Variability of Leptosphaeria maculans in Relation to Blackleg of Oilseed Rape

D. C. McGee and G. A. Petrie

Plant Pathologists: Dept. of Agriculture, Victorian Plant Research Institute, Victoria, Australia; and Research Station, Research Branch, Agriculture Canada, 107 Science Cres., Saskatoon, Saskatchewan, Canada, S7N 0X2. Present address of the senior author: Dept. of Botany and Plant Pathology, University of Maine, Orono, ME 04473.

We wish to thank Marjorie Richardson for technical assistance.

This work was carried out during the tenure of a National Research Council of Canada Postdoctorate Fellowship held by D. C. McGee.

Accepted for publication 7 October 1977.

ABSTRACT

MC GEE, D. C., and G. A. PETRIE. 1978. Variability of Leptosphaeria maculans in relation to blackleg of oilseed rape. Phytopathology 68: 625-630.

Virulent and avirulent strains of *Leptosphaeria maculans* isolated from rapeseed plants in Saskatchewan were differentiated on the basis of cultural characteristics and virulence to rapeseed ("rapeseed" here refers to rape plants grown for oil extraction from the seed). Surveys of field populations showed that the avirulent strain was more prevalent. An isolate from cabbage in Wisconsin was identical to the virulent strain. Several isolates from rapeseed in Australia all were virulent on rapeseed but showed variability in culture. There was a significant correlation between disease rankings on one set of *Brassica* sp. seed lines tested against the Wisconsin isolate and an Australian isolate. Similar results were obtained with a second set tested against the Wisconsin isolate and a field population of *L. maculans* in Australia. A strain of *L. maculans* also was detected on stinkweed (*Thlaspi arvense* L.) collected from fields in Saskatchewan. This strain could be distinguished from others by the differential reactions of rapeseed and stinkweed in pathogenicity tests. Blackleg was more severe in rapeseed field plots containing residues of rapeseed infected with blackleg than in those containing infected residues of stinkweed.

Additional key words: Phoma lingam, cruciferous crops, oilseeds.

Early work on blackleg of crucifers (1, 2) suggested that strains of the causal organism *Phoma lingam* (Tode ex Schw.) Desm., the asexual stage of *Leptosphaeria maculans* (Desm.) Ces. et de Not., could be separated on the basis of virulence, although the evidence was not conclusive. Later, Pound (10) considered that the organism which caused mild symptoms of blackleg on cabbage in the Puget Sound area of Washington was an avirulent strain of *Phoma lingam*, the cause of severe blackleg on cabbage (*Brassica oleraceae* L.) in eastern states of the U.S. This strain also was characterized by a brownish-yellow pigment produced in culture.

In recent years, blackleg has become a major disease of rapeseed (*Brassica napus* L., *B. campestris* L.) in France (3) and Australia (7). In this article "rapeseed" refers to rape plants grown for oil extraction from the seed. Blackleg also occurs on rapeseed in Canada, but is considered to be a minor problem (8). McGee (5) suggested that blackleg was more severe in Australia than Canada because the relationship between periods of availability of inoculum and crop susceptibility to infection was more favorable for disease development in Australia and the most prevalent strain of the pathogen in Australia was more virulent than that in Canada.

Leptosphaeria maculans also occurs on cruciferous weeds in Canada (9), but the epidemiological significance of infected weeds in relation to blackleg of rapeseed has not been determined.

In this paper the variability of *L. maculans* isolated from different geographical regions and cruciferous hosts is examined and its significance to blackleg of rapeseed is considered.

MATERIALS AND METHODS

Isolates.—Collection of plant residues was undertaken to obtain different isolates. In April 1975, residues of rapeseed and stinkweed plants grown in 1974 were obtained from fields in different parts of Saskatchewan. Concurrently, residues of rapeseed crops grown in 1974 were collected from fields in Victoria, Australia. Small samples of the latter were packaged in dry condition and airmailed to the laboratory at Saskatoon.

