

Pathogenicity and Host-specificity of *Pleospora papaveracea*, a Candidate for Biological Control of Poppy (*Papaver rhoeas*)

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Abstract

The influence of growth stage of poppy plants, dew period, dew temperature and inoculum concentration on disease expression caused by the *Dendryphion* state of *Pleospora papaveracea* was investigated. A 24 h dew period at 25°C, and an inoculum concentration of 1.5×10^6 spores/ml killed 100% of plants 3 d after inoculation. Poppy seedlings at the seven- to eleven-true leaf stage were the most susceptible to the fungal pathogen. The host range of the fungus was also studied. Thirty-three varieties of cereal crops were found to be immune, but some *Papaver* species gave a hypersensitive or susceptible reaction.

Introduction

In Italy 52% of the arable land is cultivated with cereals, of which wheat represents 30%. Weed management is mainly by chemical control; 37 herbicides are registered for weed control in wheat (Anonymous 1983).

To reduce the use of chemicals in cereal systems, alternative strategies, such as biological control, are being investigated. In 1986 a research programme began to seek fungal pathogens for use as mycoherbicides against problem weeds in cereal crops. This work is supported by the European Communities Commission and the Italian Ministry of Agriculture. One of the selected target weeds was poppy (*Papaver rhoeas* L.; Papaveraceae), an important annual weed in cereal crops, especially wheat. This weed is reported as the greatest producer of biomass among broadleaf weeds in wheat (Covarelli 1981, Sgattoni *et al.* 1984) and is widespread in Italy (Pignatti 1982).

Among the fungal pathogens recorded on poppy, the *Dendryphion* state of *Pleospora papaveracea* (De Not.) Sacc. (Pleosporaceae) was selected as a candidate mycoherbicide for biological control of this weed. This paper deals with the pathogenicity and host-specificity of this fungus.

Materials and Methods

Influence of Environmental Factors on Disease Development

The influence of different factors on disease development in controlled conditions were investigated, including: plant growth stage; inoculum concentration; dew period; and dew temperature.

From diseased poppy plants from different areas of Central Italy, several fungi were recorded. All of them were tested using Koch's postulates. The anamorph of *P. papaveracea* was found to be pathogenic and damaging on poppy plants. The fungus causes small, black, rounded spots on the upper and lower leaf surfaces and petiole.

Inoculum of the fungus was produced on malt extract agar plates (30% maltose) incubated at 25°C for 9 d under fluorescent lights (12 h light; 12 h dark). A spore suspension was prepared by adding about 10 ml of 0.001% Teepol solution to each plate and scraping the culture with a glass slide. The suspension was adjusted according to counts made with a Neubauer hemacytometer.

Poppy seeds, placed in Petri dishes on three filter papers drenched with tap water, germinated after 10 d in a programmable incubator (12 h light, 15°C / 12 h dark, 20°C). When the seedlings had fully expanded cotyledon leaves, they were transplanted to trays containing a commercial potting medium.

By sowing on four dates, poppy plants of different growth stages were obtained: cotyledon leaves to three true leaves; 4 to 7; 8 to 11; and 12 to 16 leaves. They were sprayed to runoff with a water spore suspension of the fungus (1.5×10^6 spores/ml; 0.001% Teepol added), followed by a 24 h dew period, then incubated in a growth chamber (12 h light, 23°C / 12 h dark, 15°C). Control plants were sprayed with a solution of water and surfactant only.

The age at which the plants were most affected by the fungus was investigated. Dew periods of 6, 12, 18 and 24 h were used (other conditions as above). RH of about 100% was obtained by keeping inoculated plants in plastic bags previously sprayed with demineralized water. Influence of dew temperatures (15, 20, 25 and 30°C), and inoculum concentrations (7.5×10^4 , 1.5×10^5 , 7.5×10^5 , 1.5×10^6 spores/ml) was investigated at the conditions described above.

All treatments were replicated three times using 10 plants/replicate. Every experiment was repeated twice. Number of plants killed and number of infected leaves were recorded every 2 d. Plants were cut off at crown level 12 d after inoculation, and dried at 80°C for 48 h.

Host-specificity of Pleospora papaveracea

Several varieties of cereal crops and three *Papaver* spp. were inoculated with *P. papaveracea*. Thirty, 14- to 20-d-old, plants of each variety or species tested were sprayed to runoff with the optimum spore suspension described above, and placed at the best conditions for disease development. Poppy plants were used as controls.

Parts of plants with symptoms or symptom-less, randomly chosen, were cut off to reisolate the fungus. They were washed and disinfected in mercuric chloride (HgCl_2) for 30 secs, then placed in Petri dishes containing 12% agar.

Results

Influence of Environmental Factors on Disease Development

From diseased poppy plants, in addition to the *Dendryphion* state of *P. papaveracea*, the following fungi were isolated and identified: *Phoma exigua* Desm.; *Alternaria alternata* (Fr.) Keissl. (Hyphomycetes); *Fusarium solani* (Mart.) Sacc. (Hyphomycetes); *F. moniliforme* v. *subglutinans* Woll. and Reik.; the first record of *Verticillium dahliae* Kleb. occurring on *Papaver*; and *Scopulariopsis fusca* Zac.

The characteristic disease symptom caused by the *P. papaveracea* is water-soaking of petiole tissues. Later the petioles become dry and narrow with consequent wilt and decay of the whole leaves (Del Serrone *et al.* 1987).

On the basis the Student Newman Keuls test (Least Significant Difference) the biomass reduction of plants treated with the *Dendryphion* state of *P. papaveracea* in comparison with untreated plants, was always significantly different at all plant ages considered.

