Canadian Plant Disease Survey

Inventaire des maladies des plantes au Canada

Vol. 74, No. 2, 1994

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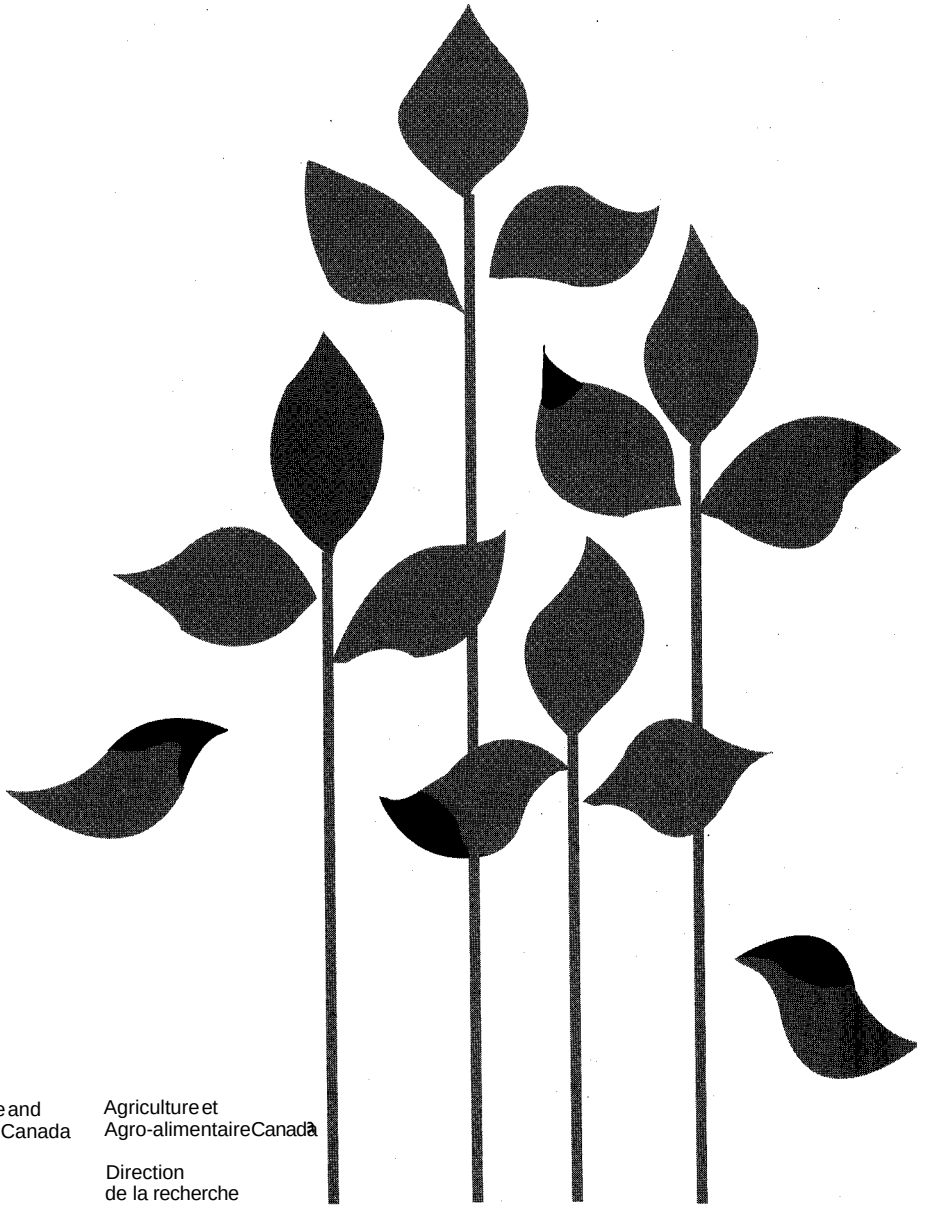

Agriculture and Agri-Food Canada
Research Branch

Agriculture et Agro-alimentaire Canada
Direction de la recherche

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Contents

The *Canadian Plant Disease Survey* is a periodical of information and record on the occurrence and severity of plant diseases in Canada and on the assessment of losses from disease. Other original information such as the development of methods of investigation and control, including the evaluation of new materials, will also be accepted. Review papers and compilations of practical value to plant pathologists will be included from time to time.

Research Branch, Agriculture and Agri-Food Canada

Editor: R.M. McNeil, B.Sc.
Compiler: B.A. Morrison
Information and Planning Services
Agriculture and Agri-Food Canada
Ottawa, Ontario K1A 0C6

L'inventaire des maladies des plantes au Canada est un periodique d'information sur la frequence des maladies des plantes au Canada, leur gravite, et les pertes qu'elles occasionnent. La redaction accepte d'autres communications originales notamment sur la mise au point de nouvelles methodes d'enqête et de lutte ainsi que sur l'évaluation des nouveaux produits. De temps a autre, il inclut des revues et des syntheses de rapports d'intérêt immediat pour les phytopathologistes.

Direction de la recherche, Agriculture et Agro-alimentaire Canada

Editrice: R.M. McNeil, B.Sc.
Compilatrice : B.A. Morrison
Services d'informations et de planification
Agriculture et Agro-alimentaire Canada
Ottawa (Ontario) K1A 0C6

Erratum

Volume 74:1, 115–116, 1994

A typographical error was introduced when Table 1 was reset. The heading within the table should read as follow:

Under the heading SURVEY RESULTS, the sub-heading TREES INFESTED, should read TREES INSPECTED.

Please correct your copy to reflect this change.

Errata

Volume 74:1, 115–116, 1994

Une erreur dans le titre du Table 1 s'est produite au moment de la recomposition.

Sous le titre SURVEY RESULTS, on devrait lire TREES INSPECTED plutôt que TREES INFESTED.

Veuillez corriger votre exemplaire en consequence.

Note from the Editor

Your response to the recent survey for the "Canadian Plant Disease Survey" was very positive. Thank you for taking the time to complete the questionnaire and for including your comments. The information you supplied is being analyzed and recommendations with regard to production locus and methods, content (including overlaps or gaps), potential funding sources and/or partnerships are expected to be made in the near future. In short, your recommendations will be incorporated to future formats. I would like to take this opportunity to thank you for your efforts.

N.B. Other publications included in this review were: the "Pest Management News", the "Canadian Agricultural Insect Pest Review", "Biocontrol News", "Insect Liberations in Canada - for classical biological control purposes", and the "Pest Management Research Report" publication on diskette.

Note de l'éditrice

Votre participation au sondage sur la brochure « l'Inventaire des maladies des plantes au Canada » a ete tres encourageante. Merci d'avoir pris le temps de remplir ce questionnaire et d'y avoir inclus des commentaires. Nous sommes en train d'analyser l'information que vous nous avez fournie. Des recommandations seront faites d'ici peu en ce qui concerne les methodes et le lieu de production, le contenu (incluant les chevauchements et les omissions) ainsi que les sources de financement ou le partenariat. Vos recommandations seront donc prises en compte dans l'elaboration de la presentation future de ce document. Je profite donc de l'occasion pour vous remercier de votre precieuse collaboration.

Nota : D'autres publications ont fait partie de cet examen. Il s'agit des « Nouvelles en lutte dirigee », de « La revue canadienne des insectes nuisibles aux cultures », des « Nouvelles en lutte biologique », des « Lâchers d'insectes au Canada - A des fins de lutte biologique classique » et du « Rapport de recherche sur la lutte dirigee » qui a ete produit sur disquette.

Fusarium stem and fruit rot of sweet pepper in Ontario greenhouses

W.R. Jarvis[1], *S.K. Khosla*[2] *and S.D. Barrie*[1]

The causal agent of a stem and fruit rot disease of greenhouse sweet peppers was found to be *Nectria haematococca* (anamorph *Fusarium solani).* It infected wounds caused by salt damage at the stem base and scars left by careless leaf removal and fruit picking. No fungicides were considered suitable for field trials after in vitro screening; none is registered in Canada for sweet peppers. Very good control was achieved by scrupulous greenhouse hygiene and improved ventilation and nutrition, and by clean-cutting leaves, axillary shoots and fruit with a sharp knife rather than breaking and tearing, which left very susceptible wounds.

