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Effect of pea cultivar, pathogen isolate, inoculum concentration and leaf wetness duration on Ascochyta blight caused by *Mycosphaerella pinodes*

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Summary. The effect of host leaf wetness duration, *Mycosphaerella pinodes* inoculum concentration and pathogen isolate on the latent period and the incubation period of the pathogen or disease severity were quantified on pea (*Pisum sativum* L.). Seedlings of two widely grown pea cultivars, Onward and Merveille de Kelvedon, respectively susceptible and moderately resistant to *M. pinodes* were subjected to six leaf wetness durations of 6, 12, 24, 48 and 72 h, and inoculated with five inoculum concentrations, 2.5×10^3, 4×10^4, 3.5×10^5, 4×10^6, and 5.2×10^7, in order to determine whether the cultivars reacted differently to *M. pinodes* isolates inoculated under identical conditions. Increasing the duration of leaf wetness and inoculum concentration caused significant ($P<0.001$) increases in disease severity within each cultivar. Both the incubation period and the latent period decreased with increasing conidial concentration and leaf wetness duration. Generally, the cv. Onward had a significantly shorter incubation period, and latent period and higher disease severity than cv. Merveille de Kelvedon. Isolates differed in aggressiveness at higher levels of leaf wetness (48 h) duration and of inoculum concentration (4×10^6), but there was no significant interaction between isolates and leaf wetness duration, or between isolates and inoculum concentration. The optimum levels for obtaining a consistent infection and for readily separating the susceptible and the partially resistant cultivars were a leaf wetness of 48 h and an inoculum concentration of 4×10^6. The study also showed that continuous leaf wetness for 48 h was a threshold for application of fungicides to control the fungus in the susceptible cultivar.

Key words: disease severity, incubation period, *Pisum sativum*.

Introduction

In Algeria, little information is available on the factors affecting the disease severity (DS) and the yield losses caused by Ascochyta blight from *Mycosphaerella pinodes* (Berk & Blox) Vesterg on peas (*Pisum sativum* L.) (Bouznad,1998). According to Van der Plank's theorem, quantitative information on how environmental factors affect specific disease components is useful to determine the quantitative impact that plant genotypes with partial resistance have on the rate of disease progress in the field. Ascochyta blight from *M. pinodes* has become more common in the recent years because of the large amount of inoculum left in the field, which is then spread by wind and rain splash.

The pea cultivars widely grown in Algeria are not all totally resistant to *M. pinodes,* but no information is available on how these cultivars react to epidemiological factors such as temperature, leaf wetness duration (LWD) and inoculum concentration (IC). Typically, the first symptoms of *M. pinodes* occur on the plant stems from where they progress to other parts of the plants (Raynal, 1977; Bouznad, 1988; Maufras, 1996; Xue *et al*., 1997; Tivoli, 1999; Bretag, 2006; Tivoli and Banniza, 2007). Symptoms consist of lesions surrounded by a yellowish chlorotic ring

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which evolves into dark-brown spots lacking distinct margins on the leaves, and to reddish spots with a necrotic centre on the pods (Champion *et al*., 1984; Pederson and Morrall, 1994; Maufras, 1996; Vloutoglou and Kalogerakis, 2000). Allard *et al*. (1993), Tivoli and Lemarchand (1998) stated that *M. pinodes* is strongly correlated with temperature and LWD. Singh and Reddy (1990) suggested that temperature below than 6°C impedes the infection process. Whereas, temperatures between 18 and 21°C and a LWD of 12 h or more favour infection initiation.

Previous studies also report that moisture affects all stages of the disease process. Hence Shaner (1981), Royle and Butler (1986) and Van Ginkel and Scharen (1988), suggested that moisture parameters should be investigated as a means to predict the likelihood of the infection of airborne spores.

