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Inventaire des maladies des plantes au Canada

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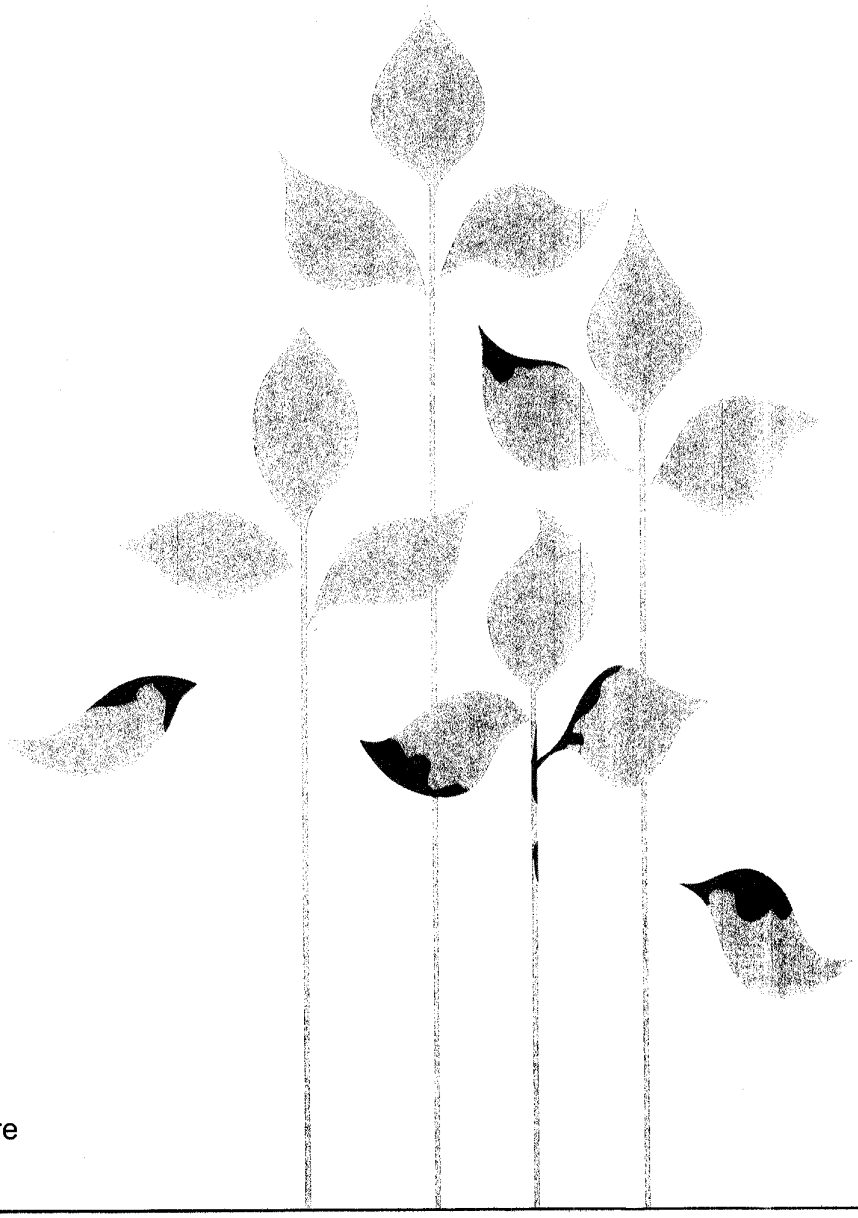

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Contents/Contenu

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.

L'Inventaire des maladies des plantes au Canada est un périodique d'information sur la fréquence des maladies des plantes au Canada, leur gravité, et les pertes qu'elles occasionnent. La rédaction accepte d'autres communications originales notamment sur la mise au point de nouvelles méthodes d'enquête et de lutte ainsi que sur l'évaluation des nouveaux produits. De temps à autre, il inclut des revues et des synthèses de rapports d'intérêt immediat pour les phytopathologistes.

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A REMINDER

All regular, occasional and potential contributors of survey and disease loss articles and annual disease occurrence compilations are reminded that the Canadian Plant Disease Survey is published twice yearly in the spring and fall by Research Branch, Agriculture Canada. The Canadian Phytopathological Society through an *ad hoc* Advisory Committee is responsible for editorial policy. At the present time there is a shortage of papers for the next issue. This reminder is a call for additional papers and authors are requested to submit their material for publication now. Furthermore, those with appropriate data and observations are urged to submit articles on a regular and ongoing basis so there will be adequate material available to warrant continued publication. Authors are reminded that they are responsible for correctness of both articles and galley proofs.

C.P.S.
Advisory Committee

UN RAPPEL

Tous les rédacteurs réguliers, occasionnels ou éventuels d'articles traitant d'inventaires, de pertes dues aux maladies des plantes ainsi que de compilations de fréquence annuelle de ces mêmes maladies sont priés de noter que l'Inventaire canadien des maladies des plantes est publié deux fois l'an, au printemps et à l'automne, par la Direction générale de la recherche d'Agriculture Canada. Un comité consultatif *ad hoc* de la Société canadienne de phytopathologie est responsable de la politique éditoriale. Présentement, il manque des articles pour le prochain numéro. Par ce rappel nous demandons aux auteurs de bien vouloir soumettre immédiatement leurs articles pour publication. De plus, ceux qui disposeraient de données et d'observations appropriées sont encouragés à soumettre leurs articles sur une base régulière et continuelle afin d'assurer la poursuite de la publication de l'Inventaire. Veuillez noter que les auteurs sont responsables de la correction des articles et des épreuves.

S.C.P.
Comité consultatif

Fungi recovered from diseased roots and crowns of alfalfa in north central Alberta and the relationship between disease severity and soil nutrient levels

R. D. Reeleder[1]

A survey of alfalfa fields in north central Alberta was undertaken in 1980-81 in order to determine which soilborne pathogens were most prevalent. Fields with "alfalfa sickness" symptoms were generally luvisolic and sulfur-deficient. Root and crown damage tended to be more severe on sulfur-deficient soils. *Cylindrocarpon gracile* and *Fusarium roseum* were the fungi recovered most frequently from diseased tissue. Soil type, sampling date, and symptom type all affected frequency of recovery of fungal genera. Symptoms of *Plenodomus meliloti* were frequently observed but the fungus was only rarely recovered. *Phytophthora megasperma* var. *megasperma* was not recovered from "alfalfa sickness" affected plants or field soils. There appeared to be a relationship between disease severity and the concentration of soil nutrients.

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En 1980-81 un inventaire des champs de luzerne dans le nord de la région centrale de l'Alberta fut entrepris afin d'y déterminer l'identité des principaux pathogènes du sol. Les champs présentant des symptômes de «maladie de la luzerne» étaient généralement luvisoliques et pauvres en soufre. Les dommages aux racines et au collet tendaient à être plus sévères dans les sols pauvres en soufre. *Cylindrocarpon gracile* et *Fusarium roseum*, deux champignons, ont été isolés le plus fréquemment à partir de tissus malades. Le type de sol, la date d'échantillonnage et le type de symptômes ont influencé la fréquence d'isolation des différents genres de champignons. Les symptômes causés par *Plenodomus meliloti* ont souvent été observés mais celui-ci a rarement été isolé. *Phytophthora megasperma* var. *megasperma* n'a pas été isolé à partir de plants présentant des symptômes de «maladie de la luzerne» ni à partir des échantillons de sol. Il semble exister une relation entre la sévérité de la maladie et la concentration en éléments nutritifs du sol.

Introduction

Poor growth of alfalfa in north central Alberta, characterized by yellowing, stunted plants, has been referred to as "alfalfa sickness" (17). Several attempts have been made to identify the cause of this problem and, most recently, *Phytophthora megasperma* has been implicated (3). Other researchers have presented conflicting results (6, 13) and further evaluation of the problem appeared necessary. In this regard, a disease survey was carried out in 1980-81 in order to further assess the prevalence of various root and crown pathogens in north central Alberta alfalfa fields. This report summarizes the results of that survey.

Materials and methods

Alfalfa fields were selected randomly from lists of contract fields obtained from alfalfa dehydration plants. Fifty-five fields were sampled between May 5 and June 4, 1980. The fields were located within the counties of Lac Ste. Anne, Barrhead, Smoky Lake and Athabasca, and the Municipal District of Sturgeon. Within each field, four sites representative of the terrain were selected and several plants (4-10) were collected from each site. Usually, plants were dug in such a way that a block of soil containing the top 6-12 inches of the taproot was removed and placed in a plastic bag. This insured that most lateral and tertiary roots would be retained. Fields were classified as either "alfalfa sick" or "healthy" on the basis of the presence or absence of characteristic symptoms (17). Samples were kept cool until examination and, generally, diseased tissue was selected and plated out within 48 hr of collection.

Root and crown damage was assessed by dividing the symptoms into several groups, each with its own severity scale (Table 1, Figs. 11 - 14). Tissue from each type of symptom was then surface sterilized in 1% NaOCl for 3 min. or 30 sec., rinsed 3X in sterile distilled H_2O, and blotted dry before plating. Most isolations were made on acidified potato dextrose agar (PDA), acidified Malt agar, and PDA amended with 300 ppm streptomycin. Corn meal agar, amended with 200 ppm vancomycin and 5 ppm penicillin, and VYS-PBNC agar (12) were used to a lesser extent. Plates were incubated in the dark at 2° or 15°C for up to 2 weeks and examined regularly for the appearance of fungal colonies. Cultures were retained for identification.

A selected group of the fields examined in May was sampled again in July and November, 1980, and in March, 1981. The same procedure was followed except that for the latter two sampling dates plants were not rated for disease severity.

Samples of root tissue from plants collected in July were placed in a Baerman funnel apparatus (14). After 24 hr of incubation, suspensions were examined for the presence of nematodes possessing stylets (7). Soil samples collected in July, 1980, were analyzed by the Provincial Soil and Feed Testing Laboratory, Alberta Agriculture. Linear regression equations were derived to show relationships between root disease severities and levels of soil minerals. The regressions were examined with the analysis of variance (ANOVA) procedure.

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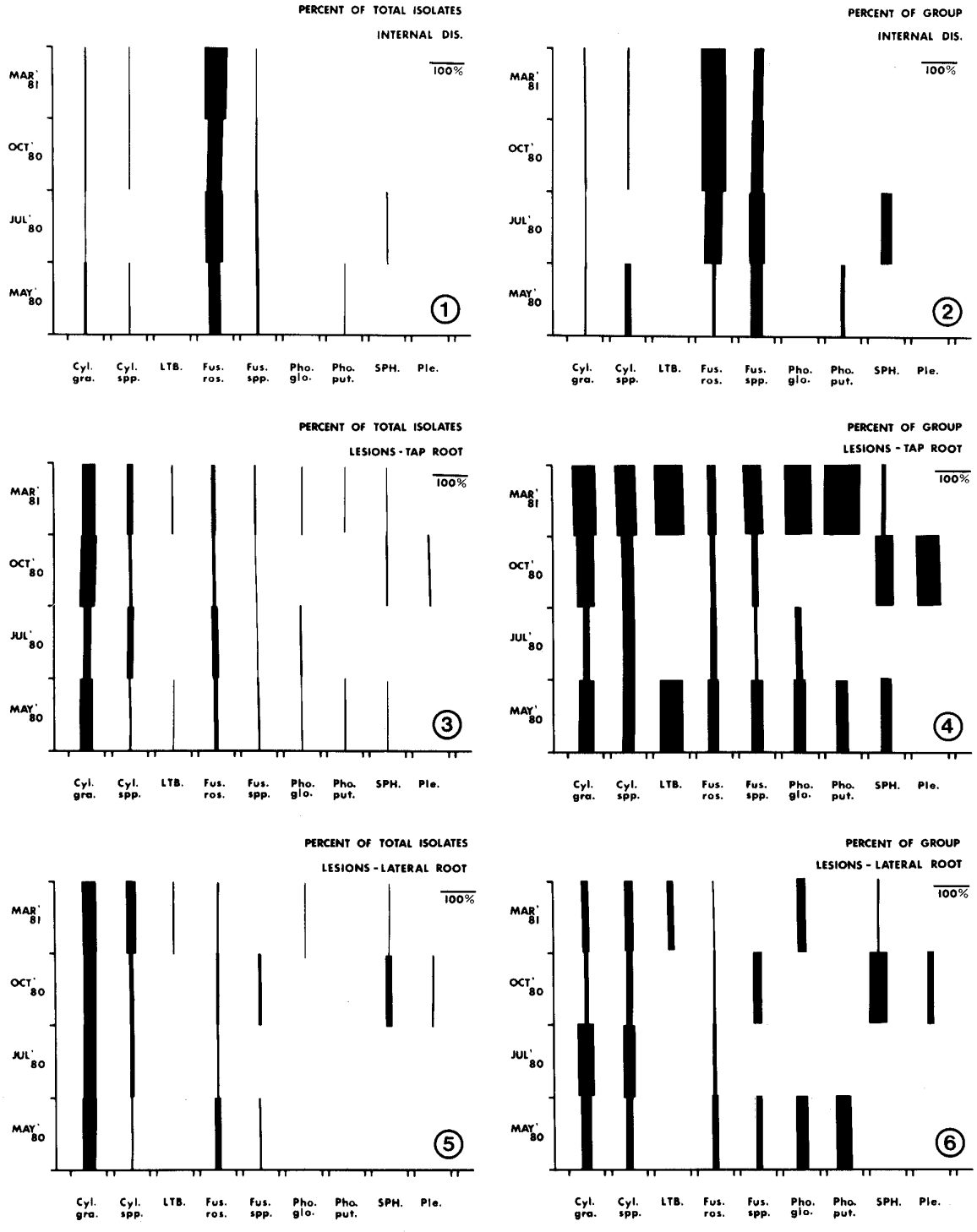


Figs. 1 - 6 Effect of sampling date on recovery of fungi. "Percent of total" indicates percent that each fungal group represents out of all fungal isolates recovered from that symptom type for a given sampling date. "Percent of group" indicates percent of all isolates of a fungal group which were recovered from that symptom type for a given sampling date. Fungal groups: Cyl. gra. = *Cylindrocarpon gracile*, Cyl. spp. = *Cylindrocarpon* spp., LTB = Low temperature basidomycete, Fus. ros. = *Fusarium roseum*, Fus. spp. = *Fusarium* spp., Pho. glo. = *Phoma glomerata*, Pho. put. = *P. putaminum*, SPH = unidentified Sphaeropsidales, Ple. = *Plenodomus meliloti*.

