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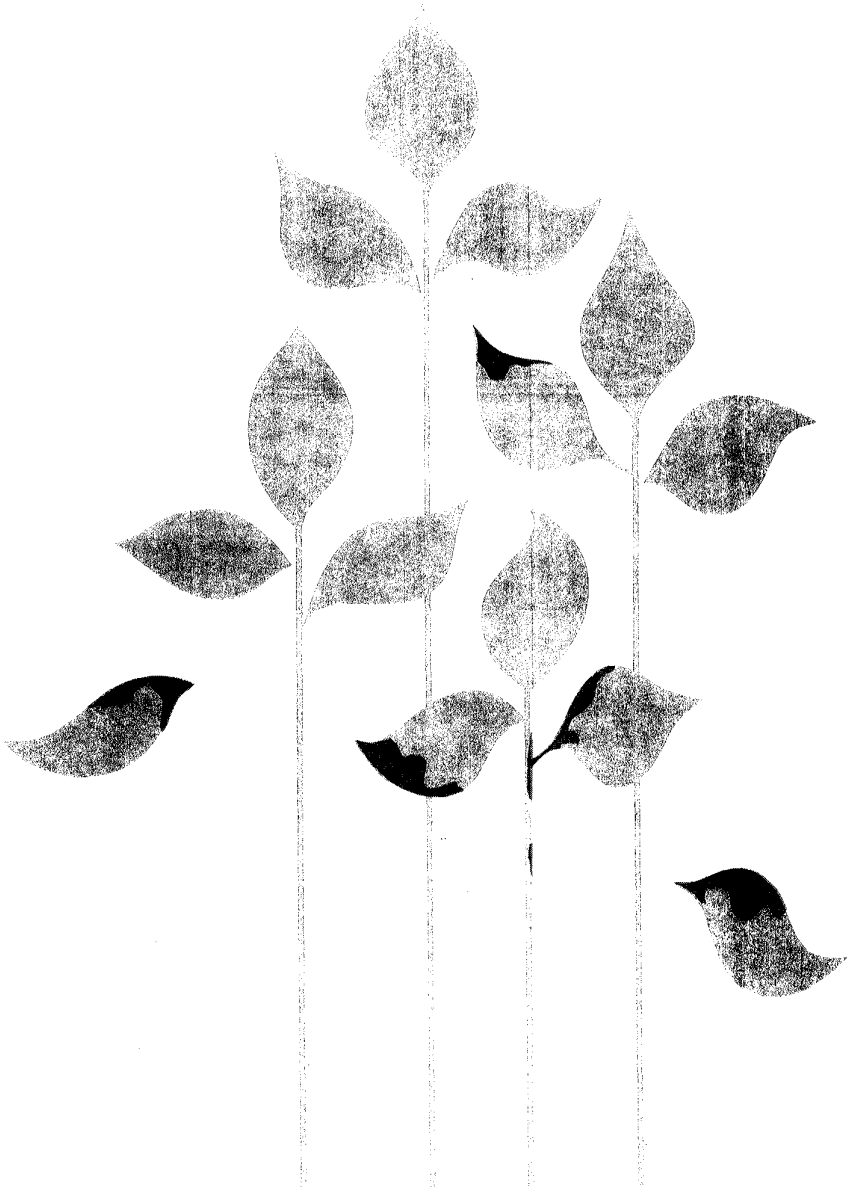

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Contents

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.

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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Influence of Paratylenchus projectus on alfalfa sickness in Alberta[1]

E. J. Hawn and G. C. Kozub

Despite high populations of *Paratylenchus projectus* Jenkins (pin nematodes), healthy samples of grey wooded Luvisolic soil from central Alberta were significantly more productive of Grimm alfalfa growth than were samples of virgin or alfalfa-sick soils tested. The difference was due to higher fertility and pH. Grimm alfalfa consistently reduced populations of pin nematodes that had been increased by preplanting with *Lotus corniculatus* L. (bird's-foot trefoil). Comparably high populations of pin nematodes elsewhere in Alberta did not produce alfalfa sickness.

Can. Plant Dis. Surv. 58:1, 1-4. 1978

Malgré la présence de fortes populations de *Paratylenchus projectus* Jenkins (nématode acuminé), on a constaté que des échantillons de sols Luvisols gris boisés du centre de l'Alberta produisaient davantage de luzerne Grimm que des échantillons de sols vierges ou montrant des signes de "fatigue" de la luzerne. Cette différence se justifie par le meilleur état de fertilité et le pH plus élevé de ces sols. La culture de luzerne Grimm a régulièrement réduit les populations de nématodes qu'avait accrues une plantation antérieure de *Lotus corniculatus* L. (Lotier corniculé). Ailleurs en Alberta, des populations relativement fortes de nématodes n'ont pas provoqué de symptômes de "fatigue" de la luzerne.

Introduction

Alfalfa sickness, reported by Webster et al. in 1967 (13), occurred in alfalfa (*Medicago sativa* L.) growing in Dark Grey Luvisolic soils of central Alberta. Affected plants were short, spindly, and yellowish green and had little or no nodulation and poorly developed root systems. In affected stands, growth was retarded but healthy plants were frequently found in irregular patches throughout the fields.

The Dark Grey Luvisolic soils were characteristically of low pH (5.8-6.0), low in nitrogen (N), phosphorus (P), sulfur (S), and humus (7, 9). Neither fertility level nor moisture content were critical factors in disease development in field tests (12, 13). None of a wide range of alfalfa varieties tested was resistant to alfalfa sickness (3). Mild heat (51°C) frequently eliminated the disease (3) and indicated that a toxin might be an incitant.

A pin nematode, *Paratylenchus projectus* Jenkins (5), was found in alfalfa-sick soils by the late W. R. Orchard (14) and identified by L. Y. Wu of Biosystematics Research Institute, Agriculture Canada. Populations were generally greater in the rhizospheres of 'sick' plants (11).

Coursen et al. (1) listed 89 hosts of *P. projectus* in 1958. These included alfalfa, red clover, oats, bromegrass, and timothy -- all grown in central Alberta areas of alfalfa sickness. The host range of the pin nematode and its association with alfalfa sickness in the Grey Luvisolic soils of central Alberta (4, 9, 11, 14) suggested that the nematode might be closely involved in this serious plant disease.

In this study of alfalfa sickness, the effect of initial pin nematode population, soil source, pH, and *Rhizobium* was examined.

Materials and Methods

Soils were selected from the University of Alberta soil science plots at Breton, Alberta, where the soils are luvisolic and alfalfa sickness has been reported (13). The plots sampled were virgin (BH), healthy (BD7), and sick (BD3). The "Virgin" soil was low in N, P, and S (7), unfertilized, and pH 5.2 and contained 752 pin nematodes/kg (6, 8). "Healthy" and "Sick" soils came from adjacent plots of 2-year-old Grimm alfalfa. The "Healthy" plot was on a complete fertilizer (N, P, and K) program with additional lime (L) and sulfur (S), was pH 5.8, and contained 41,300 pin nematodes/kg. The "Sick" plot was on a complete fertilizer program (excluding L), was pH 5.4, and contained 74,400 pin nematodes/kg.

Half of the soil taken from each plot was planted to *Lotus corniculatus* L. cv. Leo (bird's-foot trefoil) for 3 months to increase the populations of pin nematodes. The counts per kilogram of soil were: virgin - 768, healthy - 6,280, sick - 145,880. All plant debris was removed before seeding with alfalfa was begun. The remaining soil was stored at ambient room temperature without watering.

Elements or factors considered in this study of alfalfa sickness were type of soil, effect of pH, effect of initial pin nematode population, effect of preplanting with bird's-foot trefoil, and influence of inoculating the soils

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Table 1. Means* and standard errors for total yield of Grimm alfalfa top growth, plant weight, and final *Paratylenchus projectus* population** for the three soil sources and influence of preplanting with bird's-foot trefoil

	Variables and bird's-foot trefoil levels					
	Top growth (g)		Plant root and crown weight (g)		No. of *P. projectus* per kg of soil	
Soil source	Not preplanted	Preplanted	Not preplanted	Preplanted	Not preplanted	Preplanted
Virgin (BH)	11.82a	13.33a	13.44bc	14.76ab	280c (740)	770b (1,610)
Healthy (BD7)	13.21a	13.44a	16.94a	14.80ab	1,450b (1,970)	85,680a (92,620)
Sick (BD3)	12.44a	10.06b	11.73cd	11.01d	1,230b (2,090)	227,720a (264,450)
Standard errors (71 df)	0.397		0.570			0.127

*For each variable, means followed by the same letter do not differ significantly ($P > 0.05$) using Tukey's test (2).
**For numbers of *P. projectus*, geometric means are given and the standard error is in $\log_{10}$ units. Arithmetic means are in brackets.

