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The epidemiology of purple leaf blotch on leeks in Victoria, Australia

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Abstract

The incidence of purple leaf blotch disease was investigated on seven successive commercial leek crops grown at Cranbourne, Victoria between 1996 and 1997. First symptoms occurred on older leaves, 54–69 days after transplanting. Lesions with typical symptoms were colonised by either *Alternaria porri* (6%), *Stemphylium vesicarium* (42%) or mixtures of both pathogens (52%). Purple leaf blotch was caused by a disease complex and was endemic at Cranbourne due to the continuous cropping of leeks. Disease incidence in all monitored crops increased as plants matured (123–158 days after transplanting) until harvest but never exceeded 11% due to fortnightly applications of mancozeb. Disease levels showed no significant correlation with weekly temperature, precipitation, relative humidity or leaf wetness duration. Disease levels were significantly ($P < 0.05$) higher on autumn/winter (May/June) 1997 crops when 38 periods of leaf wetness $\geq$8 h because of dew and low temperatures (10–13 °C). The weekly rate of increase of disease incidence was significantly ($P < 0.01$) correlated with days after transplantation. Concentrations of airborne *A. porri* and *S. vesicarium* conidia within leek crops showed a diurnal periodicity and maximum numbers were trapped between 11:00 and 15:00 h. The concentration of airborne *S. vesicarium* conidia was three to six times the concentration of airborne *A. porri* conidia. Conidia were more abundant during spring/summer (September–February). Ascospores of *Pleospora allii* were found during May–September. The greater concentrations of airborne *S. vesicarium* conidia suggest that it may be the dominant pathogen in the purple leaf blight complex. Fungicide sprays were unnecessary until 8–10 weeks after transplanting, and regular protectant sprays curtailed but did not eradicate purple leaf blight. The results indicated that predictive models, based on temperature and the frequency of leaf wetness periods $\geq$8 h, will assist in reducing fungicide inputs as plants mature and, in southern Victoria, fungicide applications on leeks should be timed for autumn/winter when infection periods occur.

Abbreviations: PLB – Purple leaf blotch; DAT – days after transplanting; LAD – leaf area diseased.

Introduction

Purple leaf blotch (PLB) is an important disease of *Allium* spp. worldwide (Aveling, 1998) and is more prevalent in warm and humid environments (Maude, 1990; Miller and Lacy, 1995). Early symptoms appear as white flecks on the older leaves. Under suitable environmental conditions the white flecks enlarge and produce sunken purple lesions, which are often elliptical, sometimes with a yellow to pale brown border. The pathogen usually associated with the PLB symptom is *Alternaria porri* (Ellis) Cif. The symptoms of PLB are, however, generally indistinguishable from those of stemphylium leaf blight caused by *Stemphylium vesicarium* (Wallr.) Simmons and PLB is considered to be a complex caused by both pathogens (Suheri and Price, 2000a,b). In Australia, *A. porri* has been recorded on onion and leek (Herbarium, Institute for Horticultural Development, Knoxfield, Victoria; Warcup and Talbot, 1981; Simmonds, 1984).

PLB causes considerable losses in Allium production. On onions it reduced foliar production by 62–92%

(Utikar and Padule, 1980); yield in Kenya by 44% (Bock, 1964); bulb yield by 59% (Gupta and Pathak, 1988); and seed yield by 97% (Lakra, 1999). In Indonesia, yield losses of 60–70% of shallots occur during the wet season due to a high incidence and severity of PLB (Triwidodo et al., 1999).

Most information on the epidemiology of PLB is based on studies of the interaction of *A. porri* on onion. These include the effects of environmental factors, such as temperature, humidity or leaf wetness, on infection and lesion development (Bock, 1964; Everts and Lacy, 1990a; 1996; Suheri and Price, 2000a), conidial formation (Everts and Lacy, 1990a) and sporulation and dispersal (Meredith, 1966; Everts and Lacy, 1990b). The epidemiology of PLB on leeks or other Allium crops under Australian conditions has not been previously studied and there is limited information on the development of PLB under the cool conditions which typically occur at the time when crops such as leek and onion are grown by vegetable farmers in southern Victoria. Recent studies (Suheri and Price, 2000a,b) showed that Victorian isolates of *A. porri* and *S. vesicarium* from leeks were pathogenic on leeks and onion and South Australian isolates of *S. vesicarium* from garlic were pathogenic on onion. Infection occurred at 5 °C following leaf wetness periods $\geq$16 h.

