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Role of seed infection by the Ascochyta blight pathogen of dried pea (*Mycosphaerella pinodes*) in seedling emergence, early disease development and transmission of the disease to aerial plant parts

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Abstract

The role of infected seed in the epidemiology of Ascochyta blight of pea, caused by *Mycosphaerella pinodes*, was studied both under growth chamber and field conditions, using healthy seeds, naturally infected seeds and artificially infected seeds. Results suggest that infected seeds caused serious losses, as a result of poor germination and high transmission of the disease, to parts of the plants under soil level. Foot rot symptoms often caused the death of young seedlings. Losses were increased by low temperatures during the early stage of crop development. *M. pinodes* progressed from seeds to aerial parts of the plants, but no Ascochyta blight symptoms occurred, the disease remaining near to the basal parts of the plants as a foot rot symptom. This suggests that seeds cannot be regarded as a source of contamination in the epidemiology of the disease.

Introduction

Ascochyta blight, caused by *Mycosphaerella pinodes* (Berk. and Blox.) Vestergr., the teleomorph of *Ascochyta pinodes* (Berk. and Blox.) Jones, is one of the most important disease of pea (*Pisum sativum*). It is widespread throughout the major pea growing areas of the world (Wallen, 1965; Lawyer, 1984). The pathogen, which infects all aerial parts of the plant, is responsible for serious yield losses of up to 20% (Tivoli et al., 1996). High incidences of *M. pinodes* are also found on commercial seed samples. According to Deneufbourg et al. (1994), this fungus is the most common pathogen detected on pea seed; however, the levels of seed infestation vary considerably from year to year and between seed samples, depending on local climatic conditions.

Gilchrist (1926) first described a serious foot rot of pea caused by *M. pinodes*. Later, Lindford and Sprague (1927) and Hare and Walker (1944) showed that seed transmission was an important factor in the spread and survival of the pathogen. Infected seeds could carry the disease to new areas and act as primary sources of infection for initiating the disease under favourable environmental conditions. Furthermore, it is well established that using infected seeds induces a significant reduction in emergence, resulting in poor crop establishment (Wallen, 1965; Maude, 1966; Wallen et al., 1967; Bretag, 1991; Bretag et al., 1995). Many plants from infected seeds do not emerge because of severe infection resulting in embryo death; sometimes, plants die soon after emergence, because of infection from diseased seeds producing a foot rot. However, although it has been reported that sowing infested seed may serve to establish the fungus in the resulting crop of peas (Lindford and Sprague, 1927), little information is available on the importance of transmission from seed to aerial parts of the plants. According to Maude (1966), only 5 to 10% of plants growing from infected seeds had lesions evident on the stem above-ground level. Bretag et al. (1995), studying the correlation between seed-borne inoculum and disease development, concluded that seed inoculum appeared to be more important by its effect on emer-

Table 1. Percentage seed with *M. pinodes* on 5 samples of pea seed with varying degrees of infection

Surface sterilisation	Percentage coat area discoloured				
	0	1–25	26–50	51–75	76–100
No	0.0	65.0	72.0	52.0	35.0
Yes	0.0	79.0	71.0	90.0	80.0

gence than as a source of aerial infection. Therefore, serious gaps exist in the literature on the significance of seed transmission in the epidemiology of the disease.

The aim of this study was to determine if seed-borne infection of *M. pinodes* could induce the development of an epidemic of Ascochyta blight on aerial plants parts. A number of experiments were carried out in the field and growth chamber. The nature of seed-borne infection and the effect of seed infection on transmission of the disease were studied, as well as the influence of temperature on the severity of the disease. Fungal progress in the plant was investigated over a long period. The paper also presents results of field experiments conducted in 1995 and 1996 to establish the relationship of seed infection to subsequent infection in the crop.

Materials and methods

Nature of seed-borne infection and effect of seed infection on seedling infection

Seeds of pea cv. Solara were harvested from plants artificially infected with *M. pinodes*. The discoloured area of the seed coat, which was assumed to be due to infection by *M. pinodes*, was estimated visually and the seeds were classified into four categories according to the proportion of lesions on the seeds: less than 25%; 26 to 50%; 51 to 75% and 76 to 100% of coat area with lesions. Healthy seeds were used as controls. From each sample, 50 seeds were selected at random and tested for infection, using an agar plate method under two sets of conditions (after surface sterilisation with 1.2% sodium hypochlorite for 10 min and without surface sterilisation). Seeds were aseptically plated onto malt agar in Petri dishes and incubated at laboratory temperature (18–22 °C). Seven days later, dishes were examined and the fungi were identified. This methodology allowed us to conclude that *M. pinodes* was the only pathogen detected on seed (Table 1). Surface sterilisation did not suppress *M. pinodes* growth from stained seeds, which were apparently internally infected. However, sterilisation eliminated most of the non-pathogenic fungi (*Mucor, Penicillium, Fusarium*. .) that often prevented the detection of *M. pinodes*.

