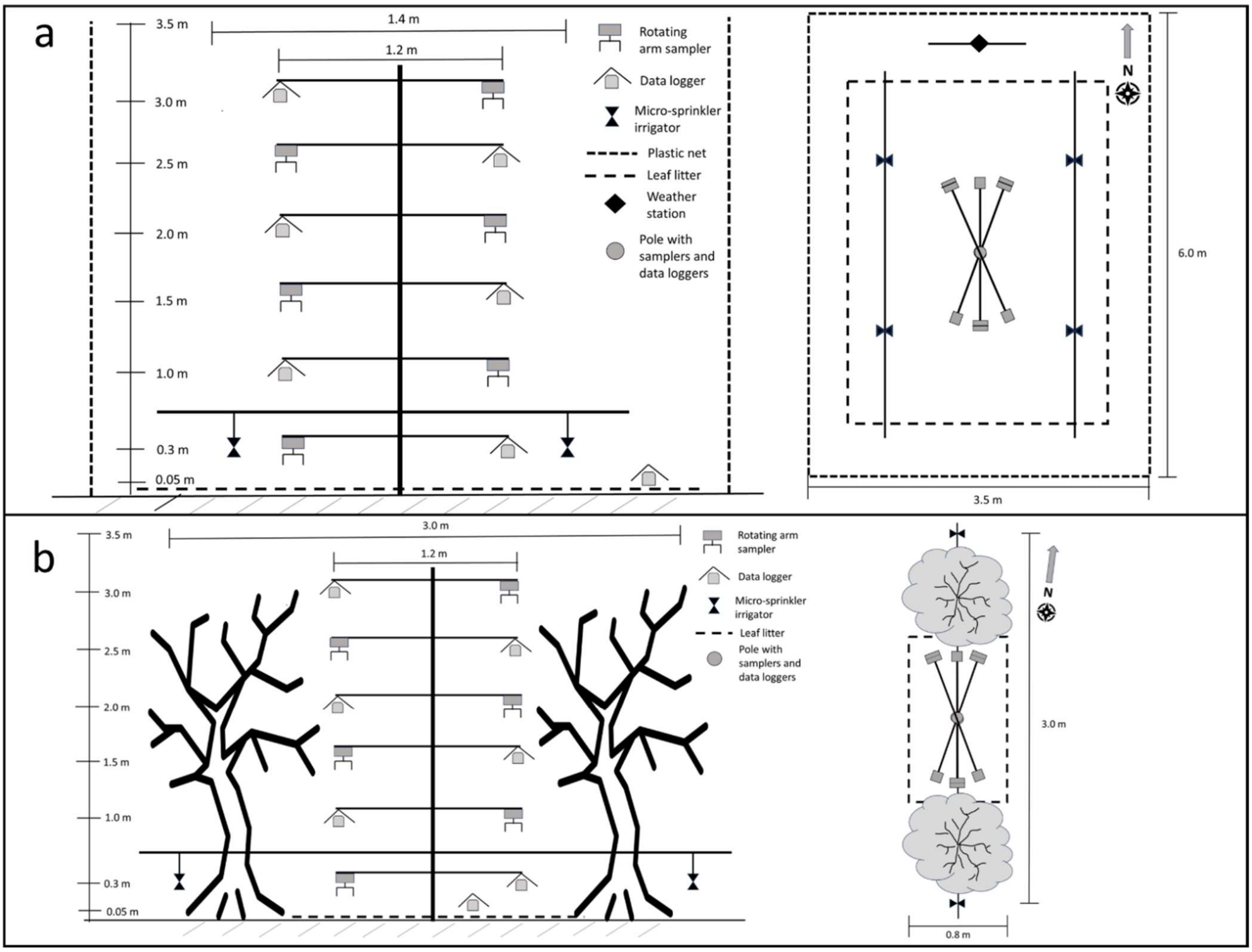

Fig. 1 a) Lateral and overhead view of the experimental set-ups with a wind fence in San Michele all'Adige (NO WIND) and b) exposed to natural wind in an orchard in Cles (WIND). The dimensions of the fence, of the structure bringing data loggers and rotating arm samplers and of irrigator distance are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

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 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 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.

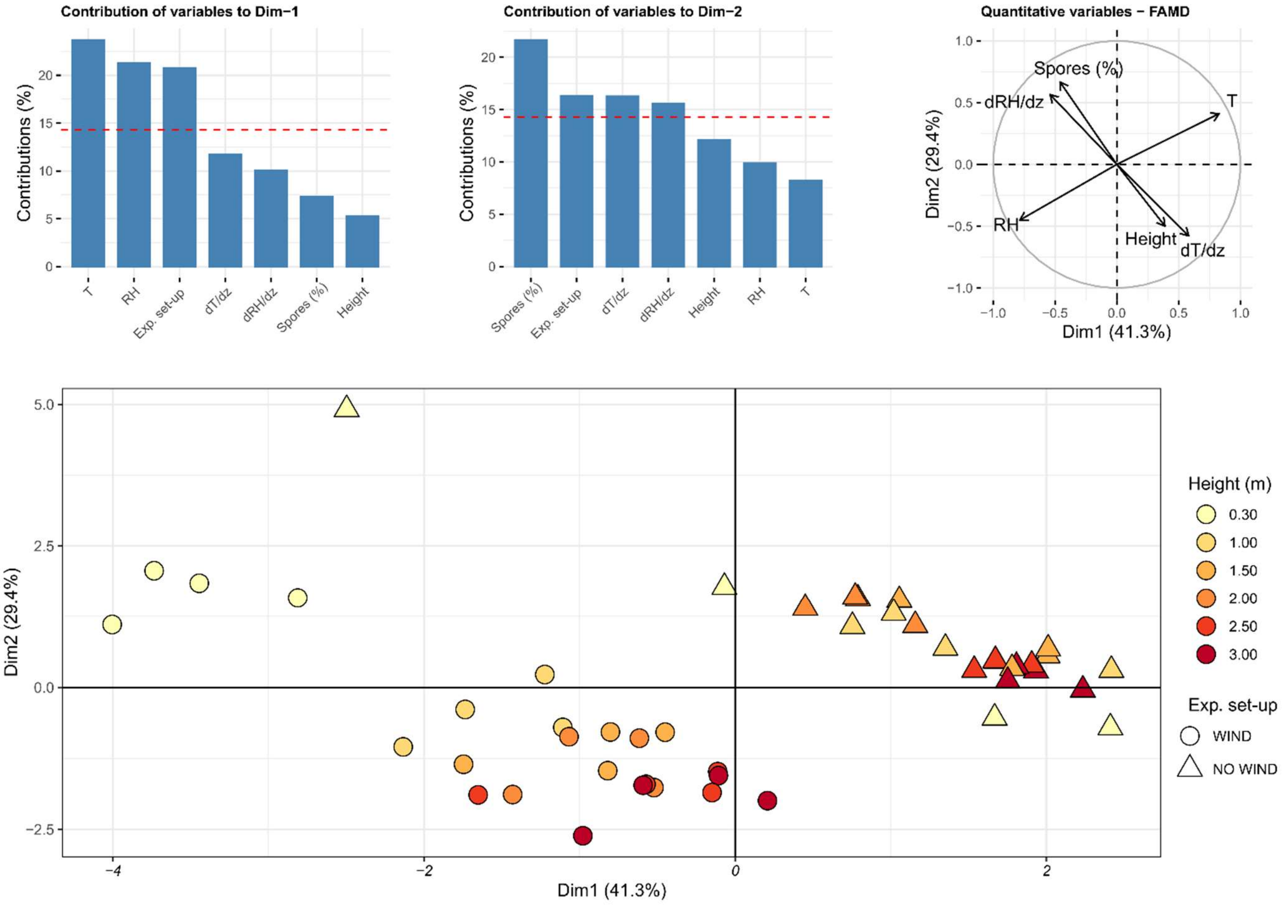

Fig. 2 Factor Analysis of Mixed Data (FAMD) of all factors involved in the study: percentage of ascospores of *Venturia inaequalis* (Spores %), temperature (T), relative humidity (RH), T and RH vertical gradients (dT/dz and dRH/dz, respectively), height (m) and experimental set-up (WIND and NO WIND). 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

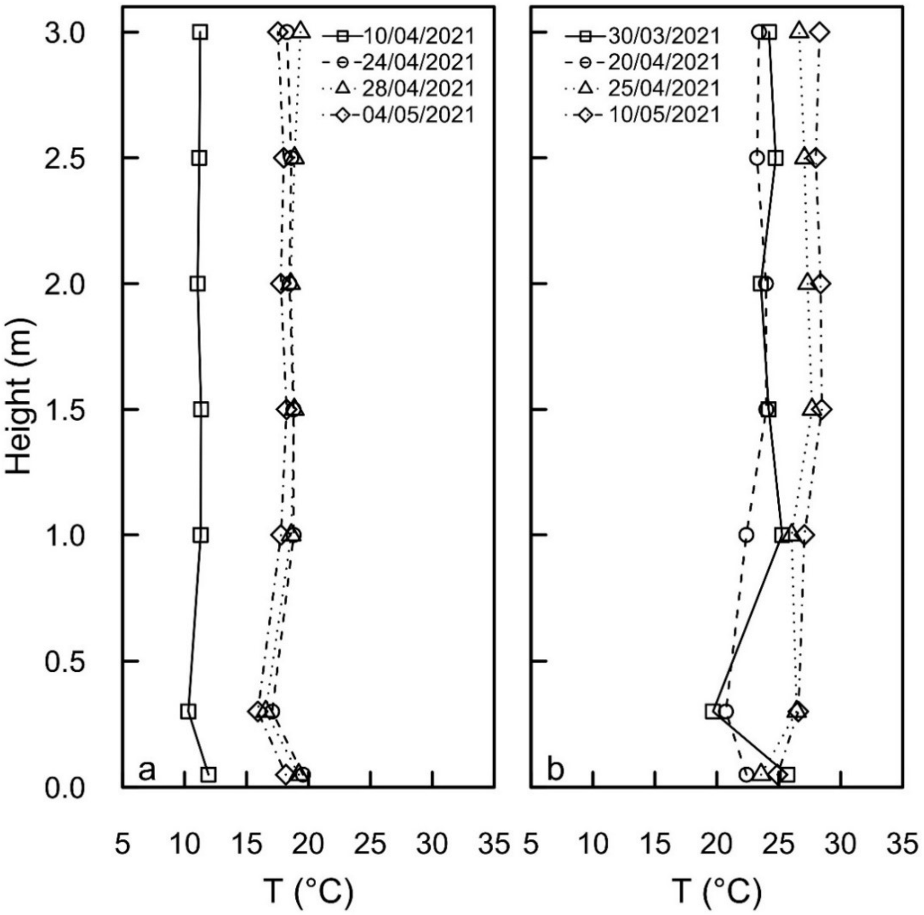

Fig. 3 Temperatures (T) recorded during the four trials (marked with dates) with under-canopy sprinkling to release ascospores of *Venturia inaequalis,* in presence of natural wind (WIND, a) and in absence of wind (NO WIND, b) at different heights from the ground. Data represents the average temperature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