The management of PLB on Allium crops has primarily depended on frequent applications of fungicides (Sugha, 1995) and host resistance (Bock, 1964; Bisht and Thomas, 1992). Management strategies based on epidemiological principles can help reduce the number of fungicide sprays required to control PLB. This is especially desirable in tropical countries, such as Indonesia, where fungicides are often sprayed at 2-day intervals throughout the growing season to control PLB (Triwidodo et al., 1999). Disease prediction models based on temperature, leaf wetness duration and spore concentration have been developed for *Botrytis squamosa* on onion (Sutton, 1990) and for leaf spot of oilseed rape caused by *Alternaria brassicae* (Kennedy and Graham, 1995). A predictive model for PLB is not available currently although data for development of such a model is available (Meredith, 1966; Everts and Lacy, l990a,b; 1996; Suheri and Price, 2000a).

The aims of this study were: (i) to study the progress of PLB on successive leek crops during different growing seasons in southern Victoria, (ii) to determine the relationship between disease incidence in a commercial crop and weather parameters, (iii) to determine the periodicity of airborne inoculum of *A. porri* and *S. vesicarium* over a PLB infected leek field and (iv) to utilise the information from these studies to prevent the onset of PLB epidemics and develop strategies that control PLB more effectively by reducing the input of fungicides to periods when the crops are at greatest risk of infection.

Materials and methods

Preliminary disease survey

A preliminary survey of the incidence of PLB on leek was initiated after samples of leek leaves, showing typical PLB symptoms were collected on 9 May 1995 from a commercial leek farm in Orbost, Victoria (385 km east of Melbourne, 148°30′ E, 37°45′ S). Three commercial onion and leek farms in south-eastern Victoria (Dandenong, Five Ways and Cranbourne) were surveyed for PLB during June 1995. Leaves with PLB symptoms were detached from plants. The pathogens associated with the diseased lesions were isolated using a single spore technique (Suheri and Price, 2000b) and identified. Herbarium specimens and isolates were deposited at the Herbarium, Institute of Horticultural Development, Knoxfield, Victoria.

Experimental site

This study was conducted at a commercial leek farm at Cranbourne, Victoria (approximately 70 km SE of Melbourne, 145°20′ E, 38°15′ S). Leek is a major crop on this farm, and is grown throughout the year to provide a continuous supply for local, interstate and export markets. Therefore leek crops at various growth stages were available throughout the year. The crops were established by transplanting seedlings into beds when they were at the 4-leaf stage (10- to 12-week-old). Each bed (60 m × 1 m) consisted of two rows of leek plants spaced 80 cm between rows and 15 cm within the row. Within each crop a single plot, consisting of six raised beds (running north–south) situated between two rows of fixed overhead sprinkler irrigation stands spaced 10 m apart along the rows with different planting dates and containing approximately 9600 plants, was randomly selected for disease assessment.

Regular crop maintenance, conducted by the farm staff, included fortnightly applications of mancozeb fungicide (2 kg/ha). As these were commercial crops it was not possible to include control plots without fungicide treatment in this study.

Disease assessment

The incidence (%) of PLB in each plot was monitored from transplanting to harvest on seven successive crops

planted between July 1996 and June 1997. A total of 720 plants per plot (7.5% of the plot population) were chosen by systematically selecting 20 plants in six staggered sections along each of the six beds, with the irrigation stands serving as the base point for each section. Each bed was treated as a replicate.