Table 2. Means* and standard errors for total yield of Grimm alfalfa top growth, plant weight, and final *Paratylenchus projectus* population** for three soil sources and two lime treatments

	Variables and lime treatments					
	Top growth (g)		Plant root and crown weight (g)		No. of *P. projectus* per kg of soil	
Soil sources	None	pH 6.5	None	pH 6.5	None	pH 6.5
Virgin (BH)	10.01d	15.15a	12.63b	15.57a	430b (1,290)	505b (1,060)
Healthy (BD7)	12.17c	14.49ab	15.49a	16.25a	10,510a (53,100)	11,820a (41,490)
Sick (BD3)	9.46d	13.03bc	10.98b	11.76b	18,480a (123,000)	15,170a (143,540)
Standard error (71 df)		0.397		0.570		0.127

*For each variable, means followed by the same letter do not differ significantly ($P > 0.05$) using Tukey's test (2).
**For numbers of *P. projectus*, geometric means are given and the standard error is in $\log_{10}$ units. Arithmetic means are in brackets.

with the recommended strain of *Rhizobium meliloti* Dangar.

Each combination of soil and nematode population level was placed in sixteen 15-cm clay pots and seeded with five Grimm alfalfa seeds per pot. Treatments were: (1) control, (2) lime to raise soil pH to 6.5, (3) inoculate the soil with *Rhizobium*, (4) lime to raise soil pH to 6.5 and inoculate with *Rhizobium*.

All combinations of the soil types and treatments formed a 3 (soils) X 2 (nematode levels) X 2 (lime) X 2 (*Rhizobium* inoculum) factorial experiment that was set out in four randomized blocks on a greenhouse bench. Watering was controlled to maintain the soil at 25% of field capacity. Uniform light was supplied by fluorescent light banks regulated to give illumination for 16 h/day at 21,530 lux. The temperature was maintained at 20°C ± 1°.

Top growth from each test plot was collected at six regularly-spaced intervals from 23 January to 22 August 1973. It was bagged, dried at 75°C for 48 h, and then weighed. On completion of the test, data were compiled on top growth, total weight including roots and crowns, soil pH (Fisher Accumet), populations of pin nematodes, and extent of *Rhizobium*-induced nodule growth. Analyses of variance (2) were carried out on the above data. A logarithmic transformation was applied to

Table 3. Means* and standard errors of *Rhizobium meliloti* levels on total yield of top growth, plant weight, *Paratylenchus projectus* populations**, and pH

	Variables			
R. meliloti level	Top growth (g)	Plant weight (g)	No. of *P. projectus*/kg	Soil pH
Control	12.38a	13.54a	4,765a (65,290)	6.53a
Inoculated	12.39a	14.01a	4,110a (55,870)	6.53a
Standard error (71 df)	0.229	0.329	0.073	0.012

*For each variable, means followed by the same letter do not differ significantly ($P > 0.05$) using Tukey's test (2).
**For numbers of *P. projectus*, geometric means are given and the standard error is in $\log_{10}$ units. Arithmetic means are in brackets.

Table 4. Soil pH means* and standard error for three soils, two nematode population levels, and two lime treatments

		Lime treatment	
Soil	Pin nematode populations	None	To pH 6.5
Virgin (BH)	Low	5.83i	6.94abc
	High	5.96hi	6.91bc
Healthy (BD7)	Low	6.25f	7.08a
	High	6.62e	6.72de
Sick (BD3)	Low	6.12fg	7.04ab
	High	6.08gh	6.82ce
Standard error (71 df)		0.029	

*Means followed by the same letter do not differ significantly ($P > 0.05$) using Tukey's test (2).

the pin nematode counts to make the treatment means and variance independent.

Results and discussion

Although the total alfalfa yields of top growth and weights of plant roots and crowns were not significantly affected by preplanting in virgin or healthy soils, top growth yields were lower ($P < 0.05$) in pots of sick soil that had been preplanted with bird's-foot trefoil (Table 1). Populations of pin nematodes after alfalfa growth were higher ($P < 0.05$) in soils that had been preplanted to bird's-foot trefoil, especially the sick soil, which had low pH and no previous history of liming.

The contrasting yields of top growth in healthy and sick soils showed that the former owed its high productivity to a residual influence of NPKSL fertilizers that resulted in pH 5.8 and production of good yield despite high populations of pin nematodes in preplanted test portions of this soil. When compared to virgin soil, weights of plant roots and crowns were reduced in sick soil but not in healthy, despite significantly greater populations of pin nematodes (Tables 1 and 2).

Lime treatment increased total yield and plant weight (Table 2). The largest increase (51%) in total yield due to lime treatment occurred in virgin soil, which had no previous NPKS or L fertilizers and had the lowest pH. In contrast, the healthy soil, which had had prolonged NPKSL treatment, showed the least increase (19%) and the sick soil, which had received NPKS, gave an intermediate increase (37%).

Lime treatment had no significant effect on the numbers of pin nematodes (Table 2) but its effect on pH was greater in virgin than in healthy soil (Table 4) probably because of previous use of lime in the healthy plot.

Inoculation of alfalfa seed with *R. meliloti* neither promoted nodulation nor significantly increased yield of alfalfa grown in the test soils (Table 3). This indicated a

need for a viable strain of the bacterium better able to cope with the Grey Luvisolic soil environment.

High populations of pin nematodes were found in the rhizospheres of healthy alfalfa in the Leduc district (11) and, in southern Alberta, pin nematodes were detected in 52% of the irrigated stands examined. Also, in a survey of irrigated alfalfa-grass pasture soils at the Lethbridge Research Station extending from 1973 through 1975, numerous pin nematodes were present in 71% of the samples examined.

Alfalfa consistently reduced pin nematode populations in this study, a result confirmed by Townshend and Potter (10), who found bird's-foot trefoil, timothy, and clovers were good hosts. These hosts are grown in and out of the areas of alfalfa sickness in Alberta and would, if the pin nematode was the principal cause of the disease, contribute greatly to disease development and spread through increase in nematode population.

The reduced yield of alfalfa from preplanted sick soil ($P < 0.05$) compared to preplanted healthy soil ($P < 0.05$) showed the value of NPKSL fertilizers (Table 1). It is evident that such management together with the poor host capabilities of the alfalfa minimized the role of the pin namatode in alfalfa sickness in Alberta.

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A yield loss conversion factor for peas moderately affected by fusarium root rot [1]

P. K. Basu[2]

Losses in oven-dry seed weight from moderate root rot (*Fusarium solani* f. sp. *pisi*) in several commercial pea (*Pisum sativum*) cultivars grown in a *Fusarium*-infested field at Ottawa were 27.4%, 20.5% and 17.3% in 1974, 1975 and 1976, respectively. Despite variation in cultivar yield response over the three-year period, it was concluded that a yield loss conversion factor of 0.23 was reasonable for moderately affected plants which usually do not show distinct aboveground disease symptoms.

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Les pertes de rendement en graines (poids sec) attribuables à une infection modérée de pourridié fusarien (*Fusarium solani* f. sp. *pisi*) sur plusieurs cultivars commerciaux de pois (*Pisum sativum*), cultivés à Ottawa dans un champ infesté, se sont établies à 27.4% en 1974, 20.5 en 1975 et 17.3 en 1976. Malgré les différences variétales observées durant ces trois années, on peut raisonnablement proposer un facteur de conversion de 0.23 pour évaluer les pertes de rendement de plantes modérément atteintes par la maladie, mais dont les parties aériennes ne manifestent pas de symptômes nets.

Pea (*Pisum sativum* L.) root rot caused by *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (F. R. Jones) Snyd. & Hans. is widely distributed in Canada (1). In an earlier study (2) it was found that plants had to be grouped into severe and moderate categories in order to estimate pea yield loss. The symptoms of the moderately affected plants ranged from a trace to 5 cm brown to black discoloration of the lower stem and tap root region, lateral roots were usually clean and plants apparently healthy except for normal senescence of a few lower leaves. The average yield reduction in the severe category was about 60% (2,3). However, the effects of moderate infection on pea yield remained inconclusive largely because of insufficient data. Hence further experimental work was undertaken to determine the yield loss of field-grown peas showing moderate levels of disease.

Materials and methods

In a continuation of the pea root rot study initiated earlier at Ottawa (2), several commercial pea cultivars were tested in 1975 and 1976 in the same *Fusarium*-infested field (0.28 ha) where root rot had developed each year since 1971.