Can. Plant Dis. Surv. 74:2, 131–134, ***1994.***

Nectria haematococca (l'anamorphe *Fusarium solani)* est l'agent causal de la pourriture du fruit et de la tige du poivron cultive en serre. Les blessures causees par le sel a la base de la tige et les cicatrices laissées par une cueillette et un effeuillage négligents ont ete infectees par l'agent causal. Aucun fongicide n'a semble convenir pour les essais au champ apres les tests in vitro; il n'existe pas au Canada de fongicide homologue pour les poivrons. Une tres bonne prevention contre l'infection a pu être assuree grâce aux mesures suivantes : proprete rigoureuse dans la serre; ventilation et nutrition ameliorees; coupe rase des feuilles, des nouvelles pousses et des fruits a l'aide d'un couteau bien aiguise, plutôt que d'avoir recours a l'arrachage et ainsi risquer fortement qu'une infection ne survienne.

In the summer of 1990, a severe disease appeared in a 1-ha sweet pepper crop *(Capsicum annuum* L.) cv. *Cubico* grown hydroponically in rockwool in a greenhouse. The estimated yield loss was about 50%. In subsequent years a similar disease, but less severe was noted in nearby greenhouse pepper crops. In all cases, dark brown, sunken lesions occurred at the base of the stem, at leaf-scars and at wounds left by axillary shoot removal. Infection also occurred where periderm over the scars was breached by newly emerging axillary shoots. Later in the season infection also occurred where wound periderm was breached by emerging new shoots.

Growth rate was slowed and affected plants tended to wilt on hot days. The upper leaves became characteristically slightly mottled between the veins, a symptom reminiscent of magnesium deficiency. Interveinal tissue later became necrotic. Fruits on affected plants had fine, longitudinal, pale or brown striations, and the shoulders were markedly Flattened. The calyx lobes became brown and curled upwards. Fruits rotted usually from beneath the calyx, and occasionally from the flower end. Affected fruits contained few seeds which were discoloured brown around the margin. The fruit cavity often contained whitish-pink mycelium.

Both stem and fruit lesions often had superficial whitish mycelium and sporodochia of *Fusarium solani* (Mart.) Sacc., and reddish-brown perithecia of *Nectria haematococca* Berk. & Broome.

Isolates from surface-sterilized lesions and from superficial mycelium all yielded cultures of *F. solani* and almost all isolates developed perithecia of *N. haematococca.* Reinoculation of stem wounds and of picked fruit with conidia reproduced the symptoms. Of 40 surface-sterilized discoloured seeds taken from rotting fruit, four yielded cultures of *F. solani.* No isolates were recovered from apparently healthy seed taken from fruit with the striate symptoms.

This disease resembles one reported from England and the Netherlands (1) and from Hungary (3).

1 Agriculture and Agri-Food Canada, Harrow Research Centre, Harrow, Ontario NOR 1G0.
2 Ontario Ministry of Agriculture, Food and Rural Affairs, Harrow, Ontario NOR 1G0.
Accepted for publication January 24, 1994.

Control: Fungicides

No fungicide is registered for greenhouse peppers in Canada. The results of a preliminary screening of some fungicides incorporated into potato-dextrose agar (Difco) are summarized in Table 1. Two isolates of *N. haematococca,* 268 and 269, were used but there was no significant difference between them in response to the treatments and the data for the two isolates were pooled for a multiple mean comparison. The data were transformed by a ln - 0.1 transformation.

Table 1. Radial growth (mean of four replicate measurements) of two isolates, 268 and 269, of *Nectria haematococca* on potato dextrose agar incorporating commercial fungicides.

Fungicide and rate g product L^{-1}	Radial growth of colony cm		SE
Benlate 50 WP 0.55	1.285	a*	0.075
Benlate 50 WP 0.55 + Manzate 200 2.25	1.300	ab	0.050
Benlate 75 WP 1.25	1.505	b	0.055
Ferbam 76 WP 1.5	2.415	c	0.115
Manzate 80 WP 2.25	2.525	c	0.025
Dithane M45 2.0	2.610	c	0.030
Maneb 80 WP 2.0	2.625	c	0.015
Botran 75 WP 1.25	2.650	c	0.110
Rovral 50 WP 1.0	2.700	c	0.600
Maneb 80 WP 0.75	2.960	cd	0.180
Check, no fungicide	3.980	de	0.150
Micro-Niasul 90 0.75	4.810	e	1.470

* Values followed by the same letter are not significantly different, P=0.05. All fungicides except Micro-Niasul 90 and Maneb 80 WP inhibited radial growth, the Benlate formulations significantly more so than the others.

Control: Cultural Practices

A rigorous clean-up program was established from *a priori* principles of greenhouse hygiene (2). It comprised complete removal of all crop debris, removal and steam-sterilization of rockwool bags, replacement of torn plastic sheeting on the soil surface, and complete surface sterilization of the superstructure with high pressure water spray followed by a spray of 1% sodium hypochlorite. When the new crop was planted in rockwool blocks on rockwool slabs, the fertigation emitter was placed so as to eliminate the tissue-damaging accumulation of salts left at the stem base by evaporation, where infection apparently first occurred. Pruning of excess and axillary shoots was done with a sharp knife, not by breaking and tearing which also had been observed to cause large, poorly-healing wounds very susceptible to infection. Similarly, fruit was cut, not pulled off. As the crop matured, new axillary shoots breaking through the wound periderm were cut off cleanly, not broken off, permitting new wounds to heal quickly.

In addition to these revised hygiene and pruning procedures, the environment control computer was reprogrammed to ensure that dew was never deposited, and ventilation through the crop was improved. These improvements in the environment and operating procedures resulted in insignificant levels of infection in the next two crops. The crop fertigation was also adjusted to provide a slightly lower nitrogen supply and a higher EC (electrical conductivity, reflecting the matric potential of the nutrient solution). The water supply was also adjusted to enhance root activity.

Discussion

Fusarium stem and fruit rot caused by *Nectria haematococca* (anamorph *Fusarium solani*) presents a serious threat to the rapidly expanding sweet pepper production in Ontario greenhouses. However, a strict program of crop hygiene and environment control has brought an acceptable level of disease control without recourse to fungicides.

That seed from infected fruit was found to be infested is of concern but we do not know if commercial seed is a source of inoculum. *N. haematococca,* however, is a common soilborne fungus with airborne ascospores, so that inoculum sources could be in the greenhouse or close by outside. Covering all the soil in the greenhouse with intact plastic sheeting, however, eliminates the soil source within the greenhouse, and keeping the headerhouse clean and as soil-free as possible also reduces the inoculum.

Of the fungicides assayed in vitro, only benomyl inhibited growth appreciably of two isolates of the fungus. No greenhouse trials were done and no conclusions can be drawn about fungicidal control.

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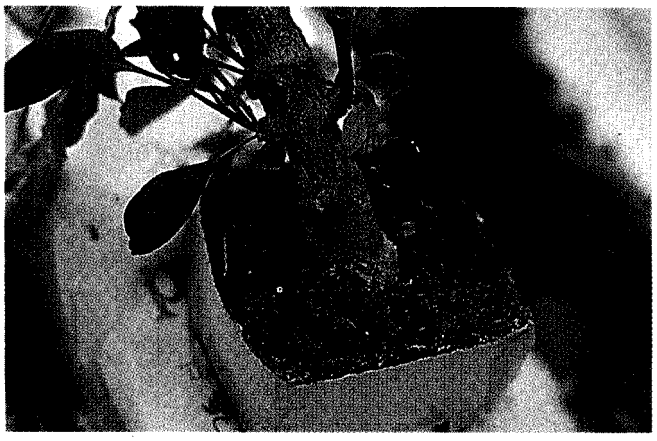

Figure 1. Fusarium stem and fruit rot of greenhouse pepper: lesion at the base of the stem at the site of salt damage.

