Virulence of *Phoma lingam* to Cabbage

J. M. BONMAN, Graduate Research Assistant, R. L. GABRIELSON, Plant Pathologist, Western Washington Research and Extension Center, Puyallup 98371; P. H. WILLIAMS, Professor, and P. A. DELWICHE, Graduate Assistant, Department of Plant Pathology, University of Wisconsin, Madison 53706

ABSTRACT

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Isolates of *Phoma lingam* obtained from cabbage tissues were differentiated by symptoms on wound-inoculated cabbage cotyledons and by pigment production in vitro. Isolates that produced pigment caused only cotyledonary tissue darkening and limited tissue collapse, did not spread significantly in seedbeds, and produced only superficial lesions on transplanted cabbage. Isolates that did not produce pigment caused cotyledonary tissue collapse and were able to spread in cabbage seedbeds and damage transplanted cabbage grown to maturity. A range of virulence was observed within the latter isolates.

Additional key words: *Leptosphaeria maculans*

In blackleg epidemics caused by *Phoma lingam* (Tode ex Fr.) Desm. on cabbage (*Brassica oleracea* L. var. *capitata* L.), infected seed is the usual source of primary inoculum (9). Seed is densely sown in beds where the pathogen is readily disseminated by splashing water. Infected seedlings are transplanted, and later in the season they become severely diseased and die.

In Washington, Pound (6) described a Puget Sound strain of *P. lingam* that differed from eastern U.S. isolates on the basis of symptom development, virulence in greenhouse studies, and production of a yellow brown pigment in culture (6,7). Pound et al (7) concluded that only the eastern strain of *P. lingam* was important in causing blackleg epidemics, but this was not proven in field studies in eastern cabbage-producing areas. This study examined the ability of selected *P. lingam* isolates to spread in cabbage seedbeds and to cause disease in cabbage transplants grown to maturity in the field in Wisconsin. The correlation between field virulence and pigment production in vitro and virulence level on cabbage seedlings was also examined.

Present address of senior author: The Rockefeller Foundation, G.P.O. Box 2453, Bangkok, Thailand.

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MATERIALS AND METHODS

Market Prize cabbage seed, obtained from the Alf. Christianson Seed Co. of Mount Vernon, WA, was used in all experiments. A direct test (4) of 10,000 seeds uncovered no *P. lingam* in this lot.

Conidial suspensions of *P. lingam* were derived from pure cultures and naturally infested cabbage residues (Table 1). *P. lingam* cultures maintained on potato-dextrose agar were flooded with sterile distilled water, and the resulting conidial suspension was plated on V-8 juice agar in petri plates. Conidia were harvested after 7–10 days at room temperature (about 21 C) under constant fluorescent light. Infested cabbage seed crop residues, obtained from western Washington in the fall of 1978, were placed in a moist chamber at 20 C for several days to induce sporulation. Conidia oozing from pycnidia on the residue were removed by washing and used as inocula. Spore concentrations were adjusted to 10^7 spores per milliliter with distilled water. Cabbage tissues were inoculated by wounding with a sterile needle and adding a 10-μl drop of spore suspension. Sterile water inoculations were included as controls.

Laboratory tests. To test the virulence of *P. lingam* isolates, we sowed cabbage seed in Jiffy Mix and allowed it to grow at room temperature on a laboratory growth bench with a 12-hr photoperiod. Cotyledons of plants were inoculated 7–10 days after planting, and symptoms and signs were evaluated about 10 days later (Fig. 1). Pigment production by the various isolates was observed after 30 days of growth at 21 C in Czapek-Dox broth plus yeast extract at 2 g/L (5).

Field tests. To measure the ability of *P. lingam* isolates to spread in seedbeds, we sowed 100 seeds per row in 1-m^2 plots consisting of five 1-m rows spaced 20 cm apart. Plots were placed at least 8 m apart, with oats sown between to prevent fungus movement between plots by wind-driven rain. Trials were located at the University of Wisconsin Agricultural Experiment Station in Arlington.

When the first true leaves began to appear, the cotyledons and hypocotyls of 10 plants per plot were inoculated as previously described. In the first experiment, planted 10 May 1979, a randomized complete block design was used with five replicates per treatment. Ten plants in the southwest corner of each plot were inoculated. In a second experiment, planted 14 June 1979, a completely randomized design was used with four replicates per treatment. Ten plants were inoculated at random locations in each plot. We measured disease spread by recording the number of infected plants in each plot when the plants had attained commercial transplant size, which was 39 days after inoculation in the first experiment and 51 days in the second experiment.

