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Growth and sporulation of *Stemphylium vesicarium*, the causal agent of brown spot of pear, on herb plants of orchard lawns

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Abstract

The inoculum sources of ascospores of *Pleospora allii* and of conidia of its anamorph *Stemphylium vesicarium* were investigated in relation to the brown spot disease epidemiology on pear. Dead and living leaves of three pear varieties (Abate Fétel, Conference and William), seven grasses (*Poa pratensis, Festuca rubra, Festuca ovina, Lolium perenne, Digitaria sanguinalis* and *Setaria glauca*) and *Trifolium repens*, which are used in pear orchard lawns, were inoculated with conidia of *Stemphylium vesicarium* virulent on pear and incubated under controlled-environment. *Stemphylium vesicarium* was always re-isolated from dead leaves of the considered plants, but not from symptomless green or yellowish living leaves. The fungus was occasionally re-isolated from leaf segments showing unspecific necrosis. Inoculation of pear leaves with isolates from grasses demonstrated that the fungus did not lose pathogenicity. Pseudothecia, ascospores and conidia were produced on all the dead inoculated leaves; differences between specimens were found for phenology of pseudothecia, their density and size, and for the number of conidia produced. Pseudothecia were produced faster in the lawn species than in pear leaves, and their density was higher, especially for *S. glauca*, *L. perenne* and *P. pratensis*. Ascospore maturation and ejection was more concentrated for the pseudothecia developed on pear leaves than for those on *F. ovina* and *S. glauca*. All the lawn species produced more conidia than pear leaves.

Introduction

Brown spot of pear (*Pyrus communis*), caused by *Stemphylium vesicarium* (Ellis and Ellis, 1985), is a disease of economic importance in fruit-growing areas of southern Europe: the Po Valley (Italy), Catalunya (Spain) and Bouches du Rhone (France) (Ponti et al., 1982; Villardel, 1988; Blancard et al., 1989; Realise et al., 2002). In recent years, the disease has spread to The Netherlands (Polfliet, 2002; Van Dijke, 2002), Portugal (Llorente and Montesinos, 2002) and Belgium (P. Creemers, RSF-Research Station of Gorsem, pers. comm.).

Disease symptoms consist of extended necrotic areas on leaves and shoots. Fruits show small necrotic spots that progressively enlarge and deepen in round-shaped brown areas that can rot. Infected fruits are unmarketable.

In Italy, the epidemics begin in late spring, when fruits are highly susceptible (Montesinos et al., 1995a), and progressively increase until harvest, when 80–90% of fruits can be affected (Ponti and Laffi, 1993). Disease development is faster on fruit just before harvest (Montesinos and Vilardell, 1992) because of a combination of both high spore densities and suitable environmental conditions for infection (Montesinos et al., 1995a).

Two kinds of spores are produced by the pathogen: the teleomorph, *Pleospora allii* produces ascospores within pseudothecia, while the anamorph *S. vesicarium* produces conidia.

Cavanni and Ponti (1994) stated that the role of ascospores and conidia in the epidemiology of brown spot on pear was not completely understood. In recent years, pseudothecia of *P. allii* were observed in infected pear leaves on the orchard ground (Llorente et al., 2003; Maccaferri et al., 2003), maturing ascospores from February to May (Llorente et al., 2003). First disease symptoms usually appear in June (Ponti et al., 1982), but at this moment, few or no ascospores are present in the orchard air (Picco et al., 1996; Maccaferri et al., 2003), while conidia are usually abundant (Bugiani et al., 2004). Conidia continue to be airborne during the pear-growing season, though sporulation rarely occurs on affected pear tissue (Maccaferri et al., 2003). So, some epidemiological aspects of this disease remain unclear.

Considering that *S. vesicarium* is basically a very common saprophyte on grasses (Ellis, 1971) and that disease incidence is higher in pear orchards managed on grassy soils than on bare soils (Cavanni and Ponti, 1994), it was postulated that fungal strains infecting pear develop and produce spores on the herbaceous plants growing in pear orchard lawn. This study was undertaken to verify this hypothesis.

Materials and methods

Stemphylium vesicarium *strains and preparation of conidial suspensions for artificial inoculations*

Pear fruits and leaves showing typical brown spot symptoms were collected during summer from several orchards in Emilia-Romagna (North Italy). Fruit or leaf segments were washed in running tap water for 20 min, sterilised with ethyl alcohol (70%) for 15 s and in sodium hypochlorite (2% of available chlorine) for 2.5 min, rinsed 3 times in sterile water and dried on absorbent paper under a sterile air flow. Segments were placed in Petri dishes containing water agar (1.2%) adjusted to pH 5.2 and incubated for 6–8 days at 25 °C. Fungal colonies growing from segments were transferred to potato dextrose agar (PDA) containing streptomycin sulphate (50 mg l^{-1}) and incubated at 15–25 °C, with 12 h daylength. *Stemphylium vesicarium* colonies were identified according to Ellis (1971). Single-spore isolates were obtained from these colonies and maintained.

