Fusarium Species Associated with Crown Rot of Alfalfa in Nevada

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ABSTRACT

Uddin, W., and Knous, T. R. 1991. *Fusarium* species associated with crown rot of alfalfa in Nevada. Plant Dis. 75:51-56.

The distribution, pathogenicity, and phytotoxicity of *Fusarium* species associated with crown rot of alfalfa (*Medicago sativa*) was examined in 14 fields in the northern Nevada counties of Churchill, Pershing, and Lyon. The five species recovered, in descending order of frequency, were *F. solani*, *F. acuminatum*, *F. oxysporum*, *F. sambucinum*, and *F. avenaceum*. Only *F. sambucinum* and *F. avenaceum* were not isolated at all locations. Isolates of *F. solani* and *F. acuminatum* were the most pathogenic on 4-mo-old alfalfa, followed by *F. sambucinum* and *F. oxysporum*. An isolate of *F. avenaceum* was the least pathogenic. However, there were interactions between the *Fusarium* species and the alfalfa cultivars. Pathogenicity and the frequency of recovery were positively correlated. Synergism was observed when all five species were present. Cell-free culture filtrates of *F. sambucinum* and *F. acuminatum* caused local and translocatable phytotoxicity in an alfalfa leaf bioassay, while filtrates of the other three species did not. There were no significant differences among eight alfalfa cultivars in the phytotoxic reaction caused by the culture filtrates of these *Fusarium* species.

Crown rot of alfalfa (*Medicago sativa* L.) is widespread throughout the world. The disease is characterized by the presence of dark brown to black, wedge-shaped necrotic lesions that spread from the center of affected crowns downward into vascular tissue. Species of *Fusarium* are the most consistently isolated fungi from necrotic crowns and roots; however great variations in species and pathotypes occur, and several species may serve as primary pathogens (5,6,14,17, 19,21). Additionally, some species of *Fusarium* produce substances that are toxic to living plant tissues (1,3,4,7,13, 16,22). These species include *F. oxysporum* Schlechtend.:Fr. f. sp. *medicaginis* (J. L. Weimer) W. C. Snyder & H. N. Hans., *F. o.* f. sp. *lycopersici* (Sacc.) W. C. Snyder & H. N. Hans., *F. sambucinum* Fuckel, and *F. solani* (Mart.) Sacc. (1,2,8,10).

Although investigators in many regions have previously determined that fusaria were the dominant pathogenic fungi associated with the crown rot complex and that they occur in various combinations in different geographic locations, the etiological components of crown rot in commercial alfalfa-producing regions of Nevada have not been investigated. Studies of the influences of crop management on the incidence of crown rot in experimental plots at Reno have been performed (15,20), but the identities of the fusaria isolated were not precisely determined. Also, information on the relative phytotoxic activities of these fungi is required for subsequent toxicological studies on the fusaria associated with this complex.

The objectives of this study were to identify the fusaria associated with crown rot in commercially grown alfalfa in northern Nevada and to examine the pathogenicity and phytotoxicity of these *Fusarium* species.

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MATERIALS AND METHODS

Sample collection. Alfalfa plants with crown rot were collected from a total of 14 commercial fields at three major alfalfa-producing counties in Nevada—Churchill, Pershing, and Lyon. Three- to five-year-old hay or seed fields of the cultivars Lahonton, Washoe, Gladiator, Armour NAPB, L720, WL318, WL512, WL545, and AS13-R were selected. Some cultivars were not present at all locations. Crowns with few stems were selected for collection because this is one of the typical symptoms of plants affected by crown rot. Ten plants (two from the center and two near each corner) were removed from each field. The fields ranged in size from 0.1 to 2 ha. Tap roots at least 25 cm long were removed from the soil and shaken to remove most of the adhering soil. Foliage 5 cm or more above the crown was removed, and the plants were placed in plastic bags and stored on ice in an ice chest for transport to the laboratory. Collections were performed three times in each field at 4-wk intervals during the growing season (May–August) of 1986.