These naturally infected seeds were tested for the presence of *M. pinodes* within the tissue of seed components. For each infection category, three replicates of 50 seeds were soaked and divided into seed coat, cotyledon and embryo. The external (situated against the coat) and internal (situated against the embryo) parts of cotyledons were scored separately. The localisation of inoculum in seed components was determined by observation of discoloration and fruiting bodies under a light microscope.

The influence of infected seeds on seedling infection was assessed, for each infection category using a subsample of 25 seeds in three replications. Seeds were sown in plastic pots containing sterile compost. The experiment was conducted in a climate chamber at 20 °C, with 14 h illumination using sodium high-pressure lamps, giving a light level at plant height of 300 μE/m^{2}s^{-1}. High relative humidity was maintained by covering the pots with plastic. Three weeks after sowing, the young plants were carefully removed from the substrate and washed in water. Emergence counts were made and transmission frequency from seed to seedlings was calculated. The seedlings were examined for foot rot infection, and disease severity was estimated using the following scale of 0 to 5, modified from Maumene et al. (1992), indicating increasing infection by *M. pinodes*:

0: healthy plants;
1: streaks on the hypocotyl or on the epicotyl;
2: streaks on the hypocotyl and on the epicotyl;
3: lesions girdling the hypocotyl and streaks on the epicotyl;
4: lesions girdling the hypocotyl and the epicotyl;
5: weak plants with lesions girdling the hypocotyl and the epicotyl.

A Necrotic Index (NI) was also calculated as the weighted average of the disease by the equation:

$$NI = \sum_{i=0}^{5} n_i(d_i)/N$$

Where n_i is the number of plants in the diseased class i, d_i the value of the diseased class (0–5) and N the total number of plants assessed.

Isolations were made from lesions by surface sterilising the tissue in 96% ethanol for 45 sec and then placing pieces of tissue on malt agar. Seven days later, *M. pinodes* growing from pieces of tissue was identified as described previously.

Effect of temperature on transmission of infection from seed to seedling

The influence of temperature on infection of pea seedlings from diseased seeds was investigated using 3 seed samples; naturally infected seeds (seeds with less than 25% coat area with lesions were chosen because of their good emergence and their rate of transmission of infection from seed to seedling); artificially infected seeds, produced by dipping healthy seeds for 35 min in a conidial suspension of *M. pinodes* at 5×10^5 spores ml^{-1}; healthy seeds, used as controls. Seeds were sown in plastic pots containing sterile compost (1.1.1 soil : sand : peat mixture). For each seed sample, three replicates of 100 seeds were taken at random for testing, at each temperature. The experiment was conducted in growth chambers at 8, 13 and 20 °C, under the light regime described previously. The high humidity required was maintained by the use of plastic covers.

Young plants were carefully removed from the substrate 420 degree-days after sowing (three weeks at 20 °C, five weeks at 13 °C and 8 weeks at 8 °C) and washed in water. Emergence counts were made and the percentage of diseased seedlings was determined. For disease examination, plants were scored for foot rot lesions (0–5 scale) and the Necrotic Index was calculated. The presence of fruiting-bodies was determined on lesions by microscopic observation. Confirmation of *M. pinodes* infection was made by plating hypocotyl and epicotyl sections on agar (after surface sterilisation for 45 sec in a solution of 1.2% sodium hypochlorite) and scoring for growth of *M. pinodes*.

Study of pathogen progress in plants and the spread of disease

The transmission of *M. pinodes* from infected seeds to aerial plant parts was studied in growth chambers and in field trials. Artificially infected seeds and healthy seeds were used in the growth chamber experiment. Seeds were sown separately in plastic pots containing sterile compost. The experiment was conducted at 20 °C and 8 °C in growth chambers, under the light regime described previously. A high humidity was maintained by covering the pots with plastic. Observations on plant infection were recorded at 280 degree-day intervals for 5 weeks at 20 °C and 12 weeks at 8 °C. Eighteen plants from each sample were carefully removed from the soil and washed in water and the Necrotic Index was calculated. The transmission of *M. pinodes* from infected seeds to aerial plant parts was studied by observing the presence of the fungus in various parts of the healthy as well as infected plants raised from infected seeds. Each plant was aseptically divided into hypocotyl, epicotyl, internodes and stipules, up to the tenth node. Segments of plant parts were surface sterilised for 45 sec in a solution of 1.2% sodium hypochlorite and plated onto Petri dishes on agar. After incubation for 7 days at laboratory temperature, Petri dishes were observed for growth of *M. pinodes*.