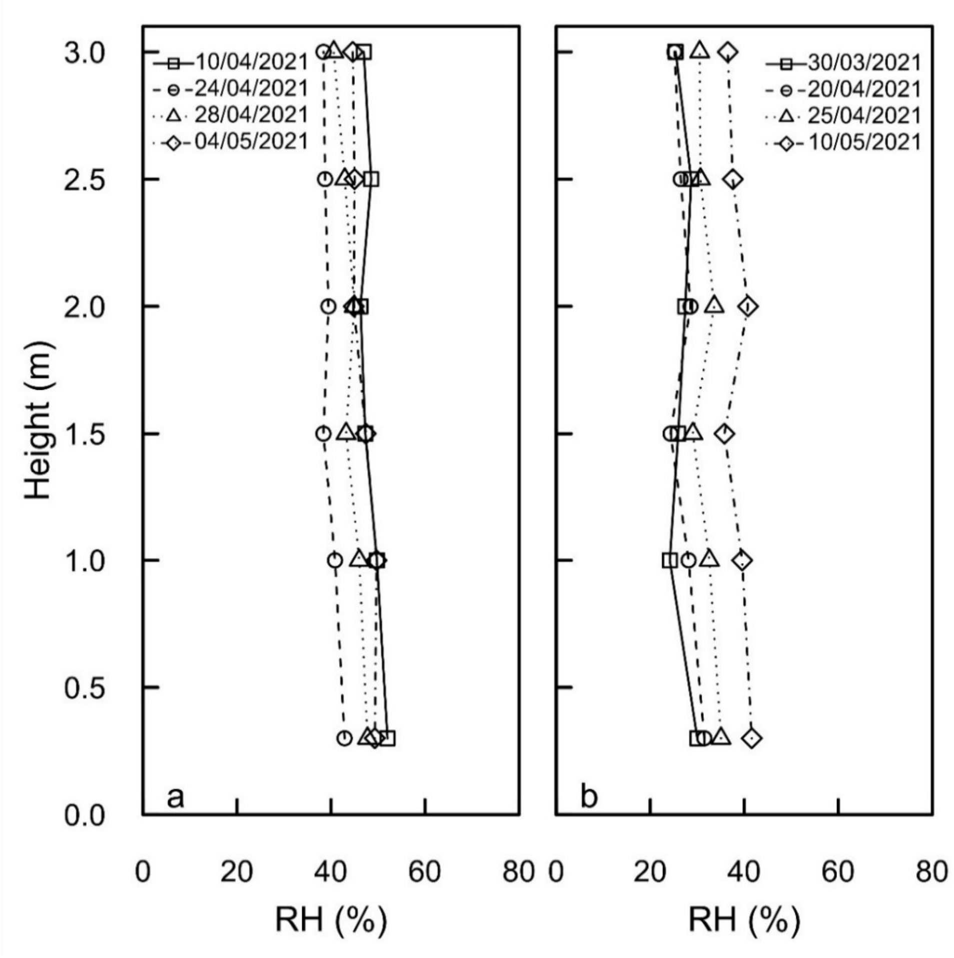

Fig. 4 Relative humidity (RH) recorded during the four trials (marked with dates) with under-canopy sprinkling to release ascospores of *Venturia inaequalis,* in presence of natural wind (WIND, a) and in absence of wind (NO WIND, b) at different heights from the ground. Data represents the average relative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

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). 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.

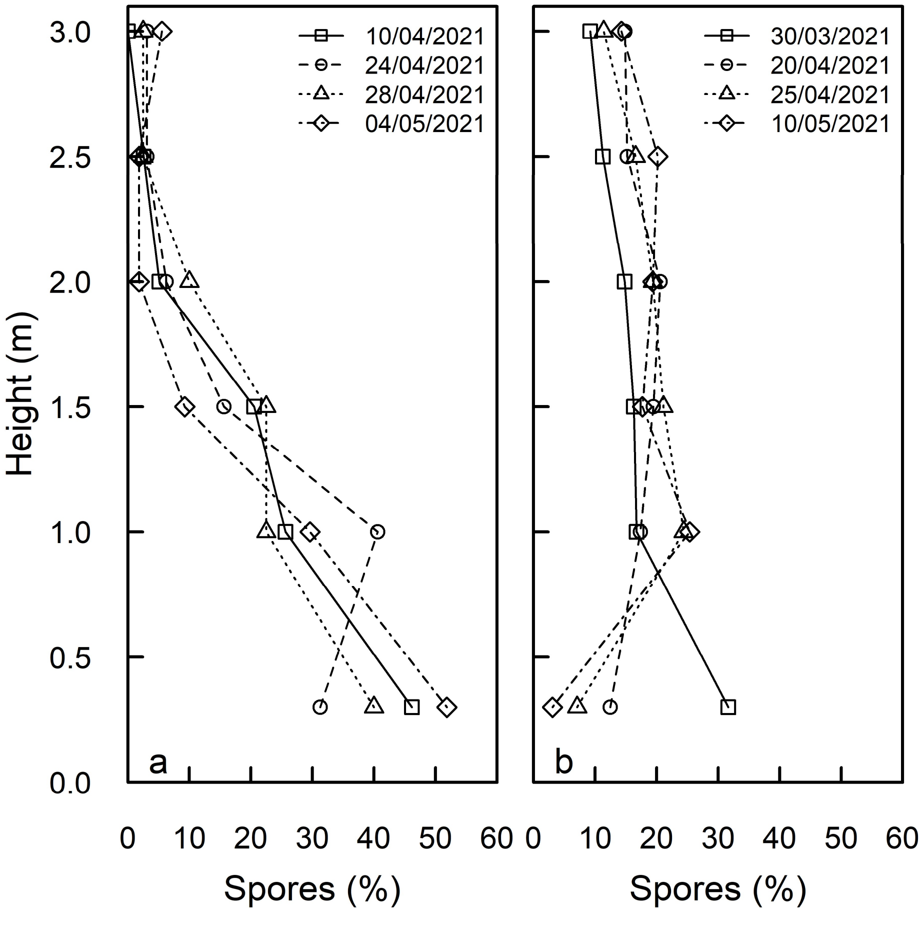

Fig. 5 a) Vertical distribution of the percentage of ascospores of *Venturia inaequalis* in presence of natural wind (WIND) and b) without wind (NO WIND) in each of four trials (marked with dates). Irrigation was carried out from 11 am to 1 pm and spore trapping from 11 am to 3 pm

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).

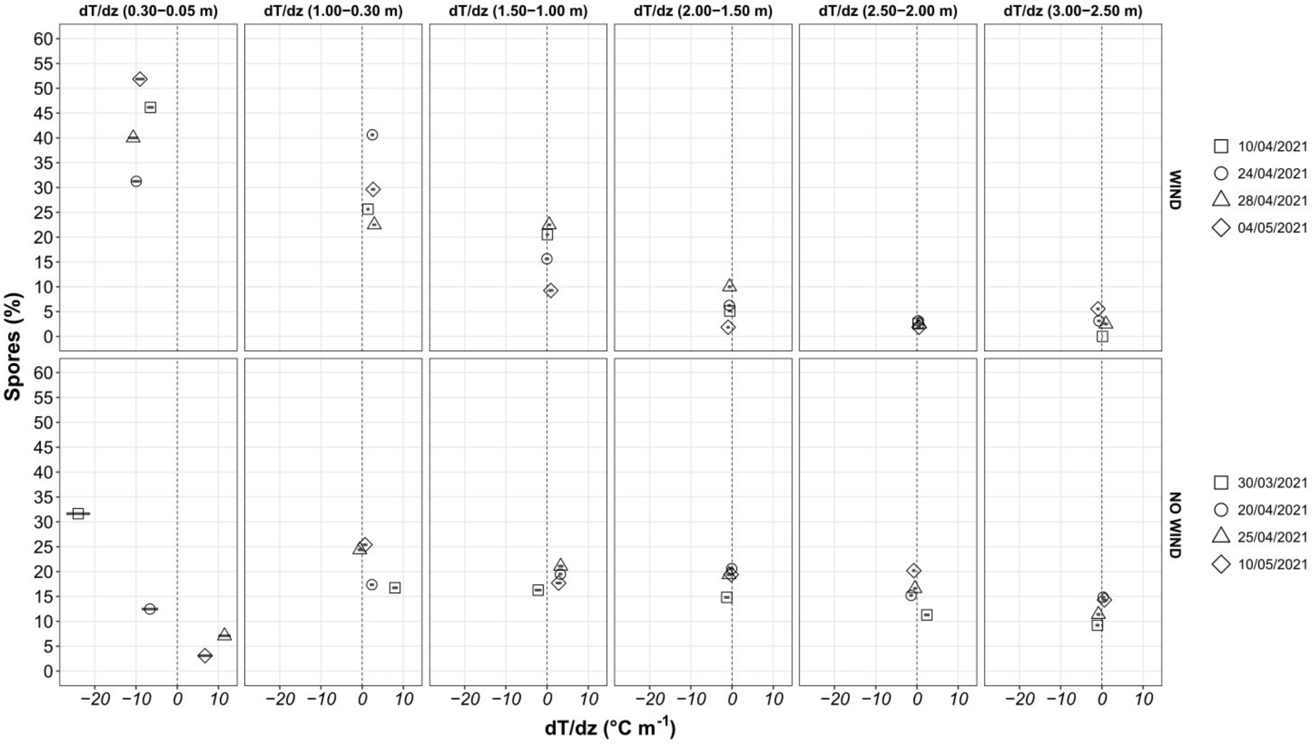

Fig. 6 Vertical temperature gradients (dT/dz, average of the four-hour trial) in comparison with the percentage of ascospores of *Venturia inaequalis* at different heights, in presence of natural wind (WIND, top) and in absence of wind (NO WIND, bottom). Different symbols represent the four trials (marked 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

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 pm 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 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 minutes (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 hours of the trial (Fig. 9).

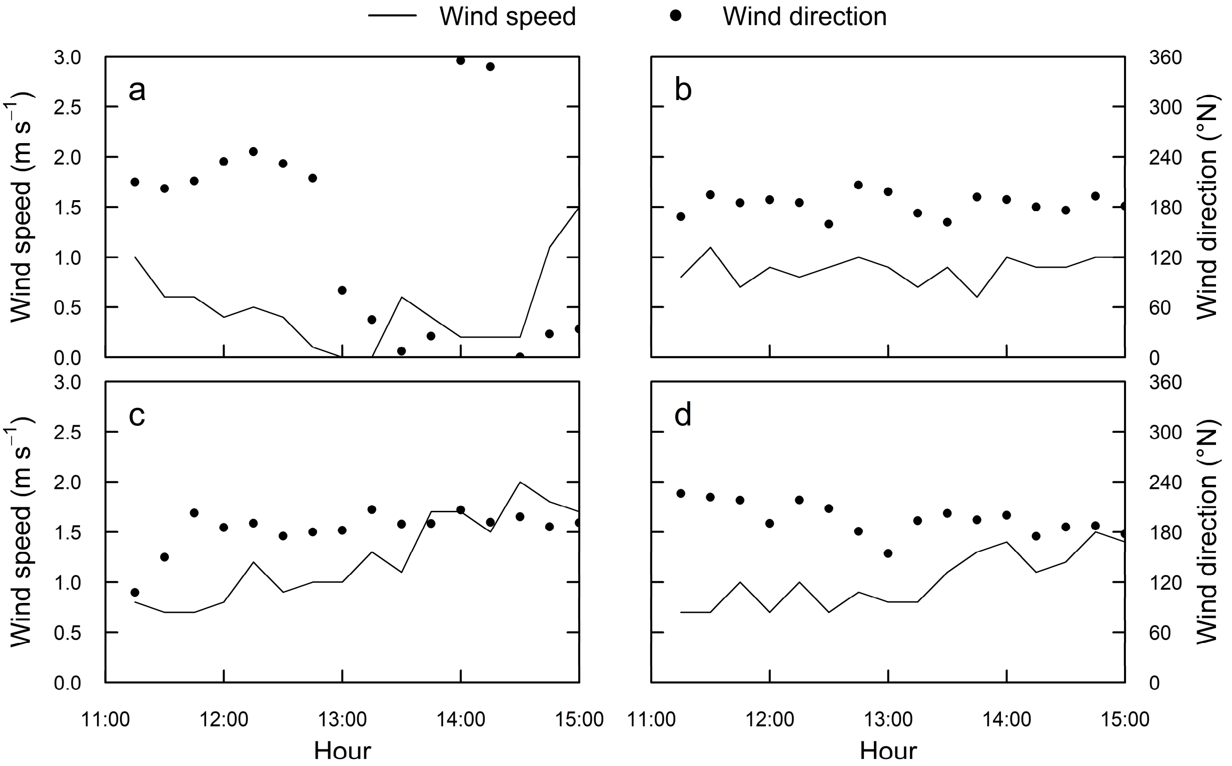

Fig. 7 Average wind speed (m/s) and wind direction (0° = north) measured every 15 minutes at 3.0 m above the ground from 11 am to 3 pm in the four trial dates in presence of natural wind in Cles (WIND). Lowercase letters represent the trial dates: a) 10 April; b) 24 April; c) 28 April; d) 4 May