Isolation, identification, and preparation of cultures. Root samples were trimmed, washed in running tap water and then with diluted household detergent to remove all soil, blot dried, and cut into segments about 5 cm long. Segments were soaked in 0.5% NaOCl for 5 min, rinsed with sterile, distilled water, dipped in 95% ethanol, and flamed. Segments were cleaved longitudinally with a sterilized razor blade. Crown tissue samples (approximately 1 $\times$ 2 mm) were removed from the leading edge of necrotic lesions. Three tissue samples were removed from each plant, for a total of 1,260 tissue samples from 420 plants. The tissue samples were transferred to a water agar (WA) medium.

After 2–3 days of incubation on WA, mycelial tips from fungal colonies that originated from tissue pieces were transferred to freshly prepared potato-dextrose agar, which was also used to identify all fungal isolates. Single-conidia isolates of *Fusarium* species were identified according to the classification system of Nelson et al (18). Macro- and microconidia, conidiophores, and colony morphology were the main criteria used for species identification. The identity of each species was confirmed by T. A. Toussoun (Fusarium Research Center, The Pennsylvania State University, University Park, PA).

Representative cultures of each *Fusarium* species that was derived from single conidia and their culture filtrates were prepared for pathogenicity and phytotoxicity studies, respectively. Three 5-mm-diameter plugs from the advancing margin of a selected *Fusarium* isolate from each species were placed into 200 ml of Czapek-Dox agar in 1-L Erlenmeyer flasks. The stationary flasks were maintained at 24 C and 85% RH in a controlled environment. After 18 days, the agar from each flask was passed through a series of filters to collect the conidia and remove mycelia. Vacuum filtration was performed through Whatman No. 541, 1, and 3; Schleicher & Schuell (S & S) No. 24; and Millipore 8.0 μm, 3.0 μm, and 0.45 μm filters. The filtering sequence ensured the availability of conidia-free culture filtrates for use in the phytotoxicity study. Conidia were collected in a beaker by rinsing the S & S and Millipore filters with sterile, distilled water. The Whatman filters retained the mycelia. Conidia concentrations were estimated with a hemacytometer and adjusted to 1×10^6 conidia per milliliter.

Pathogenicity tests. Alfalfa seedlings were grown in sand treated with aerated

Table 1. *Fusarium* species isolated during the survey of commercial alfalfa fields in northern Nevada

County Farm	Alfalfa cultivars	*Fusarium* isolates				
		F. solani	*F. acuminatum*	*F. oxysporum*	*F. sambucinum*	*F. avenaceum*
Churchill						
Frosty Acres	WL318	X	X	X	-	-
	Gladiator	X	X	X	-	-
	WL512	X	X	X	X	-
	AS13-R	X	X	X	-	-
Frey	Lahontan	X	X	X	-	-
Pershing						
List	WL318	X	X	X	X	-
Nevada Nile South	Armour NAPB	X	X	X	X	X
Lovelock Seed Co.	WL318	X	X	X	-	-
	Gladiator	X	X	X	-	-
Brinkerhoff	L720	X	X	X	-	X
Lyon						
Jones	Washoe	X	X	X	-	-
Frade	WL318	X	X	X	-	-
	Lahontan	X	X	X	-	-
	WL545	X	X	X	X	-

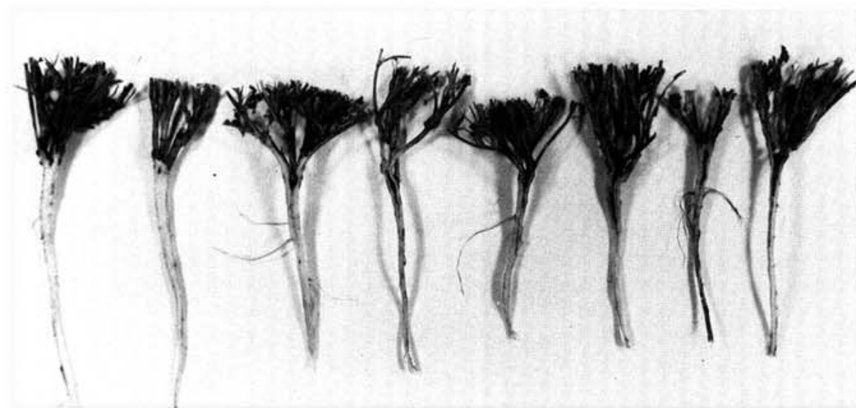

Fig. 1. Necrotic crown lesions of 6-mo-old Washoe alfalfa 2 mo after inoculation. Left to right: control (Czapek-Dox agar), control (distilled water), *F. avenaceum*, *F. oxysporum*, *F. sambucinum*, *F. acuminatum*, *F. solani*, and all five species combined.

