Effect of Rust on Yield and Digestibility of Pearl Millet Forage

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We thank Richard Layton for preparing the graphs.

Accepted for publication 1 October 1990.

ABSTRACT

Wilson, J. P., Gates, R. N., and Hanna, W. W. 1991. Effect of rust on yield and digestibility of pearl millet forage. Phytopathology 81:233-236.

The effects of infection by *Puccinia substriata* var. *indica* on yield and digestibility of pearl millet forage was examined in 1988 and 1989. Dwarf hybrids Tifleaf 1 (susceptible) and Tifleaf 2 (resistant), and tall hybrids Gahi 3 (susceptible) and Tift 85DA×186 (experimental cultivar Gahi 4, resistant) were inoculated or treated with fungicide after the first harvest to establish different levels of disease. For both dwarf and tall hybrids, there were no consistent differences in yield or digestibility between the disease-free plots of susceptible and resistant cultivars. Green yield, dry-matter yield, and forage quality as measured by in vitro digestibility were negatively correlated with final rust severity and area under the disease progress curve of dwarf and tall hybrids. Dry-matter concentration was unaffected by disease. Loss of digestible dry-matter yield could be expressed by either curvilinear or linear functions of final disease severity. In three of four experiments, the rate of loss of digestible dry-matter yield was greater at low rust severities than at higher rust severities, which indicated that highly effective resistance is necessary to reduce losses attributable to rust.

Additional keywords: *Pennisetum glaucum*, yield loss.

Rust of pearl millet (*Pennisetum glaucum* (L.) R. Br. K. Schum.) has occurred annually in the southeastern United States since a severe epidemic developed in 1972 (10). At Tifton, GA, infection foci of the disease, caused by *Puccinia substriata* Ellis & Barth. var. *indica* Ramachar & Cummins, are first observed from early July to mid-August. The extent of damage can vary, depending on the time of initial infection, the age of the crop at infection, and weather conditions.

The detrimental effects of rust on pearl millet have not been well documented. A study conducted for 1 yr indicated that rust severities of 29% or greater on the flag leaf reduced grain yield and thousand-grain weight (8). In that experiment, inoculations were applied when the plants were in the boot stage; it was not indicated whether this was typical of natural rust development.

Potential damage by diseases of forage plants includes both yield reduction and loss of nutritional value. Nutritional value is assessed primarily by digestibility and may be considered for each individual nutrient (protein, soluble carbohydrate, cell wall, etc.) or for the dry-matter aggregate. Digestibility may be determined in an animal-feeding trial. Alternatively, in vitro digestibility may be quantified with a two-stage procedure in the laboratory. Dry-matter loss after 48 hr of anaerobic incubation with rumen fluid and sequential incubation with acid pepsin (9) provides precise estimation of digestibility, generally in good agreement with live-animal evaluation.

The effects of rust infection on pearl millet forage have been examined in segregating families from a program aimed at backcrossing rust resistance into a susceptible inbred (4). Rust infection had a detrimental effect on pearl millet forage. Dry-matter concentration, dry-matter yields, in vitro dry-matter digestibility, and digestible dry-matter yields were reduced in rust-infected plants compared with rust-free plants. A 51% reduction in digestible dry-matter yield was measured. However, the relationship to rust severity was not quantified. In addition, the test determined effects of rust on near-isogenic inbreds; however, no information is available about losses from rust sustained by commercial hybrids. The present experiments were conducted to determine the response relationship between yield and digestibility of pearl millet forage hybrids and rust severity.

