Interaction of Fusarium Root Rot with Pea Aphid and Potato Leafhopper Feeding on Forage Legumes

K. T. Leath and R. A. Byers

Research Plant Pathologist, Agricultural Research Service, U. S. Department of Agriculture (also Adjunct Associate Proffesor Plant Pathology, Pennsylvania, State University); and Research Entomologist, Agricultural Research Service, U. S. Department of Agriculture (also Adjunct Assistant Professor of Entomology, Penn. State Univ.), respectively, U. S. Regional Pasture Research Laboratory, University Park, PA 16802. Contribution No. 390 from the U. S. Regional Pasture Research Laboratory.

We thank R. R. Hill, Jr. for statistical advice, and E. H. Quigley and W. E. Priddy for technical assistance.

Accepted for publication 11 August 1976.

ABSTRACT

LEATH, K. T., and R. A. BYERS. 1977. Interaction of Fusarium root rot with pea aphid and potato leafhopper feeding on forage legumes. Phytopathology 67: 226-229.

The development of root rot caused by *Fusarium roseum* was determined in alfalfa and red and white clover grown with and without stress caused by feeding of pea aphids (*Acyrthosiphon pisum*) on the foliage. Significantly more root rot developed in all forage species whenever plants were subjected to aphid feeding. Longevity of red clover plants with roots inoculated with *F. roseum* and of alfalfa plants inoculated with *F. oxysporum* was reduced significantly whenever the plants were subjected to aphid feeding. Significantly more plants died after subjection to the combined stress of feeding by potato leafhoppers (*Empoasca* fabae), root inoculations with *F. roseum*, and exposure to winter conditions.

Additional key words: *Trifolium pratense*, *Trifolium repens*, *Medicago sativa*, plant stress.

Fusarium root and crown rot which is caused by several *Fusarium* species is a chronic disease problem afflicting red clover, alfalfa, and other forage legumes. The fungi colonize roots shortly after seeding (8), but usually do not cause severe rot until the plant is under stress from some other agent (6, 8), for example increased levels of foliar disease (8). Similar relationships have been shown in red clover with red clover vein mosaic virus (2) and with increased frequency of clipping (9).

One of the most serious stresses that occurs each year on forage legumes grown in the northeastern United States is that caused by the feeding of leafhoppers, aphids, weevils, spittlebugs, and plant bugs. Insect feeding essentially is continuous from spring growth until autumn frost. The interaction of leaf and stem feeding injury by insects with an increased severity of root rot of forages has not been investigated.

The objective of the present investigation was to evaluate interactions between root rot-causing *Fusarium* species and insect feeding on the leaves and stems of plants.

MATERIALS AND METHODS

General.—All plants were grown singly in a peat-soil mix in 10-cm-diameter clay pots in the greenhouse. Before the plants were used in experiments insects were controlled by weekly sprays of malathion and oxythioquinox. Plant tops were clipped to a height of 5-cm at the start of each test. Cages used in the greenhouse were of wood frame-plastic screen construction, and each held 30 plants. Plexiglass tubular cages previously described (7) were used to cage individual plants in the growth chamber. Insects used to stress the plants were the pea aphid [*Acyrthosiphon pisum* (Harris)] on all plant species and the potato leafhopper [*Empoasca fabae* (Harris)] on alfalfa.

Roots were inoculated by wounding the taproot about 1 cm below the soil surface. This was done with a single cut, about 1 cm long, made longitudinally with a scalpel through the cortex. Two milliliters of a fungal suspension were pipetted over the wounded area and the soil was replaced. Roots of control and aphid-infested plants also were wounded. Isolates used were not the same in all tests and are described with the individual experiments. Fungi were stored in sterile soil between experiments.

Fresh weights of harvested plant tops were used to determine the degree of plant stress imposed by the individual treatments. In the red clover experiment the plants were clipped twice, once during- and again at the end of the test. In other experiments, the plants were clipped only at the end of the test. Aphid counts were made at the end of each experiment (twice during the red clover experiment) to establish the level of aphid activity.