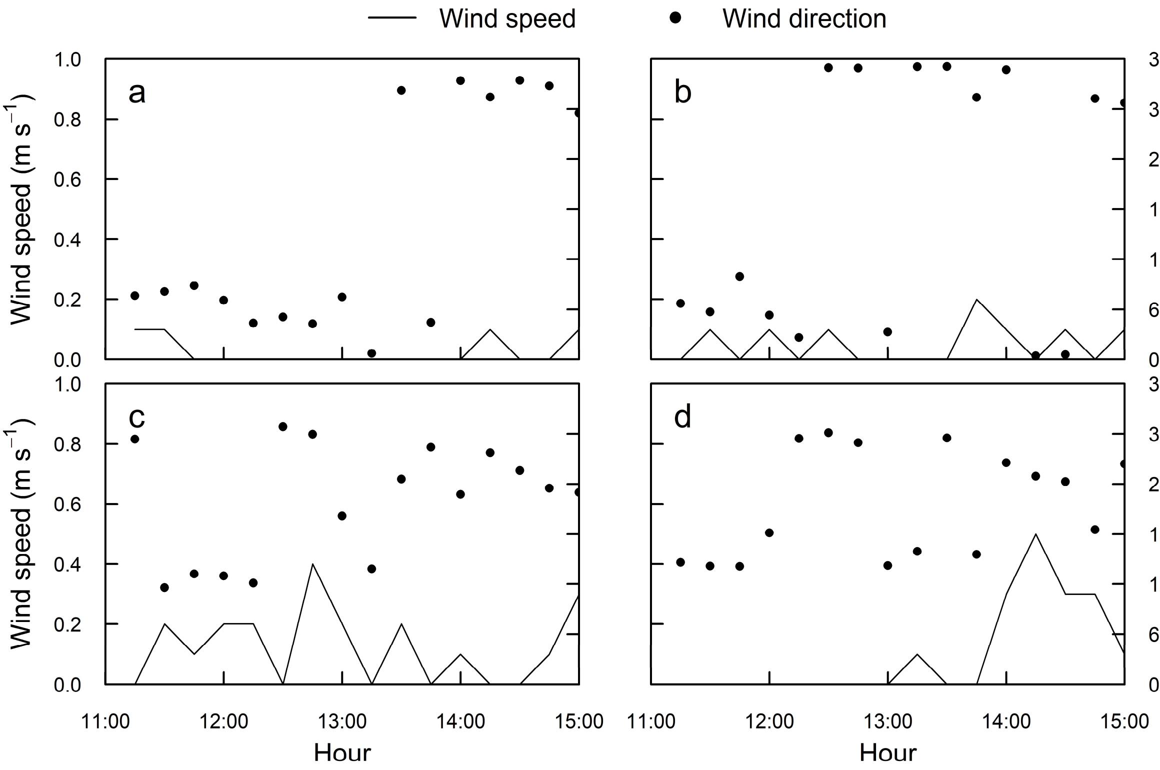

Fig. 8 Average wind speed (m/s) and wind direction (0° = north) measured every 15 minutes at 3.0 m above the ground from 11 am to 3 pm in the four trial dates in San Michele all'Adige inside the testing area protected by the wind fence (NO WIND). Lowercase letters represent the trial dates: a) 30 March;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

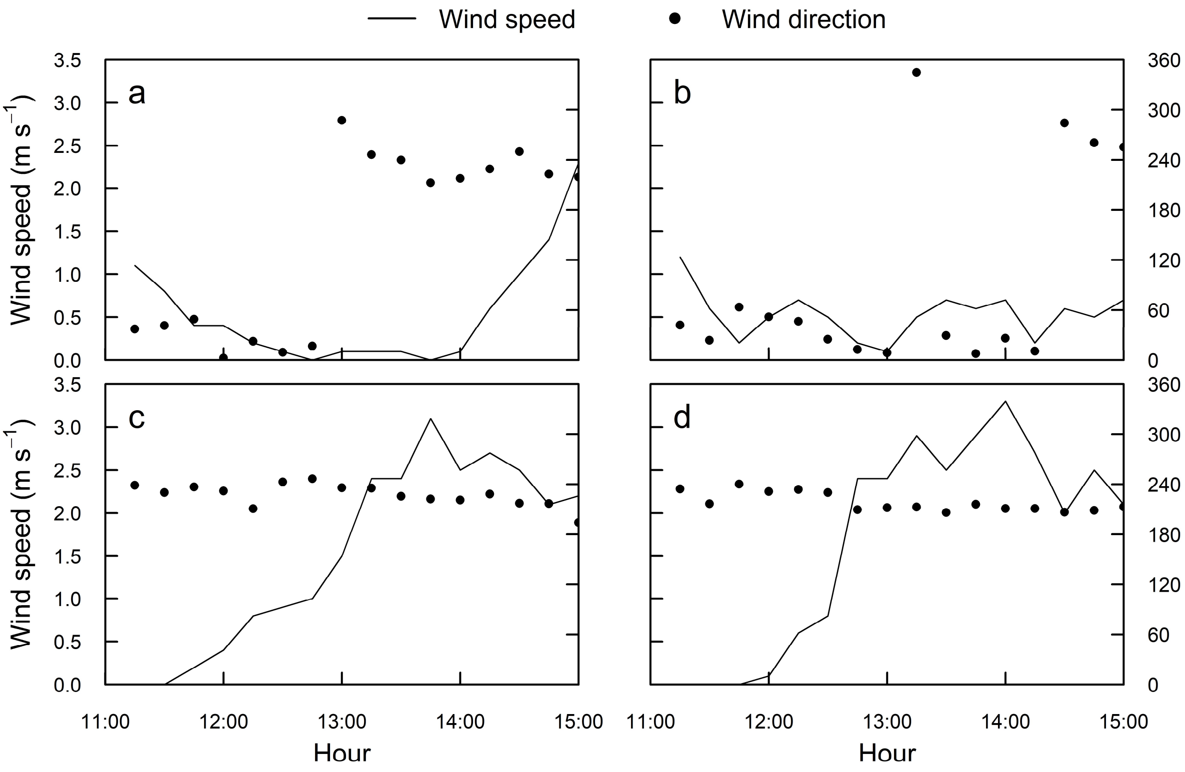

Fig. 9 Average wind speed (m/s) and wind direction (0° = north) measured every 15 minutes at 3.0 m above the ground from 11 am to 3 pm in the four trial dates in San Michele all'Adige, in the open field outside the fence. Lowercase letters represent the trial dates: a) 30 March; b) 20 April; c) 25 April;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

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 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 and 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. 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 and 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 hours 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 hours 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 hours 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 hours under sunny conditions or less than 24 hours 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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PSEUDOTHECIUM DEVELOPMENT AND ASCOSPORE DISCHARGE IN *VENTURIA ASPERATA* AND *V. INAEQUALIS*: RELATION TO ENVIRONMENTAL TRIGGERS

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Summary. *Venturia asperata* (*Ascomycetes*) was first described in 1975, as a saprotroph on overwintered apple leaf litter, and then, in 2007, as the cause of atypical apple scab symptoms on scab-resistant apple cultivars in southern France, and later in northern Italy and China. Information on *V. asperata* is limited. This study expanded knowledge by comparing development of pseudothecia and ascospore discharge in *V. asperata* and *V. inaequalis*. Leaf litters with pseudothecia of *V. asperata* or *V. inaequalis* were prepared, and a spore trap was placed above each litter. Over the 2-year study, pseudothecia of the two pathogens developed differently: *V. asperata* had delayed pseudothecium maturation and emptying in relation to degree day accumulation, compared to *V. inaequalis*. The ascospore release for *V. asperata* was also delayed, commencing and ending later than *V. inaequalis*. The delayed spore ejection and pseudothecium development of *V. asperata* compared to *V. inaequalis* may partly explain the late onset of symptoms in orchards during each growing season. These results have implications for plant protection strategies on scab-resistant apple cultivars, in particular under warm climates that occur in the Mediterranean region.

Keywords. Apple scab, disease management, epidemiology, light, resistant cultivars.

Introduction

Use of apple cultivars resistant to scab, caused by *Venturia inaequalis* (Cke.) Wint., is increasing worldwide, with clear advantage in reductions in use of fungicides with potential negative impacts on human health and the environment (Didelot *et al*., 2016). However, reduced fungicide applications could create favourable conditions for other diseases, including fruit rots, sooty blotch, or Marssonina blotch, which all thrive in the absence of regular fungicide applications (Ellis *et al*., 1998; Boutry *et al*., 2023).

The main source of resistance to scab in apple cultivars for more than 50 years has been the *Rvi6* (=*Vf*) gene (Caffier *et al*., 2012, Gessler and Pertot, 2012; Didelot *et al*., 2016). An important drawback of monogenic host resistance is that it may be overcome, and virulent, resistance breaking strains of *V. inaequalis* have become dominant in several countries in Europe (Parisi *et al*., 1993, 2004; Gessler and Pertot, 2012; Patocchi *et al*., 2020). In 2007, atypical apple scab symptoms were observed for the first time in southern France, on fruit of cultivars carrying the resistance gene *Rvi6*. Initially, these symptoms were reported on the cultivar Ariane/Les Naturianes®, and in the following years on the cultivars Co-op 38/GoldRush® and Prima. Morphological and molecular analyses and pathogenicity tests showed that the causal agent of the scab-like symptoms in France was not *V. inaequalis* but was *Venturia asperata* Samuels & Sivan. (Caffier *et al.*, 2012).

Venturia asperata (*Ascomycetes*) was first described in New Zealand by Samuels and Sivanesan (1975), and was later reported in Canada by Cortlett (1985). In both locations, *V. asperata* was reported as a saprotroph, growing on overwintered apple leaves on the ground, with no symptoms observed on fruit and leaves in growing seasons. In 2012, atypical apple scab symptoms were recorded in Italy (Cesena, Emilia-Romagna Region), on the fruit of cultivar CIVG198/Modì®, a resistant cultivar carrying the *Rvi6* gene (Turan *et al.*, 2019). Symptoms were more severe at fruit harvest, and became established in the following years. Morphological and molecular analyses of isolates from conidia and ascospores confirmed that *V. asperata* was the causal agent of these atypical scab symptoms (Turan *et al.*, 2019). In 2018, *V. asperata* was reported on the *Rvi6* gene resistant cultivar in a commercial orchard in the province of Trento, Italy (Gualandri *et al.*, 2021; Prodorutti *et al.,* 2023). *Venturia asperata* was also reported in the nearby province South-Tyrol in 2019 (Öttl, 2021), and in other regions of northern Italy (Piemonte, Veneto, Friuli Venezia Giulia; Erschbamer, 2024). In 2018, *V. asperata* was reported from the Heilongjiang Province in China (Zhou *et al.*, 2021).