Proportion of PLB lesions containing A. porri *or* S. vesicarium *or mixtures of both*

A total of 36 plants was sampled by randomly selecting one plant, bearing at least one sporulating lesion, from each plot scored for disease incidence. Conidia in each lesion were detached by briefly pressing the lesion surface onto a piece of double sided adhesive tape (1 cm × 2 cm, Scotch 3M, St. Paul, Minnesota, USA) attached to a microscopic slide. The lesion imprints were mounted in clear lactophenol, examined under a compound microscope at 100× magnification and the total numbers of detached *A. porri* and *S. vesicarium* conidia counted.

Environmental data

Temperature, relative humidity and precipitation data for 1995–1996 were obtained from the National Bureau of Meteorology station (Royal Botanic Garden, Cranbourne), situated about 1.5 km from the experimental site. For the period 24–30 June 1996 and throughout 1997, a data logger (Department of Agricultural Sciences, La Trobe University, Bundoora, Vic. Australia) was installed in a Stevenson screen placed adjacent to leek crops on the farm. The data logger was fitted with 5 mm diam. temperature sensors, a flat leaf wetness sensor, a cup anemometer, and a tipping-bucket rain gauge. The leaf wetness sensor was calibrated before use and the leaf was recorded as wet with a sensor reading of >100 mv. The data logger was programmed to record wet and dry bulb temperature, leaf wetness duration, rainfall and wind speed at 5 min intervals for the period 24–30 June 1996 when the Burkard spore trap was operating and the results averaged for each hour of the seven day period. The data logger was programmed to record at hourly intervals from 10 February 1997 until the final disease observation in June 1997. The daily maximum, minimum and mean temperatures, precipitation, hours of leaf wetness and wind speed were calculated.

Relationship of disease incidence with environmental data

The rate of increase of disease incidence was calculated weekly in each plot and the average weekly disease increase was calculated. The relationship between disease increase per week and weekly average temperature, cumulative weekly precipitation and weekly total occurrence of relative humidity (RH > 90%) or weekly cumulative hours of leaf wetness were tested using correlation and regression analyses. The differences in disease incidence at harvest between different plots was tested by analysis of variance.

Concentration of airborne A. porri *and* S. vesicarium *spores in leek crops*

The optimum height required to trap airborne conidia of *A. porri* and *S. vesicarium* was determined using 'rotorod' samplers (Department of Agricultural Sciences, La Trobe University) placed 25, 50, 75, and l00 cm above ground level in the experimental plots. The results revealed that the optimum height for maximum catch of *Alternaria* and *Stemphylium* conidia was at the upper leek canopy level (50 cm above ground level).

The periodicity of air-borne conidia of *A. porri* and *S. vesicarium* within leek crops between 24 June and 30 June 1996 was determined using a battery operated Burkard volumetric spore sampler (Burkard Manufacturing Co., U.K.) placed at ground level. Two rotorod samplers were placed 50 cm above ground level within the plot, on the day of disease assessment each week throughout the experimental period and air was sampled continuously from 10:30 h to 14:30 h. The rotorods were changed every 30 min in order to avoid overloading of the deposits on the trapping surfaces. The adhesive tapes were detached, mounted on microscopic slides in clear lactophenol, examined microscopically for *Alternaria* and *Stemphylium* conidia and the total number of trapped conidia were recorded. Following the first observation of *Pleospora* ascospores on these slides, their numbers were also recorded.

Results

Preliminary disease survey

The PLB lesions on leek samples collected from farms in Orbost, Five Ways and Cranbourne in 1995 contained either *A. porri* alone (VPRI 20609, VPRI 21960, VPRI 21961, VPRI 21962) or mixtures with *S. vesicarium* (VPRI 20610, VPRI 20612, VPRI 20613, VPRI 21963); leek samples from farms in Dandenong contained either *S. vesicarium* or other *Stemphylium* spp. (VPRI 21965). Disease severity on

infected plants ranged from very light (1–5 lesions per plant) to approximately 25% leaf area diseased (LAD).

