Low-temperature fungi associated with Alfalfa root and crown rot in central Alberta

D. Stelfox[1] and M. Bertsch[2]

Samples of 2, 3, and 4-year old alfalfa were examined in early spring and late autumn in 1978 to determine the relative prevalence of low-temperature fungi associated with the root and crown rot disease complex. The most frequently isolated fungi were *Fusarium* sp. and *Cylindrocarpon* sp. Plenodomus sp. was isolated more frequently in spring than in autumn.

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Des echantillons de luzerne plantée depuis 2, 3 et 4 ans furent examinees tôt au printemps et tard a l'automne 1983, afin de determiner l'abondance relative des champignons de basse temperature associés au complexe de maladies causant la pourriture de la racine et du collet. *Fusarium* sp. et *Cylindrocarpon* sp. furent isoles le plus souvent alors que Plenodomus sp. fut isolé plus fréquemment au printemps qu'a l'automne.

Introduction

Winter injury with reduced vigour is one of the main factors involved in lowering yields of alfalfa (*Medicago sativa* L.) in central and north central Alberta. Previous reports associate various low temperature fungal pathogens with alfalfa winter injury and subsequent decline in this region (2, 3, 4, 5, 7, 10, 11). The major fungi are involved with winter crown rot (5), crown rot (7), and various root rots (2, 3, 9). Some, such as *Fusarium* sp. and *Cylindrocarpon* sp. were also found to be prevalent in studies of crown and root rotting fungi of alfalfa in Manitoba and Quebec (1, 8). During a 6-year period over 60% of alfalfa stands in west central Alberta (5) were shown to be affected by winter crown rot. In the Peace River area, a survey (9) revealed a severe level of root rot in 68% of fields inspected.

Increased requests for diagnosis of damage to legumes and perennial grasses in central Alberta led to field and laboratory investigations from the Plant Industry Laboratory, Edmonton in 1977 and 1978. Severe depletion in 3 and 4-year old alfalfa stands was noticeable in the early summer both years. Greatest damage appeared to occur in the Barrhead – Westlock area (Fig. 1) where several thousand acres of uneconomical stands were plowed under in June 1978. Winter of 1977-78 was characterized by a rather light and short-lived snow cover. Affected regions were subjected to extremely low temperatures in early December 1977.

The objective of this study was to determine the identity and prevalence of fungi associated, in spring and late autumn, with crown and root rots of mature alfalfa plants in two regions of central Alberta. Samples were lifted in late May to early June and again in late October to early November 1978. The plant material was refrigerator-stored in the laboratory and cultures obtained were studied over a period of 10 months.

1 Box 1, Site 1, R.R. 1, Edmonton, Alberta T6H 4N6

2 67 Grandin Village, St. Albert, Alberta T8N 1R9

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Materials and Methods

Two, 3 and 4-year old alfalfa plants showing symptoms of foliage yellowing and stunting were selected for the study from 42 fields in the grey-wooded and black soil zones of central Alberta. Tissue from specimens with obvious crown and root lesions, but not in advanced stages of decay, was used for plating purposes. Most samples were transported from the field in portable coolers, then stored in the laboratory at 5°C. A few samples were mailed to the laboratory by extension workers.

Estimates of the top growth of plants were recorded. Top growth was then removed just above the crown area. Surface soil was manually removed, then roots were washed for 15-30 minutes under running tap water to remove soil remnants. After excess water was absorbed by paper towels, outer cortical tissue was peeled away at lesion sites using a sterile scalpel. Small portions of underlying root or crown tissue from lesion margins were selected for plating. Outer cortical tissue was plated, where lesions were shallow or superficial.