Venturia spp. are hemibiotrophic fungal pathogens. They overwinter as saprophytes in host plant leaf litter on orchard soil, where pseudothecia mature in late winter and spring, producing ascospores that cause primary infections. Subcuticular mycelia develop in fruit and leaves, producing conidiophores and conidia that emerge through the cuticles. During this parasitic stage, *Venturia* spp. can only infect particular hosts, and *V. inaequalis* and *V. asperata* are the only *Venturia* spp. known to cause scab symptoms on apple (Caffier *et al.*, 2012; Turan *et al.*, 2019). Conidia and ascospores of *V. inaequalis* and *V. asperata* differ in shape and size and can be distinguished morphologically (Samuels and Sivanesan, 1975, Caffier *et al.*, 2012; Turan *et al.*, 2019).

Little information is available on the epidemiology of *V. asperata*. Caffier *et al.* (2012) carried out a single season trial comparing release of ascospores from *V. asperata* and *V. inaequalis*. The first ascospores of both fungi *V. inaequalis* and *V. asperata* were detected in March, but those of *V. inaequalis* were detected at the beginning of that month, and those of *V. asperata* at the end. However, both species had coincident peak release and depletion of ascospores. Pathogenicity tests in France and Italy revealed difficulties in reproducing symptoms on apple leaves and fruit following artificial inoculations of conidia of *V. asperata* (Caffier *et al.*, 2012; Turan *et al.*, 2019). The symptoms were weak and appeared later than those caused by *V. inaequalis*, and few or no conidia were recovered from lesions developed on fruit and leaves. This suggests that climatic conditions for infection and sporulation of *V. asperata* differ from those for *V. inaequalis* (Caffier *et al.,* 2012), or that *V. asperata* may be a weaker parasite than *V. inaequalis*.

Daylight has been shown to affect ascospore discharge of *V. inaequalis* (Brook, 1969, 1975; MacHardy and Gadoury, 1986; Gadoury *et al.*, 1998; Rossi *et al.,* 2001; Stensvand *et al.*, 2009), but no information is available for light effects on *V. asperata*. If rain started during night-time, and there was continuous leaf wetness for the next 24 hours, most ascospores of *V. inaequalis* (98%) were discharged between 7:00 and 18:00 (MacHardy and Gadoury, 1986). The peak of ascospore numbers was reached between 11:00 and noon, with a noticeable increase in ascospore discharge beginning at approx. 7:00, i.e., 2-3 hours after sunrise (MacHardy and Gadoury, 1986). Similar results were reported by Rossi *et al.* (2001) in northern Italy, where 93% of the ascospores of *V. inaequalis* were ejected during daylight, with most became airborne within the first two hours after sunrise.

Venturia asperata should be considered an emerging pathogen of apples, spreading in key apple-growing regions of Europe, with symptoms appearing on cultivars carrying the *Rvi6* gene. For this reason, there is a need for epidemiology on this pathogen, particularly to determine whether the conditions and timing for infections align with, or differ from, those of *V. inaequalis*. Increasing knowledge on the epidemiology of *V. asperata* also has practical implications for management of apple scab, because this may influence the timing of fungicide treatments for disease control. Future breeding programmes and selection of scab-resistant cultivars should also consider host susceptibility to *V. asperata*, alongside *V. inaequalis.*

The present study aimed to fill knowledge on the epidemiology of *V. asperata*, by comparing the development of pseudothecia and ascospore discharge with those of *V. inaequalis.* The objectives were to characterize: i) development of pseudothecia, and ii) ascospore discharge in relation to rainfall and degree-day accumulation; and iii) assess effects of daylight on ascospore discharge.

Materials and methods

Apple fruit and leaves with suspected symptoms of *V. asperata* infections were collected on 3 September 2018 from an organic commercial orchard of cultivar Modì, located in Romagnano municipality (Trento Province, Italy; coordinates 45.995053°N, 11.118136°E). Symptoms and fungal propagules on the host samples were described, and compared with those caused by *V. inaequalis,* on fruit and leaves collected from an organic commercial orchard of 'Golden Delicious' in the same general location (46.010059°N, 11.112870°E). The samples from fruit and leaves were then processed for molecular and morphological identification of the putative causal agent. To obtain monosporic isolates, fruit skin was cut from the margins of brownish suberose patches on fruit, or small pieces were cut from dusty patches on leaves, for these sample sources resembling symptoms and signs of *V. asperata*, and a drop of water was put on each host lesion. The drop was then transferred to, and plated on, potato dextrose agar (PDA; Oxoid) containing chloramphenicol (100 mg mL^{-1}; Sigma), and then incubated at 18°C. After 24 h, individually germinated conidia were picked up under stereomicroscope observation, and transferred to PDA + chloramphenicol, and incubated at 18°C.

After development of isolates, two monosporic cultures obtained from fruit and two from leaves were used in molecular analyses. Total genomic DNA was extracted from mycelia using Nucleospin Plant II (Macherey-Nagel), and the ITS region was amplified using the primer Vasp (5′-GTCTGAGAAACAAGTAATAG-3′), specific for *V. asperata* (Stehmann *et al*., 2001), in combination with ITS4 (White *et al*., 1990). After sequencing of the PCR products of the two isolates from fruit, a BLAST search was carried out in the NCBI database.

To confirm presence of *V. asperata* in the field, fruit and leaves from the same 'Modì' orchard were sampled in 2019 and 2020, from August to October each year, and assessed for molecular identification of this fungus.

To assess evolution of disease incidence, the 'Modì' apple orchard was monitored at the end of each growing season in 2019, 2020 and 2021. The proportions of diseased fruit and shoots were calculated by randomly checking symptoms on 500 fruit immediately before harvest, and on 50 shoots before start of leaf fall. Just before leaf fall, on 31 October 2019 and 5 November 2020, apple leaves with symptoms of *V. asperata* were collected in the 'Modì' orchard, from trees where no fungicides had been applied during the growing seasons. At the same time, apple leaves with symptoms of *V. inaequalis* were collected from untreated trees in the 'Golden Delicious' orchard.

Leaves from the two cultivars were immediately placed on the ground in two separated plots (each plot containing leaves of one cultivar), in San Michele all'Adige (Trento Province, Italy; 46.189922°N, 11.135227°E). A sample of the collected leaves from each sampled orchard ('Modì' or 'Golden Delicious') was observed under a light microscope to confirm presence of infections of, respectively, *V. asperata* or *V. inaequalis*, by assessing morphological characteristics of conidia and conidiophores (Figure 2). At an experimental site in San Michele all'Adige, the collected leaves were placed in two plots in a grass meadow. The plots each measured 2 × 2 m, and were 100 m apart from each other and at least 100 m away from any apple trees, to avoid cross-contamination (MacHardy, 1996). Additionally, a plot (2 × 1 m) of leaf litter was placed adjacent to each of the 2 × 2 m plots, and was used for monitoring of pseudothecia. To confirm that *V. asperata* pseudothecia developed in the leaf litter of 'Modì', in 2020 maturing pseudothecia were collected from leaves overwintered in the leaf litter, and were directly subjected to PCR with specific primers for *V. asperata*, using the methods described above.

The plots were prepared as described in Prodorutti *et al.* (2024). Grass was removed by light soil tillage, and a layer of leaves was placed on the soil above a white non-woven fabric (permeable polypropylene, Ortoclima, Tenax s.p.a.), which prevented earthworms from degrading the leaves. Each plot was then covered with a wire mesh (1 × 1 cm) to keep the leaves in place. The leaves (approx. 150 m^{-2}) were placed in a single layer, avoiding overlapping.

Development of pseudothecia of *V. asperata* and *V. inaequalis* was evaluated weekly, in 2020 and 2021, from the first week of March to mid-July, in order to cover the complete primary season for ascospore maturation and release. Each week, ten leaves were randomly selected from the leaf litter from each cultivar (Modì or Golden Delicious), and 60 pseudothecia were randomly harvested and observed under a light microscope. Pseudothecia were harvested from leaves previously soaked in water for 10 min, and were then crushed on glass microscope slides and classified in three groups ("immature", "mature", or "empty") according to their stages of development. The immature pseudothecia included stages from pseudothecium initials to pseudothecia with most asci containing non-pigmented (immature) ascospores. For the "mature" group, most asci contained septate and pigmented (mature) ascospores. Pseudothecia were classified as empty if most of the asci were empty or aborted (Prodorutti *et al.*, 2024). The percentages of immature, mature, or empty pesudothecia (out of 60) were calculated for each assessment.

On 27 February 2020 and 24 February 2021, a volumetric spore trap (Myco-trap, Paul Illi Mech.) was placed above each leaf litter, in the middle of each plot. During the entire primary season for ascospore maturation and release, ascospores of the two *Venturia* spp. (Figure 2) captured by the spore traps were counted on each rainy day (≥ 0.2 mm rain d^{-1}). A microscope tape was mounted on a 7-d rotating clock cylinder in each spore trap. The microscope tapes were cut in pieces representing single days, placed on glass slides, and ascospores were counted using a light microscope (200 × magnification). Ascospore counting on the tapes was carried out by assessing along three parallel horizontal lines of each glass slide (Mandrioli, 2000). The daily total number of counted ascospores (sum of the three horizontal lines per slide) for each spore trap was calculated, and was used to compute total seasonal ascospore ejection and percentage of the total numbers of ascospores trapped during each season.

To study the effect of daylight on the ascospore release of *V. asperata*, hourly counting of spores was carried out in the days when rain started before sunrise (https://www.timeanddate.com/sun/) and continued after sunrise. In these days, ascospore counting on each tape (at 400 × magnification) was carried out by assessing 24 vertical lines of the slide, corresponding to each hour of the day.

Weather data were recorded by a weather station (model TMF 500, Nesa s.r.l.), located at the San Michele all'Adige experiment site. Tree budbreak of the two cultivars (Modì or Golden Delicious) was assessed in the 2020 and 2021. Because budbreak of 'Modì' (27 February, 2020; 24 February, 2021) was earlier than for 'Golden Delicious' (3 March, 2020; 1 March, 2021), summation of degree days (DDs, base 0°C) from budbreak of cultivar 'Modì' to the last day of ascospore ejection and to the emptying of pseudothecia was used to compare data of the two *Venturia* species. Hours and related hourly data reported here refer to solar hour (Central European Time, CET). A leaf wetness (LW, min h^{-1}) sensor (Vantage Pro, Davis Instruments Corporation) was placed on the ground to assess hourly wetting of the leaf litter. Total solar irradiation (TSI, MJ m^{-2}) was recorded each hour, at 2 m above the ground.

‘Tidyverse’ packages (Wickham *et al*., 2019) of R language (version 4.4.1; R Core Team 2024) were used to handle data and generate plots. Cumulative proportions of empty pseudothecia of the two *Venturia* spp. were modeled as a function of scaled accumulated DDs since ‘Modì’ budbreak, using binomial regression. A mixed effect model (glmer, package lme4) was used, with sampling date as random effect. Different link functions were compared using the Akaike information criterion (AIC). The contribution of additional fixed effects (*Venturia* species, year, and their interactions) were assessed using likelihood ratio tests (LRTs). Scaled DDs were back transformed for interpretation.

The probability of observing ascospores on any given hour was modeled as a function of presence of light (TSI above zero) and LW at the ground level, using binomial regression. Similarly, hourly ascospore ejection intensity was modelled with count regression models using TSI. In all cases, the mixed effect model (glmer, package lme4) was used with sampling date as random effect.

