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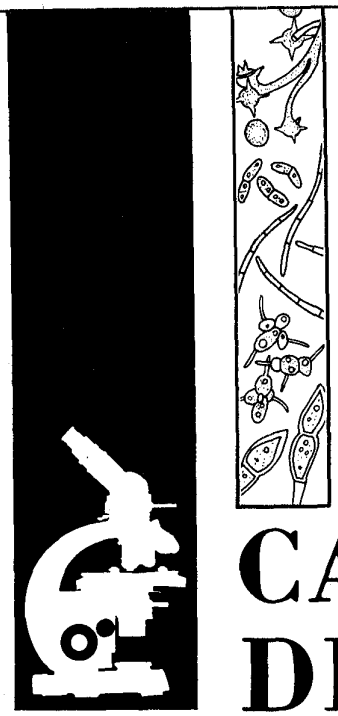

CANADIAN PLANT DISEASE SURVEY

EDITOR: D.W. CREELMAN

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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. It will also accept other original information such as the development of methods of investigation and control, including the evaluation of new materials. Review papers and compilations of practical value to phytopathologists will be included from time to time. It will not accept results of original research suitable for publication in more formal scientific journals".

EFFECT OF FUNGICIDES ON GERMINATION OF LOWBUSH BLUEBERRY POLLEN AND ON NUMBER OF SEEDS PER BERRY[1]

C. L. Lockhart [2]

Introduction

Rich (3) and Eaton (1) have shown that certain fungicides have a marked effect on germination of apple and sweet cherry pollen. Recently Shawa et al. (4) have shown that fruit set of cranberry was reduced by ferbam (ferric dimethyldithiocarbamate) but not by zineb (zinc ethylenebis dithiocarbamate). For several years ferbam and zineb have been recommended for the control of blossom and twig blight in the lowbush blueberry, Vaccinium angustifolium Ait. (2). Because the blueberry is closely related to the cranberry and because fruit set has been a problem in the lowbush blueberry fields, it was of considerable interest to determine the effect of these fungicides on the germination of blueberry pollen. Additional data on the effect of these fungicides on the number of seeds per 50 berries per plot were determined in 1956. The fungicide plots were located at Tower Hill, N. B., and were replicated 4 times (2).

Materials and methods

Germination of pollen grains was determined by dusting the grains on a medium of 0.5% agar and 13.5% sucrose (Wood and Barker (5)). The per cent germination was recorded after 17 hours at room temperature. Fungicide concentrations for the tests were based on the rate of 15 pounds of dust per acre (2.9 kg/ha) of ferbam 7%, zineb 3.9% and ziram 7% (zinc dimethyldithiocarbamate). The fungicides were applied as follows:

(1) One-ml aliquots of microground fungicide, equivalent in concentration to a field application, were pipetted to the surface of agar medium in Petri dishes before application of pollen.

(2) For determining ED 50 values a dilution series of each fungicide was prepared and one-ml aliquots were assayed with pollen using the agar medium technique.

(3) In the greenhouse, lowbush blueberry flowers were dusted with fungicides using a small hand duster and their pollen was collected for germination tests.

(4) A fungicide-coated spatula was used to place pollen grains on the stigmas of lowbush blueberry flowers in the greenhouse.

For dry conditions the flowers were held in the greenhouse and for wet conditions the flowers were sprayed immediately following pollination and then held in a mist chamber. After 17 hours the blossoms were collected and smears of the style tips were made in a drop of water on a glass slide. Pollen germination was determined by microscopic examination. Smears of pollinated styles which received no fungicide served as controls. All tests were replicated and repeated at least twice.

Results and discussion

The ED 50 values show that the lowbush blueberry pollen will germinate on higher concentrations of zineb than that of ferbam or ziram (Table 1).

Little or no pollen germination occurred on artificial media which contained ziram or ferbam (Table 1). Moderate germination occurred on the medium that contained zineb. However, pollen from plants dusted with these fungicides germinated as well as the control (Table 1). As the pollen grains were enclosed in the anthers at time of dusting, the dust probably did not come in contact with the pollen.

In general, under dry conditions ferbam and ziram had some inhibitory effect on the germination of pollen but only ferbam resulted in a slight reduction in the percent of styles with pollen germ tubes (Table 2). Under wet conditions all 3 fungicides produced inhibition of germination, with zineb causing less inhibition of germination than ferbam or ziram. Although there was considerable variation in the number of pollen germ tubes per style, zineb appeared safer than ferbam or ziram.

Seed counts on 50 berries collected from replicated fungicide trial plots showed that the number of seeds in plots treated with fungicides were not significantly different from those in control plots (Table 3).

In view of the above experiments, the fungicide zineb appears to be safer to apply for the control of monilinia and botrytis blights of lowbush blueberry than ferbam or ziram.

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[1] Contribution No. 1257, Research Station, Canada Department of Agriculture, Kentville, Nova Scotia.

[2] Plant Pathologist.

Table 1. The germination of lowbush blueberry pollen after 17 hours at room temperature

Fungicide treatment	ED 50 in ppm	Per cent germination* of pollen on artificial medium treated with fungicide	Per cent germination* of pollen from dusted plants
Zineb	200.0	21	100
Ferbam	5.6	1	100
Ziram	8.8	0	98
Control		100	97

* Averages based on duplicate counts of 100 pollen grains per treatment and tests repeated twice.

Table 2. Per cent of lowbush blueberry styles with germinated pollen following pollination with pollen mixed with fungicide and held under dry or wet conditions

Fungicide treatment	Number of styles examined		Per cent styles with pollen germ tubes		Germ tubes per style		Per cent of pollen germinated	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Zineb	15	28	100	100	15-50	5-40	100	90
Ferbam	16	33	94	91	4-30	3-35	90	65
Ziram	15	52	100	98	5-18	5-40	90	65
Control	25	37	100	100	10-60	10-50	100	100

Table 3. Number of lowbush blueberry seeds from fungicide plots

Fungicide treatment	Average number of seeds per plot
Ferbam	1075 a*
Zineb	1207 a
Thioneb**	1240 a
Control	1069 a

* Seed counts followed by same small letter indicate Duncan's Multiple Range grouping of treatments which do not differ significantly at the 5% level.

** Thioneb (polyethylene thiuram disulphide).

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XYLEM ABERRATION, A TRANSMISSIBLE DISEASE OF STONE FRUITS[1]

T. B. Lott [2]

Introduction

Gumming of trunks and branches; distortion of shoots, leaves, and fruits; and pitting of the xylem were described (10) as associated symptoms occuring in sweet cherry, sour cherry, and apricot. Gumming and distortion were reported as transmissible. Experimental transmission of pitting was not then reported and has been difficult to demonstrate with certainty. One selected example is presented here. Wood pitting is the most distinct and general symptom in this syndrome in various host plants, and the name "xylem aberration" is suggested pending further work on relationships to other named diseases.

