

INFESTATION OF CRUCIFER SEED IN WESTERN CANADA BY THE BLACKLEG FUNGUS *LEPTOSPHAERIA MACULANS*¹

G. Allan Petrie² and T.C. Vanterpool³

Abstract

Of 1,890 seed samples of rape (*Brassica napus*) and turnip rape (*B. campestris*) produced in western Canada and plated between 1968 and 1973, 2.6% were infested with the blackleg fungus, *Leptosphaeria maculans*. The highest percentage of farm samples yielding the fungus in any year was 4.0 in 1969, and the highest infestation level in any sample, 2.7%. Contaminated samples originated in almost all major rape producing areas of the prairies from the Peace River region of Alberta to southern Manitoba. All three major strains of the fungus occurred in *Brassica* samples, with strain I (the 'brassica' strain) being by far the most common. Over 33% of the seeds in a sample of *Raphanus sativus* var. *oleifera* carried *L. maculans* (strains I and II) following surface disinfection. The pathogen was detected in seed samples of *Cheiranthus cheiri* (strain I), *Sisymbrium altissimum* (strain II), and *Thlaspi arvense* (strain III). Observations made in field plots revealed that natural infections of pods of rape and oilseed radish usually started at the stigmatic end.

Résumé

Des 1,890 échantillons de graines de colza (*Brassica napus*) et de navette (*B. campestris*) produites dans l'ouest du Canada et semées de 1968 à 1973, 2.6% a été infesté par le champignon de la jambe noire (*Leptosphaeria maculans*). Le pourcentage le plus élevé d'échantillons infestés a été de 4 en 1969 et le plus fort niveau d'infestation de tous les échantillons, de 2.7%. Les échantillons contaminés provenaient de presque toutes les principales régions productrices de colza des Prairies, depuis la région de Rivière de la Paix en Alberta jusqu'au sud du Manitoba. On a trouvé les trois principales souches du champignon dans les échantillons de *Brassica*, la souche I (trassica) étant de beaucoup la plus abondante. Plus de 33% des graines d'un échantillon de *Raphanus sativus* var. *oleifera* était infesté par *L. maculans* (souches I et II) après désinfection de surface. On a observé le champignon pathogène dans les échantillons de graines de *Cheiranthus cheiri* (souche I), de *Sisymbrium altissimum* (souche II) et de *Thlaspi arvense* (souche III). Les observations des parcelles ont révélé que l'infestation naturelle des siliques du colza et du radis oléagineux débutait généralement à l'extrémité des stigmates.

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² Plant Pathologist, Agriculture Canada, Saskatoon.

³ Professor Emeritus, Department of Biology, University of Saskatchewan, Saskatoon.

In recent years, blackleg caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not. (imperfect state: *Plenodomus lingam* (Tode ex Fr.) Hohn.] has again become a major cause for concern in certain rape and cabbage growing areas of the world (10, 23), and naturally, seed has been suspect as a reservoir of primary infection. That seed-borne infection plays a critical role in initiating field infections in rutabaga, rape, and similar crops has not been clearly demonstrated, although in the case of cabbage it appears to be important in establishing the disease in the seedbed prior to transplanting. Several workers have studied the problem of transmission of the pathogen in seed of rutabaga and turnip (1, 3, 5, 7, 9, 11), and others have conducted similar

