Seasonal Patterns of Ascospore Discharge by *Leptosphaeria maculans* in Relation to Blackleg of Oilseed Rape

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ABSTRACT

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A consistent seasonal pattern of *Leptosphaeria maculans* ascospore discharge, which started in July of the year after crop growth, was detected from residues of 40 fields of oilseed rape (*Brassica napus* and *B. campestris*) grown during 3 yr in Saskatchewan. Exceptions occurred in 1977, when ascospores were discharged in May from residues of four rape fields grown in 1976. Ninety-five percent of the *L. maculans* isolates obtained from one of the latter groups of residues were the normally infrequent, virulent strain of the pathogen, while 98% of the isolates from residues of another crop grown in 1976 from which the more common discharge pattern was detected in 1977, were the prevalent avirulent strain. A distinct seasonal pattern of ascospore discharge also was detected from a third strain of *L. maculans* which is pathogenic to stinkweed (*Thlaspi arvense* L.), but not rape. Severe blackleg only developed in rape plants inoculated at the crown before the 6-leaf stage of growth, because younger tissues were more susceptible to infection than older ones. Disease progress was slower in plants grown at 12 C than at 18 C. Symptoms of blackleg could not be detected on rape in the field until September. This supported the contention that ascospore discharge of the prevailing strain on rape residues, which starts after developing crops are past the 6-leaf stage, occurs too late to cause severe loss in Canada. A similar relationship between the times of ascospore discharge and disease development on stinkweed was found for the stinkweed strain of *L. maculans*.

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

Blackleg (caused by *Leptosphaeria maculans* [Desm.] Ces. et de Not.) was confirmed first on oilseed rape (*Brassica napus* L., *B. campestris* L.) crops in Canada in 1961 (11), but it was not considered a serious disease until recently (8). The disease has caused major crop losses in Australia, where Canadian oilseed rape cultivars were introduced in 1968 (6), and also is a serious disease of oilseed rape in France (4). In Canada, the most common strain of the pathogen found on rape is avirulent, and produces only superficial stem lesions (7). However, a virulent strain, which causes severe stem cankers also exists (7). A third strain which is not pathogenic to rape occurs on the cruciferous weed stinkweed (*Thlaspi arvense* L.) (7). The most prevalent strain of the pathogen in Australia is more virulent than the more prevalent one in Canada (7). However, growing conditions differ greatly between these two countries and it is conceivable that epidemiological factors in addition to strain differences may be associated with the difference in disease severity.

In Australia, ascospores liberated from rape plant residues are the major source of inoculum and field disease patterns were related to seasonal patterns of ascospore production (5). A seasonal pattern of ascospore discharge also occurs in France (1). Similar information is lacking in Canada, although it has been shown that ascocarps can develop on overwintered residues of rape and stinkweed (9).

Rape plants become severely diseased when infected at an early growth stage. Under field conditions in Australia, stem cankers causing severe reduction in seed yield resulted only from infections that occured before flowering (6). In France, when crowns of rape plants were inoculated in the field, the more severe were the stem cankers (3) the earlier infection took place. However, it was not established whether this phenomenon resulted from younger tissues being more susceptible to infection than older ones, from differences in time for disease progression, or from higher temperatures during disease development after early inoculations. Barbetti (2) and McGee (5) showed that, after inoculation at the cotyledonary stage of growth, blackleg was more severe in plants grown at temperatures above 12 C than at lower temperatures.

In this study, seasonal patterns of ascospore discharge of strains of *L. maculans* from rape and stinkweed residues in Canada are described and factors affecting susceptibility of rape to infection in relation to stage of growth are clarified. The findings are used to explain differences in the field disease patterns of blackleg of rape in Canada, Australia, and France.

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MATERIALS AND METHODS

Determination of seasonal patterns of ascospore discharge. The technique described by McGee (5), who used ascospore liberation tunnels, was used to determine seasonal patterns of ascospore discharge from rape and stinkweed plant residues collected in Saskatchewan. In April, collections were made of residues of plants grown in the previous year. These were cut into sections 6-cm long and, for each collection, four groups of sections were prepared, each of which would fully load one tunnel. Each group was stored outside in a plastic pot at the Agriculture Canada Research Station, Saskatoon. Periodically, the four groups were brought into the laboratory for 7 days. The residues were sprinkled with water to induce ascospore discharge and placed in the tunnels. Ascospores discharged over a 2-hr period were trapped on Vaseline-coated slides and the residues returned outside. The slides were scanned microscopically and the number of ascospores on the standard trapping area estimated as being either 0, 50, 300, 3,000, 9,000, or 18,000. The average discharge for the four groups of residues was calculated; discharges were measured from residues of four rape crops grown in 1974, 18 grown in 1975, and 22 grown in 1976. Discharges also were measured from residues of one stinkweed patch grown in 1974, two grown in 1975, and two grown in 1976.

