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Development of *Pleospora allii* on garlic debris infected by *Stemphylium vesicarium*

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Abstract

Pseudothecia of *Pleospora allii* developed best on garlic leaf debris infected by *Stemphylium vesicarium* incubated at low temperature (5–10 °C) and relative humidity (RH) close to saturation. RH of less than 96% prevented the formation of pseudothecia, while an incubation temperature of 15–20 °C led to the early degeneration of pseudothecia. Under natural conditions, colonization by pseudothecia of unburied garlic leaf debris varied between seasons from 6.0 to 15.5 pseudothecia/mm^2, whereas lower colonization levels were recorded when samples were buried. Pseudothecial maturity was reached 1–4 mo after the deposition of garlic debris on the soil surface and 15 days after the burial of residues. In the later case, pseudothecia degenerated with degradation of the plant debris. Ascospore release, which required rainfall or dew periods, occurred between late January and late April depending upon the year. A high correlation was found between pseudothecia maturation and four meteorological variables. Two of which, i.e. the number of hours with RH $\geq$ 98% and with a mean temperature of 4.5–10.5 °C, and the accumulated rainfall, explained most variability (adjusted $R^2 = 0.82$–0.98 depending upon the year). A multiple regression equation relating the pseudothecia maturity index with these two variables could be used to forecast the epidemic onset of *Stemphylium* leaf spots in Southern Spain. Temporal progress of pseudothecia maturation was best fitted by a monomolecular model.

Introduction

A new disease affecting the above-ground parts of garlic plants has been observed since the early eighties in the main areas where this crop is grown in Spain (Basallote et al., 1993). Typical symptoms of the disease are white and/or purple leaf spots, with sunken tissues and extensive necrosis of the leaf (Basallote et al., 1993). The causal organism, *Stemphylium vesicarium* (Wallr.) Simmons, as well as its teleomorph, *Pleospora allii* (Rabenh.) Ces. & de Not., have been reported on garlic (Aveling and Naude, 1993; Basallote et al., 1996), onion (Rao and Pavgi, 1975; Shishkoff and Lorbeer, 1989; Simmons, 1969) and asparagus (Evans and Stephens, 1984; Fallon et al., 1984, 1987), all affected by similar symptoms. In southern Spain rainfall and mild temperatures during the spring favour severe epidemics in garlic crops leading to significant yield losses (Basallote et al., 1996).

Numerous pseudothecia of *P. allii* developed during the winter on garlic plant debris infected by *S. vesicarium* (Basallote et al., 1993; Prados et al., 1994). Pathogenicity of these pseudothecia has been demonstrated on both garlic and onion plants (A.M. Prados, unpubl.). The teleomorph has also been observed on infected residues of asparagus (Conway, 1987; Evans and Stephens, 1984; Falloon et al., 1984, 1987; Johnson, 1990), and the importance of such infected residues in the epidemic development of leaf spots has been demonstrated. Fallon et al. (1984) showed that the severity of *Stemphylium* purple spot epidemics in asparagus was reduced when crop residues were removed after crop harvest. Disease development was effectively reduced by the burial of plant debris, thus impeding ascospore release which resulted in a reduction of primary inoculum levels. However, plant residue burial for 10–14 wk failed to completely prevent both pseudothecial development and the viability of affected ascospores (Johnson, 1990).

There is little information on the phenology of *P. allii* developing on infected plant debris, and on the influence of environmental factors. The objective of this work was to study the development of the teleomorph of *S. vesicarium* on garlic debris under both controlled and natural conditions, and to determine the relationship between its development and environmental factors.