July soil samples were also subjected to baiting tests designed to detect *Phytophthora megasperma* var. *megasperma* (10).

Results

Several different species of fungi (Table 2) were recovered during this survey but *Cylindrocarpon gracile* Bugn. and *Fusarium roseum* (LK.) emend. Snyder and Hansen were the fungi most frequently isolated from diseased plant tissue. Symptoms of brown root rot [*Plenodomus meliloti* Mark.-Let.] were commonly observed but *P. meliloti* was only rarely recovered from the lesions. Recovery of *P. meliloti* could be improved by plating pycnidia on agar media, however, this was not done routinely. *Phytophthora megasperma* var. *megasperma* was not recovered from diseased plants nor was it detected during the baiting tests. The effect of sampling date on the recovery of fungi from various types of symptoms soil types is shown in Figs. 1-10. Only the most frequently recovered fungi and others of interest are shown. Incubation temperature and soil type (luvisols vs. chernozems) had little effect on the recovery of most fungi listed in Table 2. Certain groups, however, were markedly affected. For example, 72.2 percent of the isolates of *Phoma glomerata* were recovered from plants growing in chernozemic soils whereas those recovered from luvisolic soils represented only 27.8 percent. Most *Phoma putaminum* isolates, on the other hand, came from plants growing in luvisolic soils (90.5 percent).

Symptoms observed were generally similar to those previously described (2, 8, 9, 11). The "vascular streaking" symptom has not been previously described on alfalfa by workers in this area. It typically consists of narrow reddish brown streaks which appear to be located in the vascular tissue of the taproot (Fig. 14). The streaking, which originates in the crown but is not necessarily associated with significant crown damage, may extend down the entire taproot or only partway. Survey results indicated that this symptom was more widespread in M.D. Sturgeon fields (chernozemic soils) than in Lac Ste. Anne county fields (luvisolic soils). The fungi recovered most frequently from tissue with this symptom were *Phoma medicaginis* Malbr. and Roum. (44%) and *Fusarium roseum* (31%). Similar symptoms have been described elsewhere (4, 16).

Table 1. Alfalfa root and crown symptom severity scales.

Symptom*	Code	Disease Severity Scale**
Crown Rot	CR	0 - 4
Lateral Root Damage	LR	0 - 5
Tap Root Damage	TR	0 - 5
Internal Discolouration	ID	0 - 6
Vascular Streaking		%§

* Discoloured fine roots were plated out but the degree of damage was not assessed.

** "0" indicates the absence of disease and the maximum value indicates that either 100 percent of the tissue was discoloured (CR, LR, TR) or, in the case of ID, that the discolouration extended down the taproot for a distance greater than 12 cm.

§ This symptom was recorded as the percent of plants in the sample which had vascular streaking.

Table 2. Fungi recovered from roots and crowns of diseased alfalfa plants, 1980-81.

Fungi Recovered	Percent of Total Isolates	Fungi Recovered	Percent of Total Isolates
Acremonium	0.2	*Phoma glomerata*	1.0
Aspergillus	0.4	*Phoma putaminum*	1.1
Low Temp. Basidiomycete	0.9	*Phytophthora*	0.2
Cylindrocarpon gracile	24.6	*Pythium*	0.6
Cylindrocarpon spp. *	9.1	*Rhizoctonia*	1.3
Fusarium roseum	19.7	*Plenodomus meliloti*	0.3
Fusarium spp. **	4.6	*Trichoderma*	0.5
Geotrichum	0.2	DHB§	7.5
Gliocladium	0.9	Mucorales	1.3
Papulaspora	0.3	Sphaeropsidales	1.8
Penicillium	0.3	Misc. identified	0.5
Phialophora	3.2	Misc. unidentified	12.9
Phoma medicaginis	6.7		

* *Cylindrocarpon* isolates belonging to species other than *C. gracile.*

** *Fusarium* isolates belonging to species other than *F. roseum.*

§ Non-sporulating isolates with dark-grey mycelium and compact growth habit.

Table 3. Relationship of root disease severity values to soil nutrient levels.

	Equation	R^2	Age of Stand (Years)
May+	ID§ = 0.798 + 0.082 (P)	0.86**	4 - 6
	ID = 0.027 (K) - 4.250	0.92**	4 - 6
	ID = 1.635 (N) - 0.167	0.65*	2 - 3
	TR = 1.576 + 0.071 (P)	0.67*	4 - 6
	LR = 1.093 + 0.030 (P)	0.77*	4 - 6
	LR = 0.10 (K) - 0.833	0.87**	4 - 6
	LR = 1.364 + 0.024 (P) - 0.046 (S)	0.94*	4 - 6
July+	LR = 1.480 - 0.095 (S) - 0.079 (N)	0.88*	4 - 6
	LR = 0.110 + 0.055 (N) - 0.001 (K)	0.99**	1

+ Equations were derived from severity values (Table 1) obtained from plants collected in May or July, 1981.
§ ID = internal discolouration, TR = exterior damage to tap root, LR = exterior damage to lateral root, N = nitrogen (kg/ha), P = phosphorous (kg/ha), K = potassium (kg/ha), S = sulfur (ppm).
* The regression is significant at P = .05 (ANOVA).
** The regression is significant at P = .01 (ANOVA).

Significant relationships were found to exist between disease severity values and the nutrient status of field soils (Table 3). The results of soil analyses indicated that fields with the symptoms of "alfalfa sickness" had soils that were either deficient in sulfur (0.55-2.10 ppm), acid (pH $<$ 6.0), or both. Fields with such symptoms generally occurred on luvisolic soils.

Disease severity values recorded for plants collected in M. D. Sturgeon fields (chernozemic) tended to be lower than those recorded for plants originating in Lac Ste. Anne Cty. (luvosolic). These differences were usually not statistically significant, however (Table 4).

Nematodes possessing stylets were detected in root samples from most fields surveyed. *Tylenchus* sp., found in 10 out of 15 fields, was the genus most frequently recovered. *Paratylenchus* sp. was observed in samples from 5 out of 15 fields.

Discussion

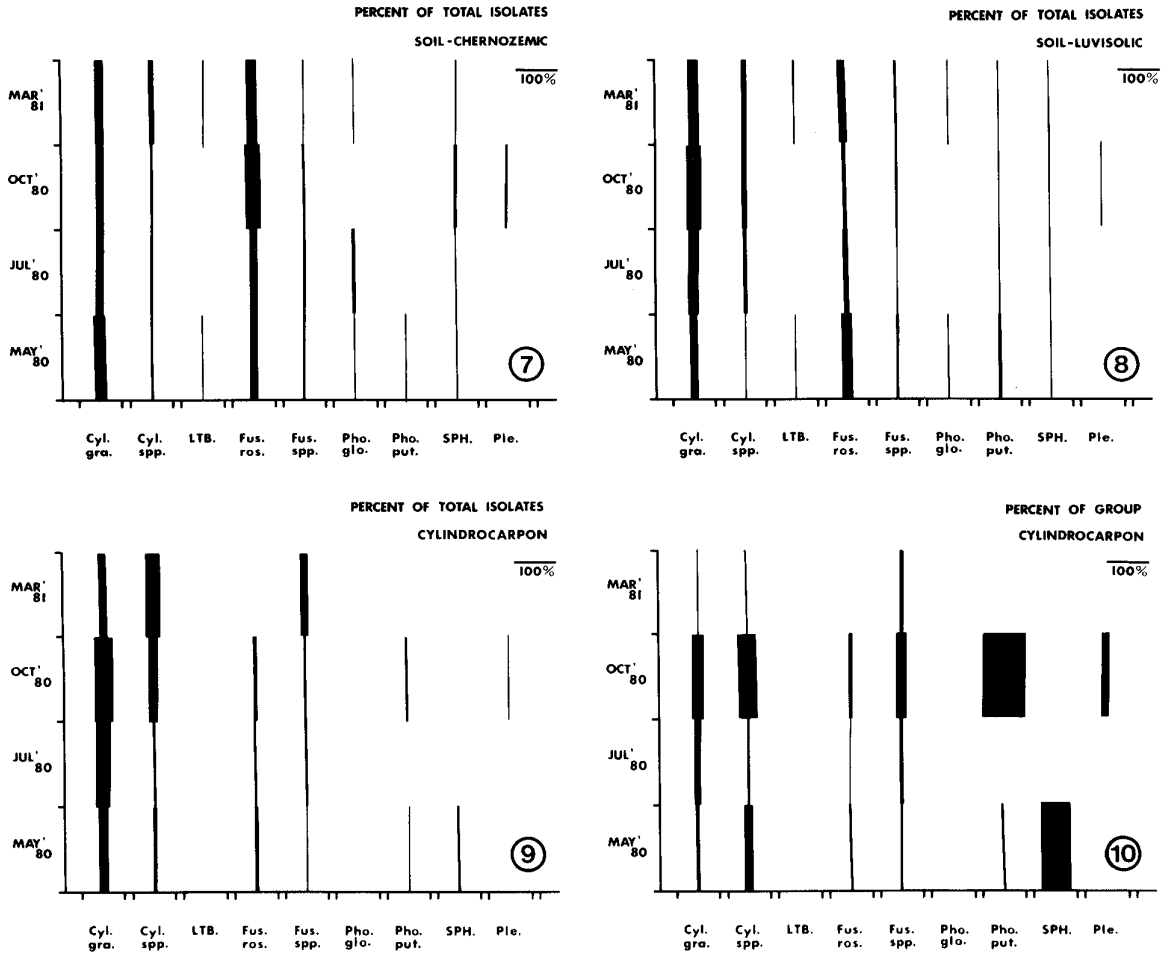
Previous reports have indicated that *Phytophthora megasperma* is the cause of "alfalfa sickness" (3). Only two isolates of *Phytophthora megasperma* were recovered during this survey. Both isolates were nonpathogenic to alfalfa seedlings (Reeleder, unpublished data) and appear to belong to *P. megasperma* var. *sojae*. It is unlikely, therefore, that *P. megasperma* var. *megasperma* is the cause of "alfalfa sickness", although it has previously been detected in Alberta (15). In contrast, sulfur deficiency and soil acidity appear to be strongly associated with "alfalfa sickness". Hawn and Kozub (6) concluded that differences between "alfalfa sick" and healthy soils were due to differences in fertility and pH levels. Field experimentation is needed to verify these relationships.

Symptoms of brown root rot (*Plenodomus meliloti*) were frequently observed during the survey. Most of the damage on tap and lateral roots could be attributed to brown root rot on the basis of the typical symptoms observed (11). *Cylindrocarpon* symptoms (2) were observed much less frequently than those of *Plenodomus meliloti* but *Cylindrocarpon gracile* was nevertheless the fungus most often recovered during this survey. Cormack (2) attributed *Cylindrocarpon* root rot to *C. erhenbergi* Wr. but this species was rejected by Booth (1) in his revision of the genus. More work is needed to clarify the relationship of the various *Cylindrocarpon* species recovered to *Cylindrocarpon* root rot and other root diseases.

