

Use of Near-Isogenic Host Populations to Estimate the Effect of Three Foliage Diseases on Pearl Millet Forage Yield

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ABSTRACT

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Forage yields of F₂ and F₃ near-isogenic populations of pearl millet, *Pennisetum americanum*, were used to estimate the effect of *Cercospora* leaf spot (caused by *Cercospora penniseti*) red leaf spot, and brown leaf mottle (the latter two of unknown etiology). Pearl millet inbreds used to create the populations were: Tift 18 (susceptible to brown mottle); Tift 13 (resistant to brown mottle); 442 (susceptible to red spot and resistant to *C. penniseti*); and 664 (resistant to red spot and susceptible *C. penniseti*). The F₂ near-isogenic populations of varying degrees of resistance were established based on disease data collected from about 1,000 spaced F₂ plants of a cross between resistant and susceptible inbreds. Mean yields of plants in five disease severity classes were compared to determine the effect of *Cercospora* leaf spot on yield. The F₃ near-isogenic populations were prepared by mixing equal quantities of selfed seed from F₂ plants (of a resistant × susceptible cross) having the same F₂ disease classification.

These seed mixtures were planted in a replicated small plot trial in which three forage yields were taken. Both procedures for establishing near-isogenic populations require the grouping of enough F₂ plants or selfed seed to create populations with similar frequencies for all genes affecting yield except the genes controlling the disease and those closely linked to them. The F₂ near-isogenic population tests showed that red spot had no effect on forage yield, whereas *Cercospora* leaf spot, when severe, reduced forage yields 20–25%. In the F₃ near-isogenic population test, brown mottle had no effect on the first forage yield, but reduced the second harvest by 23% and the third harvest by 30%. Thus, red spot susceptibility or resistance may become a useful genetic marker, whereas *Cercospora* leaf spot and brown mottle are diseases that should be controlled by the breeding of resistant lines.

Diseases that kill leaf tissue reduce forage quality by lowering the plant's protein content, acceptability, and digestibility, and by increasing its lignin content (1,4). Chemical and in vitro dry matter digestibility analyses of small forage samples can be used for such quality assessments.

The effect of foliage diseases on forage yield is more difficult to ascertain. A comparison of plot yields of a susceptible cultivar

protected by a fungicide with unprotected plots will give useful information if it can be established that the fungicide does not affect yields in the absence of the disease. If cultivars with a high degree of resistance to the disease in question can be found, a direct yield comparison with susceptible cultivars that are affected will give a rough approximation of the yield loss caused by the disease. Genetic differences in material so compared, however, can greatly confound results. Isogenic lines developed to overcome such genetic differences are costly and time-consuming to produce. James (6) recognized these problems in his 1974 assessment of plant diseases and losses. Simons (7) showed that transfer of genes for

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resistance to *Puccinia coronata* from *Avena sterilis* to cultivated oats reduced the incidence of rust and increased yield.

The research reported here was designed to discover more rapid and less costly methods for estimating the effect of foliage diseases on forage yield of pearl millet (*Pennisetum americanum* (L.) Leeke.).

MATERIALS AND METHODS

The three foliage diseases used as models in this study were *Cercospora* leaf spot (caused by *Cercospora penniseti* Chupp) and red leaf spot and brown leaf mottle (both of unknown etiology). The *Cercospora* leaf does not appear in significant amounts until the millet initiates flowering and then becomes progressively more severe until the plants mature. The red leaf spot and brown leaf mottle have not been described previously. Red leaf spot first appears about the time of flower initiation. First symptoms appear as irregular or zonate water-soaked blotches on the leaves; these soon become mahogany in color, and as the plant matures the affected areas coalesce and become necrotic in advance of surrounding tissue. The brown leaf mottle first appears as a faint brown irregular mottle of the leaf and sheath at about the time of rapid stem elongation. The symptoms gradually become more pronounced as the plant matures. The affected tissue becomes necrotic before normal senescence, and leaves on affected plants appear to senesce sooner than healthy plants. For both the red leaf spot and brown leaf mottle, numerous attempts to isolate pathogens or to identify them through histological studies have been unsuccessful in determining the cause of the disease. All plants

TABLE 1. The effect of five severity classes of leaf spot (caused by *Cercospora penniseti*) on the yield of F₂ plants from a cross of pearl millet inbreds 664 (susceptible) and 442 (resistant)

Severity of leaf spot on F ₂ plants		No. of F ₂ plants	Mean green yield of F ₂ plants (g/plants)
Class	Degree		
1	Very slight	367	1,184
2	Slight	387	1,315
3	Medium	211	1,360
4	Medium severe	22	953 ^a
5	Severe	12	857

^aYields of plants in severity groups 4 and 5 were significantly ($P=0.05$) less than those of groups 1, 2, and 3.

carrying the genetic components for susceptibility to these diseases, whether grown under field or greenhouse conditions, always have the same symptoms for the respective diseases. Thus, these diseases appear to result from genetically controlled biochemical activities and not to be caused by plant pathogens.

