Dendryphon penicillatum and *Pleospora papaveracea*, Destructive Seedborne Pathogens and Potential Mycoherbicides for *Papaver somniferum*

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ABSTRACT

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Dendryphon penicillatum and *Pleospora papaveracea* were isolated from blighted *Papaver somniferum* and *Papaver bracteatum* plants grown in growth chambers and the field in Beltsville, MD. The etiology of the diseases was determined, and the fungi are being investigated as potential mycoherbicides to control the narcotic opium poppy plant. *P. papaveracea* is known to be a highly destructive seedborne pathogen of *Papaver somniferum*, causing seedling blight, leaf blight, crown rot, and capsule rot. Single conidia and ascospores were isolated and cultures established from naturally infested seed and diseased foliage and pods of opium poppy from Iran, Colombia, Venezuela, Sweden, India, and the United States (Maryland and Washington). Mycelia and conidia of *P. papaveracea* and *D. penicillatum* produced on necrotic leaf tissues appear morphologically similar, and the fungi were previously considered to be anamorph and teleomorph. However, no anamorph/teleomorph connection could be

established, and the fungi appear to be distinct taxa. *P. papaveracea* produced conidia, mature pseudothecia, and chlamydospores in vitro and on infected stems. *D. penicillatum* produced conidia, microsclerotia, and macronematous conidiophores. Although both fungi were pathogenic to three poppy cultivars, conidial inoculum from *P. papaveracea* cultures was more virulent than conidial inoculum from *D. penicillatum*. Eight-week-old plants became necrotic and died 8 days after inoculation with a conidial suspension of *P. papaveracea* at 2×10^5 spores per ml. Disease severity was significantly enhanced by inoculum formulations that contained corn oil, by higher conidial inoculum concentrations, and by increased wetness periods. Symptoms on plants inoculated with either pathogen included leaf and stem necrosis, stem girdling, stunting, necrotic leaf spots, and foliar and pod blight. Inoculated seedlings exhibited wire stem, damping-off, and root rot. Conidia, and less frequently pseudothecia, of *P. papaveracea* and conidia of *D. penicillatum* were produced abundantly on inoculated, necrotic foliage, pods, and seedlings. Cultures from conidia or ascospores reisolated from these tissues consistently produced fungi whose morphologies were typical of the fungus from which the inoculum was derived.

In 1996, opium poppy (*Papaver somniferum* L.) seedlings and mature plants produced from Plant Introduction (PI) seed accessions grown in greenhouses and growth chambers in Beltsville, MD, were dying of an unknown disease that spread rapidly throughout the collection, killing the majority of over 200 accessions. Symptoms included seedling wire stem, foliage and stem necrosis, and blight of mature plants. Conidia were produced abundantly on seed, diseased leaves, and symptomatic plants from the field. Microsclerotia were observed in water-soaked roots of seedlings, on seed coats, and in infected and water-soaked mature plant tissues. A few accessions grown in the field exhibited foliar leaf spot and necrotic spots on pods.

Dendryphon penicillatum (Corda) Fr. (synanamorph *Helminthosporium papaveris* K. Sawada) is a seedborne pathogen that is known to cause a highly destructive disease of opium poppy in countries in Europe and Asia where *Papaver somniferum* is grown (9,13,23). The teleomorph of the fungus has been considered to be *Pleospora papaveracea* (de Not.) Sacc. Work on the disease has been described from several European countries, as well as the United States, India, and Japan (5,7,16,17,20,24-26). Leaf blight disease of opium poppy caused by *Helminthosporium* species was reported

to be one of the most destructive diseases in Bulgaria, Switzerland, Czechoslovakia, the Netherlands, Romania, and Yugoslavia (23). The disease is known by several names: capsule rot, capsule blight, poppy fire, leaf blight disease, Helminthosporiosis, and spotted poppy Helminthosporium disease. Symptoms and signs have been found on all plant parts and at all stages of plant development (seedlings, crowns, roots, stems, leaves, pods, and capsules), although a few studies report that capsules are either more susceptible or are the only susceptible tissues (2,23). One study reports that the pathogen is strictly a saprophyte of dying tissues of *Papaver somniferum* (14).

Helminthosporium disease on poppies was described in 1917 by Sawada (21) and was subsequently investigated by Girzitska (10), Christoff (4), and Barbacka (2). In these reports, *D. penicillatum* was considered the conidial form of *P. calvescens* (Fr. ex Desmaz.) Tul. (= *P. papaveracea*). Barbacka (2) noted that two fungi, *H. papaveri* and *D. penicillatum*, have been associated with the disease, but considers that references to the latter in the literature really refer to the former. Girzitska (10) reported a connection of *D. penicillatum* with *P. papaveracea* (= *P. calvescens*) and, thus, considered them the same fungus. Christoff (4) reported *P. calvescens* to be the perfect stage of *H. papaveri*.

