Foliar Diseases Alter Carbohydrate and Protein Levels in Leaves of Alfalfa and Orchardgrass

A. Mainer and K. T. Leath

Former Graduate Assistant, Department of Plant Pathology, The Pennsylvania State University; and Research Plant Pathologist, Science and Education Administration, Federal Research, U.S. Department of Agriculture (also Assoc. Prof. of Plant Pathology, The Pennsylvania State University), respectively, U.S. Regional Pasture Research Laboratory, University Park, PA 16802. Present address of senior author: Centro Regional de Investigaciones Agrarias, Apartado 10, La Coruña, Spain.

The authors thank R. R. Hill, Jr. for statistical advice and R. Kerns and E. Quigley for technical assistance.

Authorized for publication 26 September 1977 as Journal Series Paper 5388 of the Pennsylvania Agricultural Experiment Station and Contribution No. 445 from the U.S. Regional Pasture Research Laboratory.

Accepted for publication 6 March 1978.

ABSTRACT

MAINER, A., and K. T. LEATH. 1978. Foliar diseases alter carbohydrate and protein levels in leaves of alfalfa and orchardgrass. Phytopathology 68: 1252-1255.

The percentage of crude protein (CP), total nonstructural carbohydrate (TNC), and dry matter (DM) levels were investigated with alfalfa and orchardgrass infected with *Phoma medicaginis* and *Stagonospora arenaria*, respectively. In alfalfa, the TNC and CP were decreased and the DM increased when the infection severity level was greater than 80%. Crude protein was significantly reduced in orchardgrass leaves at the 20% disease level, TNC at the 50% disease level, and DM was increased at all levels of disease. Crude protein was higher in healthy new growth from diseased alfalfa plants than CP in regrowth from healthy plants. In bulk-harvested orchardgrass the TNC percentage, TNC yield, and DM yield were reduced. We concluded that foliar diseases reduced the TNC and CP of both alfalfa and orchardgrass leaf tissues sufficiently to adversely affect the quality of the forage and that the loss of TNC was greater than that of CP.

Additional key words: *Medicago sativa*, *Dactylis glomerata*, forage quality.

Foliar diseases of forage crops occur worldwide and are a major factor contributing to poor quality forage (9, 10). Foliar diseases reduce forage quality by defoliation, by inducing higher levels of undesirable constituents, and by reducing the amount of desirable constituents.

Different effects of foliar disease on forage quality constituents have been reported. For example, Carr (5, 6) and Isawa et al. (12, 13) reported that carbohydrates were reduced by disease, whereas Carr (5, 6) and Davies and Williams (7) found that carbohydrate levels were higher in diseased leaves than in healthy leaves. Protein levels were reduced by some foliar diseases (3, 4, 12, 13), but were unaffected by other diseases (19, 20). Burton (4) and Isawa et al. (12, 13) reported that foliar diseases caused the dry matter percentage of leaves to increase. Only Brigham (3), Carr (5, 6) and Isawa et al. (12, 13) studied the effects of different severities of disease on quality.

The objective of this research was to compare, over a range of severities and under controlled conditions, the effect of specific foliar diseases on the protein and carbohydrate levels in alfalfa and orchardgrass leaf tissues.

MATERIALS AND METHODS

Alfalfa (*Medicago sativa* L. 'Arc'), and orchardgrass (*Dactylis glomerata* L. 'Pennlate') were used in all experiments. Plants were grown in metal utility carts in the greenhouse, as described previously (14). Ninety-nine alfalfa and 66 orchardgrass plants were seeded per cart, fertilized as needed with a soluble complete fertilizer, and cut back to a 5-cm height after each evaluation. Insects were controlled with stubble sprays of diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] and oxythioquinox [cyclic *S.S*-(6-methyl-2,3-quinoxalinedithiol) dithiocarbonate] after plants were cut back, and with weekly foliar sprays of oxythioquinox and malathion (*O,O*-dimethyl phosphorodithioate of diethyl mercaptosuccinate). No insecticides were applied for at least 1 wk before inoculation.