Inoculations

Trees of the native chokecherry (Prunus virginiana L. var. demissa (Torr. & Grey) Torr.) were grown in an experimental plot. Two trees were inoculated in July, 1959. They were separated by an untreated tree. Each received two inoculations by budding. The inoculum was from an apricot seedling. In other work, similar inoculum produced severe twisted leaf in 'Bing' cherry, and also necrosis, distortion, and gumming of twigs, and fruit symptoms and severe wood pitting. It produced slight ring pox in 'Wenatchee' apricot, a slight reaction in 'Shiro-fugen' flowering cherry, and no effect in 'Italian' prune, 'Elberta' peach, and 'Montmorency' sour cherry. This virus selection was originally obtained from a 'Wenatchee' apricot naturally infected with ring pox, but not examined for wood pitting.

Results

Five years after inoculation, considerable swelling had occurred at all the four inoculation sites in the two inoculated chokecherry trees. No other such swellings were found elsewhere in the inoculated trees or in the intermediate untreated tree or in six other untreated trees. Removal of the bark revealed almost continuous pitting in the wood at both inoculation sites in one tree and slight pitting at both inoculation sites in the other tree. Inside the swellings there was darkening of the wood and many small gum pockets, some of them radial, and some of them between growth rings. In both trees there was irregularity in the wood of five annual growth rings. Irregularity was slight in the ring of the year following inoculation and progressively more severe in rings of later years. The first tree also showed slight wood pitting up to three feet from the ground in a streak on one side, but the other tree showed no pitting at the base. Figure 1 shows the four swellings after stripping with some of the pits still filled with bark.

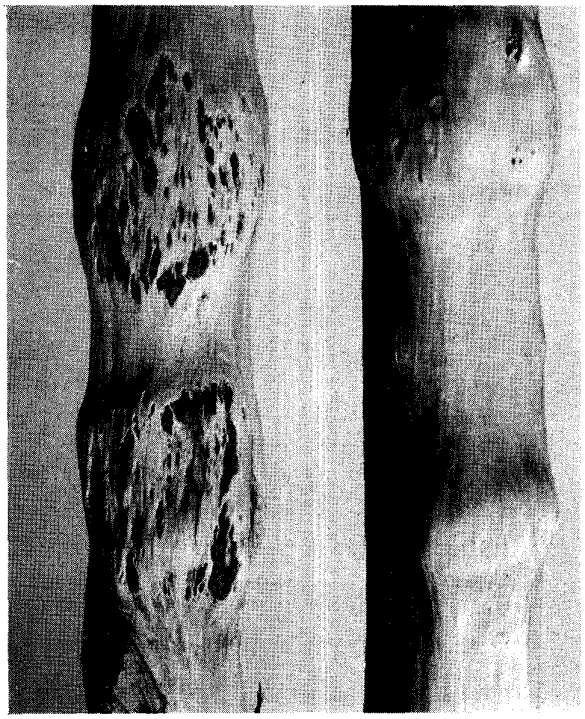

Figure 1. Swelling and wood pitting in chokecherry at the sites of inoculations.

Discussion

This paper presents only one example of the definite transmission of the wood-pitting symptom

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[2] Plant Pathologist (retired)

of the syndrome of xylem aberration. Leaf symptoms and gumming have long been known to be transmissible. Wood pitting was noticed in 1962, by Mr. J. May, in an injured cherry tree. It is now recognized as the most reliable and generally present symptom in the snydrome of xylem aberration in various hosts. The wood of many hundreds of stone-fruit trees has been examined after removal of the bark. Some of these trees had not been inoculated. Some had been used in studies of various virus diseases. Many had been used in the indexing of propagation materials and in the testing of wild plants of native species. Hundreds of trees showed wood pitting, apparently as a result of experimental inoculations but there was usually some slight uncertainty. Wood pitting was found in a few uninoculated trees and in a few inoculated trees where the virus of xylem aberration was not expected. These occurrences could have been due to insect transmission. They could have been caused by natural root grafting which has been demonstrated in these experimental plantings. They could have been due to transmission by nematodes. Xiphinema species were identified, by Dr. R. Stace-Smith, in soil from the plots but their significance here is not known. Wood pitting is usually most severe at the base of the tree and progressively less severe further up. Thus, inoculations by budding are made in the part of the tree which is least likely to show symptoms. Transmission of wood pitting is free from the usual uncertainties when severe pitting is localized at the sites of inoculations as reported here. Some apricot trees have shown pitting more severe at the sites of inoculations than in the adjacent branches. Proof of the transmissibility of the wood-pitting symptom of the syndrome of xylem aberration will increase the reliability of much other work.

Xylem aberration is considered to be different from twisted leaf (4, 5, 6, 7, 8, 9) and from ring pox of apricot (2, 3, 6, 7, 8, 9) with which it has sometimes occurred. Many selections of twisted leaf and ring pox have been transmitted without xylem aberration. In 'Bing' sweet cherry the leaf symptoms of twisted leaf and xylem aberration are difficult or impossible to differentiate. Leaf symptoms in the 'Sam' variety are likely to be those of xylem aberration and not of twisted leaf. Fruit symptoms reported as those of twisted leaf in sweet cherry (5) are not usually found in British Columbia. They appear to be part of the xylem aberration syndrome and not of twisted leaf as it occurs here. In British Columbia, the 'Wenatchee' apricot is best for the study of ring pox. In experimental work here, ring pox is not closely associated with xylem aberration as it is with twisted leaf.

Xylem aberration is considered to be different from virus gummosis of apricot (1) which has probably occurred here for many years.

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DISTRIBUTION IN ONTARIO OF VERTICILLIUM STRAINS CAUSING WILT OF POTATOES

L.V. Busch[1]

Introduction

Surveys of the important potato-growing areas in Ontario were made during the three growing seasons of 1964, 1965, and 1966 to determine the distribution and the relative prevalence of the strains, dark mycelial (DM) and microsclerotial (MS), of Verticillium albo-atrum Reinke and Berth within the province. Samples of stems from potatoes suspected of having wilt were collected from all fields visited and at the same time, where possible, the variety of potato involved, the seed source, and the previous cropping history of the field for the last two years was noted. Personnel of the Ontario and Canada Departments of Agriculture also sent in many samples.