Isolations were made on potato sucrose agar (PSA), on acidified potato sucrose agar (PSA-A), and on a selective antibiotic medium (PP) containing pimaricin and pentachloro-nitrobenzene (PCNB). Subculturing utilized PSA, PSA-A, PP and cornmeal agar (CMA). Plates were incubated at 0.5, 10, 15, and 20°C for a minimum of 2 weeks. 0" and 5°C plates were incubated for as long as 20 weeks. At 3-day intervals initial plates were examined and fungal colonies were subcultured. Some cultures remained in a controlled temperature chamber for several months. Duplicates were maintained on PSA or CMA slants at 10°C.

Results

Fusarium spp. were among the most common isolates associated with damaged alfalfa crown and root tissue. They were isolated from 50% or more of rotted tissue, and from nearly 40% of tissue showing punctures, splits, brown flecks, and dark necrotic bands (Table 1). They occurred in 76 and 38% of samples from Regions I and II respectively. One type of

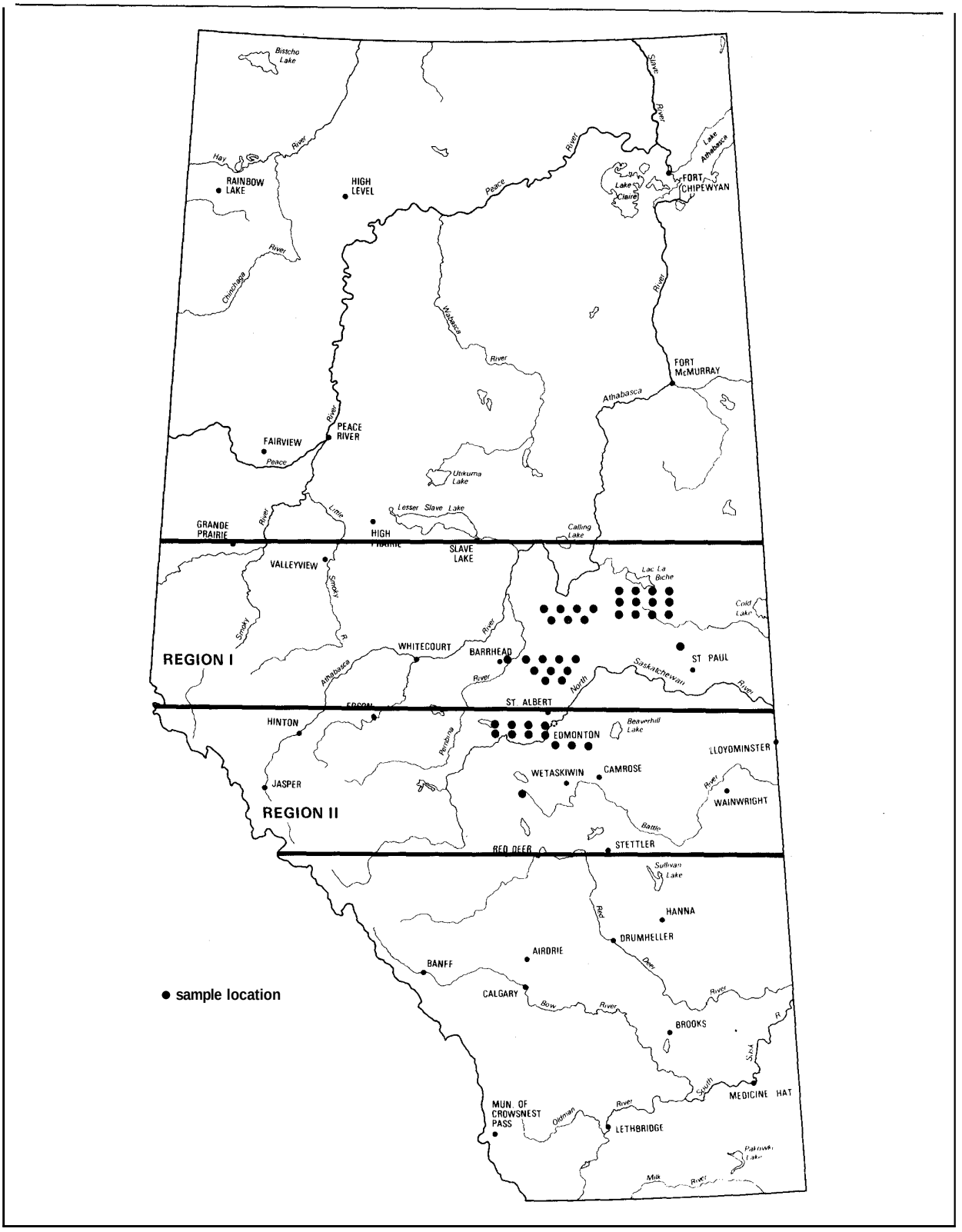

Figure 1. The areas of sampling locations in Regions I and II of central Alberta.

Table 1. Percentage of 42 alfalfa samples from which specific fungi were isolated from crown and root lesions in two regions of central Alberta in 1978

Types of Lesion	Fusarium	Cylindrocarpon	Plenodomus	Pythium	Rhizoctonia	LTB	Botrytis
Crown rot	63	6	10	22	2	2	8
Root rot	50	64	28	0	14	0	0
Root punctures, splits, flecks, bands	37	84	0	5	5	0	0

Fusarium sp. formed tiny sclerotia on host tissue and in culture. In 13 of 34 samples involving rotted crowns, only ***Fusarium*** sp. was isolated. In 10 instances it was associated in crown tissue with other pathogenic fungi such as ***Plenodomus*** sp., ***Cylindrocapon*** sp., ***Pythium*** sp., and ***Rhizoctonia*** sp. In damaged root tissue it was isolated alone from one sample. One of the disorders from which ***Fusarium*** sp. alone was isolated is crown rot of a 2-year old plant (Fig. 2A). All isolates developed well on plates in the laboratory at near-freezing temperatures.