MATERIALS AND METHODS

Plot establishment. Dwarf hybrids Tifleaf 1 and Tifleaf 2 and tall hybrids Gahi 3 and the experimental cultivar Tift85DA×186 (Gahi 4) were evaluated. Tifleaf 1 and Gahi 3 are susceptible to *P. s. indica*, and Tifleaf 2 and Gahi 4 are resistant. Dwarf and tall cultivars were evaluated in different experiments and planted in solid-seeded, 4-m long, single row plots spaced 2 m apart. Plots were planted 22 June 1988 and 24 June 1989. Fertilizer (5-10-15 NPK) was applied in-furrow at planting at the rate of 280 kg/ha. Plots were arranged in a randomized complete block design with six replications. Within each replication, three plots of the resistant hybrids were planted, and either seven (1988) or nine (1989) plots of the susceptible hybrids were sown. To prevent early infection by *Pyricularia grisea* (Cooke) Sacc., plots of the dwarf cultivars were sprayed to runoff by using a hand-sprayer with the fungicide chlorothalonil (Bravo 720, 8 ml/L) 2 wk after emergence. Plots were harvested with a forage chopper, 11 August 1988 and 15 August 1989. Green yield was determined for each plot.

Disease establishment. Approximately 2 wk after the first harvest, plots were either protected with chlorothalonil (applied as described), left untreated, or inoculated at intervals to establish different rust severities among the plots. The treatments are described in Table 1.

The first inoculation of each season was performed about 3 wk after natural infection was first observed in the field so that disease would develop at near-natural conditions. A urediniospore suspension (2×10^4 spores per milliliter) was misted onto approximately 15 culms in the center of the plot; they were then covered overnight (16 hr) with a plastic bag held in place by a rubber band. In subsequent inoculations, the urediniospore suspension was sprayed into five whorls distributed along the length of treated plots.

As infections developed, the percentage of foliage with uredinia or killed by rust infection was estimated at 7- to 8-day intervals with the aid of standard diagrams (7). Final disease ratings were made 10 days before harvest. Area under the disease progress curve (AUDPC) was calculated by the equation

$$\text{AUDPC} = \Sigma\{[Y_{i+1} + Y_i]/2\}[X_{i+1} - X_i],$$

in which Y_i is the percentage of disease severity at time X_i.

Second harvests were taken on 18 October 1988 and 22 October 1989, when grain was in the late-milk stage of development. Green yield, dry-matter yield, and digestibility were determined for each plot.

Digestibility of whole plant samples was estimated with a two-stage in vitro incubation (9). Samples retained from each plot at harvest were oven-dried (60 C) and ground to pass a 1-mm screen. Rumen fluid, obtained from a cannulated steer fed bermudagrass hay, was mixed in a 1:1 ratio with McDougall's buffer. Samples of known dry weight (0.4 g) were inoculated with 35 ml of rumen-fluid buffer, gassed with carbon dioxide, and incubated at 39 C for 48 hr in 50-ml centrifuge tubes with rubber stopper seals and gas escape valves to maintain anaerobic conditions. Control tubes were included to account for dry matter added in the rumen fluid. Tubes were centrifuged, the supernatant was decanted, and the tubes were incubated for an additional 48 hr after the addition of 35 ml of acid pepsin. Residual dry matter was determined after centrifuging, decanting, and oven-drying. The proportion of dry matter digested in vitro was calculated as 1 − [(residue + control residue)/initial sample weight].

Data were analyzed by analysis of variance and regression analysis. Before analysis of variance, green yield and dry-matter yield were transformed to log(kg yield + 1), final rust severities were transformed to log(% rust + 1), and AUDPCs were transformed to $\sqrt{(\text{AUDPC} + 1)}$ to reduce associations between

TABLE 1. Treatments used to establish different rust severities on plots of forage pearl millet hybrids

Treatment number	Cultivar type[a]	1988		1989	
		Treatment[b]	Dates	Treatment	Dates
1	Resistant	F	8-25,9-2,9-12,9-18	F	8-25,9-5,9-14,9-21,10-2,10-13
2	Resistant	U		U	
3	Resistant	I	8-21,8-28	I	8-23,9-1,9-8
4	Suceptible	F	8-25,9-2,9-12,9-18	F	8-25,9-5,9-14,9-21,10-2,10-13
5	Susceptible	F	8-25,9-2,9-12	F	8-25,9-5,9-14,9-21
6	Susceptible	F	8-25,9-2	F	8-25,9-5,9-14
7	Susceptible	F	8-25	F	8-25,9-5
8	Susceptible	U		F	8-25
9	Susceptible	I	8-21	U	
10	Susceptible	I	8-21,8-28	I	9-8
11	Susceptible	...		I	9-1,9-8
12	Susceptible	...		I	8-23,9-1,9-8