Materials and methods

Sections of stems 2-3" long were surface sterilized, cut into small pieces and placed on acidified plates of potato-dextrose agar (PDA) plus streptomycin. Fungal colonies tentatively identified as Verticillium were transferred from the isolation plates and grown in pure culture on PDA at 20°C and 29.5°C for final confirmation as to strain. The cultures were classified as either DM or MS strains of V. albo-atrum and V. nigrescens, the presence of chlamydospores being used as the criterion for the latter species.

Table 1. Results of a survey during 1964, 1965, and 1966 of potato areas in Ontario. All figures refer to number of fields involved.

Variety	Fields Examined	Culture Type MS	DM	MS/DM	Nig.	Seed Source Maritimes	Ontario	Potatoes in last 2 years
Sebago	17	8	13	4	1	5	1	-
Kennebec	10	5	7	2	-	6	1	5
Cobbler	6	3	3	-	-	1	2	-
Cherokee	5	2	3	-	-	1	-	-
Other	17	6	10	-	2	-	3	2
Total 1964	55	24	36	6	3	11	7	7
Sebago	9	5	5	2	1	2	5	-
Kennebec	16	5	12	2	-	7	4	6
Cobbler	6	-	5	-	1	4	2	2
Cherokee	6	4	4	2	-	4	1	4
Netted Gem	1	-	-	-	1	-	-	-
Other	5	3	2	-	-	-	3	2
Total 1965	43	17	28	6	3	17	15	14
Sebago	19	12	6	1	2	9	7	8
Kennebec	26	10	19	3	2	14	9	16
Cobbler	10	9	2	1	-	4	4	4
Cherokee	6	3	3	-	1	4	2	3
Netted Gem	6	6	2	2	-	3	2	3
Kathadin	5	4	1	-	-	4	1	3
Other	4	3	1	-	-	2	2	2
Total 1966	76	47	34	7	5	40	27	39

[1] Associate Professor of Plant Pathology, Dept. of Botany, University of Guelph, Guelph, Ontario

Results and discussion

Table 1 shows the number of fields of each potato variety examined, the distribution of Verticillium strains in each variety, the seed source and the cropping practice. Note that in all three years a few fields yielded both MS and DM cultures.

No correlation is apparent in Table 1 between variety, seed source, or cropping practice and the strain of V. albo-atrum present, with the possible exception of 'Kennebec'. This variety yielded a larger number of DM than MS cultures in all three years. Since 'Kennebec' is not resistant to the MS strain (1), no ready explanation of the bias is apparent. However, Table 1 indicates that more 'Kennebec' seed of Maritime than of Ontario origin is planted each year; since DM is the only strain present in potatoes in the Maritimes this could possibly account for the results noted herein.

Table II shows the distribution of strains by year within the 10 most important potato-growing counties of Ontario. Several additional counties, not shown in Table II, were also visited and examined during 1964 and 1965.

Three of the ten counties surveyed showed a strong trend in favor of one or the other strain of Verticillium. Dufferin and Durham produced far more DM cultures than MS, and Wentworth, more MS than DM. There are, of course, many factors which could account for this; cropping practice and temperature are the two most obvious.

It is interesting to note that during 1964 and 1965 the total number of DM cultures isolated was greater than the total number of MS isolates obtained. However, this trend was reversed in 1966 with the MS cultures being more numerous.

It is generally conceded that while the optimum temperature for growth on a petri dish for both DM and MS cultures is the same, the MS strain will grow at a higher temperature than DM strains, the latter strain being inhibited at 29-30°C.

Table III shows the weekly maximum air-temperature means from the second week of June to the last week of August. Table IV shows the weekly maximum soil-temperature means at the 10-cm level under sod for the same period of time. The data for both tables were obtained from the Guelph Weather Station.

An examination of Table II reveals that during 1964 the air temperature mean exceeded 81°F (27°C) only during the week of July 25 with nine days from the last week in June to the end of July recording a maximum of 86°F (30°C) or higher. At no time during 1965 did the maximum air-temperature mean come close to 81°F and only one day during June and July exceeded 86°F. However, during 1966 the air

Table 2. Distribution by strains of V. albo-atrum in the 10 important potato growing counties of Ontario

County	MS			Total	DM			Total	nigrescens			Total
	64	65	66		64	65	66		64	65	66	
Dufferin	-	2	-	2	2	4	9	15	1	-	2	3
Durham	-	1	8	9	4	12	5	21	-	-	-	-
Elgin	3	-	6	9	2	-	2	4	-	-	-	-
Middlesex	2	-	5	7	1	2	2	5	1	-	-	1
Norfolk	1	2	2	5	1	2	1	4	-	1	1	2
Simcoe	4	2	7	13	9	3	7	19	-	-	-	-
Waterloo	2	-	1	3	3	1	1	5	-	-	1	1
Wellington	1	-	4	5	-	-	-	0	-	-	-	-
Wentworth	5	5	6	16	1	1	2	4				
York	3	4	7	14	3	3	5	11				
	21	16	46	83	26	28	34	88	2	1	4	7