Results

Symptoms and signs on apple fruit were atypical apple scab spots, being less intense compared to scab lesions caused by *V. inaequalis* (Figure 1). The lesions on fruit developed first as small slightly grey spots, that slowly enlarged and then evolved into necrotic suberose spots, each surrounded with a light and smooth ring. Spots on fruit usually had less clear edges and were lighter (brownish) in appearance compared to those caused by *V. inaequalis* (grey to black; Figure 1). Leaf lesions caused by *V. asperata* were less distinct and more irregular compared to leaf lesions caused by *V. inaequalis*. Leaf lesions caused by *V. asperata* appeared only on abaxial leaf surfaces as velvety-grey spots, in contrast to those caused by *V. inaequalis* which were present on both surfaces of infected leaves (Figure 1). Observations with a light microscope showed that conidiophores and conidia, and pseudothecia and ascospores, collected from infected fruit and leaves in Romagnano, matched well with previous descriptions of *V. asperata* (Samuels and Sivanesan, 1975; Caffier *et al.,* 2012; Turan *et al*., 2019; Shen *et al*., 2020), that these structures differed from those of *V. inaequalis* (Figure 2). Conidiophores and conidia of *V. asperata* emerging on fruit or leaf epidermides were observed using stereo and light microscopes, developing from the margins of necrotic suberose spots on fruit or from spots on leaves (Figure 2). PCR with species-specific primers for *V. asperata* from monospore isolates obtained from fruit and leaves each gave the expected 450 bp product, and BLAST analyses of the sequences of the two isolates from fruit were 100% identical to the published sequence of *V. asperata* (KX156341). Consensus sequences of the two isolates were submitted and deposited in GenBank nucleotide database (accession nos. MT459450 and MT459451). The PCR analysis of pseudothecia collected on overwintered leaves of 'Modì' showed DNA amplification at 450 bp, and the PCR from symptomatic fruit and leaves samples collected in 2019 and 2020 confirmed establishment of *V. asperata* in the 'Modì' orchard.

Symptoms of *V. asperata* on apple fruit and leaves appeared later in the growing seasons than those caused by *V. inaequalis*. Symptoms caused by *V. asperata* on fruit occurred from the end of July to early August, and on leaves from September to October. The first symptoms of *V. inaequalis* infections on leaves appeared in April, and on fruit in May–June.

Monitoring of symptoms of *V. asperata* in the orchard showed increases in percentage of infected fruit at harvest (early September), from 1.2 to 9.4%, from 2019 to 2021 (Table 1). On 'Modì', only one to two spots per infected fruit were typically observed, and three to four or more spots were found on approx. 20% of symptomatic fruit. At the end of the growing seasons (late October to early November), high proportions of shoots were symptomatic (i.e., at least one leaf with scab per shoot), resulting in disease incidence of 40% in 2019, and 100% in 2020 and 2021 (Table 1). In the three years of monitoring, all fruit and shoots in the untreated trees of 'Golden Delicious' showed symptoms of *V. inaequalis* (100% incidence).

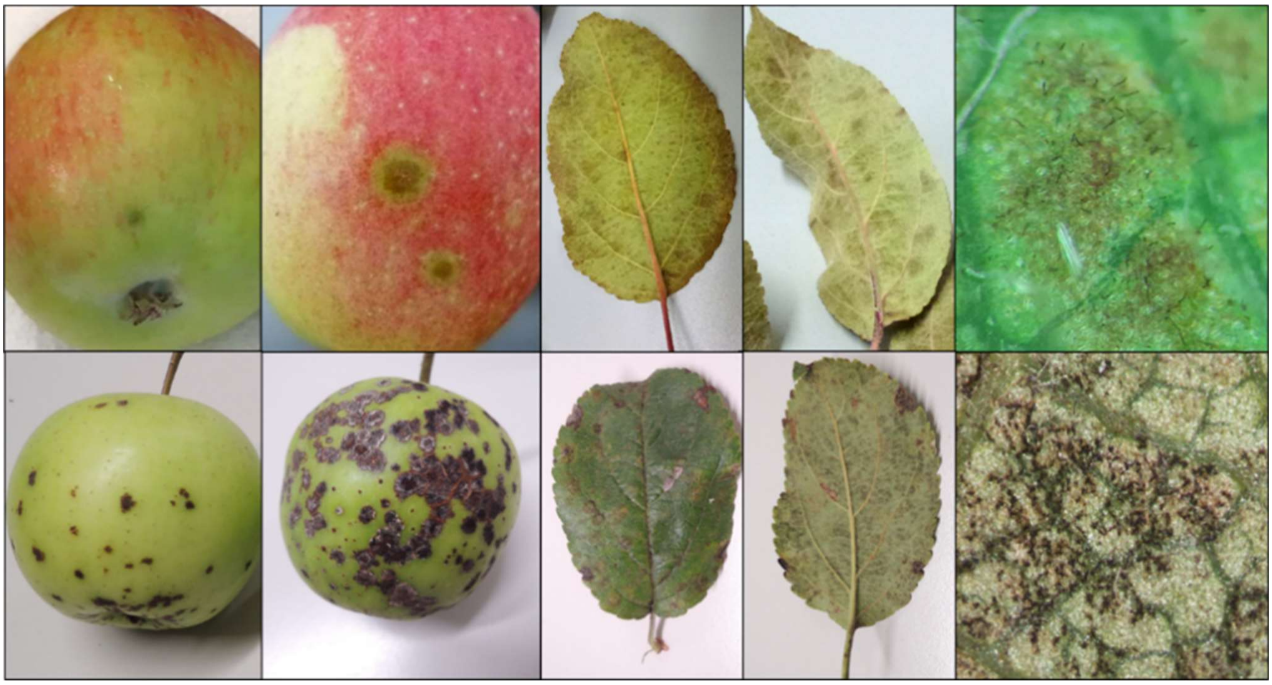

Figure 1. Fruit and leaves of apple cultivar Modì (top row) with (from left to right) sporulating lesions of *Venturia asperata* and leaf lesion detail, and of cultivar Golden Delicious (bottom row) with (left to right) sporulating lesions of *V. inaequalis* and leaf lesion detail.

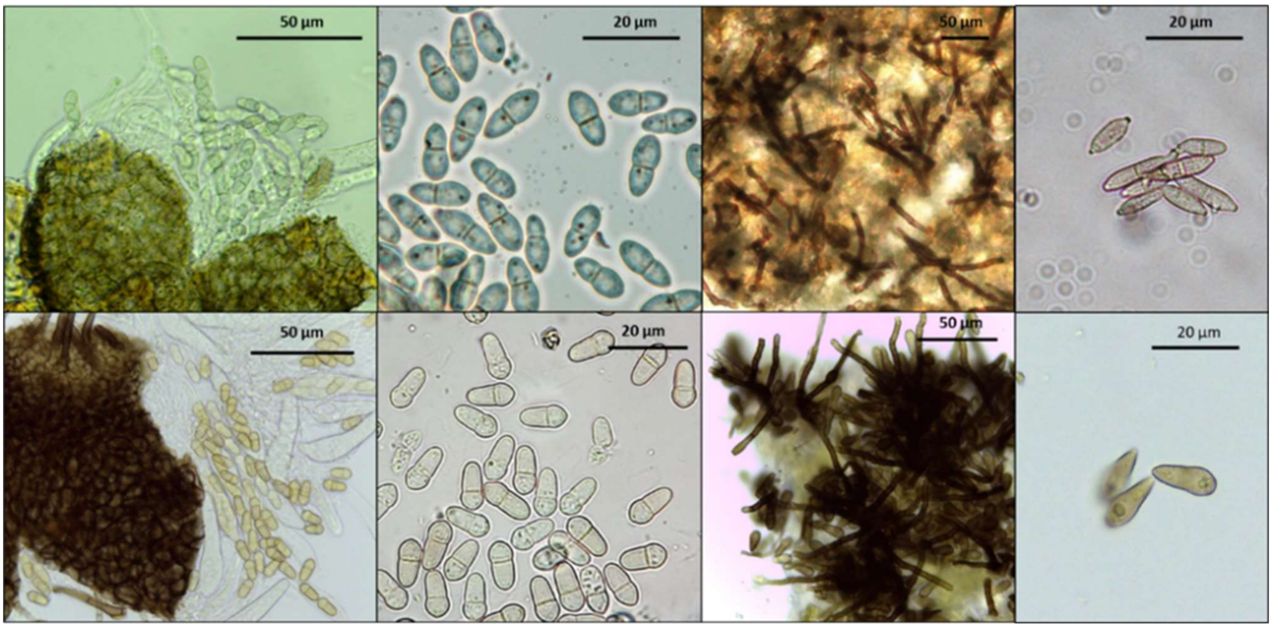

Figure 2. Pseudothecium with asci, ascospores, conidiophores, and conidia (left to right) of *Venturia asperata* (top) and *Venturia inaequalis* (bottom).

Table 1. Incidence of *Venturia asperata* symptoms on apple fruit and shoots during 2019, 2020 and 2021 in an orchard of cultivar Modì located in Romagnano (Italy).

Year	Day/month	Symptomatic fruit (%)[a]	Day/month	Symptomatic shoots (%)[b]
2019	06/09	1.2	23/10	40.0
2020	04/09	5.6	06/11	100.0
2021	03/09	9.4	22/10	100.0

[a] 500 fruit assessed.

[b] 50 shoots assessed.

Table 2. Development of pseudothecia of *Venturia inaequalis* and *V. asperata* in San Michele all'Adige (Italy) in 2020 and 2021.

Year	Period with mature pseudothecia (day/month)		Peak of maturation (day/month)		DDs of 95% empty pseudothecia[a]	
	V. inaequalis	*V. asperata*	*V. inaequalis*	*V. asperata*	*V. inaequalis*	*V. asperata*
2020	13/03 to 29/05	08/05 to 26/06	24/04	22/05	1,181	1,744
2021	10/03 to 26/05	27/04 to 08/07	27/04	03/06	1,009	1,941

[a] Degree days (DDs, base temperature 0°C) from budbreak of cultivar Modì to 95% of the pseudothecia empty (data predicted by the probit model).

Table 3. Rainfall data, numbers of ascospores of *Venturia inaequalis* and *V. asperata* trapped, and degree days recorded, in San Michele all'Adige (Italy) in 2020 and 2021.

Year	Total rainfall (mm)[a]	Total numbers of ascospores[b]		Period of ascospore release (day/month)		DDs for 95% ascospore release[c]	
		V. inaequalis	*V. asperata*	*V. inaequalis*	*V. asperata*	*V. inaequalis*	*V. asperata*
2020	452.2	5,474	6,742	14/03 to 06/06	26/04 to 03/07	965	1,400
2021	333.2	9,823	28,350	11/04 to 23/06	28/04 to 08/07	1,048	1,104

[a] Rainfall was recorded from budbreak of cultivar Modì to the last day of ascospore ejection from *V. asperata*.

[b] Total number of ascospores collected by volumetric spore traps in the season.

[c] Degree days (DDs, base temperature 0°C) from budbreak of cultivar Modì to the time of 95% of seasonal ascospore trap count.

Pseudothecia of the two *Venturia* spp. developed differently in the 2-year trial. Pseudothecia of *V. asperata* had delayed maturation and emptying of ascospores compared to pseudothecia of *V. inaequalis* (Figure 3, C and D). The periods of detection of mature *V. inaequalis* pseudothecia were similar in the 2 years (13 March to 29 May in 2020; 10 March to 26 May in 2021). Mature pseudothecia of *V. asperata* were present from 8 May to 26 June in 2020, and from 27 April to 8 July in 2021 (Table 2). The peaks of proportions of mature pseudothecia of *V. inaequalis* were observed on 24 April (37% of mature pseudothecia) in 2020, and 27 April (32%) in 2021. *Venturia asperata* had greatest proportions of mature pseudothecia on 22 May (23%) in 2020, and on 3 June (15%) in 2021 (Table 2). These dates were approx. 1 month after equivalent dates for *V. inaequalis*.