Figure 2. Nodal lesion at a removed leaf scar.

Figure 3. Leaf symptoms resemble those of magnesium deficiency.

Figure 4. Striate symptoms on fruit from an affected plant.

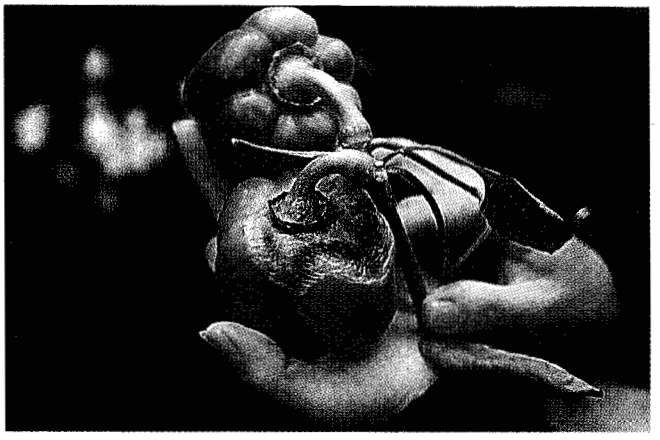

Figure 5. Fruit usually rots from the calyx end.

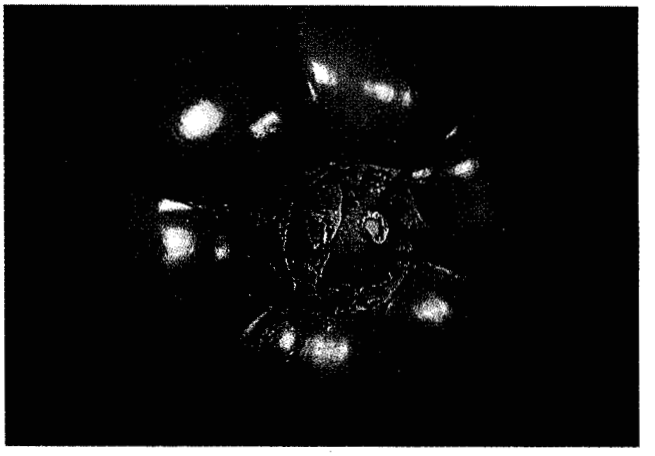

Figure 6. Incipient fruit rot beneath the calyx lobes.

Figure 7. Nodal lesion at the site of an infected fruit peduncle.

Relationship between a greenhouse and field assay for biological control of common root rot of spring wheat and barley

L.J. Duczek[1]

Bacteria and fungi, isolated from coleoptiles of seedlings of spring wheat and barley collected from commercial fields in Saskatchewan, were evaluated as seed treatments to reduce common root rot. There was no relationship between greenhouse and field trials in effectiveness of isolates in controlling common root rot. Some isolates reduced symptoms in the field, with the best showing a 61% decrease in disease compared to the control. Some isolates also reduced symptoms in the greenhouse assay. As currently described, the greenhouse assay is unsuitable as a screening technique to evaluate potential efficacy in the field.

Can. Plant Dis. Surv. 74:2, 135–140, 1994.

Des bactéries et des champignons, isoles a partir de coleoptiles de semis de ble de printemps et d'orge cueillis dans des champs commerciaux de la Saskatchewan, ont ete evalues comme traitement de semence en vue de reduire le pietin commun. Aucun lien n'a ete etabli entre les essais faits au champs et ceux en serre pour ce qui est de l'efficacite des isolats dans la lutte contre le pietin commun. Certains isolats ont reduit les symptômes au champ et, au mieux, ont fait chuter les cas de maladie jusqu'a 61 % comparativement aux sujets temoins. Des isolats ont egalement reduit les symptômes dans les essais en serre. Selon les donnees que nous possedons, l'essai en serre ne convient pas en tant que technique de depistage pour evaluer le rendement au champ.

Introduction

In the Canadian prairies, common root rot, caused mainly by *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur, anamorph *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., has been estimated to annually reduce yield of spring wheat (*Triticum aestivum* L.) by 6% *(5)* and spring barley (*Hordeum vulgare* L.) by 10% **(9).** Antagonistic microorganisms have been evaluated as biological controls for this disease. Porter (10) reported a reduction of disease in aseptically grown seedlings with a bacterium that also inhibited the growth of C. *sativus* in culture. Later reports indicated that some bacteria and/or fungi were antagonistic to C. *sativus* in vitro (1,2,4,8,11), but these organisms were not evaluated in the field.

Although several reviews indicate organisms selected from in vitro tests generally fail as biological control agents under field conditions (6,7), a rapid and reliable screening test would permit an efficient evaluation of large numbers of microorganisms. The purpose of this study was to compare the results of greenhouse screening tests with field evaluations to determine if a greenhouse test is a suitable assay.

Materials and methods

Fungi and bacteria were isolated from plants collected from commercial spring wheat or spring barley fields in Saskatchewan. In each field, plants were removed from four sites 10 m apart. In **1990,** plants were collected at the two-leaf to tillering stage (June 6-21) from 79 fields. In **1991,** plants were collected over the same growth stages (June **5–19)** from 44 fields. The coleoptiles were removed from the plants, washed in running tap water, and surface treated for 2 min in 0.6% sodium hypochlorite, and rinsed twice in sterile distilled water. For the isolation of fungi, coleoptiles were plated on Difco Potato Dextrose Agar (PDA) supplemented with 100 mg/L streptomycin sulphate and 50 mg/L of vancomycin hydrochloride. Plates were incubated at 20°C in the dark for 2 to 6 days and then hyphal tips of randomly selected fungi were transferred onto slants of PDA. Cultures of known pathogens and of mucoraceous fungi were discarded and 177 isolates from the 1990 collection and 101 isolates from **1991** were retained for the assays.

1 Agriculture and Agri-Food Canada, Saskatoon Research Centre, **107** Science Place, Saskatoon, Saskatchewan **S7N** 0X2.
Contribution No. **1096,** Saskatoon Research Centre.
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Bacteria were isolated from coleoptiles by grinding ten washed coleoptiles in 2 mL of sterile distilled water in a glass tube and pestle tissue grinder. The homogenate was poured into 100 mL of sterile distilled water, mixed by shaking and 4 mL was pipetted into 100 mL of warm molten Difco Nutrient Agar (NA). The agar-homogenate suspension was poured into six plates. After one or two days of incubation at 20°C, individual randomly selected colonies were transferred to NA slants. Eighty-two unidentified bacterial isolates were selected from the 1991 plant collections and maintained for the assays.

For the greenhouse assays, inocula from the fungal slants were placed on PDA plates and incubated for about four weeks. Most of the fungi produced myceliogenic colonies. A 2.5 mL aliquot of 2% methocel and 80 seeds of the spring barley cultivar cv. Melvin were added to a single plate of each isolate. The surface colony was scraped with a spatula and the fungal suspension was mixed with the seeds. Bacterial isolates were grown for about one week on NA plates and 80 barley seeds were added to each plate and stirred with a spatula. The seeds inoculated with the fungal or bacterial suspensions were air dried for 16 h before planting. Twenty seeds were placed at a 6 cm depth in 13-cm diameter pots containing a soilless mix (12) and covered with a 6 cm layer of the *C. sativus* inoculated sand and soilless mix. The *C. sativus* culture was isolated from a naturally infected subcrown internode of wheat. The pathogen was incubated for 10 or more days on minimal medium agar (13) in 9-cm petri plates. A 0.1% Tween 20 solution was added to each plate and the colony was scraped with a bent glass rod. The suspension was filtered through two layers of cheesecloth and the spore concentration estimated with a haemocytometer. Conidia were sprayed onto a soilless mix and sand (2:1 ratio) mixture as it was being tumbled in an electric cement mixer to a concentration of 200 *C. sativus* conidia/cm^3. There were four replicate pots of each test isolate. Because of space limitations, 20 isolates were screened concurrently, along with controls (untreated or methocel treated seed) in a greenhouse at 15–25°C with 16 h of natural light supplemented with high pressure sodium lighting at 9000 lux. After five weeks, disease symptoms on subcrown internodes were rated using the Horsfall-Barratt rating system (3).

