Effects of temperature and moisture on the number, size and septation of ascospores produced by *Leptosphaeria maculans* (blackleg) on rapeseed stubble

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The optimum temperature for ascospore production by the virulent strain of Lepfosphaeria maculans (blackleg) on naturally infected rapeseed (Brassica napus) stubble was 15°C, although peak sporulation occurred earlier at 20°C and sporulation continued longer at 10°C. In the year after crop growth, sporulation occurred earlier as the frequency of moistening of infected stubble between April and June increased. Freezing of infected 1992 stubble from 4 September to 30 October 1992, did not affect the number of ascospores caught in 1993. The number of days with maximum temperatures of 20°C or more in the 21 days prior to ascospore discharge was negatively correlated with ascospore length and width in the virulent "rape" strain. In the "thlaspi" strain from *Thlaspi* arvense, number of days with temperature maxima over 20°C was negatively correlated with number of 5-septate ascospores. Number of days with mean temperatures between 9 and 16°C was positively correlated with ascospore length and width. LOG (spore number + 0.5) was negatively correlated with ascospore length and width. Strains of *L. maculans* from cruciferous weeds produced more ascospores per trapping date over a three year period than did the virulent strain on rapeseed stubble.

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La temperature optimale pour que la souche virulente Lepfosphaeria maculans (jambe noire) puisse produire des ascospores dans le colza (Brassica napus) sur chaume, naturellement infecte, etait de 15 °C. Cependant, la sporulation de pointe s'est deja produite a 20 "C et s'est poursuivie jusqu'a 10 "C. Dans l'année qui a suivi la recolte, la sporulation s'est produite plus t6t lorsqu'il y a eu augmentation de la frequence d'humectation du chaume infect6 entre avril et juin. Le gel du chaume infecte en 1992, entre le 4 septembre et le 30 octobre, n'a pas modifie le nombre d'ascospores preleves en 1993. Le nombre de jours, ou les temperatures maximales ont atteint 20 °C ou plus dans les 21 premiers jours qui ont precede la sortie des ascospores, est anticorrele a la longueur et a la largeur de ces derniers dans la souche virulente presente dans le colza. Dans la souche « thlaspi » originaire du *Thlaspi* arvense, le nombre de jours, ou des temperatures maximales de plus de 20 °C ont ete enregistrees, était anticorrele au nombre d'ascospores à 5 cloisons d'ascospores. Il existait une correlation positive ehtre le nombre de jours où la temperature a varie entre 9 et 16 °C et la longueur et la largeur des ascospores. LOG (nombre de spores + 0,5) etait anticorrele a la longueur et la largeur des ascospores. Les souches de *L.* maculans provenant des cruciferes (mauvaises herbes) ont produit plus d'ascospores par seance de prélèvement sur une periode de trois ans que ne l'a fait la souche virulente dans le colza sur chaume.

Introduction

A study of the initiation of ascospore production by *Leptosphaeria maculans* (Desm.) Ces. & De Not. (blackleg) on 9-month-old and older rapeseed (*Brassica napus* L. and *B. rapa* L.) stubble in Saskatchewan was started in 1975 (10). Subsequently, departures from the "typical" seasonal pattern of ascospore discharge were attributed largely to the effects of temperature and precipitation. This study further explores environmental influences on the pathogen's ascospore production and morphology.

Materials and Methods

Effect of temperature on sporulation

Stubble from a 1990 *B. napus* crop infected by the virulent "rape" strain of *L. maculans* was collected in April 1991 and stored outdoors until March 1993. During this period a relatively low level of ascospore discharge (100/10 g stubble/hour) was naturally induced in the material. This level was the background rate of discharge for the experiment. Seven stem pieces 12–14 cm long were placed

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in each of 30 plastic containers (13 cm high x 11 cm in diameter) having tight-fitting lids. A 9 cm disc of filter paper was placed in the bottom of each container and kept moistened with water. Five replicate containers of stubble were placed in each of six unlighted incubators maintained at 10, 15, 20, 24, 28, and 32°C. At intervals the stubble was transferred to five ascospore liberation tunnels (9). Air flow was adjusted to 13,000 cc/min using a Rotameter (Brooks Rotameter Co., Lansdale, PA) and ascospores caught for one hour on vaseline-coated microscope slides. Spores were stained with dilute cotton blue in lactophenol (1) prior to being counted.

Effect of frequency of moistening on sporulation

Stubble from a 1991 crop naturally infected by the virulent "rape" strain was collected in late March 1992. One 7 cm piece including a portion of the lower stem and upper taproot was cut from each plant. Random samples of 18 stem pieces were placed in each of 65 open transparent containers 13 cm high x 11 cm in diameter. There were 13 treatments each replicated five times. For 12 of the treatments, deionized water was atomized onto the stems to runoff periodically between 2 April and 30 June of 1992, and the containers drained of water. The last treatment was an unmoistened control. In six treatments the stubble was moistened 7, 13, 19, 26, 45, or 90 times at equally spaced intervals. In another six treatments the stubble was moistened on 26 consecutive days or 13 alternate days from 10 April to 5 May, May 6 to 31, or June 1–26. Between April and June the containers were kept in a greenhouse with a daily mean temperature of 15°C. After 30 June the material was transferred into well-drained plastic containers 8 cm x 11 cm in diameter and placed in a shaded location outdoors until the end of October, 1992. The samples were moistened 45 times by rainfall, 15 in July and approximately 10 times monthly from August to October. The mean temperatures were 17, 16, 10 and 4°C, respectively, for the four months from July to October. At the end of October, the samples were transferred to an unheated greenhouse until April 1993, when they were again placed outdoors. In 1993, the samples received rainfall 10 times monthly in April, May and June. Mean temperatures in April, May and June were 5, 11 and 14°C, respectively. Sporulation was tested in July, August and September 1992, and in June 1993 using ascospore liberation tunnels.