In 1975, the infested area was planted with 29 pea cultivars (Table 1) using four replications in plots of 1.8 × 2.1 m size in a randomized block design. In 1976, 12 cultivars (Table 2) were planted using eight replications with the same size of plots. Commercially fungicide-treated pea seeds, obtained from Asgrow Seed Co., Kalamazu, Michigan, U.S.A., were sown with a grain drill at a spacing of 5 × 15 cm in early June each year. Plants were rated for disease and harvested when the majority of the pods of apparently healthy plants had filled approximately 60 to 70 days after planting. One hundred plants were dug along the diagonals of each plot, their roots were washed and classified into healthy, moderate (root rot 1-3) and severe (root rot 4) categories (2). In addition, oven-dry (48 h at 80°C) seed weights (g) were obtained from 25 healthy and 25 moderately affected plants from each plot to determine the yield loss of the latter group.

Results and discussion

The effects of moderate root rot development on pea yield loss will be discussed on the basis of data obtained in the *Fusarium*-infested field at Ottawa during three years (1974-1976). The average yield loss of 10 cultivars with moderate root rot was 27.4 ± 2.6% in 1974 as reported earlier (2). The loss was 20.4 ± 2.5% for 29 cultivars in 1975 (Table 1) and 17.5 ± 2.6% for 12 cultivars in 1976. (Table 2). It is noteworthy that the standard error values, 2.6, 2.5 and 2.6 were within 15% of their respective means. Such values are considered acceptable for crop-disease-loss assessment (4). However, the yield loss of moderately affected plants of different cultivars ranged from zero to nearly 50% (Table 3). This makes the grouping of cultivars based on the percent yield loss of the moderately affected plants inadequate. Six of the cultivars tested each year also showed large variation in yield loss from moderate root rot (Table 4). The loss values of cultivars, such as, Asgrow #4683 and Charger remained reasonably consistent, but those of Anoka, Trojan and Venus decreased while the losses in Nugget increased during the three-year period. The causes of these changes and also the presence of two negative loss

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Table 1. Percentage of plants in healthy and moderate root rot categories, their yield* and yield loss of moderately affected plants of 29 pea cultivars grown in a fusarium-infested field at Ottawa, 1975

Cultivar	Healthy		Moderate		Per plant yield loss of moderate (%)
	%	Yield	%	Yield	
Anoka	29.5	5.6	40.8	3.5	37.5
Beagle	14.3	2.5	54.0	2.3	8.0
Canjoy	29.3	1.0	67.8	0.9	10.0
Charger	30.0	3.1	40.5	2.4	22.6
Dark Skin Perfection	8.3	3.5	59.0	3.2	8.6
Dart	47.5	2.0	20.5	1.3	35.0
Dot	25.5	0.9	37.5	0.9	0.0
Esquire	33.0	1.5	52.0	1.2	20.0
Green Arrow	21.5	3.4	51.5	3.4	0.0
Green Bay	8.3	3.0	56.0	2.9	3.3
Hyalite	21.5	2.9	47.0	1.7	41.8
Jade	16.8	3.1	72.5	2.1	32.6
Mars	26.0	4.7	49.5	2.6	46.8
Medalist	33.3	1.8	60.7	1.6	11.1
Nugget	8.0	2.7	64.3	2.4	11.1
Pacemaker	17.8	3.8	28.8	2.8	26.3
Scout	36.2	1.8	50.8	2.1	-16.6
Signet	38.3	3.9	39.3	2.4	38.5
Small Sieve Freezer	20.4	2.6	69.3	1.9	26.9
Sparkle	18.0	1.5	55.5	2.6	36.6
Target	31.0	1.5	44.0	1.2	20.0
Trojan	9.8	3.9	62.7	3.2	17.9
Trumpet	4.0	3.5	22.0	3.7	-5.7
Venus	22.3	4.4	42.4	3.3	25.0
Viking	20.2	2.2	45.0	1.6	27.8
Wyola	17.8	4.2	30.3	2.9	31.0
#4683 (Asgrow)	42.0	2.6	29.0	2.5	3.8
#A-45 (Asgrow)	38.3	1.8	53.8	1.6	11.1
#XPF3007 (Asgrow)	4.5	1.7	54.8	1.4	17.6
Mean ± SE	23.2 ± 2.2	2.9 ± 0.2	48.3 ± 2.6	2.3 ± 0.2	20.5 ± 2.5

*Yield represents oven-dry seed weight (g) per plant derived from 25 plants from each of four replications.

Table 2. Percentage of plants in healthy and moderate root rot categories, their yield* and yield loss of moderately affected plant of 12 pea cultivars grown in a fusarium-infested field at Ottawa, 1976

Cultivar	Healthy		Moderate		Per plant yield loss of moderate (%)
	%	Yield	%	Yield	
Alaska	53.3	1.3	37.0	1.1	15.4
Anoka	10.8	2.3	73.3	2.2	4.3
Charger	34.0	1.5	57.3	1.2	20.0
Dart	25.3	1.3	55.5	1.0	23.1
Medalist	29.8	1.6	63.3	1.5	6.3
Nugget	14.8	1.7	65.8	1.1	35.3
Target	29.5	2.2	52.0	1.7	22.7
Trojan	25.3	1.7	62.5	1.5	11.8
Trumpet	39.5	2.2	48.0	1.7	22.7
Venus	24.3	2.7	64.0	2.3	14.8
Wyola	27.5	1.3	62.8	1.0	23.1
Mean ± SE	31.2 ± 4.1	1.8 ± 0.1	55.9 ± 3.6	1.5 ± 0.1	17.3 ± 2.6

*Yield represents oven-dry seed weight (g) per plant derived from 25 plants from each of eight replications.

Table 3. Tentative grouping of several pea cultivars based on the range of percent yield loss* of moderately affected plants grown in a fusarium-infested field at Ottawa in 1974, 1975 and 1976

	Range of percent yield loss				
Year	0-10	11-20	21-30	31-40	41-50
1974	#4683 (Asgrow)	D.S.P.†	Charger	Jade	Anoka
				Trojan	#XPF 3007 (Asgrow)
	Nugget	Mars		Venus	
1975	#4683 (Asgrow)	#A-45 (Asgrow)	Charger	Anoka	Hyalite
			Pacemaker	Dart	Mars
	Beagle	#XPF 3007 (Asgrow)	Small Sieve Freezer		Jade
	Canjoy			Signet	
	D.S.P.†	Esquire	Venus	Sparkle	
	Dot	Medalist	Viking	Wyola	
	Green Arrow	Nugget			
	Green Bay	Target			
		Trojan			
1976	#4683 (Asgrow)	Alaska	Dart		
		Charger	Target		
	Anoka	Trojan	Trumpet		
	Medalist	Venus	Wyola		

*Percent yield loss based on oven-dry seed weights (g) of healthy and moderately affected plants.
†Dark Skin Perfection.

Table 4. Percent yield loss* of moderately affected plants of six pea cultivars grown in a fusarium-infested field at Ottawa in 1974, 1975 and 1976

Cultivar	1974	1975	1976
#4683 (Asgrow)	6.6	3.8	7.7
Anoka	48.8	37.5	4.3
Charger	20.8	22.6	20.0
Nugget	7.7	11.1	35.3
Trojan	25.9	17.9	11.8
Venus	34.0	25.0	14.8

*Percent yield loss based on oven-dry seed weights (g) of healthy and moderately affected plants.

values have not been further investigated. Notwithstanding such (natural) variations, it seemed appropriate to derive an average loss value for the moderate root rot category with the available data. The three-year average loss for all cultivars tested was 23% which can be expressed as a yield loss conversion factor of 0.23. This factor multiplied by the percentage of plants in the moderate category (Tables 1 and 2) would provide an estimate of yield loss in field plots or in growers' fields having predominantly moderately infected plants. For example, 71.6% of the plants sampled showed moderate root rot symptoms during the 1971 pea disease survey in Ontario (1); the remaining plants were healthy. Using the factor of 0.23, the estimated pea yield loss in Ontario in that year would have been 16.5%. Similar loss estimates could also be made for other provinces where the percentage of moderately affected plants were known (1). In order to estimate an

overall loss from both moderate and severe root rot the loss conversion factors of 0.23 and 0.61 (2,3) should be used, respectively, for the two categories of diseased plants. It is noteworthy that, unlike severely affected plants, moderately affected ones do not usually show aboveground symptoms. Consequently the percentage of such plants or the area occupied by them in a field would have to be determined from ground surveys (1). The aerial photographic methods, as employed for the determination of severe root rot or drought affected areas (3), may not be applicable for moderately affected plants.

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The author wishes to thank N. J. Brown for his excellent technical assistance.

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Incidence and severity of net blotch of barley and distribution of Pyrenophora teres biotypes in the Canadian prairies in 1976[1]

A. Tekauz

Net blotch was found in most barley (*Hordeum distichum, H. vulgare*) fields sampled in three prairie provinces. Disease severity was generally very light in six-rowed barley throughout the area sampled and in two-rowed barley in Alberta. Two-rowed barleys in Manitoba and Saskatchewan had moderate levels of disease. One biotype of *Pyrenophora teres* predominated in Manitoba, another in Alberta; and both were common in Saskatchewan. A third biotype was found only in eastern Manitoba and in two fields in Alberta.