To measure the ability of *P. lingam* isolates to cause disease in transplanted

Table 1. Sources of *Phoma lingam*

WI1	Isolate from hybrid cabbage seed, 1973. Wisconsin isolate PHW 100.
WI2	Isolate from hybrid cabbage seed, 1975. Wisconsin isolate PHW 171.
WI3	Isolate from storage cabbage grown in Racine County, WI, in 1972, isolated in 1973. Wisconsin isolate PHW 104.
OR1	Isolate from cabbage seed plant stem, Oregon, 1978.
WA1	Isolate from cabbage seed plant leaf spot, Washington, 1978.
WA2	Isolate from single ascospore from cabbage seed crop residue, Washington, 1978.
WA3	Combined infested residues from four cabbage fields, Washington, 1978.

cabbage, we grew seedlings to transplant size in multipot trays in the greenhouse and wound inoculated them at the cotyledonary node 2 days before transplanting. One group of 720 plants was transplanted to a field at the University of Wisconsin Agricultural Experiment Station, Hancock, on 23 May 1979, and a second group with 1,020 plants on 12 June 1979. A randomized complete block design with four replications was used in each experiment. After transplanting, inoculation sites were below the soil line. Experimental plots were 5 × 5 m, spaced at 5-m intervals with oats sown between them.

In mid-August, the plants were harvested whole and weighed, and the lower stem and roots were rated for disease severity. A five-point disease rating scale was used, with 0 = no symptoms; 1 = slight, superficial blackening around inoculation site; 2 = moderate, superficial blackening around inoculation site; 3 = moderate blackening, with some blackening and necrosis extending inward; 4 = severe blackening and necrosis extending well inward, roots intact; 5 = severe blackening and necrosis throughout the lower stem, roots severely damaged.

A two-way analysis of variance indicated a significant interaction between planting date and treatment on the basis of disease ratings; consequently, a one-way analysis of variance was done for each planting date. Two-way analysis of variance indicated no significant effect of planting date and no significant interaction between planting date and treatment on the basis of plant weight; weight data for both plantings were thus pooled.

Table 2. Virulence of *Phoma lingam* isolates from different sources on cabbage cotyledons and pigment production in vitro[x]

Inoculum	Virulence[y]	Pigment production
WI1	HV	−
WI2	HV	−
WI3	MV	−
OR1	WV	+
WA1	WV	+
WA2	WV	+
WA3	WV	+[z]

[x] Cotyledons of 10-day-old seedlings were punctured and inoculated with 10 μl of a suspension containing 10^7 spores per milliliter. Symptoms were recorded 10 days after inoculation. Cultures were observed for pigment production after 30 days of growth in Czapek-Dox broth plus yeast extract at 2 g/L, after McGee and Petrie (5).

[y] HV = highly virulent, causing expanding tissue collapse and dense sporulation after moist incubation; MV = moderately virulent, causing tissue collapse with a sharp border between collapsed and healthy tissue and dense sporulation after moist incubation; WV = weakly virulent, causing darkened necrosis but very limited tissue collapse and sparse or no sporulation after moist incubation.

[z] Pigment production by pure culture isolates from WA3 residues.

Table 3. Blackleg disease spread in cabbage seedbeds following inoculation with different *Phoma lingam* isolates

Inoculum	Diseased plants (%)[w]	
	1st experiment[x]	2nd experiment[y]
WI1	...[z]	51.4
WI2	20.8	2.5
WI3	<0.5	0
OR1	0	<0.5
WA1	0	0
WA3	0	0
Check	0	0

[w] Ten plants inoculated at first true leaf stage. Data taken after plants attained commercial transplant stage.

[x] Data averaged from five replicates per treatment.

[y] Data averaged from four replicates per treatment.

[z] Not tested.