Effect of freezing on sporulation

The objective was to arrest pseudothecial development at various stages during the spring and autumn to determine the period(s) when interruption of the process caused the greatest delay and/or reduction in ascospore production. In the first experiment, stems naturally infected by virulent *L. maculans* in 1991 were frozen at -7°C for either 3 or 6 weeks between 1 April and 15 July 1992. The five 3-week freezing periods were: 1–22 April, 22 April to 13 May, 13 May to 3 June, 3–24 June, and 24 June to 15 July. The four 6-week freezing periods were: 1 April to 13 May, 22 April to 3 June, 13 May to 24 June, and 3 June to 15 July. There were five replicate pots per treatment and five unfrozen controls. When not frozen or being tested for sporulation, samples were stored outdoors. Conditions for the periods 1 July to 31 October 1992, and 1 April to 30 June 1993, were described previously. From April to June, 1992, mean monthly temperatures were 5, 10 and 15°C, respectively. Sporulation was tested as previously described in mid-October 1992, and in early June 1993. The material was left outdoors during the winter of 1992–1993 under snow cover.

The second freezing experiment of four treatments by five replicates was conducted in September and October 1992, using stubble from a 1992 crop infected by the virulent strain. One set of stems was frozen at -7°C from 4 September to 2 October, a second set from 2–30 October, and a third from 4 September to 30 October. A fourth set was not frozen. When material was not frozen, it was left outdoors, and remained outdoors from 30 October to late August 1993, when it was tested for sporulation.

Effects of temperature and moisture on ascospore numbers, size and septation

Sporulation was followed from 1991–1993 in a collection of rape (*B. napus*) stubble infected by the virulent "rape" strain of *L. maculans,* a collection of stinkweed *(Thlaspi arvense* L.) stems infected by the "thlaspi" strain, and a collection of flixweed (*Descurainia sophia* (L.) Webb) stems infected by the "sisymbrium" strain. Sporulation was tested at least once per month from April to October each year using ascospore liberation tunnels. For each collection there were five replicate samples, each consisting of eight 12 cm stem segments, which were stored outdoors in wooden flats. Shortly after each trapping date the lengths and widths of 25 ascospores taken at random were measured for each of the five replicates per collection and the number of 5-septate (normal) ascospores was recorded.

Temperature and moisture records and statistical analyses

At the site near Saskatoon where stubble samples were kept, rainfall was recorded using a Springfield rain gauge and temperature extremes recorded using a Springfield maximum/minimum thermometer (Springfield Instrument Co., Woodridge, New Jersey). Reference also was made to the Monthly Meteorological Summary for Saskatoon prepared by the Atmospheric Environment Service of Environment Canada. The following meteorological data were compiled for both the ten- and 21-day periods immediately preceding each date on which spores were collected: number of days on which the maximum

temperature reached or exceeded 20, 25 or 30°C, and the number of days on which the mean daily temperature fell between 9 and 16°C. Preliminary experiments indicated that mean daily temperatures of 9–16°C are optimal for initiation of ascospores. Total precipitation and number of days with measurable rainfall were recorded for the 16-day period from 5–21 days preceding ascospore collection. During the four days prior to trapping, the stems were moved indoors temporarily whenever there was a threat of rain. This allowed them to dry out prior to being tested.

Statistical analyses were performed using the SAS GLM statistical package procedure (SAS Institute Inc., 1989) after a LOG (no. spores + 0.5) transformation of ascospore numbers.

Results and Discussion

Effect of temperature on sporulation

Samples held at 10 and 15°C produced large numbers of ascospores over periods of 92 and 85 days, respectively (Table 1). The 10°C material required 27 days to reach maximum sporulation compared to 13 days for that at 15°C, but sporulation was maintained above the initial base level of 100 spores/h, seven days longer at 10°C. Cumulative sporulation, or total mean numbers of ascospores produced/10 g residue over all dates tested, was higher at 15°C. The samples at 20°C were much less productive than those at the lower temperatures. The sporulation maximum was reached in only six days at 20°C but sporulation exceeding the base level was maintained for only 30 days. Continuous temperatures in excess of 20°C had a profound adverse effect on ascospore production (Table 1). At 24 and 28°C there was a brief burst of sporulation after two days which lasted less than a week. At 32°C no increase in sporulation above the base level was detected.

Effect of frequency of moistening on sporulation

As the frequency of moistening of infected stubble was increased from April to June, the time intervening before the first ascospore discharge decreased (Table 2). The earliest sporulation above a trace level detected in any treatment occurred in July 1992, in series 45 and 90. Abundant sporulation began in August on stems moistened daily from 6–31 May, and in September on the other material moistened for 26 days. Material moistened 13 times in April, May, or June 1992, produced abundant spores only in June 1993. Material moistened intermittently for 19 days or less also sporulated in June 1993, with ascospore numbers decreasing as the number of moistenings decreased.