Cylindrocarpon sp. occurred in 64% of the cases involving root rot and 84% involving root banding, splits and punctures (Table 1). This fungus was isolated from 6% of tissues involving crown rot. It occurred in 69 and 85% of samples from Regions I and II respectively. The most commonly isolated species was ***C. ehrenbergi*** with its reddish-brown sclerotia-like stromata on the surface of advanced root-rot tissue (Fig 2B). ***C. destructans,*** forming no stromata in culture or on host tissue, also was obtained from rotted root tissue. Both species were associated with more than two-thirds of alfalfa samples from Regions I and II. There was a noticeable effect of season on incidence of ***Cylindrocarpon*** spp. isolations (Table 2).

Plenodomus meliloti, which usually required 8 to 12 weeks plate incubation to induce pycnidial spore formation, was isolated from 29 and 23% of samples from Regions I and II respectively. It occurred most commonly in root-rotted tissue (Fig. 2D), but sometimes in connection with crown rot (Fig. 2E). The numerous tiny dark brown to black pycnidia formed on or within affected tissue. Beaks frequently developed (Fig. 2C) and extruded spores, following several weeks of low temperature incubation. The fungus was always associated in brown root rot tissue, with ***Fusarium*** sp. and ***Cylindrocarpon*** sp. and its prevalence was about the same in Region I (29%) as in Region II (23%) samples. As in the case of ***Cylindrocarpon*** sp. it was isolated with much greater frequency in spring than in autumn.

Pythium spp. were isolated from damaged crown tissue but not from root rot samples (Table 1). No attempt was made to identify the several different isolates originating from 8 samples most of which came from Region II. Other phycomycetes, generally considered to be soil saprophytes, were isolated.

Rhizoctonia sp. was less prevalent than ***Pythium*** spp. in spring and autumn samples, and occurred in none from Region II. In the 4 instances where the fungus was present, it was always associated with either ***Cylindrocarpon*** sp. or ***Fusarium*** sp. On only one occasion was a low-temperature basidiomycete (LTB) retrieved, and it occurred with other pathogens in crown rot tissue. LTB was recently identified as ***Coprinus*** sp. (13). A ***Botryris*** sp. was isolated once from severely rotted crown tissue and once from reddish-brown flecks on taproot tissue. Several isolates of miscellaneous unidentified fungi were obtained during the study.