[a]Resistant cultivars, Tifleaf 2 (dwarf) and Gahi 4 (tall); susceptible cultivars, Tifleaf 1 and Gahi 3.
[b]F = fungicide, U = untreated, and I = inoculated.

TABLE 2. Treatment means for yield, digestibility, and rust severities of forage pearl millet hybrids

	Dwarf hybrids[a]						Tall hybrids[a]					
Treatment	GY log(kg+1)	DMC (%)	DMY log(kg+1)	IVDMD (%)	Rust log(rust+1)	AUDPC $\sqrt{(\text{AUDPC}+1)}$	GY log(kg+1)	DMC (%)	DMY log(kg+1)	IVDMD (%)	Rust log(rust+1)	AUDPC $\sqrt{(\text{AUDPC}+1)}$
1988												
1	1.30	23.8	0.74	58.2	0.20	3.6	1.27	26.2	0.75	58.0	0.06	2.5
2	1.21	24.6	0.67	56.7	0.36	4.6	1.24	25.1	0.72	53.5	0.06	3.0
3	1.26	23.8	0.71	55.7	0.30	4.8	1.28	24.7	0.73	51.8	0.03	3.1
4	1.19	24.3	0.65	58.2	0.62	8.5	1.31	25.6	0.77	52.9	0.13	3.5
5	1.25	24.2	0.70	56.2	0.63	8.9	1.31	25.4	0.78	55.7	0.38	5.3
6	1.20	24.6	0.67	54.4	0.86	10.9	1.26	24.6	0.72	52.4	0.91	7.7
7	1.20	25.9	0.65	51.6	1.07	14.6	1.26	25.3	0.73	50.1	1.27	13.5
8	1.12	25.0	0.61	49.0	1.36	17.9	1.20	25.0	0.67	48.3	1.33	16.5
9	1.02	25.2	0.53	46.7	1.80	27.4	1.14	24.6	0.62	46.4	1.54	20.9
10	1.03	25.6	0.54	50.3	1.84	28.4	1.19	24.3	0.65	46.0	1.56	22.2
LSD (P=0.05)	0.08	1.5	0.07	0.8	0.17	2.6	0.07	1.3	0.06	0.8	0.24	2.1
1989												
1	1.21	25.6	0.69	58.9	0.01	1.1	1.22	24.1	0.68	56.2	0.04	1.2
2	1.18	26.5	0.67	56.4	0.03	1.3	1.16	24.2	0.63	53.9	0.06	1.6
3	1.19	25.7	0.67	57.3	0.01	1.3	1.19	23.6	0.64	54.9	0.19	2.5
4	1.15	24.7	0.63	58.6	0.28	2.4	1.15	24.2	0.63	55.8	0.49	4.7
5	1.15	26.6	0.65	58.7	0.35	3.1	1.11	24.3	0.59	54.7	0.64	5.2
6	1.13	27.6	0.65	55.4	0.58	4.4	1.12	24.4	0.59	54.0	0.70	5.2
7	1.08	25.4	0.58	51.4	0.98	7.2	1.08	23.7	0.56	48.5	1.17	9.2
8	1.07	24.1	0.55	48.8	1.45	15.1	1.02	23.5	0.51	47.3	1.59	18.5
9	1.05	25.2	0.56	48.4	1.49	15.3	1.02	23.2	0.50	47.0	1.69	20.2
10	1.02	25.5	0.53	47.7	1.67	18.2	0.99	24.0	0.49	48.0	1.71	20.2
11	1.03	25.2	0.54	48.2	1.60	17.7	0.96	24.1	0.47	47.4	1.67	20.1
12	1.01	24.8	0.52	48.0	1.67	19.6	1.01	23.9	0.51	46.5	1.69	20.8
LSD (P=0.05)	0.05	2.2	0.06	0.6	0.18	1.6	0.06	1.4	0.05	0.7	0.16	1.8