Fitt *et al*. (1989, 1998), Huber and Gillespie (1992), also reported that free water on the leaves affects sporulation. On the other hand, Makowski (1993), Tivoli *et al*. (1997) and Rogger *et al*. (1999) stated that IC had a strong impact on the life cycle of *M. pinodes* and hence on all the components of Ascochyta blight. The aim of the present work was to examine the conditions favouring infection, including LWD and IC, and that enable the susceptible cultivars to be separated from the moderately resistant cultivars. This is expected to lead to an accurate timing of fungicide application, compared with a strict calendar approach which rarely takes the cultivar susceptibility into account.

Materials and methods

Plant material

Pea plants cv. Onward and cv. Merveille de Kelvedon (MK) were used in all experiments. The two cultivars are the most commonly cultivated varieties in Algeria, the first one being highly susceptible and the second one moderately resistant to the blight.

Seeds of each cultivar were sown in 20-cm diameter pots (10 seeds per pot) containing an unsterilized soil/compost mixture. The seedlings were thinned to five per pot. The plants were maintained in a glasshouse. Three replicates were used for each combination.

Fungal material and inoculum production

Two isolates of *M. pinodes*, md0202 and tn0203 were used in the study. The isolates came from two localitions in the Chellif region and presented respectively a low and a high score of aggressiveness on 'Onward'.

Strains were raised on potato dextrose agar (PDA) for 10 days at 21°C. Conidia from 10 day-old cultures were collected by adding 10 ml of sterile distilled water to dislodge the spores. The spore suspension was filtered through two layers of cheesecloth to remove mycelium and agar fragments. The spore concentration was determined using a haemocytometer. The conidial suspension was diluted with sterile distilled water to the concentration required for each experiment.

Leaf wetness duration

Two week old pea seedlings of 'Onward' and 'MK' were sprayed to run-off with a conidial suspension of 4×10^6 spores ml^{-1}. The seedlings were then subjected to LWD of 6, 12, 24, 48 or 72 h. To maintain the wetness, the seedlings were covered with transparent polyethylene bags sprayed inside with distilled water. Unbagged seedlings were taken not to have been exposed to leaf wetness. At the end of the LWD, seedlings were uncovered and kept in a glasshouse at temperature from 15 to 25°C.

Inoculum concentration

The IC effect was investigated on 15 day-old (three-leaf stage) pea plants of 'Onward' and 'MK'. Plants were inoculated by spraying to runoff with spore suspensions containing 2.5×10^3, 4×10^4, 3.5×10^5, 4×10^6, and 5.2×10^7 spores ml^{-1}. Control plants were sprayed with sterile distilled water. Suspensions were applied with a spray atomiser with an adjustable nozzle to form a high density of fine droplets on the aerial parts of the plants. Immediately after inoculation, the plants were covered for 48 h with transparent polyethylene bags sprayed inside with distilled water. After the incubation period (IP), the plants were uncovered and kept in a glasshouse at temperature from 15 to 25°C.

Disease assessment

Mycosphaerella pinodes infection on the leaves was recorded 21 days after inoculation using a 0–5 disease scale following to Tivoli *et al*. (1996), where 0, no lesion; 1, a few scattered flecks; 2, numerous flecks; 3, 10–15% leaf area necrotic and appearance of flecks; 4, 50% of leaf area covered by lesions; 5, 75–100% of leaf area dehydrated or necrotic. To

determine the IP and latent period (LP), plants were inspected daily for up to 20 days.

The IP was defined as the period (in days) from host inoculation to the appearance of the first symptoms on the leaves, and the LP as the time (in days) from inoculation to the first formation of pycnidia in the lesions. Each lesion with pycnidia on leaves was recorded with the aid of a hand lens (10×).

Statistical analysis

Data analyzed were subjected to analysis of variance (ANOVA) considering the effect of factors individually and the interaction of factors using the R Statistics software (Version 2.5.0). ANOVA was carried out to detect the effect of IC, LWD, isolate and host susceptibility on IP, LP and DS. Data for IC were transformed to the natural logarithmic scale before analysis.