Leptosphaeria maculans was isolated from fresh plant material or plant residues by surface sterilizing diseased tissue in 1.0% sodium hypochlorite for 2 min and plating on V-8 juice agar containing 40 μg/ml of rose bengal and 100 μg/ml of streptomycin sulfate. Direct isolations from ascocarps of *L. maculans* also were made by attaching pieces of tissue with ascocarps to the underside of petri dish lids over water agar. Groups of ascospores discharged onto the agar then were transferred to V-8 juice agar. An isolate of *L. maculans* from cabbage (*Brassica oleraceae* L.) in Wisconsin was obtained from P. H. Williams.

Pathogenicity tests.—Isolates of *L. maculans* were tested for pathogenicity to rapeseed in the greenhouse or

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growth chamber. Safeguards to prevent escape were employed whenever imported cultures were used. Inoculum was prepared by incubating isolates of the fungus on autoclaved oat kernels for 3 wk at 25 C. The kernels then were air-dried and stored in paper envelopes. Rapeseed plants, grown on a soil-less mix (11), were inoculated by placing three kernels at the crown of individual plants at the one-leaf stage of growth. At maturity, when all pods were formed, crowns were rated for stem-canker severity on a 0 to 5 scale as described (6). Isolates with ratings ranging from 0 to 1.5 were considered avirulent and those ranging from 1.5 to 5.0 were considered virulent. In all tests each treatment consisted of four pots, each containing three plants.

Single Australian and Canadian isolates from rapeseed and the Wisconsin isolate were tested on seven *B. napus* and *B. campestris* seed lines known to vary in susceptibility to the Wisconsin isolate. Another group of 18 *Brassica* spp. seed lines were tested for susceptibility to the Wisconsin isolate in the greenhouse and to field populations of *L. maculans* in Australia in 1975 by growing single 2-m rows of each line in the field at Werribee, Victoria. The severity of blackleg infection on adult plants in the Australian test was determined using the method described by McGee (4).

Infectivity of isolates to rapeseed and stinkweed plants was compared. Plants were grown in soil-less mix, five plants per pot. At the 3- to 4-leaf stage of growth, plants in six pots for each host kind were sprayed with a conidium suspension of an isolate containing between 1×10^7 and 3×10^7 spores/ml and applied with a hand atomizer. After 3 wk of growth in the greenhouse severity of blackleg symptoms on individual plants was rated as follows: no symptoms (−); black flecks 0 to 1 mm in diameter on leaves and stems (+); black spots, 1 to 2 mm in diameter on leaves and stems (++); spots containing a few pycnidia, 2 to 5 mm in diameter on leaves and 1 cm long on stems (+++); lesions containing abundant pycnidia, several cm in diameter on leaves and several cm long on stems (++++). The average rating for each isolate on each host kind was determined. The infectivity of isolates on stinkweed was tested further. At the 3- to 4-leaf stage of growth approximately 10 leaves in each of five pots were pierced in the center with a needle coated with inoculum of an isolate. The needle was coated by rolling it on the surface of the culture. Controls consisted of leaves pierced with an inoculum-free needle. After 14 days in the greenhouse the plants were rated for the diameter of each leaf lesion on a scale of 0-5 as follows: no lesion, 0; lesion 0 to 2 mm, 1; lesion 2 to 4 mm, 2; lesion 4 to 8 mm 3; lesions 8 to 16 mm, 4; and lesion >16 mm, 5. The average rating was calculated for each isolate tested.

