Pythium species in alfalfa fields in central Alberta

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Five species of Pythium were isolated from seedling alfalfa roots collected during a survey of 4 alfalfa fields in central Alberta in 1976. P. sylvaticum occurred on 3 fields, P. paroecandrum on 2 fields, and P. ultimum. P. hypogynum, and P. *torulosum* were each found on 1 field.

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Cinq especes de Pythium ont ete isolees des racines de plantules de luzerne recueillies en 1979 au cours d'une enquête portant sur quatre luzernières du centre de l'Alberta. P. *sylvaticum* a été identifie dans trois champs, P. paroecandrum, dans deux et enfin P. ultimum, P. hypogynum et P. torulosum dans un champ chacun.

Introduction

Numerous alfalfa growers in central Alberta report a rapid decline in stand density of recent crops in comparison with stands established following initial sod breaking. A biological toxic agent has been postulated as one of the factors operating in "alfalfa sick soil" (6). Information has been lacking concerning the role of pythiaceous fungi in alfalfa seedling establishment and stand decline in Alberta. *Pythium* spp. have been shown to directly or indirectly affect germinating seed or seedlings of alfalfa and to retard development of plants through root girdling or root tip necrosis, especially on finer textured acid soils (1, 2).

The present study involved a survey for the presence of pythiaceous fungi on alfalfa seedlings in four central Alberta alfalfa fields. Each of the four fields was sown to a companion crop of barley, and had a history of recent poor alfalfa growth. Examination of, and isolations from, the seedlings sampled were carried out at the Plant Industry Laboratory, Edmonton. Isolates obtained in the study were not tested for ability to infect germinating alfalfa seeds and seedlings.

Materials and methods

Alfalfa seeds enclosed in nylon gauze strips to facilitate preemergence sampling were sown in the four fields listed below which were seeded to alfalfa during late May, 1976. Seedlings were randomly collected from the four fields on the following dates:

Field 1 (Bluffton)	27 May, 8 and 30 June, 19 July.
Field 2 (Millet)	8 and 30 June, 12 and 27 July.
Field 3 (Namao)	16 June, 7 and 20 July.
Field 4 (Spruce Grove)	16 June, 8 and 23 July.

Field 1 was located in the grey-wooded soil zone, and fields 2, 3 and 4 were in the black soil zone.

Specimens were placed in polyethylene bags, tied securely, and transported in a cooler to the laboratory. They were then washed under flowing tap water, surface-sterilized in 70% ethyl alcohol for 15 seconds, rinsed twice in sterile distilled water, dried between sterile filter paper, and plated on a selective antibiotic medium (PP) (5) containing *pimaricin* and Pentachloronitrobenzene (*PCNB*). The plates were incubated in darkness at 20°C for 14 days during which they were examined frequently for fungal growth. Aseptate isolates were subcultured on PP agar and retained for future identification. Any seedlings not plated immediately upon reaching the laboratory were stored overnight at 5°C. The upper green portions of larger seedlings were removed prior to surface-sterilizing. Notes were made of the number of seedlings with healthy-appearing roots, as well as those bearing brown lesions, necrotic or "pinched" areas.

Table 1. Occurrence of *Pythium* isolates in four central Alberta alfalfa fields.

Location	Number of seedlings plated	Number of *Pythium* isolates
Field 1 (Bluffton)	338	14
Field 2 (Millet)	461	28
Field 3 (Namao)	402	9
Field 4 (Spruce Grove)	343	18

Results

A total of 69 *Pythium* isolates were obtained from 1594 seedlings plated. Isolates were not obtained consistently from either sick or healthy-looking seedlings and they originated from both hypocotyl and root regions. Several seedlings yielded *Pythium* spp. although their roots showed no rotting or necrosis. No *Phytophthora* sp. was isolated from any of the seedling samples collected.

The greatest number of isolates were obtained from two of the fields located in the black soil zone, and a lesser number were obtained from the other two fields (Table 1). The percentage of isolates recovered from seedlings was highest

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during a two week period in early July on two of the black soil fields (Fig. 1), the lowest percentage recovery occurred in seedlings lifted from the remaining two fields. The percentage of isolates recovered from seedlings was low for all four fields by late July when, presumably, all field soils had warmed and/or dried considerably.