Can. Plant Dis. Surv. 58:1, 9-11. 1978

On a constaté la présence de la rayure réticulée dans la plupart des champs d'orge (*Hordeum distichum, H. vulgare*) échantillonnés dans les Prairies. La maladie était généralement très bénigne chez l'orge à six rangs et à deux rangs, partout et en Alberta respectivement, et modérée chez cette dernière au Manitoba et en Saskatchewan. Un biotype de *Pyrenophora teres* dominait au Manitoba, un autre en Alberta, et les deux se rencontraient en Saskatchewan. Un troisième n'était répandu que dans l'est du Manitoba et dans deux champs en Alberta.

Introduction

Three biotypes of *Pyrenophora teres* (Died.) Drechsl., causal agent of net blotch of barley (*Hordeum distichum* L., *H. vulgare* L.) have been reported to occur in western Canada (5, 6). The distribution of N- and VN biotypes, which produce typical net blotch symptoms on foliage, was widespread in the prairies, while the S-type which produces spot-like symptoms, was restricted to the eastern half of Manitoba. Previously (1), culture 102 of *P. teres*, an N-type isolate, was thought to be typical of this pathogen in western Canada. Since breeding for net blotch resistance in barley had concentrated only on resistance to N- biotypes, it was necessary to determine both the distribution and the relative importance of the three biotypes in the prairies to assess the need to change future breeding programs. Although the 1974 surveys gave an indication of the distribution and proportion of *P. teres* biotypes, the data were based on a relatively small number of fields found infected that year, because of the generally dry conditions. Consequently, there was a need for more comprehensive data on the distribution of *P. teres* biotypes and the surveys described herein were carried out. The severity of net blotch infection was also recorded during sampling.

Materials and methods

Surveys of commercial barley fields were conducted in 1976 to assess the incidence, severity and distribution of net blotch. The routes followed were similar to those used in 1974 (5), but a larger area of Alberta was included in the major survey, and the number of fields sampled in both Alberta and Saskatchewan was increased. Methods of disease assessment, sampling, pathogen isolation and differentiation of *P. teres* biotypes were the same as those described previously (4, 5). Herta and C.I. 9214 were used in place of Fergus and C.I. 5791, respectively, as the two-rowed and resistant checks in the four-cultivar barley series used to differentiate *P. teres* isolates. The three-province survey was carried out from July 24 to 30; the Winnipeg region was surveyed on July 27 and July 29.

Results and discussion

Manitoba, Saskatchewan and Alberta survey

The route followed, some reference points and the location of the 114 fields sampled is shown in Fig. 1. Net blotch was found in 87 (76%) of the fields examined (100% of 44 two-rowed fields and 61% of 70 six-rowed fields). Variation in symptoms was similar to that described previously (5). Disease severity ratings, based on the total area affected on the top two leaves, were primarily in the trace to very light range in six-rowed barley in all three provinces with the exception of two fields near Killam, Alberta, which had severe infections. Two-rowed barley fields had light to moderate levels of net blotch in Manitoba and Saskatchewan and very light levels in Alberta. Plants in most fields sampled were at the milky-ripe to soft dough stages of growth. N-, VN-, and S-type isolates, respectively, comprised 58%, 39% and 3% of the total number of *P. teres* isolates. N-type isolates accounted for almost all those found in Alberta, more than half of those in Saskatchewan, but they were rare in Manitoba. VN-type isolates were the predominant type found in Manitoba, were common in Saskatchewan, but were not found in

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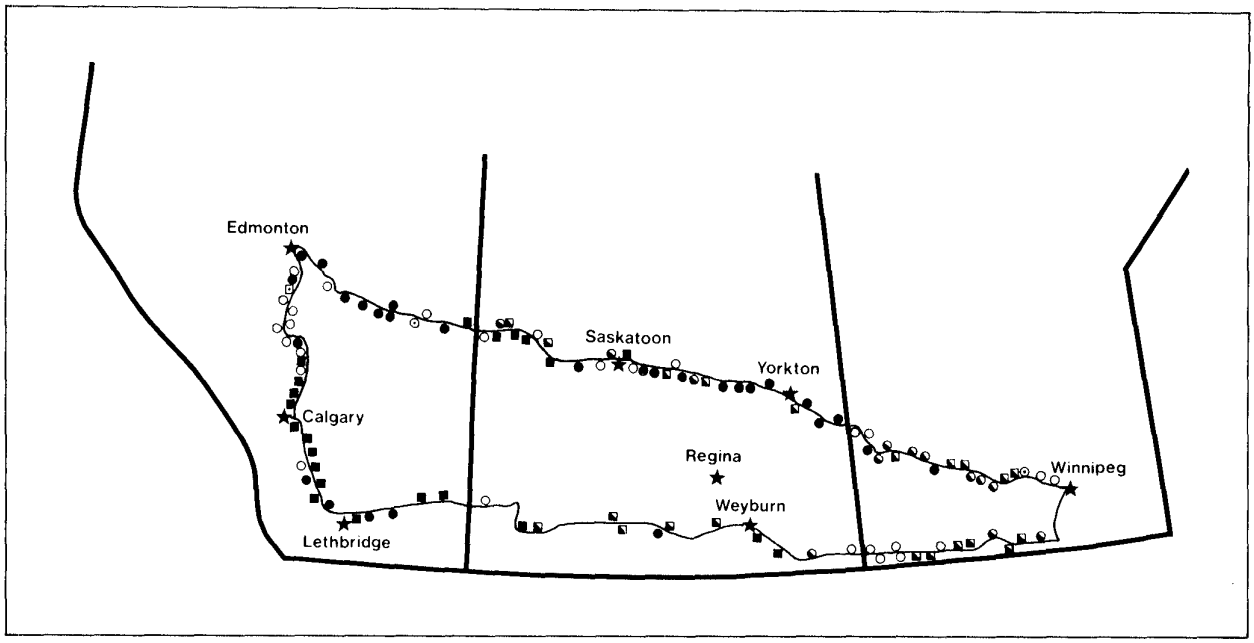

Fig. 1. Distribution of barley fields infected with net blotch (*Pyrenophora teres*) in Manitoba, Saskatchewan, and Alberta. Two-rowed barley – □ no net blotch found; ■ N-type isolate found; ◪ VN-type isolate found; ⊡ S-type isolate found. Six-rowed barley – ○ no net blotch found; ● N-type isolate found; ◐ VN-type isolate found; ⊙ S-type isolate found.

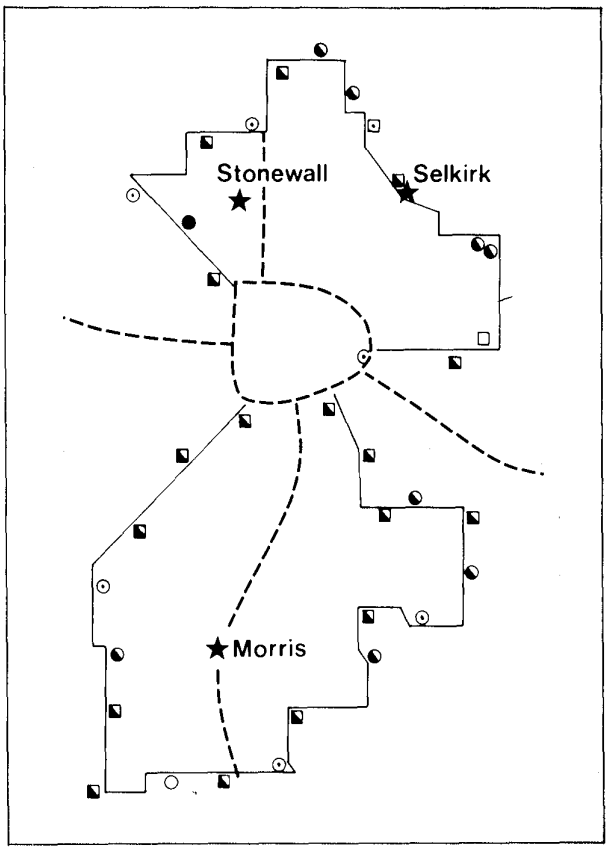

Fig. 2. Distribution of barley fields infected with net blotch (*Pyrenophora teres*) in the Winnipeg, Manitoba region. Two-rowed barley – □ no net blotch found; ■ N-type isolate found; ◪ VN-type isolate found; ⊡ S-type isolate found. Six-rowed barley – ○ no net blotch found; ● N-type isolate found; ◐ VN-type isolate found; ⊙ S-type isolate found.

Alberta. For the first time, S-type isolates were found outside the province of Manitoba, namely in two fields in Alberta.