Table 4. Alfalfa root and crown disease severity values and soil sulfur levels in mature stands, May and July, 1980§

Symptom	M. D. Sturgeon		Lac Ste. Anne Cty	
Code**	May	July	May	July
CR	0.58*	1.79	1.65*	1.88
ID	1.42	1.79	2.88	2.21
TR	1.56	2.55	3.61	3.26
LR	1.34	1.33	1.96	2.03
Sulfur (ppm)	-	7.10	-	1.04

§ Fields were four to six years old. Severity values in newer fields were lower.
* Value for M. D. Sturgeon was significantly different (P = .05) than the value for Lac Ste. Anne Cty. (May).
** See Table 1.



Figs. 7 - 10 Effect of sampling date on recovery of fungi. "Percent of total" indicates percent that each fungal group represents out of all fungal isolates recovered from that symptom type for a given sampling date. "Percent of group" indicates percent of all isolates of a fungal group which were recovered from that symptom type for a given sampling date. Fungal groups: Cyl. gra. = *Cylindrocarpon gracile*, Cyl. spp. = *Cylindrocarpon* spp., LTB = Low temperature basidomycete, Fus. ros. = *Fusarium roseum*, Fus. spp. = *Fusarium* spp., Pho. glo. = *Phoma glomerata*, Pho. put. = *P. putaminum*, SPH = unidentified Sphaeropsidales, Ple. = *Plenodomus meliloti*. Figs. 9 - 10 illustrate fungal recovery from taproots with *Cylindrocarpon* root rot.

The significance of the presence of *Tylenchus* sp. and other plant parasitic nematodes is presently unknown. *Tylenchus* has been previously reported from Alberta (5) but little is known about its possible effects upon alfalfa roots. Hawn and Kozub (6) concluded that alfalfa was not attacked by *Paratylenchus projectus* Jenkins.

Although significant relationships appear to exist between soil nutrient levels and disease severity (Table 3), it should be noted that none of these relationships have as yet been confirmed with independent data. It is of interest, however, that higher concentrations of certain nutrients appear to increase the severity of some symptoms while other nutrients decrease severity. Also note that these relationships appear to vary with the sampling date (Table 3). Further research is required to determine whether soil nutrient levels can affect disease severity and thereby affect stand persistence and/or yield.

Work is presently underway to assess the pathogenicity of fungi recovered during this survey. Techniques to detect *Medicago* germplasm resistant to *P. meliloti* and *Cylindrocarpon* spp. are being developed.

Acknowledgements

The assistance of the following in identifying various fungal species is gratefully acknowledged: Drs. D. J. S. Barr, C. Booth and E. Punithalingam. Valuable technical assistance was provided by Lorraine Clarke and Harry Kope. Legal locations of alfalfa fields were supplied by Paddle Valley Products Ltd., Legal Alfalfa Products Ltd., and Smoky Lake Processors Ltd. This research was funded by a grant to N. Colotelo from the Farming for the Future programme - Agricultural Research Council of Alberta.

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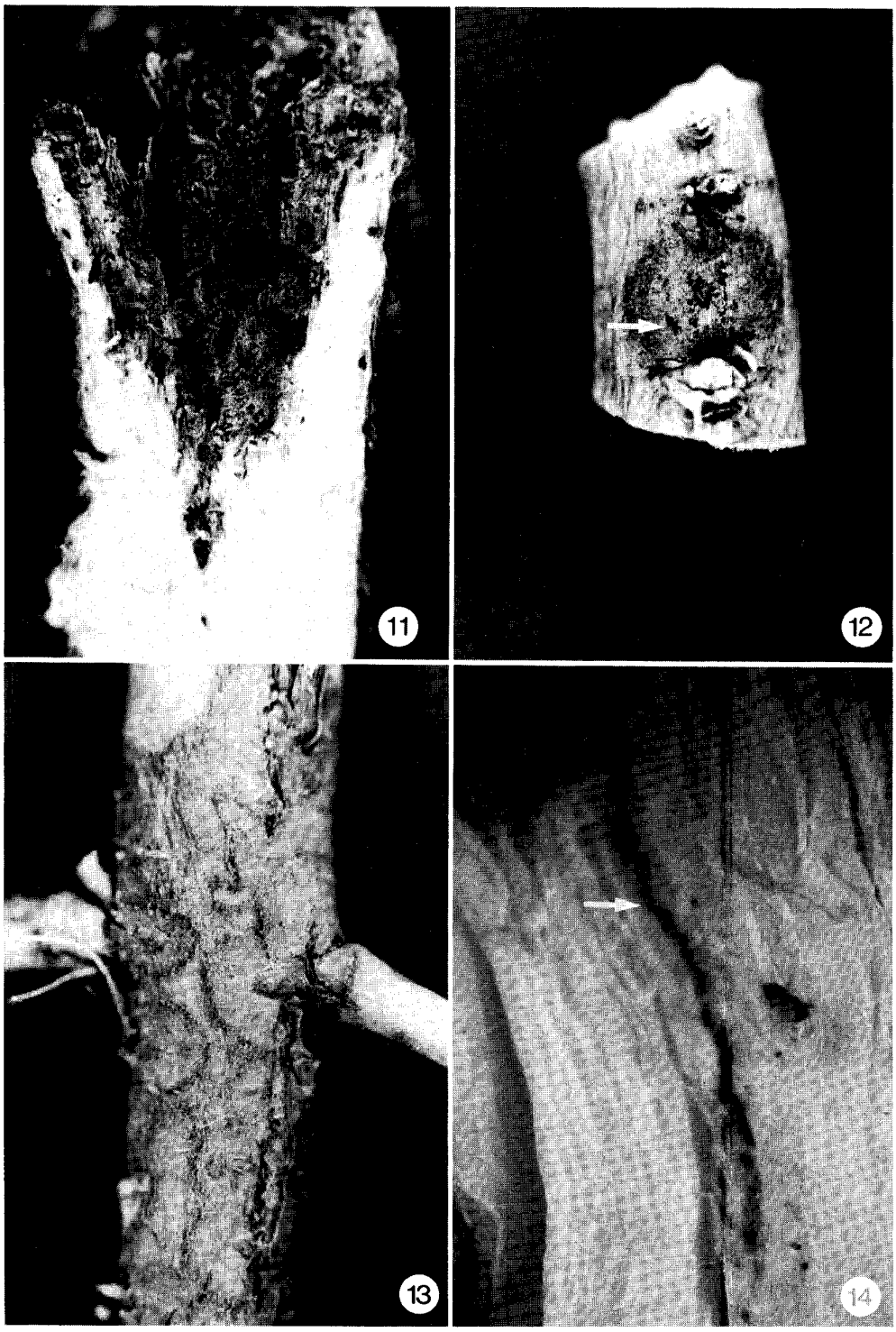

Fig. 11 Internal discolouration (ID), wedge-shaped rot extending from the crown into the taproot.

Fig. 12 Lateral root (LR) damage, caused by *Plenodomus meliloti*. Pycnidia are visible on surface of lesion (arrow).

Fig. 13 Taproot (TR) damage, caused by *Plenodomus meliloti*. Several lesions have coalesced together.

Fig. 14 Vascular streaking. Reddish-brown discolouration extending from the crown into the taproot.

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The accuracy of identifying *Bipolaris sorokiniana* conidia extracted from soils in Saskatchewan[1].

L.J. Duczek

The assignment of *Bipolaris*-type conidia extracted from Saskatchewan soils using the flotation technique, as *B. sorokiniana*, was 99% reliable. Single spore isolates of *Bipolaris*-type conidia obtained from naturally infested soil in Saskatchewan were compared to single conidium isolates of *B. sorokiniana* derived from colonies growing out of naturally infected subcrown internodes of wheat. With two exceptions the severity of spot blotch symptoms on Betzes barley as well as length of conidia was similar for isolates from each source. Of 181 isolates from soil, 179 were *B. sorokiniana* and the two exceptions were identified as *Curvularia spicata*.

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L'identification comme *Bipolaris sorokiniana* des conidies de types *Bipolaris* extraites d'échantillons de sol de la Saskatchewan à l'aide d'une technique de flottaison, s'est révélée exacte à 99%. Des isolats de conidies uniques de type *Bipolaris* extraites de sol infecté naturellement en Saskatchewan ont été comparés à des isolats de conidies uniques de *B. sorokiniana* provenant de colonies croissant sur du blé infecté naturellement au niveau des entrenoeuds sous le collet. Sauf pour deux exceptions, la sévérité des symptômes de tache helminthosporienne sur l'orge Betzes, de même que la longueur des conidies se sont avérées similaires quel que soit la provenance des isolats. Des 181 isolats de type *Bipolaris*, 179 se sont révélés être *B. sorokiniana* tandis que les deux exceptions ont été identifiées comme étant *Curvularia spicata*.

Introduction

Bipolaris sorokiniana (Sacc. in Sorok.) Shoem. (syn. *Helminthosporium sativum* Pamm., King & Bakke; *H. sorokinianum* Sacc. in Sorok.), perfect stage *Cochliobolus sativus* (Ito & Kurib). Drechsl. ex Dastur, is the main cause of common root rot of wheat and barley in the Canadian prairies. Primary infections are initiated generally by soil-borne conidia. Ledingham and Chinn (14) developed a technique to extract conidia from soil and then modified it to determine conidial viability as well as total population (7). This technique, or one slightly modified, was used to study the relationship between conidial population and severity of common root rot (8), the conidial population in different soil profiles (9), the change in conidial populations in soil over time (1), the change in conidial populations following rapeseed (3) and cereal crops (4), and the effect of fungicide sprays on conidial populations in soil (5).

The procedure involved shaking soil, oil, and water in a tube, pipetting an aliquot of the oil-emulsion phase containing conidia onto a slide, counting and then determining the number per gram by using the appropriate dilution factor. Conidia conforming morphologically to that of *B. sorokiniana* were identified at 50-100× using a compound or binocular microscope. This technique has also been used to recover conidia of *B. oryzae* (Breda de Haan) Shoem. (16) and large spores of other fungi (2, 7, 14). The similarity of *B. sorokiniana* conidia to those of other multi-septate dematiaceous hyphomycetes could result in inaccurate counts if a mixed population was extracted. Therefore, the purpose of this study was to determine the accuracy of assigning all the *Bipolaris*-type conidia extracted from soil as *B. sorokiniana*. Cultures derived from single conidia extracted from naturally infested soil were compared to cultures originating from single conidium isolations made from naturally infected plant tissue. The two variables used were conidial length and the production of leaf spot lesions on barley. These were chosen because this fungus, in addition to causing symptoms on subcrown internodes, crowns, and basal stems, causes spot blotch on leaves of cereals, and conidial size is one of the important criterion used in separating species of dematiaceous hyphomycetes (11).

Materials and methods

Conidia were recovered from soil by the oil-flotation method (9). Three or four millilitres of the oil emulsion layer were mixed with a sufficient amount of warm melted potato dextrose agar (Difco), supplemented with 1% molasses, 100 ppm streptomycin sulfate, and 50 ppm vancomycin hydrochloride, to cover the bottom of a 9 cm diameter plastic petri plate. After incubation for 8-16 hr at 20-22°C, single germinated *Bipolaris*-type conidia, identified with a binocular microscope at 50-100×, were plated on minimal agar medium (18). After a further 16-24 hr, hyphal tips were transferred to the medium in test tubes. Each culture originated from a separate single conidium. Isolates from a plant source originated from single conidium cultures obtained from *B. sorokiniana* colonies growing from naturally infected subcrown internodes of wheat plated on minimal agar medium (12). Isolates from both sources were from various locations throughout the agricultural area of Saskatchewan. Soil was collected in 1979 and 1980, and plants (internodes) were collected in 1979.

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For the leaf spot test, 15 seeds of Betzes barley (*Hordeum vulgare* L.) were planted 2 cm deep in soil-free mix (17) in a 10 cm square plastic pot. Pots were placed in a growth chamber where the regime was 20°C, 16 hr light and 15°C, 8 hr dark. Plants were inoculated 11 days later at the early three leaf growth stage. Inoculum was prepared by scraping and suspending spores of 10+ day old cultures, grown on minimal agar medium, in 0.1% Tween 20 solution, filtering through four layers of cheesecloth, and adjusting the concentration using a haemocytometer. For each isolate, plants in four pots were sprayed simultaneously with 30 ml of a 2×10^4 spore/ml suspension using a soda straw sprayer (10). The spray mist was delivered from a 1 m distance with the 10 cm cut end of the straw immersed in the 50 ml erylenmeyer flask spray vessel. Pots were turned 180° when half the spore suspension remained. For the control only 0.1% Tween 20 solution was used. A plastic bag (13 × 8 × 34 cm) was placed over the plants in a pot to form a humidity chamber. The bag was supported by a wire frame and secured to the pot exterior by an elastic band. The plastic bags were removed after 3 days. The severity of spot blotch (percentage of leaf area lesioned) was estimated on the first leaf of 10 plants/pot 7 days after inoculation. Leaf tip discoloration was not included in the estimate of coverage since some leaf tip discoloration occurred in the control as well as the other treatments

One hundred and ten isolates from soil and from plants were compared. Eleven trials each of 20 isolates comprising 10 from each source were conducted. The plant and soil isolates originates from 85 and 75 locations, respectively. Subsequently, an additional six isolates from plants and 81 from soil were examined. A control (0.1% Tween 20 solution) and one standard culture (No. 1133) were also included in each trial.