The greatest reduction of dry weight was obtained at the third stage; 100% and 90% of plants of this age were killed by the fungus 3 to 4 d after inoculation with a 24 h dew period and 25

to 30°C of temperature, respectively. In conditions of lower temperature and dew duration (12 h dew at 23°C and 24 h dew at 15°C) plants of third stage did not show high biomass reduction; however the percent of infected leaves which resulted were 59.1 and 72.2%. The 1.5×10^6 spores/ml concentration was the best one for which a significant result was obtained (Tables 1 to 3).

Table 1. Effect of growth stage of poppy plants artificially-inoculated with the anamorph of *Pleospora papaveracea* (De Not.) Sacc. (1.5×10^6 spores/ml, 23°C, 24 h dew) on biomass reduction and number of infected leaves.

Growth stage	Dry weight reduction (%)	Significance ¹ ($P = 0.01$)	Infected leaves (%)	Significance ¹ ($P = 0.01$)
Cotyledon to 3 true leaves	35	A	98.9	B
4 to 7 true leaves	88.2	B	95.0	B
8 to 11 true leaves	42.8	A	93.8	B
12 to 16 true leaves	40.5	A	81.6	A

¹ Same capital letters stand for a non-statistically significant difference.

Table 2. Effect of dew period and temperature on biomass reduction and number of infected leaves of poppy plants artificially-inoculated at the 4- to 7-leaf stage with the anamorph of *Pleospora papaveracea* (De Not.) Sacc. (1.5×10^6 spores/ml, 23°C).

Parameter	% Dry weight reduction	Significance ¹ ($P = 0.01$)	% Infected leaves	Significance ¹ ($P = 0.01$)
Dew period (h)				
6	0.1	A	25.8	A
12	0.1	A	59.1	B
18	49.2	B	93.5	C
24	92.1	C	99.5	D
Temperature (°C)				
15	28.4	A	72.2	A
20	26.3	A	86.2	B
25	91.5	B	99.9	C
30	86.1	B	100	C

¹ Same capital letters stand for a non-statistically significant difference.

Table 3. Effect of inoculum concentration on biomass reduction and number of infected leaves of poppy plants artificially-inoculated at the 4- to 7-leaf stage with the anamorph of *Pleospora papaveracea* (De Not.) Sacc. (25°C, 24 h dew).

Inoculum concentration (spores/ml)	% Dry weight reduction	Significance ¹ (P = 0.01)	% Infected leaves	Significance ¹ (P = 0.01)
7.5 x 10 ⁴	2.0	A	5.8	A
1.5 x 10 ⁵	4.3	A	8.0	A
7.5 x 10 ⁵	26.1	B	50.1	B
1.5 x 10 ⁶	84.1	C	99.0	C

¹ Same capital letters stand for a non-statistically significant difference.

Host-specificity of Pleospora papaveracea (Table 4)

None of the cereal crops tested developed disease symptoms 14 d after inoculation, and the fungus was never reisolated from them. *Papaver dubium* L. and *P. nudicaule* L., a wild and a cultivated species respectively, gave a hypersensitive reaction. A few small, black, rounded spots appeared on the surface of the leaves 5 d after inoculation, but the fungus was not reisolated. *P. somniferum* was susceptible (30% of the plants died 5 d after inoculation).

Conclusions

To get 100% plant mortality with *P. papaveracea*, a dew period of about 24 h was required. This dew period is too long for use of this pathogen as a mycoherbicide, and was probably due to the need for penetration through stomata. This type of penetration mechanism requires a long period of RH to allow germ tube formation and penetration through the stomatal opening. Host-specificity testing showed that the anamorph of *P. papaveracea* has a restricted host range within the genus *Papaver*. All cultivars of the crops tested were immune.

Furthermore, the laboratory trials on the effectiveness of *Dendryphion* state of *P. papaveracea* showed that this fungal pathogen was able to drastically reduce the biomass of poppy plants in suitable conditions just 3 d after inoculation. Although the isolates tested did not provide satisfactory indications in terms of the dew period it would be useful to conduct an additional investigation to discover more adaptable isolates to be used under field conditions, and additional studies on the telomorph of the fungus and on its pathogenic variability towards poppy.

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Table 4. Host-specificity of the *Dendryphion* state of *Pleospora papaveracea* (De Not.) Sacc.

Plant name	Reaction ¹	Plant name	Reaction
Durum wheat	immune ²	Barley	
'Procace'	"	'Protidor'	immune
'Valgerardo'	"	'Pirate'	"
'Creso'	"	'Selvaggio'	"
'Latino'	"	'Panda'	"
'Karel'	"	'Fior'	"
'Valnova'	"	'Gerbel'	"
'Valforte'	"		
Bread wheat		Sorghum	
'Mec'	"	'Esquival'	"
'Manital'	"	'DK 18'	"
'Costantino'	"	'Arno'	"
'Pandas'	"	'Orion'	"
'Salomone'	"	'M 518 G'	"
Maize		Oat	
'Roberta'	"	'Karlott'	"
'LG 1'	"	'Astra'	"
'Rx 42'	"	'Angelica'	"
'Anjou'	"	'Condor'	"
'Ennio'	"		
'Lorena'	"		
<i>Papaver</i> spp.			
<i>P. nudicaule</i> L.	hypersensitive ³		
<i>P. dubium</i> L.	hypersensitive		
<i>P. somniferum</i> L.	susceptible ⁴		

¹ 14 d after inoculation;

² Plants did not show symptoms, the fungus was not reisolated;

³ Plants showed few lesions, the fungus was not reisolated;

⁴ Plants with symptoms or killed, the fungus was, reisolated.