A review of the literature on the poppy disease pathogen describes strains, races, and a wide range of conidial dimensions, as well as the presence or absence of microsclerotia, chlamydospores, stromata, perithecia, and a *Dendryphon*-type of conidiophore (7). Reports suggested that production of different fungal structures varied, depending on cultural substrate and plant parts on which

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the fungus was growing, including seed (1,15,16,22). Among the 26 isolates studied, Meffert (15) distinguished seven races or strains based on conidial morphology. In that report, a form of *D. penicillatum* was isolated that produced numerous, small, black sclerotia on seeds and young infected seedlings and a dark brown, penicillium-like, branching conidiophore different from *H. papaveris* conidiophores. Anamorphic strains of *H. papaveris* (teleomorph *P. papaveracea*) and *D. penicillatum* were recognized, and the sclerotia-producing strain was named *D. penicillatum* var. *sclerotiale*. Naumann (18) investigated the inhibition of different morphological strains of *H. papaveris* by actinomycete strains. Schmiedeknecht (22) evaluated 170 single-spore isolates from different locations in Germany and found differences in conidial size, aerial mycelia, spore production, and pigmentation. Czyżewska and Zarzycka (6) concluded that *H. papaveris* is probably not a uniform species, but composed of a few races differing chiefly in cultural appearance and spore size. Nevertheless, the causal agent of the poppy blight disease has been considered to be one morphologically variable species with an anamorphic and teleomorphic state (7,24). Recent morphological and molecular genetic studies of isolates used in the current study show that *D. penicillatum* is a distinct fungus and not a part of the life cycle of *P. papaveracea* (8).

Although there are numerous reports describing the occurrence of poppy blight and resulting yield and seed losses (23), few investigations include information regarding the virulence or pathogenicity of isolates from artificial inoculations (11,17,20). Research papers on the poppy blight disease generally report the use of one strain and do not adequately describe reproductive morphology or give a clear description of the morphological characteristics of their isolate. A distinction is not generally made between the disease caused by (or the cultures and inoculum derived from) different strains of the pathogen and there is no indication that conidial inoculum was derived from the anamorphic versus the teleomorphic state. Thus, the relative virulence or pathogenicity of strains or isolates reported in the literature is difficult to assess, and disease etiology is uncertain.

A further difficulty in determining the etiology of the poppy disease from previous reports is that the pathogen(s) are referred to by different names that may not be synonymous. The teleomorph synonym includes *Pyrenophora pelli* (Fr.) Rab., *P. calvescens* (Fr. ex Desmaz.) Tul., and *P. papaveracea* (de Not.) Sacc. The anamorph has been referred to as *D. penicillatum* (Corda) Fr., *Dendryphium penicillatum* (Corda) Fr., *Brachycladium penicillatum* Corda, *D. papaveris* (Sawada) Sawada, *D. penicillatum* var. *sclerotiale*, *H. papaveris* K. Sawada, and *H. papaveri* K. Sawada (7, 15,25,27).

Paramount to plant pathology investigations and the development of a biocontrol agent is the need to accurately determine the

etiology of a disease. Demonstration that the organism isolated causes the observed symptoms is usually achieved by completing Koch's postulates. The objective of this study was to determine the etiology of poppy blight occurring in PI accessions grown in the greenhouse, growth chambers, and field and to conduct growth chamber studies to evaluate the potential for using the pathogens as bioherbicides for *Papaver somniferum*. Preliminary reports have been published (12,19).

MATERIALS AND METHODS

Single-spore isolates were obtained by isolating single conidia from diseased poppy foliage and pods of plants growing in the field and growth chambers in Beltsville, MD. Isolates also were obtained from seed of five PI accessions, from dried infected capsules from Venezuela and Colombia, and from dried stem stubble of *Papaver bracteatum* and *Papaver somniferum*. Fungi were obtained from seed by the blotter method. Approximately 300 seeds per PI accession were soaked in 0.5% NaOCl for 5 min and rinsed with sterile distilled water. Seeds were incubated on sterile moist filter paper in petri dishes at 24°C and evaluated daily for the appearance of fungi. Fungi were cultured on potato dextrose agar (PDA) (Difco Laboratories, Detroit) or half-strength V8 juice agar (100 ml of V8 juice, 2.0 g of CaCO₃, 16 g of agar, and 1 liter of distilled water). In April and May 1998, dry stems were collected from field stubble of poppy plants inoculated the previous spring. Fungi from this and other dried samples were obtained by placing tissue in a moist chamber, incubating as described above, and examined daily for the presence of fungi. Morphological descriptions of *D. penicillatum* and *P. papaveracea* were made from foliage and stems of inoculated poppy plants exhibiting symptoms of the disease. To determine whether there was an anamorph/teleomorph connection between the fungi, morphological characteristics were examined in at least 50 single-ascospore cultures obtained from pseudothecia produced by each *P. papaveracea* isolate and in a similar number of conidia from each fungus and isolate listed in Table 1.