Two to five leaves infected by the individual isolates were removed, placed in a 9 cm diameter glass petri plate fitted with moist filter paper and incubated at 20-22°C. After 3-4 days, sporulation occurred on leaf tips, and the length of 20 randomly selected spores was measured at 250×. No sporulation occurred on the leaves of plants sprayed with 0.1% Tween 20 solution.

Results and discussion

The mean ± standard error for percent leaf spot and conidial length for the standard culture (No. 1133) used in all 11 tests was 3.8 ± 0.91 and 80.4 μ ± 4.98, respectively. The variation between trials was not significant for percent leaf spot, but the variation between trials was significant at the 0.1% level for conidial length.

For percent leaf spot on Betzes barley the mean ± standard error for the 110 isolates from each of the plant source and soil source was 2.8 ± 1.37 and 2.7 ± 1.12, respectively. Values from both sources were normally distributed according to the Kolmogorov-Smirov Test for goodness of fit. Data were analyzed as a three-level nested analysis of variance (Table 1). This shows that there was a significant added variance among isolates within trials and among trials within the source (plant or soil), but no significant difference between the plant or soil source.

The mean ± standard error for conidium length for the 110 isolates from the plant and the soil source was 76.5 μ ± 6.16 and 77.1 μ ± 5.65, respectively. The range for the means of isolates and for readings, given in brackets, was 63.8 to 97.5 μ (49.4 to 106.4 μ) and 64.0 to 93.9 μ (41.8 to 117.8 μ), respectively, for the plant and soil source. The means of isolates from both sources were normally distributed according to the Kolmogorov-Smirov Test for goodness of fit. Data were analyzed as a three-level nested analysis of variance (Table 2). This shows that there was a significant added variance among isolates within trials and among trials within the source (plant or soil), but no significant difference between the plant or soil source.

The correlation coefficient of spore length and leaf spot based on means of all isolates (pairs = 220) was -0.110. This value was not significant.

The additional six plant derived isolates and 81 soil derived isolates formed colonies typical of *B. sorokiniana*. All but two isolates from soil produced spot blotch symptoms on Betzes barley and conidia having the typical *B. sorokiniana* shape, dimension, and coloration.

The conidium length of isolates was within the range for *B. sorokiniana* given by Ellis as 40-120 μ (11) and by Luttrell as 36 to 129 μ (15). All but two isolates caused spot blotch of barley. The data show that isolates from soil are similar to isolates from infected plant tissue.

Considerable variability occurred between trials for both variables. No doubt conditions varied between trials over the time period of the study even though procedures were standardized. Harding (13) noted conidium size was influenced by different cultural conditions.

Table 1. Three-level nested analysis of variance table for percent leaf spot on Betzes barley for 11 trials of 10 isolates of *Bipolaris sorokiniana* from two sources (plant or soil) with 4 readings per isolate

Sources	df	SS	MS	Fs
Among source (plant-soil)	1	2.397	2.397	0.095n.s.
Among trials within source	20	504.434	25.222	5.830***
Among isolates within trials	198	856.481	4.326	6.686***
Error	660	427.181	0.647	
Total	879	1790.493		

***Significant at the 0.1% level.

Table 2. Three-level nested analysis of variance table for conidial length for 11 trials of 10 isolates of *Bipolaris sorokiniana* from two sources (plant or soil) with 20 readings per isolate

Source	df	SS	MS	Fs
Among source (plant-soil)	1	118.871	118.871	0.186n.s.
Among trials within source	20	12801.754	640.087	4.651***
Among isolates within trials	198	27248.071	137.617	8.915***
Error	4180	64527.610	15.437	
Total	4399	104696.286		

***Significant at the 0.1% level.

Two of the isolates derived from soil were different from the rest. In culture, the colonies of both of these isolates, which were alike, were similar to those of *B. sorokiniana*. They did not produce leaf spots on barley, but they did sporulate on the necrotic leaf tips. The conidia produced in culture or on senescent leaf tissue differed from those of *B. sorokiniana* in shape, septation, and coloration. Although conidial length was within the range recorded for *B. sorokiniana*, the mean length of 44 μ was less than for *B. sorokiniana*. These isolates were identified as *Curvularia spicata* (Bainer) Boedijn.

Individual conidia of *C. spicata* extracted from soil might be confused as those of *B. sorokiniana*, particularly in view of the variability in *B. sorokiniana*. In culture, however, *C. spicata* is easily differentiated on the basis of conidial morphology. Of 181 isolates from soil that were selected as *B. sorokiniana*, 179 isolates (99%) were *B. sorokiniana* and 2 isolates (1%) were *C. spicata*. This level of reliability indicates that the oil and water flotation technique is a valuable tool for estimating *B. sorokiniana* conidial numbers in naturally infested Canadian prairie soils.

Acknowledgement

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Diseases of pulse crops in Alberta, 1978-79

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Selected fields of processing (*Pisum sativum*) and dry peas (*P. sativum* and *P. sativum* var. *arvense*), processing and dry beans (*Phaseolus vulgaris*), fababeans (*Vicia faba*), soybeans (*Glycine max*) and lentils (*Lens culinaris*) in Alberta were examined for diseases over a two-year period. Overall, root rot (*Fusarium* spp. and *Rhizoctonia solani*) was the most common disease in pea and bean crops. Other prevalent diseases recorded on peas were powdery mildew (*Erysiphe pisi*), Alternaria leaf spot (*Alternaria alternata*), bacterial blight (*Pseudomonas pisi*) and downy mildew (*Peronospora viciae*). Bacterial blights (*Pseudomonas phaseolicola* and *Xanthomonas phaseoli*) were prevalent in processing beans, while white mold (*Sclerotinia sclerotiorum*) was common in dry beans. In fababeans, Alternaria leaf spot (*Alternaria alternata*), powdery mildew (*Microsphaera penicillata* var. *ludens*) and chocolate spot (*Botrytis cinerea*) were the most common diseases. Fusarium root rot (*Fusarium oxysporum*) in lentils and bacterial blight (*Pseudomonas glycinea*) in soybeans were the only infectious diseases recorded on these crops.

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Des champs sélectionnés de pois de transformation (*Pisum sativum*), de pois secs (*P. sativum* et *P. sativum* var. *arvense*), de haricots secs et de transformation (*Phaseolus vulgaris*), de fèves des marais (*Vicia faba*), de fèves soja (*Glycine max*) et de lentilles (*Lens culinaris*) situés en Alberta furent examinés sur une période de deux ans afin d'évaluer l'importance des maladies présentes. En général, la pourriture des racines causée par *Fusarium* spp. et *Rhizoctonia solani* était la maladie la plus répandue dans les champs de pois et de haricots. Le blanc (*Erysiphe pisi*), la brûlure bactérienne (*Pseudomonnas pisi*) et le mildiou (*Peronospora viciae*) furent souvent observés sur les pois. La tache aérolée (*Pseudomonas phaseolicola*) et la brûlure bactérienne (*Xanthomonas phaseoli*) étaient répandues chez les haricots de transformation alors que la pourriture sclérotique (*Sclerotinia sclerotiorum*) était commune chez les haricots secs. Chez la fève des marais on a souvent retrouvé la brûlure alternarienne (*Alternaria alternata*), le blanc (*Microsphaera penicillata* var. *ludens*) et la tache chocolat (*Botrytis cinerea*) tandis que l'on n'a détecté que la pourriture fusarienne des racines (*Fusarium oxysporum*) chez les lentilles et la brûlure bactérienne (*Pseudomonas glycinea*) chez les fèves soja.

Introduction

The area of land devoted to pulse crop production in Alberta has steadily increased in recent years (Table 1). Most of this increase has come about as the result of the introduction and acceptance of "new" pulse crops such as fababeans and lentils. In view of the increased importance of pulse crops to Alberta's agricultural industry, comprehensive surveys were conducted during 1978 and 1979 to identify prevalent and potentially serious diseases. Diseases of pulse crops have been appraised similarly in Saskatchewan (9, 10, 11, 12) and Manitoba (14, 15).

Surveys were carried out in commercial fields in southern and central Alberta. Fewer fields were surveyed in 1979 compared to 1978, but more detailed disease assessments were made in 1979. The crops surveyed in 1978 were processing peas (garden peas, *Pisum sativum* L.), dry peas (garden peas grown for seed and field peas, *P. sativum* var. *arvense* L.), dry beans (field beans, *Phaseolus vulgaris* L.) and fababeans (*Vicia faba* L.) The 1979 survey also included processing beans (snap beans, *P. vulgaris* L.), soybeans (*Glycine max* (L.) Merr.) and lentils (*Lens culinaris* Medik.).

Methods

Varying numbers of fields of each pulse crop were selected for survey (Tables 2 and 3) so as to include the main cultivars currently being grown and to fairly represent the geographical areas of production. Between June and September of each year, efforts were made to visit each field at least twice and, where possible, three times at approximately 4 to 5 week intervals. Second visits to a few fields were not possible in 1979 because of early crop maturity. Soybean and lentil fields surveyed in 1979 were examined only once.

Fields were traversed in a large semi-circle and at ten equally spaced sites, ten plants (in 1978) or all plants (in 1979) within three metres of row length were examined. At each of the sites, various plant parts were examined. Roots were carefully dug up and checked for disease. Counts were made of the total number of plants/3 m in 1979, and disease incidence was recorded as a percentage of the total plants examined. No attempt was made to rate severity. Field diagnoses of infectious diseases were confirmed by laboratory isolation and identification of the pathogens. In some cases, attempts were made to reinfect healthy plants to reproduce the diseases under greenhouse conditons.

Results

Several diseases were found in each crop (Tables 2 and 3). Of these, Fusarium and Rhizoctonia root rots, powdery mildew, fungal leaf spots and bacterial blights were the most prevalent. Besides the diseases mentioned in Tables 2 and 3, several others were recorded at very low incidence levels (<1%); these are mentioned in the text.

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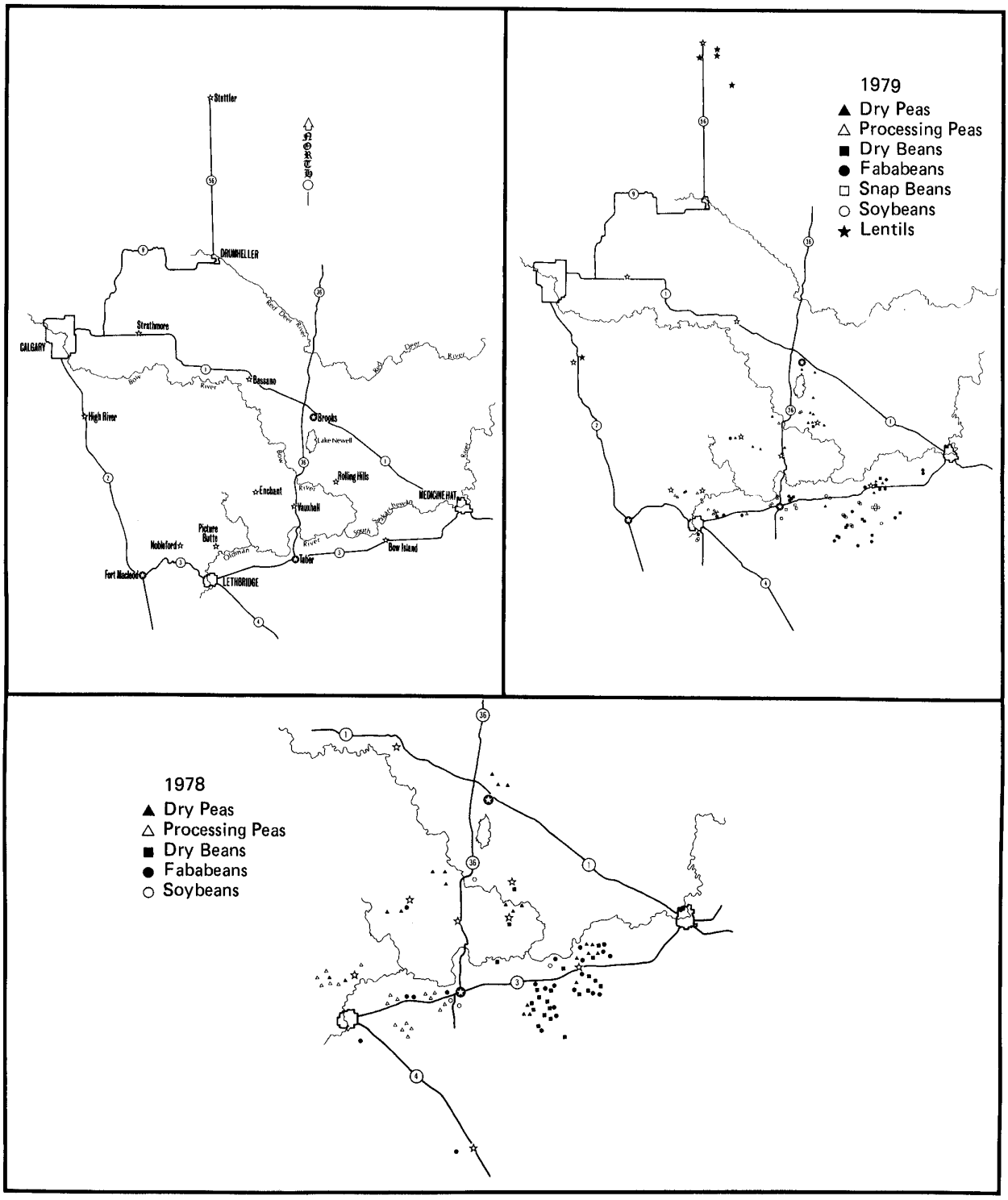

Figure 1. Distribution of pulse crop fields in southern Alberta which were surveyed for diseases in 1978 and 1979.

