The Effect of Insect Defoliation on the Presence and Severity of *Fusarium* Crown-Rot in Alfalfa[1]

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ABSTRACT Alfalfa *Medicago sativa* L. ('Fla. 77') was inoculated with three different isolates of *Fusarium* and defoliated to varying levels with yellowstriped armyworms, *Spodoptera ornithogalli* (Guenée), to determine the effect of insect defoliation on the development of crown-rot under greenhouse conditions. There were no significant interactions between short-term insect defoliation and *Fusarium* crown-rot on forage quality, yield, or root carbohydrate reserves. Although insect defoliation alone did reduce plant height, yield, and maturity (18, 33, and 30% respectively) at the first harvest, no significant effects were observed at two subsequent harvests. Of the three isolates tested, *Fusarium oxysporum* Schlecht was the most virulent. Short-term defoliation did not increase the severity of *Fusarium* crown-rot in alfalfa.

KEY WORDS Insecta, *Spodoptera ornithogalli,* alfalfa, *Medicago, Fusarium.*

Fusarium crown-rot is a serious disease associated with the production of alfalfa, *Medicago sativa* L., in the United States (Leath and Byers 1977, Graham et al. 1979, Richard et al. 1980). Although the pathogens causing the disease are abundant in most alfalfa producing areas, the disease usually manifests itself only after the plant has been stressed. Alfalfa is an extremely hardy plant capable of withstanding invasion from root-rot pathogens (Dickason et al. 1968); however, plants stressed by insect feeding may be more susceptible to pathogen invasion (Leath and Byers 1977, Godfrey and Yeargan (1987). Godfrey and Yeargan (1987) showed that when alfalfa was stressed by both root-rot fungi and clover root curculio, *Sitona hispidulus* (F.), alfalfa yield was reduced by 21% compared with an 8% loss when stressed by clover root curculio or root-rot fungi alone. Leath and Byers (1977) found significantly more root-rot development when alfalfa plants were stressed by pea aphid, *Acyrthosiphon pisum* (Harris), feeding.

Interactions between crown-rot disease and insects that are regrded as defoliators have not been reported. Further research in this area needs to be conducted to develop comprehensive Integrated Pest Management (IPM) programs for alfalfa. The objective of this investigation was to evaluate the effect of stress caused by short-term defoliation on the incidence and severity of *Fusarium* crown-rot in alfalfa. The interaction of insect defoliation and crown-rot infection on alfalfa yield, quality, and root carbohydrate storage reserves are reported.

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Materials and Methods

Plant Maintenance. Alfalfa ('Fla. 77', a variety recommended in Louisiana) was planted (n = 5 inoculated seeds per pot) on 7 August 1987 in plastic pots (15 cm diam) and maintained in the greenhouse at 16 : 8 photoperiod, 26°C ± 5°C, and > 50% RH. Alfalfa seedlings were thinned to a single plant on 14 September. The soil mixture consisted of washed river sand, perlite, and peat moss (2 : 1 : 1 ratio). Plants were checked daily for soil moisture, with water applied to the soil on the sides of the pots as needed. This watering technique allowed plant crowns to remain dry. Before the study began, plants were harvested twice (at the 20% flowering stage) and fertilized at recommended rates (O N : 20 P_2O_5: 20 K_2O) after each harvest. Whiteflies, Aleyrodidae, and twospotted spider mites, *Tetranychus urticae* Koch, were controlled in November with sprays of malathion (50% emulsifiable concentrate [EC] at 2.5 ml/liter; Ferti-lome, Voluntary Purchasing Groups, Inc., Bonham, TX.) and dicofol (18.5% EC at 2.5 ml/liter; Dexol Industries, Torrence, Ca.). On 5 February 1988, plants were clipped to a height of 7 cm, fertilized, and allowed to regrow for 1 wk.

