The Role of Bacteria in the Root and Crown Rot Complex of Irrigated Sainfoin in Montana

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ABSTRACT

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Root and crown rot limits stand longevity and forage yield in sainfoin grown in Montana. A histological investigation of decayed sainfoin roots revealed the presence of bacterial cells within discolored vessels and decayed cavities in the root xylem. The bacteria were closely associated with tissue degeneration in naturally infected 3- and 4-yr-old sainfoin roots. Four different bacteria consistently were isolated from 2-, 3-, and 5-yr-old naturally infected sainfoin roots with typical symptoms. Three of these bacteria produced typical symptoms of root and/or crown rot when reinoculated into greenhouse-grown sainfoin. The three bacteria, *Pseudomonas syringae, P. marginalis* (*P. fluorescens*), and an *Erwinia amylovora*-like bacterium, were equally capable of producing symptoms in the inoculated plants. *Fusarium solani*, previously considered the causal organism of root and crown rot of sainfoin, was not consistently isolated from symptomatic tissues. Consequently, the causal organisms involved in the root and crown deterioration in irrigated sainfoin may be one or more bacteria rather than a single fungal pathogen.

Additional key words: Onobrychis viciaefolia.

Sainfoin (*Onobrychis viciaefolia* Scop.), a deep-rooted perennial legume, was introduced as a forage crop in Montana in the early 1900s. It is nonbloating, drought resistant, winter hardy, has excellent forage quality, and is resistant to the alfalfa weevil (*Hypera postica* Hyllenhal). The release of cultivar Eski in 1964 (7) resulted in its widespread use in the northwestern USA.

Sainfoin usually is long-lived (11), but several years after the release of Eski reduced stand persistence and forage yield caused by root and crown rot were noted. Stand deterioration in irrigated fields usually occurred after the 3rd or 4th yr (3). Root and crown rot of sainfoin has been reported in New Mexico (16) and appears to be prevalent wherever sainfoin is grown in the USA.

Initial investigators concluded that root and crown rot in sainfoin was incited by the fungus, *Fusarium solani* (Mart.) Appel & Wr. (2,22) and a screening program to select for resistance was initiated. The inability to consistently isolate *F. solani* from diseased tissues (22) necessitated further investigation.

The objectives of this study were to macro- and microscopically describe the progression of root and crown deterioration in irrigated sainfoin; to identify the fungal and bacterial organisms associated with naturally infected sainfoin roots during progressive stages of deterioration; and to determine the pathogenicity in greenhouse-grown sainfoin of the organisms isolated from diseased sainfoin roots.

MATERIALS AND METHODS

Pattern of root and crown deterioration. Root specimens were selected at random from naturally infected 1- to 5-yr-old sainfoin stands located at the Field Research Laboratory, Bozeman, MT. In the laboratory, the roots were thoroughly cleaned, dissected, and the different symptom types noted. The symptom types of 50 3-mo-old and 50 15-mo-old sainfoin roots were recorded.

Histology. During the summer of 1977, five 4-yr-old sainfoin plants exhibiting root and crown rot symptoms were sampled. Tissue sections were removed for histological study at different intervals along the taproot and lateral roots 6–15 cm below the crown. Ten taproot sections from 2- and 3-yr-old sainfoin were prepared similarly for histological examination.

A histological study of sainfoin roots artificially inoculated with the suspect pathogen, *Fusarium solani*, also was undertaken. Root sections of 8-mo-old sainfoin roots which previously had been inoculated by the root-cut-soak technique (2) were sampled along the taproot at 1-cm intervals.

Root sections were vacuum infiltrated with formalin acetic-acid alcohol fixative (FAA), dehydrated in Johansen's *t*-butyl alcohol series, and embedded in Paraplast (8). Specimen blocks were softened in a solution of 60% ethanol, saturated glycerol, and glacial acetic-acid (16:1:4) for 3 wk at 30 C and then stored at −9.0 C for 4 hr prior to sectioning. Sections 8-μm thick were affixed to glass slides with Haupt's adhesive and stained by using Johansen's quadruple stain procedures (8).