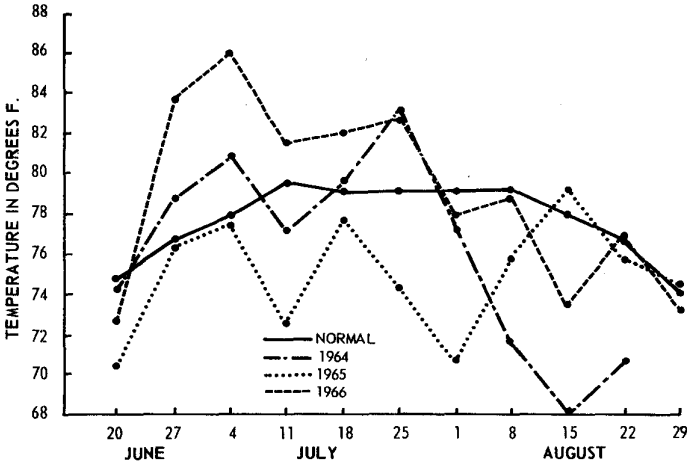

Table 3. Weekly maximum mean of air temperature

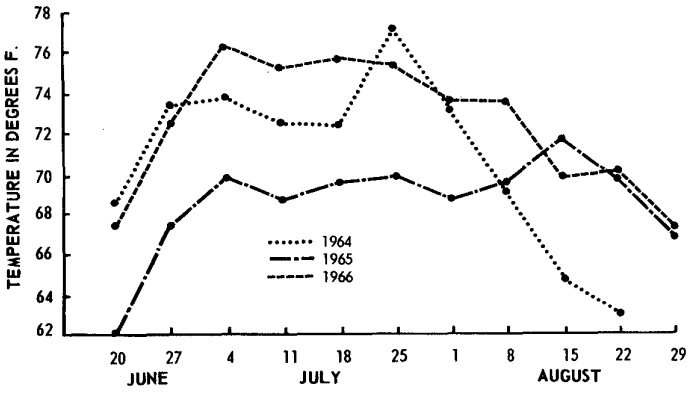

Table 4. Weekly maximum mean of soil temperature at 10 cm. level

temperature mean exceeded 81°F (27°C) from June 27 to July 25 with 16 days during this period recording a maximum of 86°F (30°C) or higher. This, and the fact that the sun can heat the stems by insolation above the ambient air temperature, (Waggoner (6) noted the stem of a tomato was 91°F 10 cm above the soil line with an ambient air temperature of 83°C) would suggest that the MS strain of V. albo-atrum would be favored over the DM strain in 1966. This would agree with Edgington and Waggoner's conclusions (2) in a parallel situation for 1964 in Connecticut.

During 1966 three of the cultures obtained from the same potato stems were mixtures of the DM and MS strains of V. albo-atrum. An examination of these isolates showed the presence of microsclerotia and dark mycelium on the same plate. This could be caused by infection of the potato plant by V. albo-atrum DM strain together with V. albo-atrum MS strain or by a species or strain of Verticillium which has both microsclerotia and dark mycelium present. When the inoculum was taken in the area where dark mycelium was present and transferred to fresh plates only a DM culture resulted. If the culture was taken where microsclerotia were present, only an MS culture resulted. Recently Schnathorst (4) demonstrated cross-protection between isolates of Verticillium in cotton plants. If this same phenomenon is exhibited in potato, then simultaneous infection of the plants by isolates of the DM and MS strains of Verticillium must have occurred in the field.

Smith (5) in his literature review refers to Verticillium tricorpus a species which normally exhibits dark mycelium, dark microsclerotia and chlamydospores. He suggests that Keyworth's "intermediate types" were actually V. tricorpus and not V. albo-atrum. However since Keyworth (3) makes no reference to chlyamdospores, Smith's conclusions may well be open to question.

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ASCOCHYTA DISEASES OF PEAS IN PRINCE EDWARD ISLAND IN 1966

W. L. Seaman[1]

Leaf and pod spot caused by Ascochyta pisi Lib. has been the most frequently encountered of the ascochyta diseases of pea in Canada and has been reported on garden, canning or field peas in every province since 1921. Mycosphaerella blight caused by Mycosphaerella pinodes (Berk. & Blox.) Vest. (Didymella pinodes (Berk. & Blox.) Petr.; imperfect state, Ascochyta pinodes L. K. Jones) has been found in the field or in seed from every province except Prince Edward Island and Newfoundland. In 1962 M. pinodes caused severe pod spot of 'Chancellor' field peas in Manitoba (4) and has since been prevalent in that province on the A. pisi-resistant variety 'Century' (formerly 'Creamette'). Mycosphaerella blight caused the complete destruction of a 20-acre field of canning peas near Florenceville, New Brunswick in 1964 (5). Footrot caused by A. pinodella L. K. Jones (Phoma medicaginis var. pinodella (Jones) Boerema) has been reported in Ontario (2, 4), British Columbia (7), Quebec (3, 7) and Alberta (5) since 1955. Jones, however, isolated all three species from pea seed grown in Eastern Canada, chiefly in Ontario, in 1925-26 (6). Records in this laboratory of seed tests performed on commercially grown peas in the years 1944 to 1949 indicated that the three Ascochyta species occurred in seed grown in every province from Quebec to British Columbia. A. pisi was the predominant species encountered.

A number of commercial fields of peas grown for freezer-processing in Prince Edward Island were surveyed for ascochyta diseases August 10-12, 1966. In a 100-acre field of Rogers 'Perfected Freezer' peas planted June 18 near Brookfield, P. E. I. and examined one week before harvest, approximately 40% of the plants examined exhibited somewhat sunken purplish lesions partially girdling the stems at the defoliated lower nodes. Incidence of the disease was less apparent in an adjoining field of 'Dark Skin Perfection' peas planted 11-12 days later. Similar symptoms were encountered in fields of 'Dark Skin Perfection' near Sherwood, P. E. I. The foliage and pods of affected plants appeared to be completely healthy except for a trace of rust on a few leaves. Symptoms of infection by A. pisi were not found in the fields examined.

Lesioned stems which were surface sterilized, sectioned and plated on pea agar consistently yielded pycnidium-forming colonies which resembled those of A. pinodes isolated from pea seed grown in Manitoba and Ontario. Isolates were obtained from both purplish lesions at the nodes and from tan-centered, dark brown, elognate lesions on the internodes. They were not, however, recovered on PDA from segments of more extensive sunken black lesions girdling the lower stems and upper roots of many plants in a field at Sherwood.

The pathogenicity of single-spored isolates was tested on peas in a growth room at 20°C with floures-cent and incandescent lighting of 2000 ft-c and a relative humidity of 80-90% (95-100% for 48hr. after inoculation). Symptoms on pycnidiospore-inoculated foliage of 2-week-old 'Improved Laxton's Progress' garden pea and 'Chancellor', 'Arthur' and 'Century' field peas were similar for all isolates and were indistinguishable from those of isolates from Manitoba-grown seed. Pycnidia formed on necrotic leaf tissues were brown to black, globose to subglobose and measured 91 × 78μ to 250 × 200μ. Pycnidiospores of one P. E. I. isolate fit Jones' (6) description of A. pinodella, measuring 6.7 - 10.5 × 2.1 - 4.2μ, av. 8.2 × 3.0μ. Pycnidiospores of the other isolates were slightly larger but were intermediate in size between those of A. pinodella and A. pinodes. Size and septation of pycnidiospores of all isolates were highly variable, with the proportion of continuous to uniseptate spores varying among pycnidia of the same isolate. Such variable intermediate-spored isolates have been frequently isolated from pea seed in recent years and have been considered to be variants of A. pinodes. All were very similar in colony growth on agar.

The source of inoculum in the P. E. I. fields is unknown; samples of the Alberta-and Idaho-grown seed used in planting the crops examined were not available for testing. The fields have been continually cropped to peas for several years, thus providing conditions highly favorable for survival and spread of the pathogen in debris.