steam (>12 hr) on benches in the greenhouse. One bench was used for each of the three replications of the split-plot design. Eight rows of 40 alfalfa plants were seeded into each bench. Each row consisted of five plants for each of eight cultivars, with cultivar positions assigned randomly within the rows. Plants were spaced at 15-cm intervals within rows with 30 cm between rows. Each row was assigned for inoculation with one isolate of each of the five species of *Fusarium*, a combination of all five species, or with control treatments of sterile, distilled water or Czapek-Dox agar. Four-month-old plants were inoculated by injecting 1 ml of a conidial suspension (1×10^6 conidia) to a depth of 5–7 mm into each crown. Control plants were injected in a similar manner with sterile, distilled water or Czapek-Dox agar. The greenhouse was maintained at about 32 C and 30–60% RH during the day (16 hr) and about 14 C and 50–80% RH at night. Water was applied twice daily to keep the entire volume of the sand uniformly moist in each bench. The pH of the sand was 7.1. Fertilizer (Miracle-Gro 10-15-10) was applied twice a month.

Plants were dug and disease development was evaluated 2 mo after inoculation. Disease severity indexing (DSI) was conducted with the crown necrosis scale proposed by Turner and Van Alfen (21): 0 = none, 1 = <2%, 2 = 2–20%, 3 = 21–50%, 4 = 51–90%, and 5 = 91–100% necrosis. Isolations were made as described previously from the field plants to detect and identify the *Fusarium* species in diseased plants. The DSI values were subjected to analysis of variance, and LSD values for the *Fusarium* species and the cultivars were determined.

Leaf infiltration assay. The same isolates of five *Fusarium* species that were previously used in the pathogenicity test were examined for their toxin-producing potential. The standard for comparison in this test was an isolate *F. o.* f. sp. *medicaginis* (GH7), which had been previously shown to be both pathogenic and to produce toxin (10). Conidia-free culture filtrates of *F. o.* f. sp. *medicaginis* isolate GH7 were obtained in the same procedure described for the other fusaria. In addition to the primary concentration of the culture filtrate, a second batch of double strength was prepared by reducing the water content by 50% with rotary evaporation.

The eight alfalfa cultivars used in the pathogenicity study were seeded in 8-L plastic pots that had been filled with pasteurized sand to 5 cm below the rim. Pots were arranged in the greenhouse in a split-plot design with three replications where each row of eight pots (a cultivar per pot) were randomly assigned to each fungus culture filtrate. Plants were maintained in the greenhouse at 32 C during the day and 10 C at night. Each pot contained an 8-mo-old plant that had been cut twice at the time of leaf selection. Plants were watered daily and fertilized (Miracle-Gro 10-15-10) twice a month. The pH of the sand was 7.3. Three-week-old fully expanded leaves from the third to the seventh node (first node the lowest) were selected for infiltration treatment.

Infiltration was done with a procedure similar to that of Ireland and Leath (12). This process was performed on a sunny day with the use of a 5-cc syringe fitted with a 21G needle. The needle was enclosed within a rubber stopper that had a concave bottom. This apparatus and procedure enabled the infiltration treatment to be performed with a slight pressure on the liquid dispensed on the leaf surface, which was supported underneath by a flat-surface rubber stopper. The blunt needle tip was projected only into the cavity to avoid contacting or puncturing the leaf surface. One leaflet of each leaf was infiltrated with a primary concentration culture filtrate containing 1% dimethyl sulfoxide (DMSO) until the leaflet was completely water soaked. Each treatment consisted of five leaves (on each plant), and a total of 120 leaves from each cultivar were treated. Sterile, distilled water and Czapek-Dox agar, with 1% DMSO, were used as control treatments. The treated leaflets were evaluated daily for 5 days. The infiltration procedure was repeated a week later in the same manner with double-concentrate culture filtrates and new sets

of plants.