Isolation of fungi and bacteria. In July 1978, 50 2-yr, 50 3-yr, and 25 5-yr-old sainfoin plants were randomly selected from irrigated sainfoin stands located at the Field Research Laboratory, Bozeman, MT. Isolations were made at the leading edge of the discolored area from each of the different symptom types encountered. Bacteria were isolated by placing a small piece of affected tissue into a test tube containing 1 ml of sterile distilled water. This material was incubated for 15–17 hrs at 21 C and streaked on BC agar plates (medium B of King et al (9) to which 100 mg/ml of cycloheximide was added prior to autoclaving). The plates were examined for bacterial colonies after 4 days at 27 C.

Because *Fusarium solani* previously had been implicated in this disease, Komada's specific medium (10) for isolation of *Fusarium* sp. was employed.

Identification. Fusarium colonies were identified to species according to the procedure outlined by Toussoun and Nelson (24). Bacteria were identified by using a wide range of biochemical and physiological tests as described in Bergey's Manual of Determinative Bacteriology (4,12). The tests employed were those outlined by Schinde and Lukezic (20,21) and by Stanier et al (23). The sole modification of standard tests was in the gelatin hydrolysis test (23) in which plates were flooded with 1 N HCl (5 ml per plate) to precipitate the unhydrolyzed gelatin. All bacterial strains were

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twice selected as single colonies, prior to storage in sterile distilled water at 4 C.

Initial tests suggested that the three prevalent bacterial types belonged to the genera *Erwinia* and *Pseudomonas*. Consequently, the tests were designed to differentiate between the major taxonomic groups of these genera.

Pathogenicity tests. The bacteria tested for pathogenicity were assayed in 2.5-mo-old greenhouse-grown Remont sainfoin plants growing in vermiculite watered with a dilute nutrient solution. The seven bacterial isolates assayed were isolated from naturally infected sainfoin roots and one strain, *P. marginalis* var. *alfalfae* (Wolfe) (*P. fluorescens* [4]), was isolated from diseased alfalfa roots in Pennsylvania by F. L. Lukezic (14). The inoculum was prepared as follows: the bacterial strains, maintained in sterile distilled water at 4.0 C, were streaked on BC agar, and incubated at 21 C for 3 days. The *F. solani* isolate judged the most virulent in previous pathogenicity tests, was cloned from a single cell on natural potato dextrose agar (NPDA) according to the procedure outlined by Toussoun and Nelson (24) and incubated at 21 C for 2 wk under a 12-hr photoperiod provided by supplemental fluorescent lighting. For inoculation, a 400-μm (26-gauge) needle was dipped into the bacterial or fungal colony and passed through the sainfoin crown. Each inoculation site received approximately 8×10^8 bacterial cells or 10^5 conidia. Both *F. solani* and a composite of four strains of an *Erwinia amylovora*-like bacterium in combination were inoculated into sainfoin crowns in one of the treatments. Controls were injected with sterile distilled water. The inoculations were carried out in two separate greenhouse trials; after inoculation, the plants were incubated in the greenhouse for 4 wk in trial 1 and for 6 wk in trial 2. The temperature during the two trials ranged 18–30 C. A 12-hr photoperiod was provided by fluorescent lights. The sainfoin seedlings, five per 15-cm-diameter pot, were arranged in a three-replicate, random block design.

After the incubation period, the plants were harvested, split longitudinally through the crowns, and scored. Sections of symptomatic roots from each treatment were sterilized 5 min in 0.5% NaOCl and placed on potato dextrose agar (PDA) and acidified PDA (HPDA). Isolations for bacteria on BC agar were carried out using the method described above. The oxidase test (23) was performed on the *Pseudomonas* sp. isolates that were isolated.