Two rating systems based on visual estimation of browning were used to evaluate the severity of root rot. The first system included classes 1 through 5 which were defined: 1 = no browning; 2 =external browning at wound site; 3 = internal browning at wound site; 4 = internal browning spreading from wound site; and 5 = browning through the complete root and plant dead. The second rating system consisted of classes 1 and 2, which were

defined as: 1 = no internal browning and 2 = internal browning.

Duncan's new multiple range test (13) was used to test for significant differences between treatments. A factorial analysis, based on orthogonal comparison (12) was used to test for significance of interaction between stresses.

Fusarium-aphid interaction.—*Root rot severity.*—1) Red clover.—Twenty-four 5-month-old plants of red clover (*Trifolium pratense* L.'Pennscott') were divided randomly into four groups, each with six replicate plants. Plants in aphid- and *Fusarium*-aphid treatments were caged in the greenhouse with aphid-infested broad beans (*Vicia faba* L.). Plants in the control and the *Fusarium* treatments were caged adjacently without broad beans or aphids. Roots of the plants in the *Fusarium* and *Fusarium* -aphid treatments were inoculated with three isolates of *F. roseum* Lk. ex Fr. known to be pathogenic to red clover. Inoculum consisted of mycelia, macroconidia, and agar, from one petri-dish culture of each isolate, comminuted for 20 seconds in 100 ml of distilled water. Two milliliters of the fungus slurry were pipetted over the wound in the taproot, and then soil was replaced over the wound. Tops were harvested 48 and 81 days later. The experiment was terminated after 133 days when the tops were weighed and root rot was scored.

2) White clover.—Two separate but similar experiments were done with white clover (*T. repens* L. 'Regal'). Three-month-old plants were used in both experiments, six plants per treatment. In the first experiment, plants were caged individually and randomly arranged in a growth chamber programmed for a 14-hour photoperiod (cool-white fluorescent and incandescent illumination at 20,000 lux) at 25 ± 1 C, alternating with a 10-hour dark period at 18 ± 1 C. At the start of the experiment, appropriate plants received 10 aphids each and root inoculations with *F. roseum* known to be pathogenic on white clover. Inoculation was made as described for red clover. The duration of the experiment was 24 days.

In the second experiment, plants were caged individually in a growth chamber programmed for a 16-hour photoperiod at 21 ± 1 C alternating with an 8-hour dark period at 18 ± 1 C. Aphid introductions and root inoculations were done as described for the first experiment. Duration of this experiment was 81 days; plants were cut back on the 51st day, and again 10 aphids were placed on each plant.

3) Alfalfa.—Alfalfa (*Medicago sativa* L. 'Saranac') was used in two similar experiments. Eight plants per treatment were caged individually in growth chambers, as in the white clover experiments. A single isolate of *F. roseum* 'Avenaceum', pathogenic to alfalfa, was used to inoculate roots. The initial aphid population in these experiments was 10 per plant. Both experiments ran for 47 days. Plants were 4 months old at the start of the first experiment and 5 months old at the start of the second experiment.

Plant survival.—In tests with Pennscott red clover and Atlantic alfalfa, aphid feeding continued until all the plants died in one treatment. The number of survival days was recorded for each individual plant and the mean survival days per treatment was determined. The maximum number of survival days equaled the duration of the test. Survival data for red clover were obtained from the experiment described in the section on root rot severity. Survival data for alfalfa were obtained in a separate experiment. Plants were 5.5 months old when they were caged with aphids in the greenhouse at a rate of three aphids per plant. Two days later, roots were inoculated with *F.* oxysporum Schlecht. known to cause root rot on alfalfa. Duration of this experiment was 48 days.