The ANOVA revealed highly significant differences among spore numbers at each of the four dates. Most of the statistical contrasts for June 1993 were highly significant (Table 2), and indicated that the relationship between the seven spaced treatments (0 to 90) was linear except for the very wet or very dry treatments. Spore numbers in the 45- and 90-day treatments had reached their maxima earlier and were declining by this time. Two contrasts that were not significant, probably as a result of variability in ascospore numbers, were 13 vs. A13, M13, J13 and 26 vs. A26, M26, J26. These results indicate that the same number of moistenings were about as effective whether applied over three months or within a single month. In the field, maximum sporulation often was reached in June of the second year after crop growth and declined thereafter. Sporulation in the 90-day treatment declined after August 1992. This material probably received too much moisture for optimal sustained ascospore production. Spore numbers were declining by June 1993. Under field conditions the May–June 1993 period would have been the first opportunity for infection at a highly susceptible stage of crop development.

Effect of freezing on sporulation

The ANOVA revealed highly significant differences in spore production among the 10 spring treatments when they were tested in October 1992, and June 1993. A significant linear relationship existed for the 3-week treatments for both dates. Such a relationship was found for the 6-week treatments only in June 1993. There were significant differences between the 3- and 6-week treatments on both dates, and both sets of treatments were significantly different from the control (Table 3). Ascospore numbers declined progressively from April to July freezing treatments, both in the 3- and 6-week series. Freezing from early or late June to mid-July resulted in relatively few spores. In years in which moisture was not limiting, this was the period in which squash mounts of pseudothecia revealed active formation of asci and ascospores. By the end of the third week of June 1992, about 25% of the stems had pseudothecia with immature asci and a relatively small number of maturing (pale yellow) ascospores. In the second half of July, mature spores were abundant.

Stubble frozen from 4 September to 30 October 1992, produced the same number of ascospores in the summer of 1993 as the unfrozen controls or material frozen only in September or in October (Table 4). Therefore, no evidence was obtained that exposure of stubble to the environmental conditions of autumn is important to ascospore formation the following year. In August 1992, temperatures reached or exceeded 20°C on 22 days, which was too warm for ascospore initiation, while the mean temperatures in October and November were 10°C and 0°C, respectively, which were too cold. The mean daily temperature most favorable for ascospore production, between 9 and 16°C, was recorded on only three days in October and 12 in September.

Effect of temperature and moisture on ascospore numbers, size, and septation

Ascospore numbers, size, and the number of 5-septate spores varied significantly with date and year in all three collections. This shows that these variables are strongly influenced by environmental conditions. In the "thlaspi" strain from stinkweed, as ascospore numbers increased from May to August 1991, spore length and width, and number of 5-septate spores declined (Figs. 1,3,4). In 1992, ascospore length increased from April to late June, declined until late September, and increased again in October (Fig. 4). The number of 5-septate ascospores followed a similar pattern (Fig. 3), as did spore numbers (Fig. 1), although in the latter case the changes were more erratic. The mean number of ascospores caught per date increased from 27.5 x 10^3 in 1991, the year sporulation started, to 43.0 x 10^3 in 1992, then dropped sharply to 4.1 x 10^3 in 1993. The highest discharge occurred in June 1992, with a mean of 108.9 x 10^3 spores/10 g stubble/1.5 h.

In the virulent "rape" strain, sporulation commenced in 1991, approximately two months tater than it had in the "thlaspi" strain (Fig. 1,5). Peak ascospore discharge (14.9 x 10^3) in the "rape" strain occurred in late July 1992, a month later than in the "thlaspi" strain. The mean annual number of spores caught per date from the "rape" strain increased from 4.5 x 10^3 in 1991, to 6.2 x 10^3 in 1992, and then declined to 0.9 x 10^3 in 1993. In 1992, spore length and number of 5-septate spores declined from April to July, increased in mid-September, then declined again by mid-October. This differed from what was observed in the "thlaspi" strain.

Separate analyses of variance were performed on data for the "rape" strain and the "thlaspi" strain. In both instances, when any one of spore number, LOG (no. spores + 0.5), spore length, spore width, spore size (L x W), or number of 5-septate spores was the dependent variable, both year and date effects were highly significant. For the "thlaspi" strain there were significant positive correlations in the combined 1991 and 1992 data, (i) between the number of days with a mean temperature of 9–16°C (in the 21-day period before ascospore trapping) and both ascospore length and width, and (ii) between the number of 5-septate ascospores and ascospore length or width (Table 5). All other significant correlations were negative, such as (i) between LOG (no. spores + 0.5), and spore width and number of 5-septate spores, and (ii) between the number of days with either a maximum temperature of 220°C (21 days prior to spore trapping), or ≥25°C (10 or 21 days prior to trapping), and the number of 5-septate ascospores. The correlations of number of days with maxima 225°C with length or width approached significance. Rainfall measurements were not significantly correlated with ascospore numbers, length, or width in the "thlaspi" strain. Number of days with measureable rainfall and LOG (no. spores + 0.5) produced the highest correlation (r = +0.45). In 1993, no significant correlations were found between spore numbers, size, or septation, and precipitation or temperature in the "thlaspi" strain. In the "rape" strain, the significant correlations in the three years' data were mainly between temperature measurements and ascospore length or width (Table 5).