Strains used in the present work were selected on the basis of their ability to cause severe disease symptoms when inoculated onto detached leaves of a susceptible pear variety 'Abate Fétel'. Leaves were detached from 2-year old potted plants grown in a glasshouse at 20 ± 2 °C, washed in running tap water for 30 min, dried under a sterile air flow and placed in sterilised plastic boxes (20 × 30 cm) over blotting paper wetted with sterile water, the upper surface touching the paper. Six 10 µl drops of a conidial suspension were placed on the lower leaf surface. Control leaves were inoculated with water. Boxes were enclosed in plastic bags and incubated at 25 °C, 12 h daylength, for 30 days. Leaves were inspected daily to determine the number of inoculated drops causing necrosis of the leaf tissue. Disease incidence was then calculated as a percentage of total inoculations showing necrosis over the total inoculations. Three strains (ISA94, BR11 and D440) which caused a rapid and severe appearance of leaf necrosis were then selected.

Inoculum suspensions were prepared by collecting conidia from 14-day old fungal colonies grown on V8 (Campbell Ltd., Italy) agar in Petri dishes incubated under fluorescent light at 20 °C with 12 h daylength. The colonies were dispersed in 10 ml of distilled water using a spatula and the resulting suspension filtered through a double layer of cheesecloth and adjusted to 1×10^5 conidia per ml. A germination rate between 90 and 95% of these conidia was found after 2 h of incubation at 25 °C.

Inoculation of dead leaves

In the first experiment, the following species common in orchard lawns pear orchards in northern Italy were used: *Poa pratensis* (Kentucky bluegrass), *Festuca rubra* (red fescue), *Festuca ovina* (sheep fescue), *Lolium perenne* (perennial ryegrass), *Digitaria sanguinalis* (tall crabgrass), *Setaria glauca Beauv.* (yellow foxtail) and *Trifolium repens* (white clover). Three pear varieties (Abate Fétel, Conference and William, in decreasing order of susceptibility to brown spot) were also included. All the plants were grown in pots in a glasshouse at 18–25 °C.

Thirty fully expanded leaves of these plants and of the pear varieties were collected, washed under running tap water for 15 min, autoclaved at 120 °C for 20 min and placed on a microscope slide in

9-cm Petri dishes containing a double layer of wetted blotting paper. Twenty-five leaves were inoculated with strain ISA 94, by uniformly distributing 0.5 ml of inoculum per dish, and incubated at 25 °C, 12 h daylength, at 100% relative humidity. Five leaves were inoculated with sterile water.

After 7 days, the leaves were washed for 15 min under running tap water, sterilised with sodium hypochlorite (1%) for 1 min, rinsed in sterile water and dried under a sterile air flow. Five leaf segments were cut from each leaf and placed in Petri dishes on water agar (1.2%), pH 5.2, and incubated at 25 °C. Fungal colonies growing from the leaf segments were transferred onto V8-agar and incubated at the same temperature. Colonies of *S. vesicarium* were identified and their frequency of isolation determined. The experiment was repeated once.

A single-spore strain from each lawn species was tested for pathogenicity on detached 'Abate Fétel' pear leaves and compared with the original strain ISA94. Four replicates (4 leaves per replicate, 6 inoculations per leaf) were used. The incidence of inoculations showing necrosis was recorded daily for 17 days. The Kolmogorov–Smirnov Test (KST) was used to analyse differences between strains in the progress of disease incidence over time: the null hypothesis that such distributions come from the same one was tested at $p \leq 0.05$. A one way analysis of variance (ANOVA) was applied to test differences between strains for disease incidence at the end of the experiment (17 days after inoculation). The data were arcsin transformed to make variances homogeneous. The Student–Newman–Keuls (SNK) Test at $p \leq 0.05$ was used to separate means.

Inoculation of living leaves

In the second experiment, plants of *L. perenne, P. pratensis, F. rubra, F. ovina* and *T. repens* were grown in 40-cm pots (4 pots per species) in a glasshouse and inoculated 6 months after sowing with 100 ml of inoculum in each pot. The inoculum was a mixture of aliquots of conidia from strains ISA94, BR11, and D440. One pot per lawn species was sprayed with sterile water as control. Plants were inoculated to run off using a compressor air atomizer in such a way to wet both leaf surfaces. Following inoculation each pot was covered for 48 h with a transparent plastic bag to preserve leaf wetness and 100% relative humidity, and incubated in the glasshouse at 18–25 °C, to ensure optimal conditions for infection (Montesinos et al., 1995b; Llorente and Montesinos, 2002).

Leaf samples containing green leaves showing necrosis, green symptomless leaves and yellowish symptomless leaves were randomly collected 7, 14 and 28 days after inoculation from each lawn species. The leaves were washed under running tap water for 15 min, sterilised with sodium hypochlorite (1%) for 1 min, rinsed in sterile water and dried under an air flow. In aggregate, about 3300 leaf segments were cut from the leaves, including necrotic tissue when present. Segments were plated until identification of *S. vesicarium* colonies. The experiment was repeated once.

Single-spore isolates obtained from the *S. vesicarium* colonies re-isolated from the leaves were tested for pathogenity on 'Abate Fétel' leaves.

Production of conidia and pseudothecia

To test the ability of the leaves of the 7 lawn species and of the 3 pear varieties in supporting spore production by *S. vesicarium*, 6 leaves were detached from each plant species or variety and their area was measured by a LI-COR 2000 meter (LI-COR Inc., Lincoln, Nebraska, U.S.A.). The leaves were treated as described in the first experiment, until inoculation and incubation.

After 24 days-incubation, 3 leaves were immerged in test-tubes containing 1.5 ml of water (1 leaf per tube) and gently shaken for 30 s to detach conidia. The concentration of spores was determined using a Burkard haemocytometer. Spore production was expressed as the mean number of conidia per square centimetre of leaf.