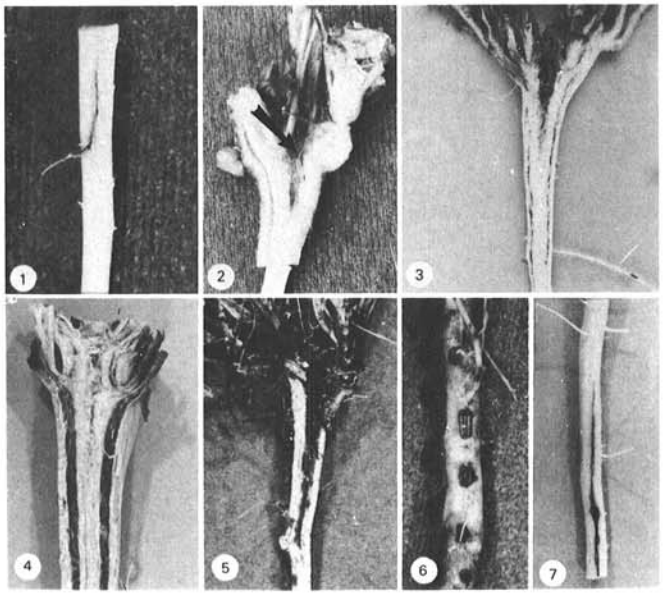

Fig. 1–7. Symptom types in naturally infected sainfoin. **1,** Discoloration originating in a lateral root and extending towards the crown in 2nd yr sainfoin. **2,3,** Crown discoloration and decay originating at the base of an old dead stem and progressing down the taproot in 2nd yr sainfoin. **4,** Vascular discoloration initiated at the base of two old dead stems, extending down the taproot in 3rd yr sainfoin. **5,** Severe discoloration and decay of the crown and taproot in 4th yr sainfoin. **6,** Larval feeding galleries of the Sitonia weevil (*Sitonia sissifrons* [Day]) in 2nd-yr sainfoin roots. **7,** Discoloration extending from an insect wound.

RESULTS

Pattern of root and crown deterioration. In sainfoin stands in their first growing season, the predominant symptom type was a black vascular discoloration of the taproot and lateral roots (Fig. 1). The discoloration appeared to move from the lateral roots into the taproot and was restricted to a small number of vessels. The vascular streaking was sporadic in most instances although severe discoloration was sometimes observed. Discoloration of the lateral roots increased from 24–48% between 3 and 15 mo of age and streaking of the taproot increased from 18–96% of the plants sampled in the same time interval.

At the end of the first and throughout the second growing season, affected plants had crown discoloration directly below the dead stems that remain after the forage is harvested (Fig. 2). These stems were hollow and continuous with the crown tissue. The affected tissue was usually red-orange in color, although black discoloration sometimes was observed. The former was coincidental with the physiologic crown splitting described by Sears et al (22). Eighty percent of the 15-mo-old sainfoin plants exhibited crown discoloration and/or decay. The red crown deterioration that progressed down the taproot (Fig. 3) appeared to be independent of the black vascular discoloration initiated at the base of secondary old dead stems produced each time the forage is harvested (Fig. 4). In 3- and 4-yr-old sainfoin plants, decay and discoloration associated with these dead stems converged in the crown and collectively produced the extensive central decay of the crown and taproot (Fig. 5). Lateral movement of the decay into newly initiated xylem and cortical tissue occurred in the advanced stages of root decline. In a survey of 25 4-yr-old sainfoin plants grown under irrigation, 50–90% of the vascular tissue in the crown was decayed and discolored.

Excepting those in the first growing season, all sainfoin roots exhibited signs of insect injury (probably caused by the Sitonia weevil [*Sitonia sissifrons* Day]) in the taproot and lateral roots (25) (Fig. 6). Xylem discoloration resulted when larval feeding activity extended through the cortex to the outer xylem. In 3rd and 4th yr sainfoin, decay and discoloration was observed in the vicinity of insect wounds (Fig. 7). Insect wounds presumably serve as entry

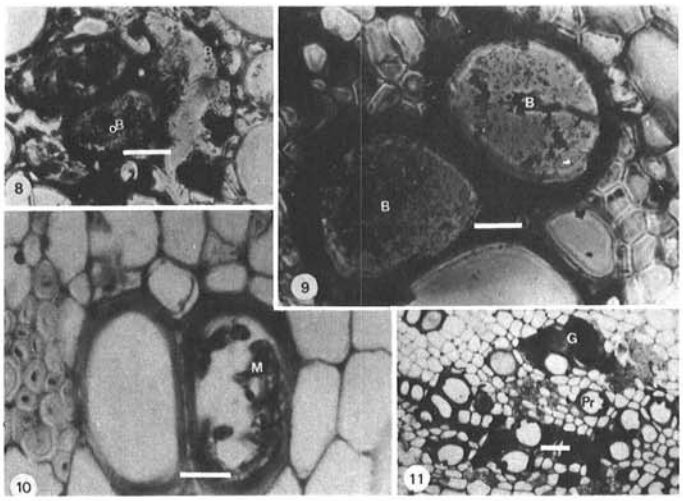

Fig. 8–11. 8–9, Photomicrographs of naturally infected 4th yr sainfoin roots and **10,11,** of greenhouse grown sainfoin roots artificially inoculated with *Fusarium solani*. **8,** Bacteria (B) in vessels and in a break in the xylem. **9,** Bacteria in vessel elements. **10,** Mycelium (M) of *F. solani* restricted to the vessels. **11,** Gels (G), which arise as protrusions (Pr) in vessel walls, eventually occlude the vessels. Bars represent 20 μm in Fig. 8–10 and 50 μm in Fig. 11.

points for pathogens.