Proportion of PLB lesions containing A. porri *or* S. vesicarium *or mixtures of both*

Of the 82 PLB lesion imprints, 5 (6%) contained only *A. porri*, 34 (42%) contained only *S. vesicarium*, whilst 43 (52%) contained a mixture of both *A. porri* and *S. vesicarium*. Of the mixed infections only 17 (21%) contained more *A. porri* conidia than *S. vesicarium*.

Disease incidence and its relationship with environmental data

All crops remained disease free for periods ranging from 54 to 69 DAT (Table 1). The incidence of disease in each plot generally increased towards the end of the growing season (Figure 1) as plants matured (123–158 DAT).

In 1996, the highest disease incidence (7.2%), with a mean weekly rate of 0.6%, was observed on plot 1. Plants in plots 2 and 3 bolted and therefore the final disease incidence in these plots was lower than in other plots where plants were harvested at maturity. Rainfall rarely occurred between October 1996 and January 1997 (Figure 1). Less irrigation water was also available and temperatures $>20\,^{\circ}$C occurred frequently during this period (Figure 1). There was no significant correlation between the weekly rate of disease increase in plots 1–5 and, weekly average temperature, cumulative weekly precipitation or weekly total occurrence of relative humidity (RH > 90%).

In 1997, disease incidence in plots 6 and 7 was initially very low at the early stages of plant growth, and by harvest had reached a maximum of 8.4% in plot 6 and 10.5% in plot 7 (Table 1, Figure 2). Final incidence levels in crops grown during 1997 were significantly higher ($P < 0.05$, Table 1) than crops grown in 1996 and this was also reflected in the increased area under the disease progress curves. The on-farm weather records at Cranbourne revealed that leaf wetness periods of $\geq$8 h occurred on 14, 11, 23, and 15 occasions during March, April, May, and early to mid-June, respectively. Increases in disease incidence coincided

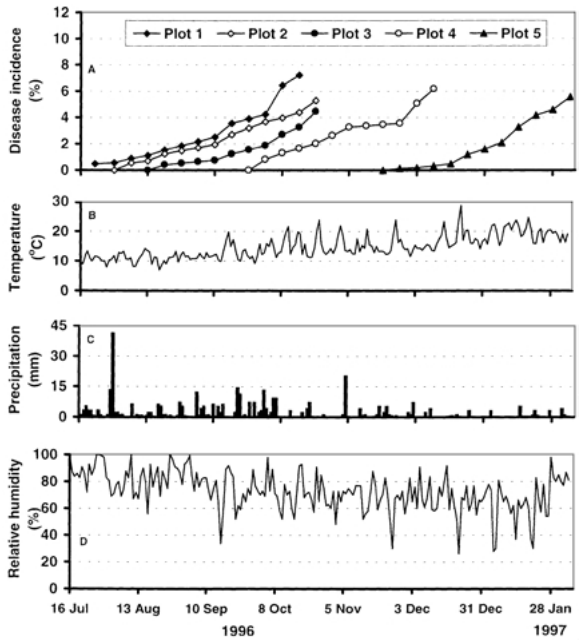

Figure 1. The incidence of purple leaf blotch in five successive leek crops grown at Cranbourne between July 1996 and February 1997 (A) and the daily mean temperature (B), precipitation (C) and relative humidity (D) recorded at the National Bureau of Meteorology station at Cranbourne.

Table 1. Incidence of PLB from transplanting to harvest on successive leek crops grown at Cranbourne, Victoria between May 1996 and June 1997

Plot	Date transplanted	Date harvested	Growing period (days)	Time to first symptoms (DAT)	Disease incidence at harvest (%) ±s.e	Weekly rate of disease increase (%)	Area under disease progress curve (AUDPC)
1	27 May 1996	15 Oct. 1996	141	56	7.2 ± 0.5	0.6	23.6
2	13 June 1996	23 Oct. 1996	132	54	5.3 ± 0.4	0.4	16.5
3	12 July 1996	23 Oct. 1996	123	60	4.4 ± 0.5	0.5	14.1
4	6 Aug. 1996	10 Dec. 1996	126	56	6.2 ± 0.4	0.5	18.7
5	18 Sept. 1996	4 Feb. 1997	139	69	5.6 ± 0.4	0.5	19.0
6	21 Dec. 1996	6 May 1997	136	59	10.5 ± 0.8	0.9	36.0
7	10 Jan. 1997	16 June 1997	158	60	8.4 ± 0.4	0.6	28.9
					LSD 1.4 $P < 0.05$		