Plant materials and fungal inoculum. Plants for the pathogenicity studies were grown from seed for 8 weeks in Jiffy Mix Plus potting medium (Jiffy Co., Shippegan, Canada) in 10-cm-diameter pots in a growth chamber with a 28 and 22°C 12-h day/night temperature. Relative humidity was 40% or less, with an 11-h photoperiod from fluorescent lights at 500 µE s⁻¹ m². Pots were watered as needed to prevent wilting. Seed lots of *Papaver somniferum* were obtained from a local grocery (designated Indian Grocery [IG]), from an accession from Venezuela used for the production of opium (VEN), from the ornamental variety White Cloud (WC), and from PI accessions.

TABLE 1. Host, origin, and morphological characteristics of fungi isolated from *Papaver somniferum*- and *Papaver bracteatum*-infected seed and diseased foliage

Species	Isolate designation ^y	Fungal structures produced in culture ^z			Host	Origin
		Microsclerotia	Dendryphion-type conidiophores	Pseudothecia		
<i>Dendryphion penicillatum</i>	Cf96	+	+	—	<i>Papaver somniferum</i>	Beltsville, MD
<i>Dendryphion penicillatum</i>	835-I-F2-1	+	+	—	<i>Papaver bracteatum</i>	Sunnyside, WA
<i>Dendryphion penicillatum</i>	381B	+	+	—	<i>Papaver bracteatum</i>	Iran, PI381488
<i>Dendryphion penicillatum</i>	415560	+	+	—	<i>Papaver somniferum</i>	Afghanistan, PI415560
<i>Pleospora papaveracea</i>	B96	—	—	+	<i>Papaver somniferum</i>	India, PI415573
<i>Pleospora papaveracea</i>	Pf96	—	—	+	<i>Papaver somniferum</i>	Beltsville, MD
<i>Pleospora papaveracea</i>	19C	—	—	+	<i>Papaver somniferum</i>	Colombia
<i>Pleospora papaveracea</i>	251083-1	—	—	+	<i>Papaver somniferum</i>	Afghanistan, PI251083
<i>Pleospora papaveracea</i>	414296	—	—	+	<i>Papaver somniferum</i>	Sweden, PI414296
<i>Pleospora papaveracea</i>	VEN	—	—	+	<i>Papaver somniferum</i>	Venezuela

^y Isolates from the personal collection of N. O'Neill. Isolate 835-I-F2-1 was isolated from dried stems of *Papaver bracteatum* collected by W. Kaiser. An isolate from the same material was deposited by W. Kaiser at the American Type Culture Collection (ATCC 46989) and at the Commonwealth Mycological Institute (CMI 246547).

^z Isolates were cultured on half-strength V8 juice agar. The abundance of Dendryphion-type conidiophores produced on V8 culture plates varied depending on the isolate.

Fungal inoculum was prepared by scraping conidia from 7- to 10-day-old V8 juice agar plates incubated at 23°C for 7 days with a 16-h photoperiod equipped with fluorescent lights. Conidia were suspended in sterile distilled water containing 0.001% Tween 20 at concentrations indicated for each experiment and then filtered through two layers of cheesecloth. Plants were sprayed at a rate of approximately 2.5 ml per pot (to runoff). Pots were placed in a mist chamber at 22 to 24°C for 24 h, or for times indicated, and returned to a growth chamber. Conditions for disease development in the growth chamber were 55 to 60% relative humidity with a 16-h photoperiod under fluorescent lights at 24°C. Disease assessments were based on a visual estimate of percent foliage blight on a 0 to 9 scale, in which 0 = 0 to 3%, 1 = 3 to 6%, 2 = 6 to 12%, 3 = 12 to 25%, 4 = 25 to 50%, 5 = 50 to 75%, 6 = 75 to 87%, 7 = 87 to 93%, 8 = 93 to 96%, and 9 = 96 to 100% foliage necrosis.