Insects. Alfalfa fields in Louisiana from June to October are usually infested by a complex of lepidopteran species (Wilson and Quisenberry 1987), all considered to be general defoliators. The yellowstriped armyworm, *Spodoptera ornithogalli* (Guenée), was used in this study because it readily feeds on alfalfa, severely injuring young stands as far north as Maryland (App and Manglitz 1972), and is also easily colonized in the laboratory.

Egg masses were collected in September 1987 from alfalfa on the Red River Research Station near Bossier City, LA. Larvae from these egg masses were maintained on a modified pinto bean diet (Perkins 1979) for six generations in a growth chamber at 14 : 10 photoperiod, 27°C ± 0.5°C, and 75% RH.

Alfalfa foliage (plants were ca. 18 cm in height) was infested with 10 to 15 neonate larvae per plant on 16 February 1988 using a small camel hair brush. Plants were observed twice daily, and when the level of insect feeding had produced the desired level of defoliation (25, 50, or 75%) the larvae were removed.

Pathogens. Three *Fusarium* spp. isolates (two *F. solani* Mart. Appel & Wr. and one *F. oxysporum* Schlecht.) from field-grown, necrotic, alfalfa roots and crowns were used. These two pathogenic species are among the most commonly associated with root- or crown-rot (Leath and Byers 1977, Graham et al. 1979, Godfrey et al. 1986, 1987). Plants were removed from the field and washed under tap water to remove soil. Necrotic tissue was excised from the crown, submerged in 70% ethanol for 30 sec, surface-disinfected in 0.5% NaOCl for 3 min, and plated on fresh potato-carrot agar (APCA) (Dade and Gunnell 1969) acidified to pH 4.0 with 50% lactic acid. Plates were incubated at 24°C for 7 d. Single spores of isolates tentatively identified as *Fusarium* spp. were tranferred to carnation leaf agar (CLA) and identified according to the classification scheme of Nelson et al. (1983).

Fungal inocula were prepared by culturing the *Fusarium* isolates from CLA plates in flasks containing 100 ml of fresh potato-carrot broth in a shaking water bath at 24°C for 4 d. The resulting fungal suspensions were filtered through cheesecloth. The filtrate was centrifuged to concentrate the conidia, the resulting

pellet resuspended in sterile distilled water, conidia counted with a hemacytometer, and adjusted to a final concentration of 1×10^5 conidia per ml.

On 12 February 1988, soil was removed to expose the plant crowns. Each plant was then injected to a depth of 5 mm using a hypodermic needle and administered either 1 ml of the appropriate fungal suspension or 1 ml of distilled water (control). Separate hypodermic needles were used for each of the three isolates and the control.

Parameters Measured. Plants were harvested on a 35 d schedule (bloom of the control plants was ca. 25%). At each harvest, several plant parameters were measured to evaluate the effects of insect feeding and disease infection including the following: stem maturity (rated on a scale of 1-3, where 1 = vegetative, 2 = bud, 3 = flowering), stem height, and dry weight (oven dried 24 h at 60°C).

Foliage from each plant was ground in a cyclone mill (Techeor Corp., Herndon, VA) to pass through a 1 mm screen. Forage quality was determined using near-infrared spectroscopy calibrated to the respective tests. Crude protein (CP) was analyzed by the improved Kjeldahl method (Association of Official Agricultural Chemists 1980), and in vitro digestible dry matter (IVDDM) was determined by the modified Van Soest procedure (Nelson et al. 1976).

Destructive root samples were taken at each harvest to determine the incidence and serverity of disease. The roots of all alfalfa plants on one bench (n = 48, 3 replications) were removed from the pots, loose soil removed, secondary roots stripped, washed under tap water, split longitudinally to expose any discolorization of root tissue, and indexed for infection. Crown infection was assessed by estimating the severity of disease on a 0-4 scale, where 0 = no discoloration; 1 = slight discoloration, 1-10% tissue affected; 2 = discoloration spreading, 11-50% tissue affected; 3 = large area discolored, 51-75% tissue affected; 4 = extensive discoloration, 75-100% tissue affected. Lengths of the discoloration also were measured.