[a]GY = green plot yield; DMC = dry-matter concentration; DMY = dry-matter yield; IVDMD = in vitro dry-matter digestibility; Rust = final rust severity; AUDPC = area under disease progress curve.

means and variances. Treatment yields were tested for differences by Fisher's least significant difference (LSD). Simple correlations between final rust severity with AUDPC, plot yields, and digestibility data were determined. Digestible dry-matter yield was calculated as the product of green yield, dry-matter concentration, and digestibility. Digestible dry-matter yields were regressed on final disease severities. Yield reductions associated with disease were determined by solving linear or logarithmic regression equations.

TABLE 3. Correlation coefficients among components of and factors that affect digestible dry-matter yield of pearl millet forage (1988 and 1989 data for dwarf and tall hybrids were pooled)[a]

	DMY	DMC	IVDMD	Rust	AUDPC
GY	0.97 **[b]	0.09	0.39 **	−0.47 **	−0.38 **
DMY		0.32 **	0.39 **	−0.45 **	−0.36 **
DMC			0.09	0.00	−0.03
IVDMD				−0.58 **	−0.52 **
Rust					0.95 **

[a]DMY = dry-matter yield; DMC = dry-matter concentration; IVDMD = in vitro dry-matter digestibility; Rust = final rust severity; AUDPC = area under the disease progress curve; GY = green plot yield.
[b]** = Significant correlation at $P = 0.01$.

TABLE 4. Regression equations fit to digestible dry-matter yield of pearl millet forage (Y) as a function of rust severity 10 days before harvest (X)

Cultivars	Year	Regression equation	r^{2*}	*df*	*P*
Dwarf	1988	$Y = 100 - 24.91 \log(X)$	0.78	59	<0.0001
	1989	$Y = 100 - 26.44 \log(X)$	0.80	71	<0.0001
Tall	1988	$Y = 100 - 0.99(X)$	0.59	59	<0.0001
	1989	$Y = 100 - 26.48 \log(X)$	0.90	63	<0.0001

*Coefficient of determination.

RESULTS

In the absence of disease in the first harvest, there were no differences ($P > 0.05$) in green yield between untreated plots of Tifleaf 1 and Tifleaf 2, or between Gahi 3 and Gahi 4, indicating that they had similar yield potentials.

Rust severities on the plots increased gradually as is found in natural epidemic development. There were no abrupt increases in the disease progress curves as a result of inoculations or termination of the fungicide treatments.

After establishing different levels of rust infection, treatments were a significant source of variation ($P < 0.01$) in green and dry-matter yields for both the dwarf and tall hybrids in the second harvest. Yields of rust-free plots of the resistant and susceptible hybrids did not consistently differ, nor did yields of inoculated and fungicide-protected plots of the resistant cultivars (Table 2). Treatments were not a significant source of variation for dry-matter concentration, but were significant for variation of in vitro dry-matter digestibility ($P < 0.01$).

Data were pooled across experiments and correlations among the factors that affected digestible dry-matter yield are presented in Table 3. Dry-matter concentration was correlated only with dry-matter yield. All other factors that contributed to digestible dry-matter yield were correlated.

