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Effects of Nitrogen, Plant Density, Moisture Stress and Artificial Inoculation with *Macrophomina phaseolina* on Charcoal Rot Incidence in Grain Sorghum

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With 3 figures

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Abstract

Effects of different levels of nitrogen, plant density, soil moisture stress and of artificial inoculation with *Macrophomina phaseolina* on charcoal rot incidence in grain sorghum were studied in a split-split-split field plot design. Nitrogen levels were applied to main plots; plant densities, moisture stresses and inoculations were replicated in sub, sub-sub and sub-sub-sub plots. A gradient of soil moisture stress was created during grain filling by line-source sprinkler irrigation. Analysis of variance indicated significant effects of moisture stress, plant density and their interactions on charcoal rot disease incidence as measured by plant lodging. Lodging increased linearly along the gradient of water supply suggesting a positive relationship between moisture stress and disease development. Lodging was also significantly ($P < 0.01$) and highly correlated with other charcoal rot disease parameters (soft stalk, number of nodes crossed, root infection and plant senescence). There were no significant differences ($P < 0.05$) in charcoal rot incidence among inoculation treatments. Lodging increased in both high nitrogen and high plant population treatments under moisture stress conditions.

Zusammenfassung

Einflüsse von Stickstoff, Bestandsdichte, Feuchtigkeitsstreß und einer künstlichen Inokulation mit *Macrophomina phaseolina* auf das Auftreten der Charcoal Rot-Krankheit der Hirse

Untersucht wurden die Einflüsse von unterschiedlichen Stickstoffmengen, Bestandsdichten, Bodenfeuchtigkeitsstreß und einer künstlichen Inokulation mit *Macrophomina phaseolina* auf das Auftreten der Charcoal Rot-Krankheit der Hirse in einer "split-split-split" Feldparzellenanlage. Unterschiedliche Stickstoffmengen wurden zu den Hauptparzellen appliziert; Bestandsdichten,

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Bodenfeuchtigkeitsstreß und Inokulationen wurden in sub, sub-sub und sub-sub-sub-Parzellen wiederholt. Ein Bodenfeuchtigkeitsstreßgradient wurde während der Körnerreife durch line-source-Bewässerungen erzielt. Eine Varianzanalyse deutete auf signifikante Einflüsse von Feuchtigkeitsstreß, der Bestandsdichte und deren Wechselwirkungen auf das Auftreten der Charcoal Rot-Krankheit, gemessen als Pflanzenlagerung, hin. Die Lagerung nahm linear mit dem Wassergradient zu, hindeutend auf eine positive Wechselwirkung zwischen dem Feuchtigkeitsstreß und der Krankheitsentwicklung. Eine signifikante ($P < 0{,}01$) und hohe Korrelation zwischen der Lagerung und anderen Charcoal Rot-Krankheitsparametern (wie weicher Stengel, Anzahl der durchquerten Knoten, Wurzelbefall und Pflanzensenescenz) wurde festgestellt. Es gab keine signifikanten Unterschiede ($P < 0{,}05$) des Charcoal Rot-Auftretens zwischen den Inokulationsbehandlungen. Eine erhöhte Lagerung wurde bei hohen Stickstoffzugaben und hohen Bestandsdichten unter Feuchtigkeitsstreßbedingungen beobachtet.

Charcoal rot of sorghum (*Sorghum bicolor* [L.] Moench) caused by *Macrophomina phaseolina* (Tassi) Goid, is an economically important disease in most sorghum-growing regions of the world (MUGHOGHO and PANDE 1984, TARR 1962). Symptoms of the disease include root rot, soft stalk and lodging of plants, premature drying of stalks and poorly developed panicles with small inferior quality grain. Charcoal rot occurs when sorghum plants are subjected to soil moisture stress during grain filling (EDMUNDS 1964). ODVODY and DUNKLE (1979) obtained 17 % charcoal stalk rot and 83 % charcoal root rot from moisture-stressed fertile plants grown in *M. phaseolina* infested soil; non-stressed plants did not develop charcoal rot symptoms. Crop management systems which influence soil moisture also affect charcoal rot development. Sorghum grown in close spacings showed more charcoal rot incidence than a wider spaced crop (WADSWORTH and SIEGLINGER 1950). PATIL *et al.* (1982) reported cultivar differences in the effect of plant densities on charcoal rot and attributed the higher disease incidence in a higher plant population to increased moisture stress.