Rapeseed and stinkweed plant residues were compared as sources of inoculum for blackleg of rapeseed in plots of *B. napus* 'Tower' grown in the field at the Forestry Farm, Saskatoon. Eight plots, 1 m apart, were laid out in a single block; each plot consisted of nine rows 2 m long and 0.5 m apart. Immediately after the seeds were planted (30 May 1975) blackleg-affected residues, collected in 1974 from a rapeseed crop and a stinkweed patch in Saskatchewan, were placed in alternate plots in the block. The residues were laid in the spaces between four consecutive rows from the center row in one half of each plot. Periodic measurements of ascospore discharge from these residues were made during the growing season, using an ascospore liberation tunnel as described (6). On 11 September, samples of 50 plants were taken in each plot from the two rows with residue on either side and from the two rows in corresponding positions in the half-plot containing no residues. The samples were taken at least 0.5 m from the ends of rows. Each plant was examined for blackleg infection, as indicated by the presence of pycnidia of *P. lingam* on the stem. The percentage of infected plants in the two parts of each plot was calculated.

Cultural tests.—*Leptosphaeria maculans* isolates were grown from mycelial disk transfers on V-8 juice agar in petri plates for 7 days at 20 C. Growth rate was determined by measuring colony diameter and production of pycnidia was estimated visually. Isolates also were tested for production of a brownish-yellow pigment by transferring small disks of mycelium to Czapek's broth (35 g/liter) plus yeast extract (2 g/liter) and growing them in still culture for 4 wk at 20 C.

Survey of field populations of Leptosphaeria maculans on rapeseed in Saskatchewan.—To obtain more extensive data on the field populations of *L. maculans*, blackleg-infected rapeseed plants, as indicated by the presence of pycnidia of *P. lingam* on the stem, were collected from 32 fields in Saskatchewan during September 1975. Isolates of *L. maculans* were obtained and tested for pathogenicity to rapeseed plants and for cultural characteristics. In November, 1975, more intensive surveys were made in one field near Star City and in another near Lake Lenore, Saskatchewan. Blackleg-infected plants were collected at equally spaced sites on a diagonal transect of each field. Isolations of *L. maculans* were made from lesions on the crowns and upper stems of plants from each site. Pathogenicity and cultural tests were made on each isolate.

TABLE 1. Pathogenicity of isolates of *Leptosphaeria maculans* from different sources on *B. napus* 'Midas'[a]

Sources of isolates		Isolates tested	Disease severity rating[b,c]
Origin	Host	(no.)	
Wisconsin	cabbage	1	3.8
Australia	rapeseed[d]	5	2.8 ± 0.1
Saskatchewan	rapeseed	7	0.7 ± 0.2
Saskatchewan	stinkweed	5	0.3 ± 0.1

[a]Inoculated by placing fungus-infested oat kernels at the crown of plants at the one-leaf stage of growth.

[b]Crowns of adult plants rated 0 were healthy with other ratings increasing in stem canker severity to 5, where stems were completely severed.

[c]Values are means and their standard errors.

[d,e]"Rapeseed" here means oilseed rape (*Brassica napas* L.) plants grown for oil extraction from the seed.

RESULTS

Pathogenicity of Leptosphaeria maculans isolates to rapeseed.—Isolates of *L. maculans* from rapeseed in Australia were more virulent on *B. napus* 'Midas' than were those from rapeseed and stinkweed plants in Saskatchewan (Table 1). The isolate from cabbage in

TABLE 2. Pathogenicity of isolates of *Leptosphaeria maculans* from different sources to different seed lines of rapeseed plants

Seed line		Disease severity rating[b,c] of isolates from:		
Brassica sp.	Cultivar or P.I. No.[d]	Wisconsin cabbage	Australia rapeseed	Saskatchewan rapeseed[e]
B. campestris	Torch	3.8	3.0	1.4
B. campestris	R1312	4.0	2.2	0.4
B. napus	Midas	3.3	1.8	0.2
B. napus	Brown Sarson	0.3	0.0	0.5
B. campestris	R1357	0.0	0.0	0.2
B. napus	Ramses	0.4	0.1	0.5
B. napus	Major	0.0	0.0	0.0

[a]Inoculated by placing fungus-infested oat kernels at the crown of plants at the one-leaf stage of growth. "Rapeseed here means oilseed rape (*Brassica napus* L.) plants grown for oil extraction from the seed.
[b]Crowns of adult plants rated 0 were healthy, with other ratings increasing in stem canker severity to 5, where stems were completely severed.
[c]Values are the means of three replicates.
[d]*Brassica* spp. collection of Agriculture Canada Research Station, Saskatoon; numbers prefixed by R are plant introduction numbers.
[e]Isolate avirulent in original test (Table 1).