Ascochyta spp. were not recovered from surface soil collected from between the rows of peas at Brookfield and Sherwood and assayed by a dilution plating method that has been used successfully in isolating A. pinodes and A. pinodella from other soils (unpublished results).

In the fields surveyed, the lack of symptoms on the foliage and pods, as well as the restricted

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nature of the lesions at the lower nodes and the absence of pycnidia in the necrotic tissues, indicated that the plants were infected during the seedling stage and that further development of the disease in the crop was prevented by the unusually dry weather that prevailed in the province during July and August. Precipitation in July was 30% below normal (1). It is significant that peas seeded in mid-June, when precipitation was higher than normal, exhibited a higher incidence of disease than those seeded at the end of the month. Samples of seed harvested from the fields in late August and plated on pea agar after surface sterilization yielded 0-2% A. pinodes.

The abundance of infected plants in the field together with the virulence of the pathogens and the prolific production of pycnidia in lesioned tissues under controlled conditions indicated that severe losses could have been encountered in the crops examined under more favorable weather conditions.

The employment of good cultural practices, including 3-or 4-year crop rotations and removal of pea vines from the fields following harvest, should be emphasized to growers in areas where peas for freezing and canning are becoming more widely grown. Seed treatment with captan or thiram where seed infection with A. pinodes is suspected is also recommended (8).

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A METHOD FOR DETECTION AND STUDY OF THE SUGAR-BEET NEMATODE IN SOIL

E.J. Hawn[1]

Introduction

A method, based on Comstock's (1) and Streu's (3) root cage, was developed for assaying soil for viable cysts of Heterodera schachtii Schmidt, the sugar-beet nematode.

Materials and methods

Narrow soil chambers (Fig. 1-A) were constructed by separating two 2.5- × 35.5-cm sheets of window glass with 0.6-cm frost-shield gasket (Richardson Manufacturing Company Limited, Winnipeg, Manitoba). Each open-topped chamber had three 2.5-cm gaps cut in the bottom gasket to provide for subirrigation. Time tape (Professional Tape Company Incorporated, 355 Burlington Avenue, Riverside, Illinois), 3.8-cm wide, was used to bind the sides of the chambers together. Sieved, air-dry soil was poured into each chamber and compacted by gentle tapping. The bottom of each chamber was immersed in water until the soil was completely moistened by capillary action.

Seeds or seedlings were placed in the moist soil at the top of each chamber which was then enclosed in black plastic to occlude light and prevent growth of algae.

The chambers were set at an angle of 30° in a combination rack and watering tray (Fig. 1-B) which forced the plant roots to grow along the lower soil-glass interface (Fig. 1-C). Water was put into the tray as required.

Results and discussion

Hatching of H. schachtii eggs is stimulated in the rhizospheres of host plants (4). After four weeks' incubation the white-cyst stage could be seen on the roots with the aid of a stereoscopic microscope (Fig. 1-D). Fully developed cysts were visible to the naked eye. The entire exposed root system was examined by drawing a grid on the glass and examining each square.

Seedlings of the following Cruciferae were transplanted to chambers filled with H. schachtii-infested soil: flixweed (Descurainia sophia (L.) Webb), stinkweed (Thlaspi arvense L.), rape (Brassica napus L.), and commercial yellow mustard (Brassica hirta Moench). The following weeds of the family Chenopodiaceae were also tested: redroot pigweed (Amaranthus retroflexus L.), A. powellii S. Wats., and kochia (Kochia scoparia (L.) Schrad.).

After four weeks' incubation in the greenhouse at 21-25°C cysts were found on the roots of stinkweed, rape, and yellow mustard. Stinkweed and rape have previously been reported as hosts for H. schachtii by Jones (2). Thus, results obtained by this method confirm the susceptibility of stinkweed, one of our more common weeds, and show the need for eradication in crop rotations that include the sugar beet and cultivated Cruciferae.

Yellow mustard proved satisfactory as a bioassay plant because of its susceptibility to H. schachtii and the rapid ramification of its roots through the soil in observation chambers. In a series of tests infestations as low as two viable cysts per 453.6 gm of soil were detected. This method is, therefore, practical for detecting infestations of H. schachtii in field soil.

Both male and female larvae of H. schachtii enter the young roots of the host near their growing tips. After approximately three weeks the males emerge from the third larval skin and leave the root to seek out and fertilize the females. Swarms of nematodes were frequently observed near the white cysts in the chambers (Fig. 1-D, a and b). Samples taken from these swarms contained only males of H. schachtii.

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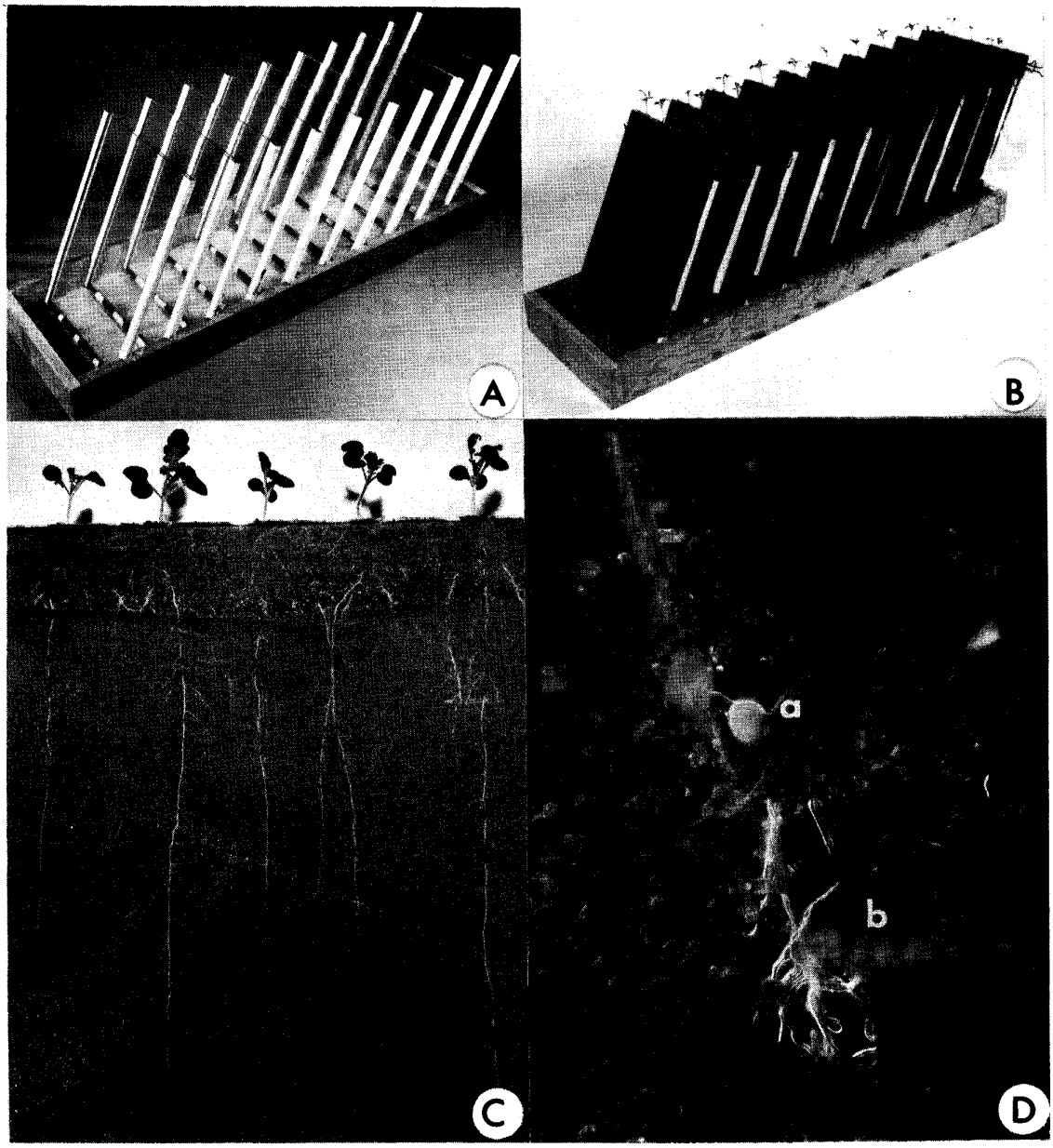