Pathogenicity of *D. penicillatum* and *P. papaveracea* to *Papaver somniferum* cultivars. Poppy cvs. IG, WC, and VEN were inoculated with *D. penicillatum* isolate Cf96 or *P. papaveracea* isolate B96. Plants were inoculated with a spore suspension of 4×10^5 spores per ml and placed in a mist chamber for 24 h. A fine mist was sprayed into the mist chamber for 1 min of every hour to maintain 100% humidity, and runoff of inoculum was not observed. The plants were returned to a growth chamber and evaluated daily for disease symptoms. The experiment consisted of four replicate pots randomized in the mist and growth chambers in complete blocks by isolate and cultivar, and the experiment was conducted twice. Control pots were inoculated with sterile distilled water containing 0.001% Tween 20. The data was analyzed as a two-factor linear model using Proc Mixed (release 6.11; SAS Institute Inc., Cary, NC). Mean comparisons were done using pairwise contrasts (Table 2).

A similar experiment was conducted to determine the susceptibility of seedlings to poppy blight. Eighteen-day-old seedlings of *Papaver somniferum* cvs. WC and IG were inoculated with *P. papaveracea* isolate B96 and *D. penicillatum* isolate Cf96 at conidial concentrations of 4×10^5 spores per ml. Plants were given a 24-h wetness period, and disease was evaluated daily. The experiment consisted of four replicate pots of five seedlings per pot for each treatment and was conducted twice.

Virulence of *P. papaveracea* and *D. penicillatum*. The virulence of conidial inoculum from three isolates of *P. papaveracea* from different sources (Pf96, B96, and 19C) (Table 1) and conidia from *D. penicillatum* (Cf96) was determined on *Papaver somniferum* cvs. IG and WC. Inoculum concentration of the isolates was 3×10^5 spores per ml, and plants were given a 24-h wetness period. Disease was evaluated 5, 8, and 10 days after inoculation. The experiment included four replications of each treatment, and the experiment was conducted twice. The severity data was analyzed using Proc Mixed (release 6.11; SAS Institute Inc.). Mean

comparisons for variables were done using pairwise contrasts and are given in Tables 3 and 4.

Effect of conidial inoculum concentration and wetness period on severity of foliage blight. The effects of inoculum concentration and wetness period on disease severity were each evaluated on *Papaver somniferum* cultivars inoculated with *D. penicillatum* isolate Cf96 and *P. papaveracea* isolate B96. In inoculum concentration experiments, conidial inoculum was prepared and applied to 8-week-old plants at conidia spore densities of 1.5×10^5 , 7.5×10^5 , 3.75×10^6 , and 1.875×10^7 conidia per ml. Plants were placed in a mist chamber for 24 h, returned to the growth chamber, and evaluated for foliage blight symptoms 3, 5, 7, and 9 days after inoculation. The influence of wetness period on disease severity was investigated by inoculating *Papaver somniferum* cultivars with 3×10^5 conidia per ml and placing plants in a mist chamber for 0, 6, 12, 24, or 48 h. Pots were returned to the growth chamber, and foliage blight assessments were made at 6, 8, 10, 12, and 14 days after inoculation. The experiments were designed as complete randomized blocks with four replications, and each experiment was conducted twice.

Analysis of variance was performed using the MIXED procedure in DAD (release 6.11; SAS Institute Inc.). Graphical examination of the residuals indicated heterogeneous variance, so the residual variance was partitioned into groups of similar variance. This procedure was used as an alternative to transforming the data (Table 5).

Disease development with conidial inoculum formulated with oil. The effect of oil inoculum formulation on severity of foliage blight in plants exposed to a short wetness period was determined using conidial inoculum formulated as a water or as a water and oil suspension. The length of dew period necessary for effective disease development in the field may be reduced by oil inoculum formulations (3). Ten-week-old WC and IG plants were inoculated with *P. papaveracea* isolate B96 and *D. penicillatum* isolate Cf96 conidia suspended in either sterile distilled water with Tween 20 or in sterile distilled water with 0, 5, 10, and 30% unrefined corn oil (Spectrum Naturals, Inc., Petaluma, CA), at a concentration of 6×10^5 conidia per ml. Plants were placed in the mist chamber for 6 h and returned to a growth chamber. Disease assessments were conducted as described above at 3, 5, 7, and 10 days after inoculation. The experiment included four replicate pots for each treatment, and the experiment was conducted twice. The severity data was analyzed using Proc Mixed (release 6.11; SAS Institute Inc.). Mean comparisons for variables were done using pairwise contrasts.