Materials and methods

Pseudothecial development under controlled-environment conditions

Garlic leaf segments (10 cm long) naturally infected by *S. vesicarium* were thoroughly washed under tap water and then surface disinfested for a period of 5 min in an aqueous solution of sodium hypochlorite containing 5 g available chlorine per litre. The leaf samples were incubated for 3 months at 5, 10, 15 and 20 °C in plastic containers with 300 ml distilled water or oversaturated saline solutions resulting in relative humidities (RH) of 35, 75, 80, 86, 88, 96, 97, 98 and 100% (Dhingra and Sinclair, 1985). Garlic leaf segments were placed on a plastic net suspended 15 mm above the liquid. This experiment was designed as a split-plot with temperature as the main factor and RH as the secondary factor and ten blocks. Analysis of variance was performed with the data using Statistix 4.0 software. Multiple comparisons of means was made by Tukey's test. There were 10 replicates (each consisting of one leaf segment), and the experiment was repeated twice. Leaf segments were sampled at 15-day intervals for each temperature-relative humidity combination. Leaf samples were air-dried for 48 h at room temperature and carefully washed under tap water. Five 1-mm^2 fields were observed in each of the ten replicates, under the stereoscopic microscope at 10× to determine the average number of pseudothecia which had formed per field.

A total of 50 pseudothecia were sampled to evaluate the stage of maturation. This was assessed by observing the phenological stage after crushing pseudothecia between two slides and staining them with 0.1% acid fuchsine in lactophenol (Dhingra and Sinclair, 1985). In accordance with previously reported scales for pseudothecia development in other ascomycetous fungi (James and Sutton, 1982; Trapero-Casas and Kaiser, 1992), the developmental stage of each pseudothecium of *P. allii* was assigned to one of the eight categories defined as follows: 1, pseudothecial primordia; 2, pseudoparaphyses filling the lumen of the pseudothecium; 3, initiation of asci differentiation; 4, asci with undifferentiated ascospores; 5, asci with ascospores in the process of formation and asci with mature ascospores; 6, pseudothecia having all asci with mature ascospores; 7, pseudothecia with some empty asci; 8, pseudothecia with empty or no asci. For each sampling date, a pseudothecial maturation index (Trapero-Casas and Kaiser, 1992) was calculated as an average of the stages observed, using the equation:

$$\mathrm{MI} = \sum n_i (st_i)/N,$$

where MI = maturity index, n_i = number of pseudothecia in stage *i*, st_i = developmental stage according to the 1–8 scale described, and N = total number of sampled pseudothecia.

Pseudothecial development under field conditions

Five field experiments were conducted over the years 1991–95 in southern Spain. One of them was established in 1991 on a plot in Granada, in silty loam soil with 1.2% organic matter and pH 7.8. The other experiments were carried out in plots of sandy loam soil, 1.4% organic matter and pH 8.5, in Córdoba during 1991–95.

In all the experiments, garlic leaf debris naturally infected by *S. vesicarium* was collected during the previous harvest period (June) and kept under room temperature until late October. Ten 10-cm long leaf segments were then placed in plastic net bags (250 × 250 mm, 1.45-mm mesh) that were fixed onto the soil surface. In the 1992–93 experiment two types of inoculum sources were used: leaf and inflorescence stalk debris, which were either located on the soil surface or buried at 10-cm depth. Bags containing infected garlic residues were sampled at 15-day intervals from mid November until stage 8 was observed. The sample bags were dried at room temperature for two days and then kept in the laboratory until processing. Four plant segments were observed for each sampling date. The number and maturity index of pseudothecia were determined as described above and the size of 100 randomly chosen pseudothecia was measured.

During all the experimental periods, measurements were made each hour of temperature using a Pt 100 thermistor (SAINCO, Madrid, Spain), relative humidity using a hair higrometer (SAINCO, Madrid, Spain), and rainfall using a tipping-bucket rain gauge (Campbell Scientific, Inc., Logan, UT, USA).

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MI for each 15-day period was related with: (a) physiological time (PT) determined by accumulated degree-days of average temperature using a threshold of 0 °C, (b) accumulated number of hours during which the recorded relative humidity was near saturation (≥98%) and mean temperatures were within the interval 4.5–10.5 °C (NH), (c) number of hours during which the minimum temperature was under 0 °C and relative humidity was ≥ 98% (NH0), and (d) accumulated rainfall (RA).

