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Phenotypic variability of *Leptosphaeria lindquistii* (anamorph: *Phoma macdonaldii*), a fungal pathogen of sunflower

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Growth of 17 isolates of *Phoma macdonaldii*, the causal agent of sunflower black stem, was investigated for response to pH and temperature, and for morphology and asexual morphogenesis (pycnidiogenesis and pycnidium size). For all isolates, the optimum pH for growth was between 4 and 5, and the optimum temperature varied between 20 and 30°C and radial growth was slowest at 5 and 35°C. Significant differences in the number and size of pycnidia were observed between isolates. Pycniospore germination was investigated under various conditions in five isolates chosen for their geographical origins, pigmentation, optimum growth temperature and pycnidogenesis. Increasing the concentration from 10^6 to 10^7 pycniospores per mL decreased the germination rate. The optimum temperature for pycniospore germination varied between 15 and 30°C, depending on the isolate, and the optimum and maximum pH values were 5 and 7, respectively. The optimum and minimum relative humidities allowing pycniospore germination were 100 and 95%, respectively. Pycniospore germination was photo-independent. An artificial inoculation method was developed and the aggressiveness of the pathogen was assessed on a susceptible sunflower cultivar, using a 1–9 scale that integrated the percentage of necrotic area on the cotyledon petiole at the stage when the first pair of leaves was fully developed. Significant differences in aggressiveness were observed among the 17 isolates. The parameters investigated clearly suggest the occurrence of a wide phenotypic variability in *Phoma macdonaldii*.

Keywords: aggressiveness, ascospores, black stem, *Helianthus annuus*, pycniospores

Introduction

The black stem disease of sunflower (*Helianthus annuus*) is caused by *Phoma macdonaldii* (Boerema, 1970), teleomorph *Leptosphaeria lindquistii* (Frezzi, 1968). The pathogen has been reported in various European countries (Yugoslavia, Bulgaria, Hungary, Romania: Maric *et al.*, 1988), Asia (Iran: Madjidieh-Ghassemi, 1988; Pakistan: Siddique-Mirza *et al.*, 1988; China: Hua & Ma 1996), the Americas (USA: Acimovic (1984); South America: Maric *et al.*, 1988) and Australia (Acimovic, 1984). In France, the disease has been spreading steadily since 1990, and the fungus is now a major component of the pathogenic complex on sunflower (Peres & Lefol, 1996).

The disease is characterized mainly by the appearance of black spots on the stem, around the petiole insertion point. Coalescing spots at the base of the stems develop into a wide black sleeve (Peres & Lefol, 1996), and black

maculae surround the back of the flower head. Early plant senescence results in yield losses of 10–30% (Penaud, 1996), and reductions in oil content of the seeds (Maric *et al.*, 1988) and thousand seed weight (Carson, 1991). Although chemical control can be considered (Penaud, 1996), its efficiency and the optimum time of application of the active ingredients remain to be ascertained. Within such a context, genetic variability for resistance of sunflower should be investigated, as it may lead to the identification of tolerant or resistant cultivars. However, such an approach requires accurate knowledge of the variability of the pathogen, which has remained insufficient so far.

This paper reports on the phenotypic variability present among *Phoma macdonaldii* isolates, as affected by abiotic factors. In particular, the effects of culture medium, pH and temperature on growth, sporulation and pycniospore germination of the pathogen were investigated. Aggressiveness of the isolates on a susceptible cultivar was evaluated by a test on cotyledon petioles. The objectives of this work were to provide information to permit selection of isolates to be included

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in a screening programme for sunflower resistance, and to devise a test suitable for such screening.

Materials and methods

Plant material

Seeds of a susceptible sunflower hybrid (Santiago) were disinfected for 5 min in a sodium hypochlorite solution (6 chlorometric degrees), rinsed three times in sterile distilled water and then sown uniformly at a depth of 2 cm in 40×30×25 cm plastic containers filled with vermiculite. Plants were raised in a growth chamber regulated at 24 ± 1°C (light period, 14 h at 200 μE m⁻² s⁻¹, provided by Osram-Vialox, Molsheim, France, NAV-T 600 W lamps) and 17 ± 1°C (dark period, 10 h), and at 75–85% relative humidity. Each container was watered every third day with 500 mL of water, containing 1 mL of a nutrient solution (NPK 6–3–6 and micronutrients; Substral, Boulogne-Billancourt, France).