RESULTS

In addition to many saprophytic genera and species of fungi, two pathogenic fungi were consistently isolated from diseased *Papaver somniferum* and *Papaver bracteatum* tissues and cultured on PDA and V8 juice agar (Table 1). Isolates of *D. penicillatum* and *P. papaveracea*, which have been shown to be different species, were obtained from infested seed, foliage, harvested infested pods, seedlings, and field stubble. Both fungi were found on symptomatic plants in the field in Maryland. Both species were isolated from seed from Venezuela, but only *P. papaveracea* was found on seed

TABLE 2. Foliage blight severity in 8-week-old *Papaver somniferum* cvs. Indian Grocery, White Cloud, and an opium poppy accession from Venezuela (VEN) 7 days after inoculation with a conidial suspension of *Dendryphion penicillatum* isolate Cf96 and *Pleospora penicillatum* isolate B96

Cultivar	Disease severity ^x	
	<i>D. penicillatum</i>	<i>P. papaveracea</i>
Indian Grocery	2.75 b ^{y,z}	9.00 a
VEN	4.25 b [*]	8.75 a
White Cloud	7.50 a	9.00 a

^x Plants were rated for disease severity based on a visual estimate of percent foliage blight on a 0 to 9 scale, in which 0 = 0 to 3%, 1 = 3 to 6%, 2 = 6 to 12%, 3 = 12 to 25%, 4 = 25 to 50%, 5 = 50 to 75%, 6 = 75 to 87%, 7 = 87 to 93%, 8 = 93 to 96%, and 9 = 96 to 100% foliage necrosis.

^y Cultivars within a fungus with different letters are different at the $P < 0.01$ significance level.

^z Asterisk indicates fungi within a cultivar are different at the $P < 0.001$ significance level.

TABLE 3. Mean comparisons of the virulence of *Pleospora papaveracea* isolates 19C, B96, and Pf96, and *Dendryphion penicillatum* isolate Cf96 to *Papaver somniferum* cvs. Indian Grocery and White Cloud

Cultivar	Isolate			
	19C	B96	Cf96	Pf96
Indian Grocery	7.7 a ^y	7.2 a ^{*,z}	2.38 b [*]	7.7 a
White Cloud	7.9 a	8.6 a	5.1 b	8.2 a

^y Fungi within a cultivar with different letters are different at the $P < 0.05$ significance level.

^z Asterisk indicates that cultivars within a fungus are different at the $P < 0.05$ significance level.

from Colombia. Isolates of *D. penicillatum* but not *P. papaveracea* were obtained from *Papaver bracteatum*. Fungal structures of both species (conidia, pseudothecia, ascospores, microsclerotia, chlamydospores, and Dendryphion-type macronematous conidiophores with conidia) were observed and isolated from stem stubble pieces left from field plots that had been inoculated with either fungus the previous fall. Both species were often found on the same stubble pieces.

Single conidia isolated from Dendryphion-type conidiophores from infested seed, *Papaver somniferum* field stubble, or isolates in culture consistently produced *D. penicillatum* cultures. On V8 juice agar, *D. penicillatum* produced abundant conidia and numerous microsclerotia submerged in the medium. *P. papaveracea* produced abundant conidia and terminal and intercalary chlamydospores, but did not produce microsclerotia. *P. papaveracea* produced pseudothecia in cultures older than 30 days, but these rarely matured to produce ascospores. Pseudothecia with mature asci and ascospores were frequently produced embedded in filter paper from infested seed in moist chambers. Numerous microsclerotia of *D. penicillatum* were often produced on filter paper surrounding each infested seed.

The two species differed in morphological characteristics and were both pathogenic to opium poppy, although they differed in virulence. Described from diseased, inoculated plants, the fungi produced similar appearing, semimacronematous to micronematous conidiophores and conidia on inoculated leaves of *Papaver somnif-*

erum. However, other morphological characters distinguished the fungi from each other. Conidiophores produced by *D. penicillatum* were 40 to 95 µm long, 4 to 5 µm thick, and yellow brown, with one to three branches along the conidiophore. Conidia were solitary or catenulate, 23.3 µm (standard deviation [SD] = 4.3) long, and 5.3 µm (SD = 0.64) wide, cylindrical, rounded at the ends or obclavate, pale to olive to light brown, and minutely granulate, with 2.95 (SD = 0.54) septa. Dendryphion-type conidiophores were occasionally produced in culture on V8 juice and PDA agar, as well as on seed surfaces and dead overwintered poppy stem debris from the field. These conidiophores were long, straight, septate, dark brown, tapering, and branched toward the apex to produce conidia at the tip (7). They arose singly or in fascicles of two to four from microsclerotia produced on substrate surfaces.

