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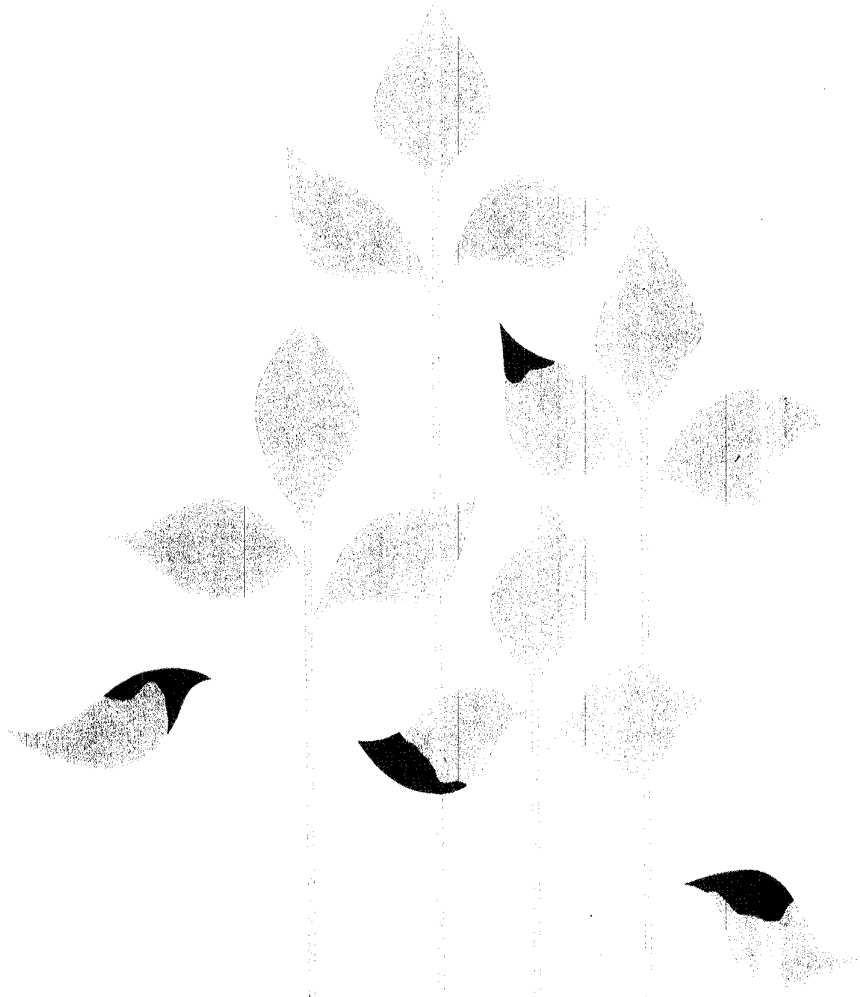

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The *Canadian Plant Disease Survey* is a periodical of information and record on the occurrence and severity of plant diseases in Canada and on the assessment of losses from disease. Other original information such as the development of methods of 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 immédiat pour les phytopathologistes.

Research Branch, Agriculture Canada

Compilers: H.S. Krehm, PhD.
P. Beauchamp, M.Sc.,
Research Program Service,
Agriculture Canada, Ottawa, Ontario K1A 0C6

Direction de la recherche, Agriculture Canada

Compilateurs: H.S. Krehm, PhD.
P. Beauchamp, M.Sc.
Services des programmes de recherche,
Agriculture Canada, Ottawa, (Ontario) K1A 0C6

Occurrence of *Myriosclerotinia borealis* on Winter Cereals in Ontario[1]

Edward F. Schneider and W.L. Seaman[2]

Myriosclerotinia borealis was isolated from winter cereals in an area near the northern limits of production in Ontario. The winter climate may determine the southern latitude for field activity of *M. borealis* in Ontario, based on results from Scandinavia at a much more northern latitude where Sclerotinia snow mold is a major problem. Although only 11 fields were affected, they were widely distributed and all of the diseased plants were necrotic. Usually plants were infected with *M. borealis* alone but in some fields other snow mold fungi also were observed on necrotic plants.

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On a isolé *Myriosclerotinia borealis* sur des céréales d'hiver cultivées dans une région situé aux limites septentrionales de production en Ontario. Le climat hivernal peut déterminer la latitude la plus au sud où *M. borealis* est actif en Ontario, d'après des données provenant de Scandinavie située à une latitude beaucoup plus au nord et où *M. borealis* est maintenant un problème majeur. Même si la moisissure nivale n'a infecté que 11 champs, ils étaient largement répartis dans toute la région et tous les plants infectés étaient nécrosés. Généralement, les plants n'étaient infectés que par *M. borealis* mais, dans cetains champs, on a observé d'autres champignons causant la moisissure des neiges sur les plants nécrosés.

Introduction

Sclerotinia snow mold of winter cereals and perennial grasses occurs in the more northerly or colder regions affected by snow mold fungi. The causal fungus, described as *Sclerotinia borealis* Bub. & Vleug. in Vleugel, 1917, was placed by Kohn (1979) in *Myriosclerotinia* as *Myriosclerotinia borealis* (Bub. & Vleug.) Kohn. *Sclerotinia graminearum* Elenev. ex Solkina was regarded as a taxonomic synonym of *S. borealis* by Schumacher and Kohn (1985), who also questioned the validity of *Myriosclerotinia* as the appropriate generic designation of this fungus.

Sclerotinia snow mold has been reported from northern Europe (Årsvoll 1975, Jamalainen 1949, Mäkelä 1981), USSR (Tupenevich and Shirko 1939), and Japan (Tomiyama 1955). In North America, sclerotinia snow mold was first reported by M.W. Cormack on grasses at Prince George, B.C., in 1951 (Conners and Savile, 1952) and on winter wheat at Vanderhoof, B.C., in 1953 (Conners and Savile, 1954); the identity of the causal fungus was confirmed by Groves and Bowerman (1955). It was subsequently found on turf grasses in the Peace River region of British Columbia and Alberta (Vaartnou and Elliott 1969), on grasses, winter rye and winter wheat in Saskatchewan (Smith 1972, 1974), and on rye in Manitoba (Smith 1974). In the USA *M. borealis* has been found in Alaska (Lebeau and Logsdon 1958), Washington (Sprague et al. 1961) and Minnesota (Stienstra 1974). In eastern Canada *M. borealis* was found in Ontario on turfgrass in 1979 (W.L. Seaman and J.D. Smith, unpublished), on winter wheat in 1982 (W.L. Seaman and E.F. Schneider, unpublished), and on winter wheat in Quebec in 1985 (L. Couture, personal communication). Observations on the distribution of *M. borealis* on winter cereals in Ontario in 1982-85 are reported herein.

Observations

In 1982 snow mold damage was extensive and severe in southern Ontario (south of latitude 45°28'), following snow cover that persisted into late spring. *M. borealis* was observed in mixed infections with *Typhula ishikariensis* Imai var. *ishikariensis* Årsvoll and Smith and *Microdochium nivale* (Fries) var. *nivale* Samuels and Hallett on turfgrass at the Arnprior site where *M. borealis* was collected in 1979. In addition *M. borealis* occurred on winter wheat (*Triticum aestivum* L.em. Thell.) in test plots at Hyndford, Ontario (Table 1); at that location snow mold damage resulted in approximately 80% plant kill; however *M. borealis* was associated with less than 1% of the necrotic plants. Also in 1982, necrotic cereal plants from test plots at Kapuskasing, which lies north of the winter wheat production area of Ontario, were conspicuously colonized by *M. borealis*, with as many as 10 sclerotia per plant. The affected winter cereals from that field included wheat (soft white cultivars Talbot and Fredrick, red cultivars Abe and Monopol), rye (*Secale cereale* L. cv. Puma), barley (*Hordeum vulgare* L. cv. Dover), and triticale (cv. Wintri) (Table 1). Apparently *M. borealis* has not previously been reported on triticale. Many of the plants from Kapuskasing also were infected with one or more of the other snow mold fungi, e.g. *T. incarnata* Lasch ex Fr., *T. ishikariensis* var. *ishikariensis*, *T. phacorrhiza* Reichard ex Fries, and *M. nivale* var. *nivale*. Mixed infections of *S. borealis* and other snow mold fungi have been reported elsewhere (Sprague et al. 1961, Smith 1974). Plants from the other fields in Ontario were infected with *M. borealis* alone. *M. borealis* also was found on necrotic plants in two fields near Elmvale in Simcoe County, a high snowfall area and the most southerly location at which it was found during the surveys.

Snow cover in 1983 was lost throughout most of southern Ontario because of mild weather and rain during January, re-

1 *Contribution number PRC M-6 Plant Research Centre, Agriculture Canada, Ottawa, Ont. K1A 0C6*

2 *Plant Research Centre, Agriculture Canada, Central Experimental Farm, Ottawa, Ontario K1A 0C6*

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Table 1. Host and distribution of *Myriosclerotinia borealis* observed during spring surveys in Ontario (1982-1985).