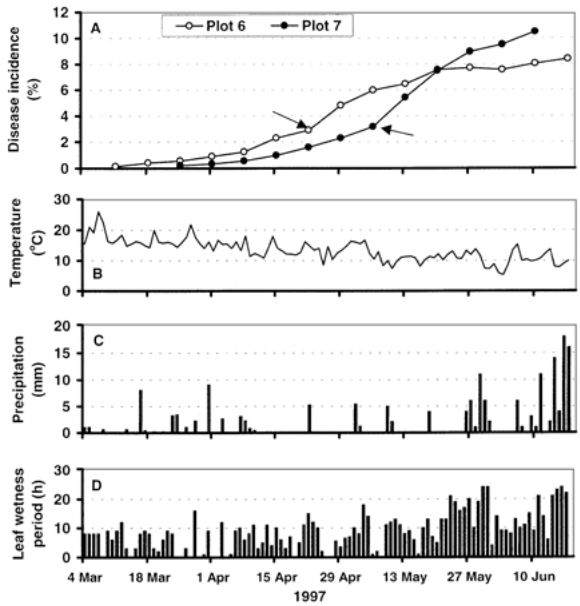

Figure 2. The incidence of purple leaf blotch in two successive leek crops grown at Cranbourne between February and June 1997 (A) and the daily on farm mean temperature (B), precipitation (C) and hours of leaf wetness (D). Arrows indicate 100 DAT.

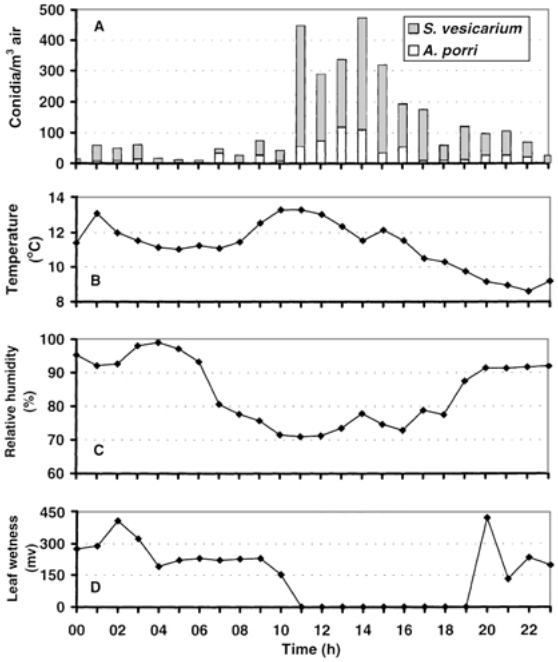

Figure 3. The mean diurnal periodicity of *A. porri* and *S. vesicarium* conidia (A) trapped in a leek crop at Cranbourne, from midnight (0 h) to 23:00 h from 24 to 30 June 1996 using a Burkard volumetric spore trap, and the on farm mean hourly temperature (B), relative humidity (C) and leaf wetness (D) measured using a data logger. The leaf was recorded as wet with a sensor reading >100 mv.

with longer periods (≥8 h) of leaf wetness, as a result of dew or precipitation, on three or more consecutive days, which occurred more frequently during these months (Figure 2). During this period the average temperatures in plots 6 and 7 were 13.3 °C and 11.2 °C respectively. The weekly rate of increase in disease incidence was not significantly correlated with individual or combined weekly average temperature, cumulative weekly precipitation and weekly cumulative hours of leaf wetness but was highly significantly correlated ($r = 0.41$, $P < 0.01$) with DAT over all seven plots. However, the maximum weekly rates of disease increase observed in the plot (rates >1.5) tended to occur in the range 108–134 DAT.