Two kinds of near-isogenic populations (2,4) were used in this study. The F₂ near-isogenic populations were created based on yield and disease incidence data collected on each F₂ plant in a large population. To be useful, F₂ near-isogenic populations must include enough F₂ plants in each population to give them similar frequencies for yield genes in the absence of the disease being studied. Two or more populations within any disease class should have similar mean yields. The population size required can be determined by adding F₂ plants within a similar disease class to two or more populations until each population has about the same mean yield. Differences between the mean yields of the different disease classes may then be assumed to be caused by the effect of the disease on yield. The variances among all F₂ plants within the populations may be used to assess the significance of the differences between disease classes.

The F₂ near-isogenic populations were used to study the effects of *Cercospora penniseti* and red leaf spot on forage yield in pearl millet. To create these populations, inbred 664 (susceptible to *C. penniseti* and resistant to red spot) was crossed with inbred 442 (resistant to *C. penniseti* but susceptible to red spot). The F₁ seed was produced in the greenhouse in the winter and approximately 1,000 F₂ plants were grown in the field the following summer. These were planted in rows 0.9 m apart, and when about 10 cm tall, plants in the row were thinned to one plant every 40–45 cm.

When F₂ plants of the cross 664 × 442 had flowered, each plant was rated from 1 to 5 for severity of *C. penniseti* injury, was classified for presence or absence of red leaf spot, and was cut and weighed. These data were used to develop F₂ near-isogenic populations as described above. The variance between the yields of individual plants was used to assess the significance of the differences among the means of the populations differing in disease class.

The F₃ near-isogenic populations used to study the effect of the brown mottle disease on pearl millet forage yield were prepared as follows: F₂ plants of a hybrid between inbred 13 (resistant to brown mottle) and inbred 18 (susceptible) were self-pollinated by bagging two heads on each plant before anthesis. When the seeds were mature, the two bagged heads on each plant were harvested and stapled together, and the presence or absence of brown mottle and trichomes was recorded. Seed from each plant was threshed and

TABLE 2. Effect of the physiologic brown spot disease on the forage yield of near-isogenic populations of pearl millet

Materials tested	Trichomes on plants		No. of F ₂ genotypes mixed	Disease class	Dry matter yield (kg/ha)			Total	
	F ₂	F ₃			7/5	8/9	10/6	actual	relative (%)
Inbred 13				Resistant	4,013	3,482	1,109	8,604	100.0
Inbred 18				Susceptible	3,976	2,470	605	7,051	82.0
13 × 18 F ₁				Resistant	6,366	4,516	2,639	13,521	157.1
13 × 18 F ₃									
near-isogenic populations ^a									
#1	few	few	17	Resistant	4,577	3,376	1,500	9,453	111.0
#2	few	few	17	Susceptible	4,251	2,481	985	7,717	89.7
#3	few	seg	19	Resistant	4,595	3,341	1,431	9,367	108.9
#4	few	seg	19	Susceptible	4,736	2,612	977	8,325	96.8
#5	many	many	88	Resistant	4,283	2,842	1,391	8,516	99.0
#6	many	many	88	Susceptible	4,259	2,276	1,079	7,614	88.5
LSD ($P=0.05$)					555	424	273	812	
Mean of 1, 3, 5				Resistant	4,485	3,186	1,441	9,112	105.9
Mean of 2, 4, 6				Susceptible	4,415	2,456	1,014	7,885	91.6
Sig. of difference					NS	** ^b	**	**	
(Susceptible × 100)/ Resistant					98.4	77.1	70.4	86.5	
Development of brown spot					little	much	much		

^aNear-isogenic populations prepared by mixing selfed seed of up to 88 F₂ plants that had few or many trichomes and brown spot resistance or susceptibility in common.

^b*** Significant at $P=0.01$.

packeted. Because the presence of trichomes is a recessive trait and because we thought this trait might be linked with disease resistance, we separated the seed packets into two groups, those from plants with trichomes and those from plants without. Samples from the latter group of seed packets were planted in the greenhouse and the packets were further divided into those with no trichomes in the F₃ seedlings and those segregating for the trichome character. Equal quantities of seed from 17 resistant plants in group #1 (no trichomes in the F₂ or F₃) were then mixed to make a seed mixture for resistant F₃ near-isogenic population #1. Seeds from 17 susceptible plants were similarly mixed for population #2. The same procedure was followed to make resistant and susceptible near-isogenic populations #3 and #4 from seed of 19 plants each and populations #5 and #6 from seed of 88 plants each.

The near-isogenic population concept assumes that blending seed of many F₂ plants will produce F₃ populations that have similar frequencies for all genes affecting yield, except genes controlling the character in question or those genes closely linked to them (2,4). To further test this assumption, another 17-plant population and two 50-plant populations were prepared from the seeds used to prepare population #1. These were also included in the yield trial.