County and Location	Crop[a] Affected	Numbers of Fields
Renfrew		
Beachburg	Wheat	3
Douglas	Wheat	1
Hyndford	Wheat	1
Ottawa-Carleton		
North Gower	Wheat	2
Simcoe		
Elmvale	Wheat	2
Cochrane		
Kapuskasing	Wheat, Barley, Rye, Triticale	1

[a] Winter wheat cvs. Fredrick, Abe, Talbot, Monopol; barley cv. Dover, rye cv. Puma, triticale cv. Wintri.

sulting in little or no snow mold damage. In 1984 *M. borealis* was found on winter wheat plants from two fields near Ottawa, and from one field at Douglas and one field near Beachburg (Table 1). In 1985 *M. borealis* was observed on winter wheat in two other fields near Beachburg and again from one of the fields near Ottawa.

M. borealis is unique among the snow mold fungi in that it grows more rapidly on a frozen culture medium than on a supercooled liquid medium (Tomiyama 1955). In nature *M. borealis* is an important pathogen of winter cereals in northern Japan at high elevations, where the soil freezes before the plants become snow covered (Tomiyama 1959), and of ley grasses at latitudes of 65-70°N in Finland (Mäkelä 1981).

Our observations of *M. borealis* in Renfrew County at Douglas and Beachburg (latitude ca. 45°28′), in the Ottawa-Carleton region (latitude 45°19′), and in Simcoe County near Elmvale (latitude 44°35′) indicate that its activity is limited to areas near or north of the present northern limit of white winter wheat production in Ontario. In the affected areas *M. borealis* occurred sporadically, usually affecting single plants in a row or in relatively small areas of the fields; plants bearing sclerotia were invariably dead. Within the main area of winter wheat production in Ontario, *M. borealis* was not one of the snow mold fungi reported on fine turf grasses by Fushtey (1980). In more northerly areas with a longer period of snow cover, such as at Kapuskasing (latitude 49°25′), where *M. borealis* caused devastating losses to winter cereals in test plots in 1982, the fungus appears to be well adapted. The possible role of *M. borealis* in overwintering damage to forage and turf grasses in that area and to hard red winter wheat in northwestern Ontario has not been determined.

Acknowledgements

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Fungi isolated from stems and roots of soybean in Ontario

T.R. Anderson[1]

Thirteen genera of fungi were isolated from stems and roots of soybean [*Glycine max* (L.) Merr.] including the plant pathogens *Corynespora cassiicola* (Berk. & Curt.) Wei, *Fusarium oxysporum* Schlecht., *Phytophthora megasperma* f. sp. *glycinea* Kuan and Erwin, *Pythium* spp., *Rhizoctonia solani* Kuhn., and *Thielaviopsis basicola* (Berk. & Br.) Ferr. *F. oxysporum* and other species of *Fusarium* were isolated more frequently than other fungi. Certain fungi such as *C. cassiicola* were common on roots and others such as *Phomopsis* spp. were common on stems. The incidence of isolation of most fungi was influenced by sample date. Seed treatments with metalaxyl and furmecyclox did not affect the incidence of fungi isolated from stems and roots.

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Treize genres de champignons ont été isolés à partir des tiges et des racines du soja [*Glycine max* (L.) Merr.] notamment les pathogènes suivants, *Corynespora cassiicola* (Berk. & Curt.) Wei, *Fusarium oxysporum* Schlecht., *Phytophthora megasperma* f. sp. *glycinea* Kuan and Erwin, *Pythium* spp., *Rhizoctonia solani* Kuhn. et *Thielaviopsis basicola* (Berk. & Br.) Ferr. *F. oxysporum* et d'autres espèces de *Fusarium* ont été relevées plus souvent que tout autre champignon. Certains champignons comme *C. cassiicola* étaient communs sur les racines et d'autres comme *Phomopsis* spp. colonisaient davantage les tiges. La fréquence d'isolement de la plupart des champignons dépendait de la date d'échantillonage. Le traitement des semences au métalaxyl et au furmécyclox n'a pas eu d'effet sur le nombre de champignons prélevés sur les tiges et les racines.

Introduction

In Ontario, poor growth and low yield of soybean [*Glycine max* (L.) Merr.] may result from root infection by several soil-borne plant pathogens. These pathogens include *Corynespora cassiicola* (Berk. & Curt.) Wei (14), *Cephalosporium* sp. (6), *Fusarium oxysporum* (8), *Phytophthora megasperma* f. sp. *glycinea* (Hildeb.) Kuan and Erwin (*Pmg*) (5), *Pythium ultimum* Trow. (7), *Rhizoctonia solani* Kuhn (16; Anderson, unpublished results), *Macrophomina phaseolina* (Tassi.) Goid. (9) and *Thielaviopsis basicola* (Berk. & Br.) Ferr (2).

Phytophthora root rot has been described as the most important disease of soybean on clay soils in southwestern Ontario. Although recommended cultivars in this area are tolerant or resistant to this disease (3), yields are low in some fields or in low-lying areas of otherwise productive fields. At these locations, plants do not show symptoms characteristic of *Pmg* infection (5) but occasionally, red or brown lesions are present on the basal stem and/or upper tap root. It is possible that low yields result from root infection by other fungi such as *Fusarium*, *Rhizoctonia* and *Pythium*.

Identification of those pathogens within the disease complex that are ultimately responsible for low yield, might be simplified by the use of seed treatment fungicides with a limited spectrum of activity. Seed treatment with metalaxyl with specific activity against Peronosporales (11) reduces infection by *Pythium* spp. and *Pmg* and seed treatment with furmecyclox with specific activity against Basidiomycetes (13) controls *R. solani*.

The purpose of this research was to determine the incidence of fungi associated with basal stems and roots of soybean during the growing season and to determine if seed treatments would influence the incidence of specific pathogens.

Materials and methods

Plots were planted 13/05/82 at Fingal, Ontario in a field previously observed to have variable yields. The soybean cultivars A1564, Coles, Evans, Hawk and Hodgson were planted in 4 row plots, 3.7 m long with a row spacing of 0.6 m at a seeding rate of 27 seeds/m. The experiment was randomized in a split-plot design with cultivars as the main plots and seed treatments as sub-plots. Chloramben at 4.5 kg a.i./ha was applied prior to emergence to control weeds.

Metalaxyl (Ridomil 25 WP, Ciba-Geigy Canada Ltd.) and furmecyclox (BAS 389 05F, Chipman Chemical Canada Ltd.) were applied at 2 g a.i./kg of seed. Seeds were treated within 1 wk prior to planting.

Fungi associated with the bases of stems were isolated from five plants selected randomly from border rows of each plot. Plants were removed carefully, stored in plastic bags under cool conditions and returned to the laboratory for isolation of fungi. Segments of the lower stem located near the soil line were surface sterilized in 1.2% sodium hypochlorite, cut into 2 mm sections and plated on potato dextrose agar (PDA). Secondary roots were obtained from random soil samples collected in a weed-free area between rows from surface soil 0-15 cm deep and 5-15 cm from the row. Five sections, 5 mm in length, were removed from each of 5 surface sterilized roots per sample plot and plated on PDA. Plates were incubated for 5 to 7 days at 25°C before fungi were identified. Samples were collected 15/06/82, 15/07/82 and 15/08/82.

Results and discussion

A total of 13 genera of fungi were isolated and identified from soybean. The incidence of fungi on each cultivar was similar, therefore the mean incidence from the five cultivars was used

[1] *Agriculture Canada, Research Station, Harrow, Ontario NOR 1GO*
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Table 1. Effect of sample date and seed treatment* on incidence(%)** of soybean roots and stems infected with fungi in Fingal, 1982.