Winnipeg region survey

The locations of the 35 fields sampled are shown in Fig. 2. Plants in most fields were sampled at the milky-ripe to soft dough stage of growth. Net blotch was found in 33 (94%) fields and the biotype distribution of *P. teres* was: N-type, 3%; VN-type, 76%; and S-type, 21%. Disease severity levels ranged from a trace to very light in six-rowed barley fields and were predominately light in two-rowed fields with several having moderate levels of infection.

The results of these surveys are in general agreement with those carried out in 1974. They indicate that net blotch of barley is common and widespread on the Canadian prairies and that distinguishable biotypes of the causal organism, *P. teres*, exist throughout this region. The much larger number of fields from which the net blotch causal agent was recovered in 1976 in comparison to 1974, gives a more complete indication of the distribution of *P. teres* biotypes: in Alberta N-types predominate, VN-types (found in the 1974 survey) and S-types can occur; in Saskatchewan both

N- and VN-types are common, no S-types have yet been found; in Manitoba VN-types predominate, S-types are relatively common in the eastern half of the province and N-types are rare. Although the distribution of VN-type isolates suggests a natural spread westward from Manitoba and an apparent displacement of N-types, it is not clear how S-type isolates became established in Alberta, as they are otherwise known to occur only in eastern Manitoba. These were likely introduced to the Winnipeg region on exotic barley seed (5), but it is uncommon for commercial barley seed to be shipped from eastern Manitoba to Alberta. It is possible that seed of experimental barley originating near Winnipeg and contaminated with the S-biotype was sent to Alberta. However, most barley seed used in the two major western Canadian co-operative tests is produced at Brandon, Manitoba and Lethbridge, Alberta, where no S-type isolates of *P. teres* have yet been found.

An estimate of yield losses due to net blotch was made by applying a formula developed for scald of barley (3). For this purpose disease severity data obtained in the surveys was converted to percent leaf area affected in the top two leaves (1). On this basis, losses in infected fields were: 3-9% and below 1% for two-rowed and six-rowed barley, respectively, in Manitoba and Saskatchewan and below 1% for both types of barley in Alberta except for two six-rowed fields with a loss of 12-17%. The 1976 growing season, like that of 1974, was much drier than normal in many parts of the barley-growing regions of the prairies and this likely had the effect of decreasing severity of leaf diseases. It did not, however, appear to have much effect on disease incidence, which was much higher than in 1974. In particular, the incidence of net blotch was virtually 100% in the two-rowed barley fields sampled. The higher incidence of net blotch and the higher levels of disease severity in two-rowed than in six-rowed fields suggests that two-rowed barley is more susceptible to net blotch, at least under the types of field conditions encountered in 1976. Although the presence and distribution of *P. teres* biotypes has been mapped in a large extent of the prairies, some important barley-growing regions such as the Peace region in Alberta and the area of Saskatchewan north of Saskatoon have not been sampled. These regions should be surveyed in the future to fully assess the occurrence and distribution of *P. teres* biotypes in western Canada.

Acknowledgements

I am greatful to E. Mueller for technical assistance and to R.W. Sims for graphics.

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Co-operative seed treatment trials - 1977[1]

J.T. Mills,[2] G.J. Pelletier,[5] J.G.N. Davidson,[3] L.J. Piening,[4] and J. Nielsen[2]

Nineteen seed treatment chemicals were tested at four stations for their efficacy in controlling bunt of wheat (*Tilletia caries* and *T. foetida*), loose smut of oats (*Ustilago avenae*), and false loose smut of barley (*U. nigra*). Infection of untreated seed was high with the exception of false loose smut of barley with 2% at Ste. Foy and 6.7% at Winnipeg. One treatment gave significantly less control of bunt and oat smut at four stations, and four other treatments of oat smut at two stations than the standard Vitaflo 280, but the remaining treatments were not significantly better than this standard.

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On a évalué à quatre stations l'efficacité de 19 traitements chimiques de la semence à prévenir l'apparition de la carie du blé (*Tilletia caries* et *T. foetida*), du charbon nu de l'avoine (*Ustilago avenae*) et du faux charbon nu de l'orge (*U. nigra*). Le taux d'infection des semences non traitées a été élevé partout, sauf dans le cas du faux charbon nu de l'orge, 2% à Ste-Foy et 6.7% à Winnipeg. Aux quatre stations, un des traitements a été significativement moins efficace que le traitement ordinaire au Vitaflo 280 contre la carie du blé et le charbon nu de l'avoine, mais quatre autres traitements se sont révélés supérieurs à deux stations contre cette dernière maladie. Les autres produits ne se sont pas montrés plus efficaces que le traitement standard.

Introduction

In 1977, 19 seed treatment chemicals were tested for their efficacy in controlling common bunt of wheat [*Tilletia foetida* (Wallr.) Liro and *T. caries* (DC.) Tul.], loose smut of oats, [*Ustilago avenae* (Pers.) Rostr.] and false loose smut of barley (*U. nigra* Tapke).

Materials and Methods

Table 1 lists source, name of the product, and the active ingredients of the materials used. Vitaflo 280 was included as a standard for comparison.

Seeds of 'Norteno M67' wheat (*Triticum aestivum* L.), 'Random' oats (*Avena sativa* L.), and 'Beacon' barley (*Hordeum vulgare* L.) were used in the smut tests.

Prior to chemical treatment wheat was inoculated with dry bunt spores at the rate of 1 g spores per 200 g of wheat. The technique for inoculation of oats and barley by partial vacuum is described by Nielsen (1). The chemical dosages used were those suggested by the manufacturer (Table 2). Each sample was hand-shaken in a glass jar to cover the seed uniformly with the chemical.

After 3 days or more, 200 seeds were removed from each jar and placed in a paper envelope. Envelopes that contained seed of the same treatment were stored in polyethylene bags at 15°C for up to 5 weeks before seeding.

The tests on bunt were planted at Beaverlodge (May 20) and Lacombe, Alberta (May 20); those on the smuts of oats and barley at Ste. Foy, Québec (May 24) and Winnipeg, Manitoba (May 18). There were four replicates per test at each location. Each replicate consisted of 200 seeds planted in a row 4 m long; all rows were planted 25 cm apart; plots were arranged in a randomized block design.

The number of smutted heads in each row was recorded after the crop had headed and are expressed as means of the number of heads in the untreated rows. The results are given as means of four replicates, at each planting site. Significance at the 0.05 level was determined from the means of the treatments at each station.

Results and Discussion

Smut infection of untreated seed varied from 9.3 to 22.5% for wheat, from 2.0 to 6.7% for barley and 17.3 to 18.4% for oats.

One treatment (No. 11) gave significantly less control of bunt and oat smut at four stations, and four other treatments (Nos. 4, 5, 9, 10) of oat smut at two stations than the standard Vitaflo 280, but the remaining treatments were not significantly better than this standard (Table 2). No obvious symptoms of phytotoxicity were observed at any station.

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Table 1. Seed treatment materials used in the cooperative tests 1977.

Treatment no.	Source*	Product name	Active ingredient(s)
1		Untreated check	
2	Chemagro	Bay-meb 6447	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone (25%)
3	Chemagro	Bay-KWG 0519	identity not available
4	Chevron	Difolatan + Vitavax 2-2	cis-N-[(1,1,2,2-Tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide + carbathiin
5	Chevron	Difolatan 4	cis-N-[(1,1,2,2-Tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide (80%)
6	Chipman	TF 3387	identity not available
7	Chipman	TF 3388	identity not available
8	Dupont	DPX-14	identity not available
9	Interprovincial	Busan 25	2-(thiocyanomethylthio) benzothiazole (25%)
10	Interprovincial	Busan 30	2-(thiocyanomethylthio) benzothiazole (30%)
11	Rohm & Haas	RH-2161	identity not available
12	Uniroyal	Vitaflo 250	identity not available
13	Uniroyal	Vitaflo 280	carbathiin 14.9% + thiram 13.2%
14	Uniroyal	UBI 2036	identity not available
15	Uniroyal	UBI 2109	identity not available
16	Uniroyal	UBI 2110	identity not available
17	Uniroyal	UBI 2111	identity not available
18	Uniroyal	UBI 2112	identity not available
19	Uniroyal	UBI 2114	identity not available
20	Uniroyal	UBI 2116	identity not available

*Chemagro Ltd., Mississauga, Ontario; Chevron Chemical (Canada) Ltd., Burlington, Ontario; Chipman Chemicals Ltd., Hamilton, Ontario; Dupont de Nemours & Co. Inc., Wilmington, Delaware; Interprovincial Cooperatives Ltd., Winnipeg, Manitoba; Rohm & Haas Co. of Canada Ltd., West Hill, Ontario; Uniroyal Chemical Division, Elmira, Ontario.

Table 2. Effect of seed-treatment chemicals on infection of wheat, oats and barley by bunt or smut at Beaverlodge (B), Lacombe (L), Ste. Foy (SF), and Winnipeg (W).