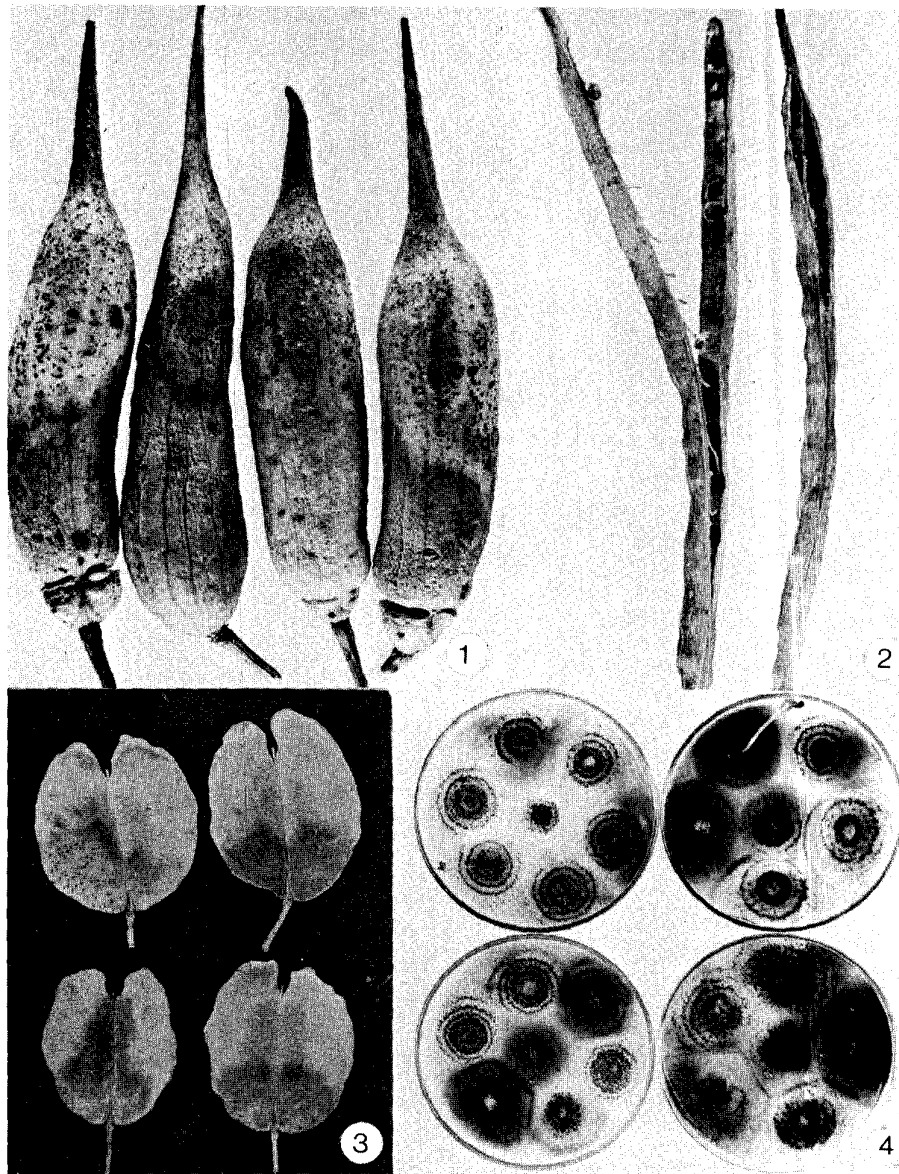


Figure 1. Lesions on pods of oilseed radish resulting from infection by *Leptosphaeria maculans* (upper portion of each pod). The small black spots were caused by *Alternaria raphani* Groves & Skolko.

Figure 2. Splitting of rape pods as a consequence of infection by *L. maculans*. Note shrunken and mycelium-covered seeds in the upper portion of pod at the left.

Figure 3. Lesions on pods of *Thlaspi arvense* resulting from infection by *L. maculans*.

Figure 4. Colonies of *L. maculans*, strain I, growing from plated surface-disinfested seed of oilseed radish. Several cultural variants are present.

investigations in relation to cabbage (8, 13, 21, 23), and rape (2, 6, 9, 19). In Canada, isolation of the pathogen from seeds of rape was first reported in 1957 (20). Van Poeteren (19) reported that in Europe 50-6043 of the seeds in rape samples sometimes carried the fungus. It also has been isolated from seed of several other cultivated members of the Cruciferae, including cauliflower (12, 13), kohlrabi (12), and brussels sprouts (4). Buddin (3) demonstrated its presence in seed of wild mustard (Brassica alba Rabenh. or B. hirta Moench), a common weed in British rutabaga fields. Neergaard (12) reported the occurrence of the blackleg pathogen on radish seed. Apart from this instance, it apparently has not been found on seed of members of cruciferous genera other than Brassica (14).