The remaining 3 leaves were observed under a stereomicroscope (20×) every 3–6 days, and the total number of pseudothecia per leaf recorded, and expressed as the average number of pseudothecia per square centimetre of leaf. Maximum diameter of 100 pseudothecia per specimen was measured after 38 days of incubation. As the fruit bodies had not yet differentiated ascospores, leaves were further incubated at 10 °C until the production of ascospores to confirm their identity as *P. allii* (Simmons, 1969).

The numbers of conidia and pseudothecia produced per square centimetre of leaf of the 10 specimens (3 replicates each) were compared using ANOVA. Data were ln-transformed to make

variances uniform. Differences between average values were tested by the SNK Test at $p \leq 0.05$. Data from ANOVA were back-transformed to produce graphs. The size of pseudothecia was also compared using ANOVA.

Overwintering of pseudothecia

To test the possibility that the pseudothecia of *P. allii* produced in leaves of the lawn species and of the 3 pear varieties overwinter under natural conditions, 20 leaves per specimen were treated as previously described and incubated at 25 °C for 15 days; at this time, several undifferentiated ascostromata were produced. The leaves were fixed to microscope slides using small tongs and exposed to natural weather conditions between early October 2002 and mid June 2003, inside the campus of the University of Piacenza (northern Italy). Meteorological conditions were continuously monitored by an electronic equipment (Weather Monitor II™, Davis Instruments, San Francisco, U.S.A.) 5 m from the experimental site.

Beginning in mid February, 20–30 pseudothecia per specimen were excised from leaves at 15-day intervals until mid-June; they were deposited on microscope slides, crushed, stained with 0.1% acid fuchsin in lactophenol, observed under a microscope and classified in 4 phenological stages, using a simplified scale from Prados-Ligero et al. (1998): (a) with undifferentiated ascospores, (b) with ascospores in the process of formation (not yet pigmented), (c) with mature ascospores, (d) with empty ascii. The frequency of pseudothecia in different stages were then calculated. Scattered observations were carried out on the viability of ascospores: they were placed in drops of water at 20 °C and observed at 2-h intervals to check germination.

Results

Inoculation of leaves with S. vesicarium

Stemphylium vesicarium strain ISA94, colonised all species tested and was re-isolated from all specimens except the control. Representative isolates were pathogenic on green leaves of 'Abate Fétel' and always caused typical brown spot symptoms. Necroses on the pear leaves inoculated with ISA94 progressed significantly faster than those inoculated with the strains re-isolated from grasses (KST significant at $p < 0.05$): disease incidence reached 100% on the 9th day after inoculation for strain ISA94 and 5 or 6 days later for the other strains (Figure 1). The *S. vesicarium* strain re-isolated from *P. pratensis* produced symptoms faster than strains from *F. rubra*, *F. ovina*, *S. glauca* and *L. pratense* (KST significant at $p < 0.05$), because more than 75% of inoculations showed necrosis 9 days after inoculation (Figure 1). There were no significant differences between strains 17 days after inoculation.

When inoculations were made on living plants, of 5 out of the 7 lawn species previously considered, *S. vesicarium* was not re-isolated from 2100 symptomless leaf segments considered in aggregate, nor from green or yellowish leaves. *Alternaria* spp. were isolated from these leaves, with frequencies of 5 and 8%, respectively, irrespective of specimens; 6% of *Alternaria* colonies were isolated from the untreated control leaves. Only 5 strains of *S. vesicarium* were isolated from leaves of *P. pratensis*, *T. repens* and *L. perenne* showing necrosis (out of 1188 leaf segments); 20% of *Alternaria* isolates were also obtained from these leaves. Necroses initially appeared as small yellowish areas irregularly distributed on the leaf blade, one or a few spots per leaf. The affected areas became larger and occupied a wide blade area; their centre became light grey with a reddish brown border and a wide yellowish and irregularly shaped halo. These symptoms were similar in all

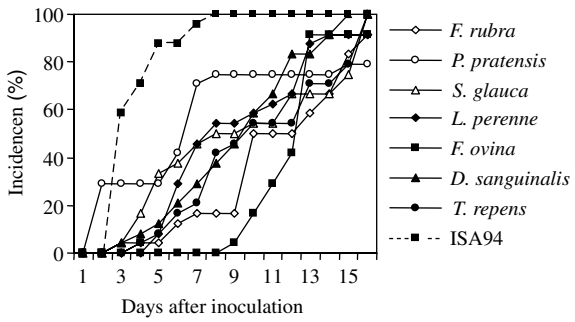

Figure 1. Incidence of necrotic spots on pear leaves (cultivar Abate Fétel) inoculated with a virulent *S. vesicarium* strain ISA94 and with isolates from dead leaves of different lawn species previously inoculated with strain ISA94. No significant differences between fungal isolates were found at the end of the experiment.

the specimens; they appeared also on the two species of *Festuca* and sometimes on the untreated control but *S. vesicarium* colonies did not grow from such affected tissues.

These five *S. vesicarium* strains re-isolated from symptomatic leaves were inoculated on the 'Abate Fétel' leaves and compared with the 3 original strains (Figure 2): one strain from *P. pratensis* did not significantly differ from the original ones; three strains, from *L. perenne*, *P. pratensis* and *T. repens*, showed a slower disease progress (KST significant at $p < 0.01$) but disease incidence at the end of experiment was not significantly different; one strain from *T. repens* also caused a significant ($p < 0.05$) reduction in the final disease incidence.