Phoma medicaginis Malbr. et Roum. was isolated from alfalfa and cultured on potato-dextrose agar. *Stagonospora arenaria* Sacc. was isolated from orchardgrass and cultured on vegetable juice agar (15). Fungi were cultured at 22 C, and irradiated for 12 hr/day with cool-white fluorescent light. Conidial suspensions were prepared by scraping the surface of the sporulating fungal cultures, comminuting the scrapings in distilled water plus surfactant (polyoxyethylene sorbitan monolaurate) for 20 sec, and filtering through cheesecloth. Alfalfa was inoculated by spraying 50 ml of inoculum on plants in each cart; orchardgrass received 100 ml of inoculum per cart. Controls received appropriate amounts of water with surfactant. Details for each inoculation are described in Table 1. After inoculation, plants were maintained for 40 to 48 hr in a large incubation chamber (14), then returned to the greenhouse.

00032-949X/78/000 225$03.00/0

Fresh plant samples were weighed, frozen in liquid nitrogen, stored in a freezer, lyophilized, weighed, ground through a 0.97-mm (20-mesh) sieve, and chemically analyzed. The percentage of dry matter (DM) per fresh weight was calculated. The percentage of crude protein (CP) (dry wt basis) was determined by the improved Kjeldahl method (1) and of total nonstructural carbohydrate (TNC) as described by Smith (17).

Two types of alfalfa samples were collected. The first type consisted of hand-picked leaflets in three disease classes, 0, 25 to 50, or 80% infection. The second type consisted of healthy terminal shoots, produced after inoculation, that were clipped as separate samples from diseased or healthy plants. One type of orchardgrass sample consisted of hand-picked leaf blades categorized as to 0, 5, 20, 50, or 80% infection. Leaves with no infection were taken only from healthy plants in the control cart. A second sample type consisted of healthy young leaf blades, produced after inoculation, and clipped in separate samples from diseased or healthy plants. A third sample type consisted of all remaining forage in each cart, bulk-harvested with shears. The fresh weight of the harvest from each cart was used to calculate the yields of DM, CP, and TNC per square meter. The analysis of variance and the LSD test were used to determine the significance of differences among treatments.

RESULTS

Typical discrete lesions appeared on lightly infected alfalfa and orchardgrass leaves but heavily infected leaf tissues became chlorotic, the lesions coalesced, and some of the most heavily infected leaf tissues collapsed and dried out in 4 to 6 days. Some heavily infected leaflets of alfalfa abscised before samples were collected, but entire alfalfa leaves rarely abscised and only when all three leaflets were severely diseased. *Phoma medicaginis* rarely caused petiole or stem lesions.

In alfalfa leaflets rated at >80% infection level, CP was 25% lower, TNC was 62% lower, and DM was 77% higher than in healthy leaflets (Table 2). Quality constituents of alfalfa with 25-50% level of infection severity were not different from those of the healthy controls. In orchardgrass, TNC was not different from the healthy leaves until infection severity reached 50% (Table 2), but crude protein was significantly reduced by an infection level of 20%. All levels of infection increased DM in orchardgrass.

Bulk-harvested, diseased orchardgrass was lower in TNC than was bulk-harvested healthy orchardgrass (Table 3). The percent protein reduction detected in the leaf samples did not occur in the bulk-harvested samples. The gram yields of TNC and DM were reduced by disease in orchardgrass, but that of CP was not.

With both alfalfa and orchardgrass, the TNC and DM of healthy regrowth was the same regardless of whether the regrowth came from noninfected or infected plants. This was not true for CP. Regrowth from infected alfalfa plants was 4.3% higher ($P = 0.05$) in CP than was regrowth from healthy plants. A similar but less significant relationship occurred in orchardgrass.

DISCUSSION

Foliar diseases reduce rates of photosynthesis and protein synthesis, increase rates of transpiration and respiration, and cause cell necrosis (2, 11, 16, 18). Gross effects on forage quality are manifested as increased DM, and decreased TNC and CP (2, 8).