Table 1. Hectares of pulse crops grown in Alberta, 1974-79.

Pulse	1974	1975	1976	1977	1978	1979
Processing peas	1,996	2,119	1,681	1,501	2,400	1,900
Dry peas	6,478	5,162	2,632	4,049	4,049	4,049
Processing beans	262	240	229	272	300	300
Dry beans	972	1,538	2,105	2,429	2,591	2,834
Fababeans	400	1,215	810	1,619	1,923	3,644
Soybeans	0	0	0	0	0	150
Lentils	-	-	-	-	50	486
Total	10,108	10,274	7,457	9,870	11,313	13,363

Processing and dry peas

The predominant disease in processing peas in both years was root rot (*Fusarium* spp. *Rhizoctonia solani* Kühn, and *Pythium ultimum* Trow.) (Tables 2 and 3). In 1979, it occurred in all the fields visited. By contrast, dry pea crops had a relatively low root rot incidence and fewer fields were infested. *Fusarium* was the most frequently isolated pathogen in the pea root rot complex, and the species included *F. avenaceum* Schlecht., *F. culmorum* (W.G. Smith) Sacc., *F. equiseti* (Corda.) Sacc., *F. oxysporum* Schlecht., *F. oxysporum* var. *redolens* (Wr.) Gordon, and *F. solani* (Mart.) Sacc. In processing peas, powdery mildew (*Erysiphe pisi* DC.), Alternaria leaf spot (*Alternaria alternata* (Fr.) Keissl.) (Fig. 2) and Ascochyta blight (*Ascochyta pinodes* L.K. Jones) were encountered only in 1978. Bacterial blight (*Pseudomonas pisi* Sackett.) was present in processing pea fields during both years, but was more common during 1979. Conversely, Fusarium wilt (*F. oxysporum*) incidence was somewhat higher in 1978 (0.5% incidence in 1979).

In dry peas, powdery mildew incidence was invariably high towards the later part of the season (Tables 2 and 3). In 1979, all 11 fields visited in mid-August had powdery mildew with five fields having disease incidence levels of 100%. Ascochyta blight and Alternaria leaf spot were also more prevalent late in the season. In most cases, these occurred in mixed infections (Fig. 3) along with powdery mildew. Downy mildew (0.6% late season incidence in 1978), Ascochyta foot rot (*Ascochyta pinodella* L.K. Jones) and bacterial blight, in particular, were higher in incidence during 1979. Low levels of Sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) and gray mold (*Botrytis cinerea* Pers. ex Fr.) infections on stems and pods were observed in dry peas surveyed in 1979. Very low levels (<1% incidence) of Fusarium wilt were recorded in dry pea crops during both surveys.

Processing and dry beans

Root rot was by far the most prevalent disease during both years (Tables 2 and 3). In laboratory isolations, *F. acuminatum* Ell. & Ev., *F. oxysporum*, *F. solani* and *Rhizoctonia solani* were found associated with diseased roots, but no *Pythium* spp. were present. White mold (*S. sclerotiorum*) occurred on dry beans each year with the highest incidence recorded in 1978. Late season visits revealed that bacterial blight was present in several fields of both kinds of beans. In dry beans, halo blight (*Pseudomonas phaseolicola* (Burkh.) Dows.) was more common during 1978, whereas common blight (*Xanthomonas phaseoli* (E.F. Sm.) Dows.) was more prevalent in 1979 (late season incidence = 1.6%). During 1979, in one field of late-planted processing beans, frequent irrigation resulted in an epidemic of bacterial blight with clearly visible foci of infection (Fig. 4). Though both bacterial blight pathogens were present in this field, *X. phaseoli* appeared to cause greater damage. Herbicide injury from drift of 2,4-D or MCPA was observed in several dry bean crops surveyed in 1978. A similar case was recorded in 1979 on processing beans. In 1979, a few dry bean fields exhibited trifluralin- or dinitramine- type herbicide damage on roots. Low levels of Botrytis gray mold were present each year in the dry bean crops.

Fababeans

Foliar diseases, developing mostly later in the season, were the most prevalent problems during both years (Tables 2 and 3). Of these, Alternaria leaf spot (*A. alternata*) (Fig. 5) was encountered in all fababean fields. Leaf infections by *Ascochyta fabae* Speg. were recorded in a few fields in 1978, but were not found during the 1979 survey. Chocolate leaf spot incidence was markedly higher in 1979. Isolations from chocolate leaf spot lesions yielded *B. cinerea*. Several such isolates of *B. cinerea* were subsequently used in attempts to re-infect fababean seedlings in the greenhouse but were non-aggressive (sensu Jarvis, 1977) and failed to reproduce the disease. Powdery mildew was found to be caused by *Microsphaera penicillata* (Wallr. ex Fr.) Lev. var. *ludens* (Salmon) Cooke. Root rot (*Fusarium oxysporum*, *F. avenaceum* and *R. solani*) incidence was higher in 1978 (2.3%) than in 1979 (0.7%). In 1979, the disease was recorded in 40% and 22% of the fababeans surveyed during early and late season, respectively.

Table 2. Diseases of pulse crops in Alberta in 1978.

Pulses	No. fields visited*	Diseases‡	Percent fields diseased		Mean disease incidence (%)	
			Early season	Late season	Early season	Late season
Processing peas	20	Root rot	55.0	75.0	26.5	35.8
		Powdery mildew	0.0	45.0	0.0	24.4
		Alternaria leaf spot	0.0	45.0	0.0	22.6
		Ascochyta blight	0.0	25.0	0.0	11.6
		Downy mildew	0.0	30.0	0.0	7.2
		Edema	0.0	15.0	0.0	2.4
		Fusarium wilt	15.0	25.0	0.2	1.2
		Bacterial blight	0.0	20.0	0.0	1.0
Dry peas	22	Powdery mildew	0.0	81.8	0.0	59.0
		Alternaria leaf spot	0.0	45.5	0.0	22.0
		Ascochyta blight	0.0	45.5	0.0	21.8
		Bacterial blight	9.1	4.5	0.2	3.1
		Root rot	36.4	4.5	4.6	2.6
		Ascochyta foot rot	0.0	9.1	0.0	1.2
		Low temperature injury	27.3	-	2.4	-
Dry beans	22	Herbicide injury	0.0	11.4	0.0	17.0
		White mold	0.0	59.1	0.0	4.6
		Root rot	81.8	11.4	14.2	4.6
		Halo blight	4.5	13.7	0.6	4.6
		Gray mold	0.0	11.4	0.0	1.6
Fababeans	21	Alternaria leaf spot	0.0	100.0	0.0	32.0
		Powdery mildew	0.0	57.1	0.0	20.1
		Ascochyta blight	0.0	19.0	0.0	6.8
		Edema on pods	0.0	47.6	0.0	4.5
		Chocolate leaf spot	0.0	42.9	0.0	2.6
		Root rot	71.4	61.9	3.5	1.1

* Early and late season visits were made to the same fields.

‡ Includes infectious and non-infectious diseases.

A pod and seed spot disease similar to one described on fababeans in Germany by Griesbach (6) was found in three fields in 1978. The main disease symptom was sunken black spots on pods and seeds. These yielded a *Pseudomonas*-like bacterium in culture and symptoms were reproduced by culating detached fababean pods in petri dishes (Fig. 6). Physiological edema on pods, seen as small black warts (Fig. 7), was observed in several fields each year of the survey (Tables 2 and 3). In 1978, 2,4-D herbicide damage was seen along the margins of several fields. Trace levels of Sclerotinia stem rot and bean yellow mosaic were recorded during the 1979 survey. Bean yellow mosaic was the only virus disease observed in all of the pulse crops inspected over the two seasons.

Soybeans

Bacterial blight (*Pseudomonas glycinea* Coerper) was the only infectious disease observed (Table 3). In one field, over 75% of the plants had foliar infections. Dicamba-type injury was observed in two fields located side by side. Damage from over-irrigation was seen in one field.

Lentils

All fields had a low incidence of root disease (Table 3) which was characterized by cortical rot and sloughing-off. Diseased plants appeared chlorotic, somewhat wilted and were killed when infection was severe. *Fusarium solani*, *F. oxysporum* and *F. oxysporum* var. *redolens* were isolated from the necrotic root tissue.

Discussion

Root rot was a major disease on virtually all of the pulses surveyed, particularly on processing peas and beans. Our

observation of its high incidence in all processing pea producing areas in Alberta are parallel with those made in an extensive nationwide survey by Basu *et. al.* (1). *Fusarium* spp. were, by far, the most prevalent and serious root rot pathogens, except in processing beans where *R. solani* was equally damaging. In peas, *F. oxysporum* was frequently isolated from plants with root rot and appeared to be more important in promoting root decay than in inciting the near wilt disease. Dry peas were not affected by root rot as frequently as processing peas. The reasons for this difference are not completely understood, though two possible explanations can be offered. Processing pea growers, most of whom have grown canning or freezer peas for many years, often quickly return the same fields back to pea production. By contrast, dry peas are usually grown by different growers who do not crop their land to peas as frequently. Another conducive factor may be the comparatively higher frequency with which processing pea crops are irrigated, thus giving higher incidences of root rot especially where *Pythium* spp. may be present. As in Saskatchewan (9, 10, 12), our surveys of dry pea fields in Alberta have shown that, while root rot is present, other diseases are relatively more serious. In fababeans, root rot incidence was somewhat higher in 1978. In part, this may be due to different cropping patterns, since a large number of fababean fields surveyed during 1978 had had other pulse crops grown in them in previous years.

Powdery mildew was more abundant in dry pea and fababean crops during 1979, probably due to the drier, warmer weather conditions in that year. On *Vicia* spp., *Erysiphe polygoni* DC. ex Merat as well as *Microsphaera penicillata* have been reported as causes of powdery mildew (4, 17). In Alberta, our studies revealed that only *M.*

Table 3. Diseases of pulse crops in Alberta in 1979.

Pulses	No. fields visited*	Diseases‡	Percent fields diseased		Mean disease incidence (%)	
			Early season	Late season	Early season	Late season
Processing peas	15, 14	Root rot	100.0	100.0	16.1	30.1
		Downy mildew	0.0	57.1	0.0	9.5
		Bacterial blight	13.3	92.9	1.4	7.5
Dry peas	15, 11	Powdery mildew	0.0	100.0	0.0†	85.5
		Ascochyta blight	6.7	63.6	trace	34.8
		Alternaria leaf spot	0.0	63.6	0.0	26.4
		Downy mildew	0.0	63.6	0.0	16.7
		Bacterial blight	73.3	90.9	3.5	14.2
		Root rot	100.0	72.7	8.3	8.2
		Hail injury	0.0	18.2	0.0	7.2
		Ascochyta foot rot	0.0	36.4	0.0	5.3
		Sclerotinia stem rot	0.0	45.5	0.0	2.5
Processing beans	15, 10	Root rot	100.0	- ‡	16.6	-
		Common blight	0.0	60.0	0.0	22.9
		Halo blight	0.0	60.0	0.0	5.3
Dry beans	15, 15	Root rot	86.7	-	3.6	-
		White mold	0.0	66.7	0.0	3.3
Fababeans	15, 9	Chocolate leaf spot	0.0	100.0	0.0	50.0
		Powdery mildew	13.3	88.9	3.1	43.4
		Alternaria leaf spot	0.0	100.0	0.0	19.5
		Edema on pods	6.7	77.8	0.8	4.5
Soybeans	5	Bacterial blight	40.0	-	14.8	-
		Herbicide injury	40.0	-	3.1	-
Lentils	5	Fusarium root rot	100.0	-	2.7	-

* Number of fields visited, early season and late season, respectively. Soybean and lentil crops were visited just once.