Fungal isolates

Monopycniospore isolates

Stem fragments showing characteristic symptoms were cut into pieces (5×5 mm), surface-sterilized for 5 min in a sodium hypochlorite solution (6 chlorometric degrees), washed three times (5 min) in sterile distilled water, transferred to Petri dishes containing potato dextrose agar (PDA, 39 g L⁻¹) and incubated for 8 days at 25°C in the dark to allow mycelial growth. The dishes were incubated for a further 10 days under alternating periods of illumination (12 h, 37 μE m⁻² s⁻¹; Philips

TLD 15 W 33 lamps) and darkness to induce sporulation. A pycniospore suspension was obtained by placing one cm² explant from these cultures in a Petri dish containing 10 mL sterile distilled water. Monopycniospore cultures were prepared by serial dilutions (Barrault, 1989) and maintained on PDA for further subculture (Table 1).

Monoascospore isolates

Monoascospore isolates (Table 1), obtained by the dilution method (Barrault, 1989) from individual, mature perithecia that had developed on overwintering sunflower stems, were maintained on PDA.

Conservation of isolates

It is essential that the pathological and physiological characteristics of isolates remain constant during storage. The method described by Barrault (1989), which proved adequate for the conservation of *Pyrenophora teres*, was adapted for *P. macdonaldii*. A sunflower stem fragment, sterilized at 110°C for 25 min, was placed on a culture of a monospore isolate on PDA. Pycnidia were visible on the stem fragment after incubation for 15 days at 25 ± 1°C under a 12-h cycle of illumination (37 μE m⁻² s⁻¹) and darkness. When these fructifications developed, the stem fragment was placed in a sterile haemolysis tube containing CaCl₂ crystals (as a desiccant) at the bottom. Tubes were closed with an absorbent cotton plug, covered with an aluminium foil and kept in the dark at 6°C. This method, used since 1996, has proved to be efficient for long-term storage of *Phoma macdonaldii* isolates (data not published).

Table 1 Isolates of *Leptosphaeria lindquistii* collected in different regions of France

Isolates ^a	Pigmentation	Pycnidogenesis	CZ ^b	Year	Region	Locality (Département)
MA1	Citrine	++	absent	1997	Ile de France	Egreville (77)
MA2	Citrine	++	absent	1997	South-west	Tour de Faure (46)
MA3	Citrine	+++++	present	1997	South-west	Castanet (31)
MA4	Citrine	+	present	1997	South-west	Saint Lys (31)
MA5	Citrine green	+++	absent	1997	Ile de France	Egreville (77)
MA6	Citrine	++	present	1997	South-west	Saint Lys (31)
MA7	Citrine	++	present	1997	South-west	Castanet (31)
MP1	Citrine	++	present	1996	Ile de France	Egreville (77)
MP2	Citrine	++	present	1996	Ile de France	Egreville (77)
MP3	Citrine	++	present	1996	South-west	Tour de Faure (46)
MP4	Citrine	+++	present	1996	South-west	Tour de Faure (46)
MP5	Citrine green	++	present	1996	South-west	Castanet (31)
MP6	Citrine green	+	present	1996	South-west	Castanet (31)
MP7	Citrine	+++	present	1996	South-west	Saint Lys (31)
MP8	Apricot	+	present	1996	South-west	Saint Lys (31)
MP9	Citrine	+++	present	1996	Ile de France	Egreville (77)
MP10	Citrine	+++	present	1996	Ile de France	Saint Pathus (77)

^aEach monopycniospore isolate (MP) was obtained from a different lesion and a different plant. Each monoascospore isolate (MA) was from a different perithecium overwintering under field conditions.

^bCZ, concentric zonation associated with the aerial development of the mycelium and the production of pycnidia.