Fusarium-leafhopper interaction.—*Plant survival.*—Plants of experimental breeding populations MSA and MSB (4) were grown in the greenhouse for 6 months, then divided into four groups of 120 plants each. Each group received one treatment. Plants in appropriate treatments were inoculated, as described for red clover, with *F. roseum* known to be pathogenic on alfalfa. The plants were transplanted on 6 June 1974 to ground beds containing field soil in a screen-house insectary. The insectary was sprayed with malathion, and weeds were removed before transplanting. Plastic screening covered the roof and sides of the insectary and divided it into two equal rooms that measured 3.5 m × 3.5 m × 3.5 m high. There were four ground beds, each measuring 1 m wide × 1.5 m long, in each insectary room. Experimental design was as follows: one room contained potato leafhoppers; one room was maintained insect-free. Two replicates of each treatment were contained in each insectary room. A replicate consisted of 60 plants.

Plant tops were harvested, dried, and weighed on 26 July and 6 September 1974. Plant counts were made on 26 July and 6 September 1974 and again on 1 April 1975, when all plants were dug. Roots were washed, trimmed to the top 5-cm of taproot, and dried at 60 C. Total

TABLE 1. Severity of root rot, caused by *Fusarium roseum*, of forage legumes grown with and without the stress of pea-aphid feeding

Stress treatment	Root rot rating[a]			
	Red clover	White clover	Alfalfa	All species
None (control)	2.3 A[b]	1.4 A	1.5 A	1.7 A
Fusarium	3.2 AB	2.1 B	2.1 B	2.4 B
Aphids	3.8 B	1.9 B	1.8 AB	2.3 B
Fusarium + aphids	5.0 C	3.1 C	3.1 C	3.6 C

[a]Root rot rating scale: 1 = no browning; 2 = external browning at wound site; 3 = internal browning at wound site; 4 = internal browning spreading from wound site; and 5 = internal browning completely through root and plant dead.

[b]Means followed by different letters within a column are significantly different, $P = 0.01$, as determined by Duncan's new multiple range test.

nonstructural carbohydrates of these upper root segments were determined using the technique of Smith (10).

Leafhoppers were netted in a nearby alfalfa field, collected from the nets with an aspirator, and counted into groups of 200 which were released into half of the insectary three times prior to the July harvest and twice thereafter. After the July harvest, both rooms of the insectary were sprayed thoroughly with malathion because aphids had infested some of the plants. All screening was removed from the insectary on 27 September 1974 to expose the plants to normal winter conditions.

RESULTS

General.—The mean plant fresh weights of harvested tops, as derived from all data, from all experiments, were: control, 9.0 g; *Fusarium*, 7.8 g; aphids, 6.5 g; and *Fusarium* + aphids, 3.9 g. All experiments followed this pattern, which indicated that when yield was used as the criterion, the treatments imposed differential stress.

The mean numbers of aphids per plant, as derived from all counts and all experiments were: control, 0; *Fusarium*, 0; aphids, 138; and *Fusarium* + aphids, 91. In every experiment, more aphids survived per plant in the aphid treatment than in the *Fusarium* + aphid treatment.

Fusarium-aphid interaction.—*Root rot severity.*—In every case, the root rot ratings for the *Fusarium* + aphid treatment were significantly greater than those of any other treatment (Table 1). Root rot ratings for control plants were lower than those of plants in the other treatments, although these differences were not always significant. Root rot in the treatment with *Fusarium* alone never differed significantly from that in the treatment with aphids alone. Factorial analyses of internal root rot ratings showed a significant interaction for *Fusarium* + aphids (Table 2).

Plant survival.—Because of similar results with red clover and alfalfa, data were pooled from the two experiments (Table 3). Mean days until death in the *Fusarium* treatment were about the same as in the control. Mean days until death were significantly less in the aphid treatment than in the control, but did not differ from the *Fusarium* treatment. Plants subjected to the combined stress of *Fusarium* plus aphid feeding died more quickly than did plants in any other treatment. Orthogonal comparison indicated that the combined effect of *Fusarium* and aphids significantly exceeded the additive effects of these two organisms acting separately.