A multiple regression was performed on the data collected from garlic debris deposited on the soil surface in the five experiments under consideration, using NH and RA as independent variables and MI as the dependent variable. Furthermore, the regression of MI values over time was analysed by linear regression and logistic, exponential and monomolecular models.

Results

Pseudothecial development under controlled conditions

No pseudothecia of *P. allii* were found after garlic debris was incubated for a 3-month period at RH<96%, regardless of incubation temperature. Only immature or degenerated pseudothecia (lumen of pseudothecia empty or granular) were observed when RH was between 96 and 98%, at any of the incubation temperatures studied (Table 1). It was observed that after 45–75 days of incubation in a saturated atmosphere, depending on the temperature, all the asci in the pseudothecia had reached maturity (stage 6).

Average density of pseudothecia on garlic leaves was significantly ($P = 0.001$) higher when they were incubated at 5 or 10 °C as compared to those incubated at 15 or 20 °C. Relative humidity of 100% provided significantly ($P = 0.001$) better conditions for the formation of pseudothecia than at other RH's tested. Analysis of variance indicated a significant ($P = 0.05$) interaction of temperature and RH. A linear increase in the average density of pseudothecia was observed as RH increased for the incubation temperature of 5 °C. There were no significant differences within samples incubated at 10 °C. For temperatures of 15 and 20 °C, incubation at RH of 100% resulted in average density of pseudothecia significantly higher than when samples were incubated at 96–98% RH (Table 1).

The highest percentages of mature pseudothecia were obtained at lower temperature (5–10 °C), whereas incubation at 15–20 °C resulted in the highest proportion of degenerated pseudothecia. However, a high percentage of degenerated pseudothecia was observed after a 3-month incubation period at all the temperatures tested (Table 1). The degeneration of pseudothecia occurred after 45 days of incubation at 15–20 °C, whereas at 5–10 °C it was detected only after maturity stage 6 had been reached. Saprophytic colonization of garlic debris by *Chaetomium* sp. occurred at all the incubation temperatures tested, throughout the experimental period, the extent of colonization being greater at the highest incubation temperatures (15–20 °C).

Pseudothecial development under field conditions

The garlic debris affected by leaf spots which was located on the soil surface, was rapidly colonized by *P. allii*. The first sexual structures of the fungus were observed 15–30 days after experiments commenced. However, the density of fruiting bodies on leaves reached maximum values between mid February–mid March depending on the year. In contrast, density of pseudothecia on inflorescence stalks was highest by mid December. The highest colonization levels (10.0–15.5 pseudothecia/mm^2) were obtained in 1991–92 in Córdoba and Granada. Irrespective of location (on the soil surface or buried at 10-cm depth), pseudothecial density on inflorescence stalk debris in 1992–93 was higher (6.5–9.5 pseudothecia/mm^2) than on leaf debris located on the soil surface (6.0–6.5 pseudothecia/mm^2), or buried (less than 2 pseudothecia/mm^2) probably because by early December the leaf residue was degrading rapidly. The level of *P. allii* colonization of leaf debris in the 1993–94 and 1994–95 experiments was similar (6.7 and 7.0 pseudothecia/mm^2, respectively) and lower than in 1991–92.

The pseudothecia which developed on leaf debris had average sizes of 288–312 μm depending upon the years considered, with the exception of 1992–93 when they were smaller (195 μm), although on inflorescence stalks the average was higher than 320 μm (Table 2).