In 1993, the declining ascospore numbers in the "thlaspi" and "rape" strains may indicate the exhaustion of the spore-producing capacity of the samples, possibly due to the combined effects of warm temperatures and abundant rainfall (Figs. 1,4,5). However, the "sisymbrium" strain produced relatively large numbers of ascospores in 1993 (Fig. 2). It produced mean ascospore numbers per date, of 7.9 x 10^3 in 1991, 33.7 x 10^3 in 1992, and 32.5 x 10^3 in 1993. The three year average for the "sisymbrium" strain was 24.7 x 10^3 and for the "thlaspi" strain it was 24.9 x 10^3. The "rape" strain produced the smallest number of ascospores, averaging 3.87 x 10^3 per date per year.

In the present study, ascospore numbers, size, and septation in *L. maculans* were greatly influenced by environmental factors. The "rape" strain responded to temperatures over 20°C by producing smaller ascospores, whereas elevated temperatures had a greater effect on ascospore septation in the "thlaspi" strain. When the latter produced large numbers of ascospores, spore size was often reduced. The "rape" strain consistently produced fewer and larger ascospores than the "thlaspi" strain. In the "rape" strain the optimum temperature for ascospore production was close to 15°C, although peak sporulation at 20°C occurred earlier and sporulation was sustained longer at 10°C. Others have reported similar temperature optima (7). In the present study, frequency of moistening of stubble during the spring following a crop was directly related to earliness of sporulation.

Before late June, ascocarp development could be interrupted by 3-week periods of freezing without catastrophic reductions in ascospore numbers. Longer periods of freezing suppressed sporulation. Interventions other than low temperature, such as chemical treatment, might be effective if applied during the period of ascospore maturation. In Ontario, where ascospores are produced in autumn of the year of crop growth (11), temperatures below freezing in September could, if prolonged, decrease ascospore numbers and perhaps delay pseudothecial development until the following spring. Reductions in ascospore numbers, often observed following overwintering of two year–old and older stubble residue in Saskatchewan (Petrie, unpublished), may be due to the harmful effects of protracted periods of freezing or repeated freezing and thawing. However, in the present study, freezing of fresh infected stubble from early September to late October, before sporulation had started, had no effect on ascospore numbers produced the following year.

The "thlaspi" strain began to produce ascospores earlier than the "rape" strain. This is likely related to the fact that *T. arvense* is a winter annual that often overwinters in the rosette stage of growth. Infection by the "thlaspi" strain can occur earlier, as can development of pseudothecia.

The seasonal pattern of ascospore initiation in *L. maculans* in the commercial cabbage-growing region of the midwestern United States resembles that described in Saskatchewan (10). Blackleg-infected stems from a 1978 cabbage (*B. oleracea* var. *capitata* **L.)** crop collected in April, 1979, and kept outdoors produced ascospores in July, 1979 (6). In western Canada spring rapeseed is usually sown in late May and takes 85–101 days to mature, depending upon the species and cultivar. In Ontario, spring rape is sown in early May and harvested in mid-August. Pseudothecia form on the current season's crop within a month of harvest (11). Mean annual precipitation in southern Ontario is approximately double that at Saskatoon (3), as is mean September and November precipitation (2). Mean daily autumn temperatures are higher and nights with frost much fewer in southern Ontario (2,4). In Saskatchewan, winter intervenes before sporulation begins, although there is no requirement in *L. maculans* for a cold period prior to ascospore formation.

In Australia, spring rape cultivars are often sown in July and grow through the mild wet winter and early spring, reaching maturity in about six months (5). In Europe, winter cultivars are sown in September and harvested 10 months later. Although ascocarps are usually produced from August onwards, they have been found in early June in the growing crop on exposed vascular tissue of severely cankered plants (8). In Australia and Europe the pathogen has ample time to develop in the crop, often under conditions ideal for pseudothecial development. Often in western Canada, a hot, dry summer is followed by a relatively short period of favorable September weather before falling temperatures retard development of the pathogen. The first ascospores are usually seen on stubble in June in the year following crop growth, but a hot spring may cause sporulation on stubble to be delayed until 12 or even 22 months after harvest of a crop (10, Petrie unpublished).

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Table 1. Effects of temperature on production of ascospores by the virulent strain of *Leptosphaeria maculans* on naturally infected rape (*Brassica napus*) stubble.

Temperature of incubation	Days taken to reach peak sporulation	Maximum no. spores produced/ 10g rape stubble/h ± s.d. (best trapping date)	No. days above base level*	Cumulative sporulation/10 g stubble
10°C	27	63,628 ± 15,442	92	3.40×10^5
15°C	13	68,447 ± 14,809	85	4.93×10^5
20°C	6	8,400 ± 2,698	30	0.25×10^5
24°C	2	206 ± 104	5	230
28°C	2	585 ± 201	5	748
32°C	0	67 ± 48	0	67**

* Number of days sporulation was maintained above the base level of approximately 100 spores (see text).
** This was the base level. No spores were produced after day 0 at 32°C.

Table 2. Effect of frequency of 1992 moistening of naturally infected 1991 rape stubble on ascospore production by *Leptosphaeria maculans* in 1992 and 1993.