Conidiophores produced by the anamorph of *P. papaveracea* were 45 to 100 µm long, 4 to 5 µm thick, and pale yellow brown, with one to two branches along the conidiophore. Conidia were solitary or catenulate, 45.6 µm (SD = 12.6) long, 7.2 µm (SD = 0.68) wide, cylindrical, rounded at the ends or obclavate, pale olive to light brown, and minutely granulate, with 5.08 (SD = 1.85) septa and transverse walls becoming darker than the outer wall. Ascospores were 200 to 400 µm, black, globose, and smooth. Ascospores were brown and 18 to 23 × 7 to 9 µm, with three septa and the middle cells containing a single vertical septum. *P. papaveracea* produced chlamydospores infrequently in culture, and microsclerotia or Dendryphion-type conidiophores were not observed. Conidial dimensions of both fungi grown in culture media were considerably smaller and more variable than conidia from diseased material (data not shown).

Single conidia from isolates of either species and single ascospores from pseudothecia of *P. papaveracea* (Table 1) consistently produced cultures exhibiting characteristics of the parent isolate. All single-ascospore cultures and conidia from the *P. papaveracea* anamorph exhibited cultural characteristics of this species. Conidia from *D. penicillatum* and from Dendryphion-type conidiophores produced cultures characteristic of *D. penicillatum*. Thus, no anamorph/teleomorph connection was established. *P. papaveracea* was homothallic, producing pseudothecia in all single-ascospore cultures.

Pathogenicity of *D. penicillatum* and *P. papaveracea* to *Papaver somniferum* cultivars. Both *D. penicillatum* (Cf96) and *P. papaveracea* (B96) were pathogenic and highly virulent to *Papaver somniferum* cvs. IG, WC, and an accession from Venezuela (VEN) used for the production of opium (Table 2). Symptoms began as

TABLE 4. Mean comparisons of the virulence of *Pleospora papaveracea* isolates 19C, B96, and Pf96, and *Dendryphion penicillatum* isolate Cf96 under different incubation periods

Incubation period (days)	Isolate			
	19C	B96	Cf96	Pf96
5	6.1 b ^y a ^z	6.6 ba	3.3 bb	6.8 ba
8	8.5 aa	8.3 aa	4.0 ab	8.3 aa
10	9.0 aa	8.7 aa	3.8 abb	8.7 aa

^y Days within an isolate with different letters are different at the $P < 0.05$ significance level.

^z Fungi within an incubation period with different italicized letters are different at the $P < 0.05$ significance level. Data are presented graphically in Figure 1.

TABLE 5. Analysis of variance of the influence of inoculum concentration and wetness period on severity of foliar blighting by *Pleospora papaveracea* isolate Pf96 and *Dendryphion penicillatum* isolate Cf96 to opium poppy (*Papaver somniferum*) cvs. White Cloud and Indian Grocery^z

Source	Degrees of freedom	Type III F	P
Inoculum concentration, experiment 1			
Cultivar	1	171.4	0.0001
Isolate	1	403.62	0.0001
Cultivar × isolate	1	0.45	0.5014
Inoculum concentration	3	2,319.48	0.0001
Cultivar × inoculum concentration	3	9.97	0.0001
Isolate × inoculum concentration	3	42.58	0.0001
Cultivar × isolate × inoculum concentration	3	29.99	0.0001
Incubation period	3	319.63	0.0001
Cultivar × incubation period	3	8.75	0.0001
Isolate × incubation period	3	18.76	0.0001
Cultivar × isolate × incubation period	3	0.38	0.7651
Wetness period, experiment 1			
Cultivar	1	301.97	0.0001
Isolate	1	615.20	0.0001
Cultivar × isolate	1	2.67	0.1034
Wetness period	4	3,925.64	0.0001
Cultivar × wetness period	4	53.84	0.0001
Isolate × wetness period	4	140.81	0.0001
Cultivar × isolate × wetness period	4	11.39	0.0001
Incubation period	4	415.82	0.0001
Wetness period × incubation period	16	62.61	0.0001

^z Data are presented graphically in Figures 2 and 3.