Production of conidia and pseudothecia on dead leaves

Conidia of *S. vesicarium* were produced on all the dead inoculated leaves. Characteristics of conidiophores and conidia agreed with the descriptions of Simmons (1969), irrespective of the specimen, but significant differences in the amount of spores produced between specimens were observed. *F. rubra* and *F. ovina* produced about 3 times more conidia than *L. perenne* and *P. pratensis*, 10 times more than *S. glauca*, *D. sanguinalis* and *T. repens*, and 26 times more than the 3 pear varieties (Figure 3). Production of conidia on pear leaves was significantly lower than that observed on the herbaceous plants (Figure 3).

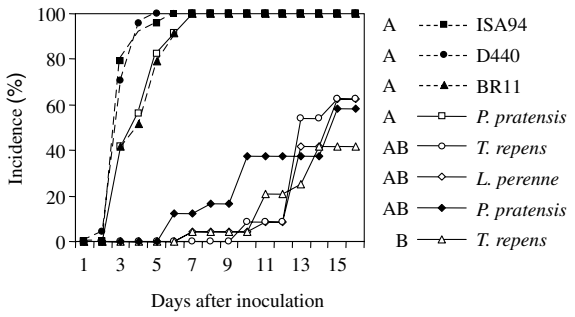

Figure 2. Incidence of necrotic spots on pear leaves (cultivar Abate Fétel) inoculated with virulent *S. vesicarium* strains ISA94, BR11 and D440 and with isolates from necrotic leaf tissue of different lawn species previously inoculated *in planta* with a mixture of the three strains. Figures followed by a different letter are significantly different (at $p \leq 0.05$) at the end of the experiment.

Pseudothecia were produced abundantly in all the dead inoculated leaves and ascospore dimensions confirmed that these were *P. allii*. Fruit bodies did not develop on the uninoculated leaves. On the 3 pear varieties, pseudothecia first began to appear after 10–13 days-incubation, and their number increased till 31 days (Figure 4a). On leaves of the lawn plants, production of pseudothecia was faster, and the maximum number appeared after 13–24 days (Figure 4b). The highest number of pseudothecia was recorded on *S. glauca*, followed by *L. perenne*, *P. pratensis*, *T. repens*, *F. rubra*, *F. ovina* and 'Abate Fétel'. Lower numbers were observed on 'Conference', 'William' and *D. sanguinalis* (Figure 4). The size of pseudothecia produced on 'Conference' and 'William' was significantly higher than that on 'Abate Fétel', *T. repens*, *L. perenne* and *P. pratensis*; smaller fruit bodies were produced on *D. sanguinalis* and on the two *Festuca* species (Figure 5).

Overwintering of S. vesicarium *in dead leaves*

Considering all the pseudothecia observed, irrespective of the specimen, the first mature ascospores were observed in late February and the proportion of pseudothecia containing mature ascospores increased till mid-April (Figure 6). The first empty pseudothecia appeared in early April on 'Conference' and by mid-June all the fruit bodies were

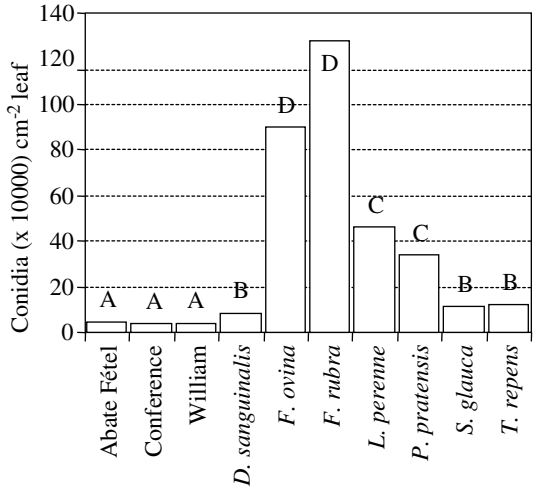

Figure 3. Conidial production by *S. vesicarium* on dead leaves of 3 pear varieties and 7 lawn species inoculated with the virulent strain ISA94, after 24 days of incubation. Bars followed by a different letter are significantly different at $p \leq 0.05$.

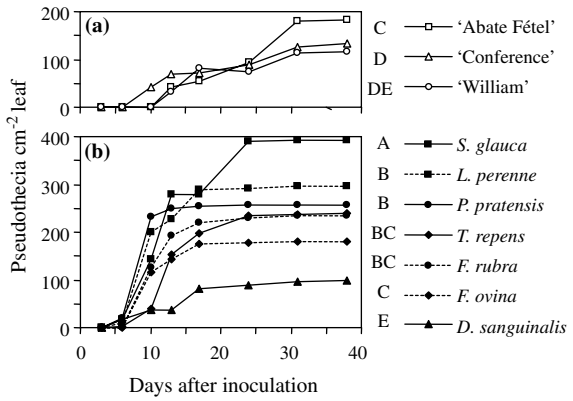

Figure 4. Numbers of *P. allii* pseudothecia produced on dead leaves of 3 pear varieties (a) and 7 lawn species (b) inoculated with the virulent strain ISA94 and incubated for 38 days. Figures followed by a different letter are significantly different (at $p \leq 0.05$) at the end of the experiment.

