Development of *Fusarium* Crown-rot in Alfalfa Stressed by Multiple Defoliations by the Yellowstriped Armyworm (Lepidoptera: Noctuidae)[1]

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J. Entomol. Sci. 26(1): 85-94 (January 1991)

ABSTRACT Alfalfa (*Medicago sativa* L.) plants (var 'Florida 77' and 'Cimarron') were injected with *Fusarium oxysporum* Schlecht., and stressed for four consecutive harvests by yellowstriped armyworms, *Spodoptera ornithogalli* (Gueneé), under greenhouse conditions. No significant interactions existed between insect defoliation and inoculation with *F. oxysporum*. Insect defoliation alone had significant effects on dry matter yield, stem height, and maturity, but forage quality, root weight, and root carbohydrate levels were not significantly affected. In vitro digestible dry matter (IVDDM) concentration in Cimarron was the only forage parameter affected by *F. oxysporum*.

KEY WORDS Insecta, *Spodoptera ornithogalli,* alfalfa, *Medicago, Fusarium oxysporum,* yellowstriped armyworm.

Several biotic factors are known to simultaneously affect production of alfalfa in the southeastern United States. Although numerous authors have reported on single factors severely affecting alfalfa yield, quality, and stand persistence, studies involving the interactive effects of two or more biotic factors are limited. Because of alfalfa's perennial nature and the wide diversity of insect, disease, and weed pests known to affect it, a more detailed evaluation of plant pest interactions is needed to develop comprehensive Integrated Pest Management (IPM) programs for the southeast.

It is not uncommon for alfalfa fields in the southeast to be infested by a complex of lepidopterous species from June until October (Wilson and Quisenberry 1987). This complex includes several different species; the corn earworm, *Heliothis zea* [Boddie]; alfalfa webworm, *Loxostege cereralis* [Walker]; alfalfa caterpillar, *Colias eurytheme* Boisduval; the green cloverworm, *Plathypena scabra* [F.]; the yellowstriped armyworm, *Spodoptera ornithogalli* (Gueneé); and occasionally others (Lee 1989). Populations of these insects usually increase on other crops (cotton, corn, and soybean), then migrate to alfalfa as these crops start maturing.

High populations of pathogenic fungi are known to infest most soils in alfalfa production throughout the southeast. Some of the most important are a complex of fungi (including *Fusarium oxysporum* Schlecht.) responsible for causing root and crown-rot (Leath et al. 1971). Although these fungi are almost always present, it is

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thought that crown-rot develops only after plants have been stressed by other factors (O'Rourke and Millar 1966, Graham et al. 1979, Godfrey et al. 1987).

This study was undertaken to determine the effect multiple defoliations by the yellowstriped armyworm had upon the development of *Fusarium* crown-rot in two alfalfa varieties ('Florida 77' and 'Cimarron'). The effects of these stresses on alfalfa dry matter yield, forage quality, and root carbohydrate reserves also were evaluated.

Materials and Methods

Plant Maintenance. Soil used for this study ('Fisons Sunshine Mix,' Fisons Western Inc., Downers Grove, IL) was sterilized in an autoclave for 8 h (110°C at 15 psi). Two alfalfa varieties (Florida 77 and Cimarron) were planted on 1 July 1988 in plastic pots (15 cm diam, 20 cm depth) and maintained in a greenhouse with a photoperiod of 16:8 (L:D), 26°C ± 5°C, and > 50% RH. These two varieties are recommended for use in Louisiana (Faw and Mason 1983). Plants were harvested before the defoliation study began at a 20% flowering stage to provide adequate levels of root carbohydrates for regrowth. After each harvest, plants were fertilized with 16.9 kg/ha of P_2O_5 and K_2O using a 0:20:20 commercial fertilizer.