Results

Inoculum concentration effects

Analysis of variance detected that IC, cultivar and isolate had significant effects on IP, LP and DS ($P<0.001$) (Table 1). No significant interactions were found between either isolate × IC, or between cultivar × isolate × IC (Table 1).

tn0203 inoculated on Onward at an IC of 5.2×10^7 spores ml^{-1} and the lowest DS (mean: 1.0, SD: 0.25) was found when isolate md0202 was inoculated on MK at an IC of 2.5×10^3 spores ml^{-1} (Fig. 1a).

The IP was also affected by the IC. The shortest IP (mean: 3.3 days, SD: 0.52) was found when tn0203 was inoculated on 'Onward' with an IC of 5.2×10^7 and the longest (mean: 8 days, SD: 0.86) occurred when md0202 was inoculated on 'Onward' with an IC of 2.5×10^3 (Fig. 1b).

Similarly, the LP was shortest when the IC was highest. The LP for md0202 inoculated on 'Onward' ranged from 11 days (SD: 0.47) respectively for the highest IC (5.2×10^7) to 18 days (SD: 1.12) for the lowest IC (2.5×10^3). When md0202 was inoculated on 'MK', the LP ranged from 10.5 (SD: 0.45) days for the highest IC to 18.6 (SD: 0.63) days for the lowest IC (Fig. 1c).

Leaf wetness effects

Analysis of variance detected that LWD, cultivar and isolates all had a significant effect on DS, IP and LP ($P<0.05$) (Table 2). For DS and IP, there were also significant differences between cultivars × LWD. The interaction between the cultivar × isolate was also significant for DS and LP. However, there was no interaction between isolate × LWD or between isolate × cultivar × LWD (Table 2).

Table 1. Analysis of variance on the effect of cultivar, isolate and inoculum concentration on some epidemiological components of Ascochyta blight evaluated on three- leaf stage pea seedlings.

Source	Disease severity[a]				Incubation period[a]				Latent period[a]			
	DF	MS	F	P>F	DF	MS	F	P>F	DF	MS	F	P>F
Cultivar	1	25.741	278.2865	<0.001	1	3.267	21.7778	<0.001	1	3.75	8.0357	<0.01
IC	4	10.019	108.3171	<0.001	4	29.358	195.7222	<0.001	4	122.15	261.7500	<0.001
Isolate	1	2.774	29.9838	<0.001	1	8.067	53.7778	<0.001	1	6.02	12.8929	<0.001
Cultivar × IC	4	0.655	7.0793	<0.001	4	2.808	18.7222	<0.001	4	0.25	0.5357	<0.05
Cultivar × isolate	1	0.840	9.0829	<0.01	1	0.267	1.7778	<0.05	1	2.82	6.0357	
Isolate × IC	4	0.192	2.0739		4	0.525	3.5000		4	0.18	0.3929	
Cultivar × isolate × IC	4	0.109	1.1820		4	0.308	2.0556		4	0.82	1.7500	

[a] DF, degree of freedom; MS, mean square; F, F ratio; *P*, probability level.

Symptoms were more severe with higher IC on both the sensitive 'Onward' and the moderately resistant 'MK' (Fig.1a–c). Dead plants, particularly from 'Onward' were found at the higher ICs of 4×10^6 and 5.2×10^7 spores ml^{-1}. No blight symptoms were seen in the control plants. The highest DS (mean:4.9, SD: 0.45) occurred with the isolate

The DS increased with the increasing LWD (Fig. 2a–c). The highest DS (mean: 4.90, SD: 0.69) was recorded at a LWD of 72 h when tn0203 was inoculated on 'Onward', and the lowest DS was 1.0 (SD: 0.19) at a LWD of 6 h with md0202 inoculated on 'Onward'. When there was no leaf wetting, the DS was generally lower than 1 (Fig. 2a).