TABLE 3. Incidence of blackleg in 1975 in field subplots of rapeseed[a] (*Brassica napus* 'Tower') plants that were infested with rapeseed[a] or stinkweed residues, or noninfested[b]

Treatment		Percentage of plants infected[c,d]
Plots containing rapeseed residue	Residue between rows	44
	No residue between rows	30
Plots containing stinkweed residue	Residue between rows	4
	No residue between rows	3

[a]"Rapeseed" here means oilseed rape (*Brassica napus* L.) plants grown for oil extraction from the seed.
[b]Residue placed between four consecutive rows from the center row of each nine-row plot at planting time.
[c]Samples of 50 adult plants taken from each plot from the two rows with residues on either side and the two rows in corresponding positions in the subplot containing no residue. Infection determined by the presence of pycnidia of *P. lingam* on the stems.
[d]Values are the means of four replicates.

TABLE 4. Severity of blackleg infection on stinkweed and rapeseed[a] plants as determined by two inoculation techniques and for various sources of isolates of *Leptosphaeria maculans*

Source of isolates		Disease severity rating[b] on plants inoculated by spraying with conidium suspensions		Disease severity rating[c] on stinkweed leaves pierced with needles coated with inoculum.
Origin	Host	Rapeseed	Stinkweed	
Wisconsin	cabbage	+++[d]	+	0.4
Saskatchewan	rapeseed[e]	++	+	1.2 ± 0.2[f]
Saskatchewan	stinkweed	++	++++	3.2 ± 0.3[g]
Noninoculated controls				0.0

[a]"Rapeseed" here means oilseed rape (*Brassica napus* L.) plants grown for oil extraction from the seed.
[b]Disease severity ratings on stems and leaves were: no symptom (−); black flecks, 0 to 1 mm in diameter on leaves and stems (+); black spots, 1 to 2 mm in diameter on leaves and stems (++); spots containing a few pycnidia, 2 to 5 mm in diameter on leaves and 1 cm long on stems (+++); lesions containing many pycnidia, several cm in diameter on leaves and several cm long on stems (++++).
[c]Disease severity rating based on lesion diameter: no lesion, 0; 0−2 mm, 1; 2−4 mm, 2; 4−8 mm, 3; 8−16 mm, 4; and >16 mm, 5.
[d]Average rating in six pots containing five plants per pot.
[e]Avirulent on rapeseed in original test (Table 1).
[f]Mean value and standard error of three isolates.
[g]Mean value and standard error of two isolates.

Wisconsin also proved to be virulent.

When single Australian and Saskatchewan isolates from rapeseed and the Wisconsin isolate from cabbage were tested against a group of *B. napus* and *B. campestris* seed lines, three lines were susceptible to the Australian and Wisconsin isolates whereas the remaining four lines were resistant. All lines were resistant to the Saskatchewan isolate, although the cultivar Torch was slightly susceptible (Table 2).

In another group of 18 *B. napus* and *B. campestris* seed lines tested against the Wisconsin isolate in the greenhouse and against a field population of *L. maculans* in Australia, a highly significant correlation ($r = 0.9$, $P = 0.001$) was obtained between disease rankings in each test.

Pathogenicity of populations of Leptosphaeria maculans on rapeseed and stinkweed.—Severe blackleg symptoms, as evidenced by stem cankers, did not develop in rapeseed field plots containing residues of rapeseed and stinkweed. However, mild stem lesions containing pycnidia of *P. lingam* were apparent and were found to be more extensive in plots containing rapeseed residues than in those containing stinkweed residues (Table 3). Moreover, the level of disease was greater in plot areas with rapeseed residue than in areas containing no residue, while there was no difference in disease severity within plots containing stinkweed residue. Measurements of ascospore discharge showed that ascospores of *L. maculans* were liberated from the stinkweed residue, both earlier in the growing season and in larger quantities than from rapeseed residue.