Pathogens. *F. oxysporum* Schlecht., the most virulent *Fusarium* isolate tested in a previous study (Lee 1989), was selected for use in this study. The *F. oxysporum* isolate was obtained from a one-year-old stand of alfalfa located at the Red River Research Station near Bossier City, LA. Alfalfa plants were removed from the field and washed under tap water to remove adhering 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 transferred to carnation leaf agar (CLA) and identified according to the classification scheme of Nelson et al. (1983). Although several pathogens can be associated with crown-rot, *Fusarium* tends to be the pathogenic genus isolated most frequently (Graham et al. 1979).

Fungal inocula were prepared by culturing the isolates of *F. oxysporum* 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, and the resulting pellet was resuspended in sterile distilled water. The conidia then were counted with a hemacytometer and adjusted with sterile, distilled water to a final concentration of 1×10^5 conidia per ml.

On 16 February 1989, soil was removed to expose the plant crowns. Each plant then was injected 1 cm below the crown to a depth of 5 mm using a hypodermic needle and administered either 1 ml of a conidial suspension of *F. oxysporum* or 1 ml of distilled water (control). Separate hypodermic needles were used for the controls. Because of the long period of time required for most pathogens to enter root tissue under normal circumstances, wounding of the root tissue in this manner is an accepted practice (Leath and Byers 1977, Richard et al. 1980).

Insects. The yellowstriped armyworm was selected for use in this study because it readily feeds on alfalfa, severely injuring young stands as far north as Maryland (App and Manglitz 1972), and because of its easy colonization 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) in a growth chamber with a photoperiod of 14:10 (L:D), 27°C ± 0.5°C, and 75% RH.

On 20 March 1989, nine days after harvest, 5-10 second instar larvae were placed on the plants that had treatments requiring defoliation. This was one harvest cycle after plants were injected with the pathogen. Plants were monitored daily, and insects were removed when a visual observation determined the desired level of defoliation (25 - 35% [light], or 50 - 75% [heavy] defoliation) had been obtained. Additional insects were applied weekly as needed to maintain the defoliation at the desired level until harvest. Insects were reapplied in the same manner after each harvest for the duration of the study.

Parameters Measured. Plants were harvested when the control plants were between the 10 - 25% flowering stage (30 - 35 d). Several plant parameters were measured at each harvest to evaluate the impact of insect defoliation and inoculation of *F. oxysporum.* Plant parameters measured included: stem maturity (rated on a scale of 1-4, where 1 = vegetative, 2 = bud, 3 = flowering, 4 = seed set; Kalu and Fick 1981), stem height, and dry weight (oven dried 30 h at 60°C).

Above ground foliage from each plant was ground in a cyclone micro-mill (Techeor Corp., Herndon, VA) to pass through a 1 mm screen. Forage quality was determined using near-infrared spectroscopy calibrated to the appropriate 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 (tissue from the base of the crown to 15 cm below) were taken at each harvest to determine the incidence and severity of disease. The roots of all alfalfa plants on one bench (n = 36, 3 replications) were removed from the pots, loose soil removed, and secondary roots stripped. The remaining tap root was washed under tap water, split longitudinally to expose any necrosis 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 necrosis; 1 = slight necrosis, 1-10% tissue affected; 2 = necrosis spreading, 11 - 50% tissue affected; 3 = large amount of necrosis, 51 - 75% tissue affected; 4 = extensive necrosis, 75 - 100% tissue affected. Necrosis was determined by measuring the length of discoloration at the point of inoculation. At each harvest, random samples (1 cm^2) from diseased root tissue were taken and plated on APCA to verify the presence of the *F. oxysporum.* Any controls showing signs of disease were also cultured.

Roots were oven dried (48 h at 60°C), weighed, and ground in a cyclone micro-mill to pass through a 1 mm screen. The phenol-sulfuric acid technique described by Whistler and Wolfrom (1962) was performed on duplicate samples of root tissue. Absorbance of each sample then was measured with a spectrophotometer (Beckman Instruments Inc., Irvine, CA) at 490 nm. Results were compared to glucose standards analyzed simultaneously to determine total available carbohydrate (TAC) levels.