Concentration of airborne A. porri *and* S. vesicarium *spores in leek crops*

The concentrations of airborne *A. porri* and *S. vesicarium* conidia followed a diurnal pattern with most of the conidia being trapped between 11:00 and 15:00 h (Figure 3). The number of conidia of both species trapped per hour was negatively correlated with relative humidity and leaf wetness, but not with temperature and wind speed (Table 2). The number of *S. vesicarium* conidia trapped exceeded that of *A. porri* (Figure 3) and was positively correlated ($P < 0.05$) with wind speed (Table 2).

The numbers of *S. vesicarium* conidia trapped each week from July 1996 to June 1997 greatly exceeded (three to six times) the numbers of *A. porri* conidia trapped (Figure 4). Populations of *S. vesicarium* conidia increased during the warmer spring and summer months (September 1996–February 1997) and

Table 2. Correlation between the number of conidia of *A. porri* and *S. vesicarium* trapped in a leek field at Cranbourne, over a 24-h period and mean hourly environmental parameters

Mean hourly	Correlation coefficient (r)		
	A. porri	*S. vesicarium*	Total conidia
Temperature (°C)	0.29	0.23	0.26
Relative humidity (%)	−0.63*	−0.65*	−0.68*
Leaf wetness (mv)	−0.52*	−0.70*	−0.68*
Wind speed (km h^{-1})	0.07	0.46*	0.38

*Significant ($P < 0.05$).

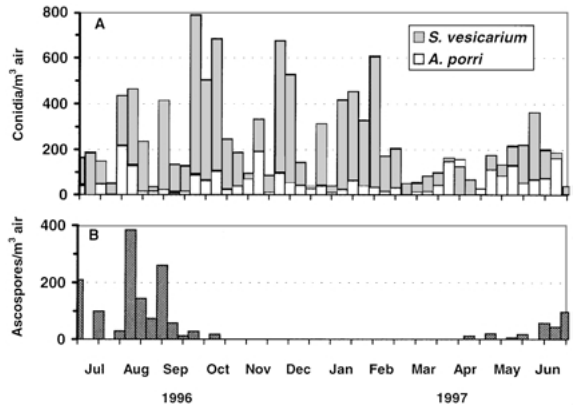

Figure 4. The seasonal periodicity of *A. porri* and *S. vesicarium* conidia (A) and *Pleospora allii* ascospores (B) trapped in leek crops at Cranbourne, between July 1996 and June 1997 using rotorod samplers.

decreased during the cooler autumn months (March–April 1997) but populations of *A. porri* did not show a similar trend. Multicellular ascospores, (30–37 μm long × 13–17 μm wide) pointed at both ends, with numerous longitudinal septa and 5–7 incomplete transverse septa, were trapped during the cooler months of May–September. Based on their morphology they were presumed to be *Pleospora allii* (Rabenh.) Ces and De Not., the teleomorph of *S. vesicarium* (Simmons, 1969).

Discussion

The results indicate that PLB was endemic on the farm at Cranbourne due to the continuous cropping of leeks. However, disease incidence and severity in each crop varied between years and seasons. In 1995, disease severity levels of up to 25% LAD were recorded whereas levels were <5% LAD on crops grown during 1996/1997. This may have been due to differences in cultivar susceptibility as cv. Grandina was grown in 1995 and cv. Otina was grown during 1996/1997. Disease incidence in all experimental plots never exceeded 11%. It was not possible to compare the rates of disease development between these plots and an unsprayed control plot on this commercial farm. However, incidence of stemphylium leaf blight on a garlic crop in South Australia, reached 100% within 3 weeks of the first symptoms being observed (Suheri and Price, 2000b). This suggests that the low incidence of PLB in the leek crops at Cranbourne was probably due to the regular fortnightly application of mancozeb, which curtailed but did not eradicate the disease.