Plant segment	Date	Treatment	Fungi+ Alt	Cc	Chc	Chs	Ct	Fo	Fs	Mu	Ps	Pmg	Py	Rs	Tb	Tv	U
Root	15/6	control	0	0	19 ± 2	19 ±19	0	27 ±27	4 ± 0	3 ± 2	0	0	3 ±5	0	16 ±11	11 ± 8	0
		furmecyclox	4 ± 4	0	13 ±14	13 ±12	0	25 ±27	12 ± 8	3 ± 5	0	0	4 ±0	0	4 ± 0	9 ±16	0
		metalaxyl	4 ± 0	0	19 ± 8	27 ± 6	0	13 ±12	12 ±11	3 ± 5	3 ± 5	0	0	0	5 ± 2	7 ± 2	0
			3 ± 2	0	17 ± 3	20 ± 7	0	22 ± 8	9 ± 5	3 ± 0	1 ± 2	0	2 ±2	0	8 ± 7	9 ± 2	0
	15/7	control	2 ± 2	7 ± 7	6 ± 7	7 ± 4	0	37 ±17	17 ±13	1 ± 2	0	2 ±4	0	0	14 ±13	0	0
		furmecyclox	7 ± 2	17 ± 7	13 ± 5	8 ± 6	0	23 ± 6	24 ± 3	0	0	1 ±2	0	0	6 ± 3	3 ± 4	0
		metalaxyl	5 ± 5	12 ±10	8 ± 6	12 ± 6	0	17 ± 9	15 ±11	0	0	3 ±4	0	0	9 ± 5	4 ± 3	0
			5 ± 3	12 ± 5	9 ± 4	9 ± 3	0	26 ±10	19 ± 5	0	0	2 ±1	0	0	10 ± 4	2 ± 2	0
	15/8	control	2 ± 2	31 ±33	5 ± 6	1 ± 2	0	42 ±10	38 ± 9	0	0	2 ±4	0	1 ±2	6 ± 5	8 ± 6	0
		furmecyclox	6 ± 2	19 ± 9	3 ± 2	3 ± 2	0	44 ±12	35 ±24	0	0	0	1 ±2	2 ±2	6 ± 5	7 ± 7	0
		metalaxyl	3 ± 4	12 ±14	4 ± 3	3 ± 2	0	30 ± 8	46 ±11	1 ± 2	0	2 ±2	2 ±2	2 ±2	6 ± 6	13 ± 4	0
			4 ± 2	21 ±10	4 ± 1	2 ± 1	0	39 ± 8	40 ± 6	0	0	1 ±1	1 ±1	1 ±1	6 ± 0	9 ± 3	0
Stem	15/6	control	1 ± 2	0	0	7 ± 4	0	68 ±18	63 ±11	1 ± 2	0	1 ±2	0	0	6 ± 2	8 ±11	5 ± 5
		furmecyclox	5 ± 8	0	0	8 ± 6	0	70 ±17	63 ±12	0	0	0	0	2 ±2	15 ±11	12 ± 9	3 ± 4
		metalaxyl	4 ± 6	0	0	12 ± 6	0	62 ±17	73 ±33	0	0	1 ±2	0	0	8 ± 7	3 ± 4	2 ± 4
			3 ± 2	0	0	9 ± 3	0	67 ± 4	66 ± 6	0	0	1 ±1	0	1 ±1	10 ± 5	8 ± 5	3 ± 2
	15/7	control	30 ± 5	0	0	0	2 ±4	83 ± 7	94 ± 4	2 ± 2	34 ± 7	0	1 ±2	0	0	10 ±10	19 ± 9
		furmecyclox	37 ± 7	0	0	0	2 ±4	73 ±24	95 ± 5	12 ± 7	39 ±14	0	2 ±4	0	1 ± 2	11 ± 8	25 ±16
		metalaxyl	24 ± 9	0	0	0	3 ±4	70 ±12	96 ± 4	4 ± 3	29 ± 4	0	1 ±2	0	1 ± 2	3 ± 2	22 ± 9
			30 ± 7	0	0	0	2 ±1	75 ± 7	95 ± 1	6 ± 5	34 ± 5	0	1 ±1	0	1 ± 1	8 ± 4	22 ± 3
	15/8	control	16 ± 6	0	0	0	1 ±2	72 ±11	100 ± 0	14 ±10	56 ±16	0	3 ±4	2 ±2	1 ± 2	23 ±19	34 ± 7
		furmecyclox	18 ±12	0	0	0	2 ±4	68 ± 9	96 ± 7	12 ± 6	62 ±19	0	4 ±3	6 ±4	0	29 ±13	45 ±10
		metalaxyl	22 ± 5	0	0	0	3 ±6	71 ±20	98 ± 3	12 ± 6	45 ±20	0	3 ±2	7 ±4	0	32 ±3	51 ±17
			19 ± 3	0	0	0	2 ±1	70 ± 2	98 ± 2	12 ± 1	54 ± 3	0	3 ±1	5 ±3	0	28 ± 5	43 ± 9

* furmecyclox and metalaxyl applied at 2 g a.i./kg of seed

** means of 5 cultivars, 5 plants/cultivar

+ Alt = *Alternaria* spp., Cc = *Corynespora cassiicola,* Chc = *Chaetomium cochloides,* Chs = *Chaetomium* spp., Ct = *Colletotrichum dematium* var. *truncatum,* Fo = *Fusarium oxysporum,* Fs = *Fusarium* spp., Mu = *Mucor* sp., Ps = *Phomopsis* sp., Pmg = *Phytophthora megasperma* f. sp. *glycinea,* Py = *Pythium* spp., Rs = *Rhizoctonia solani,* Tb = *Thielaviopsis basicola,* Tv = *Trichoderma viride,* U = species of *Aspergillus, Cladosporium, Rhizopus* and unidentified fungi.

to compile the results (Table 1). *Fusarium oxysporum* and *Fusarium* spp. were isolated frequently from both roots and stems at all sample dates. The frequency of isolation was similar to the results from a study in Delaware in which the incidence of isolating *F. oxysporum* from soybean roots and stems was 84% (4). Although *F. oxysporum* was isolated frequently from stems and roots, symptoms of Fusarium wilt (4) were not observed in the present study. *Pmg, Pythium* spp. and *Rhizoctonia solani* were isolated infrequently. The incidence of specific fungi on stems and roots did not reflect the reported spectrum of activity of metalaxyl and furmecyclox. Furmecyclox did not reduce the incidence of Fusarium isolations compared to untreated checks at any sample date. Results may have been influenced by the low incidence of *Pmg, Pythium* spp. and *R. solani* at the site based on infrequent isolation from check plants. Although *Pmg* was isolated infrequently from stems and lateral roots, it is possible that root tips were infected. Root tips of soybean are known to attract (10) and support reproduction of *Pmg* (1, 15). Determination of the incidence of *Pmg* in root tips in field grown plants would be difficult because of the fragile nature of infected root material. Plant segment and sample date affected the incidence of isolated fungi. *Corynespora cassiicola, Chaetomium cochloides, Chaetomium* spp. and *Thielaviopsis basicola* were isolated infrequently from the basal portion of stem but they were common on roots in all treatments. The incidence ot *T. basicola* was not affected by furmecyclox. Seed treatment trials in infested greenhouse soil suggest this compound has limited effect on infection by *T. basicola* (12). Seed treatment with furmecyclox at 2 g a.i./kg of cotton seed increased emergence and stand and reduced disease severity in greenhouse soil infested with *R. solani* and *Fusarium* spp. but not in field soil where the predominent pathogens were *R. solani, T. basicola* and *Fusarium* spp. (12). *Colletotrichum dematium* var. *truncatum* and *Phomopsis* sp. were isolated from stem bases more frequently than roots. Incidence of *C. cassiicola* and *Phomopsis* sp. increased with later sampling dates but the incidence of *C. cochloides* and *Chaetomium* spp. decreased at later sample dates. *Corynespora cassiicola* was isolated frequently from roots and has been reported to cause root necrosis and seedling stunting under cool (20°C) temperatures (14). *Chaetomium cochloides* and *Chaetomium* spp. were isolated from roots but the role of these organisms in the root rot complex is unknown.

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A new host and distribution record of *Pythium irregulare* Buisman, in Canada

D.D. Orr and P. Martin[1]

Pythium irregulare Buisman was identified as the causal agent of basal stem rot of greenhouse geraniums at Lacombe, Alberta. This note is the first report of this fungus causing a disease on *Pelargonium* in Canada.

Can. Plant Dis. Surv. 67:1, 7, 1987.

Pythium irregulare Buisman a été identifié comme la cause de la pourriture du collet des géraniums de serre à Lacombe, en Alberta. Cet article est le premier à faire mention de ce champignon comme cause d'une maladie chez *Pelargonium* au Canada.

In the spring of 1986, two month old greenhouse geranium seedlings (*Pelargonium zonale* cv *Sprinter*) grown at Agriculture Canada, Lacombe, Alberta exhibited signs of basal stem rot. The rot appeared as black lesions on the stems at the soil surface. The lesions expanded rapidly until the stem was girdled to a height of 2.5 cm above the soil surface. By the time the lesion had progressed to this point, which was only a matter of 2-3 days, the plant was dead.

The plants exhibiting symptoms were found to be infected with *Pythium irregulare* Buisman (confirmed by Biosystematic Research Institute, Ottawa), an organism mainly associated with damping-off of vegetables (1,2,3) and root rot of vegetables and ornamentals (2,4,5). This is the first report of *P. irregulare* causing basal stem rot on geraniums in Canada (2).

The geraniums were raised from seed in a steam pasturized soil mixture (sand, peat moss, vermiculite and soil). They were maintained at 18-21°C day and 13-18°C night temperatures and were watered with deionized water when soil moisture was low. Four different colours of the "Sprinter" geranium were sown, each exhibiting a different disease reaction to *P. irregulare*. From most to least resistant, they were "Show Girl Sprinter", "White Sprinter", "Scarlet Sprinter" and "Salmon Sprinter". These disease reactions remained through the growing season and surfaced again in autumn 1986 when cuttings of the original survivors were accidently over watered in the greenhouse.