The probit model gave overall better fits for pseudothecium emptying rate than logistic regression, and the probit model better represented data for *V. inaequalis* than for *V. asperata* (Figure 3, C and D). The triple interaction *Venturia* species × year × cumulative DDs was statistically significant (LRT = 9.7, df = 1, $P = 0.0019$), indicating that both the onset and rate of pseudothecium emptying were different for the two species for the two years. In both years, onset of emptying and the time of 95% empty pseudothecia predicted by the probit model were reached later for *V. asperata* than *V. inaequalis* (563 DDs later in 2020, 932 DDs later in 2021; Table 2). The difference in accumulated DDs between the two species when pseudothecia started emptying was similar in 2020 and 2021 ($z = 0.8$, $P = 0.85$). Rates of pseudothecium emptying were also similar for the two species in 2020 ($z = 0.7$, $P = 0.5$), but yearly variations in the rates of pseudothecium emptying were detected for *V. asperata* ($z = 3.4$, $P = 0.0008$), as the rate for *V. asperata* was slower in 2021, while a similar emptying rate was observed for *V. inaequalis* in 2020 and 2021 ($z = 1.7$, $P = 0.09$).

The periods of ascospore release were different for the two *Venturia* species, and were later for *V. asperata* than for *V. inaequalis* (Table 3; Figure 3, A and B). The first ascospores of *V. asperata* were trapped at the end of April in both years, and continued until early July. Ascospore discharge of

V. inaequalis commenced in mid-March and ended in early June in 2020, and commenced in early April and ended in the second half of June in 2021 (Table 3). Total numbers of ascospores released during entire seasons were greater in both years for *V. asperata* than for *V. inaequalis*. The difference was most noticeable in 2021, with *V. asperata* releasing almost three times the number of ascospores compared to *V. inaequalis* (Table 3). The peaks of ascospore ejection occurred later for *V. asperata*. In 2020, this was reached 26 April (at 667 DDs) for *V. inaequalis* and 11 May (at 911 DDs) for *V. asperata*, corresponding to 33.1% of the seasonal ascospore amount for *V. inaequalis* and 31.3% for *V. asperata* (Figure 3 B). In 2021, the peak of ascospore release for *V. inaequalis* was reached on 11 April (21% of the seasonal ascospores were trapped; 486 DDs), and that for *V. asperata* was on 15 May (24% of the seasonal ascospores were trapped, 935 DDs) (Figure 3 B). The time of 95% of trapped ascospores was reached earlier in *V. inaequalis* (435 DDs earlier in 2020 and 56 DDs earlier in 2021) than the equivalent periods for *V. asperata* (Figure 3 A; Table 3). The 95% DD accumulations for empty pseudothecia and for ascospore ejection were similar in both years for *V. inaequalis,* and for *V. asperata* in 2020. However, in 2021 *V. asperata* reached 95% empty pseudothecia at 1,941 DDs compared to 1,104 DDs for 95% ascospore ejection (Table 2; Table 3). The last ascospores were trapped ca. 4 weeks later for *V. asperata* than *V. inaequalis* in 2020 and ca. 2 weeks later in 2021 (Table 3).

The total rainfall measured during the primary season was greater in 2020 (452 mm) than in 2021 (333 mm; Table 3). The rainfall conditions necessary to observe the potential diurnal periodicity of ascospore release of *V. asperata* (rain started before and continued after sunrise) were fulfilled during 4 d of substantial ascospore trapping, i.e., on 15 and 17 May 2020, and 12 and 25 May 2021 (Figure 4). Sunrise occurred at 4:42 and 4:40 on 15 and 17 May 2020, and at 4:46 and 4:32 am on 12 and 25 May 2021. The patterns of ascospore release were similar in the four days, but total numbers of ascospores counted in 2020 were less than those counted in 2021. Very few ascospores were trapped from midnight to 4:00 am each day, and ascospore trapping began to increase after sunrise (from 5:00 to 7:00 am). From 7:00 to 9:00-10:00 am each day numbers of trapped ascospores rapidly increased, with exponential trends. Greatest numbers were detected at 10:00 am on 15 May and 17 May 2020, and 25 May 2021, and at 9:00 am on 12 May 2021. After these times, there were rapid declines in ascospore numbers, with values close to zero after 5:00-6:00 pm each day (Figure 4). In each of the four days, the TSI was above zero from 6:00 am to 8:00 pm. In these time intervals, the proportions of ascospores counted per 24 h ranged from 96.4% on 15 May 2020 to 98.9% on 25 May 2021.

Similar rainfall conditions were recorded in the four days when hourly counting of ascospores were carried out. Rain started during the night and continued until early-mid morning. Small rainfall amounts (0.2 – 0.8 mm h^{-1}), as occurred on 25 April 2021, induced relevant ascospore ejection. The LW measured at soil surface was recorded continuously through 24 h on 15 May 2020. In the other three events, LW was recorded for 2 to 5 h after rainfall ceased (Figure 4).

Both LW at the soil surface (LRT = 6.2, $P = 0.01$) and light (LRT = 14.4, $P < 0.001$) significantly increased probability of detecting ascospores in the spore traps. The odds ratio (OR) of observing ascospores during light hours, as opposed to dark hours, was 37 ($z = 2.624$, $P = 0.009$), while the OR for wet hours compared to dry hours was 16 ($z = 2$, $P = 0.045$). Hourly ascospore ejection intensity was over-dispersed, and was adequately modelled using a negative binomial distribution. Light had an exponential effect on the numbers of ascospores ejected, with an Incidence Rate Ratio (IRR) indicating that ejection was 38 times more intense during light hours compared to dark hours ($z = 12$, $P < 0.001$), and seven times more intense during wet hours compared to dry hours ($z = 5.5$, $P < 0.001$). Within the limited dataset, ascospore release intensity was better modelled as a function of light intensity, increasing linearly with the log of TSI (LRT = 38, df = 0). This relationship can be expressed as: Ejection intensity = $28 \times (\text{TSI} + 0.01)^{0.82}$, for TSI values ranging from 0 to 3.03 MJ m^{-2} during dry hours. For wet hours, ascospore release intensity was eight times greater than for dry hours. There was no interaction between the effect of TSI and wetness on ascospore release ($P = 0.96$).

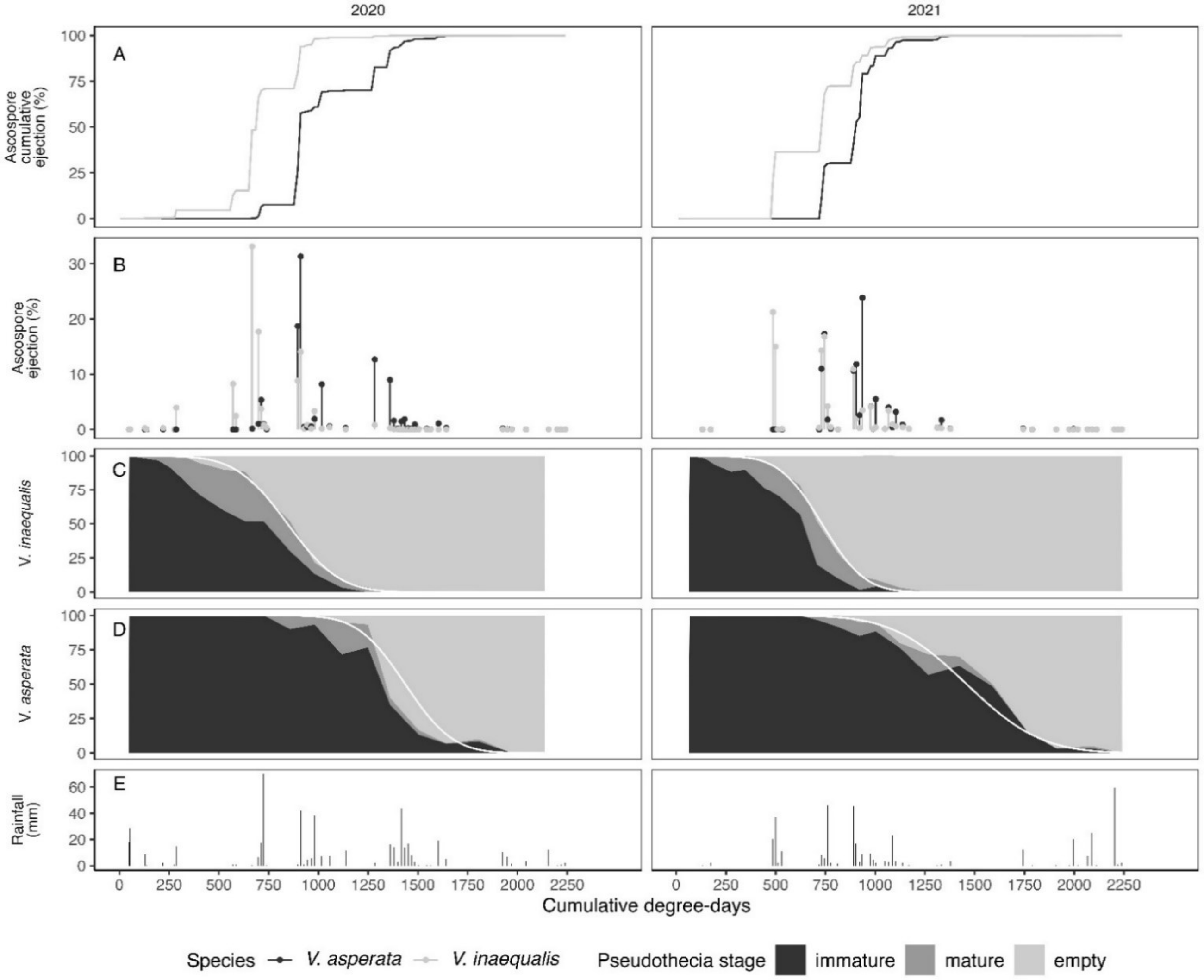

Figure 3. Degree-day accumulation (base temperature 0°C) since budbreak of apple cultivar Modì in 2020 and 2021 (San Michele all'Adige, Italy), in relation to the following. A, percentage of cumulated seasonal trapping of ascospores of *Venturia asperata* (black line) and *V. inaequalis* (grey line). B, percentages of the seasonal ascospores of *V. asperata* (black bars) and *V. inaequalis* (grey bars) released per day on days with rain (≥ 0.2 mm). C and D, percentages of immature, mature, and empty pseudothecia during the season for ascospore release of *V. inaequalis* (C) and *V. asperata* (D). Weekly assessments of 60 pseudothecia per species were evaluated in each year. Pseudothecia emptying was modelled using the probit model, represented by the white line. E, Daily rainfall (mm).