This paper presents 6 years' data for seed infestation of rape (Brassica napus L.), turnip rape (B. campestris L), and other Cruciferae by L. maculans in Western Canada. It is part of a larger study of the seed health of Brassica spp., flax, and safflower, the remainder which will be published shortly.

Materials and methods

Samples of western Canadian rape and turnip rape seed produced between 1968 and 1972 were obtained from the Plant Products Division of Agriculture-Canada and from the Canadian Grain Commission. Seed from the 1973 western Canadian cooperative rapeseed tests was also plated. Untreated seeds were transferred by means of a vacuum seeder to plates of V8 juice agar containing 40 ppm rose bengal and 100 ppm streptomycin sulfate. For each sample 200-300 seeds were examined in lots of 15-20 per plate. Records of colony numbers were made after 7-10 days' incubation under diffuse light at room temperature. Fresh subsamples from heavily-infested lots of seed were treated for 20 minutes in a 10% solution of Javex and plated as before to determine the extent to which the fungus occurred within the seed coat. Naturally infected pods of a few cruciferous species were collected in field plots and a photographic record made of symptoms produced by L. maculans.

Results and discussion

The siliques of Brassica napus and oilseed radish (Raphanus sativus L. var. oleiferus Metzg.) exhibited varying degrees of infection. The pathogen had gained entry at the stigmatic end in almost every case (Fig. 1). A brownish to whitish lesion bearing

scattered pycnidia had then spread downward symmetrically from the point of invasion to engulf from about 1/3 to over 1/2 of the pod. Although insect-transmitted conidia would appear to be a likely source of this infection, no confirmation of this has been obtained. Premature splitting of rape pods with consequent loss of seed resulted from unequal drying out of infected and uninfected portions of the valves (Fig. 2). When intact infected pods were opened, the presence of a grayish mycelium was revealed on their inner surfaces. seeds beneath lesions were shrunken, unsound and pale gray in color. Those from Raphanus pods often bore large black discolorations. Plating of surface-disinfested seeds from lesioned rape pods revealed the presence of L. maculans in almost all of them, even apparently healthy ones not adjacent to lesions. Lesions on siliques of Thlaspi arvense usually appeared to have originated basally rather than apically (Fig. 3).

Table 1. Prevalence of Leptosphaeria maculans seed infestation of rape and turnip rape in western Canada

Year	No. of samples plated	Samples infested (%)	Highest infestation recorded (% of seeds per sample)
1968	141	3.6	0.3
1969	353	4.0	2.7
1970	1,027	1.6	1.0
1971	284	3.2	0.6
1972	32	0.0	0.0
1973	53	9.4	1.0
Total	1,890	Overall % 2.6	Highest level 2.7

The seed plating data are presented in Table 1. Those for 1973 represent 53 samples from the cooperative varietal tests from five locations in Saskatchewan and a few in Alberta and Manitoba. Over 9% of these samples carried L. maculans. In Saskatchewan infested seed was obtained from the regional tests at Kelvington, Lake Lenroe, and Parkside. Seed infestation by the fungus was not detected in a number of farm samples that were plated between 1961 and 1967. From 1968 to 1972, no more than 4.0% of the growers' samples were infested in any year. However, contamination occurred in seed lots from across the prairies, from Beaverlodge, Alberta, in the northwest to Darlingford, Manitoba, near the United States border, a distance of 1000 miles. An indication of the geographical distribution of infested samples is given in Table 2. Most of these were from Saskatchewan, but this was not unexpected as 76% of all the samples plated originated in this province. Within Saskatchewan, crop district 8 had many more infested seed lots than did the others, but when the numbers

Table 2. Distribution by crop district of Brassica seed samples infested by *Leptosphaesia maculans*, 1968-1973