[1] *Agriculture Canada, Bag Service 5000, Lacombe, Alberta T0C 1S0*
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Incidence of pathogenic *Mucor* spp. in Anjou pear orchard soils in the Okanagan Valley of British Columbia

P.L. Sholberg and G.R. Owen[1]

Fifty-one Anjou pear orchards were surveyed for *Mucor* spp. in the Naramata area of the Okanagan Valley of British Columbia by sampling the top 5 cm of soil beneath pear tree canopies within the orchards. *Mucor* spp. were found in 49 of the 51 orchards sampled. A study of *Mucor* spp. levels of propagule per gram of dry soil (p/gds) within 50 of the 51 orchards showed that 22 had less than 100 (p/gds) whereas 28 had higher counts. Single spore isolates which grew at 10°C were made from the cultures isolated from the soil of 49 orchards. These isolates were tested for pathogenicity on Anjou pear fruit. Thirty-six of the 49 isolates were pathogenic *Mucor* spp. The pathogenic species formed distinct groups, having either tall, short or intermediate sporangial height. The short isolates have been positively identified as *Mucor piriformis* Fisher.

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Une enquête a été effectuée dans 51 vergers de poiriers d'Anjou situés dans la région de Naramata dans la vallée de l'Okanagan (Colombie-Britannique), afin de déterminer la présence de *Mucor* spp. Pour ce faire, on a échantillonné les cinq premiers cm de sol sous la frondaison des poiriers. On a retrouvé *Mucor* spp. dans 49 des 51 vergers. Une étude des densités de propagules par gramme de sol sec (p/gss) dans 50 des 51 vergers, a montré que 22 avaient moins de 100 (p/gss) tandis que 28 en avaient davantage. Des isolats de spore unique germant à 10°C ont été prélevés à partir des cultures isolées du sol de 49 vergers et leur pathogénicité déterminée sur des poires d'Anjou. Trente-six des 49 isolats de *Mucor* spp. étaient pathogènes. Les espèces pathogènes se répartissaient en groupes distincts selon la hauteur des sporanges. Les isolats à sporanges courts ont pu être identifiés comme appartenant à *Mucor piriformis* Fisher.

Introduction

Mucor piriformis Fischer causes a stem-end rot of Anjou pear fruit. Losses due to this fungus have been recorded in the Okanagan Valley since 1971 when it was first reported (2). *Mucor piriformis* has also been a problem on Anjou pears in the mid-Columbia area of Oregon and Washington since 1975 (1). Recent studies in Oregon have shown that the organism is confined to the soil and is spread by contaminated soil which adheres to fruit bins and harvested fruit (3).

The objectives of this study were to determine: a) how widespread this organism was in Anjou pear orchards of one area in the Okanagan Valley; b) propagule numbers in the orchard soils surveyed and c) pathogenicity of the isolates from the various orchards. An abstract of this work has been published (4).

Materials and methods

Field survey. Fifty-one Anjou pear orchards consisting of approximately 35 ha were surveyed in the Naramata area of the Okanagan Valley of British Columbia. Soil was sampled approximately 2 weeks after Anjou pear harvest from the top 5 cm of soil under the tree canopy at several locations within the orchard to produce one composite sample for each orchard. The soil samples were processed by a method based on that of Bertrand and Saulie-Carter (1). Fifty grams of soil from each sample were placed in 100 mL of water and thoroughly mixed with a blender for 1 minute. The mixture was allowed to settle for approximately 5 min., and then 0.1 mL of the soil solution was placed on acidified potato dextrose agar (pH 4.8) in 50 mm diameter plates and spread over the surface of the plate with a glass rod. Two plates were made for each sample. This procedure was followed each time plates were incubated at 2, 10 and 20°C. Plates incubated at 20°C were examined for *Mucor* spp. colonies after 3 days, at 10°C after 6 days and at 2°C after 14 days. Precise counts were made only on plates incubated at 2° because these *Mucor* spp. would likely be those which cause rot in cold storage. Counting Mucor colonies with the aid of a stereo-microscope shortly after they had germinated gave the most accurate counts. For each orchard in which *Mucor* spp. were found representative colonies were isolated from one or two plates.

Pathogenicity test. *Mucor* spp. that had been isolated from the 49 orchard soil samples were single-spored to obtain pure cultures. The single spore isolates were grown at 10°C for several days in 150 mL flasks containing 50 mL of potato dextrose agar (PDA). One culture from each orchard was transferred to a 50 mm petri plate containing PDA and tested on Anjou pear fruit for pathogenicity.

A cork borer was used to remove 5-mm diameter mycelial plugs from the petri plates on which the fungus had been growing. The plug was placed on the fruit over an injury in the epidermis made by a glass rod. Two pears were inoculated with each isolate and incubated at 23°C for 7 days. Pathogenicity was considered positive if visible rot was present at this time.

The pathogenic isolates were grouped according to sporangial height above the media. Ten representative isolates were sent to an expert in *Mucor* spp. identification.

[1] *Research Station, Agriculture Canada, Summerland, British Columbia, V0H 1Z0. Contribution No. 662*

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Results and discussion

Field survey. *Mucor* spp. were isolated from the soil of 49 of the 51 orchards surveyed when isolations incubated at 2, 10, and 20°C were considered together. Propagule levels counted in 50 orchards varied widely between the different orchards (Table 1). Twenty-two orchards had a hundred or less propagules per gram of dry soil (p/gds) whereas 28 had more than one hundred when *Mucor* spp. were accurately counted at the 2°C incubation temperature. Spotts (5) has shown that the percent infection of *M. piriformis* increases the fastest at concentrations under about 1000 spores per milliliter. Thus, it is important that packinghouse managers reduce spore concentrations in dump tanks and flumes to the lowest possible level, because any viable inoculum will lead to decay when wounds are present.

Table 1. Propagule levels of *Mucor* spp. in soil from 50 orchards plated on potato dextrose agar and incubated at 2°C for 14 days.

Propagules per gram of dry soil	No. of orchards in this range
0	12
1-100	10
101-200	17
201-1000	3
1001-10,000	5
10,001-100,000	2
100,001-346,000	1

One orchard stood apart from all the other orchards because of its extremely high count of 345,821 (p/gds). This orchard had a history of neglect and perhaps the higher than usual number of propagules were due to the buildup of inoculum by fruit allowed to rot on the orchard floor. Very little soil from this orchard would be necessary to contaminate the dump water.

Pathogenicity test. Thirty-six of the 49 single-spore isolates from 49 different orchards were pathogenic. Six of the 13 orchards which did not have a pathogenic isolate had very low counts of *Mucor* spp. in the soil. Pathogenic *Mucor* spp. would likely have been detected in the remaining seven orchards if more isolates would have been tested for pathogenicity.

The pathogenic isolates could be divided into distinct groups based on the sporangial height above the medium (Fig.1). Thirteen were 0-10mm, 11 were 11-20 mm, six were 21-30 mm and seven were 31-40 mm. The pathogenic *Mucor* spp. whether short, intermediate or tall are most likely strains of *Mucor piriformis* Fischer, however only the group with short sporangial height has been identified conclusively by mating tests to be *M. piriformis*.

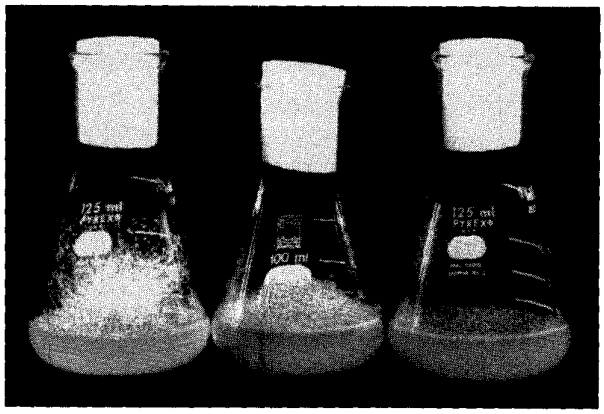

Figure 1. Pathogenic *Mucor* spp. isolated from the soil of Anjou pear orchards growing on potato dextrose agar after 1 month at 2°C in 125 ml flasks. The isolate with short sporangial height on the right has been positively identified as *M. piriformis* whereas the other two cultures are thought to be *M. piriformis* but await further confirmation to their identity.

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We thank Norm Filipenko of Naramata Co-op Growers for collecting the soil samples and Themis J. Michailides, Dept. of Plant Pathology, University of California, Davis, CA, for identifying the *Mucor* spp. cultures.

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Pathogenicity testing of three fungi recovered from roots and crowns of diseased alfalfa in central Alberta

Kan-Fa Chang and Peter V. Blenis[1]

The pathogenicity of three species of fungi, isolated from diseased alfalfa in north-central Alberta, was tested. A factorial design was used to determine the effect of variety (Algonquin vs. Beaver), plant age (1, 6, 10 and 14 mos.), fungal species (*Plenodomus meliloti, Cylindrocladium gracile* and *Fusarium roseum*) and chilling (chilled vs. non-chilled). Mortality was high, even among the control plants. A single isolate of *C. gracile* was used to inoculate 30 alfalfa seedlings growing in test tubes. Only one seedling, from which the fungus was not reisolated, died.