‡ Includes infectious and non-infectious diseases.

† Incidence 0.01%.

‡ No disease assessments were made.

penicillata var. *ludens* was present, as has been shown elsewhere in Western Canada (7, 11, 13). In processing peas, crops are harvested several weeks earlier than peas grown for seed and thus escape high powdery mildew infection. The practice of less frequent irrigations in dry pea crops may also explain the higher powdery mildew incidence (3).

In spite of a very dry summer during 1979, incidence of chocolate leaf spot on fababeans was surprisingly high and was markedly greater than that recorded in 1978. Favorable conditions for infection may have occurred during periods of high humidity following irrigation. Though both *Botrytis fabae* Sard. and *B. cinerea* have been reported as pathogens on *Vicia faba* (4, 11, 16), the former, a more aggressive and damaging species, was not encountered either during field surveys or in laboratory isolations. Also, *B. fabae* infections were not observed in fababean fields during extension visits and in seed tests done in 1979 (S. P. Sumar, unpubl. data) on samples from commercial fababean seed lots. Seed tests also revealed that Alberta-grown seed stocks were essentially free of infection by *A. fabae*, which was reflected by the very low field incidence of *Ascochyta* blight. This disease was found only in 1978, likely resulting from a single infected seed lot. At the present time, *A. fabae*, like *B. fabae*, is thought not to be established in Alberta. *Ascochyta fabae*, however, is known to occur elsewhere in Western Canada (2, 9, 11 and P.D. Kharbanda, pers. comm). Isolates of *Alternaria alternata* from diseased fababean leaf tissue did not reinfect young fababean plants in greenhouse tests. Though this fungus was frequently isolated from fababean seed in laboratory tests (unpubl. data), its mild pathogenicity and predominance in the late season, especially on senescing leaves, lead us to conclude that the fungus is a relatively unimportant pathogen.

The frequent occurrence of bacterial blights in pea and bean crops in Alberta suggests that infected seed provides most of the primary inoculum. Sprinkler irrigation was observed to be a major means of spread within fields. In soybeans, contaminated seed lots (S. P. Sumar, unpubl. data) resulted in a very high field incidence of bacterial blight (*P. glycinea*).

Lentils have become a well-established crop in Alberta. Although Fusarium root rot was present in all lentil fields surveyed, its incidence was low and neither yields nor stands appeared to be significantly affected. Although lentils grown in Alberta do not appear, at the present time, to be as seriously affected by diseases as elsewhere on the Prairies (9, 10, 12), diseases will likely become more important as production becomes more intensive.

Acknowledgements

The technical assistance of Mr. T. Isakeit, Ms. D. Fujimoto and Mrs. J. Ferguson is gratefully acknowledged. Invaluable assistance in the identification and verification of specimens was provided by Dr. C. Booth and Mr. P. M. Kirk of the Commonwealth Mycological Insititute, Kew, England, Dr. V. R. Wallen, Research Station, Ottawa, and Dr. G. A. Neish, Biosystematics Research Institute, Ottawa. The assistance of Messrs. L. Ablonczy and E. Moskaluk in preparation of the figures is also apprciated.

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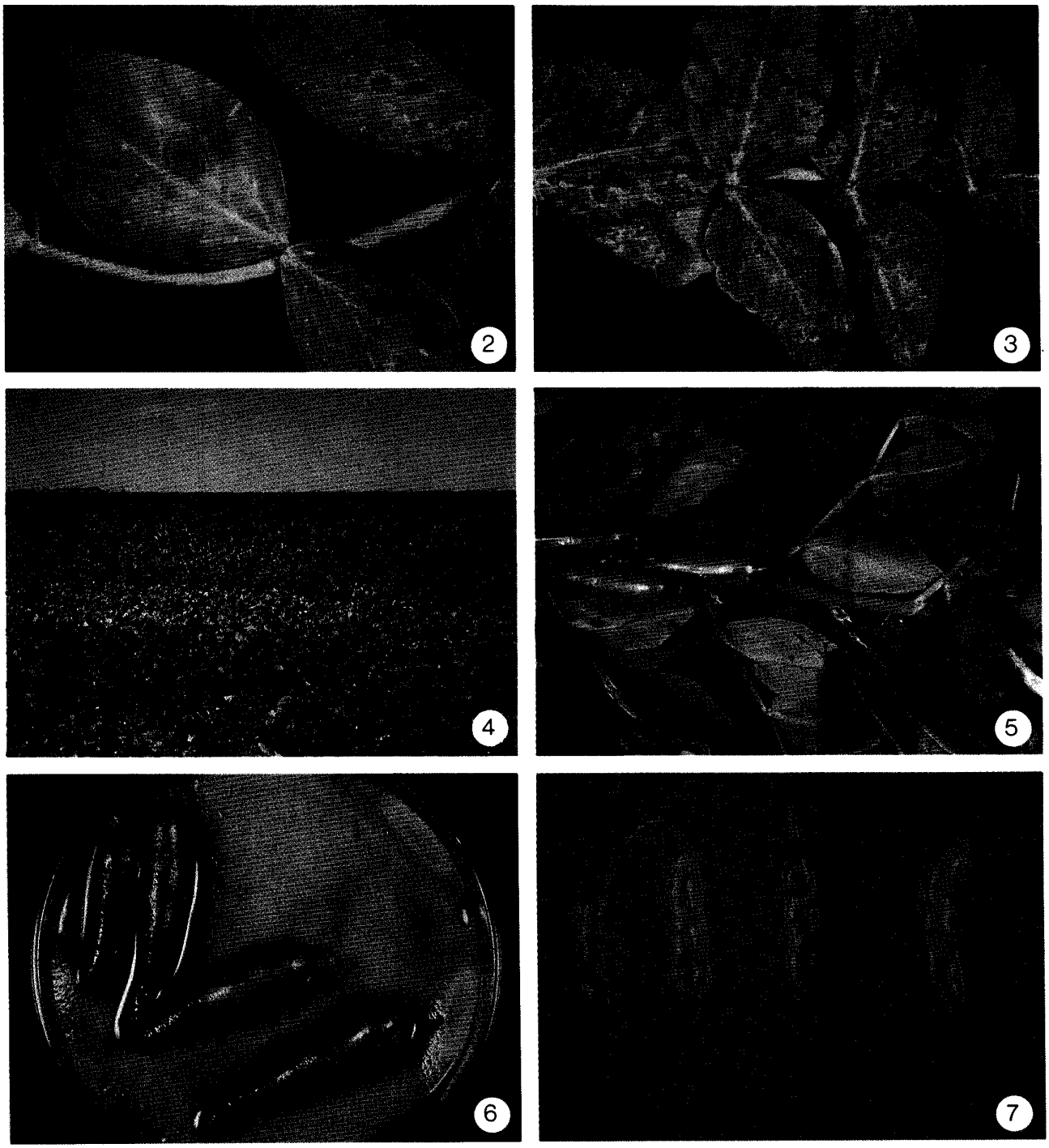

Figure 2 Alternaria leaf spot (*Alternaria alternata*) on the leaves of processing pea. **Figure 3** Mixed infections of *Alternaria alternata* and *Ascochyta pinodes* on pea leaves. **Figure 4** Foci of infection by *Xanthomonas phaseoli* and *Pseudomonas phaseolicola* in a field of processing beans. **Figure 5** Alternaria leaf spot (*Alternaria alternata*) on fababean. **Figure 6** Bacterial pod spot (*Pseudomonas* sp.) on fababean. **Figure 7** Black wart – like growths (edema) on fababean pods.

Evaluation of procedures for the detection of potato spindle tuber viroid by polyacrylamide gel electrophoresis[1]

R.P. Singh[2]

Two procedures for the extraction of nucleic acids from tissues infected with potato spindle tuber viroid (PSTV) were evaluated. A '1-day' procedure consisted of homogenization in buffer-phenol-lithium chloride solution followed by precipitation of the nucleic acids with ethanol. A '2-day' procedure involved homogenization of tissues in buffer-phenol mixture, stirring in chloroform-amyl alcohol, lithium chloride precipitation, overnight dialysis and precipitation of nucleic acid with ethanol. The final detection of PSTV was by polyacrylamide gel electrophoresis of preparations using both extraction procedures. Both procedures were equally sensitive; however, band intensity was much stronger with the '2-day' procedure. The band intensity in the '1-day' procedure was improved by varying the tissue to buffer ratio and by reducing the amount of water in which nucleic acids were dissolved prior to electrophoresis. Using this procedure, the following conclusions were made: 1) PSTV was detected more reliably from foliage than from sprouts, 2) PSTV was detected more reliably from potato plants grown at 25°C than at 15°C, 3) PSTV detection was unreliable from 2 to 3 month-old plants.

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Deux procédures pour l'extraction d'acides nucléiques à partir de tissus infectés par le viroide de la filosité des tubercules de pomme de terre (PSTV) ont été évaluées. La première procédure dite "1 jour" consiste en l'homogénéisation des tissus dans une solution de tampon-phénol-chlorure de lithium suivie par la précipitation des acides nucléiques dans de l'éthanol, la seconde dite "2 jours" implique l'homogénéisation des tissus dans un mélange tampon-phénol, agitation dans un mélange chloroforme-alcool amylique, précipitation au chlorure de lithium, dialyse au cours de la nuit et précipitation de l'acide nucléique dans de l'éthanol. La détection du PSTV est effectuée par électrophorèse sur gel de polyacrylamide des extraits obtenus à l'aide de chaque procédure. Les deux procédures ont montré une sensibilité égale toutefois, l'intensité de la bande était beaucoup plus forte avec les extraits "2 jours". L'intensité de la bande obtenue avec la procédure "1 jour" a été améliorée en changeant le rapport tissu-tampon et en réduisant la quantité d'eau utilisée pour dissoudre les acides nucléiques avant l'électrophorèse. A l'aide de cette procédure, les conclusions suivantes ont été tirées: 1) Le PSTV est détecté plus surement à partir du feuillage que des pousses, 2) PSTV est détecté plus surement chez des plants de pommes de terre cultivé à 25°C au lieu de 15°C. 3) La détection du PSTV n'est pas constante chez les plants agés de 2 à 3 mois.

Introduction

The report that potato spindle tuber viroid (PSTV) was a free-ribonucleic acid of low molecular weight (1, 15) and separated as a distinct band on polyacrylamide gel electrophoresis (PAGE) (2) resulted in the development of a PAGE procedure for its routine detection from potato plants (7, 8, 12). The PAGE procedures initially took 2-3 days to complete, but recently the procedure has been modified and can be completed in one day (10).

Potato spindle tuber viroid is carried through the pollen and seeds of potato (3, 6, 13, 14) and has been noted to occur in the major potato germ plasm collections, e.g., the Commonwealth Potato Collection in Scotland (4, 5), the International Potato Center in Peru (5), and the United States Department of Agriculture Potato Collection at Sturgeon Bay, Wisconsin (5, 9). Thus, there is apparent danger of its spreading through the exchange of potato germplasm, either in the form of tuber or as true seed. There is a need for routine testing of large numbers of potato breeding lines and the modified procedure of PAGE (10) appeared worth evaluating. However, when compared with the 2-day procedure the PSTV band appeared weaker. The improvements of this procedure and its suitability for testing sprouts and tubers are the subject of this paper. A preliminary report has appeared elsewhere (16).