Carbohydrates are readily utilized by foliar pathogens (16) and were decreased in both alfalfa and orchardgrass at the higher levels of infection. This reduction of carbohydrates caused by disease not only lowered the

TABLE 1. Details of inoculations of alfalfa with *Phoma medicaginis* and of orchardgrass with *Stagonospora arenaria*

Host and inoculation	Previous harvests (no.)	Age of plant regrowth (days)	Spore concentration (per ml)	Plant material sampled	Days from inoculation	No. of replications
Alfalfa						
1	3	24	2.5×10^6	Leaflets	10	3
				Healthy regrowth	10	3
2	6	37	1.0×10^6	Leaflets	8	3
				Healthy regrowth	8	3
3	4	35	1.0×10^6	Leaflets	9	3
				Healthy regrowth	9	3
Orchardgrass						
1	0	55	5.0×10^5	Leaf blades	11	3
				Bulk harvest	15	2
				Healthy regrowth	15	3
2	1	38	7.5×10^5	Leaf blades	10	3
				Bulk harvest	10	2
3	3	43	6.6×10^5	Leaf blades	11	3
				Bulk harvest	13	3
4	4	67	9.7×10^5	Leaf blades	14	3
				Bulk harvest	20	2
				Healthy regrowth	20	3

energy content of the forage, but also likely would cut forage yields, as evidenced by the reduced dry matter production of bulk-harvested, diseased orchardgrass.

Crude protein was reduced in proportion to disease severity, though to a smaller degree than was TNC. Possibly proteins were not readily available to the fungus and, thus, not utilized to the same extent as was the TNC. Reduction in CP was significant at lower levels of infection in orchardgrass than in alfalfa. The higher CP percentage in new growth from diseased plants than in new growth from healthy plants could have resulted from a reduced demand for nitrates in diseased leaves, making more nitrates available for protein synthesis in the new growth. Crude protein was reduced by disease in the orchardgrass individual leaf samples but not in the bulk-harvested samples. Higher levels of CP in the healthy new leaves from diseased plants could have compensated for the lower CP levels of the diseased leaves.

In general, diseased leaves had a higher dry matter percentage. From the standpoint of forage quality, the higher DM is probably important because of its effect on palatability and intake of green forage.

We concluded that foliar disease of both alfalfa and orchardgrass reduced the TNC and CP of leaf tissue sufficiently to adversely affect the quality of the forage and that the loss of TNC was greater than was the loss of CP. These losses reflect only changes in constituent levels and would be much higher if defoliation were included.

Our results support the reports of adverse effects by disease on forage quality (3, 5). Different sampling methods or disease levels could easily have accounted for the lack of agreement as to the effects of foliar disease on forage quality that is found in the literature. We would expect similar reductions in TNC and CP for most foliar pathogens because of their similar modes of pathogenicity and nutritional requirements.

TABLE 2. Comparison of three quality factors in leaves of alfalfa and orchardgrass at various levels of infection caused by *Phoma medicaginis* or *Stagonospora arenaria*, respectively

Forage species and infection level (%)	Total nonstructural carbohydrate (%)	Crude protein (%)	Dry matter (%)
Alfalfa[x]			
0	13.4[z] a	29.8 a	21.9 a
25-50	10.7 a	27.6 a	23.3 a
>80	5.0 b	22.3 b	38.6 b
Orchardgrass[y]			
0	4.6 a	24.7 a	17.1 a
5	4.1 ab	23.4 ab	21.0 b
20	3.6 ab	22.1 b	21.2 bc
50	2.8 bc	19.7 c	24.4 c
80	1.5 c	16.8 d	33.9 d

[x]Data are means of nine replicates from inoculations 1, 2, and 3.

[y]Data are means of 12 replicates from inoculations 1, 2, 3, and 4.

[z]Means in the same column for a single plant species followed by different letters are significantly different, $P = 0.05$.