Discussion

The results of this study agree with reports (5, 7) of a complex of low-temperature fungi being associated with alfalfa crown and root rot in central Alberta. The virtual absence of LTB isolates from these 1978 samples was likely due to the relatively light and short-lived snow cover during the winter of 1977-78 (5). LTB is reported to be the most destructive fungus attacking alfalfa in spring (2). Very low temperatures in early December 1977, following a prolonged spell of mild weather, may have predisposed alfalfa stands to

Table 2. Percentage of alfalfa samples from which specific fungi were isolated in spring and autumn in two regions of central Alberta in 1978

Season	Fusarium	Cylindrocarpon	Plenodomus	Pythium	Rhizoctonia	LTB	Botrytis	No. Samples
Spring	61	74	39	17	9	4	4	24
Autumn	61	17	11	6	11	0	5	18

late winter-early spring infections by other low temperature pathogens.

Cylindrocarpon sp. is reported (3) as one of the most virulent pathogens attacking alfalfa during early spring in Alberta. Elsewhere in Canada (1, **8)** the fungus is considered to be of importance on alfalfa after the first year's growth. Root infections begin at the first sign of soil thawing and proceed through 3 stages from water-soaked tissue to light brown and, finally, to dark brown necrosis (3).

The brown root rot incitant, ***Plenodomus meliloti,*** has been reported (11) to be one of the most important pathogens associated with "winter-kill". It is native to the Peace River Region as well as to central and north-central Alberta. Infected lesions appear on taproots as soon as surface soil thaws in the spring, or even during mild spells in late winter. Growth and development of pycnidia of the fungus in Alberta have been studied and illustrated (10). Partial recovery of plants severely affected by brown root rot is due to the formation of new roots produced near the crown, a condition frequently observed on samples collected in the Barrhead-Westlock region. This was one of the root abnormalities associated with stunted plants.

At least five pathogenic low-temperature ***Fusarium*** spp. have been found to cause root injury of alfalfa in Alberta (4). The two which are widespread and cause serious damage in early spring are *F.* ***avenaceum*** and *F.* ***arthrosporioides.*** In early stages of infection root symptoms can readily be confused with those attributed to ***Plenodomus*** sp. and ***Cylindrocarpon*** sp. In the present study, all three of these genera have been isolated from a single root lesion. Where ***Fusarium*** sp. alone was isolated from the margin of rotted crown tissue, it may have overrun tissue originally invaded by another pathogen. Early spring and late autumn sampling were chosen, since low temperature fungal pathogens are difficult to isolate during summer months. ***Plenodomus*** spp., in particular, is responsive to isolation attempts in early spring.

Some of the ***Pythium*** spp. reported (12) to be associated with seedling infection in alfalfa in central Alberta were similar in appearance to isolates of the present survey. The fact that no isolates were obtained from below-ground portions of these mature plants may have been due to loss of rootlets and necrotic root tips when lifting samples.

Rhizoctonia solani Kuhn is known to be part of a complex associated with ***Fusarium*** spp. in crown bud rot in Quebec, Manitoba, and Alberta (1, 8, 6). Crown rot was evident on all samples from which ***Rhizoctonia*** sp. was isolated in the present study, and typical taproot symptoms characterized below-ground tissue from which the fungus was retrieved. Damage apparently had progaessed over a period of one or two growing seasons, judging by the extent and color of root lesions.