Treatment no.	Product name	Formulation*	Dosage (g or ml/kg)	% smutted heads†					
				Wheat		Barley		Oats	
				B	L	SF	W	SF	W
1	Untreated check			22.5	9.3	2.0	6.7	17.3	18.4
2	Bay-meb 6447	WP	2.52	0.0	0.0	0.0	0.0	0.0	0.0
3	Bay-KWG 0519	WP	1.25	0.0	0.0	0.0	0.0	0.0	0.0
			2.52	0.0	0.0	0.0	0.0	0.0	0.0
			5.04	0.0	0.0	0.0	0.0	0.4	0.0
4	Difolatan +	SL	1.25	0.4	0.5	0.0	0.0	3.1	0.9
	Vitavax 2-2		1.87	0.0	0.3	0.0	0.0	1.2	0.1
			2.50	0.6	0.1	0.0	0.0	0.8	0.0
			3.12	0.0	0.0	0.0	0.1	0.7	0.0
5	Difolatan 4	SL	1.25	0.4	0.5	0.0	0.0	7.6	7.1
			1.87	0.8	0.1	0.0	0.2	9.9	7.3
			2.50	0.2	0.3	0.0	0.1	7.6	6.8
6	TF 3387	SN	1.70	1.8	2.4				
			1.90			0.0	0.0		
			3.10					1.2	0.0
7	TF 3388	SN	1.60	0.9	2.4				
			1.70			0.0	0.0		
			2.80					0.0	0.0
8	DPX-14	WP	1.56	0.4	0.0				
			1.95			0.0	0.0		
			2.60	0.1	0.1				
			2.75					0.4	0.0
			3.25			0.0	0.1		
			4.59					0.0	0.0
9	Busan 25	D	2.10	0.9	0.6	0.0	0.0	2.6	0.9
10	Busan 30	SN	0.78	0.3	2.0	0.0	0.0	3.7	0.7

(continued)

Table 2. Effect of seed-treatment chemicals on infection of wheat, oats and barley by bunt or smut at Beaverlodge (B), Lacombe (L), Ste. Foy (SF), and Winnipeg (W). (concluded)

Treatment no.	Product name	Formulation*	Dosage (g or ml/kg)	% smutted heads†					
				Wheat		Barley		Oats	
				B	L	SF	W	SF	W
11	RH-2161	SN	1.28	8.4	1.3	0.1	0.0	2.3	1.5
			5.12	0.2	0.1	0.0	0.0	0.1	0.0
12	Vitaflo 250	SL	1.56	0.4	0.8				
			1.95			0.0	0.0		
			2.76					0.9	0.0
13	Vitaflo 280	SL	1.82	0.6	0.3				
			2.28			0.0	0.0		
			3.22					0.6	0.0
14	UBI 2036	WP	1.56	0.2	0.1				
			1.95			0.0	0.0		
			2.75					0.1	0.0
15	UBI 2109	SN	1.56	0.6	0.5				
			1.95			0.0	0.0		
			2.76					0.9	0.0
16	UBI 2110	SN	1.82	0.0	0.1				
			2.28			0.0	0.0		
			3.22					0.8	0.0
17	UBI 2111	SN	1.82	0.1	0.3				
			2.28			0.0	0.0		
			3.22					0.9	0.0
18	UBI 2112	SN	1.56	0.1	0.0				
			1.95			0.3	0.0		
			2.76					1.8	0.0
19	UBI 2114	SN	1.82	0.3	0.3				
			2.28			0.0	0.0		
			3.22					1.1	0.0
20	UBI 2116	SL	1.56	0.6	0.4				
			1.95			0.0	0.0		
			2.76					0.6	0.0
†† Significance limit (0.05)				8.4	1.3	NS	NS	2.3	0.7
Mean no. of heads				223	372	275	290	228	251

* Formulation code: D = dust; SL = slurry; SN = solution; WP = wettable powder.

† % smut = $\frac{\text{mean number of smutted heads}}{\text{mean number of heads}} \times 100$

†† Treatments significantly not as good as Vitaflo 280 have values equal to or higher than the significance limit.

NS = not significant

Damping-off in tobacco seedbeds caused by Rhizoctonia solani and Pythium ultimum[1]

S.K. Gayed[2], D.J.S. Barr[3], L.K. Weresub[3]

Pre-emergence damping-off of flue-cured tobacco (*Nicotiana tabacum* L.) is negligible in steam sterilized seedbeds due to the lack of damping-off organisms either on or in tobacco seed produced in Ontario. Post-emergence damping-off was differentiated into: a) seedling rot initiated early where infection starts on the young leaves in touch with and spreading over the organic soil "muck", b) typical damping-off resulting from the infection of the stem of erect seedlings either directly from the soil or indirectly from already infected leaves. *Pythium ultimum* Trow is reported for the first time in Canada as a causal organism of damping-off in tobacco seedbeds. It caused infection at both stages without necessarily penetrating the roots, and was involved in 10% of the seedling rot cases and 25% of those of typical damping-off. However, *Rhizoctonia solani* Kühn was more frequently implicated since it caused 90% of seedling rot cases and 75% of those manifesting typical damping-off symptoms at the later stage. The lethal temperature for *R. solani* was 60°C and for *P. ultimum* 50°C, well below the temperature reached during seedbed steam sterilization, hence the probability that seedbeds are reinfested by these organisms. Isolates of each organism varied considerably in growth, cultural characteristics, and virulence. Virulence of all isolates are partially or totally lost during 15 months in culture at 5-10°C. One isolate of *R. solani* produced basidia on water agar and was identified as *Thanatephorus cucumeris* (Frank) Donk.

Can. Plant Dis. Surv. 58:1, 15-19. 1978

La fonte des semis du tabac jaune (*Nicotiana tabacum* L.) en prélevée est négligeable dans les couches stérilisées à la vapeur, du fait que les semences produites en Ontario sont quasi-exemptes des pathogènes responsables. En post-levée, la maladie se présente sous deux formes, une pourriture précoce des plantules qui se manifeste par l'infection des jeunes feuilles en contact avec le sol organique et une fonte typique provoquée par l'infection de la tige des plantules dressées, directement à partir du sol ou indirectement à partir des feuilles déjà infectées. On signale pour la première fois au Canada la présence de *Pythium ultimum* Trow comme agent responsable de la fonte des semis du tabac. Cet organisme a pu infecter les deux stades de croissance sans nécessairement pénétrer dans les racines et a causé 10% des cas de pourriture des plantules et 25% de ceux de la fonte typique, mais *Rhizoctonia solani* Kühn s'est révélé plus virulent, provoquant 90% des cas de pourriture et 75% de ceux de fonte au stade de développement plus avancé. Les températures mortelles de *R. solani* et de *P. ultimum* étaient de 60 et 50°C respectivement, soit bien en deçà des températures de stérilisation des couches à la vapeur, d'où la probabilité que ces pathogènes réinfestent les couches. La croissance, les caractéristiques culturales et la virulence d'isolats de chaque organisme ont considérablement varié. On a observé une perte partielle ou totale de virulence de tous les isolats après 15 mois de culture à 5-10°C. Un isolat de *R. solani* qui a produit des basides sur gélose aqueuse a été identifié comme étant *Thanatephorus cucumeris* (Frank) Donk.

Introduction

Damping-off of flue-cured tobacco (*Nicotiana tabacum* L.) seedlings in the greenhouse is a common disease in Canada. Despite the use of steam in seedbed sterilization or the applications of chemical sterilants, losses of tobacco seedlings in Ontario were estimated at 3-10% (5). Previous reports attributed damping-off of tobacco seedlings to *Rhizoctonia solani* Kühn and *Pythium* spp. (5) including *P. debaryanum* Hesse (3).

This communication establishes the involvement of another *Pythium* species in this disease, describes in some detail the different disease symptoms, and reports on the relative occurrence of these organisms in relation to tobacco seed and seedlings. It also reports on the heat tolerance of both organisms and the possible variation among their isolates.

Materials and Methods

In pre-emergence damping-off trials, 100 tobacco seeds produced in Ontario of cultivars Delhi 34 and White Mammoth were placed in a petri dish on moist autoclaved filter paper and on moist autoclaved muck (mixture of partially decomposed plant material, sand, silt, and clay). After incubation at room temperature for 12 days, the germinated seeds were counted. The trial was repeated twice.