Table 1. Influence of temperature and relative humidity on the development and maturation of pseudothecia of *Pleospora allii* on leaf garlic debris naturally infested by *Stemphylium vesicarium*[a]

Temperature (°C)	Relative humidity(%)	Density of pseudothecia (number/mm^2)[b]	Pseudothecia (%)[c]		
			Immature	Mature	Degenerated
5	96	1.0[a]	100	—	—
	98	2.1[b]	—	—	100
	100	3.0[c]	—	50	50
10	96	1.7[a]	100	—	—
	98	1.9[a]	100	—	—
	100	2.3[a]	5	57	38
15	96	0.0[a]	100	—	—
	97	0.0[a]	100	—	—
	100	1.4[b]	26	2	72
20	96	0.5[a]	—	—	100
	98	0.6[a]	—	—	100
	100	1.7[b]	17	23	60

[a] Leaf garlic debris were incubated for 3 months. The experiment was repeated twice, and average values are given.
[b] Mean of ten groups of five 1-mm^2 microscope fields. Within each temperature, values followed by same letter are not significantly ($P = 0.05$) different according to Tukey's test.
[c] For each temperature-relative humidity combination, 50 pseudothecia were assessed as immature (lumen of pseudothecia filled with pseudoparapyses or immature asci), mature (pseudothecia having all asci with mature ascospores) or degenerated (lumen of pseudothecia granular or lumen empty).

Table 2. Effect of location and type of garlic debris on the diameter of pseudothecia of *P. allii* formed

Place and year	Location and debris type[a]	Pseudothecia diameter (μm)[b]
Granada 91–92	surface, leaf	312.5[b,c]
Córdoba 91–92	surface, leaf	305.1[b,c]
Córdoba 92–93	buried, inflorescence stalk	326.5[b,c]
Córdoba 92–93	surface, leaf	194.9[a]
Córdoba 92–93	surface, inflorescence stalk	346.2[c]
Córdoba 93–94	surface, leaf	288.1[b]
Córdoba 94–95	surface, leaf	287.7[b]

[a]Leaf and inflorescence stalk of garlic affected by *S. vesicarium* were enclosed in plastic net bags and fixed to the soil surface or buried at 10 cm depth by early November.
[b]Each value is the average of 100 randomly chosen pseudothecia. Values followed by same letter are not significantly ($P = 0.001$) different according to Tukey's test.

The date at which pseudothecial maturity of *P. allii* on garlic debris was reached varied according to the year, the location of the experiment (Table 3). Pseudothecial maturity (stage 6) on garlic debris located on the soil surface was reached earlier in 1993–94 and 1994–95 than in the other years. The position of the residues also seemed to play a role as mature pseudothecia were observed on plant debris buried at 10-cm depth from early December, 15 days after the burial of the residues. In successive samplings, it was observed that the degradation of leaf debris was progressive and correlated to increases in the percentage of degenerated pseudothecia. Ascospore release (stage 7) occurred between late January and late April,

Table 3. Development of *Pleospora allii* on garlic debris[a] naturally infected by *Stemphylium vesicarium* deposited on the soil surface in two locations of Southern Spain

Location	Year	Debris type	Date of the begining of developmental stage[b]		
			6	7	8
Granada	1991–92	Leaf	2/March/92	16/March/92	31/March/92
Córdoba	1991–92	Leaf	14/February/92	30/April/92	30/April/92
	1992–93	Leaf	18/February/93	18/March/93	2/April/93
	1992–93	Inflorescence stalk	19/January/93	30/April/93	30/April/93
	1993–94	Leaf	30/November/93	29/January/94	11/February/94
	1994–95	Leaf	16/December/94	9/February/95	9/February/95

[a] Leaf and inflorescence stalk of garlic affected by *S. vesicarium* were enclosed in plastic net bags and fixed on the soil surface by early November.
[b] Bags containing garlic residues were sampled at 15-day intervals until ascospores were released, and phenological stage (6 = pseudothecia having all asci with mature ascospores; 7 = pseudothecia with some empty asci and, 8 = pseudothecia with empty asci or no asci) of pseudothecia was assessed for each sampling date.