Examination of field populations of *L. maculans*. Two distinct ascospore discharge patterns were detected from rape residues. Therefore, an analysis was made of the strains of *L. maculans* present in single fields from which each discharge pattern was detected. Isolates of *L. maculans* were obtained from pieces of

diseased rape plant parts from each field by surface sterilizing the pieces in 1.0% sodium hypochlorite for 2 min and plating them on V-8 juice agar containing 40 μg/ml rose bengal and 100 μg/ml streptomycin sulfate. Isolates then were transferred to V-8 juice agar and virulent and avirulent strains were differentiated by pigment production and growth rate in culture, as previously described (7).

Infection studies on rape. A growth chamber test was conducted to assess various factors influencing infection of rape plants inoculated at different stages of growth. Equal numbers of pots, each containing three *B. napus* 'Midas' plants, growing in soil-less mix (10), were placed in growth chambers maintained at 18 C and 12 C, respectively. A virulent isolate of *L. maculans* on oat kernels was used to inoculate crowns of individual rape plants as previously described (7). Plants in groups of eight pots were inoculated at the one-, three-, and six- leaf, budding, and flowering stages of development in each chamber. Four pots of plants for each inoculation were harvested when pods were fully developed, but still green. This was 8 and 16 wk after the one-leaf stage at 18 C and 12 C, respectively. The remaining plants at 18 C were harvested 8 wk after inoculation and the plants at 12 C after time periods identical to those between inoculation and full pod development of plants at 18 C. Each plant was rated on a scale from 0, (plants healthy) increasing in severity to 5 (crowns completely severed) (5) and the average disease severity rating was calculated for each treatment.

Field disease surveys. Blackleg surveys were made in 28, 30, and 16 rape fields, chosen at random, in the Lake Lenore region of Saskatchewan on 24 June, 31 July, and 10 September 1975, respectively. The fields were examined by walking through sections of the crop and looking for symptoms of blackleg on the stems and foliage. Stinkweed plants growing either in or near the field also were examined for blackleg infection.

RESULTS

Seasonal patterns of ascospore discharge. Ascospore discharges did not start until July of the year following crop growth and continued until December from residues of 40 rape crops, grown during 3 yr in Saskatchewan. Typical data obtained from individual crops grown in each of these years are presented (Table 1). Exceptions to this consistent pattern occurred in 1977 when ascospore discharges were detected in May and June from residues of four rape crops grown in 1976. Typical data from one of these crops also are presented (Table 1). Of the populations of *L. maculans* on the residues of the two crops grown in 1976 (referred to in Table 1) 94% of 91 isolates obtained from the residues with the early discharge pattern were virulent and 6% avirulent, while 2% were virulent and 98% avirulent of 80 isolates obtained from residues with the later discharge pattern.

A consistent seasonal pattern of ascospore discharge was detected from residues of stinkweed plants grown in 1974, 1975, and 1976, in which sporulation occurred in May and June and continued into the winter months in the year following plant growth (Table 1). The same pattern also was detected in 1976 and 1977 from residues of two other groups of stinkweed grown in 1975 and 1976, respectively.

Ascospores were discharged in April 1976 from residues of a rape crop grown 2 yr earlier. Spore discharge increased during the summer and was fairly constant in the fall months (Table 2). This pattern also was obtained in 1976 from residues of three other rape crops grown in 1974. Large discharges were detected in April, May, and June, 1976, from residues of stinkweed plants grown in 1974, but they declined later in the summer (Table 2).

Factors affecting the susceptibility of rape plants to infection. Disease severity declined with age at the time of inoculation for plants grown at either 18 C or 12 C (Fig. 1A). This effect also was apparent in plants grown at 18 C which were harvested 8 wk after inoculation (Fig. 1B). Disease severity in plants inoculated after the three-leaf stage of growth, and grown at 18 C, tended to diminish as time between plant inoculation and harvest decreased (Fig. 1B).