The presence of fungi known to cause pre-emergence damping-off was determined by plating 100 whole or crushed Canadian seeds of flue-cured tobacco cv Virginia 115, Hicks Broadleaf, White Mammoth, and a breeding line with a germination capacity of only 44%

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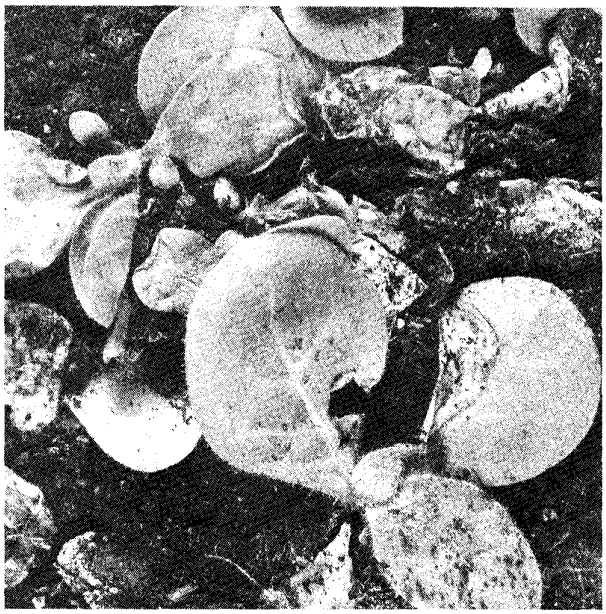

Fig. 1. Seedling rot of tobacco caused by *R. solani* attacking the leaves spreading on muck in a seedbed in the greenhouse.

on PDA and water agar and incubated at 25°C for 10 days. Samples of fungal growth from the seeds, seed fragments, and the sprouting seeds were examined daily. Whole seeds which failed to germinate on agar were crushed on glass slides, stained with cotton blue in lactophenol, and examined for the presence of *Pythium*, or *Rhizoctonia*.

In the case of seedlings showing post-emergence damping-off, diseased and bordering healthy tissue were excised, washed with water to rid them of adhering muck particles, dipped in a 0.5% calcium hypochlorite suspension for 5 minutes for surface sterilization, and rinsed in sterile water. Fragments of this tissue were transferred to PDA, and to corn meal agar (CMA) prepared with 100 ppm pimaricin, 50 ppm polymixin B and 50 ppm penicillin G (4). Tissue fragments were stained with cotton blue in lactophenol and examined for *Pythium* and *Rhizoctonia*. *R. solani* could be readily identified by its coarse, broad, septate and frequently brown mycelium as compared with the fine nonseptate colorless mycelium of *Pythium* which carried zoosporangia and/or sexual reproductive bodies.

Soil inoculation technique was used to compare the virulence of the different isolates. *R. solani* was grown on 40 ml of potato dextrose broth, and *Pythium* on corn meal broth in 250-ml Erlenmyer flasks. After 10 days incubation at 25°C, mycelial mats were filtered off, washed, blended in 200 ml water and mixed with 500 g steam-sterilized muck. Ten 3-week old seedlings of tobacco cv Delhi 34 were transplanted into two 4-inch pots of the inoculated muck. Seedlings transplanted into steamed non-inoculated muck served as check. All seedlings were kept under moist conditions for 2 weeks during which the seedlings were observed for damage.

For the determination of the lethal temperature for *P. ultimum*, cultures grown on 2.4% V-8 juice agar in test tubes for 3-5 weeks and rich in oospores, were flooded with water and placed in a water bath set at various temperatures. For the determination of the lethal temperature of *R. solani*, cultures were grown on PDA and immature, freshly mature, and air dried sclerotial pads were similarly immersed in water in test tubes placed in a water bath. The viability of the cultures was tested by plating on PDA.

Results

Pre-emergence damping-off:

The most common fungus isolated from flue-cured tobacco seed was *Alternaria alternata* (Fr.) Keissler. Several species of *Aspergillus* and *Penicillium* were also present but were less abundant. Of significance to our study was the absence of *Rhizoctonia* and *Pythium*, for neither was isolated or observed in the crushed seed by direct microscopic examination. Moreover, in the 3 trials on Delhi 34 and White Mammoth the average percent germination on sterilized filter paper in petri dishes was 85, and 75; on plain agar 84 and 75; and on steam-sterilized muck 84, and 77, respectively. This indicates that pre-emergence damping-off in steamed muck is of no significance.

Post-emergence damping-off:

Damping-off usually starts to show in steam-sterilized tobacco seedbeds about 3 weeks after seeding. At this stage, the seedlings have 3-4 leaves, including the cotyledonary leaves, which spread out on the muck for up to 15 mm. During this period and for one or two weeks later when each seedling spreads out to 25-30 mm, the leaves remain in direct contact with the muck. Stem infection at soil level by damping-off organisms occasionally takes place but leaf infection is very common at this stage. Water soaked spots start to show on the leaves and gradually extend to the stem and other leaves including the growing point (Fig. 1). Diseased areas on the seedbed are in the form of circular or irregular patches of poorly-growing, yellowish, or macerated seedlings. This type of infection that starts on the spreading leaves, can hardly be considered as typical "damping-off", and "seedling rot" is proposed as a more accurate term. As the seedlings grow, typical damping-off symptoms appear, infection becomes more restricted to the base of the stem at or below soil level. Infection of the stem may take place directly from the soil, or indirectly through infection from infected leaves. Infected leaves at the base of the stem gradually shrivel and the growing mycelium extends into the stem, causing damping-off (Fig. 2). The lesion formed on the stem varies in color from brown to black, and a total separation of the top may result with a slight pull.

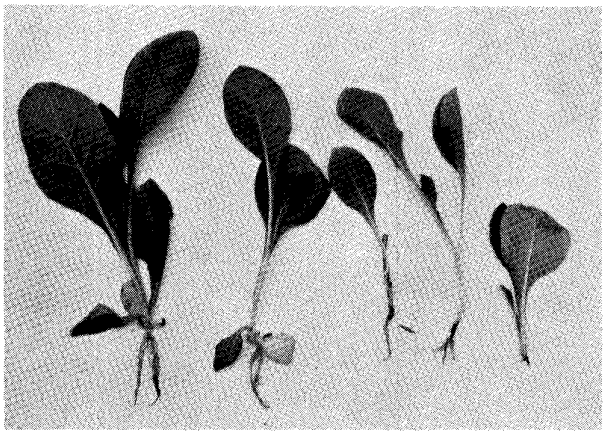

Fig. 2. From left to right, stages of leaf infection by *R. solani* leading to infection of the basal part of the stem and the separation of root from shoot.

Survey of seedling rot and damping-off organisms:
Samples of diseased tobacco seedlings mainly from greenhouses in the flue-cured tobacco area in Ontario were collected between 1967-77, including a large-scale survey carried out between 1969-71 covering 130 problem farms. Microscopic examination of diseased tissue and isolations on culture media indicated that *Rhizoctonia* was far more common than *Pythium* in young tobacco seedlings suffering from seedling rot. Of 150 samples of diseased tissue examined, *Rhizoctonia* was identified by direct microscopic examination and was isolated on PDA in 135 samples and *Pythium* only in 15 samples. The ratio of *Rhizoctonia* to *Pythium* at this early stage of seedling development was 9:1.

Later when typical damping-off symptoms were manifested on the elongated tobacco seedlings the incidence of *Pythium* increased slightly. Out of 163 samples, *Pythium* was microscopically detected and isolated from 40 samples and the remainder were caused by *Rhizoctonia*, thus the ratio of *Rhizoctonia* to *Pythium* was 3:1.

The causal organisms:
1. *Pythium*: For full identification of *Pythium*, 20 isolates were examined on water agar, CMA, 2.4% V-8 juice agar medium and on hemp seed in water after 7-10 days' incubation at various temperatures. Nine isolates produced oospores and were definitely identified as *P. ultimum* Trow. The remaining isolates were otherwise morphologically identical to *P. ultimum*, their response to temperature was similar, however they could not be fully identified because of the absence of the sexual state.

Considerable variation in the growth rate in culture was noted among the isolates of *Pythium* including those identified as *P. ultimum*. The average growth on PDA over a 24 hr period at 25°C varied from 29-69 mm. The growth rate was generally higher at 25 and 30°C than at 20 and 35°C with most isolates growing more rapidly at 30 than at 25°C. The optimum temperature for *P. ultimum* is therefore between 25-30°C and is in agreement with Lucas (6) who reported 28°C as optimum temperature for *P. ultimum*.

No correlation was found between the rate of growth in culture and virulence of the isolates. One of the most virulent isolates grew poorly on PDA and CMA.

P. ultimum survived 46°C for 45 min. but not for 90 min., survived 50°C for only 3 min. and for less than 1 minute at temperatures higher than 50°C.

After incubation for 15 months at 5-10°C, having been subcultured three times, isolates of *P. ultimum* partially or totally lost their capacity to infect tobacco seedlings, although their viability and cultural characteristics were not noticeably changed.

2. *Rhizoctonia*: Only one isolate R1-71, of six isolates of *Rhizoctonia*, when transferred to water agar produced a few fertile basidia which were adequate for its determination to *Thanatephorus cucumeris* (Frank) Donk. The non-sporulating isolates, although highly variable in color and morphology including the size, abundance and distribution of sclerotia (Fig. 3) all demonstrated the microscopic characteristics outlined by Parmeter and Whitney (9) for *R. solani* Kuhn.

