Bacteria associated with crown and root rot of sainfoin in southern Alberta

S.F. Hwang[1], R.J. Howard2 and L. Goatcher[1]

Thirty sainfoin fields in southern Alberta were surveyed in 1983 for the incidence and severity of crown and root rot disease. Mean incidence and severity were 87% and 1.9 (on a scale of 0-3), respectively. This is the first report that strains of Pseudornonas fluorescens, *Erwinia* carotovora subsp. carotovora and P. *syringae* are associated with crown and root rot of sainfoin in southern Alberta.

Can. Plant *Dis. Surv. 69:1,* 5-8, *1989,*

Trente champs de sainfoin du sud de l'Alberta ont fait l'objet d'une btude en 1983 pour determiner la fréquence et la gravité de la pourriture du collet et des racines. La fréquence et la gravitb moyennes sont de 87 % et de 1,9 (sur une échelle de 0 à 3) respectivement. C'est le premier rapport de l'existence d'une association entre des souches de Pseudornonas fluorescens, *Erwinia* carotovora sous-espèce carotovora et *P. syringae* et la pourriture du collet et des racines de sainfoin dans le sud de l'Alberta.

Introduction

Sainfoin (*Onobrychis viciaefolia* Scop.) is a perennial legume which has been grown as a forage crop in Europe and Asia for several centuries (7). In recent years it has received a lot of attention in southern Alberta, but it has never become a widely grown crop, despite having many merits as a forage (7). Crown and root rot disease has been considered one of the major limiting factors restricting the cultivation of sainfoin (3, 5, 6, 8).

The nature of crown and root deterioration in forage crops is very complex (5, 6, 10, 11). Previous studies have indicated that certain groups of bacteria, rather than a single fungus, are closely associated with the crown and root rot complex of sainfoin (5, 9, 12, 13). To obtain more information on crown and root rot of sainfoin in southern Alberta, a comprehensive field survey was carried out to determine the incidence and severity of crown and root rot; to isolate and identify the bacteria from discolored crown and root tissues; and to determine the pathogenicity of the isolated bacteria on sainfoin seedlings.

Materials and methods

Thirty sainfoin fields in southern Alberta were surveyed in 1983 for the incidence and severity of crown and root rot disease. Twenty-five plants dug at random using a sharpshooter shovel were shaken free of soil, placed in a paper bag, and stored in a cooler until processing. Plants were rinsed with tap water and split longitudinally to visually assess the severity of crown and root rot. Severity scores assigned were 0, no disease; 1, slight; 2, moderate; 3, severe.

For bacterial isolation, diseased sainfoin plants were randomly collected again from seven fields in the Nanton-Claresholm-Fort MacLeod area of southern Alberta in the fall of 1985 and stored at -5°C. Two g of tap-root tissue taken 4 cm below the crown from each of five plants per field were surface sterilized for 2 min in 0.6% NaOCl and rinsed three times with distilled water. The five root samples from each field were pooled and placed in a test tube containing 20 ml sterile distilled water and allowed to soak overnight.

A series of dilutions from the tissue-soaked solution was made on nutrient agar (NA) plates. Observations of the growth of bacterial colonies and subcultures of representative colony types were made after incubation for 7 days in the dark at room temperature. Purification of the isolated bacterial colonies was achieved by streaking them onto fresh NA plates. This process was repeated twice. All morphologically similar isolates were stored in sterile distilled water at 4°C, then submitted to the Microbiology Group at the Alberta Environmental Centre for identification to species level (1).

Once the isolates had been positively identified based on a range of biochemical and physiological tests, the virulence of the isolates was tested on 2.5-month-old sainfoin seedlings, cv. Melrose. Inoculum consisted of 1 ml aqueous suspension of approximately 10^8 cells/ml obtained from a 24-hr-old culture on NA. This was injected into the crown area using a sterile 23-gauge needle (4). Seedlings injected with sterile distilled water were used as a control. After inoculation, the pots were arranged randomly in the growth chamber at 20°C (16 hr day) and 15°C (8 hr night) with a light intensity of 300 $\mu em^{-2}s^{-1}$, which was provided by cool white fluorescent tubes. Five pots with four seedlings each were inoculated with bacteria or water. Symptoms were recorded 4 months after inoculation. The length of discoloration of tap roots at the inoculation site was measured and the severity of crown and root rot was assessed as previously described.