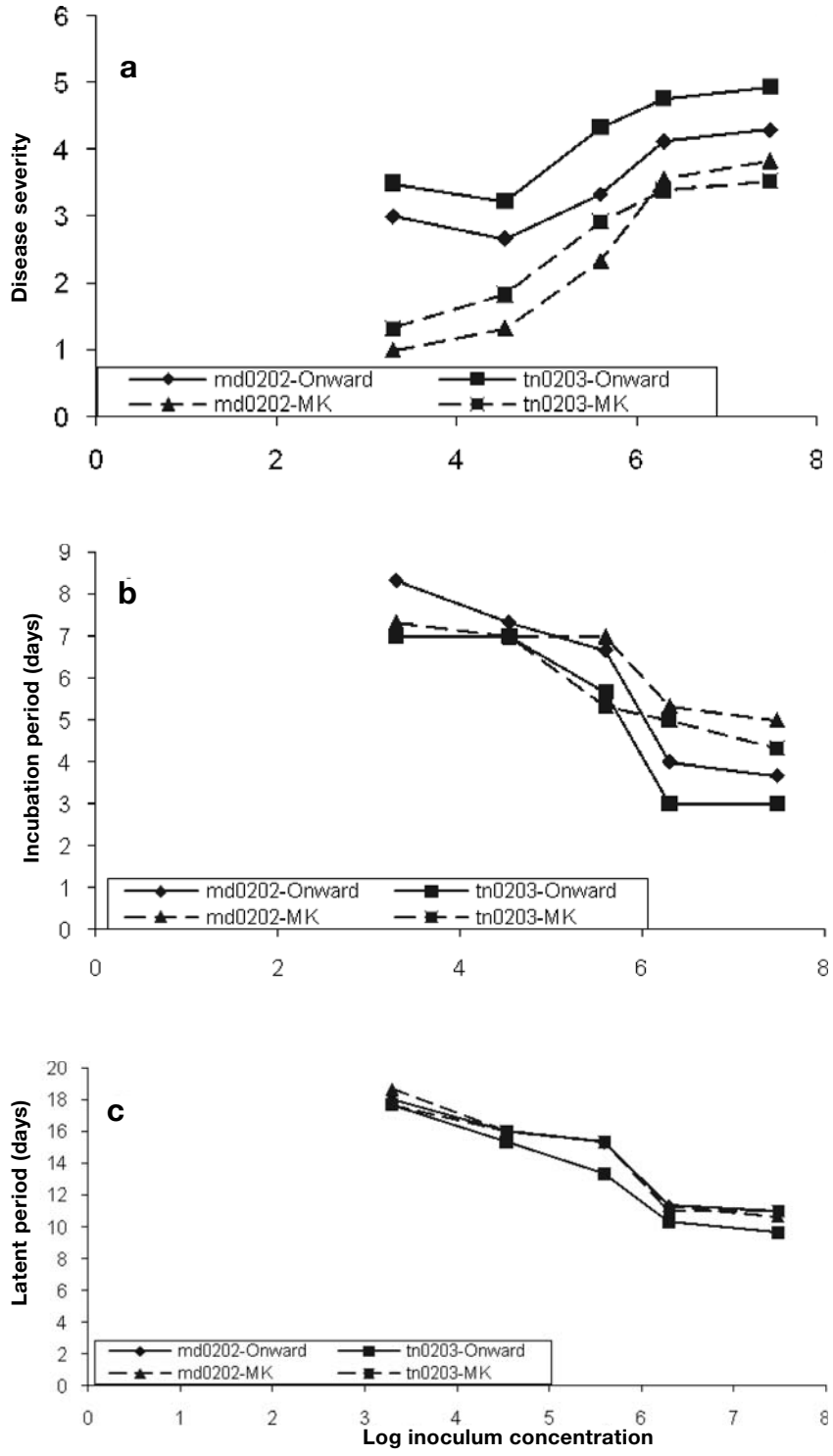

Fig. 1. Effect of log inoculum concentration in *Mycosphaerella pinodes* isolates md0202 and tn0203 inoculated on pea cv. Onward and Merveille de Kelvedon: a) disease severity; b) latent period; c) incubation period.

Table 2. Analysis of variance on the effect of cultivar, isolate and leaf wetness duration on some epidemiological components of Ascochyta blight evaluated on three- leaf stage pea seedlings.