Fig. 1. Virulence of *Pleospora papaveracea* isolates Pf96, B96, 19C, and *Dendryphion penicillatum* isolate Cf96 to *Papaver somniferum* cvs. Indian Grocery (IG) and White Cloud (WC). Plants were rated for disease severity based on a visual estimate of percent foliage blight on a 0 to 9 scale, in which 0 = 0 to 3%, 1 = 3 to 6%, 2 = 6 to 12%, 3 = 12 to 25%, 4 = 25 to 50%, 5 = 50 to 75%, 6 = 75 to 87%, 7 = 87 to 93%, 8 = 93 to 96%, and 9 = 96 to 100% foliage necrosis.

chlorotic spots and leaf margin chlorosis, progressed to water-soaked areas on stems and foliage, and finally to necrosis of all tissues. Necrotic areas became covered with a dense layer of conidiophores and conidia beginning 48 h after inoculation. No symptoms occurred in control plants. Ratings taken 7 days after inoculation revealed that *P. papaveracea* was more virulent to IG and VEN than was *D. penicillatum* and that there were significant differences in susceptibility among cultivars to *D. penicillatum*. Cultures derived from conidia reisolated from diseased tissues consistently produced cultures of the respective fungus from which the inoculum was derived.

Seedlings were highly susceptible to both pathogens. Eighteen-day-old WC and IG seedlings exhibited necrotic spots 48 h after inoculation. By 5 days, *P. papaveracea*-inoculated seedlings were killed, and seedlings inoculated with *D. penicillatum* were 87 to 93% necrotic. There was no significant difference in susceptibility between cultivars (data not shown).

Virulence of *P. papaveracea* and *D. penicillatum*. Isolates of *P. papaveracea* from different sources (Pf96, B96, and 19C) were equally virulent, causing over 90% foliage blight in WC and IG 8 days after inoculation (Fig. 1; Tables 3 and 4). *P. papaveracea* isolates were more virulent to *Papaver somniferum* cultivars than were *D. penicillatum* isolates throughout the 10-day incubation period. IG was less susceptible to *D. penicillatum* than was WC.

Effect of conidial inoculum concentration and wetness period on severity of foliage blight. Varying inoculum concentrations and wetness periods resulted in significant differences in foliage blight disease severity in *Papaver somniferum* cvs. IG and WC inoculated with *D. penicillatum* isolate Cf96 and *P. penicillatum* isolate B96 (Figs. 2 and 3; Table 5). Low inoculum concentrations and the use of cultivars differing in susceptibility revealed that *P. papaveracea* is more virulent to opium poppy than is *D. penicillatum* and that WC is more susceptible than IG. However, all plants were dead 9 days after inoculation with inoculum con-

Fig. 2. Effect of conidial inoculum concentration and incubation period on severity of foliage blight in *Papaver somniferum* cvs. Indian Grocery and White Cloud inoculated with *Dendryphion penicillatum* isolate Cf96 and *Pleospora penicillatum* isolate B96. **A**, White Cloud inoculated with Cf96; **B**, White Cloud inoculated with B96; **C**, Indian Grocery inoculated with Cf96; and **D**, Indian Grocery inoculated with B96. Plants were rated for disease severity based on a visual estimate of percent foliage blight on a 0 to 9 scale, in which 0 = 0 to 3%, 1 = 3 to 6%, 2 = 6 to 12%, 3 = 12 to 25%, 4 = 25 to 50%, 5 = 50 to 75%, 6 = 75 to 87%, 7 = 87 to 93%, 8 = 93 to 96%, and 9 = 96 to 100% foliage necrosis.

centrations of 1.8×10^6 spores per ml of either pathogen (Fig. 2) or with longer wetness periods (Fig. 3). A wetness period of at least 6 h was necessary to achieve 25 to 50% foliage necrosis (disease rating of 4) 12 days after inoculation with B96. Similar results were obtained when the experiments were repeated.

Disease development with conidial inoculum formulated with oil. Oil inoculum formulation enhanced disease severity in *Papaver somniferum* caused by *P. papaveracea* and *D. penicillatum* (Fig. 4; Table 6). Plants of WC and IG inoculated with conidia of either pathogen in 30% oil and given a 6-h wetness period exhibited 25 to 50% foliage necrosis 3 days after inoculation. Under these conditions, *D. penicillatum* was almost as virulent as *P. papaveracea*, and WC and IG were equally susceptible. Disease severity using oil formulations of 5 and 10% were proportionally less effective than 30% oil (data not shown). Oil formulation facilitated an even distribution of inoculum over leaf surfaces, resulting in more uniform necrosis.