The viability of isolates on seeds was only determined for some of the isolates by plating treated seeds on PDA for fungal isolates and NA for bacterial isolates. Only isolates which grew from all seeds were retained for further testing.

From the 1990 collection, 24 fungal isolates which showed a wide range of reaction$ in the greenhouse assays were selected for testing in the field in 1991 and of the 1991 collection, all 101 isolates were tested in the field in 1992. All 82 bacterial isolates from the 1991 collection were tested in the greenhouse and then in the field in 1992.

For the 1991 field test, 3 mL of 2% methocel along with 250 seeds of the spring wheat cv. Leader were added to each of two plates of each fungal isolate. The seeds were coated as previously described, air-dried for one to three days and 125 seeds were planted on May 30 in a single 2-m row plot. There were four replicates of seeds inoculated with each organism in a completely randomized block design. Seed was planted about 6 cm deep in a common root rot disease nursery site at the Saskatoon Research Centre farm. Untreated seed and methocel treated seed were planted as controls. At the firm dough stage (August 26–29), subcrown internodes of 40 plants per plot were rated for disease. Disease reaction was expressed as the percentage of plants with more than 50% of the subcrown internode covered with brown lesions typical of *C. sativus.* A similar test using seeds of the barley cv. Melvin was included in the field assays.

For the 1992 field test, only the wheat cv. Leader was used. Because of the large number of isolates, both the fungal and bacterial isolates were divided among three tests, each with an untreated control and an additional methocel control in the case of the fungal tests. Planting occurred on May 14, 1992 and subcrown internodes were rated August 13–26 at the soft dough stage.

Data were analyzed using the analysis of variance procedure and the least significant difference was used to determine isolates which differed significantly from the control (SAS Statistics, Version 6.07, SAS Institute, Box 8000, Cary NC 27511-8000). Isolates of both fungi and bacteria were then ranked based on the percentage control over the untreated control for bacteria and the methocel control for fungi. Values obtained from the greenhouse tests were compared with the results from the field tests using Pearson product-moment correlation.

Results and discussion

In the greenhouse assay, values for disease severity varied from 6 to 143% of the methocel control for the 1990 collection of fungal isolates (Fig. 1). For isolates 441A, 441B, 443-2, 443A, 444C, 445A, 447A and 471, disease severity was significantly ($P=0.05$ or 0.01) lower than the methocel control. In the 1991 field tests, disease severity values ranged from 68–128% of the control on wheat and from 89 to 99% on barley. There were no differences between the disease rating of the methocel control to that of any of the isolates in the field tests. The correlation between the greenhouse barley assay and the field test was -0.274 for wheat and 0.027 for barley ($n=24$). These correlations were not significant.

This study and others (Duczek unpublished) show a narrow range of disease reaction to C. *sativus* on barley in the field relative to wheat. In contrast, disease reaction in the greenhouse was more consistent on barley, and barley checks had higher levels of disease than wheat checks. For this reason in further studies, barley was used in the greenhouse and wheat in the field.

In the greenhouse assay, values for disease severity ranged from 27 to 165% of the methocel control for fungal isolates from 1991 (Fig. 2). Disease severity for 17 isolates was significantly (P=0.05) lower than the methocel control and for three isolates disease severity was significantly higher. Significance and level of significance does not appear consistent by rank in Fig. 2 perhaps because isolates were measured in several separate tests. For example, isolate 541, which was used in one test, was not significantly lower than the control, whereas isolate 546, which was used in another test, was significantly lower but its value was higher than for isolate 541. In the field tests of the same isolates, the disease severity range was 39 to 211%. For one isolate, 544A, disease levels were significantly (P=0.05) lower than the methocel control and for seven isolates significantly higher. Disease was significantly reduced compared to the methocel control in both the greenhouse and the field tests for only isolate 544A. For isolates 528A and 543, disease was significantly reduced in the greenhouse compared to the control but significantly increased in the field. The correlation between the greenhouse assay and the field test was -0.061 (n=101). This correlation was not significant. The overall mean disease rating of the methocel control in all the greenhouse tests was 43 and in the three field tests it was 24. The difference between the untreated and the methocel treated control was not significant.

For the bacterial isolates, disease severity values ranged from 42 to 151% of the controls in the greenhouse assay and from 51 to 191% in the field (Fig. 3). For six isolates disease severity was significantly (P=0.05) lower and for one isolate significantly higher than the control in the greenhouse assay. In the field, only isolate B553B, was significantly lower than the control and three isolates were significantly higher. For two isolates, B554B and B562, disease levels were significantly lower than the control in the greenhouse but significantly higher in the field. The correlation between the greenhouse assay and the field test was -0.144 (n=82). This correlation was not significant. The mean disease rating of the methocel control overall greenhouse tests was 43 and overall three field tests it was 28.

To confirm the causal organism in the field tests, five discoloured subcrown internodes were taken from randomly selected plots in each test; C. *sativus* was isolated from over 90% of the internodes.

Candidate organisms for biocontrol were isolated from the coleoptiles of young plants, since this is the site where interactions will determine success of primary infections by C. *safivus*. Some of the isolates of bacteria and fungi significantly decreased common root rot symptoms in the greenhouse. However, there was no relationship between the reaction in the greenhouse with that in the field, indicating that the greenhouse assay was unsuitable as a primary screening technique. In the field, some isolates also reduced common root rot and the lowest rating in 1991 was 68% of the control in 1991, while in 1992 it was 39%. Based on these results, all future screening will be done in the field until an appropriate greenhouse or laboratory test is developed.

Acknowledgements

The technical assistance of **L.L.** Jones-Flory and S.L. Reed is gratefully appreciated.

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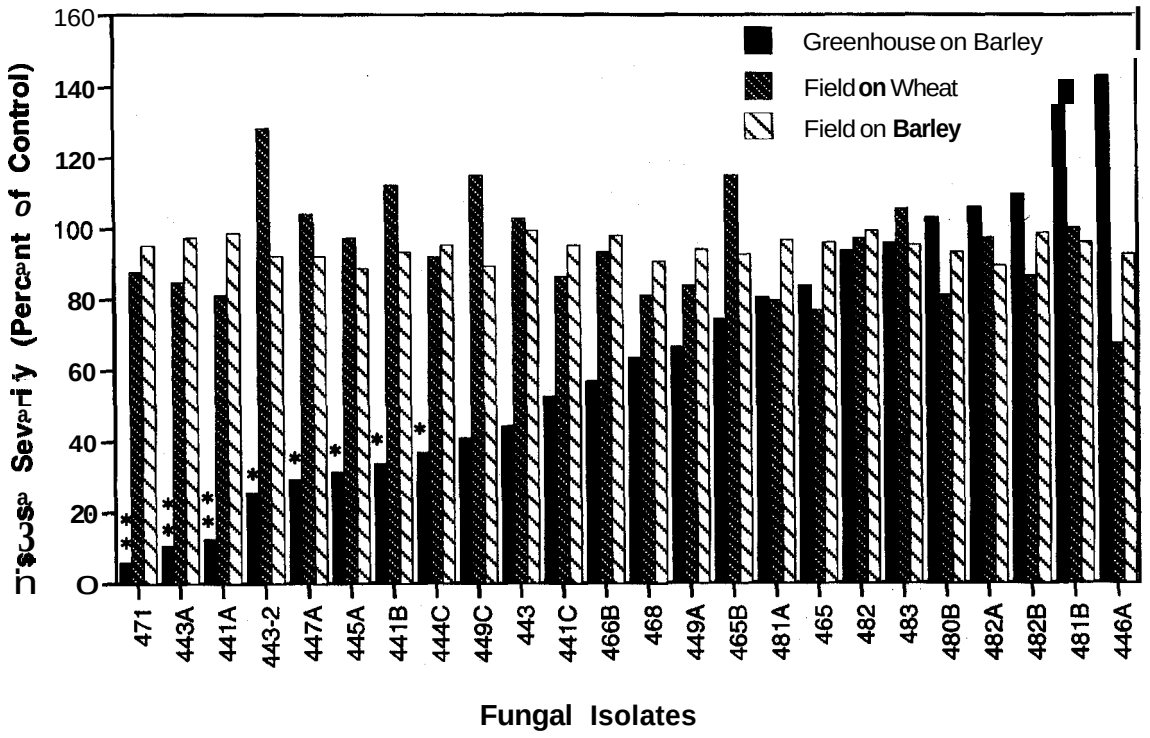

Figure 1. Effect of fungi isolated from cereal coleoptiles in 1990 on common root rot of wheat and barley in the greenhouse, and in the field in 1991. Fungi were applied to the seed. Asterisks over columns indicate a significant difference from the methocel control (* indicates P=0.05, ** indicates P=0.01).