Source	Disease severity[a]				Incubation period[a]				Latent period[a]			
	DF	MS	F	P>F	DF	MS	F	P>F	DF	MS	F	P>F
Cultivar	1	2.840	27.4115	<0.001	1	8.681	69.4444	<0.001	1	1.68	5.2609	<0.05
LWD	5	25.740	248.4335	<0.001	5	26.792	214.3333	<0.001	5	658.79	412.4609	<0.001
Isolate	1	0.867	8.3660	<0.01	1	0.681	5.4444	<0.05	1	5.01	15.6957	<0.001
Cultivar × LWD	5	0.769	7.4233	<0.001	5	2.414	19.3111	<0.001	5	3.57	2.2348	
Cultivar ×isolate	1	0.661	6.3820	<0.05	1	0.014	0.1111		1	7.35	23.0000	<0.001
Isolate × LWD	5	0.023	0.2233	<0.001	5	0.081	0.6444		5	0.24	0.1478	
Cultivar × isolate × LWD	5	0.134	1.2957	<0.001	5	0.014	0.1111		5	1.90	1.1913	

[a], see Table 1.

The IP of *M. pinodes* was also affected by the LWD. It was shortest (mean: 3 days, SD: 0.27) for the isolate tn0203 inoculated on 'Onward' at a LWD of 72 h. At a LWD of 6 h, on the other hand, the IP ranged from 6.5 to 7 days (SD: 0.47) (Fig. 2b).

The LP ranged from 11 to 20 days with md0202 inoculated on 'Onward' and from 10 to 19 days with tn0203 inoculated on 'Onward'. The shortest LP (10 days) was with tn0203 inoculated on 'Onward'. There was only slight difference in LP between a LWD of 48 h and a LWD of 72 h (Fig. 2c).

Cultivar and isolate effect

The two cultivars differed in their DS for all ICs (Fig. 1a) and for all LWDS (Fig. 2a). The highest DS (mean: 4.9, SD: 0.69) was achieved with the tn0203 inoculated on 'Onward'. The two cultivars were equally resistant at low levels of both IC and LWD. Hence, with an IC of 3.5×10^5 or less and with a LWD of up to 24h the two cultivars exhibited the same level of resistance. At higher IC and LWD, however, the cv. Onward had a higher DS (>4.1) than the cv. MK (DS<3.56).

The IP and the LP differed significantly between cultivars, the lowest IP (mean: 3 days, SD: 0.27) and the lowest LP (mean: 10.33 days, SD: 0.63) were found with tn0203 inoculated on Onward (Fig. 1). The interaction between cultivar and LWD was also significant for both DS and LP.

Effect of isolates

Significant differences were found between the isolates for DS, IP and LP (Tables 1, 2), but not for the IC. On the other hand, isolate and cultivar interacted significantly for DS with both IC and LWD (Table 2). But this effect of isolate aggressiveness with any of the epidemiological components tested was not visible for IC concentrations below 3.5×10^5 or with a LWD of 24 h. At higher IC and longer LWD, however, their effect became more pronounced. The highest DS (4.9) was found with the isolate tn0203 on 'Onward' followed by isolate md0202 also on 'Onward' (Fig.1a).

Discussion

Leaf wetness effects

Leaf wetness duration had a significant effect on DS, IP and LP. The wetness of leaf in general is an important factor enabling the fungal pathogens to infect the aerial parts of plants. When the temperature is favourable and the moisture requirements for a pathogen on a susceptible host are met for a sufficient length of time, an epidemic occurs. Weather moisture is frequently used as an indicator of the likelihood of an epidemic (Royle and Butler, 1986).