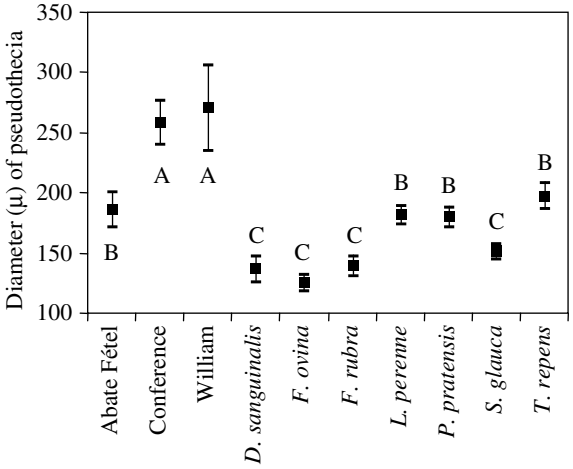

Figure 5. Diameter of *P. allii* pseudothecia on dead leaves of 3 pear varieties and 7 lawn species inoculated with the virulent strain ISA94, after 38 days of incubation. Bars indicate means ± standard errors; points with a different letter are significantly different at $p \leq 0.05$.

empty (Figure 6). Air temperature decreased from October (14.1 °C) to February (1.3 °C), then increased in March (9.4 °C) till June (25.4 °C). During winter there were 13 days with freezing temperatures, with a min of −3 °C. Total rainfall between October and mid-June was 440 mm; during the period of ascospore ejection total rainfall was 40 mm, with 17 rainy days.

By mid-February many pseudothecia already contained immature ascospores; their frequency was 100% for 'William', *D. sanguinalis*, *F. rubra*, *L. perenne* and *S. glauca*, while in the other specimens it ranged between 60 and 85% (Figure 6). Pseudothecia with mature ascospores appeared between late February and early March and their frequency increased to 100% between mid-March and late April, depending on the specimen. Ascospores germinated in a few hours, irrespective of the specimen. Empty pseudothecia first appeared on 'Conference' leaves in early April, then their frequencies increased for all the specimens (Figure 6). In *F. rubra* and *T. repens* all the fruit bodies discharged their ascospores in the first 10 days of May, while in *F. ovina* and especially in *S. glauca* several pseudothecia contained ascospores at the end of May. Further observations showed that in these lawn species ascospore ejection was complete at mid June.

Discussion

Most fungi belonging to the genus *Stemphylium* are saprophytes growing on dead plants and cellulose materials (Simmons, 1969; Ellis, 1971; Onions et al., 1981). This work showed that strains *S. vesicarium* which cause brown spot of pear are able to colonize dead leaf tissues of 7 lawn species.

Stemphylium species are able to grow as endophytes in the living leaves of various plants (Larran et al., 2000; Sultanova et al., 2002). In this work, *S. vesicarium* was not re-isolated from surface-sterilised lawn leaves which had been inoculated *in planta*. This indicated that these hosts do not support the endophytic growth of the fungus, at least within a period of 4 weeks after inoculation. Few isolates of *S. vesicarium* were obtained from leaf pieces of *L. perenne*, *P. pratensis* and *T. repens* showing unspecific leaf necrosis. Hetherington et al. (1996) isolated the fungus from diseased samples of *Avena* spp. and *L. rigidum*, and Pataky (1992) found it was a secondary colonizer or a saprophyte of the weakened grass plants in summer patches and necrotic ring spots. *S. sarcinaeforme*, *S. botryosum* and *S. trifolii* were frequently isolated from *Trifolium* species (Duke, 1981; Berg and Leath, 1996; Peat and Fitter, 2001; Tadayuki, 2003). *S. botryosum* is the main causal agent of *Stemphylium* leaf spot on alfalfa (Graham et al., 1979), but other species are pathogenic,