	Mean no. ascospores discharged / 10 g stubble / h			
Code*	July, 1992	August, 1992	September, 1992	June, 1993
0	1	0	1	188 ± 99
7	1	<1	<1	91 ± 60
13	1	1	6	408 ± 258
19	1	2	1	1,130 ± 209
26	1	8 ± 2	176 ± 99	1,430 ± 935
45	52 ± 27	220 ± 86	546 ± 127	1,223 ± 318
90	262 ± 130	1,242 ± 513	961 ± 457	731 ± 395
A13	<1	0	0	243 ± 155
M13	<1	1	10 ± 11	1,038 ± 498
J13	1	<1	18 ± 30	983 ± 367
A26	3 ± 3	11 ± 8	280 ± 111	2,175 ± 680
M26	2 ± 2	633 ± 227	632 ± 200	1,471 ± 440
J26	1	14 ± 6	338 ± 143	1,094 ± 518

Statistical contrasts**			df	Mean square	F value	Pr > F
0,7,13	vs	26,45,90 (linear)	1	20.25945	80.46	0.0001
0,90	vs	13,19,26 (quadratic)	1	4.59908	18.27	0.0001
0	vs	A26	1	15.60331	61.97	0.0001
0,A26	vs	A13	1	4.39652	17.46	0.0001
0	vs	M26	1	11.11938	44.16	0.0001
0,M26	vs	M13	1	1.47861	5.87	0.0189
0	vs	J26	1	7.73220	30.71	0.0001
0,J26	vs	J13	1	2.18326	8.67	0.0048
A26	vs	J26	1	1.36754	5.43	0.0237
A26,J26	vs	M26	1	0.00127	0.01	0.9437
A13	vs	J13	1	6.30406	25.04	0.0001
A13,J13	vs	M13	1	2.27343	9.03	0.0041
13	vs	A13,M13,J13	1	0.72493	2.88	0.0957
26	vs	A26,M26,J26	1	0.11473	0.46	0.5027

* Number of times stubble moistened between 2 April and 30 June 1992.
A,M,J = all the moistenings carried out in April, May, or June on 13 or 26 days.

** Based on June 1993 data using log transformed ascospore numbers (number + 0.5).

Table 3. Effect of freezing naturally infected 1991 rape stubble for varying periods in the spring of 1992 on ascospore production by *Lepfosphaeria maculans* in 1992 and 1993.

Code	Duration of freezing*	Mean no. ascospores discharged / 10 g stubble / h	
		October, 1992	June, 1993
0	0 control	163 ± 188	2,046 ± 690
3-week treatments			
1	April 1–22	348 ± 157	1,762 ± 585
2	April 22–May 13	44 ± 15	1,324 ± 356
3	May 13–June 13	120 ± 155	1,037 ± 556
4	June 3–24	37 ± 27	722 ± 448
5	June 24–July 15	50 ± 40	81 ± 39
6-week treatments			
12	April 1–May 13	27 + 21	797 ± 685
23	April 22–June 3	10 ± 8	357 ± 369
34	May 13–June 24	1	123 ± 101
45	June 3–July 15	0	9 ± 8

Linear contrasts**			df	Mean square	F value	Pr > F
0	vs	remainder	1	17.17737	45.13	0.0001
0	vs	3-week treatments	1	5.46780	14.37	0.0005
0	vs	6-week treatments	1	35.14207	92.33	0.0001
0, 1	vs	3, 4, 5	1	26.97082	70.86	0.0001
12, 23	vs	34, 45	1	52.14598	137.01	0.0001
3-weeks	vs	6-weeks	1	36.74351	96.54	0.0001

* Stubble frozen at -7°C.
** Based on June 1993 data using log transformed (number + 0.5) spore numbers.

Table 4. Effect of freezing of blackleg-infected 1992 rape stubble at -7°C in the autumn of 1992 on ascospore production by *Lepfosphaeria maculans* in August, 1993.

Period of freezing at -7°C	No. of ascospores / 10 g rape stubble / 1.5 h, August 1993 (±s.d.)
Control (not frozen)	21,040 ± 3,118
4 September to 2 October	20,447 ± 8,285
2 – 30 October	22,303 ± 10,879
4 September to 30 October	21,079 ± 7,198

Table 5. Correlations between selected ascospore number, ascospore size and septation, temperature and moisture variables obtained from a three year study involving *Leptosphaeria maculans* from *Brassica napus* (virulent "rape" strain) and *Thlaspi arvense* ("thlaspi" strain).

Variables	Correlation coefficient, r†	
	"rape" strain	"thlaspi" strain
No. days/21 with mean temperature between 9 and 16°C and:		
ascospore length	-0.18	+0.67 *
ascospore width	-0.40	+0.75 **
No. days/21 with maximum temperature of 20°C or higher and:		
number of 5-septate ascospores	-0.44	-0.62 *
ascospore length	-0.72 *	-0.40
spore size (length x width)	-0.88 **	-0.38
No. days/10 with max. temp. ≥ 20°C and:		
ascospore length	-0.69 *	-0.46
ascospore size	-0.79 **	-0.42
No. days/21 with max. temp. ≥ 25°C and:		
number 5-septate ascospores	-0.54	-0.75 **
spore width	-0.83 **	-0.53
spore length	-0.48	-0.55
No. days/10 with max. temp, ≥ 25°C and:		
number 5-septate ascospores	-0.48	-0.68 *
spore width	-0.66 *	-0.34
spore size	-0.67 *	-0.42
No. days/16 with measurable rainfall and:		
LOG (no. spores + 0.5)	+0.64 *	+0.45
LOG (no. spores + 0.5) and:		
ascospore length	-0.47	-0.52
spore width	-0.04	-0.61 *
Spore length and:		
spore width	+0.18	+0.79 **
no. 5-septate spores	+0.46	+0.85 **
Spore width and:		
number of 5-septate spores	+0.35	+0.70 **