Despite longer periods between inoculation and disease assessment, disease severity in plants inoculated before the bud stage of growth was slightly lower in those grown at 12 C than in those grown at 18 C (Fig. 1A). This slower rate of disease development at the lower temperature also was indicated by the difference in disease severity between plants inoculated at the same stage of growth and grown at 12 C and 18 C, but subjected to the same time periods between inoculation and disease assessment at both temperature regimes (Fig. 1C).

Field patterns of blackleg in 1975. Blackleg was not detected on rape plants in any of the fields surveyed in June or July, but infected

TABLE 1. Seasonal discharge patterns of ascospores of *Leptosphaeria maculans* from residues of rape[a] and stinkweed[b] in the year after plant growth

Plant residue[c]	Year of plant growth	Periodic discharges of ascospores of *L. maculans* in the year after plant growth[d]							
		May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Rape	1974	0	0	12	237	975	...	3,000	1,650
	1975	0	0	1,200	2,100	1,200	3,000	3,000	...
	1976	...	0	17	217	300	2,100	...	...
	1976	17	300	1,200	1,650	..	...	...	...
Stinkweed	1974	...	975	7,500	4,500	10,000	9,700	2,500	6,000
	1975	1,666	9,500	7,000	8,000	3,000	2,550	...	...
	1976	25	3,000	3,000	...	9,000	...	...	...

[a] *Brassica napus* L., *B. campestris* L.
[b] *Thlaspi arvense* L.
[c] Residue from the previous year stored in the open.
[d] Ascospore discharges measured in ascospore liberation tunnels in the laboratory. Values are the means of four replicates.

TABLE 2. Seasonal discharge patterns of ascospores of *Leptosphaeria maculans* from residues of rape[a] and stinkweed[b] 2 yr after plant growth

Plant residue[c]	Year of plant growth	Periodic discharges of ascospores of *L. maculans* 2 yr after plant growth[d]							
		Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Rape	1974	112	50	975	1,650	2,325	300	2,325	2,325
Stinkweed	1974	3,825	6,750	4,500	2,325	237	300	112	...

[a] *Brassica napus* L., *B. campestris.*
[b] *Thlaspi arvense* L.
[c] Residue from two years previous stored in the open.
[d] Ascospore discharges measured in ascopsore liberation tunnels in the laboratory. Values are the means of four replicates.

plants were found in 10 of 16 fields surveyed on 10 September. On each of these dates, stinkweed plants infected with blackleg were found at several locations.

DISCUSSION

Ascospores of *L. maculans* were discharged from plant residues in distinct seasonal patterns in Canada. The technique used to detect these patterns did not measure natural field discharges. However, in Australia, the same seasonal pattern of ascospore discharge was detected by this technique as that detected by a Burkard spore trap continually measuring natural ascospore discharges under field conditions (5). Moreover, the consistency of the discharge patterns from many groups of plant residues from different parts of Saskatchewan, over 3 yr, suggests that the results reflect natural field discharge patterns. The various discharge patterns can be related to strains of *L. maculans* existing on rape and stinkweed plants. The rare occurrence of early discharge of the virulent strain is consistent with previous findings (7,8) that this strain occurs infrequently.