Marked differences in cross infectivity of isolates from rapeseed and stinkweed occurred on these hosts after they were inoculated with suspensions of conidia in the greenhouse (Table 4). Whereas, an isolate from rapeseed caused slight disease on both hosts, that from stinkweed caused slight disease on rapeseed and leaf collapse on stinkweed. The Wisconsin isolate was almost avirulent on stinkweed. Similar results were obtained with these isolates on stinkweed when the needle-inoculation method was used.

Survey of field populations of Leptosphaeria maculans in Saskatchewan.—Thirty-one of 32 isolates of *L. maculans*, obtained from different rapeseed fields in Saskatchewan in the fall of 1975, were avirulent in pathogenicity tests against the rapeseed cultivar Midas; disease severity ratings ranging from 0.5 to 1.4. For one isolate that was virulent, a disease severity rating of 2.9 was obtained. In a more intensive survey made in November 1975, eight virulent and nine avirulent isolates were detected in a collection from the field at Star City, where the original virulent isolate was found. The virulent isolates were obtained from different sampling sites. Seven virulent and 36 avirulent isolates were obtained from a randomly selected field near Lake Lenore. However, six of the virulent isolates were from plants at one sampling site and the remaining virulent isolate was from a plant in an adjacent site. No differences were apparent in the virulence of isolates from crowns and upper stems of individual plants.

Cultural differences between virulent and avirulent isolates of Leptosphaeria maculans.—Certain cultural characteristics of the isolates were associated with their virulence to rapeseed (Table 5). In Czapek's broth medium, a very distinct brownish-yellow pigment developed in cultures of avirulent isolates, whereas no pigment was produced in cultures of virulent isolates. This difference also was apparent when isolates were grown on V-8 juice agar, as illustrated in Fig. 1. Avirulent isolates grew faster on V-8 juice agar than did virulent types. Avirulent isolates from rapeseed formed colonies with few pycnidia on V-8 juice agar (Fig. 1-A), whereas virulent isolates from rapeseed in Saskatchewan and the isolate from cabbage in Wisconsin produced masses of pycnidia (Fig. 1-B). The Australian isolates and the isolates from stinkweed were inconsistent with respect to production of pycnidia.

DISCUSSION

Strains of *L. maculans* could be differentiated among the isolates studied in this work on the basis of virulence to rapeseed and cultural tests. Two strains, one virulent and one avirulent, were detected on rapeseed in the field in Saskatchewan. These were clearly different in cultural characteristics and all isolates of a strain tested were extremely uniform. The isolate from cabbage in

TABLE 5. Cultural differences for isolates of *Leptosphaeria maculans* from different sources grouped according to virulence on rapeseed[a]

Pathogenicity to rapeseed	Source of isolates: Origin	Source of isolates: Host	Number tested	Pigment production[c]	Pycnidia production[b]	Diameter of colony (mm)[d,e]
virulent	Wisconsin	cabbage	1	−	m	3.7
	Australia	rapeseed	14	−	v	3.4 ± 0.3
	Saskatchewan	rapeseed	15	−	m	3.5 ± 0.1
avirulent	Saskatchewan	rapeseed	76	+	f	5.8 ± 0.1
	Saskatchewan	stinkweed	5	+	v	6.7 ± 0.8

[a]"Rapeseed" here means oilseed rape (*Brassica napus* L.) plants grown for oil extraction from the seed.

[b]Isolates incubated on still culture of Czapek's broth (35g/liter) + yeast extract (2g/liter). Symbols: + = brownish-yellow pigment produced, and − = no pigment produced.