An index of toxic symptom expression was used to quantify the phytotoxic levels of the filtrates. The index represents an estimate of the leaflet's color change and death. The leaf necrosis index (LNI) classes were as follows: 0 = no reaction, 1 = dull, pale green, 2 = pale, yellowish brown with leaf curled and drying, 3 = yellowish brown and desiccated, and 4 = leaf dead.

The LNI values were subjected to analysis of variance, and the LSD values for the culture filtrates and the cultivars were determined. Regression analysis for the culture filtrates was also computed, and the homogeneity of regression coefficients was tested to compare the slopes using the procedure described in Statistical Procedures for Agricultural Research (9).

RESULTS

F. solani, *F. acuminatum* Ellis & Everh., and *F. oxysporum* were isolated from every crown rot specimen collected from all fields surveyed (Table 1). In contrast, *F. sambucinum* and *F. avenaceum* (Fr.:Fr.) Sacc. were isolated only occasionally. The percentage of the fusaria isolated in the survey were: *F. solani*, 43%; *F. acuminatum*, 36%; *F. oxysporum*, 17%; *F. sambucinum*, 3%; and *F. avenaceum*, 1%. Other organisms isolated frequently included species of *Rhizoctonia*, *Pythium*, *Alternaria*, *Phoma*, *Curvularia*, *Penicillium*, and unidentified bacteria.

All five species of *Fusarium* caused crown rot when inoculated into 4-mo-old alfalfa. Crown rot in the inoculated plants was characteristic of that occurring under natural conditions (Fig. 1). The most severe disease on each cultivar was caused by the combination of all five species except for Gladiator, where the NSI value was the same as that caused by *F. solani* alone (Table 2). *F. solani* and *F. acuminatum* appeared to be the most pathogenic of the individual species, and their crown NSI values were about the same. The NSI values of *F. sambucinum* and *F. oxysporum* were also about the same but were smaller than *F. solani* and *F. acuminatum* and greater than *F. avenaceum*, which was the least pathogenic. There were some interactions between the *Fusarium* species and the alfalfa cultivars. The NSI values of *F. oxysporum* and *F. avenaceum* were greater on WL318 than other cultivars, except that *F. oxysporum* was as severe on L720 as WL318. The NSI value of *F. solani* on Gladiator was significantly greater than those on other cultivars. The NSI value of *F. oxysporum* on Moapa 69 and that of *F. sambucinum* on Lahontan were significantly smaller than those on most of the other cultivars. When all five species were present, the NSI values were higher on WL318 and L720 and lower on Thor than other cultivars.

Isolations from crown rot-affected tissues yielded (64–90%) the same species that had been introduced by inoculation, and the rest (10–36%) yielded one or more of the other species. Most (68%) of the plants inoculated with the combination also yielded all five species, and the rest yielded one to four species.

Table 2. Crown rot severity[a] in eight alfalfa cultivars inoculated with five *Fusarium* species

Fusarium isolates	Alfalfa cultivar							
	Washoe	Narragansett	Lahontan	Moapa 69	WL318	Gladiator	L720	Thor
Control (distilled water)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control (Czapek-Dox agar)	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.1
F. avenaceum	2.1	2.1	2.1	2.1	2.4	2.0	1.9	2.1
F. oxysporum	2.6	2.5	2.6	2.3	2.7	2.6	2.7	2.4
F. sambucinum	2.6	2.6	2.3	2.5	2.7	2.7	2.5	2.7
F. acuminatum	2.9	2.7	2.9	2.9	3.0	2.9	2.9	2.7
F. solani	2.8	2.9	3.0	2.9	2.9	3.3	3.0	2.9
All species combined	3.3	3.3	3.2	3.3	3.5	3.3	3.5	3.0
$LSD_{0.05}$ for *Fusarium* isolates within cultivar = 0.24								
$LSD_{0.05}$ for cultivars within *Fusarium* isolate = 0.25								

[a]Crown necrosis severity index: 0 = none, 1 = <2%, 2 = 2–20%, 3 = 21–50%, 4 = 51–90%, 5 = 91–100%.