Experimental Design. Plants were arranged by variety on four benches in a complete randomized block design with a factorial arrangement of treatments. There were six treatments per variety consisting of all possible combinations of insect defoliation level (0, 25 - 35, 50 - 75%) and the presence or absence of *F. oxysporum.* Each bench contained three replications of treatments by variety. At

each harvest, all plants on one bench were destructively sampled to measure root parameters. There were 12 replications per variety used in foliage parameter analysis at the first harvest. However, due to the destructive sampling techniques, three less replications per variety were available for analysis of foliar parameter at each of the following harvests.

All data were subjected to a univariate analysis of variance using a general linear model (SAS Institute 1985). Duncan's (1955) multiple range test was used to separate significantly different means. Unless otherwise indicated, significance was determined at the 0.05 level.

Results

Root Parameters. The effects of levels of insect defoliation and inoculation with *F. oxysporum* on root parameters are presented in Tables 1 and 2. No significant interaction between levels of insect defoliation and inoculation with *F. oxysporum* were observed on any of the root parameters measured during the study.

Based on disease index and length of necrosis, insect defoliation did not significantly affect the development of crown-rot in Florida 77 or Cimarron (Tables 1 and 2, respectively). Both varieties inoculated with *F. oxysporum* developed crown-rot. Random isolations at each harvest from necrotic tissue of plants inoculated with *F. oxysporum* revealed the presence of *F. oxysporum,* the injected pathogen. Some control plants developed necrosis; however, isolations indicated the necrosis was caused by fungi (i.e., *Rhizoctonia* and *Penicillium*) other than *F. oxysporum.*

No differences in root weights were observed between inoculated and noninoculated plants. Mean root weights of 3.2 and 2.6 g for Florida 77 and Cimarron, respectively were significantly different.

Roots of Florida 77 plants had a mean total available carbohydrate concentrations (TAC) concentration of 58.5%, while TAC for Cimarron was 53.2%. Neither the levels of insect defoliation or inoculation with *F. oxysporum* had an effect on total available root carbohydrate concentrations (Tables 1 and 2).

Foliage Parameters. The effect of insect defoliation and inoculation of *F. oxysporum* on foliar parameters are presented in Tables 3 and 4. No significant interaction between level of insect defoliation and inoculation with *F. oxysporum* was observed.

Stem height, plant maturity, and dry matter yield for both varieties were all significantly affected by the level of insect defoliation (Tables 3 and 4). Light insect defoliation (25 - 35%) reduced stem height, plant maturity, and forage yield for Florida 77 by 12.9, 12.5, and 30.4%, and 15.0, 21.7, and 36.0% for Cimarron, respectively. The same parameters were reduced with heavy insect defoliation (50 - 75%) by 21.4, 20.8, and 47.8% for Florida 77, and 21.3, 26.1, and 48.0% for Cimarron, respectively. No significant differences in stem height, plant maturity, or forage yield were found between inoculated and noninoculated plants. Mean stem height for Florida 77 was 31.4 cm, while stem height for Cimarron averaged only 29.2 cm.

Analysis of forage quality (protein, IVDDM, and ADF concentrations) indicated no significant differences due to the levels of insect defoliation (Tables 3 and 4). IVDDM concentration was significantly affected by the inoculation of *F. oxysporum*

Table 1. Effect of insect defoliation and inoculation with *F. oxysporum* on root parameters in 'Florida 77' alfalfa over four harvests.

	Interaction		Defoliation*					Inoculation†			
Parameter	*F*	*P* > *F*	*F*	*P* > *F*	0	1	2	*F*	*P* > *F*	0	1
Dis. Index†	0.6	0.55	0.3	0.72	1.4 a	1.4 a	1.4 a	11.1	<0.01	0.3	2.5¶
Necrosis Len. (mm)	0.9	0.91	0.7	0.52	6.5 a	5.7 a	6.7 a	31.4	<0.01	0.8	11.8¶
Root Wt. (g)	0.3	0.73	0.1	0.90	3.5 a	3.3 a	2.8 a	0.7	0.39	3.3	3.1
TAC (%)§	1.0	0.36	0.2	0.84	59.5 a	58.8 a	57.1 a	0.6	0.44	58.2	58.8

* Means of three levels of insect defoliation; 0 = no defoliation, 1 = light defolaition (25 - 35%), 2 = heavy defoliation (50 - 75%). Means followed by the same letter within a row are not significantly different ($P = 0.05$, Duncan's [1955] multiple range test).