† Probabilities: * P 50.05; ** P≤ 0.01

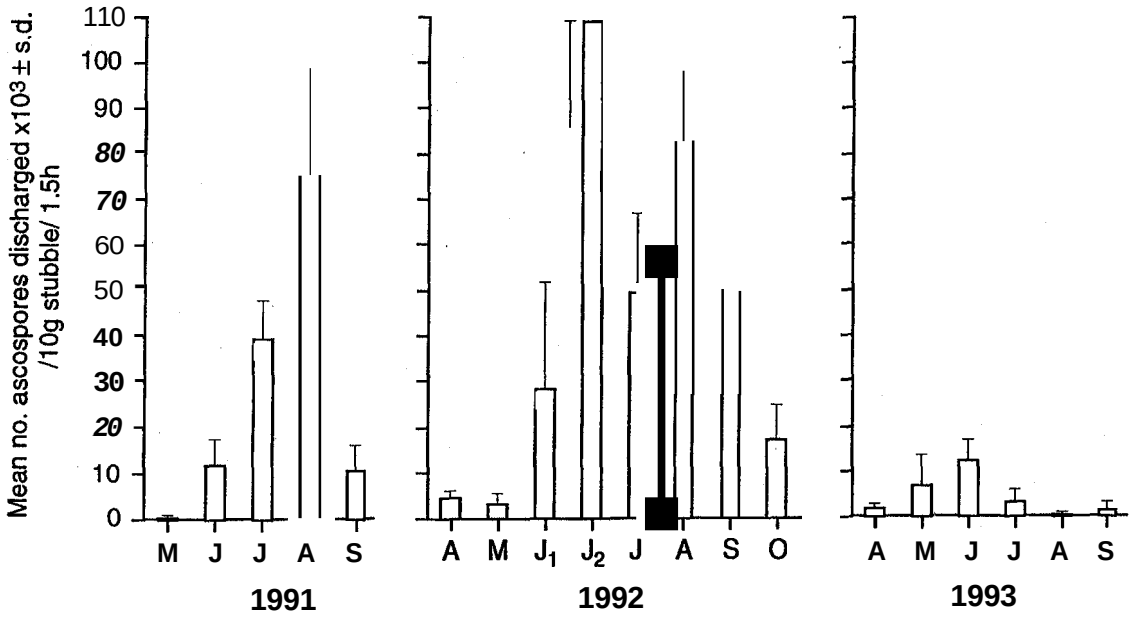

Figure 1. Mean numbers of ascospores discharged in ascospore liberation tunnels by the "thlaspi" strain of *Leptosphaeria maculans* during 1.5-h collections carried out once per month from April to September or October, 1991–93. In 1992, J_1 and J_2 represent two separate collections made in June.

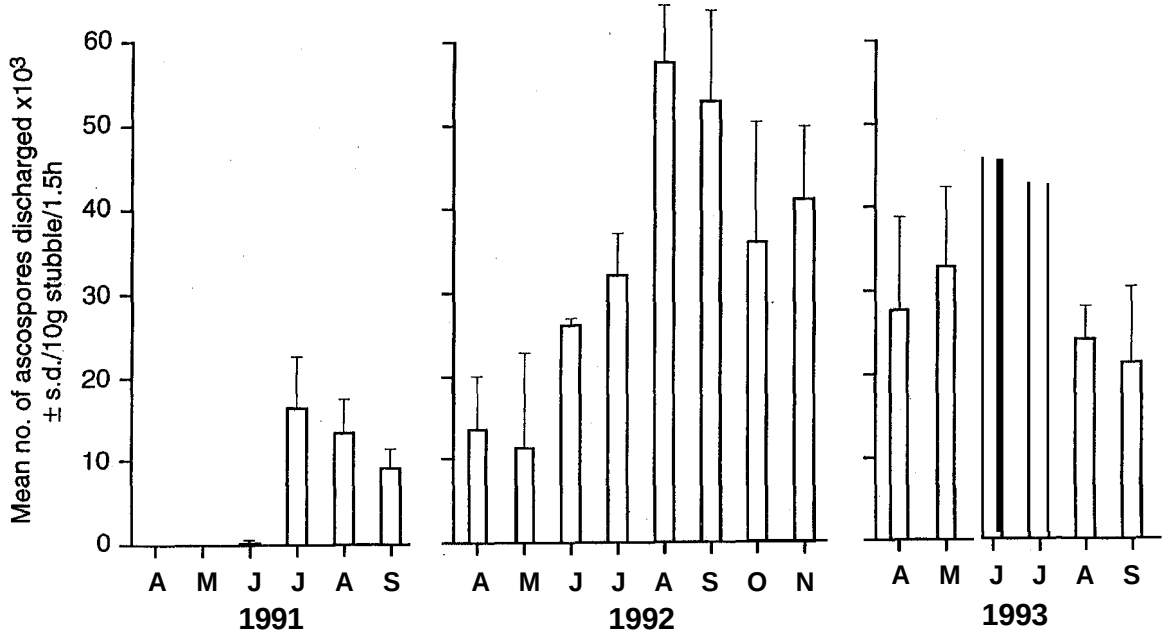

Figure 2. Mean numbers of ascospores discharged in ascospore liberation tunnels by the "sisymbrium" strain of *Leptosphaeria maculans* during 1.5-h collections made once per month from April to September or November, 1991–93.