Table 3. Necrosis of alfalfa leaves[a] treated with fungus culture filtrates applied at two different concentrations[b]

Fusarium species	Concentration	Incubation time (hours)				
		24	48	72	96	120
Control (distilled water)	Primary	0.0	0.0	0.0	0.0	0.0
Control (Czapek-Dox agar)	Primary	0.0	0.0	0.0	0.0	0.0
F. avenaceum	Primary	0.0	0.0	0.0	0.0	0.0
F. oxysporum	Primary	0.0	0.0	0.0	0.0	0.0
F. solani	Primary	0.0	0.0	0.0	0.0	0.0
F. oxysporum f. sp. *medicaginis* (GH7)	Primary	0.0	0.0	0.0	0.0	0.0
F. acuminatum	Primary	1.0	1.7	2.1	3.0	3.9
F. sambucinum	Primary	1.0	2.0	2.6	3.5	4.0
$LSD_{0.05}$ for *Fusarium* isolates within hour = 0.33						
$LSD_{0.05}$ for hours within *Fusarium* isolate = 0.36						
Control (distilled water)	Double	0.0	0.0	0.0	0.0	0.0
Control (Czapek-Dox agar)	Double	0.0	0.0	0.0	0.0	0.0
F. avenaceum	Double	0.0	0.0	0.0	0.0	0.0
F. oxysporum	Double	0.0	0.0	0.0	0.0	0.0
F. solani	Double	0.0	0.0	0.0	0.0	0.0
F. oxysporum f. sp. *medicaginis* (GH7)	Double	1.0	1.6	2.1	3.2	4.0
F. acuminatum	Double	1.0	1.6	2.3	3.3	4.0
F. sambucinum	Double	1.1	1.9	2.8	3.8	4.0
$LSD_{0.05}$ for *Fusarium* isolates within hour = 0.35						
$LSD_{0.05}$ for hours within *Fusarium* isolate = 0.36						

[a]Leaf necrosis index: 0 = no reaction, 1 = dull, pale green, 2 = pale, yellowish brown with leaf curling and drying, 3 = yellowish brown and desiccated, 4 = leaf dead.

[b]Each value represents the mean of 15 tests and eight cultivars.

Additionally, 8% of the control plants yielded *F. solani* and/or *F. acuminatum* from the stab injury zone. Because crown rot was not well established in the control plants that were affected by fusaria at the termination of the experiment, it was presumed that the pathogens were recently introduced as contaminants in the irrigation system.

Leaves treated with the filtrates from cultures of *F. sambucinum* and *F. acuminatum* developed necroses at both the primary and doubled concentration (Table 3). This phytotoxic response also occurred with the double-concentration filtrate from the test isolate of *F. o.* f. sp. *medicaginis*, GH7. In all cases, the treated leaflets became curled and discolored before they died. Filtrates of pathogenic isolates of *F. solani*, *F. oxysporum*, and *F. avenaceum* did not cause any toxic reaction in the leaves even when infiltrated at the doubled concentration. The leaf reaction to these three filtrates was the same as for the controls, with the leaflets returning to the healthy condition upon recovery from the water-soaked stage following the infiltration. There were no significant differences to leaf reactions caused by treatment with the filtrates at either concentration among the eight alfalfa cultivars.

LNI values for the primary concentration of the filtrates of *F. acuminatum* and *F. sambucinum* were not significantly different until the third day (Table 3). However, the third and fourth day of evaluation indicated that LNI values for the primary concentration of the filtrate of *F. sambucinum* was significantly greater than that of *F. acuminatum*. On the fifth day, their LNI values were not significantly different. LNI values of each day for the primary concentration of the filtrates of both fungi were significantly greater than that of the previous days. LNI values for the doubled concentration of the filtrates of *F. acuminatum* and *F. o.* f. sp. *medicaginis* isolate GH7 were not significantly different on any day during the evaluation (Table 3). However, the LNI value for the doubled concentration of the filtrate of *F. sambucinum* was significantly greater than that of *F. acuminatum* and *F. o.* f. sp. *medicaginis* isolate GH7 on the third and fourth day as in the primary concentration. They were again the same on the fifth day.