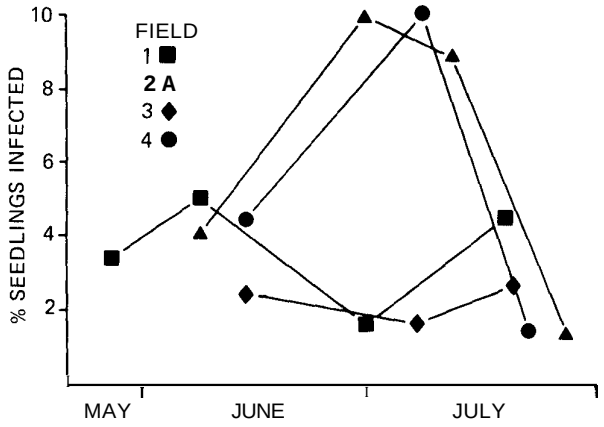

Figure 1. Occurence of *Pythium* isolates from alfalfa seedlings at four locations - 1976.

Several of the 69 *Pythium* isolates obtained were selected on the basis of reproductive structures and on growth patterns at different incubating temperatures. These were identified by Dr. D. J. S. Barr of the Biosystematics Research Unit, Ottawa, Ontario. Four of these isolates were *P. sylvaticurn* Campbell and Hendrix and they occurred on three of the test fields (Table 2). Four of the isolates in Field 2 were *P. hypogynum* Middleton. *P. paroecandrum* Drechsler came from Fields 3 and 4. *P. torulosum* Trow originated in Field 2. Two non-fruiting isolates came from Fields 1 and 4. Since not all isolates were submitted for identification it is probable that *Pythium* spp. other than those listed were isolated.

Table 2. *Pythium* isolates obtained from test fields.

Isolate	Field 1	Field 2	Field 3	Field 4
Pythium sylvaticum	X	X		X
P. hypogynum		X		
P. paroecandrum			X	X
P. torulosum		X		
P. ultimum			X	
P. spp.	X			X

Discussion

Pythium spp. were frequently isolated from roots of alfalfa seedlings with and without damping-off symptoms or root necrosis. Isolation from seedlings showing no evidence of root rot or necrosis may have been due to infections having occurred recently and disease development not being sufficiently advanced for symptoms to appear. Spores of the fungi may have survived on healthy roots during surface sterilizing. The higher percentage of seedlings in Fields 2 and 4 yielding isolates may reflect a higher inoculum level in those fields than in Fields 1 and 3. The total rainfall on the four fields surveyed during the sampling period was not appreciably different, thus soil moisture alone does not account for differing inoculum levels.

The number of species isolated and their range suggests a wide distribution of these potential alfalfa pathogens (4, 3) in central Alberta soils. *P. sylvaticum (debaryanum)* has been shown to cause rapid necrosis of germinating seed and seedlings of alfalfa in Iowa and to retard seedling development through root infection (1). The ultimate effects of the pathogen were partial or complete loss of seedling stands and dwarfed plants with root systems inadequate to carry the plants through periods of stress. Dwarfing and yellowing of many surviving mature plants are characteristic symptoms of the crop on "sick" central Alberta soils. In Ohio *P. ultimum* resulted in severe damping-off and poor emergence of alfalfa because of its high virulence, prevalence in seedlings and abundance in the soil (4). The same study indicated that *P. sylvaticum* and *P. paroecandrum* were moderately virulent to alfalfa in the cotyledon stage.

Prolonged susceptibility to fungal infection or a recurrence of susceptibility in older seedlings may be caused by stress factors such as excessive clipping and the presence of companion crops (4) which can result in low light intensity and competition for soil moisture and nutrients. The combined effect of a companion crop, harsh winter conditions, and fungal infection may weaken seedling stands and be a significant contributing factor in the current "sick" alfalfa problem on central Alberta farms.

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