Fusarium-leafhopper interaction.—*Survival of alfalfa plants.*—The effect of leafhopper feeding was apparent at the July harvest (Table 4) and was more pronounced at the September harvest. No effect of the treatments on plant numbers was evident, however, until the spring of 1975 after the plants had undergone winter conditions (Table 4). At this time, significantly fewer plants were alive in the combined *Fusarium*-leafhopper treatment than in any other treatment. Factorial analysis indicated a significant interaction between *Fusarium* and leafhopper feeding. The total nonstructural carbohydrate analysis of roots dug in April 1975 showed a relationship with the treatments imposed during the previous growing season. The percentage of carbohydrates of roots in the control treatment was 7, in the *Fusarium* treatment 4, in the leafhopper treatament 2, and in the *Fusarium*-leafhopper treatment 1.

TABLE 2. Factorial analysis, by orthogonal comparison, of internal rot caused by *Fusarium roseum*

Stress treatment	Root rot rating[a]	F-value[b]
None (control)	1.1	
Fusarium	1.3	35.25**
Aphids	1.2	22.56**
Fusarium + aphids	1.8	5.64*

[a]Root rot rating scale: 1 = internal rot absent and 2 = internal rot present. Data were pooled from aphid experiments with alfalfa, and red and white clovers.

[b] Asterisks* and **, indicate significance at $P = 0.05$ and 0.01, respectively.

TABLE 3. Mean number of survival days for red clover inoculated with *Fusarium roseum* and alfalfa inoculated with *F. oxysporum* grown with and without the stress of pea-aphid feeding

Stress treatment	Days until death[a]
None (control)	84 A[b]
Fusarium	81 AB
Aphids	77 B
Fusarium + aphids	60 C

[a]Values are means from combined results of two survival experiments.

[b]Values followed by different letters are significantly different, $P = 0.01$, as determined by Duncan's new multiple range test.

TABLE 4. Yield and stand counts of alfalfa grown under separate and combined stresses of root rot caused by *Fusarium roseum* feeding injury caused by potato leafhoppers

	Dry wt. yield (g/row)		Plants per row		
Stress treatment	26 July 74	6 Sep 74	26 July 74	6 Sep 74	1 Apr 75
None (control)	29.7 A[a]	18.8 A	14.2 A	13.0 A	8.8 A
Fusarium	32.2 A	22.4 A	14.4 A	12.6 A	8.8 A
Leafhoppers	21.6 B	10.6 B	13.8 A	12.1 A	7.4 A
Fusarium + leafhoppers	22.8 B	10.5 B	14.1 A	12.1 A	3.5 B

[a]Values followed by different letters within a column are significantly different, $P = 0.01$, as determined by Duncan's new multiple range test.

DISCUSSION

Some wound sites on roots of control plants had external browning of the callused, scar tissue. The tissues were not rotted or decayed, and the browning was quite superficial. This was most pronounced on red clover. Roots of some red clover plants in the aphid-alone treatment were slightly rotted internally, which could have been caused by extraneous soilborne pathogens and slower healing of the scalpel wounds owing to the stress of the aphid feeding. Clearly, the most severe rot developed in plants inoculated with *Fusarium* and subjected to aphid feeding, but it is not known whether this was due to the increased susceptibility of the plants or to increased pathogenicity of the fungus because of more favorable nutrition.

Insect feeding injury occurs on perennial forages in the field virtually throughout the growing severity levels equal to or greater (11, 15) than those imposed in our experiments. Feeding by leafhoppers can reduce root reserves (14), weaken root systems (3), and cause thinning of stands (1). Aphid injury has predisposed plants to winterkilling (5). None of these reports mentioned interaction with diseases, but this possibility was not eliminated.