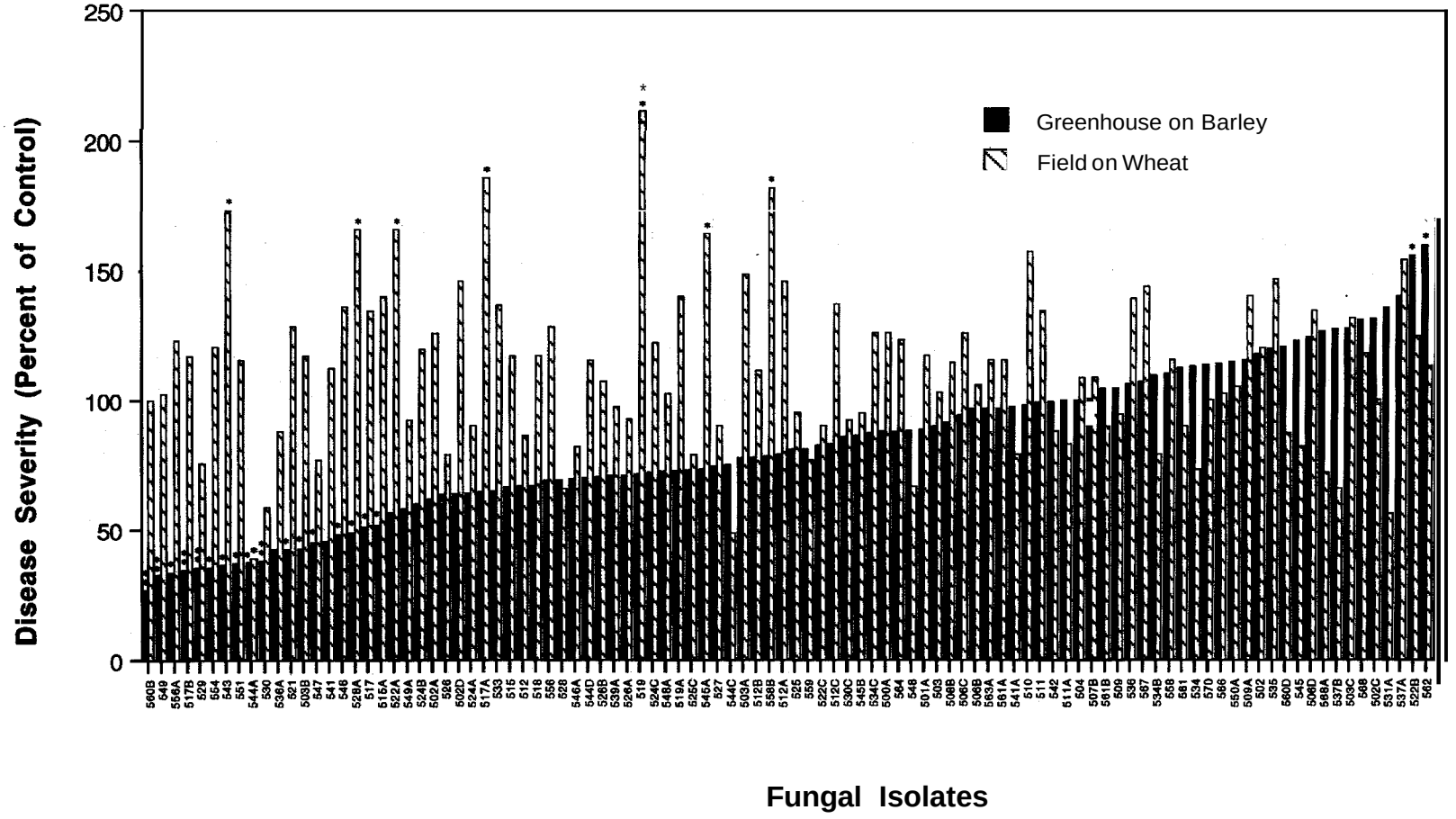

Figure 2. Effect of fungi isolated from cereal coleoptiles in 199 on common root rot of wheat and barley in the greenhouse, and in the field in 1992. Fungi were applied to the seed. Asterisks over columns indicate a significant difference from the methocel control (* indicates P=0.05, ** indicates P=0.01).