Materials and methods

Potato (*Solanum tuberosum* L. cv. Russet Burbank) plantlets infected with a mild strain of PSTV (17) and field-infected tubers of different cultivars and seedlings were used. Tubers were stored at 5°C for 3 months, then sprouted at 25°C for 1 to 3 weeks. The sprouts were either used from one tuber or from three tubers combined to make the desired weight. The 1-day procedure consisted of homogenization of tissues in buffer-phenol-lithium chloride solution (1.0 ml of distilled H_2O, 0.4 ml of 4 M NH_4OH, 0.4 ml of 0.1 M ethylenedinitrilotetraacetic acid disodium salt (adjusted to pH 7.0 with Tris), 1.2 ml of 10 M LiCl, and 4 ml of water saturated phenol containing 0.1% 8-hydroxyquinoline), followed by precipitation of the nucleic acids with ethanol (10). The 2-day procedure consisted of homogenization of the tissues in buffer-phenol solution [0.5 ml of buffer (0.2 M glycine, 0.1 M $Na_2HPO_4.7H_2O$, 0.6 M NaCl, pH 9.6), 0.1 ml of 10% sodium dodecyl sulphate and 2 ml of phenol], stirring the

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aqueous layer in chloroform-amyl alcohol (2 ml), fractionation with lithium chloride, overnight dialysis and final precipitation of the nucleic acids with ethanol (7). The tissues were homogenized with a PT-35 polytron equipped with a PT10-ST microgenerator (Brinkman Instruments, Rexdale, Canada) for 30 sec at full speed. The PAGE was performed on 5% gels [acrylamide: N,N' methylene-bis-acrylamide 40:1 W/W] in 0.04 M tris, 0.02 M sodium acetate, 0.001 M disodium EDTA pH 7.2 (7). Electrophoresis was performed in cylindrical gels (0.6 × 9 cm) at room temperature for 2.5 hr at 6-8 mA/gel. Gels were stained with Toluidine Blue 0 (7) and, after destaining, were scanned at 550 nm with a Beckman DU-8 spectrophotometer.

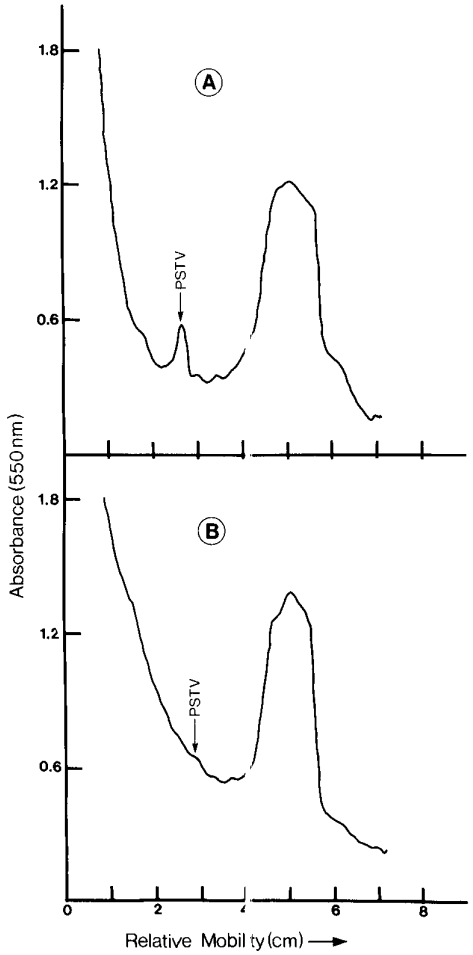

Fig. 1 Scanning profile of stained polyacrylamide gels (5%) containing nucleic acid extracted by 1-day procedure. A) potato plants grown at 25°C, B) potato plants grown at 15°C.

Results

In an initial test, 89 potato cuttings derived from a potato plant infected with a mild PSTV strain (17) and 32 cuttings from a healthy plant were evaluated by the 1-day procedure using the published procedure (10). The tests were done by two persons and the indentity of the samples was coded.

Table 1. Comparision of 1-day and 2-day procedures for the detection of potato spindle tuber viroid in potato sprouts.

Test no.	No. of samples	No. positive PSTV	
		1-day procedure	2-day procedure
1	16*	15	16
2	16*	15	15
3	16**	9	9

* Sprouts from three different tubers were combined to make one sample.

** Individual tubers were suspected to be infected with potato spindle tuber viroid.

The results indicated that all 89 PSTV samples were positive while the 32 healthy cuttings were negative on the basis of the PSTV gel banding. However, the PSTV bands were fainter in some cases and repeated testing of such samples often gave PSTV bands of variable intensity. Since the 1-day procedure was reliable, efforts to improve the PSTV band were made by manipulating buffer to tissue ratio and final volume of water to dissolve the precipitated nucleic acid. After several tests it was observed that when the tissue to extracting buffer ratio was doubled (0.5 g: 7 ml) the PSTV peak was more pronounced, compared to the single volume (0.5 g:3.5 ml) as used by Pfannenstiel *et al.* (10). Similarly, the 50, 100, and 150 µl of water used to dissolve the nucleic acid gave varying degrees of peak height. Use of 100 ul appeared suitable while those of 50 µl and 150 µl gave broader peaks. Further, we tested 72 samples of potato sprouts, 36 using modifications discussed above and 36 by the published procedure (10). Both methods detected the same number of PSTV positives (24 each), but the band's intensity was more than double of the published procedure in 20 of the 24 samples by improved procedure described here.

Table 2. Effect of potato plant parts on the detection of potato spindle tuber viroid by polyacrylamide gel electrophoresis.

Plant parts	No. samples*	No. PSTV positives
Leaves	31	30
Sprouts	31	28
Sprouts - stem end	16	15
Sprouts - mid tuber**	16	16
Sprouts -bud end	16	16
Tuber tissue***	31	24

* About 0.5 g of tissue was used for extraction of nucleic acid.

** Sprouts in the central part of the tuber, away from stem or bud ends.

*** Tuber tissue from the bud end without any sprouts.

Comparison of 1-day and 2-day procedures. Etiolated sprouts from field-infected tubers of genotypically different seedlings were combined (3 tubers/sample) for the extraction by the 1-day and 2-day procedures. The results (Table 1) showed that both procedures were equally sensitive. In the first and second test the sprouts from the same tubers were used a week apart, while in the third test suspect potato tubers were used. In contrast to Pfannenstiel *et al.* (10) we noticed again that PSTV bands were more distinct in the 2-day procedure than the 1-day published procedure (10). The seven negative sprouts from the third test were retested after growing the plants. They remained healthy.

Suitablity of 1-day procedure for testing potato plant parts. About 0.5 g of tissue from sprouts of 36 tubers were tested individually by the 1-day procedure. Thirty-one were found positive for PSTV. One eye from each of these 31 tubers was planted and leaves were tested about 1 month after emergence. The remaining tuber was used to collect 0.5 g of sprouts from various tuber parts (near stem end, mid-part and bud-end); and also tuber tissues from the bud-end were tested. The results (Table 2) showed that 30 were detected from the leaves, but only 28 from the sprouts. However, sprouts taken from various parts of the tuber were all positive and it appears that PSTV was equally distributed throughout the tuber. The PSTV detection was poor from the tuber tissues (Table 2). In addition to poor detectability of PSTV from tuber tissue, several faint bands were encountered in the vicinity of the PSTV band, which also made identification difficult.

Table 3. Effect of air temperature on the potato plants and the detection of potato spindle tuber viroid by polyacrylamide gel electrophoresis.

Age of plants	No. of samples*	15 °C	25 °C
1 week	32	31	29
4 weeks	32	16**	32
6 weeks	32	13	30
8 weeks	31	8	26
12 weeks***	30	9	21

* Only the top young growing tips were used in all the tests.

** Potato spindle tuber viroid bands were very faint.

*** The top growth was senescent and leaves were chlorotic.

Effect of air temperature on PSTV detection in potato by PAGE. The concentration of PSTV in tomato plants has been shown to increase at high temperature (4,11), but no information is available on PSTV concentration in potato plants and its effect on detectability of PSTV by PAGE. To determine this, 32 known PSTV-infected tubers were cut in two and planted one at 15 °C and the other 25 °C. The temperature range (15-25 °C) selected was that which is encountered in the greenhouses during winter-indexing of potatoes for mosaic diseases. The results (Table 3) showed that within one week of emergence 31 and 29 samples were indexed PSTV positive at 15 ° and 25 °C, respectively. However, the PSTV detection improved at 25 °C up to six weeks and then declined slowly (Table 3), while at 15 °C the PSTV detection declined sharply after the first week. When the PSTV bands of the same plants from both temperatures were compared by scanning, after four weeks of growth, it was noticed that PSTV peaks from 25 °C grown plants were very distinct while those at 15 °C were barely visible (Fig. 1A,B). The difficulty in PSTV detection could be due to low concentration of viroid present, as observed by the infectivity test (Table 4).

PSTV detection by PAGE from potato plants inoculated at various stages of growth. Twenty-four virus-free cuttings of equal age were grown at 25 °C and were inoculated with a mild strain of PSTV at 1, 2 and 3 months apart. Plants inoculated at 1 month developed symptoms within 4 weeks of inoculation and PSTV was detected from apical leaves of each plant. However, no symptoms developed in plants inoculated at 2 and 3 months growth, and only 2 of the 6 plants inoculated at 2 months growth stage were found PSTV positive, while none of the 6 plants inoculated at 3 months growth stage yielded any PSTV by PAGE.

Table 4. Effect of air temperature on viroid infectivity at various periods of growth.

	Average number of lesions**	
Age of plants*	25 °C	15 °C
1 week	376	321
3 weeks	276	162
6 weeks	143	16
9 weeks	40	0.6

* Potato cuttings 10–15 cm in height infected with mild strain of potato spindle tuber viroid were transferred to growth cabinet at specified temperatures.

** Composite samples from 5 plants were ground in glycine phosphate buffer (0.05 M glycine + 0.03 M K_2HPO_4, pH 9.2) and inoculated to 10 leaves of *Scopolia sinensis* plants.

Discussion

This study confirms that the shortened procedure of Pfannenstiel *et al.* (10) for PSTV detection is as reliable as the 2-day procedure of Morris & Smith (7), except that band intensity was variable prior to the improvements made in this study. The shortened procedure enables one worker to complete 60 to 80 tests per week using cylindrical gels and Toluidine Blue 0 staining. The efficiency could be improved further with slab-gel and ethidium bromide staining and photography. However, caution must be exercised in putting too much emphasis on the number of tests performed, rather than on the quality of nucleic acid extraction. It has been observed that steps of tissue homogenization, tissue to buffer ratio, drying of nucleic acid precipitate, and resuspension of precipitated nucleic acid could affect the results. It

was noticed that when nucleic acid precipitates were air dried instead of vacuum drying (10), the precipitates did not dissolve and PSTV bands did not separate from other nucleic acid. However, drying with N_2 gas had similar effect as vacuum drying. Strict adherence to the prescribed steps are needed for reproducible results. The negative results should be retested, if the material is for use in pollinations for potato breeding or for multiplication.

From the experiments with temperature, it is obvious that for the best results potato tubers should be sprouted or grown at 25°C rather than at low temperatures, and plants should be tested while green and young, rather than very mature plants.

Since recovery from plants inoculated at maturity was poor and such plants did not develop symptoms, there is some indication that mature plant resistance may be operative in viroid diseases also.

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Manitoba rapeseed disease survey 1978-1980

S.R. Rimmer[1] and R.G. Platford[2]

In 1980 we initiated a new surveying system involving the collaboration of agricultural representatives in a specific region of Manitoba. The data from this and our own survey are compared with the surveys of 1978/1979. Disease incidence in rapeseed in these years has been slight.

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En 1980, nous avons initié un nouveau système d'inventaire impliquant la collaboration des représentants agricoles dans une région spécifique du Manitoba. Les données de cet inventaire et du nôtre sont comparées avec les inventaires de 1978/1979. L'incidence de maladie dans le Colza a été peu élevé au cours de ces années.

Introduction

Rapeseed disease surveys have been conducted annually in Manitoba for about the last ten years (1,3,4). Generally, the survey has been conducted primarily in the region west of Winnipeg out to Brandon and northwest up to the Swan River Valley. The areas south and east of Winnipeg, the area southwest of Brandon, and the Interlake region have been neglected. In 1980, in an attempt to obtain a more representative sample, we experimented with a new procedure. This involved the cooperation of the agricultural representatives in the S.W. region of the province who, in addition to our own sampling, shipped collections of rapeseed to the MDA plant pathology laboratory where they were examined by us.

Methods

Plants were collected on the survey by sampling fields along the highways. Plants were sampled by walking into the field about 10 metres and then taking a plant every two metres until 25 plants were collected. Agricultural representatives were requested to sample five fields in their own region in this manner. Plants were individually rated for Alternaria grey leaf spot on the siliques, Sclerotinia stem rot on the stems, white rust/staghead and downy mildew, root rot and aster yellows. Ratings for Alternaria were based on percentage silique infection as follows: = 0 - 1% (trace) = 1, 1 - 5% (slight) = 2, 5 - 10% (moderate) = 3, 10% + (severe) = 4. Ratings for white rust/staghead were based on number of terminal stagheads per plant (2).