Histology. Bacteria were observed in the taproot sections of all the 4-yr-old sainfoin plants and in 90% of the 3-yr-old sainfoin roots sampled for histological study. Bacteria also were observed in most of the lateral root sections. The bacteria were restricted to vessels and broken cavities in the xylem (Figs. 8,9). Surrounding these vessels were deep-red chromophilic parenchyma, vessel, and fiber cells. These chromophilic regions corresponded to the dark vascular discoloration typically observed in diseased sainfoin roots. A survey of sections from 4-yr-old sainfoin roots revealed that 50–90% of the vascular regions had chromophilic regions and that cellular decay was directly associated with the presence of adjacent bacteria. Vessels in affected regions in which bacteria were not observed often contained gels, gums, and cell wall fragments.

Only 20% of the root sections sampled from naturally infected 2-yr-old sainfoin contained visible levels of bacteria. An unidentified fungal mycelium which appeared to be associated with discolored tissue surrounding the vessels was more frequently observed in these root sections. Extensive cellular decay was not observed in association with the fungal cells. No organisms were seen in association with the red central discoloration in the plants sampled.

Plants artificially inoculated with *F. solani* developed extensive black vascular discoloration associated with chromophilic vessels and fibers and with extensive accumulation of gells and gums (Fig. 11). Cellular degradation was associated with the fungus near the inoculation point, but further away from the inoculation point the fungal mycelium remained restricted to the vascular elements and did not appear to be associated with decay of tissues directly adjacent to infected cells (Fig. 10).

Identification. The bacteria isolated from diseased sainfoin roots initially were classified in three groups based on colony morphology, growth, and pigmentation on BC agar. Group 1 were pseudomonads which formed entire, yellow colonies and produced a yellow-green fluorescent pigment under 365 nm ultraviolet light. Group 2 bacteria produced round white colonies and a brown diffusable pigment after 8–10 days. These bacteria also solubilized the $MgSO_4$ precipitate which normally clouds the BC agar and thereby produced clear zones around the colonies after 4–6 days. Group 3 bacteria formed irregular mucoid colonies and produced a yellow nondiffusible pigment.

Fluorescent pseudomonads were characterized by means of the determinative scheme (LOPAT) proposed by Lelliott et al (13). Pseudomonads isolated from diseased sainfoin roots were either oxidase-positive or oxidase-negative.

TABLE 1. Comparison of biochemical and physiological characteristics of *Pseudomonas syringae* and the oxidase-negative pseudomonads isolated from sainfoin in Montana

Test	Oxidase-negative pseudomonads	
	Sainfoin strains[w]	*P. syringae*[x]
Oxidase	–[y]	–
Hypersensitivity	+	+
Arginine dihydrolase	–	–
Motility	+	+
Gelatin hydrolysis	+	+
Nitrate reduction	–	–
Catalase	+	+
Potato soft rot	–	–
Sodium polypectate (pH 7)	–	–
Lipase (Tween-20 and Tween-80)	+	–
Levan	+	d[z]
Carbon substrate:		
Sorbitol	+	+
Sucrose	+	+
Trehalose	–	–
Alpha-alanine	+	+
Beta-alanine	±	–
Temperature relationships:		
4 C	+	d[z]
27 C	+	+
37 C	–	–
41 C	–	–

[w]Based on 10 strains collected from diseased sainfoin roots and crowns.
[x]Results taken from Misaghi and Grogan (17).
[y]– = negative; + = positive; and ± = weakly positive.
[z]d = between 21–79% of the strains tested were positive for this test.