Table 2. Percentage seed with *M. pinodes* (*M.p*), *M. pinodes* and *Phoma medicaginis* var. *pinodella* (*M.p + P.m*) and *Phoma medicaginis* var. *pinodella* (*P.m*) on 8 seed samples of pea seed, before (−)) or after (+) surface sterilisation

Seed samples	*M.p*		*M.p + P.m*		*P.m*	
	−	+	−	+	−	+
1995						
A. Solara	0.0	0.0	0.0	0.0	0.0	0.0
B. Carrera	0.5	0.0	0.0	0.0	0.0	0.0
C. Rustic	3.5	2.0	1.0	1.5	0.5	0.5
D. Solara	19.0	19.5	17.5	9.5	1.5	1.0
E. Carrera	32.0	39.0	16.0	9.0	1.0	0.5
1996						
F. Baccara	0.0	0.0	0.0	0.0	0.0	0.0
G. Baccara	26.5	7.0	0.0	0.0	0.0	0.0
H. Carrera	37.5	43.5	0.5	1.0	0.0	0.0

During 1995 to 1996, experiments were conducted under field conditions at 3 sites. Healthy seeds were used as controls. In 1995 in Le Rheu, 5 seed samples (A, B, C, D, E), produced by growers, were tested. In 1996, in Le Rheu, Brain/l'Authion and Troyes, three other seed samples were used (F, G, H). Estimates of percent seed infection with *M. pinodes* were made on 200 to 300 seeds for each sample, using the agar plate method described previously. These seed samples were found to have different levels of infection by *M. pinodes* (Table 2). In 1996, in Le Rheu, artificially infected seeds were also used in order to have seed samples with a known high level of infection. At each site, the experimental design was a randomised block with three replicates of each seed lot. Each sub-plot consisted of 6×10 m rows, with a 17.5 cm row-spacing and 25 seeds per row. Sprinkler irrigation was applied at Brain/l'Authion and Le Rheu in 1996 in order to achieve maximum infection. Irrigation dates as well as climatic data for the period of the field trials, obtained from the records of the meteorology stations of Le Rheu, Avrille (near Brain/l'Authion) and Troyes,

Table 3. Rainfall data, irrigation dates and temperature data for the period of field trials, from March to July in Le Rheu, Brain/l'Authion and Troyes

Place	Year	Month	Rainfall data (mm)	Irrigation dates	Temperature data (°C)		
					Max	Min	Mean
Le Rheu	1995	April	27.9		12.2	5.2	9.6
		May	55.8		20.8	7.1	13.5
		June	12.0		25.5	12.2	16.3
		July	55.8		25.9	16.6	20.6
	1996	March	49.6	Everyday (10 mm)	16.1	3.0	6.9
		April	43.4	Everyday (10 mm)	14.3	3.2	13.1
		May	71.3	Everyday (10 mm)	19.2	6.1	12.1
		June	78.0	Everyday (10 mm)	22.7	12.4	16.7
		July	58.9	Everyday (10 mm)	24.3	13.0	18.1
Brain/l'Authion	1996	March	18.6		16.8	2.7	7.4
		April	22.8	22.04 (3 mm); 29.04 (11 mm)	16.5	3.9	10.4
		May	20.4	07.05 (5 mm); 15.05 (11 mm)	21.5	9.1	12.3
		June	22.5	04.06 (8 mm); 05.06 (4 mm)	25.6	13.3	18.5
		July	31.0		25.7	13.1	19.2
Troyes	1996	March	12.4		11.1	0.0	5.5
		April	24.0		16.7	2.9	9.8
		May	58.9		18.0	7.6	12.8
		June	9.0		24.5	10.8	17.7
		July	24.8		25.8	11.1	18.5

are shown in Table 3. Emerged seedlings were counted six weeks after sowing. For each treatment, plants from each row were removed from the soil at defined growth stages (3–4 leaves, 6–7 leaves, flowering and physiological maturity), and assessed for disease. The percentage of diseased plants was calculated. The severity of foot rot was estimated using the Necrotic Index, and disease severity on the leaf and stem was rated using a 0–5 scale (Tivoli, 1995).

Statistical analyses

Analyses of variance were performed using the GLM procedure of the SAS Institute (1987). The Student Newman-Keuls (SNK) test was used for comparing means when significant effects were detected.

Results

Nature of seed-borne infection in naturally infected seeds and effect of seed infection on seedling infection

The study of nature seed infection (Table 4) revealed that in seeds with less than 25% of coat area with lesions, the fungus was present only within the tissues of the seed coat. Seeds that were more stained were usually heavily infected, often deeply within the cotyledon or embryo tissues. When the discoloration reached 25%, the pathogen could be present within all the tissues.