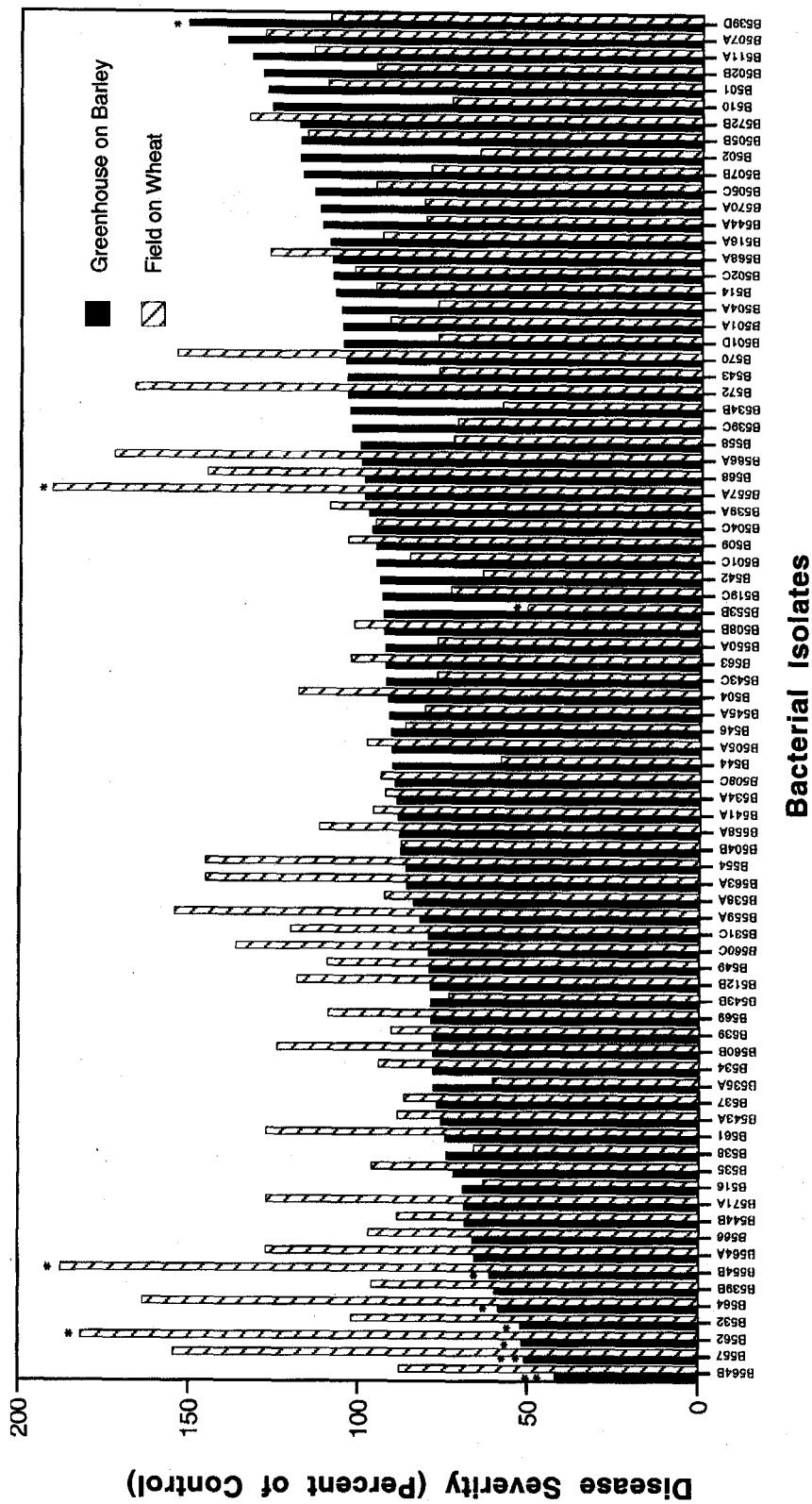

Figure 3. Effect of bacteria isolated from cereal coleoptiles in 1991 on common root rot of wheat and barley in the greenhouse, and in the field in 1992. Bacteria were applied to the seed. Asterisks over columns indicate a significant difference from the untreated control (* indicates P=0.05, ** indicates P=0.01).

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

G.A. Petrie[1]

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

1 Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, Saskatchewan S7N 0X2.
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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

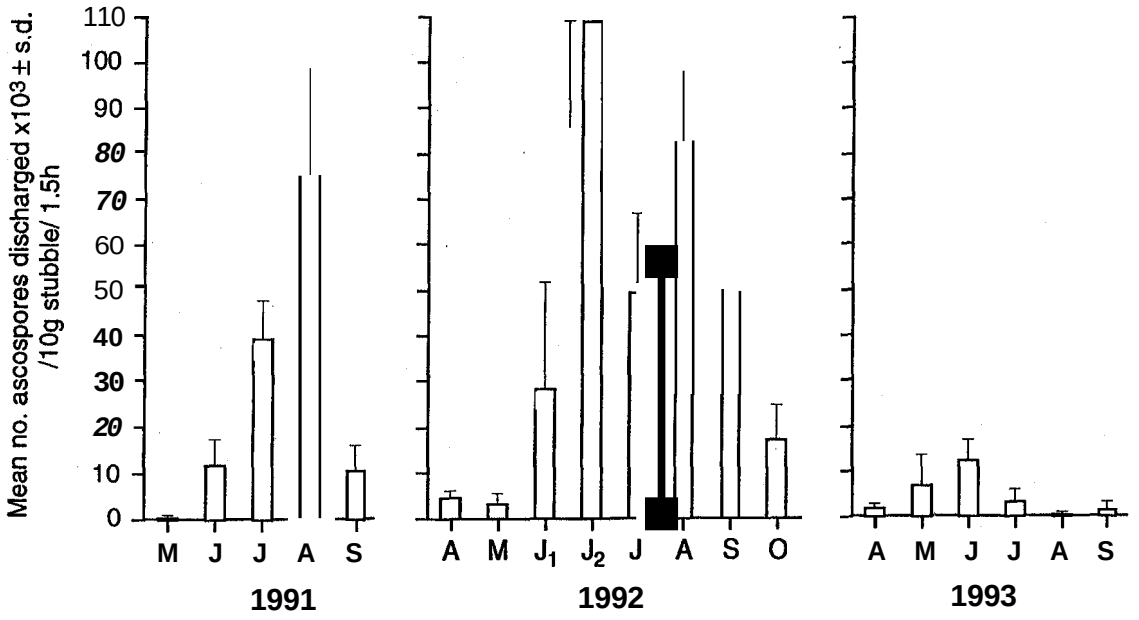

Figure 1. Mean numbers of ascospores discharged in ascospore liberation tunnels by the "thlaspi" strain of *Leptosphaeria maculans* during 1.5-h collections carried out once per month from April to September or October, 1991–93. In 1992, J_1 and J_2 represent two separate collections made in June.

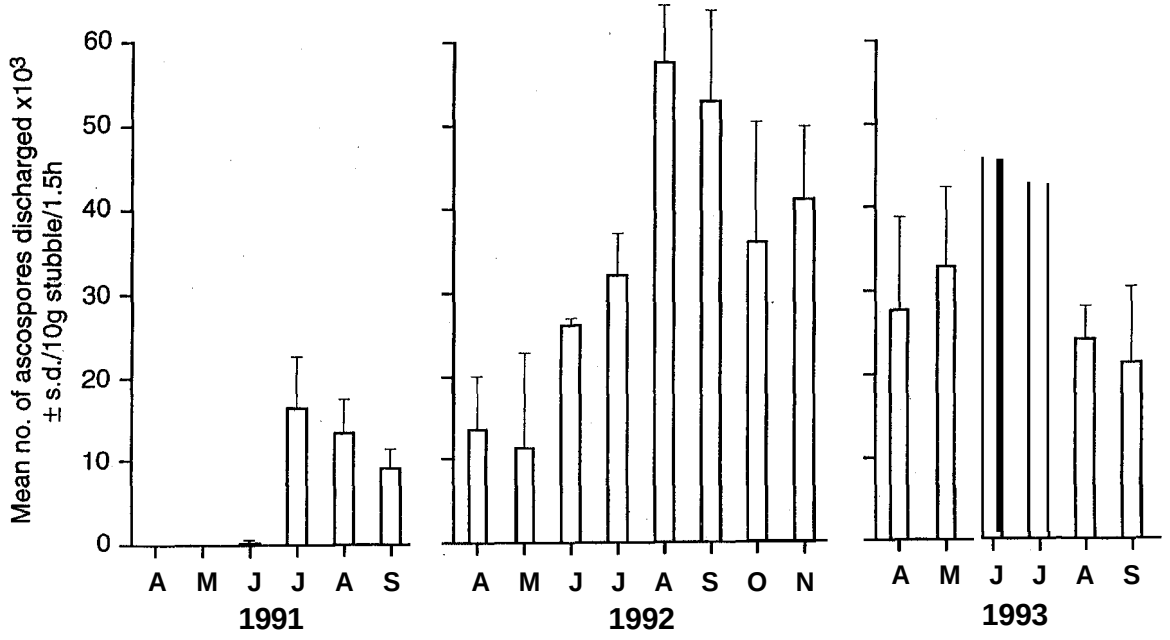

Figure 2. Mean numbers of ascospores discharged in ascospore liberation tunnels by the "sisymbrium" strain of *Leptosphaeria maculans* during 1.5-h collections made once per month from April to September or November, 1991–93.