Table 4. Correlation coefficients[a] between maturity index of *Pleospora allii* and meteorological variables during the period November–June for different locations and years

Meteorological variable	Location					
	Granada	Córdoba				
	1991–92 (leaf)	1991–92 (leaf)	1992–93 (leaf)	1992–93 (inflorescence stalk)	1993–94 (leaf)	1994–95 (leaf)
PT	0.882	0.847	0.906	0.904	0.813	0.824
NH	0.963	0.967	0.925	0.906	0.941	0.936
NHO	0.931	0.793	0.812	0.867	0.866	0.830
RA	0.942	0.889	0.924	0.877	0.824	0.836

[a] All correlations were significant at the $P = 0.001$ level.
[b] PT = physiological time determined by accumulated degree-days of average temperature using threshold 0 C; NH = number of hours in which relative humidity was $\geq$ 98% and average temperature was between 4.5–10.5 C; NH0 = number of hours during which minimum temperature was under 0 and relative humidity was $\geq$ 98%; and RA = accumulated rainfall.

depending upon the year, whereas the appearance of empty pseudothecia (stage 8) was noticed simultaneously to or shortly (2 wk) after stage 7 (Table 3).

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In all of the experiments, there was a significant correlation ($P = 0.001$) between pseudothecial MI and the four meteorological variables (PT, NH, NH0, and RA) previously described. Correlations with NH were the highest, with r ranging from 0.936–0.967 (Table 4).

Pseudothecia developed and matured faster during 1993–94 and 1994–95, in association with the highest values of NH (448 and 397 h, respectively), and of rainfall (RA) during the 15 days following plant debris deposition in the field (94 and 64-mm, respectively); however, the experimental period with the highest RA (614-mm) (Figure 1) was that of Córdoba in 1991–92.

The relationship between MI and the two meteorological variables that explained most of variability was described by the regression equation:

$$\mathrm{MI} = a + b\mathrm{NH} + c\mathrm{RA},$$

where MI = maturity index for every 15-day period; NH = accumulated hours (from the deposition of leaf debris on soil surface until the sampling date) in which the recorded relative humidity was near saturation ($\geq$ 98%) and mean temperatures were within the interval 4.5–10.5 C; and RA = accumulated rainfall (from