[c]Isolates grown on V-8 juice agar. Symbols: m = many pycnidia formed, f = few pycnidia formed, and v = pycnidium production variable.

[d]Colonies grown on V-8 juice agar for 7 days at 10 C.

[e]Mean colony diameter and the standard error of the number of isolates tested.

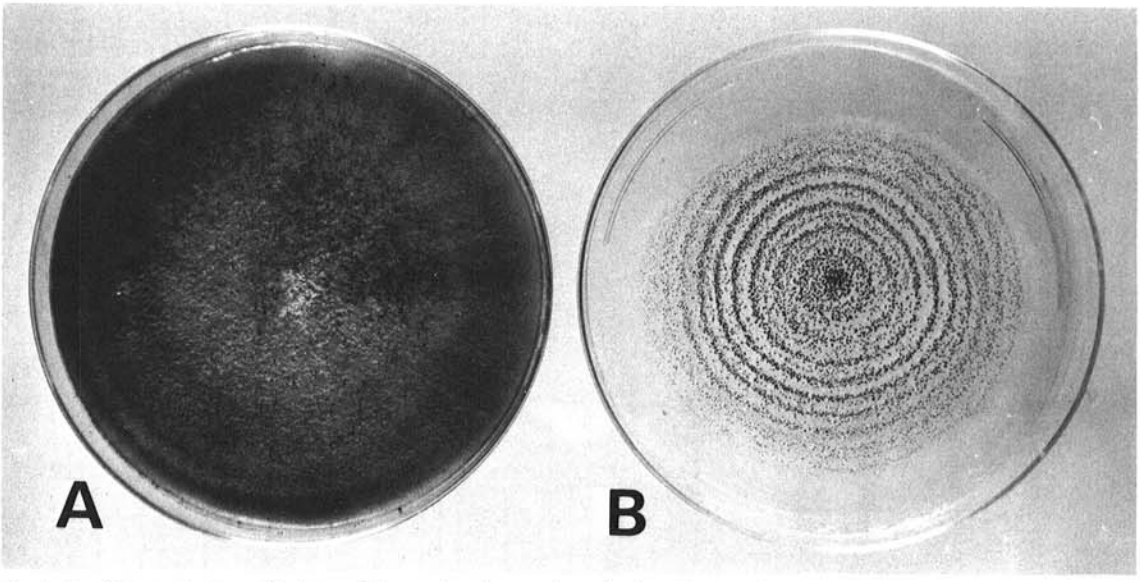

Fig. 1. Pycnidia production of isolates of *Leptosphaeria maculans*, virulent (B) and avirulent (A) on rapeseed, growing on V-8 juice agar. ["Rapeseed" refers to oilseed rape (*Brassica napus* L.) plants grown for oil extraction from the seed.]

Wisconsin gave identical responses to the virulent Saskatchewan strain in all tests, thus suggesting that it was the same strain. However, the Australian isolates, all of which were virulent, showed variability in culture and hence could not be considered representative of a single strain. A third strain was identified in the field in Saskatchewan on stinkweed. It was distinguished from the others by differential reactions on rapeseed and stinkweed in pathogenicity tests.

The isolate from cabbage in Wisconsin was typical of those obtained from the field where severe blackleg epidemics had occurred in cruciferous crops in the eastern U.S. (P. H. Williams, *personal communication*). The apparent existence of this strain in Saskatchewan thus suggests a relationship between strains of the pathogen on rapeseed and those on other cruciferous crops in North America. Further evidence for such a relationship comes from the similarity between the avirulent Canadian strain on rapeseed and the Puget Sound strain described by Pound (10), both of which produce brownish-yellow pigment in culture and cause only mild disease symptoms on the host.

Although there were differences in some cultural characteristics between the Australian isolates and the virulent North American isolates, there was evidence from pathogenicity tests that a close relationship existed between field populations in these countries. This was suggested by the significant correlations between disease rankings in one group of *Brassica* spp. seed lines tested against the Wisconsin isolate and an Australian isolate in the growth chamber, and a second group tested against the Wisconsin isolate in the greenhouse and a wild population in the field in Australia. Also, the *B. napus* cultivars Ramses and Major, which were resistant to the Wisconsin and Australian isolates in these tests, are considered to be blackleg-resistant cultivars in France.