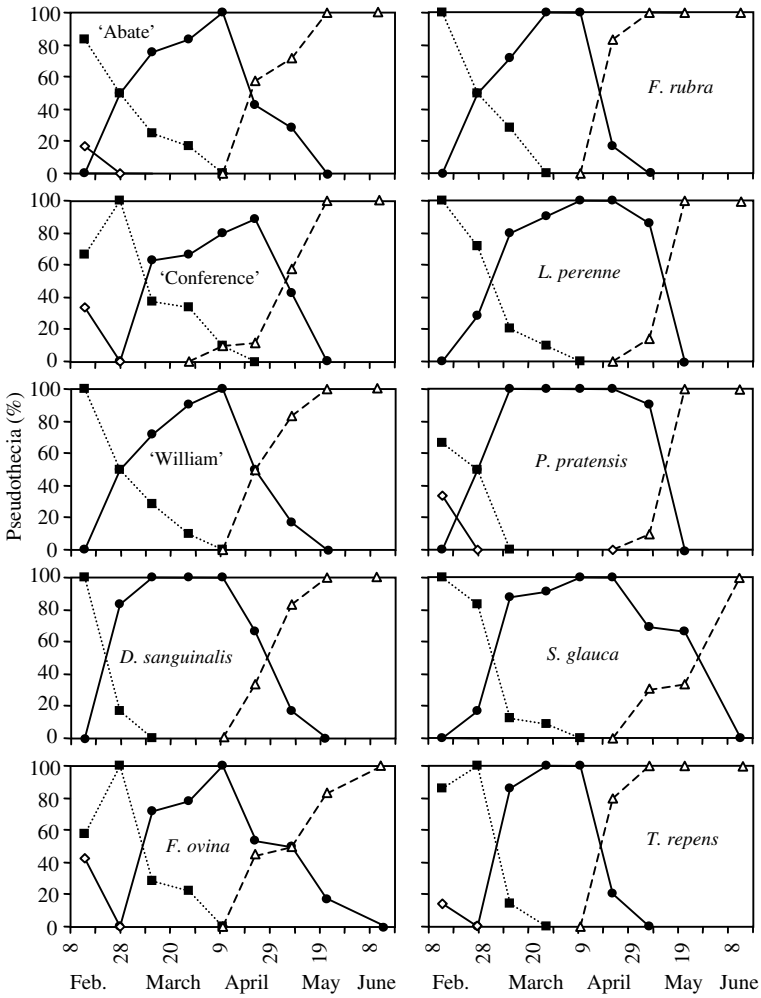

Figure 6. Phenological development of *P. allii* pseudothecia produced on dead leaves of 3 pear varieties and 7 lawn species inoculated with the virulent strain ISA94 and incubated outdoors between early October and late May. Undifferentiated ascospores (–◇–), ascospores differentiated (··■··), mature ascospores (–●–), empty ascii (- -△- -).

including *S. sarcinaeforme*, *S. vesicarium*, *S. globuliferum*, *S. herbarum* and *S. alfalfae* (Chaisrisook et al., 1995; Mackie et al., 1999; Frayssinet, 2002). In this work, the fungus inoculated on living plants was re-isolated from less than 0.1% of the specimens showing leaf necrosis; therefore, it likely developed as a saprophyte on dead leaf tissue.

This work demonstrated that strains of *S. vesicarium* virulent on pear do not lose their pathogenicity following saprophytic growth on lawn plants. It can be argued that the fungus did not lose its ability in producing phytotoxins (Singh et al., 1999, 2000) when it developed as a saprophyte, as all the fungal strains re-isolated from lawn leaves and inoculated on leaves of the susceptible pear 'Abate Fétel' produced typical disease symptoms.

Pseudothecia of *P. allii* were produced on leaves of pear, *Graminae* species and white clover. The presence and development of pseudothecia on these herbaceous plants has not been previously reported. Pseudothecium formation and maturation followed the typical process found in the *Pleosporales* (Luttrell, 1981). Density and size of pseudothecia found in the present work were similar to those observed by Prados-Ligero et al. (1998) on garlic debris naturally infected by *S. vesicarium*. Nevertheless, fewer and larger pseudothecia were produced in pear leaves than on some lawn species.

Pear leaves inoculated with *S. vesicarium*, and exposed to natural conditions in autumn 2002, showed the first mature ascospores in late February 2003 and maturation went on until about mid-May, after a period with low and frequently freezing temperatures. Ascospore ejection occurred between early April and May 20, when frequent rainfall occurred. Pseudothecia of *P. allii* mature during winter (Prados-Ligero et al., 1998) as low temperatures are necessary for this process (Aveling, 1993; Llorente and Montesinos, 2004). Under natural condition, dates of the first mature pseudothecia depend on orchard conditions ensuring suitable humidity (Llorente and Montesinos, 2004). In garlic ascospore ejection occurs between February and May, in association with rainfall (Prados-Ligero et al., 2003), in agreement with that occurring in leeks (Suheri and Price, 2000) and asparagus (Menzies et al., 1992). In this work, the dynamics of ascospore maturation and ejection in the lawn species was similar to that found in pear leaves, but in *F. ovina* and *S. glauca* the period of ascospore ejection lengthened to mid-June.

Typical conidiophores and conidia of *S. vesicarium* were produced on both lawn and pear leaves which had been autoclaved and inoculated, but on lawn leaves very high numbers of conidia were produced. Abundant sporulation by *S. vesicarium* on dead plant tissue has been previously documented (Yanez and Osada, 1990; Chowdhury et al., 1996). In garlic, onion and asparagus (Leuprecht, 1988; Menzies et al., 1992; Elena, 1996; Jakhar et al., 1996; Hausbeck et al., 1999; Suheri and Price, 2000; Suheri et al., 2001) conidia produced on affected plant tissues actively contribute to epidemics as an inoculum for secondary infections. Pear leaves and fruits subjected to natural infections produce conidia in laboratory after prolonged incubation under optimal conditions, but this rarely occurs under orchard conditions (Maccaferri et al., 2003).

Differences between species observed for both ascospores and conidia were probably due to differences in the chemical composition of the leaf tissue in the various specimens, which is crucial for promoting fungal growth and sporulation (De Ataide and Hegde, 1988; Rajani et al., 1991; Shi and Kuang, 1991).

It is known that disease incidence increases in pear orchards with a lawn of herbaceous plants compared to those on bare soils (Cavanni and Ponti, 1994). This work demonstrated that herbaceous plants covering the soil of pear orchards support saprophytic growth of *S. vesicarium* strains causing brown spot of pear, the production of pseudothecia of its teleomorph *P. allii* during winter, maturation and ejection of ascospores, and abundant production of conidia. Nevertheless, the role of these lawn plants in disease epidemiology in orchards needs to be further investigated.