At each harvest, random samples (1 cm^2) from diseased root tissue were taken and plated on APCA to verify the presence of the *Fusarium* spp. that was injected. Roots were oven dried (48 h at 60°C), ground in a cyclone mill to pass through a 1 mm screen, and subjected to near-infrared spectroscopy calibrated to the phenol-sulfuric acid technique described by Whistler and Wolfrom (1962) to determine root carbohydrate reserve levels.

Experimental Design. Plants were arranged on three benches in a complete randomized block design with a factorial arrangement of treatments. There were 16 treatments consisting of all possible combinations of insect defoliation levels (0, 25, 50 and 75%), and *Fusarium* isolates (control, two isolates of *F. solani* [isolate 1 and 2], and *F. oxysporum* [isolate 3]).

All data were subjected to analysis of variance using a general linear model (SAS Institute 1985). The Wilk's Lambda Criterion was used to determine any apparent interaction effect. Duncan's (1955) multiple range test was used to separate significant means ($P < 0.05$). Orthogonal contrasts (insects vs. controls and isolates vs. controls) also were compared (Steel and Torre 1980).

Results

Wilk's Lambda Criterion test indicated no significant interaction between insect defoliation and disease infection (harvest 1, $F = 1.30$, $P > F = 0.07$; harvest 2, $F = 0.82$, $P > F = 0.84$; and harvest 3, $F = 1.12$, $P > F = 0.27$). Because interactions

were nonsignificant, the effects of insect and disease stress on alfalfa were analyzed separately.

Stem height, yield, and rate of maturity were reduced as the level of defoliation increased (Table 1). The greatest insect damage (75% defoliation) caused significant reductions in stem height, yield, and rate of maturity of 18, 33, and 20%, respectively, compared with the controls. The 50% defoliation also significantly reduced stem height (11%), yield (30%), and rate of maturity (10%), while the 25% defoliation significantly reduced yield (15%). Protein concentrations for plants in the first harvest were significantly lower for the control plants (19%) compared with plants that were 25% defoliated (20%). Root carbohydrates were significantly reduced in the 25% defoliated plants; however, values for the 50 and 75% defoliated plants were not significantly less than the controls.

The insect defoliation obtained before the first harvest failed to produce a significant residual effect on plant growth or forage quality in either the second or third harvests. Although plants stressed by insects generally had lower yield and quality values, they were not significantly different from those for undamaged plants (orthogonal contrast, $P > 0.05$).

Table 1. Effect of insect defoliation on alfalfa, harvest 1.

Defoliation (%	Stem ht (cm)	Yield (g)	CP (%)	Carbohydrate* reduction (%)	Maturity†
0	38.1 a	1.69 a	19.1 b	____	2.2 a
25	36.4 ab	1.43 b	20.0 a	13.62 b	2.1 ab
50	33.9 bc	1.19 c	19.4 ab	7.87 a	2.0 b
75	31.3 c	1.23 c	19.8 ab	9.67 ab	1.8 c

Means followed by the same letter within a column are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

* Root carbohydrate reduction (CHO) = (% CHO control - % CHO treatment)/ % CHO control × 100.

† Maturity: 1 = vegetative; 2 = bud; 3 = flower.

Random isolations from diseased root tissue verified the presence of the *Fusarium* spp. injected. Disease indices and lesion lengths caused by the three isolates at each harvest are presented in Fig. 1. Based on disease index, isolate 3, *F. oxysporum,* was the most virulent of the three isolates tested. There were no differences in the disease indices between isolates 1 and 2, the two isolates of *F. solani,* at any of the three harvests (Fig. 1 A). There was little difference in lesion lengths, with the exception of isolate 3 at the second harvest (Fig. 1 B).