Crop district *	Saskatchewan			Alberta			Manitoba		
	No. of samples plated	Of samples infested	% infested	No. of samples plated	Of samples infested	% infested	No. of samples plated	Of samples infested	% infested
1	1	0	0.0	3	0	0.0	2	0	0.0
2	15	0	0.0	32	1	3.1	16	1	6.3
3	4	0	0.0	19	2	10.5	36	1	2.8
4	0	0	0.0	60	3	5.0	0	0	0.0
5	156	8	5.1	63	1	1.6	18	0	0.0
6	67	4	6.0	45	0	0.0	0	0	0.0
7	47	1	2.1	80	1	1.3	6	0	0.0
8	769	18	2.3				7	0	0.0
9	367	8	2.2				2	0	0.0
10							15	0	0.0
11							18	1	5.6
12-14							24	0	0.0
Unknown	0	0	0.0	18	0	0.0	0	0	0.0
Totals and averages	1,426	39	2.7	320	8	2.5	144	3	2.1

* There are 9 crop districts in Saskatchewan (disregarding subdistricts A and B), 14 in Manitoba, and 7 agricultural reporting areas in Alberta (22).

were related to the total samples plated per district, it was evident that crop districts 5 and 6 had proportionally higher rates of infestation. Affected samples were well spread across the northern half of the cultivated portion of the province. Relatively few samples from Alberta and Manitoba were infested, but they too were widely distributed. Although all three major strains of the pathogens were isolated from Brassica seed, by far the most common was strain I, the one usually associated with members of this genus (15).

seed of other genera of the Cruciferae was also examined for *L. maculans*. In a 1968 sample of oilseed radish 33.4% of the seeds were found to be infested following surface-disinfestation (Fig. 4). The seed had been harvested from plants in an "introductions nursery" at Saskatoon. Both strain I and strain II (the 'sisymbrium' strain) were recovered, the latter infrequently. A sample of wallflower (*Cheiranthus cheiri* L.) seed purchased in Saskatoon, and likely imported from abroad, had about 1.0% *L. maculans* (strain I). In a sample of tumbling mustard (*Sisymbrium altissimum* L.) seed collected near Saskatoon, 1.0% infestation by strain II was detected, and in a sample of *Thlaspi arvense* L., 0.5% infestation by strain III (15) was found.

Although geographically widespread, *L. maculans* seed infestation probably has not constituted an infection source of first importance; relatively few samples were contaminated and the incidence of infestation was low. Conidia and ascospores from overwintered stem material of cultivated crucifers and weed species likely have played the major role in initiating spring infections. However, diseased seed may have contributed significantly to the spread of blackleg into new areas of production. In the years between 1963 and 1969, field surveys conducted in the spring and fall showed blackleg of rape to be steadily increasing in prevalence in Saskatchewan (17, 18). However, from 1970 to 1972, the disease was detected in from 15 to 19% of the fields entered in this province, and its incidence generally remained low (16). At present, therefore, blackleg remains one of a number of diseases of minor importance in Western Canada. Nevertheless, its wide distribution in the region, the considerable genetic diversity of the pathogen indicated by the Occurrence of several strains, and its high virulence to cultivated varieties of rape and turnip range indicate a potentially dangerous situation. These factors, in conjunction with the sudden dramatic losses caused by the disease in other parts of the world (10), indicate a need for the development now of control measures appropriate to the Canadian Prairies.