LNI values of each day for doubled concentration of the filtrates of these three fungi were significantly greater than those of the previous days, except for that of *F. sambucinum* on the fifth day, which was not significantly greater than that of the previous day. Additionally, another difference in phytotoxicity among the fungi became strikingly apparent on the first day of evaluation. Doubled concentration of the filtrates from *F. sambucinum* and *F. acuminatum* caused a translocatable phytotoxicity in the plants whereas the filtrate of the same concentration from *F. o.* f. sp. *medicaginis* isolate GH7 did not. Untreated leaflets adjacent to treated leaflets on the same leaves and leaflets of untreated leaves up to three to four nodes above the treated leaves developed phytotoxic symptoms from the double-concentration filtrates of *F. sambucinum* and *F. acuminatum* (Fig. 2). The regression equation computed for the increase of necrosis index values over time at primary concentration for all isolates and the tests for the homogeneity of regression coefficients (9) indicated that the slopes for *F. sambucinum* and *F. acuminatum* were not different and that both were greater than the slopes for the remaining isolates, including the test isolate *F. o.* f. sp. *medicaginis*, GH7 (Fig. 3). Slopes of the regression equation for the LNI at the doubled concentration were also the same for *F. sambucinum*, *F. acuminatum*, and *F. o.* f. sp. *medicaginis* GH7, and they were greater than that of the remaining isolates (Fig. 4).

DISCUSSION

Our consistent isolation of *Fusarium* species from diseased alfalfa crown tissue

Fig. 2. Washoe alfalfa showing leaf necrosis and translocation of toxin(s) after infiltration with double concentration culture filtrate of *Fusarium sambucinum*. The leaflets (arrows) of the tagged leaves were infiltrated, and other leaves and leaflets with necrosis (no arrows) were not infiltrated.

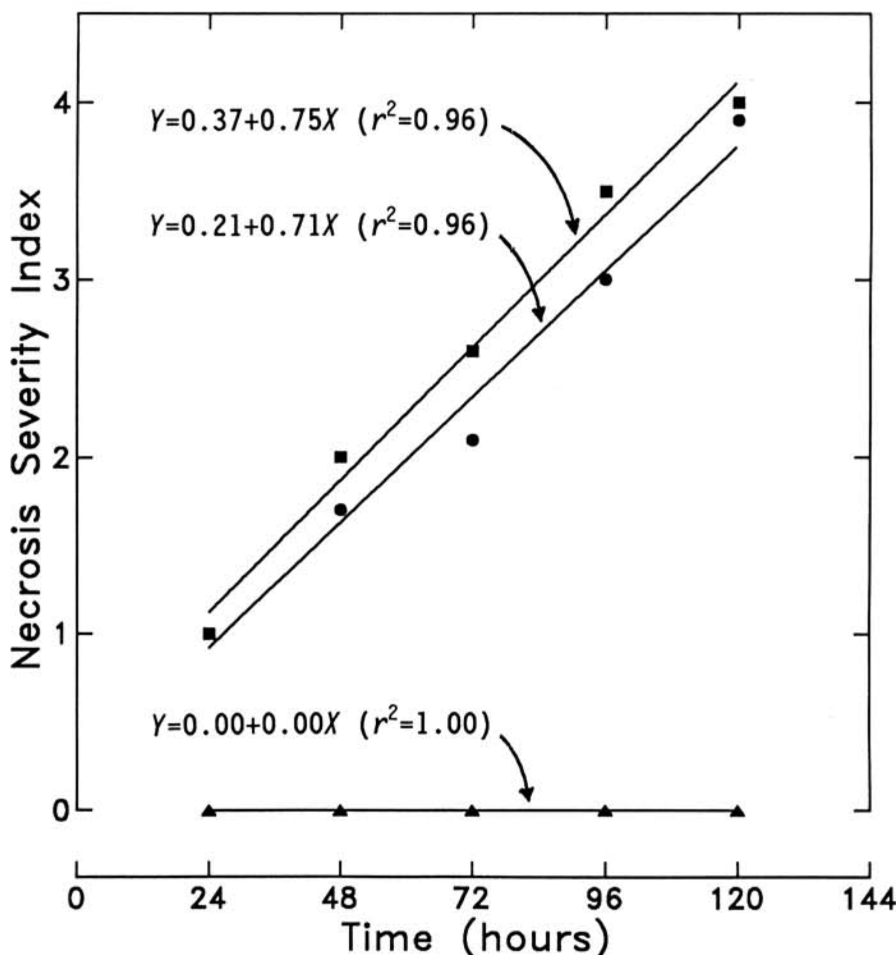

Fig. 3. Linear regression of the phytotoxicity data obtained from culture filtrates applied at primary concentration. *Fusarium sambucinum* (■), *F. acuminatum* (●), and other species and controls (▲).