Pycnidia were often detected in lesions of severely infected seeds (with more than 25% coat area with lesions). However, these fruiting bodies were mostly confined to the seed coat. These results demonstrate that selection of infected seed by external appearance is practicable.

Effect of seed infection on seedling infection was studied in growth chambers. Emergence of seedlings decreased significantly with increasing severity of seed infection (Table 5). Emergence was very badly affected in seeds with more than 25% coat area with lesions: significantly different emergence rates of 30.7%, 16.0% and 2.7% for seeds with 26–50%, 51–75% and 76–100%, respectively, of coat area with lesions were observed. Seedlings emerging from infected seeds almost always showed foot rot symptoms. Lesions varied from small streaks on the hypocotyl to lesions coalescing and completely girdling the epicotyl. Seedling infection did not depend on severity of seed infec-

Table 4. Percentage of seeds with necrosis and pycnidia on coat area, external cotyledon, internal cotyledon and embryo, for 5 infection categories

Infection Categories	Necrosis				Pycnidia			
	Coat area	External cotyledon	Internal cotyledon	Embryo	Coat area	External cotyledon	Internal cotyledon	Embryo
0	0.0 b	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0
< 25	100.0 a	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0
26– 50	100.0 a	100.0 a	12.3 ± 5.5 b	10.3 ± 6.8 b	24.2 ± 5.9 b	8.1 ± 2.3 b	0.0 a	0.0
51– 75	100.0 a	100.0 a	76.2 ± 12.3 c	54.1 ± 3.8 c	84.8 ± 14.3 c	48.5 ± 9.1 c	0.0 a	0.0
76–100	100.0 a	100.0 a	100.0 d	100.0 d	100.0 cd	94.6 ± 15.2 d	76.4 ± 7.5 b	0.0

Numbers within a column followed by the same letter do not differ significantly (Newman-Keuls test, $P \leq 0.05$).

Table 5. Effect of seed infection (percentage of discolored area) on seedling emergence, percentage diseased seedlings and Necrotic Index, three weeks after sowing in growth chamber

Percentage coat area discolored	Seedling emergence (%)	Diseased seedlings (%)	Necrotic index
0	92.0 ± 13.8 a	0.0 a	0.0 b
1– 25	57.3 ± 4.0 b	94.3 ± 6.7 b	2.5 ± 0.2 a
26– 50	30.7 ± 14.0 c	96.6 ± 5.9 b	2.7 ± 0.4 a
51– 75	16.0 ± 8.0 cd	100.0 b	3.5 ± 1.0 a
76–100	2.7 ± 2.3 d	100.0 b	3.0 ± 0.0 a

Numbers followed by the same letter do not differ significantly within a column according to Newman-Keuls test ($P \leq 0.05$).

tion, and the lesions caused by the fungus were always confined to below soil surface level. No symptoms of Ascochyta blight appeared on the aerial parts of the seedlings.

Effect of temperature on transmission of infection from seed to seedling

Effects of temperature were assessed on healthy, naturally and artificially infected seeds in growth chambers. Emergence was significantly reduced in both naturally and artificially infected seeds at 8 °C, while low temperature had no significant effect on healthy seed emergence (Table 6). It was evident that emergence was always more heavily affected when seeds were naturally infected. However, emergence rates were similar in all treatments at 13 and 20 °C. Transmission frequency of the disease from seed to seedlings was very high for the seedlings growing from naturally and artificially infected seeds. However, the number of infected seedlings was not significantly influenced by the temperature; foot rot symptoms always occurred on about 75 to 100% of the seedlings (Table 6). Examination of seedlings grown from diseased seed at various temperatures revealed visible symptoms of infection at 20 °C, 13 °C and 8 °C. However, it was evident that lesions of seedlings grown from artificially infected seeds were more severe at low temperatures; plants grown at 13 °C and 8 °C showed more extensive lesions than plants grown at 20 °C. Symptoms similar to those described above were observed, but no lesions occurred above-ground level at any temperature.

Pycnidial production sometimes occurred in lesions on heavily infected seedlings. These fruiting bodies, located above-ground level, were most frequently present at 13 °C, (21% of seedlings grown from naturally infected seeds showed fruiting bodies at 13 °C, compared to 9.6% at 20 °C and 6.11% at 8 °C; with artificially infected seeds, data were similar).

Study of pathogen progress in plants and the spread of disease

In growth chambers under high relative humidity (95–100%), no symptoms occurred on plants from healthy seeds and the pathogen could not be detected in the tissues. Foot rot symptoms caused by *M. pinodes* appeared early on plants growing from artificially infected seeds (Tables 7 and 8) but disease intensity did not increase after 420 degree days. Symptoms, as measured by the Necrotic Index, were more severe at 8 °C than 20 °C. Lesions caused by *M. pinodes* were first apparent on the cotyledon stalks from which infection spread to the hypocotyl and then upwards toward the soil surface level and to a smaller extent downwards into the radicle.