Of the plant parameters measured, no significant differences were observed between inoculated and non-inoculated plants were observed, with the exception of concentration of crude protein (Table 2). Inoculated plants had lower protein concentrations than non-inoculated plants although not significant at the first and second harvests; however, orthogonal contrasts (plants injected with *Fusarium* vs. others) indicated protein concentrtiosn were significantly lower ($P < 0.02$) at the third harvest.

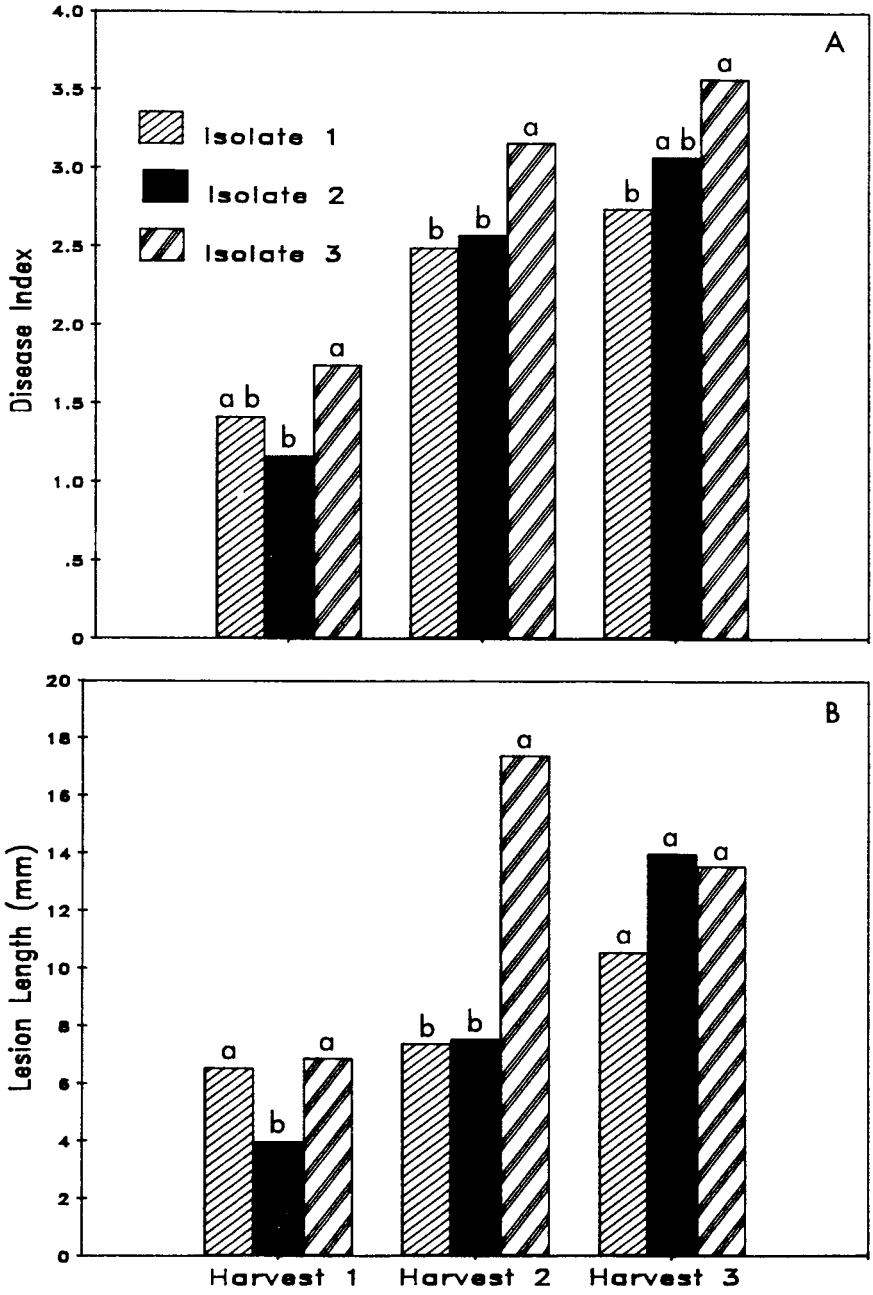

Fig. 1. Effect of the three *Fusarium* spp. isolates on the disease index (A), and lesion length (B) at harvests 1, 2 and 3. Means followed by the same letter within a harvest are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

Table 2. The effect of *Fusrium* disease incidence on crude protein concentration (%).