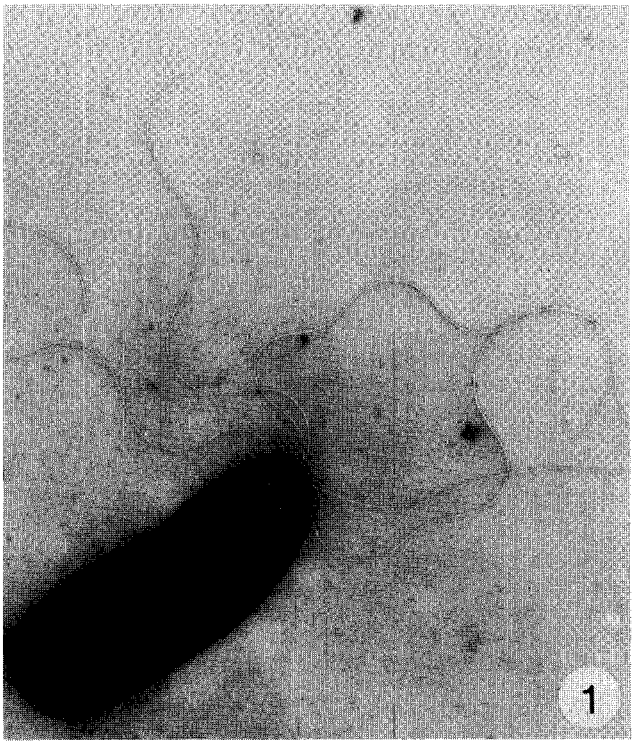
Results and discussion

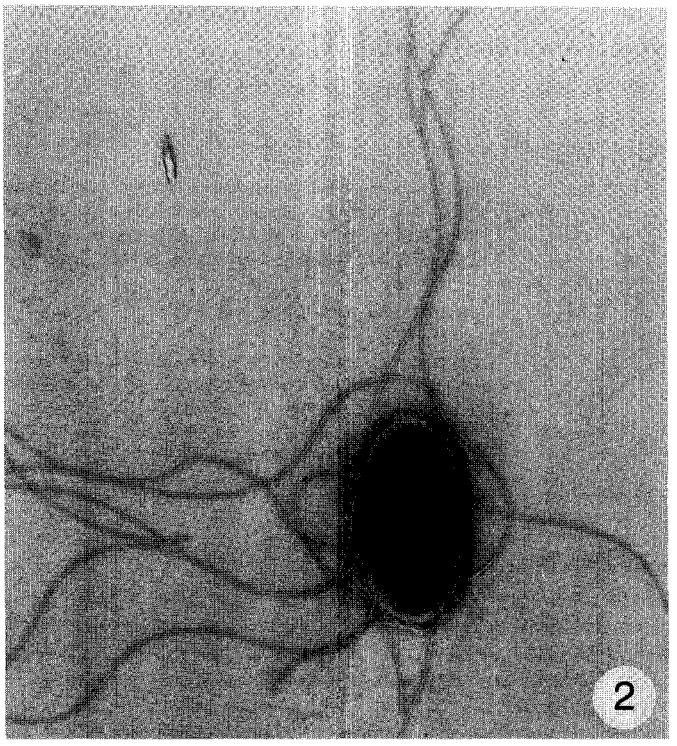
Crown and root rot was found in all of the sainfoin fields surveyed in 1983. Average disease incidence and severity of crown and root rot were 87% and 1.9, respectively (Table 1). The bacteria isolated from diseased sainfoin crown and roots were classified into the following groups: ***Pseudomonas fluorescens, Enterobacter agglomerans, Erwinia carotovora*** subsp. ***carotovora*** and ***P. syringae.*** which accounted for 66, 25, 6, and 3% of the total isolates, respectively.

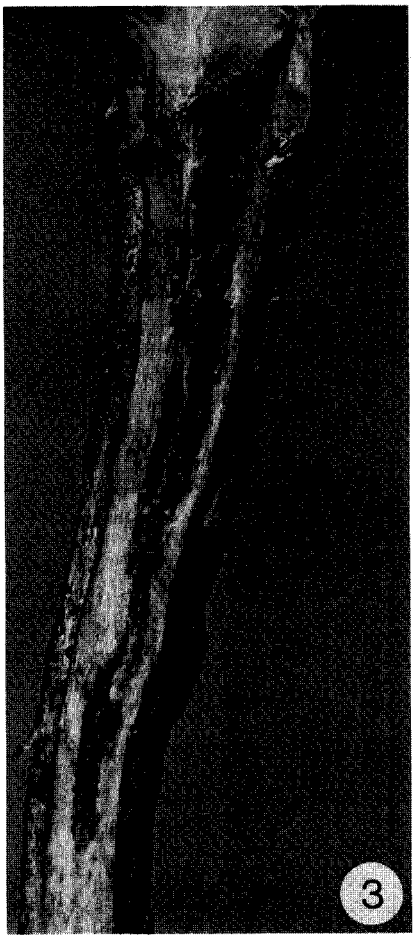
1 Alberta Environmental Centre, *Vegreville,* Alberta, Canada *TOB 4LO.*