LWD of 6 h was sufficient to initiate symptoms formation on leaves of both the susceptible and the moderately resistant cultivars but with this duration, the reaction of the two cultivars were similar when the LWD increased, however, the DS also increased up to a duration of 48 h, beyond which the DS did not increase further. Most foliar fungi can infect the leaves of a plant only while the leaves are wet. But the optimal LWD varies depending on

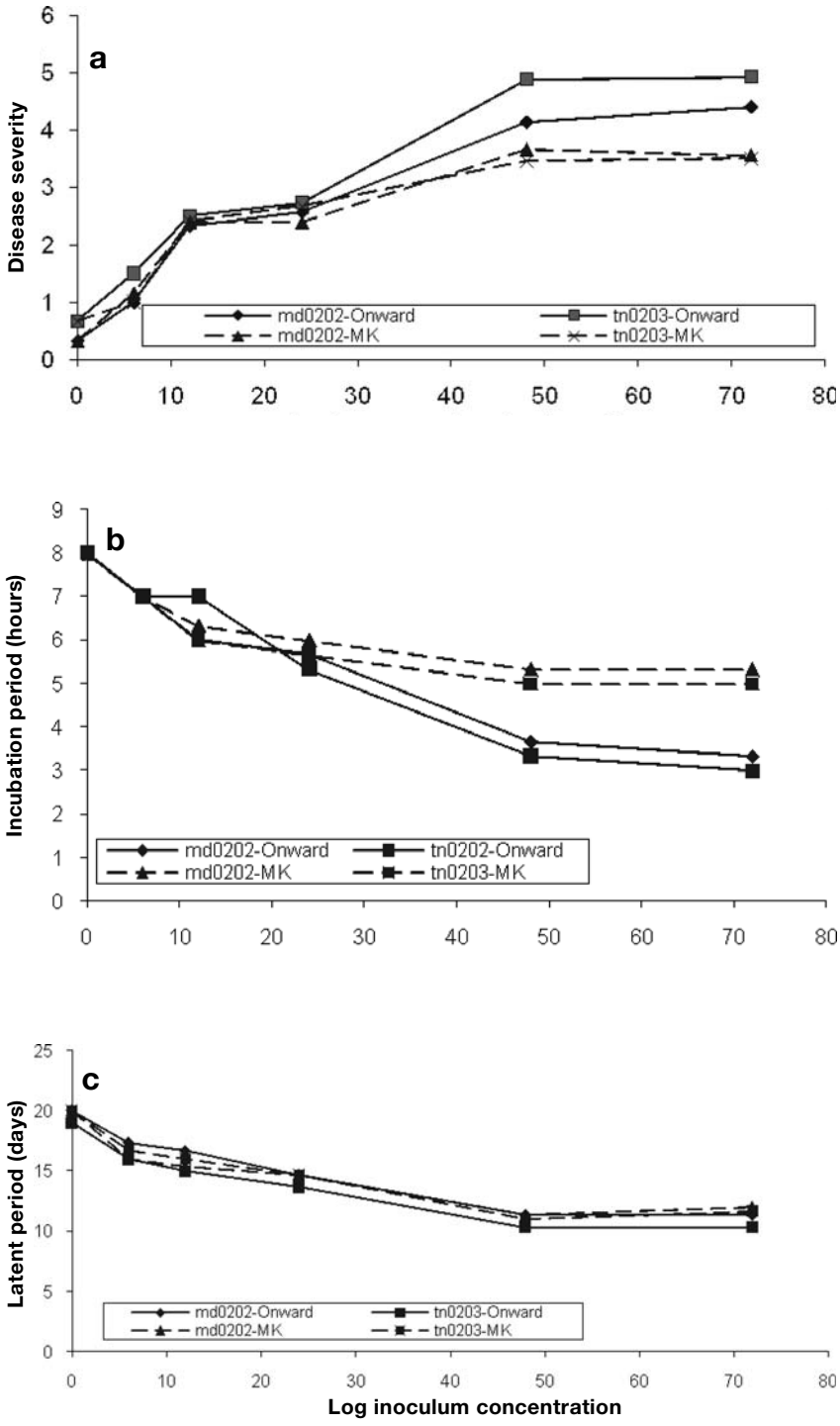

Fig. 2. Effect of leaf wetness duration on: a) disease severity, b) latent period, c) incubation period of *Mycosphaerella pinodes* isolates md0202 and tn0203 inoculated on pea cv. Onward and Merveille de Kelvedon.

the fungus group (Trapero-Casas and Kaiser 1992, Pederson and Morrall, 1994; Gilles *et al*., 2000). Many previous studies have reported that severe disease was obtained with a LWD of at least 48 h (Shew *et al*., 1988; Davis and Fitt, 1994; Scott *et al*., 1995; Rogger *et al*., 1999).