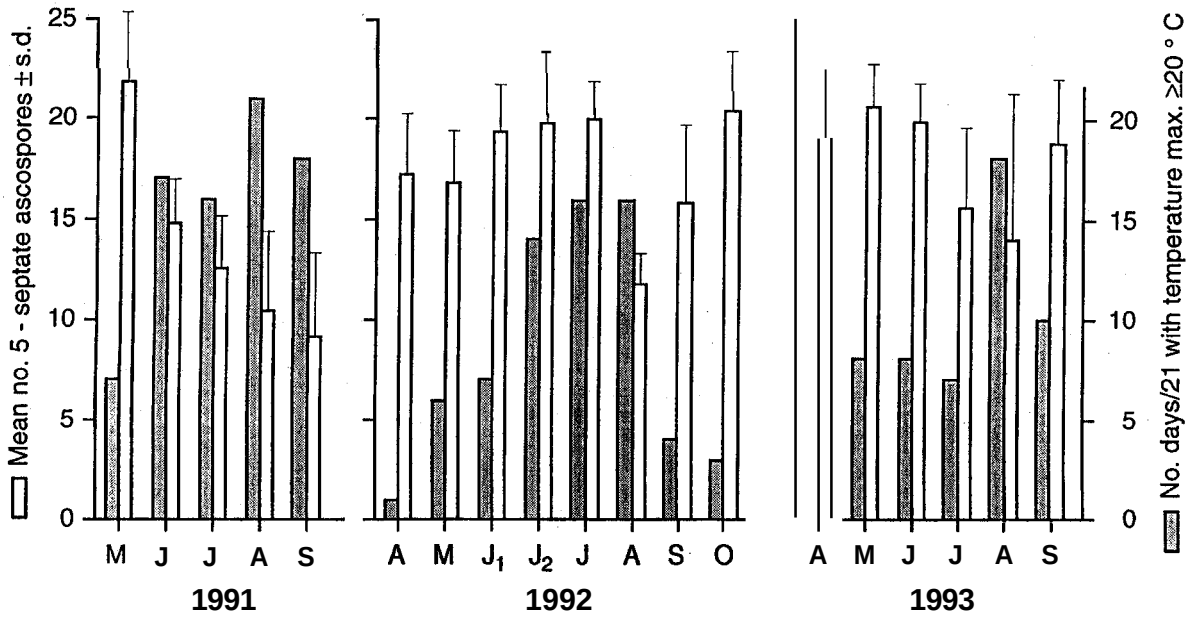

Figure 3. Variation in ascospore septation in the "thlaspi" strain of *Lepfosphaeria maculans* in relation to the number of days out of the 21 immediately preceding spore trapping that had temperature maxima of ≥20°C. In 1992, J_1 and J_2 were two separate collections in June.

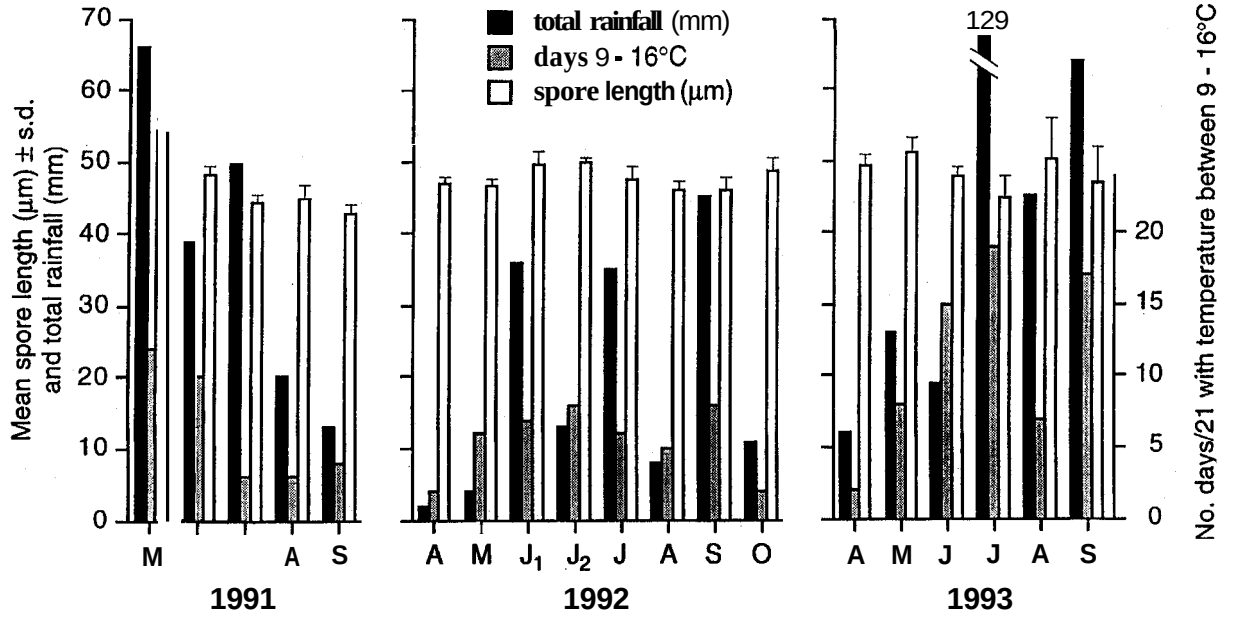

Figure 4. Variation in ascospore length in the "thlaspi" strain of *Leptosphaeria maculans* in relation to mean temperature and total rainfall. Mean temperature refers to the number of days out of the 21 immediately preceding spore collection that had a mean temperature between 9 and 16°C. Total rainfall is for the 16-day period 5 to 21 days preceding ascospore trapping.