Allowing the seedlings to grow for a long time did not result in symptoms on aerial parts of the plants. Only a few weak plants presented streaks on the first two internodes or lesions on bracts, especially at 20 °C.

Table 6. Effect of temperature on seedling emergence, percentage of diseased seedlings and Necrotic Index of plants growing in growth chamber from healthy seeds, naturally infected seeds and artificially infected seeds

Seed infection	Seedling emergence (%)			Diseased seedlings (%)			Necrotic Index		
	20 °C	13 °C	8 °C	20 °C	13 °C	8 °C	20 °C	13 °C	8 °C
Healthy	72.0±2.0 a	71.7±6.6 a	65.3±6.5 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Naturally infected	57.3±4.0 a	56.7±3.5 a	34.7±9.6 b	93.3±6.7 a	90.3±7.4 a	75.4±3.4 a	2.5±0.2 a	2.7±0.2 a	2.5±0.2 a
Artificially infected	70.3±2.1 a	64.3±5.8 a	50.7±1.1 b	100.0 a	100.0 a	100.0 a	2.5±0.5 b	3.3±0.0 a	3.5±0.2 a

Numbers within a line followed by the same letter do not differ significantly (Newman-Keuls test, $P \leq 0.05$).

Table 7. Disease development and fungal progress on plants growing from artificially infected seeds at 20 °C. Observation of necrosis and detection of the fungus were made on hypocotyl (H), epicotyl (E), bracts (B), internodes (I) and stipules (S), after 140, 420 and 700 degree-days 1–10: node number; —-: soil level; ++: presence in every plant; +: presence in at least one plant; −: absence

Plant parts	140 degree-days (Necrotic Index = 3.1)		420 degree-days (Necrotic Index = 4.1)		700 degree-days (Necrotic Index = 4.4)	
	Necrosis	Fungus	Necrosis	Fungus	Necrosis	Fungus
H	++	++	++	++	++	++
E	++	++	++	++	++	++
E	–	–	+	+	+	+
B1	–	+	+	+	+	+
I1	–	–	+	+	+	+
B2	–	+	–	+	+	+
I2	–	–	–	–	+	+
S1	–	–	–	+	–	+
I3	–	–	–	–	–	–
S2	–	–	–	–	–	+
I4	–	–	–	–	–	–
S3	–	–	–	–	–	+
I5	–	–	–	–	–	–
S4	–	–	–	–	–	–
I6	–	–	–	–	–	–
S5	–	–	–	–	–	–
I7	–	–	–	–	–	–
S6	–	–	–	–	–	–
I8	–	–	–	–	–	–
S7	–	–	–	–	–	–
I9	–	–	–	–	–	–
S8	–	–	–	–	–	–
I10	–	–	–	–	–	–
S9	–	–	–	–	–	–
I11	–	–	–	–	–	–
S10	–	–	–	–	–	–

These symptoms were not severe and never progressed above the first stipule. However, the fungus could be isolated in the absence of lesions on aerial parts of some plants more frequently from the stipules than from the internodes. For instance, the fungus was detected on the 10 first stipules of some plants 700 degree days after sowing at 8 °C, although no symptoms occurred above ground level. The proportion of plants from which *M. pinodes* could be detected increased from the beginning to the end of the experiment.

It was therefore evident that *M. pinodes* progressed from seed to aerial parts of plants, and although the

Table 8. Disease development and fungal progress on plants growing from artificially infected seeds at 8 °C. Observation of necrosis and detection of the fungus were made on hypocotyl (H), epicotyl (E), bracts (B), internodes (I) and stipules (S), after 140, 420 and 700 degree-days 1–10: node number; —-: soil level; ++: presence in every plants; +: presence in one plant at least; −: absence

Plant parts	140 degree-days (Necrotic Index = 3.1)		420 degree-days (Necrotic Index = 4.1)		700 degree-days (Necrotic Index = 4.4)	
	Necrosis	Fungus	Necrosis	Fungus	Necrosis	Fungus
H	++	++	++	++	++	++
E	++	++	++	++	++	++
E	–	–	+	+	+	+
B1	–	+	+	+	+	+
I1	–	+	+	+	+	+
B2	–	–	+	+	+	+
I2	–	–	+	+	+	+
S1	–	–	–	+	–	+
I3	–	–	–	–	–	–
S2	–	–	–	–	–	+
I4	–	–	–	–	–	+
S3	–	–	–	–	–	+
I5	–	–	–	–	–	+
S4	–	–	–	+	–	+
I6	–	–	–	–	–	+
S5	–	–	–	–	–	+
I7	–	–	–	–	–	–
S6	–	–	–	–	–	+
I8	–	–	–	–	–	–
S7	–	–	–	–	–	+
I9	–	–	–	–	–	–
S8	–	–	–	–	–	+
I10	–	–	–	–	–	–
S9	–	–	–	–	–	–
I11	–	–	–	–	–	–
S10	–	–	–	–	–	+

pathogen induced foot rot lesions, no Ascochyta blight symptoms occurred above-ground level.