Growth and morphology

The effects of temperature (5, 10, 15, 20, 25, 30 and 35°C) at pH 6 and of pH (4, 5, 6 and 7) at 25°C on the radial growth of the isolates on the PDA medium were investigated. McIlvaine buffers (Anonymous, 1963) were used to control pH. The growth medium was selected following preliminary experiments showing the superiority of PDA over 5 and 10% V8 and Czapek-Dox for the growth and sporulation of four polypycnospore isolates (data not published). The effects of temperature and pH were studied under a 12-h photoperiod. Colony diameter was measured after 8 days and the isolates were also scored for presence or absence of concentric zonation and for pigmentation of the mycelium.

Asexual morphogenesis (pycnidiogenesis)

For each isolate, pycnidia were counted on three different sectors (each sector area 1 cm²) under a binocular microscope (Wild Heerbrugg) and 50 pycnidia were measured on 10-day-old cultures grown as described above. Diameters were measured under the microscope (Biomed Leitz, Rueil-Malmaison, France). All measurements were made on three replicate plates per isolate.

Pycnospore germination

A Van Tieghem cell was used for assessing the possible effect on germination of temperature (5, 10, 15, 20, 25, 30 and 35°C), pH (4, 5, 6 and 7, using McIlvaine buffers), pycnospore concentration (10, 10², 10³, 10⁴, 10⁵, 10⁶ and 10⁷ spores per mL), light (continuous light, continuous darkness and photoperiods of 12 h light at 37 μE m⁻² s⁻¹), and relative humidity (100, 98, 95 and 90%), according to the method used by Barrault (1989). The pycnospore suspension was spread onto a glass slide, then dried under sterile conditions, at a temperature below 30°C. The slide was then put on a glass stand inside a microchamber. The addition of sterile distilled water at the bottom of the microchamber provided saturated humidity. Sterile water was replaced with saturated saline solutions at 20°C to obtain relative humidities of 98% (K₂Cr₂O₇), 95% (Na₂SO₄, 7H₂O) and 90% (ZnSO₄, 7H₂O). Humidity was controlled with a wet chromel and a dry alumel thermocouple, calibrated with standard solutions (K₂SO₄, 97% R.H.; LiCl, 13% R.H.). The voltmeter readings were used for determination of relative humidity, using calibration curves.

In each experiment, the percentage germination was assessed from the examination of 50 spores after 16 h of incubation (after 36 h for the effect of R.H.) at 25°C in the dark. A pycnospore was considered germinated when the germ tube was longer than the spore itself. Three replicate slides per experimental condition were observed.

Aggressiveness

An inoculation technique was developed to assess aggressiveness of isolates under controlled conditions. The pathogen was cultured on PDA for 10 days at 25°C under continuous light. A pycnospore suspension was prepared by flooding the plates with sterile distilled water and stirring mechanically. The concentration was adjusted to 10⁶ pycnospores per mL and 20 μL of the spore suspension were deposited with a micropipette into the pit formed by the cotyledon petiole and the epicotyl of 12-day-old sunflower seedlings grown to the first pair of developed leaves, stages 1–2 (Lancashire *et al.*, 1991). A drop of sterile distilled water was used on the control plants. During the first 72 h after inoculation, the containers in which the plants were grown were covered with a polyethylene bag to promote pathogen development. Isolate aggressiveness was assessed 10 days after inoculation, on a 1–9 scale, based on the percentage of necrotic petiole area: 1 (0–5%), 2 (5–10%), 3 (10–20%), 4 (20–30%), 5 (30–40%), 6 (40–60%), 7 (60–80%), 8 (80–100%) and 9 (100% and necrosis extending to the epicotyl).

A completely randomized block design was used with three replications (three containers per isolate tested and 20 sites of contamination per replication).

Data analysis

In each case, data were subjected to variance analysis, and means were compared with the test of Newman–Keuls ($P=0.05$). Intensity measurements relative to the aggressiveness of the isolates did not require any transformation for the standardization of distributions.

Results

Morphology

Concentric zonation was frequently associated with the aerial development of mycelium and the production of pycnidia, except for isolates MA1, MA2 and MA5. The colonies usually displayed a citrine to citrine green colour, according to the nomenclature of Rayner (1970). However, under all conditions tested, the mycelium of isolate MP8 produced apricot-coloured crystals (Table 1), which were released in the medium after mycelium autolysis.