TABLE 2. Comparison of biochemical and physiological tests between the oxidase-positive pseudomonads isolated from diseased sainfoin roots with those published for *Pseudomonas marginalis* and *P. marginalis* var. *alfalfae*

Test	Sainfoin strains					*P. marginalis* var. *alfalfae*[c]	*P. marginalis*[d]
	Group 1[a]	Group 2[b]	I	×	8		
Oxidase	+[e]	+	+	+	+	+	+
Hypersensitivity	–	–	–	–	–	+	–
Arginine dihydrolase	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	–	+	+	+
Nitrate reduction	–	–	–	–	–	+	+
Catalase	+	+	+	+	+	+	+
Potato soft rot	+	–	+	+	+	–	+
Sodium polypectate (pH 7)	+	–	–	–	+	–	+
Lipase (Tween-20 and Tween-80)	–	–	–	–	–	+	+
Levan	+	–	+	–	+	–	+
Carbon substrate:							
Sorbitol	+	+	+	+	+	+	+
Sucrose	+	–	+	+	+	+	+
Trehalose	+	–	+	+	+	+	+
Alpha-alanine	+	+	+	+	+	+	+
Beta-alanine	+	+	+	+	+	+	+
Temperature relationships in nutrient broth:							
4 C	+	+	+	+	+	0	0[f]
27 C	+	+	+	+	+	+	+
37 C	–	–	–	–	–	0	0
41 C	–	–	–	–	–	0	0

[a]Group 1 consists of four strains.
[b]Group 2 consists of three strains.
[c]Data from Schinde and Lukezic (20) and Lukezic (14).
[d]Data from Lelliott et al. (12) and Doudoroff and Palleroni (4).
[e]+ = growth; – = no growth.
[f]Data on temperature relationships not found in literature.

Oxidase-negative pseudomonads. The oxidase-negative pseudomonads were biochemically and physiologically similar to *Pseudomonas syringae* in 93% of the characterization tests published by Misaghi and Grogan (17) for that bacterium. The only difference in the tests between the sainfoin strains and the type species of *P. syringae* was the positive lipase reaction for the sainfoin strains (Table 1). When the sainfoin strains were spotted on PDA plates, incubated for 72 hr and subsequently sprayed with spore suspensions of *F. solani* and a *Rhodotorula* species, inhibition zones ranging 0.2–0.8 cm for *F. solani* and 0.8–2.0 cm for *Rhodotorula* were observed around the *P. syringae* colonies. This suggests that fungitoxic substances, possibly syringomycin, are produced by the sainfoin strains. A further distinguishing feature of the sainfoin strains was the ability to cause ice nucleation in the water suspension in which they were stored at 0 ± 1.1 C. The phenomenon did not occur in suspensions of the majority of other bacterial strains or in the distilled water blanks kept under the same storage conditions.

Oxidase-positive pseudomonads. There was considerable heterogeneity among 10 oxidase-positive pseudomonads in their reactions in the characterization tests (Table 2). Seventy percent of the strains produced a soft rot in potatoes. Reactions of these soft rotting sainfoin strains in the LOPAT tests were identical to those published for *Pseudomonas marginalis* (*P. fluorescens* [4]), but they differed from *P. marginalis* by having negative reactions in lipase and nitrate reduction. Overall, the oxidase-positive, softrotting pseudomonads from sainfoin were 88% similar to *P. marginalis* as given by Lelliott et al (13).

The sainfoin strains appear even more distinct biochemically and physiologically from *P. marginalis* var. *alfalfae* which induced hypersensitivity in tobacco, lacked arginine dihydrolase and lipase and did not cause soft rot in potatoes. Overall, the sainfoin strains were 69% similar to the published characterization tests for *P. marginalis* var. *alfalfae* (14,20).

The nonsoftrotting oxidase-positive pseudomonads (Table 2) isolated from diseased sainfoin roots probably belong to saprophytic groups associated with decaying plant residues (18).

Erwinia species. The white bacterium that produced a brown diffusable pigment and solubilized the $MgSO_4$ precipitate in BC agar, was similar to *Erwinia amylovora*. The sainfoin strains responded almost identically to the characterization tests published for *E. amylovora* var. *alfalfae*, a bacterium isolated from diseased alfalfa roots in Pennsylvania (Table 3) (21). The major differences between the sainfoin strains and *E. amylovora* (5) resided in the ability of the sainfoin strains to produce extracellular lipase, reduce nitrate, grow at 37 C, and the inability to cause the hypersensitivity reaction in tobacco.

The mucoid bacterium which produced a yellow nondiffusible pigment on BC agar tentatively was identified as *E. herbicola* var. *herbicola*, a bacterium commonly found on the surface of most plants. It is considered to be a secondary invader of decayed stems and roots and is of little importance as a primary plant pathogen (6).