Stemphylium vesicarium overwinters in fallen infected pear leaves as pseudothecia of *P. allii* (Maccaferri et al., 2003; Llorente and Montesinos, 2004), but a few ascospores are airborne in pear orchards (Picco et al., 1996), particularly in June, when first disease symptoms appear (Maccaferri et al., 2003). On the contrary, conidia are abundantly airborne from early April to September (Picco et al., 1996). A first spore peak frequently occurs in late May, with densities up to about 250 conidia m^{-3} of air per day, and further repeated peaks occur over the pear-growing season (Bugiani et al., 2004). In spite of this abundance of conidia, sporulation rarely occurs on pear leaves and fruits affected in orchards (Maccaferri et al., 2003). It can be supposed that ascospores released during winter and early spring from pseudothecia of *P. allii* on pear leaves infected by *S. vesicarium* during the growing season land on dead lawn leaves and that the fungus colonises them as a saprophyte. In spring and summer the colonised lawn leaves produce condia that become airborne and infect pears. Dead lawn leaves could also contribute to overwintering by producing pseudothecia. Studies to confirm this hypothesis are in progress.

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References

Aveling TAS (1993) *Stemphylium* leaf blight of garlic in South Africa. Phytophylactica 25: 293–294.

Berg CC and Leath KT (1996) Responses of red clover cultivars to *Stemphylium* leaf spot. Crop Science 36: 71–73.

Blancard D, Allard E and Brest P (1989) La Stemphyliose du poirier ou « macules brunes ». Phytoma 406: 37–38.

Bugiani R, Giosuè S, Rossi V and Spada G (2004) I modelli previsionali per la lotta alla maculatura bruna del pero. www.phytomagazine.com, 3(6): 43–50.

Cavanni P and Ponti I (1994) Maculatura bruna del pero: una micopatia sempre d'attualità. Rivista di Frutticoltura 56: 12, 37–42.

Chaisrisook C, Stuteville DL and Skinner DZ (1995) Five *Stemphylium* spp. pathogenic to alfalfa: Occurrence in the United States and time requirements for ascospore production. Plant Disease 79: 369–372.

Chowdhury AM, Asheque A, Zaman M, Bakr MA and Ahmed A (1996) Sporulation of *Stemphylium botryosum* Wallr. Journal of Mycopathological Research 34: 69–71.

De Ataide H and Hegde RK (1988) Vitamin requirements of *Stemphylium lycopersici* – a causal agent of leaf spot of tomato. Current Research University of Agricultural Sciences Bangalore 17: 148–149.

Duke JA (1981) Handbook of Legumes of World Economic Importance. Plenum Press, New York.

Elena K (1996) First report of *Stemphylium botryosum* causing *Stemphylium* leaf spot of asparagus in Greece. Plant Disease 80: 342.

Ellis MB (1971) Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, UK

Ellis MB and Ellis JP (1985) Microfungi on Land Plants. MacMillan, New York, USA.

Frayssinet S (2002) *Stemphylium vesicarium* Wallr. nuevo patogeno de alfalfa en Argentina. Agro Ciencia 18: 3–7.

Graham JH, Frosheiser FI, Stuteville DL and Erwin DC (1979) A Compendium of Alfalfa Diseases. American Phytopathological Society, St. Paul, Minnesota, USA.

Hausbeck MK, Hartwell J, Byrne JM and Benson B (1999) Epidemiology of *Stemphylium* leaf spot and purple spot in no-till asparagus. Acta Horticulturae 479: 205–210.

Hetherington S, Auld B, Priest M, Smith H, Van Tuat N and Van Du P (1996) Preliminary surveys and pathogenicity testing of fungi isolated from the annual grass weeds *Avena* spp. and *Lolium rigidum*. Bioherbicide Paper Plant Protection Research Institute 1: 11.

Jakhar SS, Duhan JC and Suhag LS (1996) Studies on the epidemiology and survival of *Stemphylium vesicarium* (Wallr.) Simmons in debris and seeds of onion. Seed Research 24: 135–140.

Larran S, Monaco C and Alippi HE (2000) Endophytic fungi in beet (*Beta vulgaris* var. *esculenta* L.) leaves. Advances in Horticultural Science 14: 193–196.

Leuprecht B (1988) *Stemphylium*, eine wichtige Krankheit an Spargel. Gesume München 24: 235–236.

Llorente I and Montesinos E (2002) Effect of relative humidity and interrupted wetness periods on brown spot severity of pear caused by *Stemphylium vesicarium*. Phytopathology 92: 99–104.

Llorente I and Montesinos E (2004) Development and field evaluation of a model to estimate the maturity of pseudothecia of *Pleospora allii* on pear. Plant Disease 88: 215–219.

Llorente I, Villardel P, Moragrega C, Bonaterra A and Montesinos E (2003) Biology, epidemiology and integrated control of *Stemphylium vesicarium* on pear, an emerging disease of economic impact in Europe. In: 8th International Congress of Plant Pathology (19.29 pp. 264), Christ Church, New Zealand.

Luttrell ES (1981) The pyrenomycete centrum-Loculoascomycetes. In: Reynolds DR (ed.) Ascomycete Systematics (pp. 124–137) Springer Verlag, New York.

Maccaferri E, Collina M and Brunelli A (2003) Studies on the epidemiology of *Stemphylium vesicarium* on pear. Journal of Plant Pathology 85: 310.

Mackie JM, Lloyd DL, Ryley MJ and Irwin JAG (1999) Fungal diseases of temperate annual pasture legumes in southern Queensland. Australian Journal of Experimental Agriculture 39: 699–707.