Final disease severities and AUDPCs in each experiment were significantly correlated, and the response relationships of digestible dry-matter yield to both AUDPC and final severities

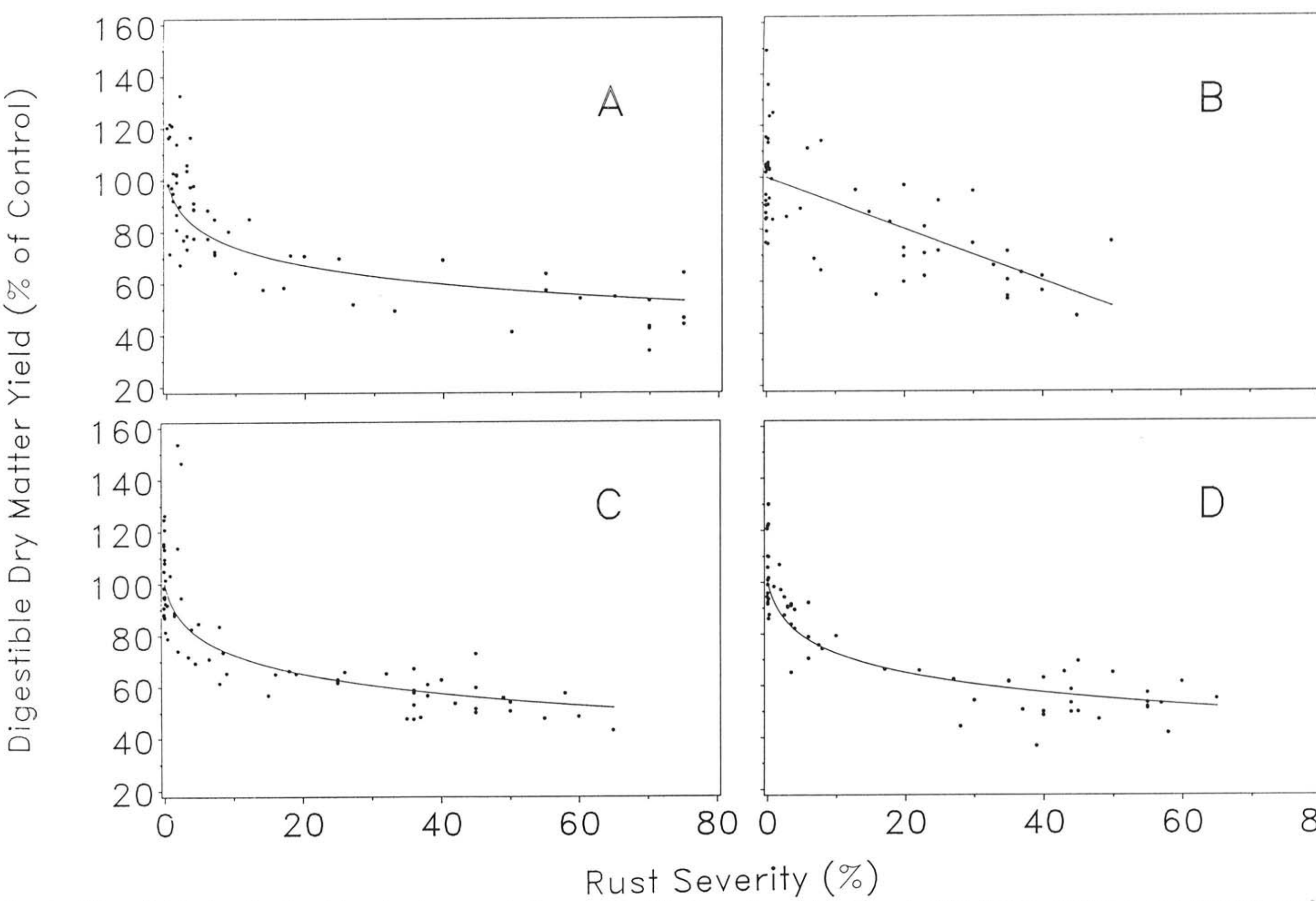

Fig. 1. Effect of infection by *Puccinia substriata* var. *indica* on digestible dry-matter yield of pearl millet forage, expressed as a percentage of the digestible dry-matter yield of disease-free plots. Dwarf hybrids (**A,C**) and tall hybrids (**B,D**) were evaluated in 1988 and 1989, respectively.

were similar. Only the relationship of digestible dry-matter yield loss to final rust severities has been presented because severities are easier to relate conceptually to a field setting. Final rust severities on dwarf and tall hybrids were correlated with in vitro dry-matter digestibility ($r \leq -0.58$, $P < 0.01$).