Field trial experiments conducted in 1995 and 1996 at three sites gave information on seedlings emergence and foot rot intensity as well as on transmission of the disease from seeds to aerial parts of plants. In 1995 in Le Rheu (Table 9), emergence of all commercial seed samples was very poor, probably because the seeds were sown too deeply. This low emergence rate could explain the lack of differences between infected seed samples. Transmission rate of the disease from infected seed to seedling and foot rot symptoms were significantly more important for plants growing from severely infected seed samples (D and E). At the end of crop growth (physiological maturity), foot rot lesions were found in both the control and infected seed plots, and disease intensity on above-ground parts of the plants was not significantly different between treatments. Several fungi, such as *Phoma medicaginis* var. *pinodella*, *Fusarium roseum* and *Fusarium oxysporum*, were also detected on these lesions (about 10%, 30% and 60% of the diseased plants, respectively). These soil fungi formed a parasitic complex, also responsible for a foot rot on pea. This could explain the transmission rate of the disease as well as the Necrotic Index which did not differ significantly for all treatments.

In the experiment conducted with artificially infected seeds in 1996 in Le Rheu (Table 10), the emergence rate was very poor in all plots, for some unknown reason. However, the percentage of emerged seedlings

Table 9. Effect of seedborne infection by *M. pinodes* from 5 seed samples on seedling emergence and seedling infection in field trials conducted in 1995 in Le Rheu (sowing date: 05/04/95)

Seed samples	Seed infection (% *M. pinodes*)		Emerged seedlings (%)	Diseased plants (%) (hypocotyl region)			Necrotic Index		
	−*	+*		3–4 S**	F**	PM**	3–4 S**	F**	PM**
A. Solara	0.0	0.0	49.9 ± 10.4 ab	3.7 ± 6.4 b	0.0 b	83.3 ± 14.9 a	0.0 b	0.0 b	1.7 ± 0.8 a
B. Carrera	0.5	0.0	65.4 ± 3.8 a	5.3 ± 9.1 b	6.6 ± 7.0 b	55.4 ± 17.0 a	0.1 ± 0.1 b	0.1 ± 0.1 b	1.4 ± 0.5 a
C. Rustic	4.5	3.5	59.2 ± 7.6 a	1.9 ± 3.2 b	1.8 ± 3.0 b	51.8 ± 37.3 a	0.1 ± 0.1 b	0.1 ± 0.1 b	1.9 ± 0.3 a
D. Solara	36.5	29.0	50.0 ± 2.0 ab	25.6 ± 1.0 a	50.0 ± 22.0 a	79.3 ± 28.8 a	0.5 ± 0.0 a	1.2 ± 0.8 a	1.5 ± 0.7 a
E. Carrera	48.0	48.0	56.8 ± 12.6 ab	27.8 ± 3.4 a	49.0 ± 8.6 a	76.2 ± 25.1 a	0.5 ± 0.1 a	1.1 ± 0.4 a	1.8 ± 0.5 a

Numbers within a column followed by the same letter do not differ significantly (Newman-Keuls test, $P \leq 0.05$).
* Total of *M. pinodes* colonies detected before (−) and after (+) sterilisation.
** 3–4 S: 3–4 Stipules; F: Flowering; PM: Physiological maturity.

Table 10. Effect of artificial infection of seed by *M. pinodes* on seedling emergence and seedling infection in field trials conducted in 1996 in Le Rheu (sowing date: 15/03/96)

Seed samples	Seed infection (% *M. pinodes*)	Emerged seedlings (%)	Diseased plants (%) (hypocotyl region)			Necrotic Index		
			F*	F + 10*	PM*	F*	F + 10*	PM*
Control Solara	0.0	57.8 ± 2.7 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
Inoc. Solara	100.0	48.3 ± 4.6 b	100.0 a	100.0 a	100.0 a	2.5 ± 0.2 a	2.6 ± 0.0 a	3.3 ± 0.1 a

Numbers within a column followed by the same letter do not differ significantly (Newman-Keuls test, $P \leq 0.05$).
* F: Flowering; F + 10: Flowering + 10 days; PM: Physiological maturity.

was significantly affected by the presence of the fungus. All infected seeds gave rise to plants with foot rot lesions caused by *M. pinodes*. Symptoms appeared early and the Necrotic Index increased during the season from 2.5 to 3.3, because of lesions girdling the hypocotyl and streaks on the epicotyl.