TABLE 3. Comparison of three quality factors in bulk-harvested orchardgrass forage with or without infection by *Stagonospora arenaria*

	Total nonstructural carbohydrate		Crude protein		Dry matter	
Forage	(%)	(g/m^2)	(%)	(g/m^2)	(%)	(g/m^2)
Noninfected	7.0[z] a	12.5 a	22.4 a	36.3 a	16.0 a	166.8 a
Infected	4.5 b	6.1 b	23.0 a	31.2 a	18.4 b	143.7 b

[z]Data are means of nine replicates from inoculations 1, 2, 3, and 4. Means in the same column followed by different letters are significantly different, $P = 0.05$.

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1970. Official methods of analysis. (Nos. 2051 and 7016) 11th ed. Washington, D.C. 1015 p.
2. BOLLARD, E. G., and R. E. F. MATTHEWS. 1966. The physiology of parasitic disease. Pages 417-550 *in* F. C. Steward, ed. Plant physiology, a treatise, Vol. 4B. Academic Press, New York. 599 p.
3. BRIGHAM, R. D. 1959. Effect of Cercospora disease on forage quality of alfalfa. Agron. J. 51:365.
4. BURTON, G. W. 1954. Does disease resistance affect forage quality? Agron. J. 46:99.
5. CARR, A. J. H. 1962. Disease assessment. Rep. Welsh Plant Breed. Stn. 1961:100-103.
6. CARR, A. J. H. 1963. Disease assessment. Rep. Welsh Plant Breed. Stn. 1962:82-84.
7. DAVIES, H., and A. E. WILLIAMS. 1970. The effect of mildew and leaf blotch on yield and quality of CV. Lior Italian ryegrass. Plant Pathol. 19:135-138.
8. GOODMAN, R. N., Z. KIRÁLY, and M. ZAITLIN. 1967. The biochemistry and physiology of infectious plant disease. Van Nostrand Co., New York. 354 p.
9. GRAHAM, J. H., K. W. KREITLOW, and L. R. FAULKNER. 1972. Diseases. Pages 497-526 *in* C. H. Hanson, ed. Alfalfa science and technology. Agronomy Monograph No. 15. 812 p.
10. HANSON, C. H. 1965. Foliar diseases and forage quality. Pages 1209-1213 *in* Proc. of the 9th Int. Grassland Congr., 7-20 January 1965, São Paulo, Brazil. 1974. p.
11. HUSAIN, A., and A. KELMAN. 1959. Tissue is disintegrated. Pages 143-188 *in* J. G. Horsfall, and A. E. Dimond, eds. Plant pathology, an advanced treatise. Vol. 1. Academic Press, New York. 674 p.
12. ISAWA, K., A. ABE, and N. NISHIHARA. 1974. Chemical composition of Italian ryegrass infected with crown rust. Ann. Phytopathol. Soc. Japn. 40:86-92.
13. ISAWA, K., and N. NISHIHARA. 1975. Chemical composition of ladino clover infected with Curvularia leaf blight. Bull. Nat. Grassland Res. Inst. 7:99-106.
14. LEATH, K. T., and R. R. HILL, JR. 1974. Large incubation chamber suited for use in selection for disease resistance. Crop Sci. 14:901-903.
15. MILLER, P. M. 1955. V-8 juice as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.
16. SEMPIO, C. 1959. The host is starved. Pages 277-312 *in* J. G. Horsfall, and A. E. Dimond, eds. Plant pathology, an advanced treatise, Vol. 1. Academic Press, New York. 674 p.
17. SMITH, D. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wis. Agric. Exp. Stn. Res. Rep. 41. 11 p.
18. SUBRAMANIAN, D., and L. SARASWATHI-DEVI. 1959. Water is deficient. Pages 313-348 *in* J. G. Horsfall, and A. E. Dimond, eds. Plant pathology, an advanced

treatise, Vol. 1. Academic Press, New York. 674 p.
19. SUMMERS, C. G., and W. D. MC CLELLAN. 1975. Effect of common leafspot on yield and quality of alfalfa in the San Joaquin Valley of California. Plant Dis. Rep. 59:504-506.
20. WILLIS, W. G., D. L. STUTEVILLE, and E. L. SORENSEN. 1969. Effects of leaf and stem diseases on yield and quality of alfalfa forage. Crop Sci. 9:637-640.