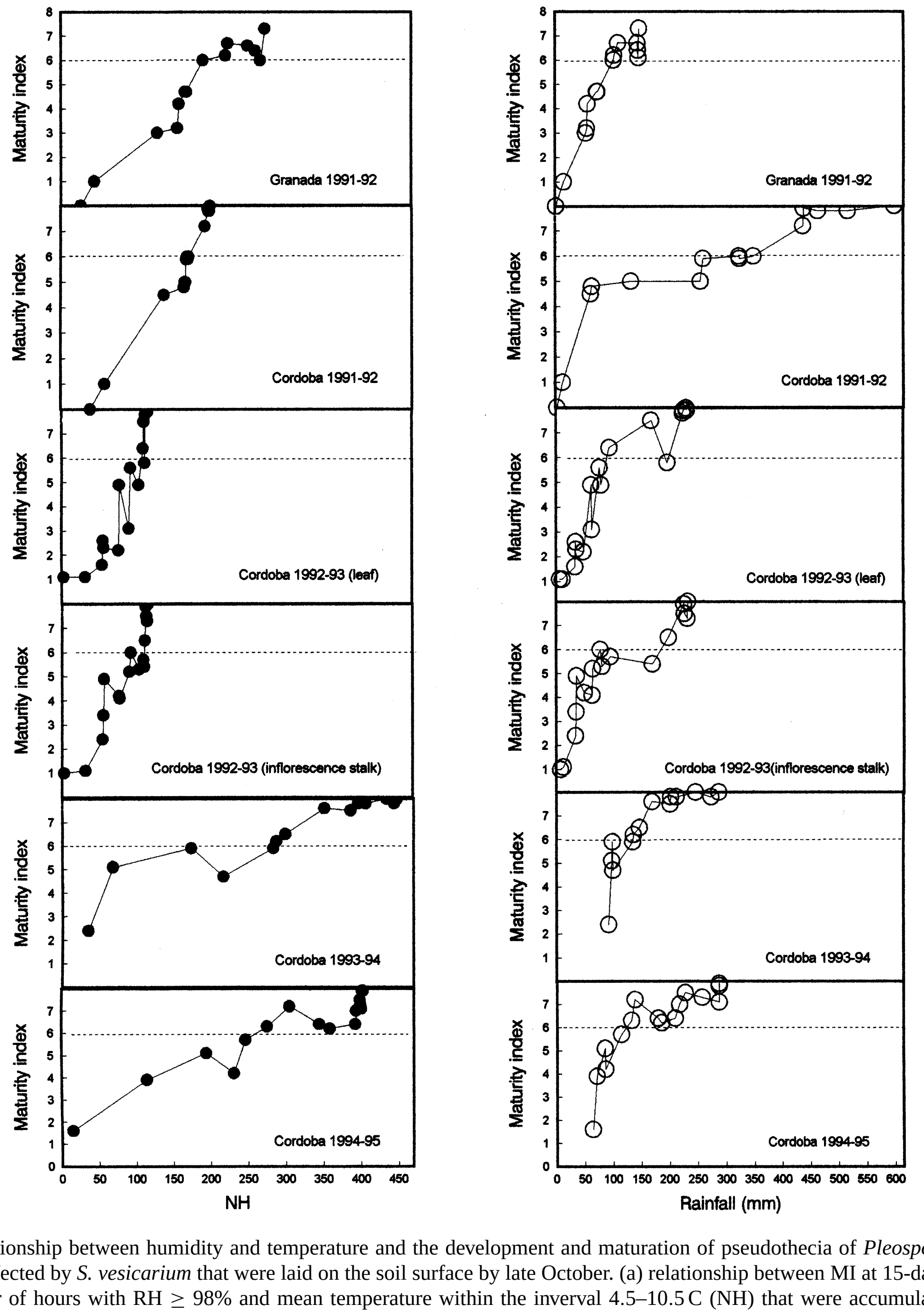

Figure 1. Relationship between humidity and temperature and the development and maturation of pseudothecia of *Pleospora allii* on garlic debris infected by *S. vesicarium* that were laid on the soil surface by late October. (a) relationship between MI at 15-day intervals and the number of hours with RH ≥ 98% and mean temperature within the inverval 4.5–10.5 C (NH) that were accumulated during the period between debris deposition and sampling. (b) relationship between MI at 15-day intervals and accumulated rainfall during the period between debris deposition and sampling (RA). The stage of pseudothecia development at which all asci had mature ascospores is indicated by a broken line.

Table 5. Regression estimates of coefficients for the relationship between maturity index and meteorological variables

Year and place	Debris type[a]	Coefficients[b]			R^2_{adj}
		a	*b*	*c*	
Granada 91–92	Leaf	−0.18 (0.42)	0.025 (0.009)	0.002 (0.001)	0.93
Córdoba 91–92	Leaf	−0.615 (0.29)	0.030 (0.002)	0.004 (0.0008)	0.98
Córdoba 92–93	Leaf	0.044 (0.51)	0.033 (0.009)	0.017 (0.003)	0.90
Córdoba 92-93	Inflorescence stalk	0.45 (0.45)	0.042 (0.008)	0.001 (0.003)	0.90
Córdoba 93-94	Leaf	2.02 (0.58)	0.011 (0.004)	0.005 (0.008)	0.82
Córdoba 94-95	Leaf	1.33 (0.43)	0.015 (0.003)	0.002 (0.005)	0.88

[a] Garlic debris was located on soil surface.
[b] Data fitted to $\text{MI} = a + b\text{NH} + c\text{RA}$, MI = maturity index, NH = number of hours in which relative humidity was ≥ 98% and average temperatures were between 4.5 and 10.5 C, and RA = accumulated rainfall (mm). Standard error is shown in parentheses. All cases were significant ($P < 0.01$).

the deposition of leaf debris on soil surface until the sampling date).

The variance accounted for by this model ranged from 0.82 (Córdoba 1993–94) to 0.98 (Córdoba 1991–92). Adjusted determination coefficients were lower in the experimental periods 1993–94 and 1994–95 (Table 5).