in northern Nevada was similar to the findings of workers in other regions (5,6,14,17,21). We found the prevalent species to be *F. solani*, *F. acuminatum*, *F. oxysporum*, *F. sambucinum*, and *F. avenaceum*, and all five species were shown to be pathogenic. When inoculated into the crowns of 4-mo-old alfalfa plants in the greenhouse, the isolates caused necrotic crown lesions that extended mainly basipetally from the point of injection. Although all five species found in Nevada have been reported by workers in other regions to be pathogenic to alfalfa (11,14,17,21), it is unusual to find all five species in one geographic location.

Studies in other regions have shown that *F. tricinctum* (Corda) Sacc., *F. semitectum* Berk. & Ravenel, *F. moniliforme* J. Sheld., *F. culmorum* (Wm. G. Sm.) Sacc., and *F. poae* (Peck) Wollenweb. have also been associated with alfalfa crown and root diseases (11,17,19,21). These species were not found in our survey. Furthermore, the report from North Dakota (19) indicates that the most frequently isolated species was *F. oxysporum* (34%), followed by *F. solani* (27%), and *F. acuminatum* and *F. avenaceum* together (22%). The species prevalence in North Dakota did not match the pattern determined in this study. Additionally, *F. sambucinum* was not recovered in their study. Similar discrepancies occurred between the results of this study and that in Utah (21). Neither the findings in our field survey nor in pathogenicity of the fungi were similar to those reported from Utah. Three of the five *Fusarium* species were common to all locations in our study. *Fusarium* species in alfalfa fields in Utah were more restricted to individual locations. There was significant pathogenic variation among the species we have isolated, but that was not true in the Utah study. Additionally, synergism was not observed when all the *Fusarium* species were combined in that study. In contrast, our isolates have indicated synergism. Previous studies in Nevada led to reports of work with a fungus or fungi identified only as *F. roseum* Link:Fr. (15,20). The taxonomic evaluation in this survey indicates that the fungi isolated in the earlier work in Nevada were likely to have been *F. acuminatum*, *F. avenaceum*, and *F. sambucinum*.

F. sambucinum was isolated from diseased crowns associated with declining alfalfa fields in Australia (17), but the pathogenicity of this fungus apparently was not studied and it was considered to be saprophytic or weakly pathogenic. *F. sambucinum* from northern Nevada is equally as pathogenic as the isolate of *F. oxysporum* and more pathogenic than *F. avenaceum*. Both *F. sambucinum* and *F. avenaceum* were isolated from diseased plants only in selected fields and regions. The latter species was the least pathogenic of the five species evaluated. We found a positive correlation between the pathogenicity of the fungus and its frequency of recovery. The apparent confinement of *F. sambucinum* and *F. avenaceum* to certain locations suggests that undetermined environmental factors may govern their distribution and/or activity. The distribution of fusaria in each field in our survey followed a similar pattern for the most species, including the most prevalent and pathogenic, *F. solani* and *F. acuminatum*. This suggests our survey of the selected fields from these major alfalfa growing counties in Nevada may generally be considered to represent the growing area. Interaction between the *Fusarium* species and the alfalfa cultivars in our study indicated that WL318 is more susceptible to most of the species than other cultivars. However, because crown rot is a complex that yielded five *Fusarium* species in our survey, the disease caused by the combination of all five species in our greenhouse test indicates that cultivar Thor may be more tolerant, and WL318 and L720 may be more susceptible to these species combined than other cultivars we have tested (Table 2).