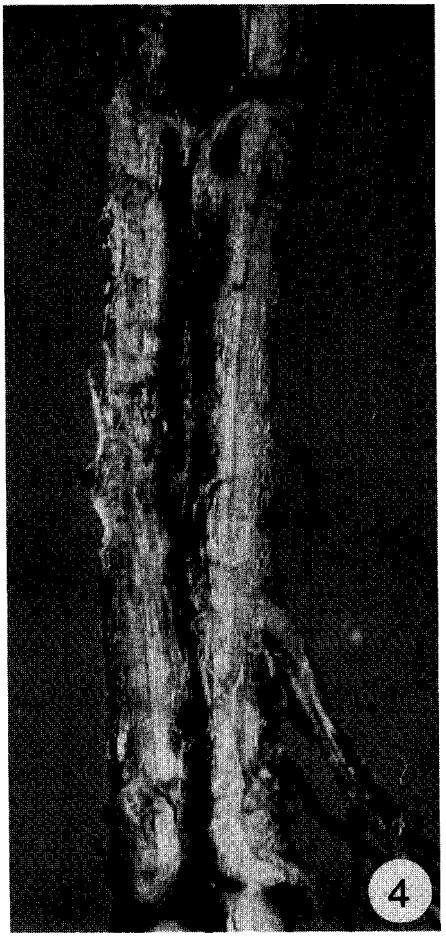
2 Alberta Special Crops and Horticultural Research Centre, Brooks, Alberta, Canada *TOJOJO.*

Accepted for publication August *24, 1988.*


1


2


3


4


5

Figure 1. Electron micrograph of *Pseudomonas fluorescens* with polar flagella (15,000 x).

Figure 2. Electron micrograph of *Erwinia carotovora* subsp. *carotovora* with peritrichous flagella (15,000 x).

Figure 3. Discoloration of sainfoin crown and root inoculated with a strain of *Pseudomonas fluorescens*.

Figure 4. Discoloration of sainfoin crown and root inoculated with a strain of *Erwinia carotovora* subsp. *carotovora*.

Figure 5. Sainfoin crown and root inoculated with sterile distilled water.

Table 1. Incidence and severity of crown and root rot of sainfoin in southern Alberta in 1983.

Location	No. of fields surveyed	Incidence %	Severity* rating
Brooks	1	100	2.28
Nanton	6	82	2.18
Claresholm	3	33	1.70
Spring Coulee	2	94	1.67
Kimball	4	100	1.69
Waterton Dam	2	98	2.31
Fort Macleod	2	82	1.89
Warner	2	98	2.07
Trout Creek Road	1	100	1.56
Granum	7	79	1.64
Total/average	30	87	1.90

***Crown and root rot severity rating scale: 0 = clean; 1 = slight, 1-20% of the crown and root discolored; 2 = moderate, 21-50% of the crown and root discolored; 3 = severe, 51-100% of the crown and root discolored.**

Based on the length and intensity of discoloration, isolates of ***P. fluorescens*** (Fig. 1) and ***E. carotovora*** subsp. ***carotovora*** (Fig. 2) were the most virulent to sainfoin seedlings, followed by P. ***syringae*** (Table 2). A range of virulence among isolates of the same ***E. carotovora*** subsp. ***carotovora*** and ***P. fluorescens*** was not detected. ***E. agglomerans*** isolates were considered nonpathogenic to sainfoin seedlings because both length of discoloration (<5 mm) and disease severity (1.0-1.1) produced by each isolate of this bacterial species was not significantly different from those in the control.

The symptoms in sainfoin seedlings inoculated with these pathogenic bacteria and grown under artificial conditions were similar to those found in naturally infected plants. In most cases, the crown tissue became necrotic and the discolored vascular tissue extended far beyond the inoculation site (Fig. 3 and Fig. 4). Control plants had dark green leaves and grew vigorously, whereas inoculated plants became chlorotic and wilted. The crown tissues of the control plants had minor necrosis and the vascular discoloration extended not more than 4 mm (Fig. 5). The discolored roots and inoculated plants yielded bacteria similar to the original inoculum.

Table 2. The pathogenicity of the different bacterial strains on sainfoin seedlings.

Organism	Length of discoloration[x] (mm)	Disease severity[y]
Pseudomonas fluorescens		
SA-2-1	17.4 ab[z]	1.8 ab
SB-1-1	17.4 ab	1.9 ab
SC-4-1	16.0 ab	1.8 ab
SD-2-4	16.1 ab	1.7 ab
SF-2-1	17.2 ab	1.9 ab
P. syringae		
SE-3-1	12.5 b	1.5 b
Erwinia carotovora		
subsp. ***carotovora***		
SC-4-2	23.0 a	2.0 a
SC-4-4	21.8 a	2.0 a
Enterobacter agglomerans		
SB-5-2	4.4 c	1.0 c
SE-3-2	4.9 c	1.1 c
Control	3.4 c	1.0 c

[x] The length of discoloration from the inoculation point.