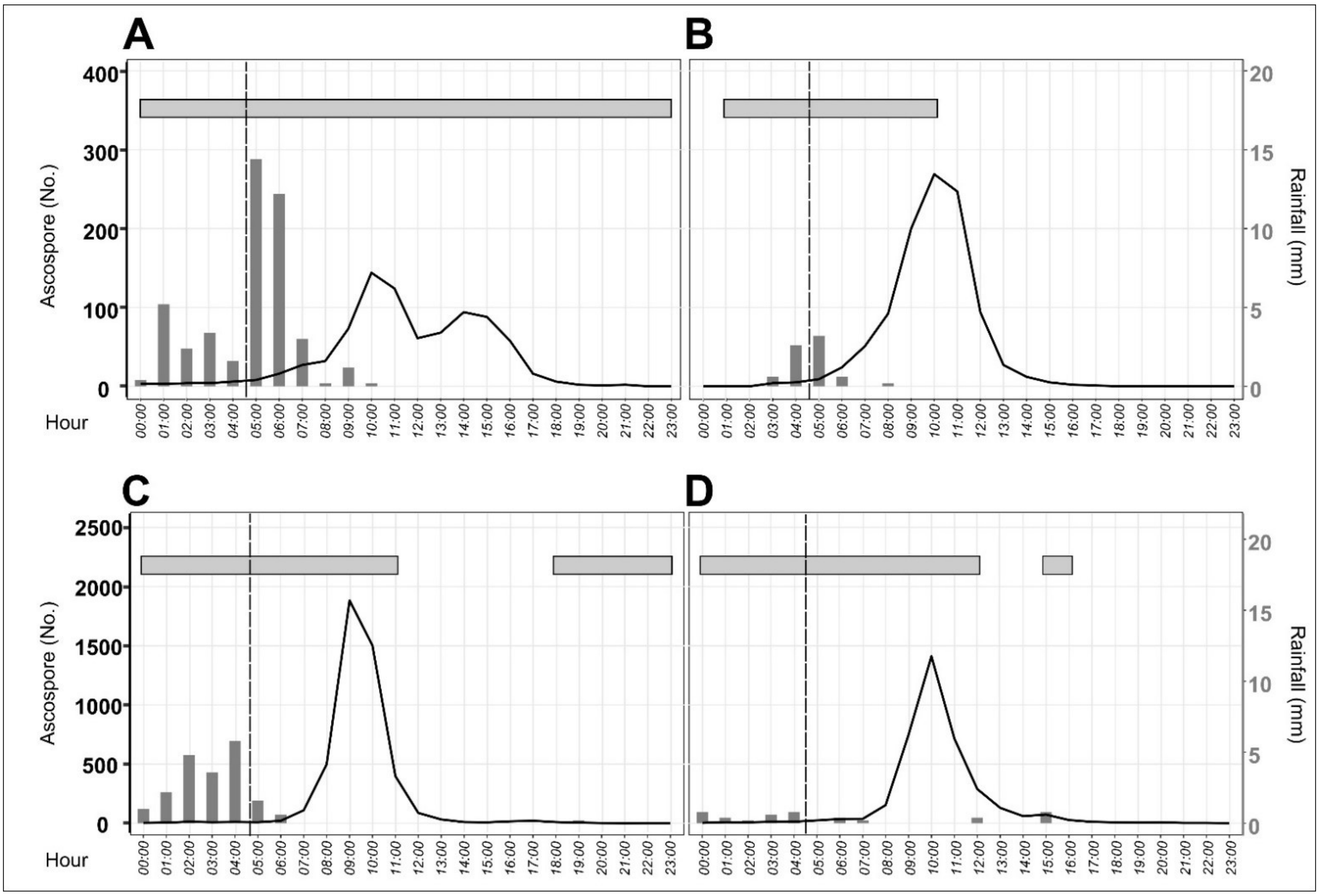

Figure 4. Hourly release of ascospores of *Venturia asperata* on four days when rainfall commenced before, and ended after sunrise. A) 15 May 2020, B) 17 May 2020, C) 12 May 2021, D) 25 May 2021. Sunrise occurred at 04:42 (Central European Time) on 15 May 2020, at 04:40 on 17 May 2020, at 04:46 on 12 May 2021 and at 04:32 on 25 May 2021. Vertical bars represent the hourly rainfall (mm), and horizontal bars indicate times when leaf wetness was recorded at ground surface. The solid line represents the hourly numbers of counted ascospores, and the vertical dashed lines indicate time of sunrise.

Discussion

In the present study, symptoms of *V. asperata* infections on apple fruit, and timing of their appearance, were similar to those previously described in France (Caffier *et al.*, 2012), and in Italy (Turan *et al.*, 2019). However, these symptoms were observed for the first time on leaves in a commercial orchard of the apple cultivar Modì. Caffier *et al.* (2012) were able to induce sporulating lesions of the pathogen on leaves of the apple cultivar Ariane, in controlled conditions and after artificial inoculations with conidia of *V. asperata*, and the symptoms they observed were weak and difficult to detect. In the present study, late appearance of leaf symptoms (September-October) was observed, with symptom intensity increasing towards leaf fall. Symptoms were confined to abaxial leaf surfaces and resembled late secondary infections of *V. inaequalis*, although lighter in appearance than for *V. inaequalis*. Because the symptoms of *V. asperata* on leaves are weak and often difficult to detect, confirmation through microscopic morphological analyses or molecular identifications are essential. The weak symptoms caused by *V. asperata*, observed both on fruit and leaves, could be due to fewer hyphae and conidia growing in each lesion, compared to *V. inaequalis* (Caffier *et al.*, 2012; Turan *et al.*, 2019).

Over the three years of observations, increases in incidence of symptoms and signs of *V. asperata* was observed in the monitored 'Modì' orchard, reaching almost 10% on the fruit in 2021. This increase over the 3 years may be partly due to gradual build-up of overwintering inoculum in infected leaf litter. The greater numbers of ascospores captured in 2021 compared to 2020, and the increasing incidence of disease, indicated increased overwintering of inoculum of the pathogen. High incidences of lesions caused by *V. asperata* on fruit of apple cultivars with *Rvi6* gene resistance to *V. inaequalis* were observed in southern France (up to 60%; Caffier *et al.,* 2012), and South Tyrol in Italy (up to 17.5% in 2023; Erschbamer, 2024). *Venturia asperata* has predominantly been reported in apple cultivars containing the *Rvi6* gene, mostly under organic management regimes. The limited use of fungicides, often only applied early in each growing season, probably facilitated outbreaks of *V. asperata* in the organic orchards with scab resistant cultivars. Little and unclear information is available about the presence of *V. asperata* on scab-susceptible (non-resistant to *V. inaequalis*) apple cultivars. Strict management of apple scab and greater competition of *V. inaequalis* in an orchard may lead to underestimation of the presence and spread of the less competitive *V. asperata* on these cultivars.

Pseudothecia of *V. asperata* had delayed maturation and ascospore emptying compared to *V. inaequalis*. The first mature pseudothecia were observed over a month and a half later than those of *V. inaequalis*, and the peak of maturation occurred about one month later for *V. asperata* than for *V. inaequalis*. The earlier onset of ascospore ejection for *V. inaequalis* in both years, and the slower rate of pseudothecium emptying for *V. asperata* in 2021, suggest that the two *Venturia* spp. have inherently different ejection patterns. As it was observed for the effect of irrigation on pseudothecium development (Prodorutti *et al*., 2024), probit regression gave a better fit for pseudothecium emptying rates, and better represented *V. inaequalis* than *V. asperata*, which is another indication that the two species have differences in seasonal development of their pseudothecia and ascospores.

In both years, the ascospore release for *V. asperata* was delayed compared to *V. inaequalis*, with initiation and peak of ascospore release occurring later in *V. asperata* than in *V. inaequalis*. In 2021, the first ascospores of *V. inaequalis* were detected almost a month later than in 2020, likely due to the prolonged dry period in March and early April of 2021. Extended dry periods delay pseudothecium maturation and extend ascospore release seasons of *V. inaequalis* (Stensvand *et al*., 2005), and this is also likely to be the case for *V. asperata*.

The less prominent difference in cease of ascospore release between the two fungi in 2021 than in 2020 may have been partly due to a more rapid breakdown of leaf litter of ‘Modì’ than ‘Golden Delicious’, although no records of leaf degradation were made in the present study.

Caffier *et al*. (2012) reported that the first ascospores of *V. asperata* were trapped approx. 20 d later than those of *V. inaequalis*, but that the peaks in ascospore release and end of ejection were observed at the same time for both species. The discrepancies between the present study results and those obtained in France may be due to the different methods used for leaf litter preparation. Caffier *et al.* (2012) placed leaves on ground covered by grass, so the leaves may have degraded more rapidly than in the present study experiments. Weekly development of pseudothecia of *V. asperata* had not been previously studied, and it was here shown that the periods of pseudothecium maturation and ascospore release overlapped.

Light triggered ascospore discharge of *V. asperata* in a manner similar reported for *V. inaequalis* (Brook, 1969; 1975; MacHardy and Gadoury, 1986; MacHardy, 1996; Gadoury *et al.,* 1998; Rossi *et al*., 2001; Stensvand *et al*., 2009). As for *V. inaequalis*, increases in *V. asperata* ascospore discharge were observed during the first 2 to 3 h after sunrise, and even small amounts of rain (0.2 mm h^{-1}) were sufficient to induce substantial ascospore ejection. Similar to previous reports for *V. inaequalis* (MacHardy and Gadoury, 1986; Rossi *et al*., 2001), on average approx. 98% of the ascospores were released during daylight hours between 6:00 am to 8:00 pm. However, in the three days when LW was recorded until 10:00 to 12:00 am (2 to 5 h after the rain stopped), more rapid decreases in ascospore numbers were detected compared to 15 May 2020, when LW was recorded continuously for 24 h.

The OR and IRR values for ascospore ejection of *V. asperata* were both high and were similar, indicating the importance of light in triggering ascospore release. This suggests that the observed inhibition of release during dark conditions may have been proportional to numbers of ascospores primed for ejection, indicating a light-dependent regulatory mechanism of ascospore release, similar to that observed for *V. inaequalis*. The observations that ejection intensity was well-represented by the log of light intensity, and that no residual pattern was observed, indicate that the light effect rapidly saturates above a certain intensity. The substantial difference between the OR and IRR for the effect of wetness indicates that while ascospore ejection is much more likely during wet than dry periods, the total number of ejected ascospores is less influenced by wetness than by light. This implies that wet conditions are a primary trigger for ascospore release, but do not significantly increase the intensity of ejection. Again, this is similar to what has been observed for *V. inaequalis*.

Conclusions

Delayed pseudothecium development and ascospore ejection in *V. asperata* compared to *V. inaequalis*, may partly explain the late appearance of symptoms caused by *V. asperata* in apple orchards during the growing seasons. Further research is necessary to identify the optimal weather conditions for primary and secondary infections by *V. asperata* in susceptible apple tissues, and to define the latency period for this pathogen. The efficacy of fungicides commonly used for management of *V. inaequalis* should also be assessed for control of disease caused by *V. asperata*.

Future apple breeding programmes should consider resistance to emerging pathogens such as *V. asperata*, and specific control strategies should be developed to address the delayed primary infections and late symptom onset caused by this pathogen. In Europe, *V. asperata* has been reported in northern Italy and southern France, areas with temperate climates. *Venturia asperata* probably has higher requirements of DD accumulation for maturation of pseudothecia compared to *V. inaequalis*. As climate change raises average temperatures, this could favor the spread and virulence of *V. asperata* in fruit-growing regions in Mediterranean areas and other areas across Europe and elsewhere, emphasizing that close monitoring of this pathogen in apple orchards is important.

Author contribution

The field experiments in this study were conducted and managed by DP, who oversaw the implementation and data collection. VG was responsible for conducting molecular analyses. VP carried out statistical analyses. DP, VG, VP, AS, EC and IP interpreted the experimental results, integrating field, molecular, and statistical data to draw conclusions. The manuscript was written by DP, with all the authors contributing to revision of the manuscript for intellectual content. All the authors read and approved the final manuscript of this paper.