Can. Plant Dis. Surv. 67:1, 11-13, 1987.

On a vérifié la pathogénicité de trois espèces de champignons isolées à partir de plants de luzerne dans le centre-nord de l'Alberta. Pour ce faire, on a utilisé un dispositif factoriel pour déterminer l'effet de la variété (Algonquin vs. Beaver), de l'âge des plants (1, 6, 10 et 14 mois), des espèces de champignons (*Plenodomus meliloti, Cylindrocladium gracile* et *Fusarium roseum*) et du refroidissement (refroidi vs. non-refroidi). La mortalité est élevée même parmi les plants témoins. On a inoculé dans 30 plantules de luzerne cultivées en éprouvette un isolat de *C. gracile*. Une seule plantule sur laquelle on n'a pas retrouvé le champignon est morte.

Introduction

In 1967, a disease, later referred to as alfalfa sickness, was reported in central Alberta (13). The disease was common on sites which previously had produced good crops of alfalfa. Affected stands were not understocked, but rather consisted of poorly-nodulated, yellow-green, and stunted plants, interspersed with occasional healthy plants. Since then, considerable effort has been made to determine the cause of this problem. Initial studies indicated that a biological agent was involved (13). Although the nematode *Paratylenchus projectus* Jenkins was more numerous in locations with poor alfalfa growth than in locations with good growth (14), subsequent inoculation experiments showed that it was unlikely to be capable of causing the disease by itself (4). Several fungi have been implicated in alfalfa sickness. Isolates of Phytophthora (including one identified as *P. megasperma* Drechs.) were recovered from seedlings inoculated with dilutions of the affected soils (5). Five of these Phytophthora isolates were pathogenic on 7-day-old seedlings. In a survey of four central Alberta alfalfa fields, 69 Pythium isolates were recovered from a total of 1594 plants (11).

In 1980-81 a survey of 55 alfalfa fields was conducted in central Alberta (9). *Cylindrocladium gracile* Bugn. (Boesew.) and *Fusarium roseum* (LK.) emend. Snyder and Hansen accounted for 44.3% of the isolates obtained from diseased alfalfa roots. Symptoms of brown root rot, caused by *Plenodomus meliloti* Mark.-Let., were frequently seen, although this fungus accounted for only 0.3% of the isolates. *Phytophthora megasperma* var. *megasperma* was not isolated. It may be significant that the inoculations that showed the pathogenicity of *P. megasperma* were done on young seedlings (5), whereas this survey included fields that were up to 6 years old. Although the results of this survey might suggest that *C. gracile, F. roseum* and *P. meliloti* are important in alfalfa sickness, the pathogenicity of these isolates was not reported, and thus their role in this disease seems uncertain. It was thus essential to do a follow-up experiment in which the pathogenicity of those isolates was tested. The primary objective of our research, therefore, was to determine whether isolates of *C. gracile, F. roseum,* and *P. meliloti,* obtained during the 1980-81 survey, were capable of causing alfalfa sickness on inoculated seedlings. Secondary objectives were to determine whether the amount of disease caused by these isolates would be influenced by plant age and variety, and exposure to chilling temperatures.

Materials and methods

Pathogenicity testing in soil. Three isolates of *C. gracile*, two isolates of *P. meliloti*, and three isolates of *F. roseum* were used. They had been collected during the 1980-81 survey and stored at 4°C for two years. They were transferred to PDA plates, and checked for viability 2 weeks before being retransferred to sterilized oat grain medium. The flasks were shaken every week to ensure uniform mycelium development in the medium. Before use, approximately equal amounts of the different isolates of the same species were combined and thoroughly mixed.

Seeds of the alfalfa cultivars Beaver and Algonquin were sown in 'Ferdinand' Rootrainer® trays (Spencer Lemaire Ind. Ltd.) in September 1983, January 1984, May 1984, and October 1984 to produce plants which were 14, 10, 6, and 1 mo. old, respectively, at the time of inoculation in November, 1984. The soil mixture used had not been sterilized and consisted of 12 kg soil, 3 kg peat, 12 kg sand, 25 g superphosphate, and 9 g calcium nitrate. The plants were kept in a greenhouse at 18°C and fertilized with 20-20-20 fertilizer (200 ppm) every two weeks.

One hundred and twenty plants in each variety × age combination were divided into four groups of 30 plants. Each group received one of the following four treatments: inoculation

[1] *Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5*

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with *P. meliloti*, *C. gracile*, *F. roseum* or sterilized oat grain medium as an uninoculated control. Replicates were obtained by having three people inoculate 10 plants in each of the treatment × variety × age combinations. The plants were removed from their trays and the soil was washed from their roots. The crown areas of the 14- and 9-month-old plants were wounded by a 22G 1/2″ syringe needle. The 6-month- and 1-month-old plants were wounded twice and once, respectively, by a 26G 3/8″ syringe needle. After wounding, the plants were transferred to 'Tinus' Rootrainers®. Nonsterilized soil, prepared as described above, was poured around the plants to a depth of 5 mm below the wound. A layer consisting of 10 ml of oat grain inoculum was placed around the wounded area. The inoculum was covered with a thin layer of soil to prevent desiccation of the inoculum.

In January 1985, half of the plants were exposed to a chilling treatment. These plants were kept at a temperature of 4°C for one month before they were returned to the greenhouse.

In April 1985, mortality was assessed for those plants that had not received the chilling treatment. In early May 1985, isolations were made from three plants in each of the 16 temperature treatment × inoculation treatment × variety combinations. The plants were chosen by randomly selecting one of the three replicates of each of the 16 combinations and then randomly selecting three plants within that replication. Modified Nash-Snyder medium (7) was used for the isolation of *C. gracile* and *F. roseum* whereas PDA was used for the isolation of *P. meliloti* and for isolations from the uninoculated controls. The isolations were repeated in mid-May, except that 1/10 strength PDA was used instead of PDA. In addition, another 38 plants, taken without particular regard to treatment, were dissected and preserved in FAA to permit visual comparison of symptoms.

Pathogenicity testing in test tubes. A second experiment was conducted in which alfalfa seedlings growing in agar in test tubes were inoculated with a single isolate of *C. gracile*. Seeds of the variety Algonquin were surface sterilized in a solution of 0.3% NaOCl, 70% ethanol, and 29.7% water for 3 min before being rinsed in sterile water and plated on PDA. Three days later, those germlings that were sterile were transferred to 18 cm test tubes containing 0.5% agar and Hoagland's solution. After growing for one month under room conditions, 30 seedlings were inoculated with a 3-wk-old isolate of *C. gracile* and 30 seedlings were left as uninoculated controls. All seedlings were placed in a growth chamber with a night temperature of 15°C, a day temperature of 20°C, and a 10-hr photoperiod. The seedlings were re-examined one month later.

Results and discussion

Many of the plants that had been grown in soil, and not exposed to chilling temperature, died (Table 1). The reason for this mortality is uncertain since many of the control plants died as well. None of the pathogens were isolated from the plants into which they had been inoculated, and there were no obvious differences in root symptoms between any of the treatments. Of the sixty seedlings grown in test tubes, only one, which had been inoculated with *C. gracile*, died. The pathogen could not be reisolated from this single specimen.

The pathogenicity of *Cylindrocarpon ehrenbergii* Wr. (later rejected as a *nomen confusum* [1]), *P. meliloti*, and *Fusarium* spp. on alfalfa have been demonstrated by other workers, but not all pathogenicity tests with these fungi have been successful. *C. ehrenbergii* and *P. meliloti* have been shown to be pathogenic to alfalfa (2, 3, 10) but there is evidence to indicate that they are more pathogenic on plants during the spring, after overwintering, than they are during the summer, when the plants are growing actively (3, 10). Nonetheless, pathogenicity of the latter fungus was demonstrated on young seedlings growing on agar medium in Ehrlenmeyer flasks (8). Similarly, although three isolates of *F. roseum* 'Acuminatum' caused rot on alfalfa cultured by the slant-board technique, a majority of the *Fusarium* spp. isolates initially tested caused no decay (6). This same species was 'weakly virulent' when inoculated into the crown of 3- to 4-month old seedlings in a separate experiment (12).

Table 1. Number of dead seedlings seven months after inoculation (non-chilled treatment)

	Seedling Age (mos)	Algonquin*	Beaver*	Total for Treatment**
P. meliloti	14	6	7	
	10	3	1	23
	1	3	2	
C. gracile	14	10	10	
	10	2	1	
	6	3	4	36
	1	3	3	
F. roseum	14	6	11	
	10	2	2	
	6	1	7	38
	1	4	5	
Control	14	9	8	
	10	1	3	
	6	4	0	30
	1	2	4	

*out of 15; **out of 60

Although our experiments failed to demonstrate the pathogenicity of any of the three fungi tested, the results must be evaluated with caution. The high rate of mortality among the uninoculated controls in the first experiment confounds interpretation of those data. Furthermore, the isolates used were several years old and may have declined in pathogenicity before they were used. Further experimentation, with fresh isolates and a wide range of environmental conditions, will be necessary to expand our knowledge of the role of these fungi in alfalfa sickness.