Growth and temperature

Variance analysis of growth was carried out separately for each temperature in all isolates and for each isolate at different temperatures. Significant differences between isolates were found for each temperature and between temperatures for each isolate (Table 2).

The data showed significant effects of temperature ($F=843.24$; $P<0.001$) and isolate ($F=9.5$; $P<0.001$) on growth, as well as a significant isolate–temperature

Table 2 Colony diameter (mm) of 13 *Phoma macdonaldii* isolates grown for eight days on PDA at pH 6 and under a 12-h photoperiod ($37 \mu\text{E m}^{-2} \text{s}^{-1}$) in growth cabinets kept at different temperatures. The values represent the mean diameters of three replicate cultures per isolate and temperature

Isolates	5°C	10°C	15°C	20°C	25°C	30°C	35°C
MP3	^e 11.25 ^b	^d 16.42 ^{cd}	^c 26.83 ^{cd}	^b 30.58 ^e	^a 44.00 ^b	^a 44.92 ^{cd}	^f 7.42 ^a
MP4	^e 9.58 ^b	^{de} 17.08 ^{cd}	^{cd} 28.17 ^{cd}	^{bc} 36.42 ^{cde}	^a 57.75 ^b	^{ab} 49.83 ^{cd}	^e 7.00 ^a
MP5	^f 7.83 ^c	^e 13.83 ^d	^d 23.00 ^d	^c 37.67 ^{cde}	^b 44.83 ^b	^a 52.00 ^{cd}	^f 6.83 ^a
MP6	^f 13.50 ^a	^e 23.00 ^b	^d 28.92 ^{cd}	^c 31.58 ^{de}	^a 47.33 ^b	^b 44.25 ^{cd}	^g 6.75 ^a
MP7	^d 7.33 ^c	^b 17.58 ^{cd}	^b 27.92 ^{cd}	^b 34.75 ^{cde}	^a 47.83 ^b	^b 29.00 ^e	^d 6.50 ^a
MP8	^e 9.83 ^b	^d 14.58 ^d	^c 30.59 ^c	^c 33.25 ^{cde}	^a 44.68 ^b	^b 38.33 ^{de}	^f 6.00 ^a
MP9	^g 9.75 ^b	^c 21.83 ^b	^b 36.83 ^b	^b 41.83 ^{bcde}	^a 57.75 ^b	^a 60.83 ^{bc}	^d 7.25 ^a
MP10	^d 10.08 ^b	^c 16.58 ^{cd}	^b 24.25 ^d	^a 51.42 ^b	^a 55.92 ^b	^a 51.92 ^{cd}	^d 7.33 ^a
MA3	^e 6.83 ^c	^d 15.00 ^d	^c 27.83 ^{cd}	^b 44.58 ^{bcde}	^b 41.17 ^b	^a 57.00 ^c	^e 7.58 ^a
MA4	^d 10.50 ^b	^c 27.42 ^a	^b 57.75 ^a	^a 75.25 ^a	^a 76.92 ^a	^a 77.42 ^a	^d 7.75 ^a
MA5	^f 7.08 ^c	^e 16.33 ^{cd}	^d 30.83 ^c	^c 38.33 ^{cde}	^b 51.75 ^b	^a 55.17 ^c	^f 6.83 ^a
MA6	^g 9.92 ^b	^c 20.00 ^{bc}	^b 31.83 ^c	^a 46.25 ^{bc}	^a 53.00 ^b	^a 55.83 ^c	^d 7.00 ^a
MA7	^e 7.08 ^c	^e 13.58 ^d	^d 24.42 ^d	^c 35.67 ^{cde}	^b 51.58 ^b	^a 70.75 ^{ab}	^e 7.00 ^a

Means with the same letter do not differ significantly, according to a Newman–Keuls test ($P=0.05$). Letters to the right of each value refer to differences between isolates (read vertically); letters to the left of each value refer to differences between temperatures (read horizontally).

interaction ($F=4.92$; $P<0.001$). Isolate MP3 grew at the lowest rate, MA4 at the highest.