Figure 1. A. Empty chambers in combination rack and watering tray; B. Chambers containing mustard seedlings. Note the black plastic covers.

C. Exposed lower side of a chamber showing root growth along the soil-glass interface;
D. White cyst of *H. schachtii* on mustard root (a) together with a swarm of males (b).

PLANT-PARASITIC NEMATODES IN GRAPE AND RASPBERRY SOILS OF ONTARIO AND A COMPARISON OF EXTRACTION TECHNIQUES[1]

J. L. Townshend [2]

Abstract

Eight plant-parasitic nematodes were associated with grape and raspberry in Ontario. Pratylenchus penetrans (Cobb) Filip. & Stekh. was harmful only to raspberry on sandy soils. Xiphinema americanum Cobb, the only species of dagger nematode found in grape and raspberry soils, was not considered to be important in itself, although it could be a vector of several soil-borne viruses. A greater number of Xiphinema americanum was extracted from soil with a combination of the Cobb and Oostenbrink methods than with the latter method alone. Extraction of Xiphinema from soil by the combined method was affected by the size and the amount of mixing of a sample.

Introduction

The development of virus-free raspberry stock and the introduction of American and European grape cultivars in Ontario necessitated the detection of plant-parasitic nematodes that might be harmful to these crops. Of particular interest were species of Xiphinema known to transmit soil-borne plant viruses (3). Grape soils were examined in 1964 and raspberry soils in 1965 by means of the Oostenbrink cottonwool filter method (9), as well as by a modification of this method to extract Xiphinema from these soils. This paper presents details of the modification and its assessment and the results of the two surveys.

Materials and methods

In the Niagara Peninsula, 90 vineyards located on 19 farms in the counties of Lincoln and Welland were surveyed in 1964. The 90 soil samples were taken from about the roots of 18 cultivars: 'Agawam', 'Canada Muscat', 'Catawba', 'Concord', 'Delaware', 'Duchess', 'Elvira', 'Foch', 'Fredonia', 'Niagara', 'Pinot Blanc', 'President', 'Seibel 1000', 'Seibel 9110', 'Seibel 10878', 'Van Buren', 'V-Port' and 'Westfield'. Three or more cultivars were grown on each farm. The soils were predominantly clay loams.

In southern and central Ontario 75 raspberry plantings located on 42 farms in the counties of Norfolk, Wentworth, Lincoln, Welland, Peel, Ontario, Durham and Prince Edward were surveyed in 1965. The 75 soil samples were taken from about the roots of 9 cultivars: 'Columbian', 'Crescent', 'Creston', 'Latham', 'Madawaska', 'Newburgh', 'September', 'Taylor' and 'Willamette'. Two or more cultivars were grown on each farm. The soils were predominately sandy loams.

Nematodes were extracted from two subsamples of 50 g each taken from each field sample. One subsample was processed by the Oostenbrink method and the other by a combined Cobb-Oostenbrink method as described below. Nematode counts were recorded as the number per 50 g of soil. The field samples were never stored longer than 2 weeks at 40° F before processing.

The Cobb (8) and Oostenbrink (9) methods were combined to extract Xiphinema from soil. The former method was the first phase: a soil sample was mixed in water in a pan and the coarse soil particles were allowed to settle out for a few seconds. The suspension was then poured through a 10-mesh sieve into a second pan. The original sample was resuspended and the preceeding steps repeated. The suspension accumulated in the second pan was stirred, debris allowed to settle momentarily, and then the suspension was poured through a 150-mesh sieve into a sink. Nematodes and fine organic debris trapped on the 150-mesh sieve were suspended in 500 ml of water in a third pan. This suspension was stirred and more debris allowed to settle out briefly before decanting into a beaker. The Oostenbrink method was incorporated at this stage. The suspension of Xiphinema and fine organic debris was poured in a serpentine manner onto #90 cheesecloth clamped in plastic hoops. The hoop with Xiphinema on the cheesecloth was placed in a pan containing a shallow layer of water. For convenience, an extraction period of 7 days was used for the combined method and for the Oostenbrink method alone.

The combined method was assessed by extracting X. americanum Cobb from a Fonthill loam. Fresh soil was collected for each of 3 experiments performed. In the first experiment 50 g samples of field soil were subjected to the following treatment: (1) the Cobb-Oostenbrink method with only cheesecloth in the hoops; (2) the Cobb-Oostenbrink method with cheesecloth and Kleenex in the hoops; (3) as in the first treatment except that the soil was first thoroughly mixed by hand; (4) the unmodified Oostenbrink method as a control.

In the remaining experiments with the combined method only cheesecloth was used in the hoops. In

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the second experiment the effect of sample size was determined by processing 25, 50, 100, 200 and 400 g samples. In the third experiment, the survival of X. americanum in Fonthill loam stored at 40° F for a prolonged period was studied. Samples of 50 g were processed weekly for 4 weeks. In each experiment the extraction period for the Cobb-Oostenbrink method was 2 days and for the Oostenbrink method 7 days at room temperature. Treatments in all experiments were replicated 8 times.