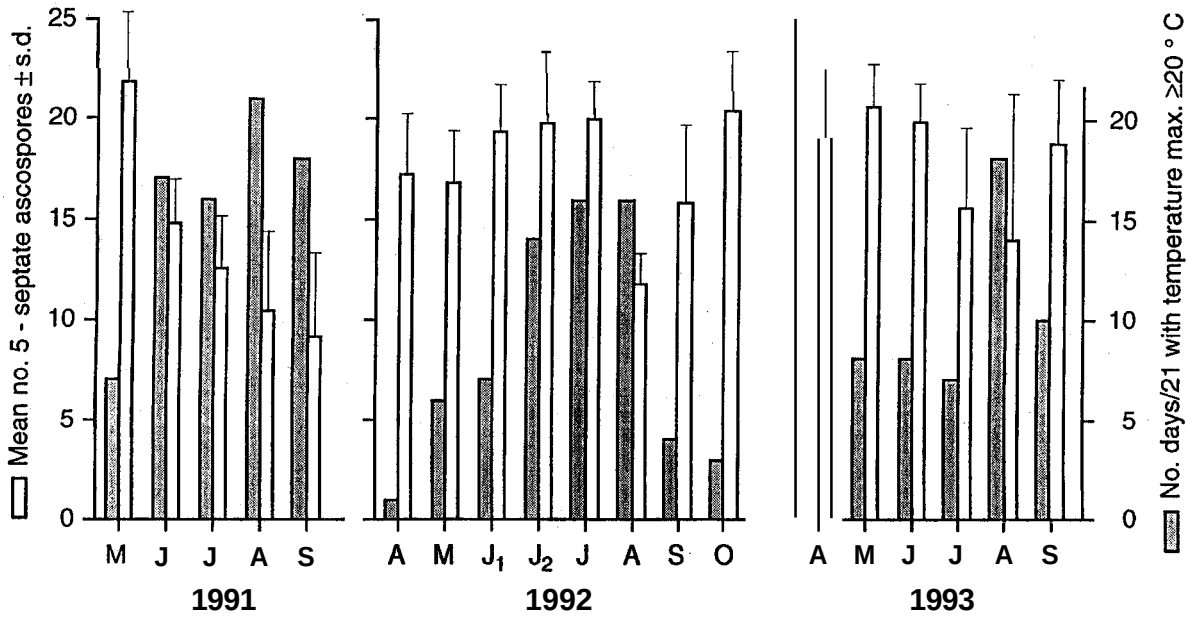

Figure 3. Variation in ascospore septation in the "thlaspi" strain of *Lepfosphaeria maculans* in relation to the number of days out of the 21 immediately preceding spore trapping that had temperature maxima of ≥20°C. In 1992, J_1 and J_2 were two separate collections in June.

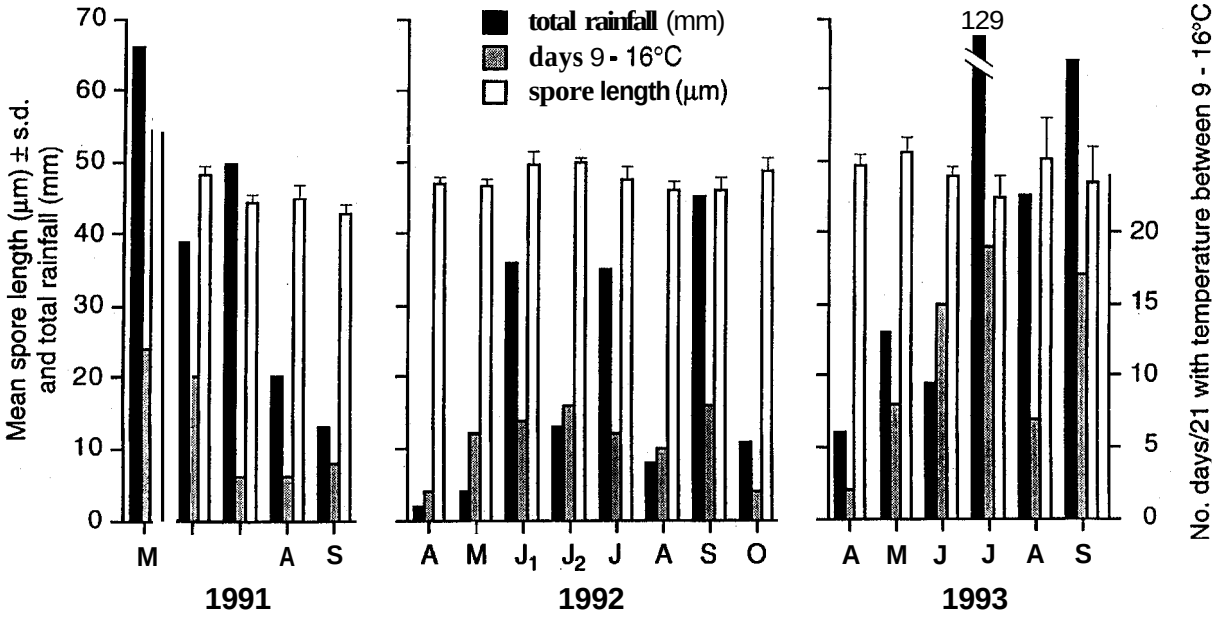

Figure 4. Variation in ascospore length in the "thlaspi" strain of *Leptosphaeria maculans* in relation to mean temperature and total rainfall. Mean temperature refers to the number of days out of the 21 immediately preceding spore collection that had a mean temperature between 9 and 16°C. Total rainfall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

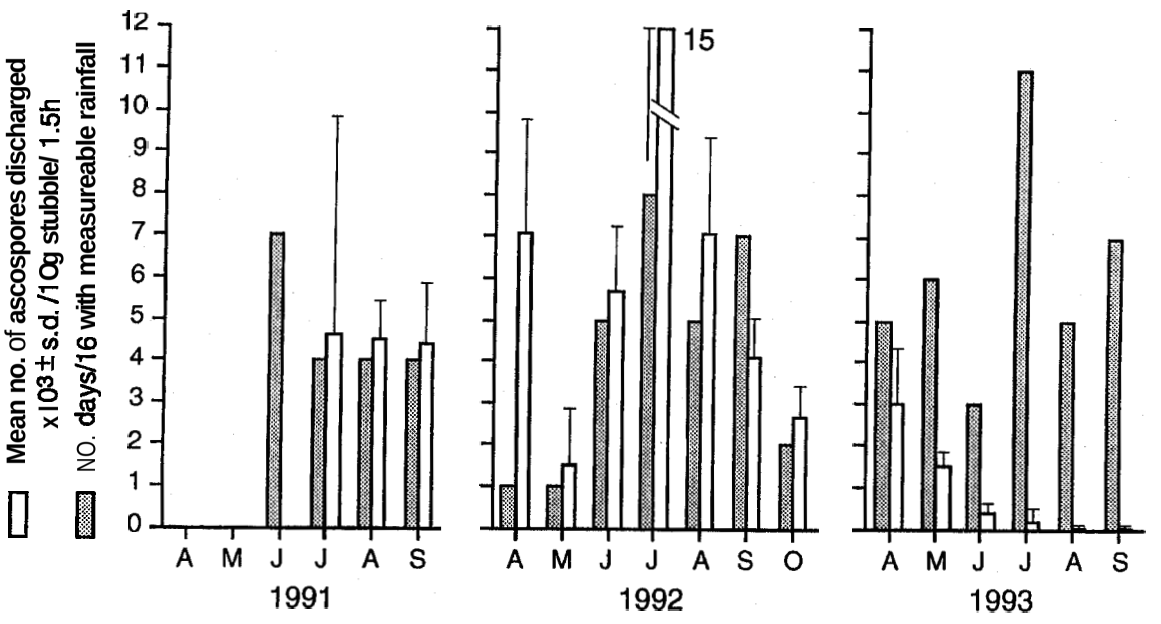

Figure 5. Mean numbers of ascospores discharged by the virulent "rape" strain of *Leptosphaeria maculans* in relation to number of days in the period 5–21 days before spore trapping with measureable rainfall.