ANAHOSUR *et al.* (1977) reported increased incidence of charcoal rot with increased application of nitrogen fertilizer. Higher levels of nitrogen fertilization needed to enhance the yields of improved sorghums increased the severity of charcoal rot (AVADHANI *et al.* 1979, MOTE and RAMSHE 1980).

The above reports indicated a relationship between moisture stress, plant density, soil fertility and charcoal rot. Data on the combined effect of these factors and their interaction on the charcoal rot development are not available. Such data would facilitate the development of an effective field screening technique for resistance to charcoal rot. This paper reports the results of investigation on the effects of different nitrogen levels, plant densities, soil moisture stresses and plant inoculations with *M. phaseolina* and their interaction on charcoal rot development under field conditions.

Materials and Methods

Seed, site and season

Certified seeds of the charcoal rot-susceptible sorghum hybrid CSH 6 obtained from National Seeds Corporation of India were sown during the postrainy season in an Alfisol at the experimental farm of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India. Negligible rainfall (13 mm) was received during the early growth stages of the crop, and subsequently it did not rain throughout the entire period of the experiment (Fig. 1). Maximum and minimum temperature records (Fig. 1) were obtained from the meteorological station situated 200 m away from the experimental plot.

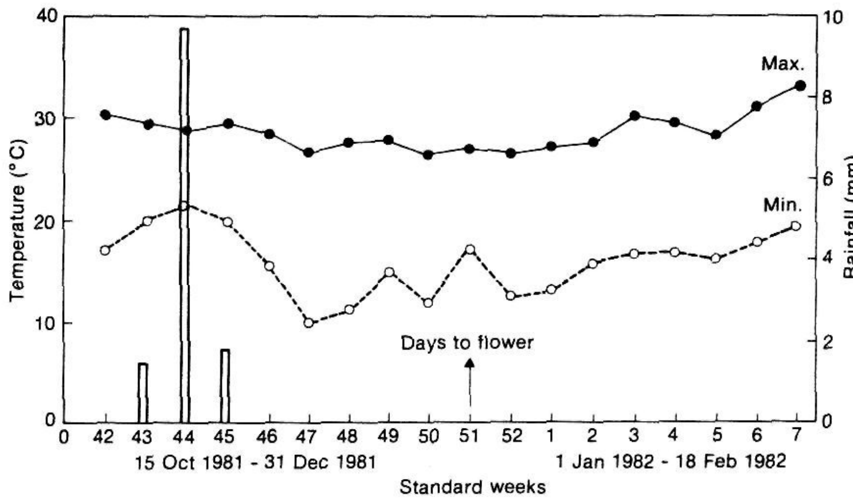

Fig. 1. Weekly average temperature (line) and rainfall (bars) at ICRISAT Center, during 1981—82 postrainy season

Experimental design

The experiment was conducted in a split-split-split plot design with four replications (GOMEZ and GOMEZ 1976). The main plots were assigned to three levels of nitrogen and three plant densities to the subplots, the sub-sub plots to the nine moisture stress levels (MSL) created by the line-source sprinkler irrigation system (LS, HANKS *et al.* 1976). The effects of natural soilborne inoculum was compared by superimposing two additional inoculation methods (sterile toothpick and pathogen infested toothpick, RAO *et al.* 1980) in sub-sub-sub plots. A 4 m wide buffer crop of CSH 6 was grown around the experiment to control border effects. A uniform distance of 0.75 m was maintained between rows to get 4 × 1.5 m sub-sub-sub plot size. The experiment was conducted under intensive fungicide and insecticide application as protection against leaf diseases and damage by insect pests.

Nitrogen fertilization

Sweet corn was thickly drilled and allowed to grow during the rainy season in the experimental area to reduce the field variability of available nitrogen. Three levels of nitrogen (N_1 = 20, N_2 = 60, and N_3 = 120 kg N ha^{-1}, as urea) were applied in two instalments: the first instalment of 20 kg N ha^{-1} in N_1 and 40 kg N ha^{-1} in N_2 and N_3 was applied 10 days after crop emergence (DAE) and thinning to the required plant densities; the remaining quantity of nitrogen in N_2 (20 kg N ha^{-1}) and N_3 (80 kg N ha^{-1}) was applied 30 DAE. A uniform application of phosphorus (26 kg P ha^{-1}, as single superphosphate) was broadcast before ridging.