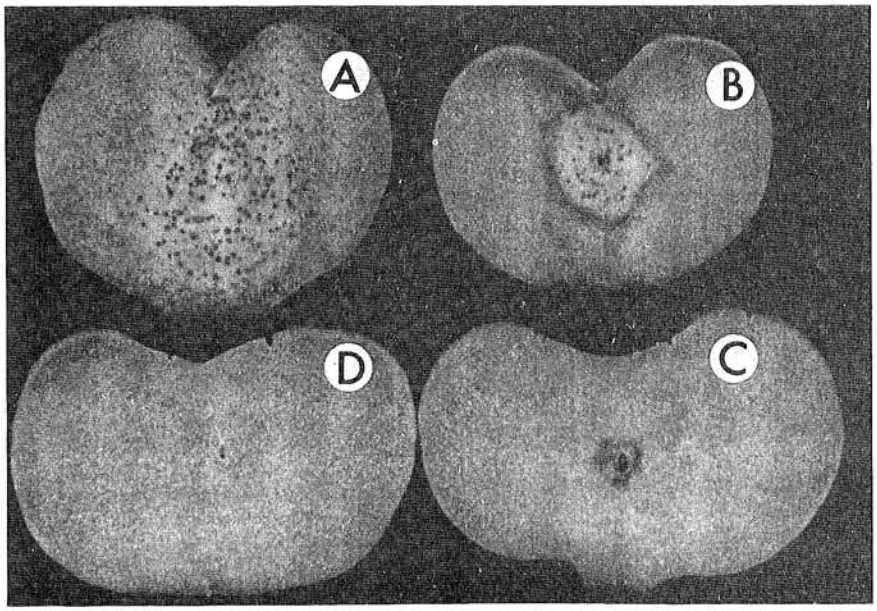

Fig. 1. Cabbage cotyledons infected by *Phoma lingam* isolates of varying virulence: **(A)** highly virulent, **(B)** moderately virulent, **(C)** weakly virulent, and **(D)** check.

RESULTS

Laboratory tests. In cotyledon inoculation tests, isolates WI1 and WI2 were highly virulent; isolate WI3 was intermediate; and isolates OR1, WA1, WA2, and the inoculum from residue WA3 were weakly virulent (Table 2). Isolates WI1, WI2, and WI3 did not produce pigment in culture, but the weakly virulent isolates did (Table 2). Although all three WI isolates caused tissue collapse, WI3 was classified as moderately virulent because it caused lesions with well-defined edges that were smaller than those caused by the highly virulent WI1 and WI2 (Fig. 1).

Field tests. In seedbed experiments, symptoms of cotyledon infection were evident after 15 days on all inoculated plants. Cotyledons inoculated with WI1 and WI2 had completely collapsed and were covered with pycnidia. Cotyledons inoculated with WI3 had sporulating lesions with well-defined edges. Cotyledons inoculated with WA1, WA3, and OR1 showed darkened necrosis but little sporulation. On seedlings at transplant size, only WI1 and WI2 had spread significantly within the seedbeds (Table 3), and only these isolates produced typical blackleg leaf spots densely studded with pycnidia. Isolates WI3 and OR1 spread very little (Table 3), and caused only a few small leaf spots with sparse sporulation. None of the other isolates spread from the inoculated plants.

In the transplant experiments, isolate WI1 caused the most severe blackleg, and it was the only isolate to reduce significantly the average plant weight (Table 4). In both plantings, isolate WI3 caused moderate symptoms and isolates OR1, WA1, and WA2 produced only superficial lesions. The maximum disease caused by each isolate is shown in Figure 2. Isolates OR1 and WA1 produced disease ratings significantly higher in the second planting than in the first, while the other isolates showed similar ratings for both plantings.

DISCUSSION

We use the term "virulence" to describe the relative disease-causing capabilities of various *P. lingam* isolates. Our highly and moderately virulent isolates correspond to Pound's eastern strain, and our weakly virulent isolates correspond to his Puget Sound strain (6,7). The virulence and disease-spreading capabilities of our eastern isolates WI1 and WI2 in field trials substantiates Pound's conclusion that only the eastern strain is important in blackleg epidemics (7). Moderately virulent isolates such as WI3 might be important in the field when