Isolates that caused seedling rot early in the season could not be differentiated by their culture characteristics from those that caused typical damping-off symptoms at a later stage of seedling growth. There was no correlation between the abundance or size of sclerotia or rate of mycelial spread, and virulence to tobacco seedlings. This virulence was substantially reduced or entirely lost after storage for 15 months under conditions described above for *P. ultimum*. The viability and culture characteristics of *R. solani* isolates were not noticeably changed.

R. solani grew less rapidly than *P. ultimum*, but still vigorously. Starting with 5 mm mycelial inoculum on PDA, the fungal growth after 24 hr at 25°C extended to 10-21 mm. Similar to *P. ultimum*, isolates of *R. solani* grew most rapidly at 25 and 30°C, many of them at 30°C. This again is in agreement with other authors including Lucas (6) who reported 28°C as optimum for *R. solani* causing sore-shin of tobacco. The lethal temperature for moistened sclerotia of *R. solani* was one minute at 60°C.

Discussion

Growers of flue-cured tobacco in Ontario prepare their seedbeds by spreading over the sandy soil a 5-cm layer of muck consisting mainly of decayed or decomposed plant material mixed with clay and sand. In order to sterilize the seedbed (7) steam is used to raise the soil temperature to 82°C at the depth of 15 cm for 30 minutes to kill disease propagules and weeds. Tobacco seeds are usually sown approximately a week after steam-sterilization of the bed.

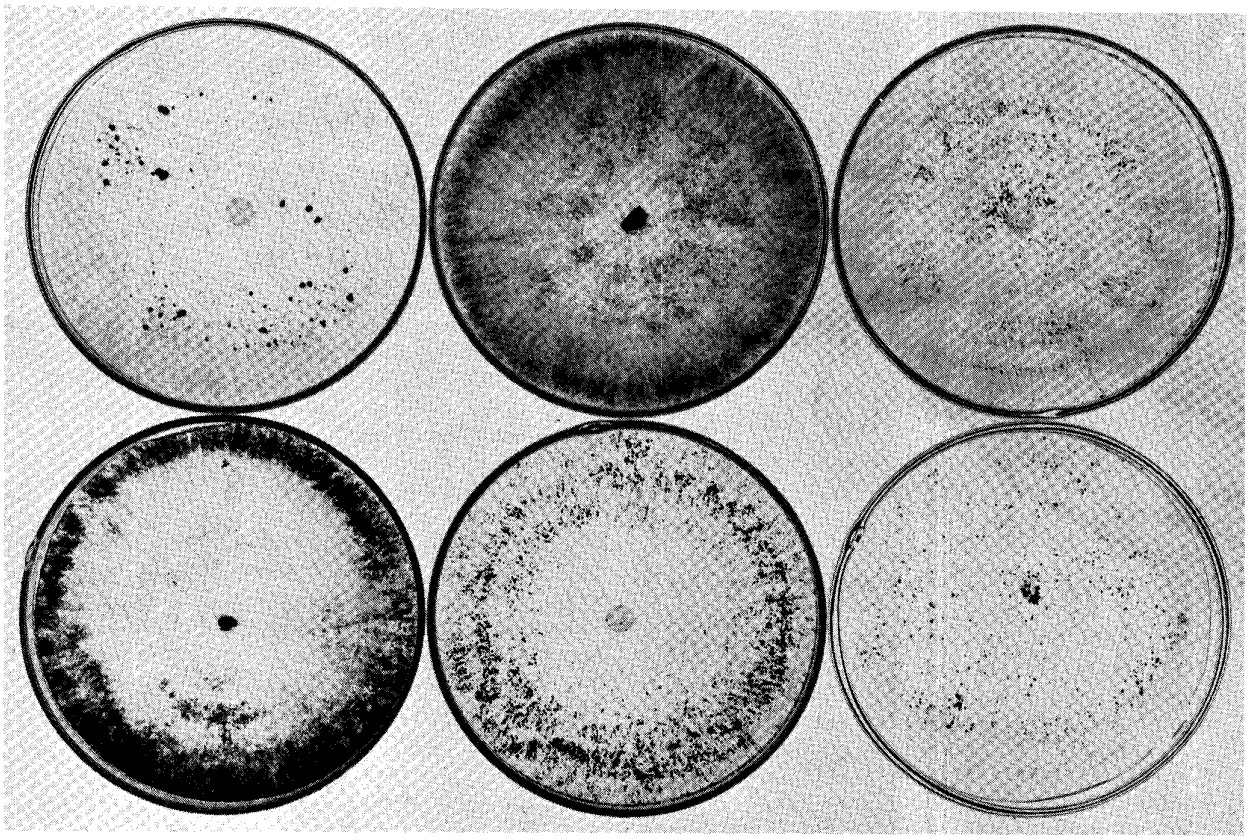

Fig. 3. Different isolates of *R. solani* on PDA showing variation in the size and the distribution of sclerotia.

In our in vitro studies exposure of less than a minute to 60°C was sufficient to inactivate not only the oospore-bearing cultures of *P. ultimum* but also the sclerotioid pads of *R. solani*. Other researchers (10) have found that sclerotia of *Rhizoctonia* are more resistant to wet heat than they were in our tests. However, in all cases reported the lethal temperature did not exceed 71°C, well below the temperature reached in steaming tobacco seedbeds. It is certain that if seedbeds are steamed according to the recommended procedures, damping-off organisms cannot survive.

Neither pathogen was among the mycoflora of tobacco seed examined, in spite of the fact that *R. solani* has been isolated in Canada from seeds of many other plants including field mustard, lettuce, flax, peas, and radish (3). Thus we have two important factors contributing to the insignificance of pre-emergence damping-off in steam-sterilized tobacco seedbeds, namely, clean seed and the low level of damping-off pathogens in the bed. Add to this the practice of tobacco growers seeding their seedbeds with soaked or sprouting seeds which reduces the period for emergence, and the advantage of the epigeal germination of tobacco seed, since evidence indicates that hypogeal germination increases risks of pre-emergence damping-off (2). Under these highly favorable circumstances, the escape of tobacco from pre-emergence damping-off in Ontario would seem to be predictable.

Post-emergence damping-off can be a serious problem in tobacco seedbeds. Within 4 weeks after seeding, disease symptoms appear on seedbed seedlings in characteristic circular or irregular patches indicating foci of re-infestation of the seedbed. It may be that the sterility of the muck itself contributes to efficient re-infestation by damping-off organisms. Baker (1) reported that the more nearly sterile the soil, the more rapid the spread of *R. solani*. He recommended soil decontamination rather than sterilization and suggested the use of aerated steam and reduced temperature that would allow the survival of antagonistic saprophytic soil microorganisms. Soil is a highly complex environment for establishing this kind of balance (8). Moreover, *Thielaviopsis basicola* (Berk & Br) Ferr. is a common pathogen in the tobacco seedbed and is controlled by steaming at 82°C as recommended by Ontario Ministry of Agriculture and Food (7).

Although previous reports show that *R. solani* is capable of infecting leaves of plants such as poinsettia (12) and China aster (13), apparently this is the first report recording the infection of leaves of tobacco seedlings with *R. solani* and *P. ultimum* directly from the soil.

Ecological studies are needed to explain why *R. solani* is more dominant that *P. ultimum* in causing damping-off in tobacco seedbeds. The relative increase in damping-off cases caused by *P. ultimum* from 10% to 25% in the later stages of seedling growth can be related to the possible increase in soil moisture in the bed under the leaf canopies of the dense and fast growing tobacco seedlings. This increase in moisture satisfies the hydrophytic needs of *Pythium*.

A detailed study on *R. solani* isolates was necessary since the name *R. solani* (9) may still be in use for a complex of species. Although isolate R1-71 conformed in its mycelial-sclerotiate stage to *R. solani*, it could be identified as *T. cucumeris* but we have not dared to refer all the isolates to this species. There is, as yet, no certainty that all isolates of the still broadly circumscribed *R. solani* necessarily belong to *T. cucumeris*.

The determination of *P. ultimum* is noteworthy. Although this species has already been implicated in the damping-off disease of many cultivated plants in Canada (3) and in tobacco in the U.S.A. (6), this is the first report of its activity in Canada on tobacco seedlings.

In respect to the site of infection, Baker (2) and others distinguish between the mode of infection of *Pythium* and *Rhizoctonia*, stressing that *Pythium* species generally infect root tips or root hairs and advance upward through the plants whereas *Rhizoctonia* causes stem decay at soil level and advances downwards. Observations made in this study and a similar observation reported by Lucas (6) indicated that *P. ultimum* is also capable of infecting the stem at the crown without any infection of the root.

Acknowledgements

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