The IP on the other hand was shortest with a LWD of 48 and 72 h and longest with no LWD or a LWD of 6 h on both the susceptible and the partially resistant cultivar. The IP was shorter on 'Onward' than on 'MK' and the IP on both cultivars decreased as LWD increased. The LP is the interval between the moment of infection and the appearance of the first pycnidium, when the infected plant becomes itself infectious. The LP was longest when the LWD had been shortest. The LP for different species of Ascochyta blight has been reported in earlier studies. It was 5 days for *A. rabiei* on chickpea (Trapero-Casas and Kaiser, 1992), 6 days for *A. fabae* f. sp. *lentis* on lentil (Pederson and Morrall, 1994) and 8 to 10 days for *A. fabae* on fabae bean (Wallen and Galway, 1977). Our study found that on pea with an optimal LWD and IC, the LP was of 11 to 12 days on 'Onward'. The long LP could be explained in part as caused by fluctuations in the temperature that had occurred in the uncontrolled glasshouse during the experimental period. These temperatures ranged from 15 to 25°C and could have disturbed and delayed pycnidia production.

Lovell *et al*. (2004) found that pycnidia development was strongly influenced by environmental conditions. This relation has already been reported by other studies (Eden *et al*., 1996; Webb *et al*., 1997; Hartman *et al*., 1999; Buloviene and Surviliene, 2006).

Effect of IC and of isolate

The IC clearly affected the DS, the IP and the LP. The DS increased when IC went up. An IC of 5.2×10^7 caused the greatest DS on the two cultivars, 4.9 on 'Onward' and 3.53 on 'MK'. Plant death in the cv. Onward was also noted with higher ICs particularly 4×10^6 and 5.2×10^7 ml^{-1}. Makowski (1993) found that increasing DS increased the risk of plant death, particularly at the juvenile stages. There was a positive correlation between IC and DS. Such a positive correlation between IC and DS was also demonstrated for other *Mycosphaerella* spp. (Scott *et al*., 1985; Shew *et al*., 1988; Rogger *et al*., 1999). In our study the IP and LP of *M. pinodes* decreased significantly as its IC increased. According to Pederson and Morrall (1994), the LP is strongly affected by the IC and the pycnidial development was lower when the IC was lower. Furthermore, Hildebrand and Sutton (1984) reported that a high IC shortened both the IP and the LP. These experiments on IC revealed significant differences between the two isolates. The more aggressive isolate caused the highest DS with the shortest IP and LP.

The study found that there were differences between the isolates. The more aggressive isolate caused a greater DS after a short IP and LP. However, no significant differences were found in the interaction between the isolates and LWD or between isolates and IC.

Both LWD and IC were important for the epidemiology of *M. pinodes*. The pea cv. Onward and MK reacted differently with both a higher IC and longer LWD. At IC of 3×10^5 spores ml^{-1} and a LWD of 24 h, the two cultivars both exhibited the equal level of moderate resistance to the isolates. But at IC of 4×10^6 spores ml^{-1} and an LWD of 48 h and longer, the cv. Onward gave susceptible reaction. It was also found that an IC of 4×10^6 spores ml^{-1}, and a LWD of 48 h, together produced a consistent infection that enabled the susceptible and moderately resistant cultivars to be readily separated. Overall, under controlled conditions, an appropriate IC and LWD are very important to evaluate the resistance of pea cultivars to *M. pinodes*. The study also found that continuous leaf wetness of 48 h was the threshold duration for the application of fungicides on susceptible cultivars to control of this disease. Furthermore, the present study highlights the importance of taking account of the resistance of the plant when devising a fungicide spraying program, in order to keep the amount of fungicide sprayed to a minimum.

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