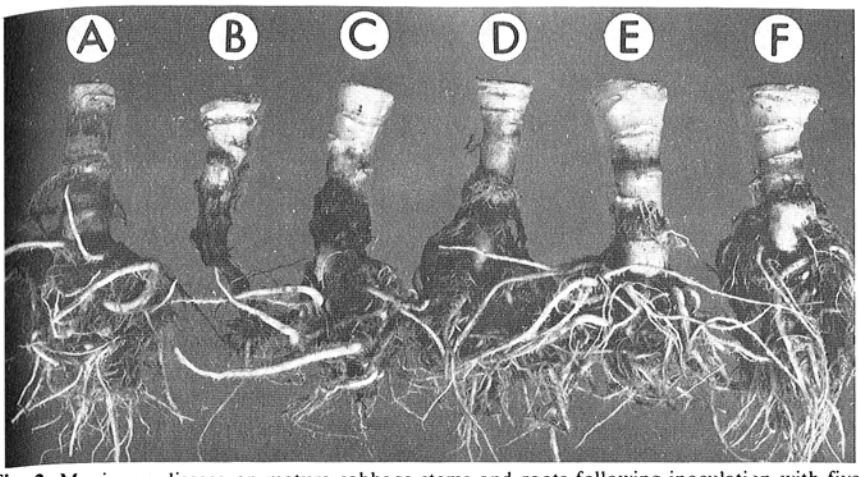

Fig. 2. Maximum disease on mature cabbage stems and roots following inoculation with five *Phoma lingam* isolates: **(A)** check, **(B)** WI1, **(C)** WI3, **(D)** WA1, **(E)** WA2, and **(F)** OR1.

timely rainfall enhances their spread to very young plants.

When seed is inoculated with weakly virulent isolates such as OR1, WA1, and WA2, damping off can occur (6). Extensive, weakly virulent, seedborne inoculum could result in stand thinning in seedbeds. Unusually high amounts of rainfall might also induce spread of weakly virulent *P. lingam* within seedbeds. Because these isolates caused only superficial lesions on transplants grown to maturity, it is unlikely that they have been important in cabbage blackleg epidemics. Two weakly virulent isolates produced statistically higher disease ratings in the June transplanting than in the May transplanting (Table 4). However, because disease ratings of 1 and 2 represent only slight damage, we believe that this increase has no practical significance.

McGee and Petrie (5) have described virulent and avirulent strains of *P. lingam* on oilseed rape in Canada that correspond to the eastern and Puget Sound strains, respectively. Our field data support their observation that avirulent isolates produce pigment in vitro, whereas virulent isolates do not. Our data further suggest that there is a range in field virulence within isolates corresponding to the eastern or virulent strain.

The present data and that of others (5–7) support the existence of two strains of *P. lingam* on cabbage, but the relationship between the strains is unclear. Based on unpublished research, R. H. Morrison (*personal communication*) believes that moderately virulent types are a distinct strain. Highly and weakly virulent *P. lingam* can occur in the same cabbage seed lot (7), and both form the perfect state, *Leptosphaeria maculans* (Desm.) Ces. & de Not. The perfect state has been found in the United States in the Pacific Northwest (2) and Wisconsin (1). Single ascospore isolates from Washington and Oregon were weakly virulent (Bonman, *unpublished*), but those from Wisconsin were highly virulent (1).

In unpublished work, we have obtained the perfect state of highly virulent isolates in culture, using methods similar to those of LaCoste (3) and Venn (8). The same methods, however, failed to produce the perfect state from weakly virulent isolates or from interstrain crosses. Because *L. maculans* is heterothallic (8), the perfect state offers great potential for clarifying the relationship between these two strains.

Progress here, however, must await the ability to produce the perfect state with weakly virulent strains under controlled conditions. Weakly virulent *P. lingam* is apparently endemic in the Pacific Northwest, but we do not know whether highly virulent *P. lingam* can also persist in this crucifer seed production region.

Table 4. Disease severity and weight of field-grown cabbages after wound inoculation of transplants at the cotyledonary node with different isolates of *Phoma lingam*

Isolate	Disease rating[w] 23 May transplants	Disease rating[w] 12 June transplants	Mean fresh weight as % of check[x]
WI1	4.16 a[y]	4.18 a	85.7 b
WI3	2.58 b	2.80 b	103.5 a
OR1	1.40 c	2.01 c[z]	109.6 a
WA1	1.04 d	1.61 d[z]	98.9 a
WA2	1.09 dc	1.11 e	104.1 a
Check	0.15 e	0.10 f	100.0 a

[w] Disease rating: 0 = no symptoms; 1 = slight, superficial blackening around inoculation site; 2 = moderate, superficial blackening around inoculation site; 3 = moderate blackening, with some blackening and necrosis extending inward; 4 = severe blackening and necrosis extending well inward, roots intact; 5 = severe blackening and necrosis throughout the lower stem, roots severely damaged.

[x] Weight data for both plantings were pooled.

[y] Means in the same columns followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] Significantly different than the value for the first planting ($P = 0.05$).

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