Radial growth was slowest at 5 and 35°C in all isolates. MA3, MA5, MA7 and MP5 grew best at 30°C, isolates MP4, MP6, MP7 and MP8 at 25°C, MA4, MA6 and MP10 between 20 and 30°C, and MP3 and MP9 between 25 and 30°C.

Growth and pH

The variance analysis of growth data showed a significant effect of pH ($F=2104.84$; $P<0.001$), with the occurrence of an isolate *pH interaction ($F=4.87$; $P<0.001$). For all isolates tested, the optimum pH for growth was between 4 and 5 (Table 3).

Production and size of pycnidia

Significant differences in the number (Fig. 1) and size (Fig. 2) of pycnidia produced by the various isolates were observed. The least-sporulating isolates (MA3, MP3 and MP6) produced 15–20 pycnidia per mm^2 vs. 45–50 in isolates MP5, MP9, MP10 and MA6. The number and size of pycnidia were significantly and negatively correlated ($r=-0.52$; $P=0.05$).

Pycniospore germination

Five isolates (MP6, MP8, MP9, MP10 and MA7) differing in morphological characteristics, radial growth rate and pycnidial production were retained for this experiment.

Pycniospore germination was maximum at concentrations of $10\text{--}10^6$ pycniospores per mL for all isolates at pH 6. Increasing the concentration to 10^7 pycniospores per mL decreased the germination rate by 20–30%, depending on the isolate (Table 4). At

optimum concentrations, germination was close to 100% for some isolates (e.g. MP8), but much lower (70–75%) for others, such as MA7. The optimum temperatures for pycniospore germination (at pH 6, 10^6 pycniospores per mL) were 15–25°C (MP6) or 15–30°C (MA7, MP8, MP9 and MP10). Germination was very low (1.3–5%) at 5°C for all isolates. The maximum temperature is likely to be above 35°C, since germination rates ranging between 32% for MA7 and 65% for MP10 were observed at that temperature

Table 3 Colony diameter (mm) of 13 *Phoma macdonaldii* isolates grown on PDA at different pH values for eight days at 25°C and under a 12-h photoperiod ($37 \mu\text{E m}^{-2} \text{s}^{-1}$). The values represent the mean diameters of three replicate cultures per isolate and pH value

Isolates	pH			
	4	5	6	7
MP3	^{abcde} 42.67 ^a	^{ab} 43.00 ^a	^a 19.67 ^b	^a 11.33 ^c
MP4	^{abcd} 44.33 ^a	^{ab} 42.67 ^a	^{ab} 18.67 ^b	^a 13.00 ^c
MP5	^{bcde} 41.00 ^a	^{ab} 42.67 ^a	^a 20.00 ^b	^a 10.67 ^c
MP6	^{ef} 35.67 ^a	^{cd} 35.00 ^a	^{bc} 15.67 ^b	^a 13.33 ^b
MP7	^{de} 38.00 ^a	^{bc} 38.00 ^a	^{cd} 11.67 ^b	^a 7.33 ^c
MP8	^{fg} 33.33 ^a	^d 31.33 ^a	^d 10.00 ^b	^a 6.33 ^c
MP9	^{ab} 47.00 ^a	^a 48.00 ^a	^{bc} 14.67 ^b	^a 13.67 ^b
MP10	^g 28.67 ^a	^d 30.67 ^a	^{cd} 12.00 ^b	^a 6.00 ^c
MA3	^{fg} 32.67 ^a	^d 31.00 ^a	^d 9.33 ^b	^a 6.00 ^b
MA4	^a 49.00 ^a	^a 48.67 ^a	^{ab} 19.00 ^b	^a 12.33 ^b
MA5	^{abc} 45.33 ^a	^{ab} 43.33 ^a	^{ab} 19.00 ^b	^a 8.00 ^c
MA6	^{bcde} 40.33 ^a	^{bc} 39.67 ^a	^{bc} 15.00 ^b	^a 6.67 ^c
MA7	^{cdef} 38.67 ^a	^{bc} 39.33 ^a	^{bc} 15.33 ^b	^a 13.00 ^b

Means with the same letter do not differ significantly, according to a Newman–Keuls test ($P=0.05$). Letters to the right of each value refer to differences between pH values (read horizontally); letters to the left of each value refer to differences between isolates (read vertically).