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Literature cited

1. Allen, J. D., and H. C. Smith. 1961. Dry-root (Leptosphaeria maculans) of Brassicas: seed transmission and treatment. *New Zealand J. Agr. Res.* 4: 676-685.
2. Anonymous. 1955. In Rapport Annuel de l'Institut National de la Recherche Agronomique, 1952. Abstr. in *Rev. Appl. Mycol.* 35:417-418.
3. Buddin, W. 1934. The canker and dry rot diseases of swedes. *Min. Agr. and Fishing, London. Bull.* 74. 47 pp.
4. Clayton, E. E. 1927. Black-leg disease of brussels sprouts, cabbage, and cauliflower. *New York State Agr. Exp. Sta. Bull.* 550. 27 pp.
5. Cunningham, G. H. 1927. Dry-rot of swedes and turnips: its cause and control. *New Zealand Dep. Agr. Bull.* 133. 51 pp.
6. Darpoux, H., J. Louvet, et J. Ponchet. 1957. Essais de traitement des semences de cruciferes contre le Phoma lingam (Toae) Desm. et l'Alternaria brassicae (Berk.) Sacc. *Ann. Epiphyt.* 8:545-557.
7. Dennis, R. W. G. 1939. Notes on seed transmission of Phoma lingam in relation to dry rot of swedes in Scotland. *Ann. Appl. Biol.* 26:627-630.
8. Henderson, M. P. 1918. The blackleg disease of cabbage caused by Phoma lingam (Tode) Desmaz. *Phytopathology* 8:379-431.
9. Lloyd, A. B. 1959. The transmission of Phoma lingam (Tode) Desm. in the seeds of swede, turnip, chou moellier, rape, and kale. *New Zealand J. Agr. Res.* 2: 649-658.
10. McGee, D. C. 1973. Losses in rapeseed caused by blackleg in Victoria, Australia. Abstr. 0825, 2nd Int. Congr. Plant Pathol., Univ. Minnesota.
11. Murphy, P. A. 1928. The connexion between dry-rot of swedes in New Zealand and British seed. *Nature* 122: 13-14.
12. Neergaard, P. 1948. Eleventh annual report from the J. E. Ohlsen *Phytopathologica I* laboratory, 1st August, 1945, to 31st July, 1947. (In Danish.) Abstr. in *Rev. Appl. Mycol.* 28: 159-160.
13. Nielson, O. 1932. Investigations on blackleg of cabbage and dry rot of swedes. (In Danish) *Tidsskr. for Planteavl*, 38:131-154. Abstr. in *Rev. Appl. Mycol.* 11:489.
14. Noble, M., and M. J. Richardsen. 1968. An annotated list of seed-borne diseases. 2nd ed. Commonwealth Mycological Inst., Kew, Surrey, and Int. seed Test. Ass., Wageningen, Netherlands.
15. Petrie, G. A. 1969. Variability in Leptosphaeria maculans (Desm.) Ces. & de Not., the cause of blackleg of rape. PhD. thesis, University of Saskatchewan, Saskatoon.
16. Petrie, G. A. 1973. Herbicide damage and infection of rape by the blackleg fungus, Leptosphaeria maculans. *Can. Plant Dis. Surv.* 53:26-28.
17. Petrie, G. A., and T. C. Vanterpool. 1968. Diseases of crucifers in Saskatchewan in 1967. *Can. Plant Dis. Surv.* 48: 25-27.
18. Petrie, G. A., and T. C. Vanterpool. 1970. Diseases of rape and other crucifers in Saskatchewan in 1969. *Can. Plant Dis. Surv.* 50:106-107.
19. Van Poeteren, N. 1931. Report on the activities of the Phytopathological service in the year 1930. (In Dutch.) *Versl. en Meded. Plantenziektenkundigen Dienst te Wageningen*, 64: 1-189. Abstr. in *Rev. Appl. Mycol.* 11:95-96.
20. Vanterpool, T. C. 1958. Rape diseases in Saskatchewan in 1957. *Can. Plant Dis. Surv.* 37:38-40.
21. Walker, J. C. 1923. The hot water treatment of cabbage seed. *Phytopathology*, 13: 251-253.
22. Williams, G. D. V. 1973. Estimates of prairie provincial wheat yields based on precipitation and potential evapotranspiration. *Can. J. Plant Sci.* 53:17-30.
23. Williams, P. H. 1967. Occurrence of Phoma lingam on cabbage seed from Australia after treatment with hot water. *Plant Dis. Rep.* 51:566-569.