During the growing season in 1995 and 1996 in Le Rheu, no typical symptoms of Ascochyta blight due to seed-borne inoculum occurred on aerial parts of adult plants, in spite of rainfall and irrigation. The first leaves of the plants growing from naturally and artificially infected seeds showed lesions at flowering with fruiting bodies (especially pycnidia) caused by *M. pinodes*, while control plants remained healthy. However, these symptoms were limited and different from those usually observed in field peas infected by *M. pinodes*. However, 15 days before physiological maturity, a few typical lesions of Ascochyta blight occurred on aerial plants parts of the plants growing from naturally infected seeds, artificially infected seeds as well as healthy seeds. At this time, it was evident that contamination was due to an external source. At harvest, disease intensity was not different between plants growing from healthy or infected seeds. This suggested that the disease did not progress from seed to aerial parts of the plants, in spite of irrigation. Experiments conducted in 1996 at 3 field sites with three other seed samples (F, G, H) gave similar results on seedling emergence, percentage of diseased plants and the Necrotic Index (data not shown).

It is therefore apparent from these results that foot rot symptoms occurred on plants growing from infected seeds, but disease did not progress from seed to aerial parts of the plants, even under irrigation.

Discussion

The aim of this study was to determine the importance of seed-borne inoculum in the epidemiology of Ascochyta blight of pea. Experiments gave results on effects of seed infection on plant emergence and development of foot rot symptoms, as well as on the transmission of disease from seed to aerial parts of the plants.

Emergence rates from discoloured seeds were significantly lower than from infected seeds which were not discoloured, and from artificially infected seeds which were only superficially contaminated. This observation, together with the lower per-

centage of emerged seedlings raised from seeds carrying deep-seated lesions, strongly indicates that emergence depends largely on the localisation of inoculum in the seed. The reduction of the proportion of emerged seedlings with increasing severity of seed infection could be explained by fungal colonisation during seed germination. In soaked seeds, latent stages of the fungus could grow from infected tissues towards the embryo. In seeds with large discoloured areas, the pathogen had either penetrated the embryo prior to seed germination, or was in close proximity to it, so that infection could spread more rapidly than for superficially infected seed so adversely affecting emergence. The highest losses were observed at 8 °C, probably because plants emerged less rapidly and the pathogen had more time to invade the developing seedling. This temperature corresponds with those which prevail in the spring; thus it could explain why a poor emergence rate was usually obtained in the field, and confirms previous reports of poor plant emergence when seed was planted under environmental conditions adverse to rapid germination, such as low temperature (Hwang et al., 1991).

Transmission frequencies from infected seed to basal parts of seedlings were very high at all temperatures, irrespective of the type of seed infection; in the growth chamber, 75 to 100% of infected seeds gave rise to plants with foot rot lesions. Seedlings could already be infected at the embryo stage. Infection could also occur during emergence, by contact with infected tissues or fruiting bodies, as well as after emergence. Thus, due to these several ways of infection, there was little chance for the seedling to escape disease. Foot rot lesions appeared early, as disease intensity was already severe 140 degree-days after sowing. However, the disease did not progress, the Necrotic Index remaining stable until the end of the experiment. It was also observed that disease intensity was more severe at low temperatures. The relative growth rates presumably favoured the pathogen and resulted in a higher intensity of infection. At higher temperatures, the hypocotyl grew away from the infected cotyledons before infection could occur. This result does not support the findings of Wallen et al. (1967), who found that *M. pinodes* was extremely active at 15 to 18 °C.

Similar results were obtained under field conditions. The percentage of diseased seedlings from infected seeds was high, but the disease intensity on above-ground parts of the plants was less than in growth chamber experiments. The smaller effect of seed infection in field plots than in growth chambers could certainly be due to the levels of seed infestation. Commercial seed samples had various percentages of seed infection but few visible symptoms, i.e. low infection intensity. Conversely, discoloured seed used for growth chamber trials, or artificially infected seed, had a high infection intensity. It is also possible that natural antagonism or competition existed between *M. pinodes* and other seed or soil-borne fungi such as *Fusarium oxysporum* or *Phoma medicaginis* var. *pinodella*, also detected in the soil and in some of the seeds. The observation of a lower impact of seed infection under field conditions raises the question of the theshold of seed infection acceptable in commercial cropping.

No Ascochyta blight symptoms occurred on aerial parts of plants growing from infected seeds in either field or growth chamber trials, in spite of favourable environmental conditions. Although fruiting bodies were sometimes formed on foot rot lesions, they could not serve to disseminate the pathogen because of their below-ground localisation.