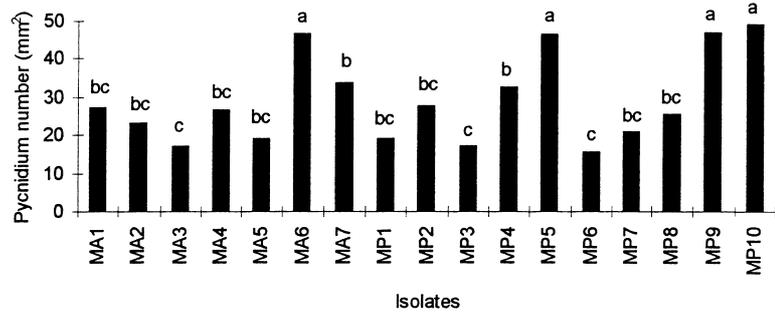


Figure 1 Pycnidia per mm² of *Phoma macdonaldii* after 10 days of culture on PDA medium, temperature 25°C, day length 12h (37 $\mu\text{E m}^{-2} \text{s}^{-1}$).

(Table 4). For all isolates at 10^6 pycniospores per mL, the optimum and maximum pH values were 5 and 7, respectively. The optimum and minimum relative humidities allowing pycniospore germination were 100 and 95%, respectively. Pycniospore germination was photo-independent (Table 4).

Aggressiveness

Significant differences in symptom production were apparent between isolates: some (e.g. MA4, MA7 and MP10) were weakly aggressive, whereas others (MP1, MP2, MP3, MP4, MP5 and MP7) were highly pathogenic (Fig. 3). Aggressiveness was not significantly correlated ($P > 0.05$) with radial growth ($r = -0.39$), pycnidia number ($r = -0.05$) or pycnidia diameter ($r = 0.20$).

Discussion

The *Leptosphaeria lindquistii* isolates investigated displayed phenotypic variability relative to growth and pycnidia production. Such variability has already been observed in related species, such as *Phoma exigua* (Decognet, 1994), *Phoma lingam* (Hassan *et al.*, 1991) or *Phoma medicaginis* (Angevain, 1984; Gray *et al.*, 1990).

The temperature optimum for pathogen growth varied with the isolate: some isolates can be called stenothermic (optimum at 25°C), whereas others are rather eurythermic (optimum between 20 and 30°C). These results are in agreement with those of Maric & Schneider (1979), who showed that the *in vitro* growth

of a *Phoma macdonaldii* isolate was characterized by minimum, optimum and maximum temperatures of 5°C, 22.5°C and 32.5°C, respectively. As observed for mycelial growth, pycniospore germination was often maximum over a wide range of temperatures (15–30°C). In *Phoma lingam*, the optimum temperature for pycniospore germination was shown to be between 20 and 25°C (Vanniasingham & Gilligan, 1988).

Phoma macdonaldii thrived at pH values in the range of 4–5. Such a peculiarity has already been reported for other species of fungi, although most phytopathogenic fungi grow best at a pH of between 5 and 6.5 (Cochrane, 1958). It should be noted that this acidophilic behaviour was also evident for pycniospore germination, which was maximum at pH 4–5.

The minimum relative humidity for spore germination is 95% and constitutes a major climatic parameter in the knowledge of disease epidemiology, because germination is essential for field infection. An increase in spore concentration to 10^7 pycniospores per mL decreased markedly the germination rate for each of the isolates. This effect could be expected since Fournet *et al.* (1970) showed that the presence of carbohydrates, proteins and a water-soluble compound in the sporiferous gels of some Sphaeropsidales and Melanconiales at high concentrations inhibited spore germination, possibly preventing spore germination within the pycnidium itself (Corbaz, 1990). Inhibition could be decreased by dilution, as was the case with other fungi (Fournet *et al.*, 1970). Boudart (1981) suggested that inhibition by sporiferous gels explains why pycniospore germination is slower than ascospore germination in *Leptosphaeria maculans*.