Results

In grape soils, eight types of nematodes occurred in small numbers only and were not related to cultivar or soil type (Table 1). Xiphinema was found in 47 of the 90 field samples from 17 of the 19 farms surveyed.

In raspberry soils seven types of nematodes were noted (Table 2). The root-lesion nematode averaged 102 per 50 g in sandy soils and only 10 in clay soils. The condition of most plantings due to bad cultural practices prevented a critical assessment of the damage caused by the root-lesion nematode. However, in a single planting, areas of stunted canes were definitely associated with large populations of root-lesion nematodes. Xiphinema was found in only 21 of the 75 field samples from 37 of the 42 farms surveyed.

The identity of the eight nematode types are noted in Table 3 along with the crops with which they were associated.

Almost twice as many X. americanum were extracted from 50 g of soil by Cobb-Oostenbrink method as by the Oostenbrink method alone (Fig. 1A). Fungi were seen in many of the dagger nematodes extracted by the latter method. Suspensions of dagger nematodes obtained by the combined method were almost

Table 1. Plant-parasitic nematodes associated with grape in Ontario

	Nematode types							
	Root-lesion	Pin	Dagger	Ring	Spiral	Stunt	Cyst	Root-knot
Average number of nematodes per 50 g of soil	31	29	7	4	18	4	2	7
Percentage of samples examined in which each nematode type occurred	83	76	52	16	18	7	4	2
Percentage of farms containing each type of nematode	100	95	89	53	58	26	21	5

Table 2. Plant-parasitic nematodes associated with raspberry in Ontario

	Nematode types						
	Root-lesion	Pin	Dagger	Ring	Spiral	Stunt	Lance
Average number of nematodes per 50 g of soil	102	65	2	6	36	2	8
Percentage of samples examined in which each nematode type occurred	68	72	28	3	39	7	3
Percentage of farms containing each type of nematode	81	83	38	2	45	7	2

Table 3. Identity of the nematodes associated with grape and raspberry

Common name	Genus and Species	Crop
Root-lesion	Pratylenchus penetrans (Cobb) Filip. & Stekh.	grape and raspberry
	P. neglectus (Rensch) Filip. & Stekh.	raspberry
Pin	Paratylenchus projectus Jenkins	grape and raspberry
Dagger	Xiphinema americanum Cobb	grape and raspberry
Ring	Criconemoides curvatum Raski	grape and raspberry
Spiral	Helicotylenchus canadensis Waseem	raspberry
	H. digonicus Perry	grape and raspberry
	H. platyurus Perry	raspberry
Stunt	Tylenchorhynchus claytoni Steiner	grape and raspberry
Cyst	Heterodera trifolii Goffart	grape
Root-knot	Meloidogyne hapla Chitwood	grape
Lance	Hoplolaimus galeatus (Cobb) Thorne	raspberry

free of debris that is present in suspensions obtained by the Cobb method alone. Both cheesecloth alone, and the Kleenex and cheesecloth trapped this debris but permitted the dagger nematode to move through. Excessive handling of soil was very destructive to X. americanum (Fig. 1A).

The number of X. americanum extracted from soil by the combined method increased linearly with the weight of the sample in the range of 25 to 200g (Fig. 1B). In large soil samples extraction was less efficient; only 840 dagger nematodes were extracted from soil samples weighing 400g when approximately 1600 were expected (Fig. 1B). The number of X. americanum in Fonthill loam stored at 40° F did not decrease significantly in 4 weeks.

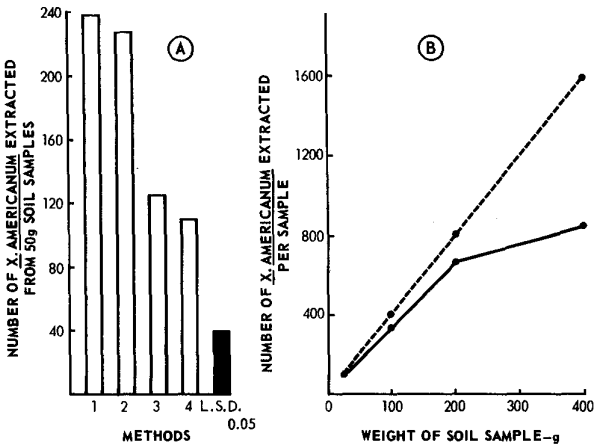

Figure 1. A. Extraction of X. *americanum* from 50g soil samples; (1) by the Cobb-Oostenbrink method with only cheesecloth in the hoops; (2) by the Cobb-Oostenbrink method with cheesecloth and Kleenex in the hoops; (3) with method (1) after the soil sample was handmixed; (4) by the unmodified Oostenbrink method. B. Effect of sample size on the extraction of X. *americanum* from soil by the Cobb-Oostenbrink method. The solid line represents the actual number of X. *americanum* extracted from the 5 samples of different weights and the broken line represents the number of nematodes expected from these samples.

Discussion

In the grape industry in Ontario, plant-parasitic nematodes, in themselves, are not important. Recently, it was proved that X. americanum could transmit grape yellow vein virus from one herbaceous host to another (7) but it was not proved that the nematode could transmit the virus from grape to grape. Moreover, grape yellow vein virus does not seem to spread in vineyards (5) nor has it been found in Ontario vineyards (H. F. Dias - personal communication). Xiphinema index Thorne & Allen, which transmits other soil-borne grape viruses, has not been found in Ontario field soils. Apparently X. index cannot survive the winters. At present, dagger nematodes as vectors of soil-borne viruses in Ontario vineyards are not important.

In raspberry production in Ontario, the root-lesion nematode Pratylenchus penetrans (Cobb) Filip. & Stekh. was the most important nematode because it damaged the crop on light sandy soils where nematode populations were frequently large. This species occurred almost as frequently in raspberry in Ontario as in the eastern United States (4). In British Columbia P. penetrans predominated in raspberry plantings on Vancouver Island whereas P. crenatus Loof predominated in the Fraser valley on the mainland (2).

Lesions were also noted on the roots (2). Pratylenchus neglectus (Rensch) Filip. & Stekh. was noted three times in raspberry in Ontario and infrequently in the eastern United States (4) as well.