Isolation. *Fusarium solani* and *Fusarium* sp. were not consistently isolated from symptomatic tissue of 2- and 3-yr-old sainfoin roots, but *F. solani* was isolated from 23% of insect wounds in 3-yr-old sainfoin roots (Table 4). Bacteria, particularly the fluorescent pseudomonads, predominated in all of the different symptom types. Although no single symptom type could be clearly associated with the presence of a particular bacterium, the black vascular discoloration in the taproots of 3-yr-old sainfoin roots appeared to be associated with the presence of the *E. amylovora*-like bacterium (Table 4).

In 5-yr-old sainfoin, *F. solani* was more frequently isolated from the decayed crown regions (33% of the crowns) than from areas further down the taproot (Table 5). Conversely, *Pseudomonas* spp.

TABLE 3. Comparison of biochemical and physiological characterisics of the white and mucoid bacteria isolated from sainfoin and two selected plant pathogenic and one saprophytic *Erwinia* spp.

Test	Sainfoin white bacteria[s]	*Erwinia amylovora* var. *alfalfae*[t]	*Erwinia amylovora*[u]	Sainfoin mucoid bacteria[v]	*Erwinia herbicola* var. *herbicola*[w]
Oxidase	–[x]	–	–	–	–
Hypersensitivity	–	–	+	–	–
Glucose fermentation	+	+	+	+	+
Motility	+	+	+	+	+
Gelatin hydrolysis	+	+	+	–	+
Nitrate reduction	+	+	–	+	+
Catalase	+	+	+	+	+
Potato soft rot	–	–	–	–	–
Sodium polypectate (pH 7)	–	–	–	–	–
Lipase	+	+	–	–	–
Levan	–	–	d[z]	–	d
Acid production on:					
Alpha-methyl-glucoside	–	0[y]	–	–	–
Glycerol	+	0	+	+	–
Cellobiose	+	0	+	–	–
Maltose	+	0	+	+	+
Xylose	+	+	+	+	+
Salicin	d	0	d	+	+
Lactose	–	–	–	–	d
Rhamnose	+	–	–	+	+
Temperature relationships in nutrient broth:					
4 C	+	0	0	+	0
27 C	+	+	+	+	+
37 C	+	0	–	+	+
41 C	–	0	–	–	–

[s] Six typical strains of this bacterium were used in the characterization tests.
[t] Data from Schinde and Lukezic (21).
[u] Data from Dye (5).
[v] Four typical strains of this bacterium were used in the characterization tests.
[w] Data from Dye (6).
[x] + = positive; – = negative; ± = weakly positive.
[y] Data concerning this test not found in literature.
[z] d = between 21–79% of the strains tested were positive for the test.

were readily isolated from all levels of the taproot, even at 20 cm below the crown where the leading edge of the downward spread of the decay frequently occurred. Both the *E. amylovora*-like strains and *E. herbicola* were isolated most frequently in the crown regions (Table 5).

The extent to which the fluorescent pseudomonads, *P. syringae* and *P. marginalis*, occur in the roots and crowns of the diseased sainfoin plants is unknown because the oxidase test was not routinely performed on all pseudomonads isolated from the field. However, in a survey of 33 bacteria randomly sampled from BC agar plates containing pseudomonads isolated from 2-, 3-, and 5-yr-old sainfoin roots, 10 strains (approximately 30%) were identified as *P. syringae*. This suggests that *P. syringae* occurs frequently in diseased sainfoin roots.

Pathogenicity tests. Symptoms in plants inoculated with the bacteria in the greenhouse were similar to those encountered in naturally infected 1- to 5-yr-old sainfoin plants grown under irrigation. In most instances, the isolates produced a black vascular

TABLE 4. Bacteria and fungi isolated from specific symptom types found in 2- and 3- yr-old sainfoin plants collected at the Montana Field Research Laboratory, Bozeman

	Number of each symptom type sampled for isolation		Percentage of the isolations producing[a]							
			Fusarium solani		*Pseudomonas* spp.		*Erwinia amylovora*-like strains		*Erwinia herbicola*	
Symptom types	2nd yr	3rd yr[b]	2nd yr	3rd yr	2nd yr	3rd yr	2nd yr	3rd yr	2nd yr	3rd yr
Red crown decay	35	35	0	0	91	60	2	60	11	24
Black discoloration beneath the old secondary stem	23	23	2	2	70	66	4	73	15	45
Vascular discoloration in the taproot	11	10	0	0	50	20	25	60	0	20
Vascular discoloration in the lateral roots	...	12	...	0	...	58	...	66	...	25
Insect wound related discoloration	14	14	0	23	70	66	7	73	0	45

[a]Based on isolations made on Komada's medium for *Fusarium* spp. and on King's B agar for the bacteria.
[b]Isolations made from 2- and 3-yr-old sainfoin plants.