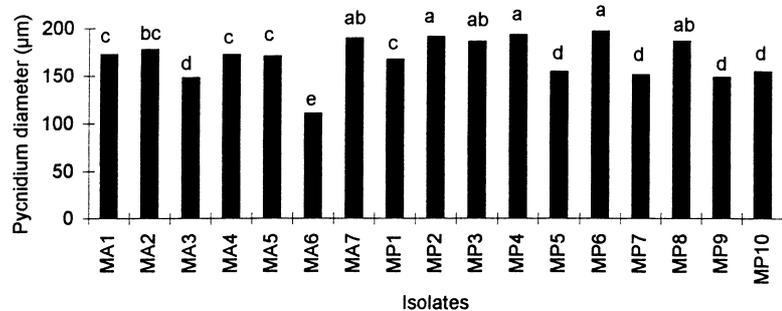


Figure 2 Pycnidium size after 10 days of culture on PDA medium, temperature 25°C, day length 12h (37 $\mu\text{E m}^{-2} \text{s}^{-1}$).

Table 4 Effects of temperature, pH, spore concentration, relative humidity and photoperiod on the percentage of pycniopore germination in five strains of *Phoma macdonaldii*, determined on 150 pycniospores (3 replications, each including 50 spores) after 16 h of incubation. Temperature effects were investigated at pH = 6, 10^6 pycniospores per mL, and in the dark; pH effects were investigated at 25°C, 10^6 pycniospores per mL, and in the dark; concentration effects were studied at 25°C, pH = 6, and in the dark; relative humidity was investigated at 25°C, pH = 6, 10^6 pycniospores per mL, and in the dark; light effects were studied at 25°C, pH = 6, and 10^6 pycniospores per mL.

Treatments		MP6	MP8	MP9	MP10	MA7
Temperature (°C)	5	^a 2.33 ^e	^a 4.33 ^d	^a 1.67 ^d	^a 5.00 ^d	^a 1.33 ^c
	10	^{ab} 32.00 ^d	^a 37.00 ^c	^b 25.33 ^c	^a 35.00 ^c	^b 25.33 ^b
	15	^a 82.33 ^a	^a 83.33 ^a	^a 73.33 ^a	^a 83.00 ^a	^a 72.33 ^a
	20	^{ab} 83.67 ^a	^a 93.33 ^a	^{ab} 78.67 ^a	^a 92.00 ^a	^b 71.33 ^a
	25	^b 87.67 ^a	^a 97.00 ^a	^c 75.33 ^a	^{ab} 93.00 ^a	^c 74.33 ^a
	30	^{abc} 74.00 ^b	^a 88.33 ^a	^{bc} 65.00 ^a	^{ab} 82.67 ^a	^c 58.33 ^a
	35	^a 51.67 ^c	^a 63.33 ^b	^b 37.33 ^b	^a 65.33 ^b	^b 32.33 ^b
pH	4	^a 31.67 ^b	^a 28.33 ^b	^a 26.33 ^b	^a 25.00 ^b	^a 18.33 ^b
	5	^a 41.67 ^a	^{bc} 31.67 ^a	^{ab} 35.00 ^a	^a 40.00 ^a	^c 26.67 ^a
	6	^a 16.67 ^c	^{ab} 11.67 ^c	^a 16.67 ^c	^{ab} 13.33 ^c	^b 8.33 ^c
	7	^a 8.33 ^d	^a 5.00 ^d	^a 8.00 ^d	^a 5.00 ^d	^a 5.00 ^c
Concentration (spores per mL)	10	^{ab} 85.00 ^a	^a 94.00 ^{ab}	^b 80.67 ^a	^a 96.67 ^a	^b 80.33 ^a
	10^2	^b 86.00 ^a	^a 99.33 ^a	^b 80.33 ^a	^a 100.00 ^a	^b 75.33 ^a
	10^3	^{bc} 84.33 ^a	^a 100.00 ^a	^c 80.00 ^a	^{ab} 93.33 ^a	^c 75.00 ^a
	10^4	^b 84.33 ^a	^a 100.00 ^a	^c 71.67 ^a	^a 100.00 ^a	^c 70.00 ^a
	10^5	^b 85.33 ^a	^b 90.67 ^b	^c 70.67 ^a	^a 98.33 ^a	^c 70.33 ^a
	10^6	^{ab} 85.33 ^a	^a 98.33 ^a	^{ab} 82.67 ^a	^a 98.33 ^a	^b 70.00 ^a
	10^7	^a 20.00 ^b	^a 30.67 ^c	^a 26.33 ^b	^a 20.00 ^b	^a 26.67 ^b
RH	100%	^a 99.33 ^a	^a 96.00 ^a	^b 83.67 ^a	^a 96.67 ^a	^b 88.33 ^a
	98%	^a 88.33 ^b	^a 81.67 ^b	^b 66.00 ^b	^a 88.67 ^b	^b 68.00 ^b
	95%	^a 15.00 ^c	^a 13.33 ^c	^b 5.00 ^c	^b 5.00 ^c	^b 5.00 ^c
	90%	^a 0.00 ^d	^a 0.00 ^d	^a 0.00 ^c	^a 0.00 ^c	^a 0.00 ^c
Light	Light	^a 87.33 ^a	^a 95.00 ^a	^b 80.00 ^a	^a 95.00 ^a	^c 71.67 ^a
	Darkness	^b 84.00 ^a	^a 100.00 ^a	^b 83.33 ^a	^a 100.00 ^a	^c 70.00 ^a
	**L-D	^{ab} 85.00 ^a	^a 96.67 ^a	^b 78.00 ^a	^a 93.33 ^a	^b 71.60 ^a
D-L	^c 83.33 ^a	^a 99.00 ^a	^d 80.00 ^a	^b 94.67 ^a	^e 68.00 ^a	