Regression equations were calculated to estimate loss of digestible dry-matter yield with increasing rust infection. Data were expressed as a percentage of the digestible dry-matter yield of the disease-free plots, which were 2.4 and 2.1 kg for the dwarf hybrids and 2.5 and 1.9 kg for the tall hybrids in 1988 and 1989, respectively. Logarithmic regression equations were fitted to all of the data except for the tall cultivars of 1988, to which a linear equation was fit (Table 4). In all experiments, except the evaluation of tall hybrids in 1988, the response relationship indicated that a small increase of rust on healthy foliage resulted in greater losses of digestible dry-matter yield than similar increases of rust on more severely diseased foliage (Fig. 1).

DISCUSSION

Tifleaf 1 and Tifleaf 2 can be considered near-isogenic hybrids of pearl millet. Their pollinator parents are the same and their cytoplasmic male-sterile parents differ in backcrossed genes for resistance to rust and Pyricularia leaf spot. Gahi 3 and Gahi 4 are also near-isogenic. They have identical pollinator parents, but their cytoplasmic male-sterile parents differ by a linkage block that contains a gene for rust resistance. Because of the similarities between the hybrids, we did not expect to find differences in the performance of resistant or disease-free susceptible cultivars. Forage yields and in vitro dry-matter digestibilities of Tifleaf 1 and Tifleaf 2 have been found to be identical in the absence of disease (2). Our research confirmed this and established that there are no discernible differences between hybrids Gahi 3 and Gahi 4 in the absence of disease.

The effects of rust on pearl millet were previously examined in segregating families during the development of inbred Tift 85DB (4). In contrast to the results of Monson et al (4), we did not observe an effect of rust on dry-matter concentration. The differences in effects on dry-matter concentration between these experiments are probably attributable to the differences in plant maturity at harvest. Plants in the Monson et al study were harvested about 2 wk after anthesis, while plants in the present study were harvested when the grain was in the late-milk stage of development (3–4 wk after anthesis). This difference in maturity has been demonstrated to decrease in vitro dry-matter digestibility of pearl millet (1). Because digestibility was already decreased by maturity, the effects of rust infection on less mature foliage was probably underestimated in our study. Isawa (3) found that dry-matter concentrations of orchardgrass, Italian ryegrass, maize, white clover, and foxtail millet were either unchanged or decreased with increasing rust severities. Dry-matter digestibilities of pearl millet (4) and of the species in Isawa's study (3) either decreased or remained the same with increasing rust infection. Our findings of decreased dry-matter yield and digestibility with increased rust infection are consistent with previous studies.

Because *P. s. indica* predominantly infects the leaves, the response relationships determined in our experiments suggest that the rapid loss of digestible dry-matter yield at low rust severities represents a loss from the more highly digestible leaves, and that the rate of loss levels off as less leaf tissue remains available for infection. However, Monson et al (4) determined that digestible dry-matter yield of stems is reduced by rust infection, but is unaffected for leaves. Based on their findings, the rapid loss of digestible dry-matter yield at low rust severities, which was found in the present study, suggests that infection of the leaves causes a reduction of components that contribute to digestible dry-matter yield in the stems.

The significant loss of digestible dry-matter yield with low rust severities indicates that highly effective resistance should be incorporated into forage pearl millet hybrids. Consequently, it may be a disadvantage to use slow-rusting or partial rust resistance (6) in forage pearl millets. Slow leaf-rusting resistance in wheat significantly reduces rust severities but is less effective on lower leaves (5). And, in the field, rust severities are greater on lower leaves than on upper leaves of slow-rusting small grains (11). Because the loss of digestible dry-matter yield of pearl millet is significant at low levels of rust, it is likely that slow-rusting resistance will not adequately protect the forage against yield and quality loss.

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SUSTAINING ASSOCIATES