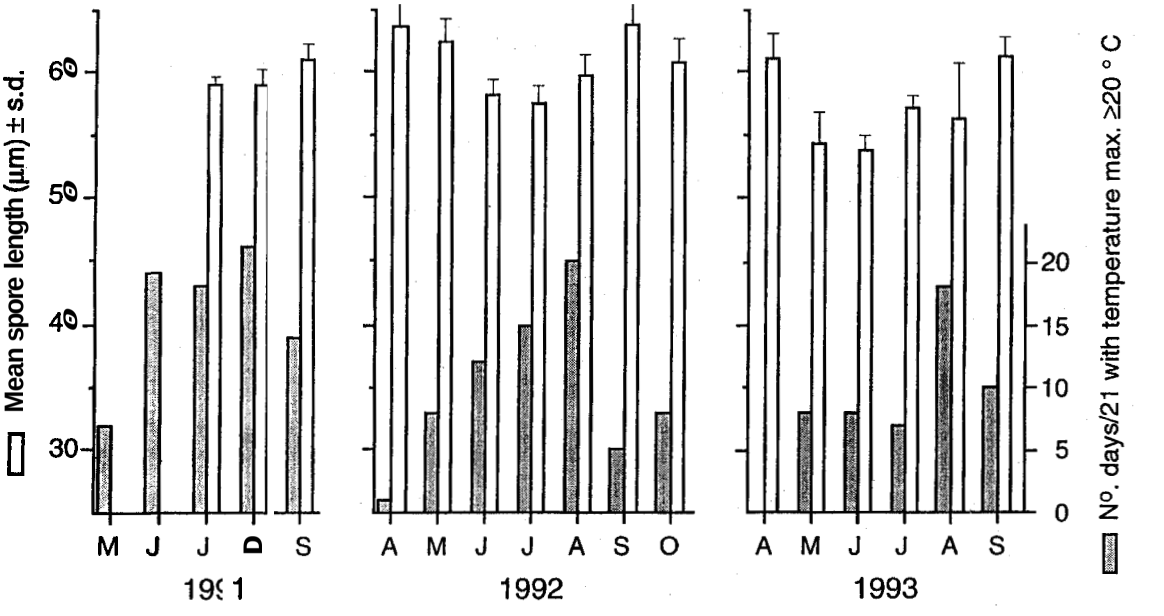

Figure 6. Variation in ascospore length in the virulent "rape" strain of *Leptosphaeria maculans* in relation to the number of days in the 21-day period immediately prior to testing that had temperature maxima of ≥20°C.

Author Index to Volume 74

Index des auteurs (volume 74)

Instructions to authors

The Canadian Plant Disease Survey is published twice a year, presenting articles on the occurrence and severity of plant diseases in Canada. Topics of interest include development of methods of investigation and control, including the evaluation of new materials. Original information, review papers and compilations of practical value to plant pathologists are accepted.

Peer reviewed articles and brief notes are published in English or French. Address the manuscript and all correspondence to Ms. Rosalyn McNeil, Information and Planning Services, Research Branch, Agriculture and Agri-Food Canada, Ottawa, Ontario K1A 0C6. Signatures of authors and the director of the establishment where the work was carried out should be supplied.

Diskette submission requirements. Please use a 3.5-inch IBM-compatible diskette. The diskette will be returned with author proofs. Send two letter-quality double-spaced printouts of the manuscript and a diskette containing all typed text, tables, figure and photo captions. Save the file, containing a single-spaced version of the article, in Wordperfect, if possible. Alternatively, save the file in ASCII format, instead of in the program's normal format. Consult your software manual for instructions on saving documents as ASCII files (sometimes called DOS files or printer files). Please label your diskette accordingly and indicate the document's full file name, including its extension.

Manuscripts should be concise and consistent in style, spelling, and use of abbreviations. They should be printed double-spaced throughout. Number all pages, including those containing abstract, tables, and legends. For general format and style, refer to recent issues of the Survey and to the *CBE* Style Manual 5th ed., 1983. Whenever possible, give numerical data in metric units (SI). Alternatively, provide the metric equivalents. Use square brackets to enclose the scientific name of a pathogen, following the common name of a disease, to denote cause.

Titles should be concise and informative, providing, with the abstract, the key words most useful for indexing and information retrieval.

Abstracts of less than 200 words should accompany each article, and should be provided in both English and French, if possible.

Figures should be planned to fit, after reduction, into one column (maximum 84 x 241 mm) or two columns (maximum 175 x 241). Trim them or add crop marks to show only essential features. Mount figures grouped in a plate tightly together, with no space between them. Provide a duplicate set of unmounted photographs and line drawings. Identify figures by number, author's name, and abbreviated legend.

Tables should be numbered using arabic numerals. Provide a concise title. Do not use vertical rules. Identify footnotes by reference marks (*†§#¶**‡), particularly when they refer to numbers.

Literature cited should be listed alphabetically in the form appearing in current issues. Either the number system or the name-and-year system may be used. For the abbreviated form of titles of periodicals, refer to the most recent issue of *Biosis* List of Serials published by Biosciences Information Service of Biological Abstracts or to the NCPTWA Word Abbreviation List, American National Standards Institute.

Recommandations aux auteurs

L'Inventaire des maladies des plantes *au* Canada est publié deux fois par année et contient des articles sur l'incidence et la gravite des maladies des plantes au Canada. Les articles portenl surtout sur la mise au point de nouvelles methodes d'investigation et de lutte comportant l'évaluation de nouveaux materiaux. Nous acceptons aussi des donnees de premiere main, des comptes rendus critiques de publications et les compilations qui peuvent être utiles aux phytopathologistes.

Les comptes rendus critiques et les courts resumes sont publiés en anglais et en franpais. Adresser le manuscrit et toute la correspondance a mademoiselle Rosalyn McNeil, Service aux programmes de recherches, Services d'information et de planification, Agricultureet Agro-alimentaire Canada, Ottawa (Ontario) K1A 0C6. Vous devez aussi nous faire parvenir la signature des auteurs et du directeur de l'etablissement oh le travail a ete effectue.

Exigences pour *la* soumission des *disquettes*. Veuillez, utiliser une disquette IBM-compatible 3.5 pouces. La disquette vous sera retournee avec les corrections de l'auteur. Envoyer deux copies du manuscrit qualite lettre tapees a double interligne et une disquette contenant tout le texte, les tableaux, les figures et les photos. Sauvegarder le fichier contenant une version de l'article a simple interligne en Wordperfect si possible. Sinon, sauvegarder le fichier en format ASCII au lieu du format normal du programme. Dans votre manuel, voir les instructions de sauvegarde de documents en fichier ASCII (parfois appeles fichiers DOS ou fichiers de l'imprimante). Veuillez etiquetter votre disquette en consequence et indiquer le nom complet du fichier du document incluant sont extension.

Les *Manuscrits* doivent être concis et faire preuve de coherence dans le style, l'orthographe et l'emploi des abreviations. Ils doivent être dactylographies a double interligne. Numeroter toutes les pages incluant celles du résumé, les tableaux et les légendes. Pour plus de renseignements sur le format des feuilles et le style, priere de consulter nos dernieres publications de L'inventaire et le *CBE Style* Manual 5ième éd., 1983. Dans la mesure du possible, soumettre les donnees numeriques en unites metriques, (SI). Sinon, fournir l'équivalent metrique. Utiliser des crochets pour identifier le nom scientifique d'un pathogene apres le nom commun de la maladie dont il est l'agent causal.

Les titres doivent être courts et revelateurs, ainsi que le resume qui les accompagne et les mots cles les plus utiles pour le classement et l'extraction de l'information.

Chaque resume de moins de 200 mots devrait accompagner chaque article et devrait être redige en anglais et en franpais si possible.

Les figures doivent pouvoir, apres reduction, entrer dans une colonne (maximum 84 x 241 mm) ou deux colonnes (maximum 175 x 241). Decoupez les figures ou indiquez par des lignes quelle est la portion essentielle de la figure. Monter les figures groupees sur une planche côte a côte sans espace entre elles. Fournir un double des photographies non montees et des graphiques. Les figures doivent etre numerotees, porter le nom de l'auteur et une legende abregee.

Les tableaux doivent être numerotes en Chiffres arabes. Fournir un titre concis. Ne pas utiliser de lignes verticales. Identifier les renvois par un signe typographique (*†§#¶**‡), particulierementlorsqu'on réfère aux nombres.

Les references *bibliographiques* devraient être citees par ordre alphabetique comme dans les livraisons courantes. On peut utiliser le systeme de numeration ou le systeme nom-et-année. Pour l'abrege du titre des periodiques, on suivra l'édition la plus recente de *Biosis* List *of* Serials publiee par les Biosciences Information Services of Biological Abstracts ou la NCTWA Word Abbreviation List et l'American National Standards Institute, Standards Committee.