[y] Severity was based on a scale of 1-3 where 1 = slight; 2 = moderate; and 3 = severe.

[z] Means within a column followed by the same letter are not significantly different using Duncan's Multiple Range Test (P = 0.05).

Most soil bacteria lack the ability to invade intact plant tissue. However, in southern Alberta, the alternate cycles of freezing and thawing within the soil can cause considerable physical stress to the roots of sainfoin and thus create avenues for the entry of non-invasive phytobacteria.

This is the first report indicating that bacteria, particularly ***P. fluorescens*** and ***E. carotovora*** subsp. ***carotovora,*** are closely associated with crown and root rot of sainfoin in southern Alberta. Although the presence of ***P. syringae*** was not consistent in our isolations, its role in the crown and root deterioration of

sainfoin cannot be ignored, mainly because of its ice nucleating properties which may lead to the formation of ice in plant tissue (2, 14). This study confirms previous research (3, 5, 8, **9, 10,** 11, 12, 13) that crown and root rot of forage legumes is a disease complex involving the interaction of soil-borne fungi and bacteria. A large-scale isolation of bacterial organisms based on a more extensive field survey is needed to fully determine the role of these bacteria in crown and root rot of sainfoin in other regions of Alberta.

Acknowledgements

We gratefully acknowledge Mr. H. Philip, Miss D. Orr, and Drs. L.J. Piening and B. Bolwyn for their valuable suggestions on the manuscript and Mr. E. Moskaluk for his technical assistance.

Literature cited

1. Alberta Environmental Centre. **1988.** Microbiological methods manual. Vegreville, Alberta. AECV88-M.
2. Arny, D.C., S.E. Lindow and C.D. Upper. **1976.** Frost sensitivity of *Zea* mays increased by application of *Pseudomonas syringae.* Nature 262:282-284.
3. Cormack. M.W. **1937.** *Fusarium* spp. as root parasites of alfalfa and sweet clover in Alberta. Can. J. Res. 15:493-510.
4. Cormack, M.W., R.W. Peake and R.K. Downey. **1957.** Studies on methods and materials for testing alfalfa for resistance to bacterial wilt. Can. J. Plant Sci. 37:1-11.
5. Gaudet, D.A., D.C. Sands, D.E. Mathre and R.L. Ditterline. **1980.** The role of bacteria in the root and crown rot complex of irrigated sainfoin in Montana. Phytopathology 70:161-167.
6. Hwang, S.F. and G. Flores. **1987.** Effects of *Cylindrocladium gracile, Fusarium roseum* and *Plenodomus meliloti* on crown and root rot, forage yield, and winterkill of alfalfa in northeastern Alberta. Can. Plant Dis. Surv. 67(2):31-33.
7. Krall, J.L., C.S. Cooper, C.S. Crowell, and A.J. Jarvi. **1971.** Evaluation of sainfoin for irrigated pasture. Mont. Agr. Exp. Sta. Bull. **658.**
8. Leath, K.T., F.L. Lukezic, H.W. Crittendon, E.S. Elliot, P.M. Halisky, F.L. Howard and S.A. Ostazsski. **1971.** The *Fusarium* root-rot complex of selected forage legumes in the northeast. Penn. Agr. Exp. Sta. Bull. **777.**
9. Lukezic, F.L., K.T. Leath and R.G. Levine. **1983.** *Pseudomonas viridiflava* associated with root and crown rot of alfalfa and wilt of birdsfoot trefoil. Plant Dis. 67:808-811.
10. Pegg, G.F. and D.W. Parry. **1983.** Infection of lucerne (*Medicago sativa*) by *Fusarium* species. Ann. Appl. Biol. 103:45-55.
11. Sears, R.G., R.L. Ditterline and D.E. Mathre. **1975.** Crown and root rotting organisms affecting sainfoin (*Onobrychis viciaefolia*) in Montana. Plant Dis. Reptr. 59:423-426.
12. Shinde, P.A. and F.L. Lukezic. **1974.** Isolation, pathogenicity and characterization of fluorescent *Pseudomonas* associated with discolored alfalfa roots. Phytopathology 64:865-871.
13. Shinde, P.A. and F.L. Lukezic. **1974.** Characterization and serological comparisons of bacteria of the genus *frwinia* associated with discolored alfalfa roots. Phytopathology 64:871-876.
14. Weaver, D.J. **1978.** Interaction of *Pseudomonas syringae* and freezing in bacterial canker on excised peach twigs. Phytopathology **68:1460-1463.**