Ascospores release (stage 7) was generally associated with rainfall or dew periods during the 15 days previous to this stage in the development of pseudothecial maturation.

The model used to describe the relationship between MI and time (D) was:

$$\text{MI} = A(1 - e^{-BD}).$$

This equation represents a monomolecular model where A is the asymptotic value and B is the maturity rate. The model was fitted to the experimental data using a Maximum Likelihood Computer Algorithm (Ross, 1982), assuming that the error term followed a normal distribution. The goodness of fit was judged by the residual mean square (RMS), the estimation of parameters, and visual examination from plots of residual against E (MI).

This equation fitted the experimental data on the temporal progress of MI of pseudothecia better than linear regression, and logistic and exponential models, particularly in the case of the field used in 1992–93 in which garlic inflorescence stalks had been buried (Table 6). However, the monomolecular model did not give a good fit for buried leaf debris in Córdoba in 1992–93 (Table 6).

Discussion

Pseudothecia of *Pleospora allii* develop on leaves and on inflorescence stalks of onion plants towards the end of the crop season (Rao and Pavgi, 1975). The formation and maturation of pseudothecia on infected onion leaf debris in an artificial medium incubated at low temperature has also been reported previously (Simmons, 1969). The present work is the first study carried out on the developmental process of pseudothecia of *P. allii* on garlic debris infected by *S. vesicarium*. It followed the typical process found in the Pleosporales (Luttrell, 1981) and resembled that described for *Didymella rabiei* (Kovacheski) v. Arx infecting chickpea residues (Trapero-Casas and Kaiser, 1992; Navas Cortés et al., 1995; Trapero-Casas et al., 1996).

The colonization of garlic leaf debris by *P. allii* was more extensive under natural conditions (6.0–15.5 pseudothecia/mm^2) than under controlled environmental conditions where a maximum of three pseudothecia/mm^2 was recorded, corresponding to debris incubated at 5 °C and a relative humidity

Table 6. Regression estimates of coefficients for the monomolecular[a] equation of MI as related to time

Place	Year	Location and debris type	*A*	*B*	RMS	df
Granada	91–92	surface, leaf	8.94 (1.14)	0.007 (0.001)	0.30	13
Córdoba	91–92	surface, leaf	8.78 (0.70)	0.096 (0.001)	0.49	14
Córdoba	92–93	surface, leaf	19.89 (8.24)	0.002 (0.001)	0.56	17
Córdoba	92–93	buried, leaf	24.2 (113.4)	0.001 (0.009)	1.86	8
Córdoba	92–93	surface, inflorescence stalk	9.62 (1.00)	0.006 (0.002)	0.30	17
Córdoba	92–93	buried, inflorescence stalk	7.98 (0.13)	0.028 (0.002)	0.17	17
Córdoba	93–94	surface, leaf	7.86 (0.31)	0.022 (0.003)	0.34	13
Córdoba	94–95	surface, leaf	7.41 (0.23)	0.021 (0.002)	0.27	15

[a] Data fitted to $MI = A(1 - e^{-BD})$, MI = maturity index and D = days after deposition garlic debris on soil surface, A = asymptotic value, B = maturity rate. Standard error is shown in parentheses, RMS = residual mean square, df = degrees of freedom.

of 100%. The low number of pseudothecia formed on infected garlic debris under controlled conditions seemed to be related to the intensive saprophytic colonization of leaves by *Chaetomium* sp. which increased with incubation temperature. Similar colonization levels were observed only on buried leaf residues in 1992–93, when heavy rains a few days prior to the garlic harvest may have favoured the development of *Penicillium* sp. and *Alternaria* sp. antagonistic to *P. allii*. This saprophytic colonization did not occur on inflorescence stalk debris cut before that rainy period. This fact could explain the larger size of pseudothecia formed on inflorescence stalks as compared to those developed on leaf tissues (Table 2).