Although inoculum of both *A. porri* and *S. vesicarium* was present all year round in leek crops, the first disease symptoms in any crop only appeared 54 DAT. This concurs with similar reports on onion (Ariosa and Herrera, 1984). The susceptibility of onion and garlic to PLB increases with leaf age and growth stage (Miller, 1983) and our results indicate that the susceptibility of leeks to PLB also follows a similar pattern.

Disease incidence was not correlated with any of the weather parameters tested and this was probably due to the low rates of disease increase in the plots. Infection of onion by both *A. porri* and *S. vesicarium* at 5 °C and 10 °C occur following leaf wetness periods of 16 h or 8–10 h respectively (Suheri and Price, 2000a). Between the autumn and winter months of March–June 1997, leaf wetness periods of ≥8 h occurred on 63 occasions and minimum temperatures often reached 0 °C. Extended leaf wetness periods were also due to dew which often remained on the leaves and leaf axils until 11:00 h. The significantly higher disease incidence of PLB on leeks during these months can therefore be attributed to conditions suitable for infection occurring more frequently than in crops grown between July–December 1996 and our results provide field evidence that supports the results of Suheri and Price (2000a). The results also indicate that autumn and winter crops are more likely to require disease management than spring and summer crops.

Spore trapping revealed that three to six times more *S. vesicarium* conidia were present in the atmosphere within leek crops compared with *A. porri* conidia. This suggests that the higher frequency of PLB lesions containing *S. vesicarium* was probably caused by a greater proportion of *S. vesicarium* conidia being deposited on the leaves and these results confirm the results of Suheri and Price (2000a) that PLB is due to a disease complex. Further studies on the interactions between different inoculum dosages of *A. porri* and *S. vesicarium* on mixed infections are desirable as the results suggest that *S. vesicarium* may be the more important of the two pathogens in the infection of leek in the field.

The diurnal pattern of airborne *A. porri* and *S. vesicarium* conidia in leek crops is similar to the periodicity of *A. porri* in onion crops (Meredith, 1966; Everts and Lacy, 1990b) and *S. vesicarium* in a garlic crop (Suheri and Price, 2000b). Populations of *S. vesicarium* conidia in leek crops were higher during the warmer months than during the cooler months and

this is similar to patterns of *S. vesicarium* conidia above an asparagus crop (Menzies et al., 1992). More conidia were trapped when precipitation or leaf wetness of $\geq$10 h occurred during the day preceding trapping although there was no significant correlation between conidia trapped and, temperature, precipitation or leaf wetness periods during the day preceding the trapping. However, increased conidial catches of *A. porri* in onion crops have been associated with rain and high RH (>60%) the previous day (Chadwa and Rajasab, 1994).

Ascospores of *P. allii* were also trapped during the cooler months of April–September when average temperatures of 10 °C occurred and highest numbers were trapped in August. Low temperatures are necessary for the production and maturation of the teleomorph stage (*Pleospora*) of *S. vesicarium* (Aveling, 1993; Prados-Ligero et al., 1998). Although the infection of leeks by ascospores was not attempted in these studies Basallotte-Ureba et al. (1999) showed that residues carrying mature pseudothecia, or ascospore suspensions of *P. allii* from pseudothecia, can infect garlic and onion. It is therefore likely that ascospores together with conidia are responsible for infection of the leek leaves, especially during the cooler months of the year.

Hosts of *S. vesicarium* include lucerne (Irwin et al., 1984), asparagus (Lacy, 1982), aster (Ichikawa and Sato, 1994), and pear (Montesinos and Vilardell, 1992), and the presence of any of these hosts in the area may also serve as a source of primary inoculum in the absence of continuous cropping of leek.

Initial infections were recorded 8–10 weeks after transplanting and this suggests that fungicide applications are not warranted until the plants reach this stage of growth. Our studies have shown that epidemics can develop provided leaf wetness periods of $\geq$8 h occur. Development of forecasting models based on leaf wetness duration and temperature will further enhance disease management by targeting applications of fungicides specifically during these periods of high disease risk. This will assist in reducing fungicide inputs and costs and the development of fungicide resistant strains of the pathogens, and provide increased disease control.

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