† Means of plants inoculated with water (0) or *F. oxysporum* (1).

‡ Disease index is based on an index of 0-4 where 0 = no necrosis, 0% tissue affected, and 4 = extensive necrosis, 75 - 100% tissue affected.

§ TAC = total available carbohydrates.

¶ Significant difference between means of plants inoculated with water and *F. oxysporum* (ANOVA, for probability value see *P* > *F* for inoculation).

Table 2. Effect of insect defoliation and inoculation with *F. oxysporum* on root parameters in 'Cimarron' alfalfa over four harvests.

	Interaction		Defoliation*					Inoculation†			
Parameter	*F*	*P* > *F*	*F*	*P* > *F*	0	1	2	*F*	*P* > *F*	0	1
Dis. Index†	0.5	0.61	0.1	0.89	1.5 a	1.3 a	1.4 a	25.9	<0.01	0.2	2.6¶
Necrosis Len. (mm)	1.0	0.39	0.2	0.82	4.9 a	7.6 a	6.5 a	5.2	0.02	0.6	11.9¶
Root Wt. (g)	0.4	0.65	2.4	0.09	2.8 a	2.6 a	2.6 a	0.3	0.57	2.6	2.7
TAC (%)§	0.1	0.87	0.5	0.62	54.3 a	54.1 a	51.2 a	0.7	0.42	52.3	54.2

* Means of three levels of insect defoliation; 0 = no defoliation, 1 = light defolaition (25 - 35%), 2 = heavy defoliation (50 - 75%). Means followed by the same letter within a row are not significantly different ($P = 0.05$, Duncan's [1955] multiple range test).

† Means of plants inoculated with water (0) or *F. oxysporum* (1).

‡ Disease index is based on an index of 0-4 where 0 = no necrosis, 0% tissue affected, and 4 = extensive necrosis, 75 - 100% tissue affected.

§ TAC = total available carbohydrates.

¶ Significant difference between means of plants inoculated with water and *F. oxysporum* (ANOVA, for probability value see *P* > *F* for inoculation).

Table 3. Effect of insect defoliation and inoculation with *F. oxysporum* on growth and forage quality parameters in 'Florida 77' alfalfa over four harvests.

	Interaction		Defoliation*					Inoculation†			
Parameter	*F*	*P* > *F*	*F*	*P* > *F*	*0*	*1*	*2*	*F*	*P* > *F*	0	1
Stem Height (cm)	0.4	0.65	3.6	0.02	35.5 a	30.9 b	27.9 c	0.2	0.65	32.0	30.9
Maturity‡	0.3	0.76	3.7	0.02	2.4 a	2.1 b	1.9 c	0.8	0.38	2.1	2.2
Forage Weight (g)	0.6	0.53	15.0	<0.01	2.3 a	1.6 b	1.2 c	0.2	0.70	1.7	1.6
CP (%)	0.7	0.52	1.0	0.38	17.8 a	17.7 a	17.3 a	1.0	0.33	17.4	17.8
IVDDM (%)	0.5	0.60	0.2	0.84	72.2 a	72.8 a	72.0 a	0.0	0.97	72.0	72.6
ADF (%)	0.1	0.94	0.6	0.57	25.0 a	24.4 a	24.4 a	0.2	0.64	24.7	24.6

* Means of three levels of insect defoliation; 0 = no defoliation, 1 = light defoliation (25 - 35%), 2 = heavy defoliation (50 - 75%). Means followed by the same letter within a row are not significantly different ($P = 0.05$, Duncan's [1955] multiple range test).

† Means of plants inoculated with water (0) or *F. oxysporum* (1).

‡ Maturity rating is based on a rating of 1-4 where 1 = vegative growth and 4 = seed pod. CP = crude protein; IVDDM = in vitro dry digestible matter; ADF = acid digestible fiber.