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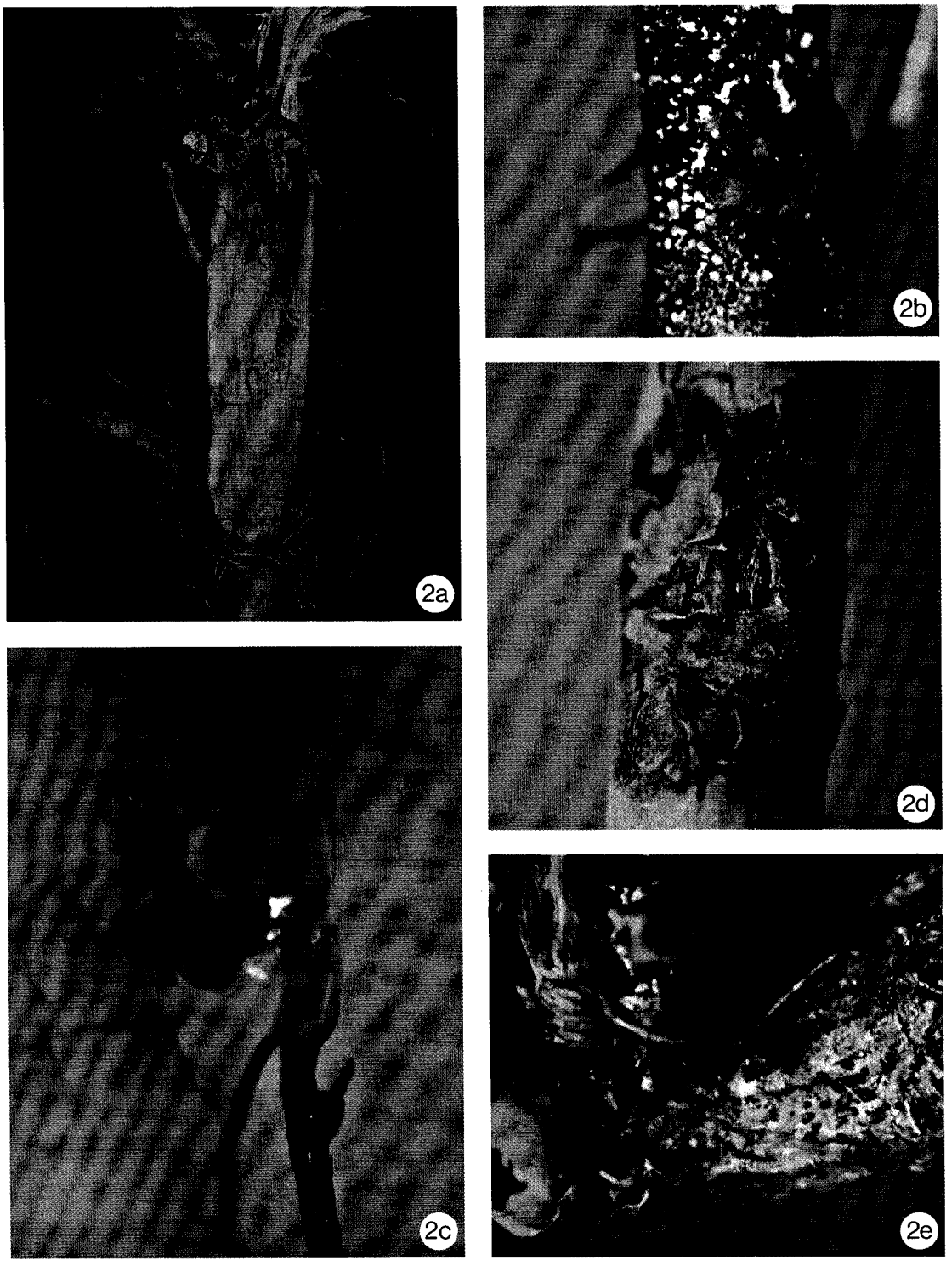

Figure 2 (A to E). Symptoms and signs of crown and root disorders: (A) 2-year old plant with crown rot, (B) reddish-brown sclerotia-like stromata of *C. ehrenbergi,* (C) beaks formed on pycnidia of *P. meliloti,* (D) typical advanced brown root rot symptoms, (E) Pycnidia formed on rotted crown tissue.