Menzies SA, Broadhurst PG and Triggs CM (1992) *Stemphylium* disease of asparagus (*Asparagus officinalis* L.) in New Zealand. New Zealand Journal of Crop and Horticultural Science 20: 427–433.

Montesinos E and Vilardell P (1992) Evaluation of FAST as a forecasting system for scheduling fungicide sprays for control of *Stemphylium vesicarium* on pear. Plant Disease 76: 1221–1226.

Montesinos E, Moragrega C, Llorente I and Vilardell P (1995a) Susceptibility of selected European pear cultivars to infection by *Stemphylium vesicarium* and influence of leaf and fruit age. Plant Disease 79: 471–473.

Montesinos E, Moragrega C, Llorente I, Vilardell P, Bonaterra A, Ponti I, Bugiani R, Cavanni P and Brunelli A (1995b) Development and evaluation of an infection model for *Stemphylium vesicarium* on pear based on temperature and wetness duration. Phytopathology 85: 586–592.

Onions AHS, Allsopp D and Eggins HOW (1981) Smith's Introduction to Industrial Mycology. 7th edn. Edward Arnold (Publishers) Ltd., London, UK.

Pataky NR (1992) Summer patch and necrotic ring spot of lawns and fine turfgrasses. Report on Plant Disease No 408, University of Illinois Extension, Urbana-Champaign, USA.

Peat H and Fitter A (2001) The ecological flora of the British Isles at the University of York. In: http://www.york.ac.uk/res/ecoflora/cfm/ecofl/

Picco AM, Betto A and Porri A (1996) *Stemphyllium*, *Pleospora* and *Alternaria* airspores in a pear tree orchard: A three year quantitative monitoring in Italy. In: 1st European Symposium on Aerobiology (pp. 156–157), Santiago de Compostela, Spain.

Polfliet M (2002) Infection of *Stemphylium* increases every year. Fruitteelt Den Haag 92: 16–17.

Ponti I and Laffi F (1993) Malattie crittogamiche delle piante da frutto. Edizioni L'informatore Agrario, Verona, Italy.

Ponti I, Cavanni P and Brunelli A (1982) Maculatura bruna delle pere: eziologia e difesa. Informatore fitopatologico 32: 3, 35–40.

Prados AM, Melero JM and Basallote MJ (1994) Development of the teleomorph of *Stemphylium vesicarium* in garlic debris affected by leaf spots. In: Proceedings of the 9th Congress Mediterranean Phytopathological Union (pp. 159–161), Kusadasi-Aydin, Turkey.

Prados-Ligero AM, Gonzalez-Andujar JL, Melero-Vara JM and Basallote-Ureba MJ (1998) Development of *Pleospora allii* on garlic debris infected by *Stemphylium vesicarium*. European Journal of Plant Pathology 104: 861–870.

Prados-Ligero AM, Melero-Vara JM, Corpas-Hervías C and Basallote-Ureba MJ (2003) Relationships between weather

variables, airborne spore concentrations and severity of leaf blight of garlic caused by *Stemphylium vesicarium* in Spain. European Journal of Plant Pathology 109: 301–310.

Rajani VV, Rawal PP and Khandar RR (1991) Cultural studies on *Stemphylium lycopersici* causing leaf spot of tomato. Indian Journal of Mycology and Plant Pathology 21: 38–42

Realise D, Castagne P, Coupard H, Kaluzny-Pinon L, Reynier C, Waligora C and Zambujo C (2002) En France: l'année 2002 se fait la poire belle. Arboriculture Fruitière 565: 25–42.

Shi SY and Kuang KY (1991) Biological characteristics of onion leaf blight fungus. Journal of Shanghai Agricultural College 9: 40–44

Simmons EG (1969) Perfect states of *Stemphylium*. Mycologia 61: 1–26.

Singh P, Bugiani R, Cavanni P, Nakajima H, Kodama M, Otani H and Kohmoto K (1999) Purification and biological characterization of host-specific SV-toxins form *Stemphylium vesicarium* causing brown spot of European pear. Phytopathology 89: 947–953.

Singh P, Park P, Bugiani R, Cavanni P, Nakajima H, Kodama M, Otani H and Kohmoto K (2000) Effects of host-selective SV-toxin from *Stemphylium vesicarium,* the cause of brown spot of European pear plants, on ultrastructure of leaf cells. Journal of Phytopathology 148: 87–93.

Suheri H and Price TV (2000) Infection of onion leaves by *Alternaria porri* and *Stemphylium vesicarium* and disease development in controlled environments. Plant Pathology 49: 375–382.

Suheri H, Price TV and Armstrong J (2001) Purple leaf blotch disease of *Allium* spp. in Australia. Acta Horticulturae 555: 171–173.

Sultanova MKH, Tashpulatov MM and Abdullaev BN (2002) Pathogenic microflora of plants damaged by cotton whitefly. Zashchita i Karantin Rastenii 3: 45.

Tadayuki S (2003) Illustrated encyclopaedia of forage crop disease. In: http://ss.ngri.affrc.go.jp/disease/detitle.html

Villardel P (1988) *Stemphylium vesicarium* en plantaciones de peral. Fruticoltura professional 18: 51–55.

Van Dijke JF (2002) Incidence of pear fruit spot can increase explosively. Fruitteelt Den Haag 92: 8–9.

Yanez MMdeJ and Osada KS (1990) Factores que estimulan la esporulacion de *Stemphylium solani in vitro*. Revista Mexicana de Fitopatologia 8: 52–58.