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GENERAL DISCUSSION AND CONCLUSIONS

Targeted irrigation on overwintering leaves on the ground, carried out in spring during periods of dry weather, may release a noticeable amount of ascospores of *V. inaequalis* under conditions unsuitable for infections, thus reducing the primary inoculum. Moreover, sprinkler irrigation caused an earlier depletion of the ascospore supply, and it significantly reduced the incidence of scab on leaves and fruit in the orchard. In the field trials, a similar scab incidence was observed between irrigated plots with a reduced fungicide schedule and non-irrigated plots with full fungicide schedule: this result suggests that if applying targeted irrigation, it is possible to reduce fungicide treatments and maintain the same efficacy as with a full fungicide schedule. On fruit, irrigation and fungicide strategy had a significant interaction, meaning that irrigation improved the efficacy of fungicides, showing a synergistic effect. The early depletion of ascospore release was another effect of irrigation allowing a reduction of fungicide applications, because it shortened the primary infection period. Targeted irrigation can therefore represent an additional tool to manage apple scab, other than fungicides, sanitation practices, DSSs, beneficial microorganisms and scab-resistant cultivars.

Targeted irrigation was adaptable to different growing conditions. In fact, it was effective both with under-canopy and over-canopy irrigation. The under-canopy irrigation system can easily be implemented in commercial orchards by placing micro-sprinklers along the rows, in order to cover the entire surface of the plot. The over-canopy irrigation system, already installed in the orchard and commonly used for irrigation and frost protection, can effectively be used to promote ascospore ejection as well, with no supplementary equipment costs for the grower. In both systems, a low water intensity was used during the present trials (2-3 mm/h) and for a period of 2 h, allowing a limited water consumption in each irrigation. However, targeted irrigation is applicable only where the water supply is not a limiting factor in spring. Under these conditions and timings, irrigation should not interfere with the development of other apple diseases. For example, attention should be paid with irrigation over the canopy during bloom of apple trees, if there is a high risk of fire blight [*Erwinia amylovora* (Burrill) Winslow et al.] during particularly warm and wet conditions. Bacterial cells can be disseminated among flowers by wind and rain but most likely by pollinators (Sundin, 2014). The short time of irrigation carried out in days of dry weather likely minimizes the risk of fire blight. Nevertheless, further research is necessary to explore the long-term effects of reducing fungicides by irrigation, such as the development of fungicide-resistant pathogen strains and the impact on secondary pathogens that could emerge in a low-fungicide environment (e.g., *Marssonina coronaria*, *Colletotrichum* spp.).

Irrigation targeted to discharge ascospores should cover the whole orchard area to achieve the highest efficacy. In this way a uniform reduction of the ascospore potential can be obtained in the orchard. As reported by Gregory (1973) and MacHardy (1996), most of released fungal spores remain within the crop, and less than 10% of them move beyond the boundary of a crop. Kaplan (1986) showed that the airborne concentration of ascospores of *V. inaequalis* was reduced by 99% at 5-6 m from their source. With these statements it is possible to assume a scarce influence on scab incidence of non-irrigated orchards located in the vicinity of irrigated orchards. Nevertheless, irrigation on extended apple growing areas should allow the highest effectiveness of this method.

In the weather conditions of northern Italy, leaf litter usually remains in the orchard and leaf degradation probably takes longer than in regions with warm-wet winters and springs. Although leaf degradation may be faster in these regions and scab inoculum may be reduced, targeted irrigation should still be effective when the right irrigation conditions are met. In our field trials, irrigation resulted in better efficacy of chemical treatments against apple scab, even in plots with a full fungicide strategy. If leaf litter remains mainly at the hedges or other vegetation surrounding the orchards, irrigation should also cover this area, but other sanitation practices such as removal or shredding of leaf litter become essential in such conditions.

The best results of targeted irrigation will be achieved at the time of highest ascospore release, usually between the pink bud and full bloom stages. At the beginning and end of the primary season, few mature spores are available to be released, and the effect of irrigation may be limited. In the present experiments, irrigation carried out around noon allowed moreover to achieve the best daily efficacy in ascospore release, because the peak of ascospore release occurs in the middle of the day (MacHardy and Gadoury, 1986). The present work demonstrated that light triggered ascospore discharge of *V. asperata* similarly to what was previously reported for *V. inaequalis* (MacHardy and Gadoury 1986; Rossi et al., 2001). This opens the possibility of using targeted irrigation to reduce infections of *V. asperata* as demonstrated for *V. inaequalis,* even if the primary infection period seems postponed compared to *V. inaequalis*. The sprinkler irrigation method may provide even better control in scab-susceptible orchards with organic management than in conventional orchards, because the active substances for organic scab control are often less effective than synthetic fungicides, and the incidence of the disease is usually higher than in conventional orchards.

The results of this thesis indicate that the use of targeted irrigation is best suited in apple growing areas with few scattered rainy days and extended dry periods during the primary infection season. Under these conditions, irrigation could induce the discharge of high number of ascospores that have matured since the previous rain. Conversely, in climatic areas and seasons where frequent and heavy rainfall occurs in spring, irrigation is likely less effective in ascospore depletion and in subsequent reduction in the need of fungicide applications. In our studies carried out in northern Italy, where spring is usually dry with spaced-out rains, we obtained a good efficacy of irrigation both on ascospore discharge and on scab control (overall higher than 50%).

Climate change, with an increase of average temperatures and rainfall frequency, could influence timing and efficacy of targeted irrigation in the future. An advance in the primary infection season of *V. inaequalis,* and earlier onset of the apple growing season may interfere with the timing of irrigations. Frequent rains in spring, as occurred in 2023 and 2024, may reduce the potential number of irrigations which can be effectively applied during the primary season. Therefore, reliable weather forecasts and the careful observation of hourly forecast data for the next 24 - 48 h and forecasting models for scab infections, are increasingly important in identifying the correct time to carry out the sprinkler irrigation.

The aerial dispersal of ascospore of *V. inaequalis* following under-canopy sprinkler irrigation was studied for the first time in the present thesis work. In fact, previous research on ascospore dispersal and deposition of *V. inaequalis* was all carried out during rain events under field conditions (Aylor, 1995; Carisse et al., 2007; Charest et al., 2002; Rossi et al., 2003). This study showed that most ascospores of *V. inaequalis* can disperse above the irrigated layer, and they were detected at all heights above the sprinklers (from 1 to 3 m above the ground). Airborne ascospores beyond the irrigated layer may therefore settle on susceptible apple tissues through dry deposition (sedimentation and impaction). Moreover, in the orchard, under-canopy irrigation had a similar effect as rain on the vertical dispersion of ascospores (exponential decline of ascospores with height), suggesting that wind played a critical role in spore transport. These results underline that targeted irrigation should be carried out in the warmest hours of the day, when higher wind speeds can decrease spore concentration in the canopy and the leaf litter on the ground can dry quickly after irrigation. For a better comprehension of the ascospore dispersal with under-canopy sprinkler irrigation, further research should be carried out by placing anemometers at different heights. Sonic anemometers would be the best option to provide accurate measurements of three-dimensional wind speed and detailed turbulence measurements.

As highlighted in this study, since ascospores dispersed above the irrigated layer could settle on susceptible apple tissues, it is essential to ensure a rain-free period of at least 24 hours after irrigation, so that spores are ejected under conditions unsuitable for scab infections and are subjected to a fast decay, without causing disease. Conversely, if an unexpected rainfall occurs shortly after irrigation, ascospores settled on the apple tissues may remain viable, and a curative fungicide may have to be applied following rain in order to reduce the risk of apple scab infection. Accurate and reliable weather forecasts are crucial in determining the effectiveness of this irrigation method. These practical recommendations are valid both for over- and under-canopy irrigation systems. A more detailed risk assessment of targeted irrigation, testing the system under different humidity and weather conditions, could make this method safer and promote its use in different apple-growing regions.

Over the two-year study, *V. asperata* showed a delayed pseudothecial maturation and emptying compared to *V. inaequalis*. The spore release was also delayed for *V. asperata*, ending about one month later than *V. inaequalis*. The different behaviour of the two species may partly explain the late onset of symptoms and signs caused by *V. asperata* in the orchards during the growing season. The present study did not quantify infection efficiency of *V. asperata* spores under different environmental conditions. To study this, experiments with artificial inoculation should be carried out to understand whether the delayed release of ascospores translates into higher or lower risk of infection. This new apple scab-like disease seems to have higher requirements of degree day accumulation for maturation of pseudothecia compared to *V. inaequalis*. Therefore, the increase of average temperatures due to the climate change, as well as the spreading of scab-resistant cultivars, may promote the development of *V. asperata* in fruit-growing areas with mediterranean besides temperate climate, across Europe and worldwide.

In the present thesis, irrigated vs. non-irrigated plots or *V. inaequalis* vs. *V. asperata* plots were compared in each year, considering the degree day accumulation for the whole primary season. Even if the degree hour accumulation might better explain the year-to-year differences in pseudothecial maturation or ascospore discharge, degree days were a good parameter to highlight seasonal differences between treatments. In any case, degree days used in these studies derived from hourly data, not by averaging the daily maximum and minimum temperature, as is often done.

To date, in the Province of Trento there are not reports on *V. inaequalis* races that have overcome resistance to the *Rvi6* gene. In fact, in the orchard of the scab-resistant cultivar Modì where assessments were carried out, only symptoms of *V. asperata* were observed. Symptoms of infections of *V. asperata* on fruit are distinguishable from those caused by *V. inaequalis*. Symptoms on leaves are more difficult to discriminate, but they can be identified since they were lighter, more irregular, and less prominent than those of V. *inaequalis*, and they only developed on the abaxial leaf surface. In any case, to confirm that lesions were caused by *V. asperata*, for each disease assessment in the Modì orchard a sample of leaves and fruit was collected and checked with microscopy.

The weak symptoms of *V. asperata*, observed both on fruit and leaves suggest that careful assessments in apple orchards are essential to monitor the presence and the spreading of this pathogen. The study of the season for ascospore release in different apple growing regions could give essential information to effectively apply the targeted irrigation and to develop effective control strategies against *V. asperata.* The increase in symptom development of *V. asperata* in the orchards over the years and the high incidence recorded in some growing areas means that the fungus can establish in apple orchards and gradually increase its overwintering inoculum in infected leaf litter on the ground.

The reduced fungicide spray schedule used in scab-resistant cultivars is likely the main factor promoting the development of *V. asperata* in the orchards. The reduction of fungicide applications in scab-susceptible cultivars during the secondary infection season of *V. inaequalis* may also promote the growth of *V. asperata,* which infections seem to occur later than *V. inaequalis*. To manage *V. asperata* it will be crucial to identify the correct timing for fungicide treatments and for targeted irrigation, which need to be prolonged during the growing season compared to *V. inaequalis*.

In conclusion, targeted irrigation could represent a sustainable and easily applicable method to reduce the incidence of apple scab, allowing to reduce the number of fungicide applications but also to increase their efficacy. Irrigation should be integrated with fungicide control strategies and with other control measures to reach an effective disease management. Incorporating the effect of irrigation on ascospore release in DSSs could improve the accuracy of information provided to the growers to predict the risk of apple scab infections.

Further research on the epidemiology of *V. asperata* is necessary to find the optimal weather conditions for ascospore discharge and for primary and secondary infections, as well as its latency period. The efficacy of fungicides commonly used against *V. inaequalis* and susceptibility of different apple cultivars to *V. asperata* should also be addressed by research. Future breeding programs for apple should take into consideration resistance to *V. asperata* and preferably also to other emerging pathogens. Specific control strategies should be developed to address the delayed primary infections and late symptom appearance caused by this fungus.

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