The burial of leaf debris resulted in its decomposition, thus reducing the amount of plant tissue available for *P. allii* development. However, pseudothecial maturation was not fully prevented by residue burial as was the case in asparagus debris infected by *S. vesicarium* (Johnson, 1990); ascospore release was however impeded. Our results confirm that the burial of plant debris is useful in the integrated control of diseases caused by fungi that overwinter on infected plant residues (Zentmyer and Bald, 1977).

In contrast, inflorescence stalk segments buried at 10-cm depth remained undecomposed throughout the duration of the experimental period and *P. allii* developed on them quickly and extensively, with a high proportion of pseudothecia reaching maturity (stage 6).

These observations suggest that the harvest of inflorescence stalks prior to the harvesting of the bulbs, a common practise in southern Spain, may be a means of reducing an important source of inoculum in fields affected by *Stemphylium* leaf spots.

Our results from controlled environment experiments indicate the importance of relative humidity and temperature on the development of pseudothecia of *P. allii* on garlic debris. Maturation of pseudothecia on garlic debris occurred in half the time of that required in infected onion residues incubated in the refrigerator (Simmons, 1969; Rao and Pavgi, 1975). This difference could be attributed to the different hosts and/or to differences in the methodology used. Our results on the development of *P. allii* under artificial conditions suggest that relative humidity is more important than temperature, since pseudothecia formation occurred at all of the temperatures tested providing the relative humidity was close to saturation. Nevertheless, pseudothecial maturation was higher at 5 and 10 °C under suitable humidity conditions. The requirement of low temperatures (5–10 °C) for long periods during pseudothecial maturation has been previously reported for *P. herbarum* (Leach, 1971) and other ascomycetes (Muller, 1979).

The number of hours with RH $\geq$ 98% and a mean temperature of between 4.5 and 10.5 °C (NH), and accumulated rainfall (RA) were the two variables that best correlated with the MI of pseudothecia (Table 4). A minimum of 97 h of NH, which occurred in 1992–93 (Figure 1a), was required for pseudothecia to reach maturity. This was achieved earlier in the experimental periods of 1993–94 and 1994–95 than in the other periods considered, coinciding with rainfall during 15-day periods prior to the sampling date (Figure 1b). Very low minimum temperatures along with scarce rainfall in Granada during the period of 1991–92 could account for the delay in pseudothecial maturity as compared with results from Córdoba during the same period (Table 3). Once pseudothecia had reached maturity, free water played a crucial role on asci bursting and subsequent ascospores release, since this was associated with rainfall or dew. This coincides with previous studies on other species of *Pleospora* (Fallon et al., 1987). Our results on the effect of temperature and rainfall on pseudothecial maturation and the release of ascospores of *P. allii* are similar to those obtained on chickpea debris infected by *D. rabiei* following an Ascochyta blight epidemic (Trapero-Casas et al., 1996).

The progress of pseudothecial maturation on garlic debris over time fitted well to a monomolecular model, except for leaf debris in 1992–93. In this case, the highest residual mean square values were found, and the asymptotic values (A) were clearly overestimated (Table 6), probably because the development of *P. allii* was prevented by competing *Penicillium* and *Alternaria* spp. The high variability in the maturity rate (B) observed, suggests it might be related to biotic and abiotic factors that change between years and locations (Table 6).

Forecasting the epidemic onset of Stemphylium garlic leaf spot could be achieved by using the multiple regression equation obtained that relates MI of pseudothecia of *P. allii* to NH and RA, thus making it possible to establish appropriate control strategies.

In conclusion, since *S. vesicarium* overwinters as its teleomorph, *P. allii*, on crop residues, integrated control programs for garlic leaf spots should include the destruction of garlic residues which can be achieved by deep ploughing. Thus, significant reduction in primary inoculum would contribute to disease control. This, combined with preventive chemical treatments applied to the crop, would provide good disease control. These treatments should be scheduled according to the prediction of infections based on the multiple regression curve that has been determined in this study.

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