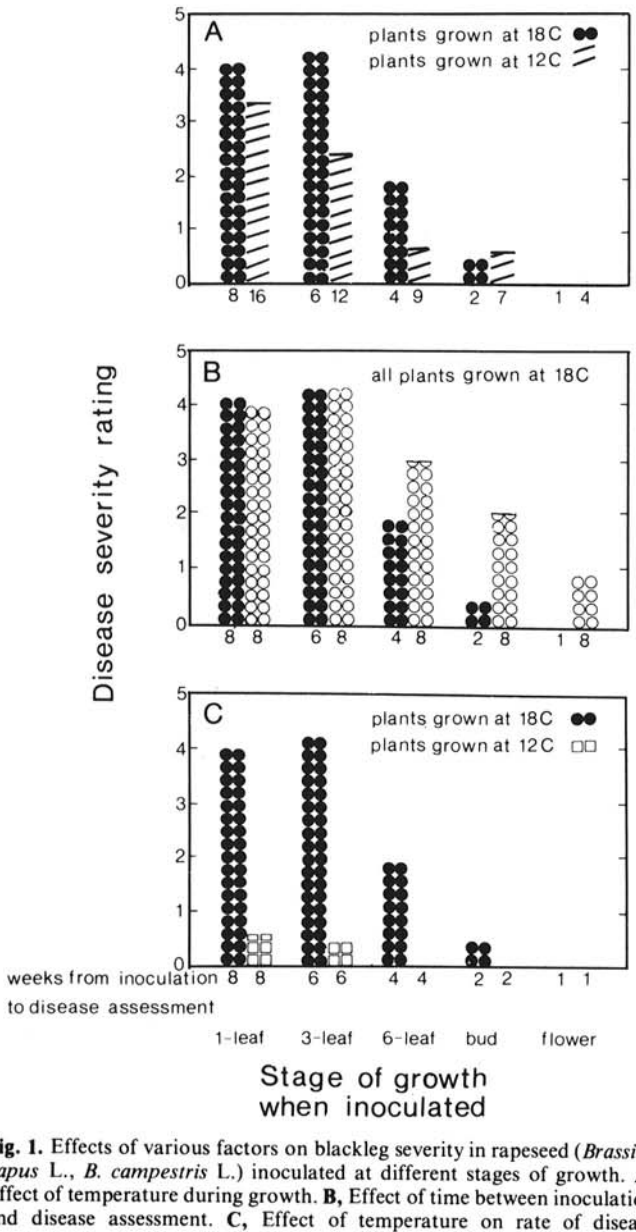

Fig. 1. Effects of various factors on blackleg severity in rapeseed (*Brassica napus* L., *B. campestris* L.) inoculated at different stages of growth. **A,** Effect of temperature during growth. **B,** Effect of time between inoculation and disease assessment. **C,** Effect of temperature on rate of disease development. Plants were inoculated at the crowns, grown in growth chambers, and rated for disease severity on a range from 0 (plants healthy) increasing in severity to 5 (crowns completely severed). Each value is the mean of four replicates.

As found by others (3,6), the earlier infection takes place, the more severe the disease in adult plants. This results primarily from crowns being more susceptible to infection on plants up to the six-leaf stage of growth than they are on older plants. Longer periods between inoculation and disease assessment were associated with increased disease severity, but this factor had little effect on the decline in disease severity with later inoculations. Although infection progressed more slowly at 12 C than at 18 C, plants were susceptible to severe blackleg infection up to the six-leaf stage of growth at both temperatures. Since average temperatures in the rape-growing districts of Canada, Australia, and France are within the range 12–18 C during the early stages of crop growth, it seems reasonable to assume that rape crops are susceptible to severe infection up to the six-leaf stage of growth in each of those countries.

Under normal cropping practices in Canada, rape is sown late in May and has six leaves by the end of June. Since ascospores of *L. maculans* are not normally discharged until July it would seem that infection would take place too late to cause severe blackleg. The field survey in 1975 supports this argument, since blackleg could not be detected in rape fields in June or July, but mild symptoms readily were found in September. The same relationship between times of ascospore production and disease development in the field occurs with stinkweed, since ascospores were discharged from stinkweed residues in April and May, and blackleg-affected plants found in June. Further evidence for the lack of pathogenicity of the stinkweed strain to rape also is apparent in these results. The sporulation pattern of the virulent strain on rape could result in plants being infected before they reach the six-leaf stage of growth. The potential therefore exists for blackleg to become more destructive in Canada. Petrie (8) noted an increase in the prevalence of the virulent strain since 1975 and observed plants with severe stem cankers in July.

Ascospore discharge from rape residues occurred earlier in the 2nd yr after crop growth than in the first. The effect of this inoculum on field disease patterns is not clear, since there are no data available regarding the persistence of rape crop residues. In Australia, however, an insignificant proportion of residue persists for more than 1 yr after crop growth (5). Possibly this is also true in Canada.

It is now possible to explain blackleg disease patterns in Australia and France on the basis of relationships between production of ascospore inoculum and susceptibility of crops to infection. In Australia rape crops sown in May or June have six leaves by mid August and ascospores are present during this period (5). These crops become severely infected whereas blackleg is less severe on crops sown later in the season when the amount of ascospore inoculum is greatly reduced (5). In France, winter cultivars normally are sown in the fall. They have six leaves by about November, and ascospores are produced during that time (1). Thus, severe blackleg infection should be expected in France.

The relationship between periods of ascospore inoculum production and susceptibility of crops to infection is more favorable for blackleg development in Australia than in Canada. This finding together with the fact that the prevalent strain of *L. maculans* in Australia is more virulent than the prevalent strain in Canada explains why blackleg has been a more serious disease of rape in Australia than in Canada.

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