	Harvest 1	Harvest 2	Harvest 3
Control	19.8 a	18.2 a	19.1 a
Isolate 1	19.4 a	17.3 a	17.3 ab
Isolate 2	19.5 a	17.5 a	16.4 b
Isolate 3	19.5 a	17.9 a	17.3 ab

Means followed by the same letter within a harvest are not significantly different ($P > 0.95$; Duncan's [1955] multiple range test).

Discussion

Alfalfa fields are usually subjected to extensive insect feeding during the growing season in Louisiana (Wilson and Quisenberry 1986, 1987). Although the 75% level of insect defoliation used in our research was somewhat severe, Wilson and Quisenberry (1987) reported reductions of up to 65% in alfalfa yields caused by a complex of lepidopteran larvae and threecornered alfalfa hoppers, *Spissistilus festinus* (Say). The largest yield reduction in our study caused by yellowstriped armyworm was only 33%.

The higher level of protein concentration at the 25% defolition level was not expected (Table 1); however, this level of defoliation was obtained long before harvest and thus, insects were removed. As a result, the plants were able to compensate adding new leaves that were higher in quality. To achieve this regrowth, plants had to rely on stored root reserves which caused significant lower levels of root carbohydrate reserves in the 25% insect defoliated plants at harvest 1.

Our data indicated that *F. oxysporum* was the most virulent of the three isolates used. Richard et al. (1980) also found that *Fusarium* crown-rot was associated with a complex of species but *F. oxysporum* was usually the predominant species involved. Leath and Kendall (1978) tested the virulence of several *Fusarium* spp known to cause root- and crown-rot in alfalfa and other legume species. Of the species tested, they concluded that *F. oxysporum* caused the most root injury followed by *F. solani.*

The inability to determine a significant interaction between short-term insect defoliation and disease infection was inconsistent with findings of Leath and Byers (1977) or Godfrey a Yeargan (1987); however, the feeding damage caused by the insects used in their studies would not be regarded as a form of defoliation. Godfrey and Yeargan indicated an interaction between disease infection and the clover root curculio (CRC); however, they speculated that the synergistic effect was caused by the CRC lesions on the alfalfa roots acting as colonization sites for the disease and thus, hastening the development of the root-rot disease. The effect was an indirect result of plant stress from insect feeding. The significant insect/disease interaction obtained in the study by Leath and Byers (1977) was obtained by using the pea aphid and the potato leafhopper. These insects have a piercing-sucking feeding habit, and in the case of the potato leafhopper, a toxin can be associated with feeding. The damage caused by the yellowstriped armyworm in our study was strictly defoliation and did not involve any insect toxins. Other research (O'Rourke and Millar 1966, Leath and Newton 1969) also indicates interactions between plant stresses (foliar diseases and insects [fungus gnats]) and root-rot

fungi; however, no previous studies have shown a synergistic effect between plant defoliators and incidence or serverity of root- or crown-rot. Godfrey and Yeargan (1987) reported no significant interaction between alfalfa weevil larval defoliation and root-rot diseases.

The results of this study show that short-term defoliation (through only one harvest period) of up to 75% failed to have a significant effect on the development of *Fusarium* crown-rot for subsequent harvests. If the experiment was extended for a longer period of time, or an additional stressing factor was incorporated (i.e. plants subjected to winter conditions), results may differ.

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