In May, seed of the parent inbreds, the F₁ hybrid, and the near-isogenic populations were planted in single-row plots 5 m long and 1.8 m apart arranged in a randomized block design with six replications. Forage on each plot was cut and weighed on 5 July, 9 August, and 6 October. The presence or absence of the brown mottle character was observed when each harvest was made.

RESULTS AND DISCUSSION

Of the 974 F₂ plants of the cross between the red leaf spot-susceptible pearl millet inbred 442 and red leaf spot-resistant 664, only 82 developed characteristic red spots. This low frequency and the absence of red spots on the F₁ plants of this hybrid indicate that resistance is completely dominant to susceptibility. When weighed, the 82 red spot plants averaged 1,261 grams per plant, which is not significantly different from the average yield of 1,275 grams for 892 plants with no red spots. Thus, this physiologic red spot on the foliage of inbred 442 does not have a deleterious effect on forage yield.

The F₁ of the cross between the *Cercospora* leaf spot-susceptible pearl millet inbred 664 and the highly resistant inbred 442 appeared to be as resistant as inbred 442. As we expected, most of the F₂ plants showed a slight or very slight incidence of the disease. Only 22 of the 999 F₂ plants studied received medium severe ratings and only 11 received severe ratings comparable to inbred 664. When weighed, the plants in severity class 1 (as resistant as 442) averaged 1,184 g per plant (Table 1). Plants in classes 2 and 3 yielded significantly more than those in class 1. The greater disease incidence of these plants indicates that they were more heterozygous at the loci controlling resistance. The heterosis expected with this greater heterozygosity could explain the increased yields of classes 2 and 3 over class 1 (3). Medium severe and severe incidence of *C. penniseti* reduced average forage yields to 80.5 and 72.4%, respectively, of the average yield of the class 1 plants. These yield reductions are significant, $P = 0.05$. It appears from this study that leaf spot caused by *C. penniseti*, when severe, can reduce the mature plant yields of susceptible pearl millet 20–25%. Resistance present in a number of lines should be incorporated into economically important lines.

The physiologic brown mottle disease is controlled by no more than one or perhaps two recessive genes. The F₁ hybrid between resistant inbred 13 and susceptible inbred 18 is completely free of brown mottle. Brown mottle is rare on young plants, but incidence increases rapidly as susceptible plants approach maturity. Little

brown mottle had appeared by 5 July when the first yield was taken from the entries in the near-isogenic population study. As a consequence, susceptible inbred 18 and resistant inbred 13 yielded the same, and the forage yields of resistant and susceptible near-isogenic populations did not differ (Table 2). The F₁ of this hybrid yielded 59% more for the season than the parental mean, and the resistant F₃ populations yielded 16.4% more than the parental means. This was 1.6% more than the expected yield for F₃ material.

Brown mottle was severe on susceptible plants on 9 August and 6 October when the second and third harvests were made. As a result, F₃ near-isogenic populations susceptible to brown mottle yielded only 77.1 and 70.4% as much as comparable resistant populations at the second and third harvests, respectively. Susceptible inbred 18 yielded only 70.9 and 54.4% as much as inbred 13 on the second and third harvests. Brown mottle is a deleterious marker and should be removed from lines of economic importance such as Tift 18.

The F₃ near-isogenic population #5 (with many trichomes) yielded less than populations #1 and #3 (with few trichomes). Dust from nearby road construction accumulated noticeably on the leaves with trichomes on several occasions during the course of this study. This could have excluded light and reduced the rate of photosynthesis in the plants with many trichomes. The exclusion of light may explain the reduced yield associated with the brown mottle physiologic disease.

The two 17-plant populations and the two 50-plant populations from the F₂ plant seed used to prepare population #1 gave total season yields of 9,453, 9,100, 9,096, and 8,892 kg/ha, respectively. The greatest difference between the two 17-plant populations, 353 kg/ha, was much less than the 5% LSD ($P = 0.05$) of 812 kg/ha. The 202 kg/ha difference between the 50-plant populations indicates that adding more F₂ plants to F₃ near-isogenic populations will reduce the differences between duplicate populations and add precision to near-isogenic population studies.

The F₃ near-isogenic population method of estimating the effect of a disease on forage yield requires 2 yr but permits replicated plot tests and management studies that supply more information than the F₂ population method. A combination of the two methods would seem to offer the best solution for the problem of estimating the effect of a disease on plant yields. It would require the production of selfed seed on the F₂ plants. If the F₂ near-isogenic population study indicated that the disease, such as red spot in this study, had no effect on forage yield, there would be no need to conduct an F₃ test. If, however, the F₂ test revealed a disease effect on yield, the selfed F₂ seed could be used to create F₃ near-isogenic populations. These populations could then be managed and tested to supply the extra information desired.

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