TABLE 5. Bacteria and fungi isolated at different intervals along the central decay region in taproots of severely diseased 5-yr-old sainfoin collected at the Montana Field Research Laboratory, Bozeman

Distance from the crown (cm)	Samples (no.)	Percentage of the isolations yielding[a]			
		Fusarium solani	*Pseudomonas* spp.	*Erwinia amylovora*-like	*Erwinia herbicola*
2	25	33	80	36	44
5	24	20	83	40	33
10	23	13	82	13	13
20	15	13	66	6	0

[a]Based on isolations made on Komada's selective medium for *Fusarium* spp. and on King's B agar for the bacteria.

TABLE 6. The pathogenicity of the different bacterial strains and a *Fusarium solani* isolate on sainfoin seedlings

Organism	Extent of discoloration mean distance (mm)[a]	Mean disease severity score[b]
Trial 1		
Pseudomonas syringae (#1)	18.5 A[c]	2.17 A
P. syringae (#2)	18.5 A	2.33 A
P. marginalis (#7)	15.3 AB	1.58 B
P. marginalis var. *alfalfae*	11.9 BC	1.50 B
Erwinia amylovora-like strain (#4)	17.2 AB	2.08 A
E. amylovora-like strain (#5)	15.2 AB	1.58 B
E. amylovora-like strain (#6)	14.8 AB	1.67 B
E. herbicola (#3)	8.5 CD	1.00 C
Control - H_2O	6.2 D	1.00 C
Trial 2		
E. amylovora-like strain		
15-5	23.8 A[c]	2.67 A
15-9	22.5 AB	2.75 A
19-1	18.6 AB	2.08 BC
20-1	18.0 AB	1.92 BC
F. solani + *E. amylovora*-like strain	16.9 B	2.40 AB
F. solani		
FS-9	8.4 C	1.58 C
Control - H_2O	7.8 C	1.00 C

[a]The extent of the black discoloration from the inoculation point.
[b]Severity was estimated as follows: 1 = light; 2 = moderate; and 3 = severe.
[c]Column means followed by the same letter do not significantly differ ($P = 0.05$).

discoloration extending from the inoculation point. One isolate, *P. syringae* #1, produced a red discoloration. Bacteria reisolated from the inoculated crowns were similar in type to those originally inoculated. In Trial One, differences in extent of discoloration were insufficient to show statistically significant differences in virulence among strains of *P. syringae, P. marginalis,* and the *E. amylovora*-like bacterium (Table 6), but symptoms produced by each of the three bacteria were significantly more extensive (as measured by disease severity score and extent of discoloration) than those in the controls. The strain of *P. marginalis* var. *alfalfae* was significantly less virulent than was *P. syringae,* but symptoms were more severe than those in the controls (Table 6). Symptoms produced by *E. herbicola* were similar to those in the controls.

In Trial Two, the four *E. amylovora*-like strains were significantly more effective in producing disease, as measured by the extent of the discoloration from the inoculation point, than either *F. solani* or the controls (Table 6). Combining both the bacteria and the fungus in the sainfoin crowns had no appreciable effect on either extent of discoloration or disease severity score indicating that neither synergistic nor antagonistic interactions exist between the two.

DISCUSSION

The most severe aspect of root and crown rot in sainfoin appears to be initiated in the crown below the old cut stem. Each time the forage is harvested a new infection site is created. The disease organism(s) appears to move down these hollow stems and to converge in the crowns of the 3- and 4-yr-old sainfoin plants resulting in severe decay of the xylem tissue. Discoloration and decay also spread down the center of the taproot and further disrupt water transport. Decay of the vascular tissue may directly kill the sainfoin plant or predispose it to environmental stresses such as drought or winterkill, or vice versa.

The amount of root discoloration and insect-wound-related discoloration and decay appear to be positively correlated with the age of the plant and are distinct from the crown decay. Their role in the actual decline of the sainfoin stands is not known, although they probably serve as infection courts.