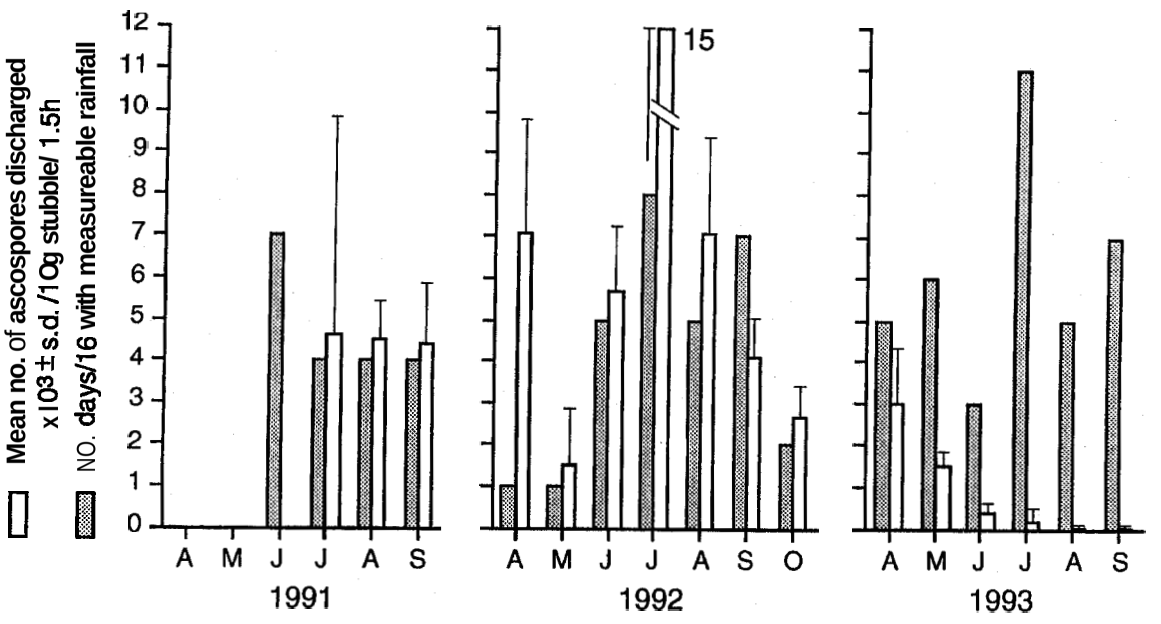

Figure 5. Mean numbers of ascospores discharged by the virulent "rape" strain of *Leptosphaeria maculans* in relation to number of days in the period 5–21 days before spore trapping with measureable rainfall.

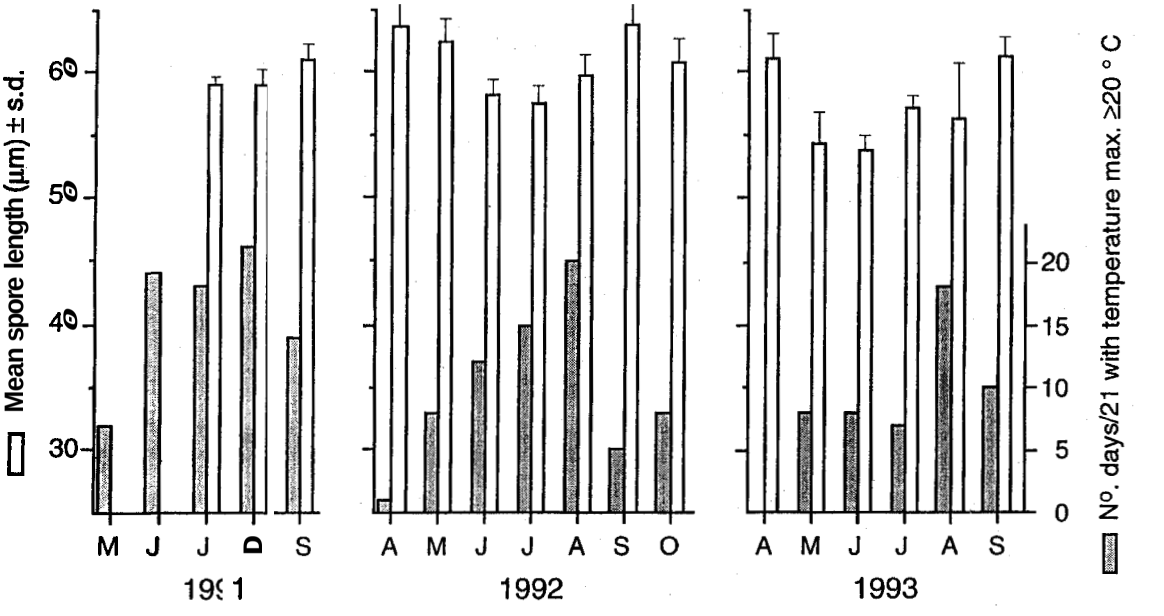

Figure 6. Variation in ascospore length in the virulent "rape" strain of *Leptosphaeria maculans* in relation to the number of days in the 21-day period immediately prior to testing that had temperature maxima of ≥20°C.