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Longevity of *Verticillium albo-atrum* within alfalfa stems buried in soil or maintained without soil at various temperatures.

P.K. Basu[1]

A large number of infected alfalfa (*Medicago sativa*) stems were produced in the greenhouse by the root-dip method of inoculation with an isolate of *Verticillium albo-atrum*. Infected stem segments (2 cm long) were buried in sterilized and non-sterilized soil or held without soil in petri plates which were incubated at -5°, 5°, 15°, 25°, 30°, and 35°C temperatures for three years. At monthly intervals stem segments were removed and plated on V-8 juice agar medium for the recovery of the pathogen. In sterile or non-sterile soil the pathogen remained viable and pathogenic throughout the test period (3 yr) only at low (-5° or 5°C) temperatures but its longevity declined with increase of temperatures. At 15°C it survived 18 months in sterile and 8 months in non-sterile soil. At 25°, 30°, and 35°C the longevity was reduced to 8, 7, and 6 months, respectively, in either kind of soil. However, the pathogen in stem segments placed in plates without soil survived 3 yrs at all temperatures tested, indicating its potential for surviving in alfalfa stems exposed to a wide range of temperatures (-5° to 35°C).

Can. Plant Dis. Surv. 67:1, 15-16, 1987.

Un grand nombre de tiges de luzerne (*Medicago sativa*) ont été infectées en serre par un isolat de *Verticillium albo-atrum* selon la méthode d'inoculation par trempage des racines. Des segments de tige infectés (2 cm de longueur) ont été enterrés dans du sol stérilisé et non-stérilisé, ou déposés hors sol dans des boîtes de Pétri et mis à incuber à des températures de -5°, 5°, 25°, 30° et 35°C durant trois ans. Tous les mois, on retirait des segments de tige que l'on mettait en culture sur un milieu d'agar et de V-8 pour récupérer le pathogène. Tant en sol stérilisé qu'en sol non-stérilisé, ce n'est qu'aux basses températures (-5° ou 5°C) que le pathogène a conservé sa viabilité et sa pathogénicité durant la période de test (3 ans) mais sa pérennité a diminué avec l'élévation de la température. À 15°C, il a survécu 18 mois en sol stérilisé et 8 mois en sol non-stérilisé. À 25°, 30° et 35°C, sa pérennité tombait, respectivement à 8, 7 et 6 mois, dans les deux types de sol. Toutefois, le pathogène renfermé dans les segments de tige déposés dans les boîtes de Pétri sans sol, a survécu 3 ans à toutes les températures d'essai, ce qui démontre son aptitude à survivre dans les tiges de luzerne exposées à un vaste écart de température (-5° à 35°C).

Introduction

A recent description of Verticillium wilt of alfalfa (*Medicago sativa* L.) caused by *Verticillium albo-atrum* Reinke & Berthold and its preventive strategies have been published (1, 2). The disease can be introduced into wilt-free areas through infected or contaminated alfalfa seeds or other plant parts (3, 4, 5, 8). In England, Heale and Issac (5) noted that *V. albo-atrum*, as resting mycelium in infected alfalfa plants, can remain viable for 5 months (mo.) at the soil surface, 7 mo. at 15 cm and 9 mo. at 30 cm below ground level. They explained that the viability of the resting mycelium decreased rapidly on the soil surface because of constantly changing conditions of moisture and temperature. Recently, Keinath and Millar (6), using two soil temperatures (6° and 21°C) and three soil matrix potentials (-0.01, -0.3 and -3.0 bars), indicated that among these factors only high soil moisture (-3.0 bars) had adversely affected the persistence of the pathogen in stems buried in soil during a 16-wk test. They (6) as well as Sewell (9) found that saprophytic growth of *V. albo-atrum* in soil was extremely limited. McKeen and Thorpe (7) noted that *V. albo-atrum* from potato (*Solanum tuberosum* L.) rarely overwintered in field soil. Experimental evidence for a long-term effect of a wide range of temperatures on the survival of the alfalfa wilt pathogen in soil is scanty.

The present work was conducted for three years under controlled conditions to determine the effect of temperature on the longevity of *V. albo-atrum* within alfalfa stems in the presence and absence of soil.

Materials and Methods

A culture of *V. albo-atrum* isolated from alfalfa grown in Saskatchewan in 1983 was used in this study. Sub-cultures were maintained on V-8 juice agar medium on which the pathogen sporulated profusely in 2-4 days at room temperature (22° ± 2°C). To obtain sufficient numbers of infected stems, about 200 Vernal alfalfa plants (5-wk-old with stems cut back) were inoculated by soaking their trimmed roots in a water suspension of conidia (6×10^6 / ml) for 15-20 minutes. Inoculated plants were then transplanted individually into 10 cm plastic pots containing a mixture of garden loam, peat, and sand (3:1:1 by volume) in the greenhouse (i.e. by the customery root-dip method of inoculation). After 5-6 wk of regrowth when the plants started showing wilt symptoms, stems were cut into 2 cm long pieces, surface-sterilized in 2% NaOCl solution (1% available chlorine) for 5 min. and plated on 2% water agar medium with 4 pieces per plate. *V. albo-atrum* grew out from more than 75% of the pieces (about 2200). The infected pieces (with the fungus visible on the surface) were either

[1] *Plant Research Centre, Agriculture Canada, Ottawa, Ontario, K1A 0C6*

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buried in sterilized and non-sterilized soil adjusted to 20 ± 2% moisture content (matric potential 0.5 bar) or kept without soil in 9 cm petri plates.

These plates (each containing 4 pieces) were sealed with air-tight tapes and placed in 6 incubators set at -5°, 5°, 15°, 25°, 30°, and 35°C. In each incubator there were 30 sets of 3 plates (sterile, non-sterile and no soil). At monthly intervals, one set from each temperature was examined for the viability of the pathogen. Stem pieces were removed from the incubated plates and plated on clarified V-8 juice agar (4 pieces per plate). In 2-4 days, if viable, the pathogen grew out from the pieces and produced typical verticillate conidiophores with numerous conidia. The result was recorded as the presence or absence of the fungus in each plate. The pathogenicity of representative surviving cultures was tested on 5-wk-old Vernal alfalfa seedlings every 6 months by the root-dip method of inoculation as described above.

Results and Discussion

During the first 6 months, *V. albo-atrum* was recovered from the stem pieces in all plates incubated at -5° to 35°C. The effect of soil temperature on the longevity of the pathogen became apparent in the following months (Table 1). At -5° and 5°C, it remained viable in sterilized or non-sterilized soil throughout the experimental period (3 yr). At 15°C, it survived 18 mo. in sterilized and 8 mo. in non-sterilized soil. At 25°, 30°, and 35°C its longevity was reduced to 8, 7, and 6 mo., respectively. Only at 15°C, a possible adverse effect of antagonistic microorganisms (in non-sterile soil) on the pathogen was indicated because it survived 10 mo. longer in sterilized soil.

Table 1. Survival (+ or —) of *V. albo-atrum* in infected alfalfa stems buried in sterile (st) and non-sterile (nst) soil incubated at -5° to 35°C from 6 to 36 months.

		Months of incubation						
Temperature (C)	Soil	6	7	8	9	18	24	36
-5°	st	+	+	+	+	+	+	+
	nst	+	+	+	+	+	+	+
5°	st	+	+	+	+	+	+	+
	nst	+	+	+	+	+	+	+
15°	st	+	+	+	+	+	—	—
	nst	+	+	+	—	—	—	—
25°	st	+	+	+	—	—	—	—
	nst	+	+	+	—	—	—	—
30°	st	+	+	—	—	—	—	—
	nst	+	+	—	—	—	—	—
35°	st	+	—	—	—	—	—	—
	nst	+	—	—	—	—	—	—

In stem pieces held in plates without soil, the pathogen remained viable for the entire period (3 yr) at all temperatures from -5° to 35°C, indicating its strong survival ability in alfalfa stems that are not buried in soil.

Pathogenicity tests showed that all representative samples of the surviving cultures remained as virulent as the initial isolate used to produce the infected stems, irrespective of incubation temperatures. It would appear that the range of temperature (-5° to 35°C) used in this work would hardly affect the survival or virulence of the pathogen. It should be mentioned that the moisture content of the soil remained nearly at the initial level (15-20% or 0.3 to 0.5 bar).