Xiphinema americanum was found in many farms where raspberries were grown. This nematode can transmit tomato ringspot virus from cucumber to cucumber (7) but it has not been proven that the nematode can transmit the virus from raspberry to raspberry. Tomato ringspot virus was found in British Columbia (6) but the virus has not been reported in Ontario in recent years. So, at present, X. americanum seems to be of little importance in Ontario raspberry plantings.

In the United States X. americanum was found in 60 percent of the raspberry soil samples collected in eight eastern states (4) whereas in Ontario the nematode was found in 28 percent of the raspberry soil samples. Improved cultural practices are essential in the raspberry industry in Ontario.

Greater numbers of X. americanum were extracted from soil by the Cobb-Oostenbrink method when the samples were of 25 to 200 g of soil. With heavier samples, a large proportion of dagger nematodes were probably carried to the bottom of the pan before the water was decanted (8) and were therefore lost. In the original Oostenbrink method the soil and endozoic fungi might be responsible for the large losses of X. americanum. The soil on the Kleenex probably obstructed the movement of the dagger nematode and also permitted endozoic fungi to more readily parasitize and kill the nematode. Ectozoic fungi may have been more active as well in such an environment.

Excessive hand mixing of soil destroyed many Xiphinema and this would cause serious reductions in the rate of recovery, regardless of the method of extraction.

The capacity of X. americanum to survive prolonged periods of storage in soil is not unusual as Bergeson and Athow found that the nematode could survive 49 weeks in soil stored at 5° C (2).

Acknowledgment

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ROOT-LESION NEMATODES ASSOCIATED WITH FORAGE LEGUMES IN THE MARITIME PROVINCES[1]

C. B. Willis and L. S. Thompson[2]

Abstract

Red, alsike and white clover, alfalfa and birdsfoot trefoil roots were examined for the presence and abundance of root-lesion nematodes, Pratylenchus spp. The numbers extracted from red clover roots in replicated plots were much higher than from alfalfa, and younger plants supported larger populations than older plants. Pratylenchus spp. were extracted from 46 of 70 root samples collected in the Maritime Provinces, and numbers extracted exceeded 500 per gram dry weight of root tissue in 13 of the 46 samples.

Introduction

Problems have been encountered in maintaining good stands of forage legumes in the Maritime Provinces. It is recognized that certain diseases (8) and insects (5) are included among factors causing reduction in forage crop yields, but an understanding of the importance of nematodes in this respect has not been developed. Preliminary examination of the nematodes associated with red clover in Prince Edward Island indicated that root lesion nematodes, Pratylenchus spp., mostly P. penetrans (Cobb, 1917) Filip. and Stekh. 1941, predominated in root samples, and were frequently more numerous in soil samples. Other plant-parasitic nematode genera found included: Tylenchorhynchus, Tylenchus, Meloidogyne, Heterodera, Paratylenchus, Helicotylenchus, Criconemoides, and Longidorus. This paper summarizes results of a survey of the prevalence of Pratylenchus spp. associated with forage legume roots in the Maritime Provinces.

Materials and methods

Samples of 25 living forage legume plants were dug at random from replicated plots near Charlottetown and from fields throughout Prince Edward Island and certain areas of Nova Scotia and New Brunswick. Samples were taken from plots or fields which had been seeded to one or more of the following: red clover, alsike clover, white clover, alfalfa and birdsfoot trefoil. Plants were not examined for nematode injury and varied considerably in age. Following washing, the root system, exclusive of the tap root, was cut into small pieces and a maximum of 20 g extracted using a modification of a technique described for soil samples (6). Numbers of Pratylenchus were counted after 1 week of extraction. The root samples were then dried at 80-90°C for 24 hours and the numbers of nematodes per gram of dry root tissue were calculated.

Results and discussion

Red clover supported a significantly higher population of Pratylenchus than alfalfa both in the seedling year and in the first year of production (Tables 1 and 2). These results were obtained from replicated field plots where the root-lesion nematode infestation was considered uniform. Younger plants supported a larger population of root-lesion nematodes than older ones, probably because of the greater abundance of small, succulent roots. Numbers recovered from alfalfa and red clover, although considerably lower in the first year of production than in the seeding year, reflect the same relative degrees of infestation between plant species. This is in agreement with results obtained in greenhouse studies (2). The numbers of root-lesion nematodes extracted from red, alsike and white clovers and from alfalfa are in agreement with those previously reported (1, 3, 4, 7).

There was a wide range in the numbers of root-lesion nematodes extracted from root samples taken throughout the Maritime Provinces (Table 3). Considering the total number of samples from each province, Pratylenchus spp. were extracted from a greater proportion of those from Prince Edward Island. The proportion of samples which yielded more than 500 Pratylenchus per gram of dry root tissue also was greater for Prince Edward Island. Red and alsike clover were more heavily infested than the other plant species. Although the numbers ranged from low to high, the large populations from some samples suggest that root-lesion nematodes should be investigated further with respect to their possible association with root injury, disease, yield reduction and lack of persistence of forage legumes in the Maritime Provinces.

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Table 1. Mean numbers of Pratylenchus spp. extracted from 4-month-old red clover and alfalfa grown in replicated field plots.

Forage legume	Variety	No. per gram of root tissue (dry wt.)
Red Clover	'LaSalle'	15,879[1]
Alfalfa	'Vernal'	4,171

[1] Mean for red clover significantly higher than for alfalfa at the 1% level.

Table 2. Mean numbers of Pratylenchus spp. extracted from 15-month-old forage legumes grown in replicated field plots

Forage legume	Variety	No. per gram of root tissue (dry wt.)
Birdsfoot trefoil	'Empire'	8,868a[1]
Alsike clover	commercial	5,461a
Red clover	'LaSalle'	3,841a
White clover	'Ladino'	3,169a
Alfalfa	'Vernal'	820

[1] Means followed by the same letter are not significantly different at the 5% level.

Table 3. Incidence of Pratylenchus spp. in forage legumes in the Maritime Provinces - 1966

Forage legume	Prince Edward Island			Nova Scotia			New Brunswick			Total		
	No. of samples	No./g dry wt. 1-500	>500	No. of samples	No./g dry wt. 1-500	>500	No. of samples	No./g dry wt. 1-500	>500	No. of samples	No./g dry wt. 1-500	>500
Red clover	17	8	7	6	3	1	4	2	0	27	13	8
Alfalfa	12	7	0	4	0	0	3	0	0	19	7	0
Birdsfoot trefoil	9	6	1	3	2	0	1	0	0	13	8	1
Alsike clover	6	2	4	0	-	-	1	1	0	7	3	4
White clover	3	1	0	0	-	-	1	1	0	4	2	0
Total	47	24	12	13	5	1	10	4	0	70	33	13

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