F. solani, previously considered the causal organism of root and crown rot in sainfoin (2,22), was not consistently associated with diseased root tissues in naturally infected sainfoin grown under irrigation in Montana. Although *F. solani* was isolated from 33% of the 5-yr-old sainfoin crowns, it was not associated with the leading edge of the decay which extends into the lower part of the tap root and contributes to the eventual death of the plant. The inability to consistently isolate *F. solani* or other *Fusarium* spp. from affected tissues and the overwhelming presence of bacteria strongly suggest that root and crown deterioration is caused by bacterial pathogens rather than by *F. solani.* This contention is further substantiated by the histological evidence of bacteria in close association with discolored and decayed vascular tissue in 3- and 4-yr-old naturally infected sainfoin roots. The presence of bacteria in 3- and 4-yr-old plants also coincides with the occurrence of pronounced yield losses in 3- and 4-yr-old irrigated fields of sainfoin grown in Montana (3,11). Conversely, no fungal mycelium was observed in the decayed root sections. Possibly, the production by *P. syringae* of substances fungitoxic to *F. solani* may account for the scarcity of fungal organisms in these root sections.

In the study of pathogenesis in sainfoin roots inoculated with *F. solani* it was apparent that the black vascular discoloration, characteristic of inoculated plants, was primarily associated with the accumulation of gels and with discoloration of vessel and fiber cells. Although macroscopically, the symptoms produced in plants inoculated with *F. solani* were similar to those in naturally infected sainfoin roots, the discoloration was not associated with the extensive root decay which is normally observed in naturally infected sainfoin stands in Montana. The coincidence in symptom types illustrates the value of histologically associating the disease organism with the symptom type, when possible.

Bacteria, particularly the fluorescent pseudomonads, are closely and consistently associated with symptoms in tissues at all stages of root and crown deterioration in irrigated sainfoin grown in Montana. Results of the biochemical and physiological characterization tests on the oxidase-negative pseudomonads suggest that the sainfoin isolates best fit the *P. syringae* group. *P. syringae*, commonly a leaf spotting and canker-causing organism, has rarely been associated with plant roots, but recently it was implicated in the vascular blackening of sugar beet taproots in Italy (15).

The ability of the sainfoin isolates of *P. syringae* to cause ice nucleation supports recent reports of this phenomenon (1,26). It is possible that ice nucleation due to the presence of *P. syringae* contributes to the destruction of crown tissue in sainfoin during the spring freeze-thaw cycle in Montana.

The LOPAT reactions proposed by Lelliott et al (13) as a determinative scheme for identification of fluorescent pseudomonads were the main criteria used in the classification of the oxidase-positive, softrotting sainfoin isolates into the *P. marginalis* group. Negative reactions in lipase and nitrate reduction may represent a further deterioration of nutritional versatility of the sainfoin strains. This is common among plant pathogenic pseudomonads (19,23).

P. marginalis var. *alfalfae*, isolated from diseased alfalfa roots in Pennsylvania, appears to be distinct from the sainfoin strains with respect to the characterization tests (14,20). The alfalfa strain also exhibited lower virulence than did the sainfoin strains of *P. marginalis* in inoculated greenhouse-grown sainfoin seedlings. Whether the sainfoin strains should be given special pathovar designation awaits further tests.

The sainfoin Erwinia (white bacteria) were included in the *E. amylovora* group because they were similar to *E. amylovora* in many characteristics and because their responses in characterization tests were almost identical to those for the *E. amylovora* var. *alfalfae* strain isolated from diseased alfalfa roots in Pennsylvania (21). The bacterium tentatively identified as *E. herbicola* var. *herbicola*, was nonpathogenic on sainfoin. This saprophytic presence is consistent with the literature published on this organism (6).

In summary, root and crown deterioration of sainfoin in irrigated fields in Montana appears to be caused by one or more bacterial organisms rather than by *F. solani* as previously reported (2,22). The extent to which these bacteria occur in irrigated sainfoin stands throughout Montana and in other regions of North America where sainfoin is grown remains to be determined. The role of these bacteria in the dryland aspect of root and crown rot of sainfoin, also, has not been studied. Sears et al (22) was consistently able to associate *F. solani* with the different root and crown rot symptoms existing in dryland sainfoin and, hence, *F. solani* may play a significant role in the disease on dryland sainfoin where conditions are less suitable for bacteria than in irrigated sainfoin.

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