Conclusions

This study provided an experimental evidence of the effect of temperature on the longevity of *V. albo-atrum* in alfalfa stems buried in soil. Low temperatures (-5° and 5°C) were most favorable for a long-term survival (3 yr or more) of the pathogen, but higher temperatures (25° to 35°C) progressively reduced its longevity. However, the pathogen within host tissues without the association of soil was remarkably tolerant to higher temperatures. This preliminary *in vitro* study indicated that infected alfalfa stems might serve as a continual source of infection in the field and that the pathogen would not be affected by -5° to 35°C air temperatures.

Acknowledgements

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

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Verticillium wilt of alfalfa in Quebec

H. Nicholls[2], C. Richard[1], and J.-G. Martin[1]

Verticillium wilt of alfalfa has been found at ten different locations in Quebec this year (1986). It is the first report of the disease in Quebec since it was first discovered in North America in 1976. The ten locations are: Sainte-Anne-de-Bellevue, Compton, Hébertville, East Hereford, La Durantaye, Lennoxville, Richelieu, Saint-Dominique, Stanstead, and Waterville. Until now, most alfalfa seed has been treated with thiram for this disease. It is not clear at this time whether infested seed and debris were the causal agent of the spread or whether the transport of alfalfa hay or natural means of dispersion were involved.

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La verticilliose de la luzerne a été trouvée à dix endroits différents au Québec cette année (1986). C'est la première fois que la maladie est détectée au Québec depuis son apparition en Amérique du Nord en 1976. Les endroits sont les suivants: Sainte-Anne-de-Bellevue, Compton, East Hereford, Hébertville, La Durantaye, Lennoxville, Richelieu, Saint-Dominique, Stanstead et Waterville. Jusqu'à maintenant, la plupart des semences de luzerne étaient traitées au thirame. On ne sait pas encore si l'apparition de la maladie est due à la semence contaminée, au transport de foin de luzerne infecté ou à des vecteurs naturels de dispersion.

Introduction

Verticillium wilt of alfalfa (*Medicago sativa* L.) is the most important disease of this crop in Europe. The notable damages it causes and its rapid dispersion in many countries of the world have lead research scientists to investigate it more thoroughly since 1950 in Europe (10).

Verticillium albo-atrum Reinke and Berth. was reported in North America for the first time in 1962, in alfalfa experimental plots at the Normandin research station in Quebec but was eradicated (4). In 1976, it reappeared in north-western United States (5, 7) and has now made its way to the eastern states where it was identified in Wisconsin in 1980 and in Wyoming, Montana, Minnesota, Pennsylvania, and New York in 1981 (8, 9, 11). Although no seed treatment with a fungicide has been required within the U.S.A (1), seeds imported into Canada from infested areas, must be treated.

After its first appearance in Quebec, the disease was not seen again in Canada until 1977 when it was found in south central British Columbia (12). At the present time, *V. albo-atrum* is widespread in Ontario, contained in Alberta, occurs in southern Saskatchewan and is absent in Manitoba (Jim Chan, personal communication). A comprehensive review of the literature, and a general view of the situation were given in 1982 (6) and 1981 (2) respectively. Agriculture Canada also produced a technical bulletin in 1982 and published a revised version in 1983 (3). Due to the importance of the disease, an extensive nation-wide survey of the Verticillium situation in Canada was coordinated by Agriculture Canada from 1980 to 1982. Since 1983, the provinces were given the responsibility to survey their own territory. In 1986, the Plant Health Division of the Agricultural Inspection Directorate (Agriculture Canada) was asked to survey uninfested areas of Canada for Verticillium wilt of alfalfa as part of their mandate under the Plant Quarantine Act.

General symptoms

Leaves of infected plants become pale yellow (Figs. 2, 3 and 4) or pinkish (Fig. 5) and later bleached and desiccated (Figs. 6 and 7). Young leaflets tend to curl inward (Fig. 7). Affected stems stay green until all the leaves are dead. Usually, a small number of stems on each plant is affected at first while the rest is intact. As the wilt progresses, the number of stems infected grows until the entire plant appears dried out. One of the most typical symptoms is the browning of the xylem easily seen on the surface of the root core when the root is cut transversely (Fig. 8) or decorticated (Fig. 9).

Isolation and identification of *V. albo-atrum*

Stems of suspected plants were cut in 3-cm-long sections, dipped in 70% ethanol during a few seconds and surface disinfected with 2% sodium hypochlorite during 2 min., plated on water agar, and incubated 3-5 days at room temperature(22°C). After microscopic examination, the *Verticillium*-like colonies were replicated on potato dextrose agar (PDA) for further identification and comparison with isolate no. 282 (our collection) isolated from an alfalfa seed lot by Jim Sheppard (Seed Pathology Laboratory, Laboratory Services Division, Agriculture Canada, Ottawa).

Pathogenicity test

One isolate of *V. albo-atrum* was inoculated by a root-dip method to 7-week-old alfalfa seedlings (cv. Saranac). After a 3-week incubation period, disease was noted using a 1-5 scale (1 = plant free of disease, 5 = plant dead) for aerial parts and roots separately after which the fungus was reisolated from roots and stems and positively identified as similar to the one inoculated.

While uninoculated plants were free of Verticillium wilt, inoculated plants were severely affected and showed typical

[1] *Agriculture Canada, Research Station, 2560 Hochelaga Blvd., Sainte-Foy, Quebec G1V 2J3*

[2] *Agriculture Canada, Agricultural Inspection Directorate, Plant Health Division, Guy Favreau Complex, 200 Dorchester Blvd. West, Montréal, Québec H2Z 1Y3*

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symptoms on leaves and stems (wilt index = 4.2), and in roots (root rot index = 4.2).

Areas affected by Verticillium wilt of alfalfa

Verticillium wilt has been found in 10 different locations in Quebec this year (Table 1). Unfortunately, no obvious pattern of infection between these places is visible. The percent incidence varies from 1 to 100%, the age of the stands varies from 2 to 7 years of age and the distribution ranges from southern Quebec, near the New York border, to northern Quebec near Lac Saint-Jean (Fig. 1). As expected, older fields are generally more infected than younger ones.

Although many alfalfa fields were surveyed during the summer months of 1986 in the whole province, the first suspected plants were observed only in mid-September. Will this be the rule in the future? If so, the disease will not affect the yield of the crop heavily. On the other hand, winter survival will certainly be low based on plant mortality and vigor this fall. It is also known that frost-hardening of plants is adversely affected by disease and consequently wilted plants are more susceptible to winter-killing.

Discussion

Generally speaking, although scattered in the southern agricultural part of Quebec, the most affected area is located in the Eastern Townships, between Lennoxville and the U.S. border (Agricultural region no. 5).

According to some producers, the disease may have been present last year in small patches, at least in Compton, since, in the fall of 1985, the same symptoms were observed in the same severely infected field in 1986.

At present, the exact origin or mechanism of introduction and dispersion of *V. albo-atrum* into Quebec is unknown. Seed was the first suspected source examined and a visit to locations such as Sainte-Anne-de-Bellevue, Lennoxville and Saint-Dominique showed that all alfalfa seed has been in fact treated with thiram prior to planting. However, the treatment is not completely reliable (6).

Table 1. Areas reported infected with *Verticillium* in 1986 in Quebec.

Location	% incidence	Age of the stand (years)	Isolation of *V. albo-atrum*
Compton			
Old stand	40	4	+
New stand	20	2	+
East Hereford			+†††
Hebertville	10	---†	+
La Durantaye	10	---	+
Lennoxville (experimental plots)	80	3	+
Sainte-Anne-de-Bellevue			
Research plot	1	2	+
Forage stand	100	7	+
Richelieu	100	5	+
Saint-Dominique	40	3	+
Stanstead	---	---	---†††
Waterville			
Old stand	70	4	---
New stand	---	2	---

† Not known
†† Isolated and identified by Le Laboratoire de diagnostic, Service de la recherche en phytotechnie, MAPAQ
††† No isolation was tried but diseased plants were positively identified

Another possible source of the disease is alfalfa hay and because of the proximity of some of these infestations to New York, a contaminated state, the disease might have been introduced into the province by means of contaminated hay and

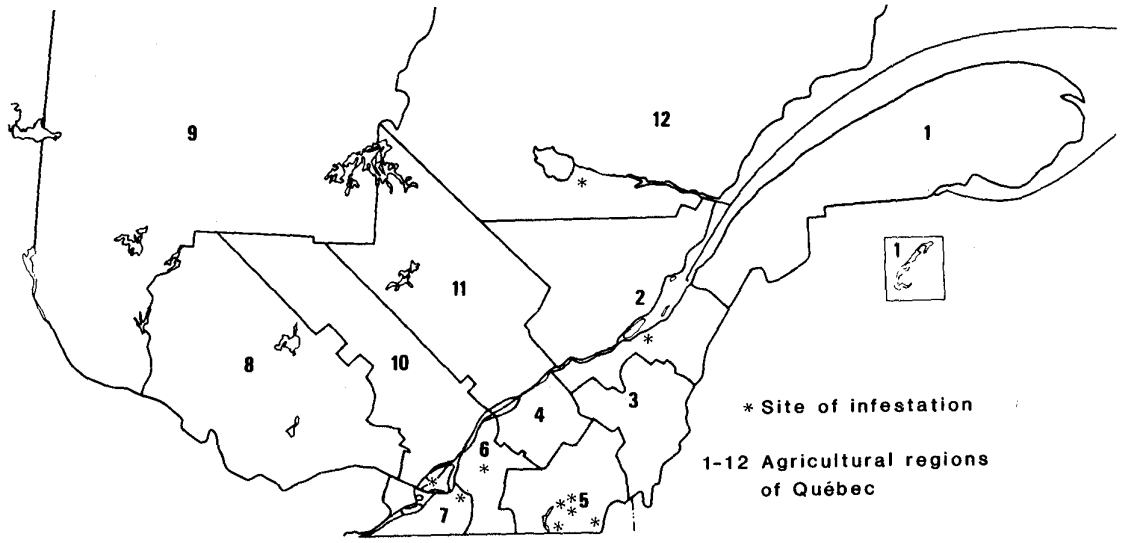

Figure 1. Locations of alfalfa crops infected by Verticillium wilt in each agricultural region of Québec.

later transmitted to locations further north. Again, this is all speculation.