Although only a small number of isolates from Australia were tested, they were obtained from different parts of Victoria and all were virulent, suggesting that the prevalent strains in the State are virulent. In Saskatchewan, on the other hand, the results of the survey of field populations showed that the avirulent strain was more prevalent. This may explain, in part, why blackleg is a more serious disease of rapeseed in Australia than in Canada.

The importance of the virulent strain in Canada is not clear. In the two fields which were surveyed intensively it was obviously a significant part of the population. Further intensive surveys are clearly necessary to determine how widely it has been disseminated. The most likely explanation for the location of the strain in only part of the field at Lake Lenore, is that it was introduced in the seed lot sown in that part of the field. This strain is obviously a potential threat to the rapeseed crop in Canada and further research to determine the significance of its presence seems warranted.

Major differences were not detected between avirulent isolates and isolates from stinkweed in pathogenicity on rapeseed in the greenhouse. However, disease patterns in rapeseed field plots, containing either rapeseed or stinkweed residues, suggested that ascospores from stinkweed did not cause any detectable disease although apparently they were produced in greater numbers than from rapeseed residue. Therefore, although stinkweed is widespread in rapeseed growing areas of Canada it does not appear to represent a significant source of inoculum for the blackleg disease of rapeseed.

LITERATURE CITED

1. CUNNINGHAM, G. H. 1927. Dry rot of swedes and turnips: its cause and control. N. Z. Dep. Agric. Bull. 133. 51 p.
2. HUGHES, W. 1933. A study of Phoma lingam (Tode) Desm. and of the "dry rot" it causes, particularly in swede

turnips. Sci, Proc., R. Dublin Soc., Ser. A 20:495-529.
3. LACOSTE, L., J. LOUVET, C. ANSELME, C. ALABOUVETTE, B. BRUNIN, and J. G. PIERRE. 1969. Rôle de Phoma lingam (Tode) Desm. et de sa forme parfair Leptosphaeria maculans (Desm.) Ces. et de Not. dans la épidemiés de nécrosé du collet de Colza (Brassica napus L. var. oleifera metzer). C. R. Seances Acad. Agric. Fr. 55:981-989.
4. MC GEE, D. C. 1973. Rapeseed: How to assess the severity of blackleg disease and predict yield. J. Agric. (Victoria) 71:241-242.
5. MC GEE, D. C. 1976. A comparison for the epidemiology of blackleg of oilseed rape in Australia and Canada. Proc. Am. Phytopathol. Soc. 3:369.
6. MC GEE, D. C. 1977. Blackleg (Leptosphaeria maculans (Desm.) Ces et de Not.) of rapeseed in Victoria: Sources of infection and relationships between inoculum, environmental factors and disease severity. Aust. J. Agric. Res. 28:53-62.
7. MC GEE, D. C., and R. W. EMMETT. 1977. Blackleg (Leptosphaeria maculans (Desm.) Ces et de Not.) of rapeseed in Victoria: Crop losses and factors which affect disease severity. Aust. J. Agric. Res. 28:47-52.
8. PETRIE, G. A. 1973. Herbicide damage and infection of rape by the blackleg fungus Leptosphaeria maculans. Can. Plant Dis. Surv. 53:26-28.
9. PETRIE, G. A., and T. C. VANTERPOOL. 1965. Diseases of rape and cruciferous weeds in Saskatchewan in 1965. Can. Plant Dis. Surv. 45:111-112.
10. POUND, G. S. 1947. Variability in Phoma lingam. J. Agric. Res. 75:113-133.
11. STRINGAM, G. A. 1971. Genetics of four hypocotyl mutants in Brassica campestris. J. Hered. 62:248-250.