DISCUSSION

We report that two different fungi cause poppy blight and that they are distinguishable by several morphological criteria. *D. penicillatum* produced conidia, abundant microsclerotia, and less frequently, Dendryphion-type macronematous conidiophores on infested seed in infected foliage, culture, and *Papaver somniferum* field stubble. *P. papaveracea* consistently produced pseudothecia, chlamydospores, and conidia that were larger than conidia of *D. penicillatum*. Except for the absence of Dendryphion-type conidiophores, the conidia and conidiophores of the anamorph of *P. papaveracea* resembled those of *D. penicillatum*. However, we found that the conidia of *D. penicillatum* were comparatively smaller, especially when taken from diseased tissues. Their similar appearance, along with the fact that both fungi are pathogenic to poppy and appear to occupy the same ecological niche, may have led to reports that the poppy fungus was a single species with races or variable spore

Fig. 3. Effect of wetness and incubation periods on severity of foliage blight in *Papaver somniferum* cvs. Indian Grocery and White Cloud inoculated with *Dendryphion penicillatum* isolate Cf96 and *Pleospora penicillatum* isolate B96. **A**, White Cloud inoculated with Cf96; **B**, White Cloud inoculated with B96; **C**, Indian Grocery inoculated with Cf96; and **D**, Indian Grocery inoculated with B96. Plants were rated for disease severity based on a visual estimate of percent foliage blight on a 0 to 9 scale, in which 0 = 0 to 3%, 1 = 3 to 6%, 2 = 6 to 12%, 3 = 12 to 25%, 4 = 25 to 50%, 5 = 50 to 75%, 6 = 75 to 87%, 7 = 87 to 93%, 8 = 93 to 96%, and 9 = 96 to 100% foliage necrosis.

sizes. We also found, as did Christoff (4) and Milatović (16), that measurements of conidia taken from culture differed from those taken from diseased tissues.

Completion of Koch's postulates (Fig. 1; Table 2) revealed that *D. penicillatum* and *P. papaveracea* are both pathogenic to opium poppy. However, *P. papaveracea* may have more potential for use as a mycoherbicide, because it is more virulent and produces ascospores in addition to conidia. Field tests are needed to determine the effectiveness of ascospores in disease development in the spring. It also is possible that microsclerotia produced by *D. penicillatum* will give this pathogen an advantage if they are shown to function as overwintering propagules. Both fungi produce abundant mycelia in liquid and solid media, but sporulation is more limited in liquid media (data not shown). Investigations on optimizing formulations are needed to determine the economic feasibility of producing large quantities of inoculum.

The influence of different factors on disease development in controlled conditions was investigated, including incubation period, wetness period, inoculum concentration, and seedling and mature plant susceptibility. Oil inoculum formulations resulted in enhanced disease under a reduced (6-h) wetness period. Results from this experiment suggest that less-susceptible cultivars may become severely diseased, even when using the less-virulent species (*D. penicillatum*) when inoculum is oil-formulated to enhance disease.

Both pathogens appear to have a good potential for development as mycoherbicides. Seedlings as well as mature plants are susceptible and, depending on environmental conditions, rapidly become completely blighted. Dense layers of new spores devel-

oped on necrotic plant surfaces, an important aspect for continued spread development of an epidemic in the field. The occurrence of both fungi on field stubble in 1998 and 1999 suggests that the fungi can successfully overwinter, at least under the climatic conditions that occurred in Maryland.

Field studies are needed to evaluate the respective potentials of each pathogen as candidates for biological control. We currently are investigating pathogen formulations for field application, host range, yield and alkaloid level losses due to disease, molecular and genetic analyses of the fungi, and disease susceptibility in *Papaver somniferum* collections.

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Fig. 4. Influence of oil conidial inoculum formulation with *Pleospora papaveracea* isolate B96 and *Dendryphon penicillatum* isolate Cf96 on disease severity in *Papaver somniferum* cvs. White Cloud and Indian Grocery. Plants were rated for disease severity based on a visual estimate of percent foliage blight on a 0 to 9 scale, in which 0 = 0 to 3%, 1 = 3 to 6%, 2 = 6 to 12%, 3 = 12 to 25%, 4 = 25 to 50%, 5 = 50 to 75%, 6 = 75 to 87%, 7 = 87 to 93%, 8 = 93 to 96%, and 9 = 96 to 100% foliage necrosis.

TABLE 6. Mean comparisons of the effect of oil formulation on the severity of disease caused by *Pleospora papaveracea* isolate B96 and *Dendryphon penicillatum* isolate Cf96

Incubation period (days)	Oil ^y		Water	
	B96	CF96	B96	CF96
3	4.3 c ^z	4.2 c	0.2 c	0.0 c
5	6.2 b*	4.5 c	0.3 c	0.3 bc
7	8.2 a*	5.7 b	1.3 b	0.8 b
10	9.0 a*	7.5 a	3.2 a*	1.7 a

^y Oil and water treatment means are different at the $P < 0.001$ significance level.

^z Incubation period within a treatment and isolate with different letters are different at the $P < 0.01$ significance level. Asterisk indicates that isolates differ within an incubation period and formulation at the $P < 0.05$ significance level. Data are presented graphically in Figure 4.

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