Roots of forage legumes are colonized by *Fusarium* species soon after plants begin to grow (8). These fungi are ubiquitous in soils where forages are grown, and the presence of at least some surface discoloration on legume roots is the rule rather than the exception. Generally, the development of severe internal rot is slow, often taking years to kill the taproot. The rate of rot development is greater when the plants are stressed (6). Our results with insect-feeding stress further substantiate this relationship.

The results of the leafhopper experiment indicate that the interaction of insect feeding and root pathogens may not be apparent immediately. In our experiment, plants did not die until they were exposed to winter conditions. Indeed, it is quite likely that if conditions during the previous season were not known, plant losses would have been attributed solely to winter injury. These results also point out that stand appearance after the final harvest in the fall may lead to the conclusion that all is well, when this is not actually the case. The poor performance of perennial forages one year may be the result of the previous year's problems.

The interaction of insect feeding with the development of root and crown rots is important because it points out the fallibility of considering insect infestation and disease incidence as separate, discrete problems. Perennial forages are subjected throughout their entire lives to a series of biotic and abiotic stresses that occur sometimes simultaneously, sometimes sequentially, but always cumulatively.

LITERATURE CITED

1. DAVIS, R. L., and M. C. WILSON. 1953. Varietal tolerance of alfalfa to the potato leafhopper. J. Econ. Entomol. 46:242-245.
2. DENIS, S. J. 1968. The red clover vein mosaic virus-Fusarium root rot complex in red clover. Ph. D. Thesis. West Va. University. Morgantown. 65 p.
3. GRANOVSKY, A. A. 1928. Alfalfa "yellow top" and leafhoppers. J. Econ. Entomol. 21:261-267.
4. HANSON, C. H. 1969. Registration of alfalfa germplasm (Reg. Nos. 1 to 7). Crop Sci. 9:526-527.
5. HARPER, A. M., and C. E. LILLY. 1966. Effects of pea aphid on alfalfa in southern Alberta. J. Econ. Entomol. 59:1426-1427.
6. LEATH, K. T., F. L. LUKEZIC, H. W. CRITTENDEN, E. S. ELLIOTT, P. M. HALISKY, F. L. HOWARD, and S. A. OSTAZESKI. 1971. The Fusarium root rot complex of selected forage legumes in the Northeast. Penn. Agric. Exp. Stn. Bull. 777. 64 p.
7. NEWTON, R. C., R. R. HILL, JR., and J. H. ELGIN, JR. 1970. Differential injury to alfalfa by male and female potato leafhoppers. J. Econ. Entomol. 63:1077-1079.
8. O'ROURKE, C. J., and R. L. MILLAR. 1966. Root rot and root microflora of alfalfa as affected by potassium nutrition, frequency of cutting, and leaf infection. Phytopathology 56:1040-1046.
9. SIDDIQUI, W. M., P. M. HALISKY, and S. LUND. 1968. Relationship of clipping frequency to root and crown deterioration in red clover. Phytopathology 58:486-488.
10. SMITH, D. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Res. Rep. No. 41, Res. Div., Coll. Agric. Life Sci. Univ. Wisconsin, Madison. 5 p.
11. SMITH, D., and J. T. MEDLER. 1959. Influence of leafhoppers on the yield and chemical composition of alfalfa hay. Agron. J. 51:118-119.
12. SNEDECOR, G. W. 1956. Chapter 12 *in* Statistical methods, 5th ed. Iowa State Univ. Press, Ames. 534 p.
13. STEEL, R. G. D., and J. H. TORRIE. 1960. Pages 107-109 *in* Principles and procedures of statistics. McGraw-Hill, New York. 481 p.
14. WILLARD, C. J. 1930. Root reserves of alfalfa with special reference to time of cutting and yield. J. Am. Soc. Agron. 22:595-602.
15. WILSON, M. C., R. L. DAVIS, and G. G. WILLIAMS. 1955. Multiple effects of leafhopper infestation on irrigated and nonirrigated alfalfa. J. Econ. Entomol. 48:323-326.