In spite of the absence of symptoms, the pathogen could be detected in apparently healthy tissues of plants grown from infected seeds. The pathogen was mainly detected in some leaves of the plants. It could be possible that sterilisation eliminated *M. pinodes* from most of the tissues. These observations lead us to believe that the fungus is non-systemic. Similar results had been found by Dey and Singh (1994), working on another Ascochyta blight pathogen; workers had shown that *Ascochyta rabiei*, the causal agent of blight of chickpea, was non-systemic. Two hypotheses could explain why *M. pinodes* progressed on the plant but did not induce disease. According to Clulow et al. (1992), epicotyl and leaf reactions involve different mechanisms of resistance; hyphae grew extensively on the epicotyls of resistant lines but rarely formed appressoria, and failed to penetrate the cuticle. It is therefore possible that epicotyl resistance would restrict the pathogen during the early stages of infection. It is also possible that during its colonisation from seed to seedling, *M. pinodes* could adapt to above-ground level tissues of plants which are different from those of aerial parts. Thus, the pathogen would have lost its ability to infect tissues beyond the epicotyl.

From these results it is evident that seed-borne inoculum can be important in its effect on crop establishment and yield losses. However, it appears to be of minor importance in the epidemiology of the disease compared with wind-borne ascospores as a source of primary inoculum (Roger and Tivoli, 1996). These findings are in agreement with the previous results of

Bretag et al. (1995). In France, seed treatments applied on commercial seed samples (carbendazim + thiram or captam) have eliminated poor crop establishment.

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References

Bretag TW (1991) Epidemiology and control of Ascochyta blight of Field Peas. Thesis, La Trobe University, Australia

Bretag TW, Price TV and Keane PJ (1995) Importance of seed-borne inoculum in the etiology of the ascochyta blight complex of field peas (*Pisum sativum* L.) grown in Victoria. Australian Journal of Experimental Agriculture 35: 525–530

Clulow SA, Lewis BG and Mattews P (1992) Expression of resistance to *Mycosphaerella pinodes* in *Pisum sativum*. Plant Pathology 41: 362–369

Deneufbourg F, Seguin B and Molinero V (1994) Les traitements de semences contre l'anthracnose. Bulletin des semences 129: 39–41

Dey SK and Singh G (1994) Seedborne infection of *Ascochyta rabiei* in Chickpea and its transmission to aerial plant parts. Phytoparasitica 22: 31–37

Gilchrist GG (1926) The nature of resistance to foot rot caused by *Ascochyta* sp. and some other fungi in the epicotyl of the pea. Phytopathology 16: 269–276

Hare WW and Walker JC (1944) Ascochyta Diseases of Canning Pea. Wisconsin Research Bulletin 150: 1–31

Hwang SF, Lopetinsky K and Evans IR (1991) Effects of seed infection by *Ascochyta* spp., fungicide seed treatment, and cultivar on yield parameters of pea under field conditions. Canadian Plant Disease Survey 71: 169–172

Lawyer AS (1984) Diseases caused by *Ascochyta* spp. In: Hagedorn DJ (eds) The compendium of pea diseases (pp. 11–15) American Phytopathological Society: St Paul, Minnesota, USA

Lindford MB and Sprague R (1927) Species of *Ascochyta* parasitic on the pea. Phytopathology 17: 381–397

Maude RB (1966) Pea seed infection by *Mycosphaerella pinodes* and *Ascochyta pisi* and its control by seed soaks in thiram and captan suspensions. Annals of Applied Biology 57: 193–200

Maumene C, Didelot D and Remuaux M (1992) Foot rot of combining pea. Proceedings of the 1st European Conference on Grain Legumes, Angers, France, pp 327–328

Roger C and Tivoli B (1996) Spatio-temporal development of pycnidia and perithecia and dissemination of spores of *Mycosphaerella pinodes* on pea (*Pisum sativum*). Plant Pathology 45: 518–528

SAS Institute (1987) SAS/STAT Guide for personal computer, version 6. Cary, NC: SAS Institute

Tivoli B (1995) Consequences des attaques parasitaires foliaires sur l'élaboration du rendement des plantes à croissance indéterminée. In: Agrophysiologie du pois protéagineux. UNIP, ITCF, INRA. pp 199–229

Tivoli B, Beasse C, Lemarchand E and Masson E (1996) Effect of ascochyta blight (*Mycosphaerella pinodes*) on yield components of single pea (*Pisum sativum*) plants under field conditions. Annals of Applied Biology 129: 207–216

Wallen VR (1965) Field evaluation of the importance of the Ascochyta complex on peas. Canadian Journal of Plant Science 45: 27–33

Wallen VR, Cuddy TF and Grainger PN (1967) Epidemiology and control of *Ascochyta pinodes* on field peas in Canada. Canadian Journal of Plant Science 47: 395–403