What is obvious though, is that once present, this disease is very easily spread. But, whether the disease first established itself in these mature stands and was then transmitted to younger ones is hard to say. Spores present on the surface of infected stems or leaves and in the soil itself can be easily transported not only by wind and insects but also by people. All kinds of agricultural machinery used in the field or even cars can serve to carry spores from infected fields to healthy ones, not to mention contaminated hay.

The disease is now becoming widely established in places in North America north of the 45th parallel. In Canada, the disease is widespread in Ontario and B.C., contained in Alberta and has been reported on two occasions in Nova Scotia in 1981 and 1986 (Jim Chan, personal communication). In Alberta, where the disease has been contained by ploughing up the fields, it is believed this strategy is a practical solution. In British Columbia, where the disease is also prevalent, a similar suggestion was made of ploughing up the fields and replacing these with resistant cultivars as a disease management practice.

For these reasons, it is believed by the authors that complete eradication of this disease in Quebec is now unlikely; however, containment is still a possibility. The development of resistant cultivars well adapted to Quebec agricultural conditions is now a high priority.

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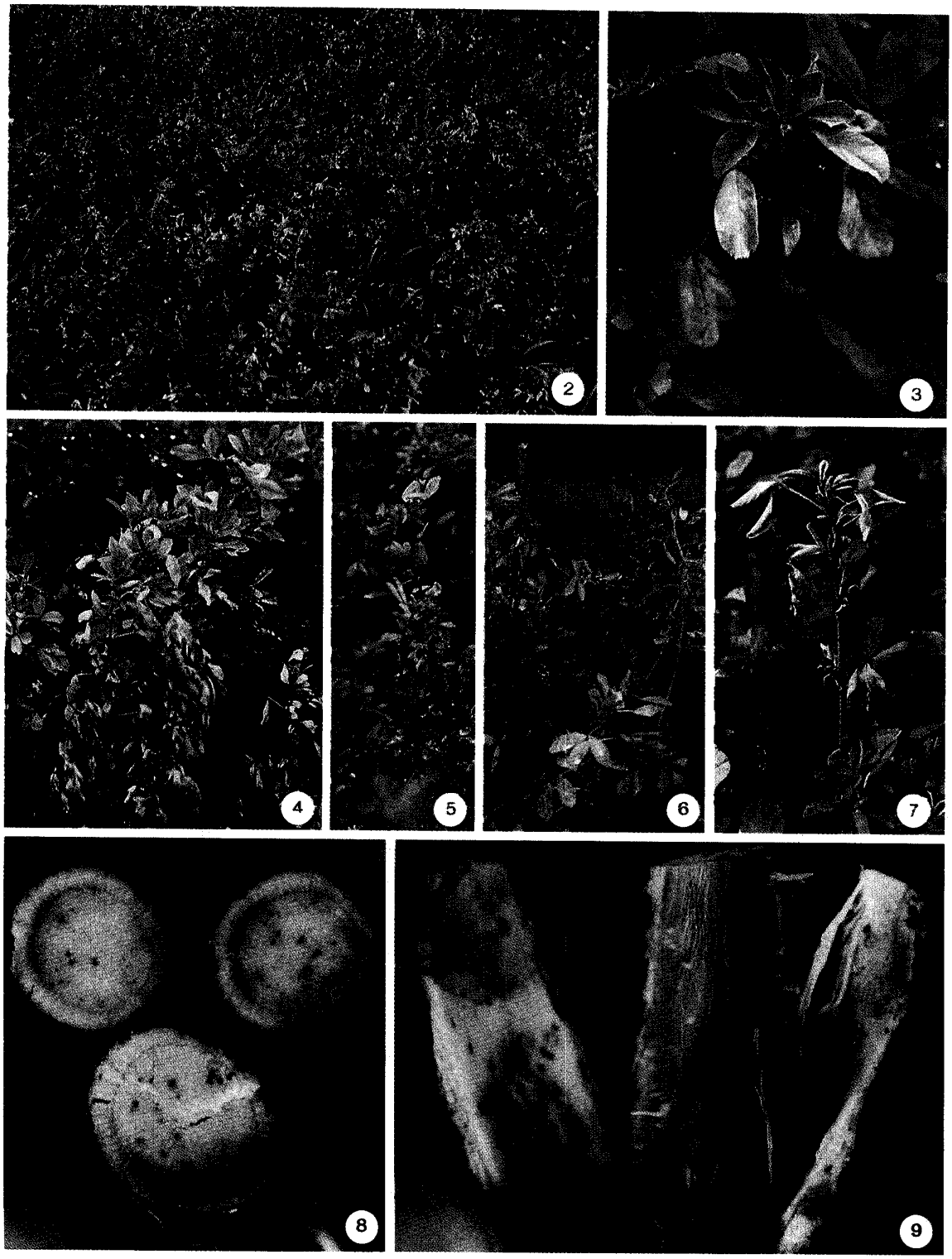

Figures 2–9. Symptoms of Verticillium wilt of alfalfa.
2. General appearance of an infected crop. 3. Early symptoms (bleached leaflet). 4. Yellow leaves. 5. Pinkish leaves. 6. Desiccated leaves. 7. Curling of the leaflet. 8. Brown ring in the root xylem. 9. Browning on the surface of the xylem.

Apostrasseria lunata associated with seedling mortality of western white pine and Douglas-fir in British Columbia

J. Hopkins and A. Funk [1]

The anamorph of *Phacidium lunatum*, *Apostrasseria lunata* has been collected on dead seedlings of western white pine and Douglas-fir from a forest site in the interior of British Columbia.

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L'anamorphe de *Phacidium lunatum*, *Apostrasseria lunata*, a été récolté sur des plantules mortes de pin argenté et de Douglas taxifolié trouvées sur un emplacement forestier à l'intérieur de la Colombie-Britannique.

Apostrasseria lunata (Shear) Nag Raj, the anamorph of *Phacidium lunatum*, Dicosmo, Nag Raj and Kendrick (1, 3), was described from collections of *Gaylussacia brachycera* Michx. Gray, *Gaultheria procumbens* L., and *Vaccinium macrocarpum* Ait. The collections were from Ontario and the eastern United states.

Dead western white pine (*Pinus monticola* Dougl.) seedlings collected in early July 1985 from a forest site near Revelstoke, British Columbia, had pycnidia of *A. lunata* scattered over the lower stem and branches. The seedlings, all natural regeneration, were believed to have died within a year of collection, probably during the previous winter. Numerous conidia were released after moistening.

A search for *A. lunata* in early September 1985 in the Revelstoke area provided a collection on dead Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings and dead western white pine seedlings. The pycnidia examined contained very few conidia at this time. No pycnidia were found on healthy tissues.

Samples of the infected seedlings of both species are deposited in the herbarium (DAVFP) at the Pacific Forestry Centre.

[1] *Canadian Forestry Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria, B.C. V8Z 1M5*

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These collections are the first ones of *A. lunata* reported from western white pine and Douglas-fir. They are also the first from a field situation in western Canada. However, *A. lunata* had been found (2) in 1984 on western larch (*Larix occidentalis* Nutt.) seedlings with damage of unknown cause; the damage, confined to necrotic needles, had developed during cold storage at the Chilliwack forest nursery.

The cause of death of the seedlings bearing *A. lunata* is unknown. No known pathogens, pests or other fungi were found on them. The seedlings were naturally regenerated and not subject to planting shock. Research into the nature of the association with *A. lunata* is required.

Acknowledgements

The authors wish to thank the staff of the Forest Insect and Disease Survey, especially R. Turnquist, for all their assistance.

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Articles and brief notes are published in English or French. Manuscripts (original and one copy) and all correspondence should be addressed to Dr. H.S. Krehm, Research Program Service, Research Branch, Agriculture Canada, Ottawa, Ontario K1A 0C6.

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

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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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