Culture filtrates of *F. sambucinum* and *F. acuminatum* were toxic when infiltrated into alfalfa leaf tissue. The degree of phytotoxicity of the filtrate of the test isolate *F. o.* f. sp. *medicaginis*, GH7, which previously was shown to produce toxin(s), was dependent upon the concentration in this study. The phytotoxicity of the filtrates of *F. sambucinum* and *F. acuminatum*, which caused more extensive and more rapid development of symptoms than the test isolate, also was affected by the concentration. This was indicated by the greater LNI values of the doubled concentration than that of the primary concentration on the third and the fourth day, and the phytotoxicity caused by the doubled concentration in the adjacent untreated leaflets and nearby upper leaves within 24 hr. The phytotoxicity that occurred in nearby untreated leaves also suggests that the toxin(s), which were produced by the fungi growing on Czapek-Dox agar, were water-soluble and translocated in the plant. Although we did not identify the toxins or their apparent concentrations, it is possible that different toxins are produced by these fungi or that the fungi produced similar compounds at different rates under the conditions of our test. The use of cell-free filtrates precluded the

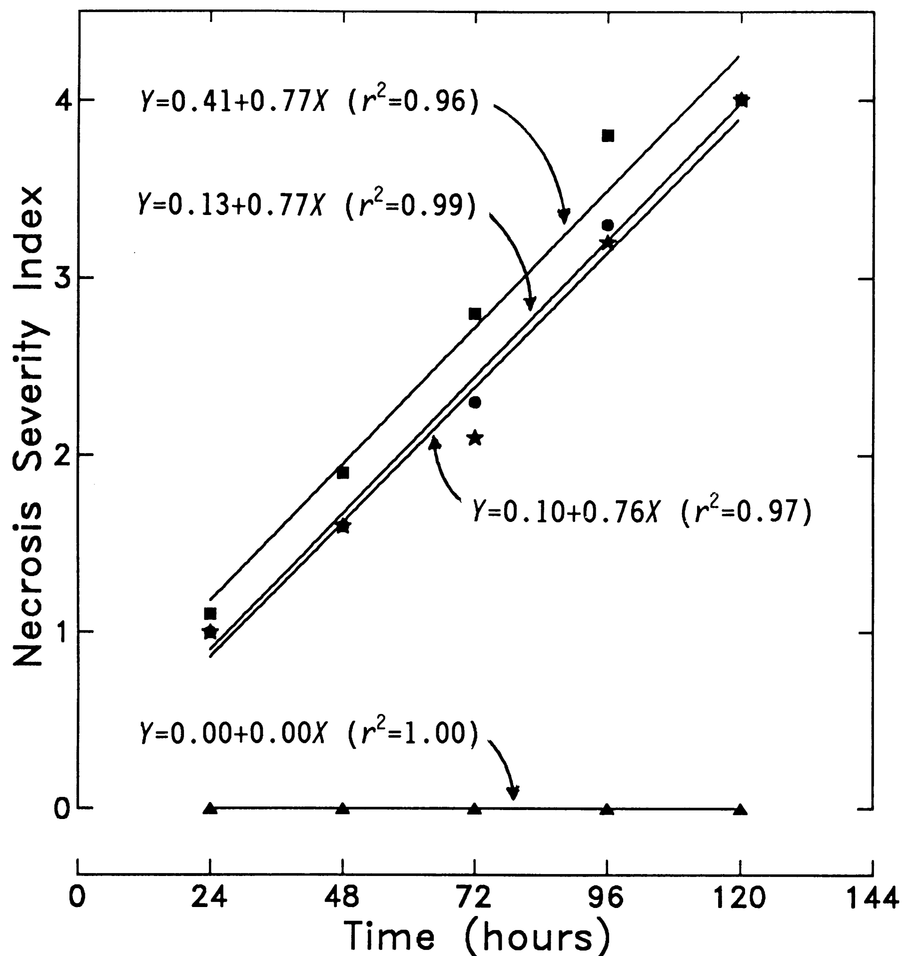

Fig. 4. Linear regression of the phytotoxicity data obtained from culture filtrate applied at double concentration. *Fusarium sambucinum* (■), *F. acuminatum* (●), *F. oxysporum* f. sp. *medicaginis* (★), and other species and controls (▲).

possibility that the toxicity could have been caused by viable fungal propagules in the filtrates.

Culture filtrates taken from pathogenic isolates of *F. solani*, *F. oxysporum*, and *F. avenaceum* did not cause a phytotoxic response in infiltrated leaflets. This indicates there was no correlation between the the apparent toxin production of the fungi and the pathogenicity. This work does not, however, suggest that these fungi are not capable of producing toxins or of having effects of toxins demonstrated under conditions other than those used in our study. A very small amount of toxin, or toxins of low toxicity, may not have become apparent with the procedure used in our study.

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