Results & Discussion

Since there are 10 agricultural representatives in Crop Districts 1, 2, and 3, (Fig. 1) we could have received from this area 50 samples of 25 plants each. In fact, 34 samples were submitted to us. These samples were compared to the samples we collected in this area and included in the over-all disease survey. These additional samples collected by agricultural representatives biases the 1980 survey for Manitoba heavily towards the south west region of the province. However, this area has not been sampled adequately in previous years. No samples were obtained from crop districts 7,9,10,11, or 12. The survey was conducted in the first week of September (Fig. 1) - a week or so later than usual - yet because of the very dry spring, even at this date very few fields were ready to swathe. Some fields were still in early or full bloom. Some fields were mixtures of *Brassica napus* and *B. campestris* where farmers had resown with *B. campestris* (due to poor emergence and flea beetle damage) fields earlier sown to *B. napus*. Samples from the agricultural representatives were received from the middle of September until the beginning of October and some of these samples were frost damaged.

Fig. 1 shows the route taken for the disease survey. Numbers on the map indicate Manitoba Crop Districts. In order to compare our sampling with that of an independent sample, Tables 1, and 2, compile information for 1978-1980 of the rapeseed acreage in Manitoba. These tables were prepared from data published by the Canadian Cooperative Wheat Producers. The acreage of each of the commonly grown varieties and types of rapeseed is shown in hectares and in proportion to the whole. Note that *B. campestris* (turnip rape) accounted for about 25% of the total in 1980 (Table 1). This proportion has increased in the last three years, though the total acreage has fluctuated considerably. The samples we collected in 1980 consisted of 36% *B. campestris* fields. Table 2 gives the proportion of *B. napus* to *B. campestris* for groups of crop districts. The S.W. region (crop districts 1,2,3) had 20% of the acreage sown to turnip rape. Our own samples consisted of 29% turnip rape from this area, again a higher proportion than the data from the Cooperative Survey. The S.W. region had 27% of Manitoba's rape production. The highest proportion of turnip rape production was in crop region 11,12, - the Interlake district. This area has not been surveyed in recent years, but as the disease situation may be different on *B. campestris* than on *B. napus* this is an area that should be surveyed.

Table 3 presents the results of the disease surveys for 1978-1980 on *B. napus*. In 1980, 40 samples were examined, 16 in the field and 24 submitted by agricultural representatives.

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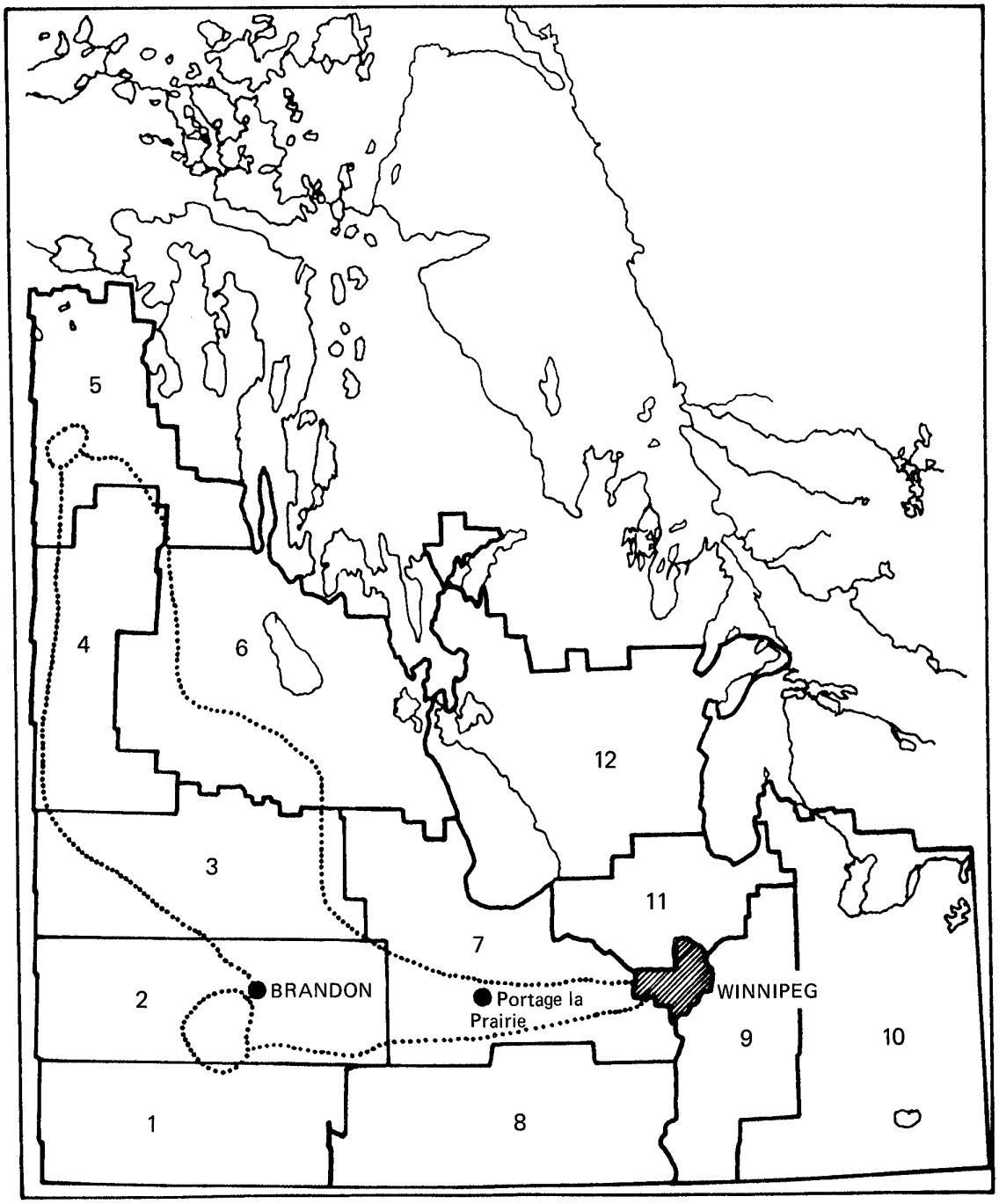

Fig. 1 1980 Rapeseed survey

Grey leaf spot (caused by *Alternara spp.*) was the most prevalent, occurring in all fields and on 92% of the plants. A few samples were moderately to severely infected but overall the disease severity (1.2) was only a trace amount. Nevertheless this is the highest level it has been for the last three years. Other diseases were observed including foot rot, aster yellows and *Sclerotinia* stem rot but these occurred at very low levels in only a few fields.

The results for *B. campestris* are given in Table 4. Grey leaf spot was again the most prevalent disease, occurring in 100% of the samples on 93% of the plants. Again the

average disease severity amounted to only a trace (1.2). White rust (staghead) and downy mildew were also commonly present but again at very low levels (1.1 and 0.4 respectively).

Diseases, then, were not a problem on rapeseed in 1980. Little disease has, in fact, occurred in the last three years but weather conditions during this period have not been favourable. No blackleg was observed in any of the samples and it seems that Manitoba is still free of this disease.

Table 1. Manitoba Rapeseed Production 1978-80 Hectarage and percentage of each variety.

	1978		1979		1980	
Variety	%	Hectares (,000s)	%	Hectares (,000s)	%	Hectares (,000s)
Brassica napus						
Altex					3.7	11.9
Midas	26.7	113.5	6.0	33.9		
Oro	2.0	8.6				
Regent			41.3	234.0	47.0	152.2
Tower	54.3	230.9	29.2	165.6	21.3	69.2
Zephyr	1.2	5.1				
	84.2	358.1	76.5	433.6	72.0	233.3
Brassica campestris						
Candle			2.1	12.0	9.8	31.6
Span	1.9	8.0	0.6	3.4		
Torch	13.7	58.1	20.6	116.7	15.8	51.2
	15.6	66.1	23.3	132.1	25.6	82.8
Other (High Erucic)	0.2	1.0	0.2	1.4	2.4	7.9
	100.0	425.2	100.0	567.0	100.0	324.0

Table 2. Manitoba Rapeseed Production 1978-80. Percentage of *Brassica Campestris* and *Brassica Napus* by Crop Districts.

	1978				1979				1980			
Crop Districts	Camp.	Napus	%+	Hect.*	Camp.	Napus	%+	Hect.*	Camp.	Napus	%+	Hect.*
1,2,3	12	88	30.3	129	15	85	26.9	152	20	78	27.1	88
4, 5, 6	16	84	25.8	110	30	70	27.4	155	27	73	22.5	73
7	18	82	15.3	65	14	86	17.5	99	26	73	20.1	65
8	11	88	19.2	82	15	84	18.7	106	24	68	20.4	66
9, 10	17	80	1.9	8	33	64	2.1	12	28	72	2.2	7
11, 12	35	65	7.5	32	67	33	7.4	42	43	56	7.7	25
Total	16	84	100.0	426	23	77	100.0	566	26	74	100.0	324

+ % production of total

* Hectarage in ,000s.

Table 5 compares the data obtained from samples in the field by us to those submitted by the agricultural representatives. Only samples from related areas were compared. The data are very similar except that the severity of grey leaf spot is higher (1.9, 1.5 compared to 1.2) for samples submitted by the agricultural representatives. This probably reflects the later sampling date of these collections (2-4 weeks later than the survey samples) and during this period disease build up occurred. This data encourages us that this form of sampling is as effective as our own field sampling, and probably is a better sampling method statistically than our own. We are limited by the number of samples we can handle this way but it will be continued as the larger sample overall is more representative and this method permits us to sample areas we are unable to visit.

Table 3. Manitoba Rapeseed Disease Survey 1978-1980. *B. napus.* - Summer rape

	1978			1979			1980		
Disease	% Fields	% Plants	Severity	% Fields	% Plants	Severity	% Fields	% Plants	Severity
Grey Leaf Spot	92	30	0.3	68	47	0.5	100	92	1.2
Foot & Root Rots	16	3	-	-	-	-	10	4	-
Sclerotinia Stem Rot	12	4	0.1	-	-	-	5	2	-
Aster Yellows	16	1	-	-	-	-	10	4	-
No. of Fields Surveyed	?			18			16 24 (Ag. Reps.)		

Table 4. Manitoba Rapeseed Disease Survey 1978-1980 *B. campestris* - Summer Turnip rape

	1978			1979			1980		
Disease	% Fields	% Plants	Severity	% Fields	% Plants	Severity	% Fields	% Plants	Severity
Grey Leaf Spot	75	25	0.5	75	36	0.1	100	93	1.2
Root and Foot Rots	12	0.5	-	14	2	-	13	5	-
Staghead White Rust	87	7	0.2	88	67	1.2	86	62	1.1
Sclerotinia Stem Rot	-	-	-	-	-	-	-	-	-
Aster Yellows	12	0.5	-	-	-	-	-	-	-
Downy Mildew	25	5	-	75	52	0.8	100	38	0.4
No. of fields surveyed	8			7			14 9 (Ag. Reps.)		

Table 5. Manitoba Rapeseed Disease Survey 1980 Crop Districts 1,2,3

	Fields sampled by GP, SRR			Fields sampled by Ag. Reps.		
	% Fields	% Plants	Severity	% Fields	% Plants	Severity
B. campestris						
Grey Leaf Spot	100	99	1.2	100	99	1.9
Staghead White Rust	100	65	1.2	100	49	1.0
B. napus						
Grey Leaf Spot	100	95	1.2	100	99	1.5

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

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Figures should be planned to fit, after reduction, one column (maximum 84 × 241 mm) or two columns (maximum 175 × 241 mm), and should be trimmed or marked with crop marks to show only essential features. Figures grouped in a plate should be butt-mounted with no space between them. A duplicate set of unmounted photographs and line drawings is required. Figures should be identified by number, author's name, and abbreviated legend.

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Les titres doivent être courts et révélateurs en contenant, avec le résumé, les mots clés les plus utiles pour le classement et l'extraction de l'information.

Chaque article doit être accompagné d'un *résumé* d'au plus 200 mots en anglais et en français, si possible.

Les figures doivent pouvoir, après réduction, remplir une colonne (maximum 84 × 241 mm) ou deux colonnes (maximum 175 × 241 mm) et devraient être taillées ou montrer les parties essentielles à garder. Les figures groupées sur une même planche doivent être montées côte à côte, sans intervalle. L'article doit être accompagné d'un double des photographies non montées et des graphiques. Les figures doivent être numérotées, porter le nom de l'auteur et une légende abrégée.

Les tableaux doivent être numérotés en chiffres arabes et avoir un titre concis. Ils ne devraient pas avoir de lignes verticales. Les renvois doivent être identifiés par un signe typographique particulier (* † § # ¶ ** ††) surtout lorsqu'il s'agit de nombres.

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