Table 4. Effect of insect defoliation and inoculation with *F. oxysporum* on growth and forage quality parameters in 'Cimarron' alfalfa over four harvests.

	Interaction		Defoliation*					Inoculation†			
Parameter	*F*	*P* > *F*	*F*	*P* > *F*	*0*	*1*	*2*	*F*	*P* > *F*	0	1
Stem Height (cm)	2.0	0.13	3.8	0.02	33.3 a	28.3 b	26.2 c	0.0	0.89	29.2	29.3
Maturity‡	0.1	0.95	4.2	0.01	2.3 a	1.8 b	1.7 b	0.1	0.83	2.0	1.9
Forage Weight (g)	2.3	0.09	14.9	<0.01	2.5 a	1.6 b	1.3 c	1.5	0.22	1.9	1.7
CP (%)	0.1	0.91	1.1	0.35	18.1 a	17.6 a	17.9 a	0.0	0.95	17.7	18.1
IVDDM (%)	0.1	0.90	0.9	0.41	73.0 a	72.6 a	72.9 a	4.1	0.04	72.7	73.0§
ADF (%)	0.4	0.65	0.7	0.50	23.7 a	24.0 a	23.8 a	3.4	0.06	23.7	23.9

* Means of three levels of insect defoliation; 0 = no defoliation, 1 = light defoliation (25 - 35%), 2 = heavy defoliation (50 - 75%). Means followed by the same letter within a row are not significantly different ($P = 0.05$, Duncan's [1955] multiple range test).

† Means of plants inoculated with water (0) or *F. oxysporum* (1).

‡ Maturity rating is based on a rating of 1-4 where 1 = vegative growth and 4 = seed pod. CP = crude protein; IVDDM = in vitro dry digestible matter; ADF = acid digestible fiber.

§ Significant difference between means of plants inoculated with water and *F. oxysporum* (ANOVA, for probability value see *P* > *F* for inoculation).

in Cimarron; however, protein and ADF concentrations were not significantly different from the uninoculated plants. Inoculation with *F. oxysporum* in Florida 77 had no significant effects on forage quality.

Discussion

No significant interaction between the levels of insect defoliation and the inoculation of *F. oxysporum* on either the root or foliar parameters were observed. Insect induced stress has been shown to hasten the development of crown-rot (*Empoasca fabae* [Harris], Leath and Byers 1977; *Sitona hispidulus* [F.], Godfrey and Yeargan 1987), but studies involving interactions between defoliating insects and disease development are limited. These results indicate that under greenhouse growing conditions, where no other plant stresses are involved, repeated defoliations did not increase the severity of crown-rot. A previous study by Lee (1989) indicated similar results when plants were stressed by a single defoliation. Development of crown-rot under field conditions may be attributable to interactions from several different factors, both biotic and abiotic stresses.

Results indicated differences between alfalfa varieties in certain plant parameters. TAC concentration, root weight, and stem heights were all higher in Florida 77 than Cimarron. Neither variety had a significant tolerance or susceptibility to the plant stresses tested.

Because of the limited amount of crown-rot development, even after five harvests periods, it was not surprising the inoculation with *F. oxysporum* had little impact on foliar parameters. The disease progressed slowly throughout the study, and if the study was extended for a longer period of time (i.e., 1 yr or more), the effect of the pathogen might have been more dramatic.

Results may have varied dramatically if other plant stresses, such as a foliar pathogen or drought stress, were involved. To better understand the process involving crown-rot development, further research in the area of plant interactions needs to be conducted.

Acknowledgments

We gratefully acknowledge the technical assistance of B. D. Nelson, Southeast Research Station, Franklinton, LA., in determining forage quality analysis. Appreciation is given to R. Bessin and D. Moellenbeck for their advice on statistical analysis. The authors wish to thank T. J. Riley and J. B. Graves for critical review of this manuscript. Approved for publication by the Director, Louisiana Agricultural Experiment Station as Manuscript Number 89-17-3516.

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