**L-D: 8 h of light then 8 h of darkness, D-L: 8 h of darkness then 8 h of light.

Means with the same letter do not differ significantly, according to a Newman-Keuls test ($P=0.05$). Letters to the right of each value refer to differences between treatments (read vertically); letters to the left of each value refer to differences between isolates (read horizontally).

Variation in pathogenicity of isolates has been observed in *Phoma lingam* (Cunningham, 1927; Williams, 1985, 1992; Mengistu *et al.*, 1991), *Phoma medicaginis* (Angevain, 1984) and *Phoma exigua*

var. linicola (Decognet, 1994), and is now reported in *Phoma macdonaldii*. No correlation between aggressiveness and growth or other phenotypic characteristics could be detected, in contrast with the observations

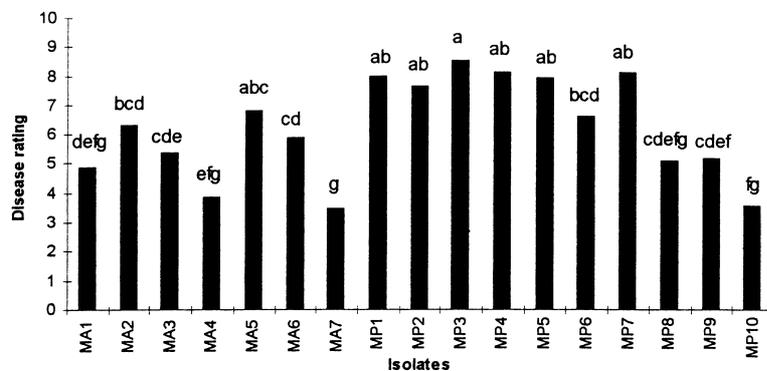


Figure 3 Aggressiveness of *Phoma macdonaldii* isolates recorded 10 days after inoculation; 25/18°C day/night temperature, relative humidity 75–85% and day length 14 h ($200 \mu\text{E m}^{-2} \text{s}^{-1}$).

on *Phoma lingam* (Pound, 1947; Delwiche, 1980; Hill *et al.*, 1984; Petrie, 1988; Koch *et al.*, 1989). However, before any decisive conclusion can be drawn, it will be necessary to increase the isolate pool to include nonaggressive isolates characterized for their phenotypes including biochemical and/or molecular characters, as already reported for *Phoma lingam* (Koch *et al.*, 1991; Williams, 1992).

The investigation of isolate aggressiveness led to the development of a method of inoculation under controlled conditions that will be a valuable tool, not only for the study of sunflower tolerance or resistance, but also for more fundamental approaches to the genetics of the interaction.

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