Plant densities

A thick crop stand was initially obtained by planting the hybrid sorghum CSH 6 at a higher seed rate (35 kg ha^{-1}) than normal (10 kg ha^{-1}) in 18 rows parallel to the LS on either side. Three plant densities (D_1 = 66,675, D_2 = 133,350 and D_3 = 266,700 plants ha^{-1}) were created by adjusting the plant-plant distance by thinning.

Moisture stress levels and their measurement

Experimental plots were furrow-irrigated to field capacity at planting and 10 DAE. Three more irrigations each of 40 mm were provided using the ordinary overhead sprinkler irrigation up to boot leaf plant growth stage. Thereafter plots were irrigated by the LS at 10 days interval up to physiological maturity (black layer formed at the proximal end of the grain) to create a gradient of water supply, in eighteen rows parallel to the LS. Each pair of rows parallel to and away from the LS was identified as a MSL. Nine MSL, S_1 nearest and S_9 farthest from the LS, were considered as observational units.

The amount of water supplied during grain filling in the different MSL was measured at four times (69, 83, 93 and 103 DAE) by collecting the water, at crop height, in cans placed at right angles on either side of the LS at 2.3, 4.5, 6.8, 9.0, 11.3 and 13.5 m. Plant growth stages at the four times were anthesis, soft dough, hard dough and physiological maturity. Transpiration rate was measured as leaf-air temperature differential (HOWELL *et al.* 1984) and used to correlate the linear application and availability of moisture at different stress levels.

Inoculation methods

In each MSL three sets of an equal number of plants within a plant density were marked for comparing inoculation treatments. There were 10 plants in the D_1, 20 plants in the D_2 and 40 plants in the D_3 treatments. The first set was left uninoculated (I_0) and charcoal rot was allowed to develop from natural soilborne inoculum. In the second set a sterile toothpick (I_1) was inserted in each plant at the second internode from the soil level. The third set was inoculated with *M. phaseolina*-infested toothpicks (I_2). Inoculations were carried out as described by RAO *et al.* (1980).

Plant lodging as a measure of charcoal rot in root and stalk

Total plants in each plot were counted before the initiation of lodging, the first apparent symptom of charcoal rot. Lodged plants were counted at intervals of 5—7 days from first incidence to physiological maturity and at harvest. Percent lodging was calculated (number of lodged plants divided by total number of plants × 100) for each interval. Nine days after physiological maturity the experiment was harvested and other charcoal rot disease parameters (soft stalks, progress of infection up the stalk as no. of nodes crossed, root infection and foliage senescence) were recorded to confirm stalk rot infection in lodged and nonlodged plants in each treatment.

Soft stalks were determined by pressing the internodes of plants between two fingers and thumb, and the percent soft stalks calculated (number of soft-stalked plants divided by total number of plants × 100). Plants were carefully uprooted and scored for percent root infection on a 1—5 rating scale (1 = no infection, 2 = less than 10 % roots infected, 3 = 11 to 25 % roots infected, 4 = 26 to 50 % roots infected, and 5 = more than 50 % roots infected. Stems were then split open to measure the extent of *M. phaseolina* colonization in the stem. This was determined by counting the number of nodes crossed by the infection. Plant senescence was measured on a 1—5 rating scale (1 = foliage completely green, 2 = less than 10 % foliage brown and dry, 3 = 25 % foliage brown and dry, 4 = 50 % foliage brown and dry, and 5 = complete death of the foliage).

Statistical analysis

The experiment was arranged for the nitrogen levels, plant densities, moisture stress levels and artificial inoculation with *M. phaseolina* in a split-split-split plot design. Data on each charcoal rot disease parameter were analysed separately by the technique of analysis of variance for three-or-more factor experiments (GOMEZ and GOMEZ 1976). Correlation coefficients among different parameters of charcoal rot disease were calculated using simple linear correlation analysis procedures (GOMEZ and GOMEZ 1976, LITTLE and HILLS 1978).

Results

Charcoal rot development in roots and stalks

Lodging started 101 DAE when the crop was nearing physiological maturity. Data on periodical lodging (%) are presented in Fig. 2. Differences among plant densities in the initial number of lodged plants were recorded across nitrogen and moisture stress levels. Lodging began earlier and was highest at $N_3 \times D_3 \times S_9$ treatment. No plant lodged in $N_1 \times D_1 \times S_1$ and $N_1 \times D_1 \times S_2$ treatments. In between these two extremes a range in lodging was recorded. The progress of lodging was higher in D_3 plant density in all the three nitrogen levels, and in MSL S_7 — S_9 situated farthest from the LS (Fig. 2).

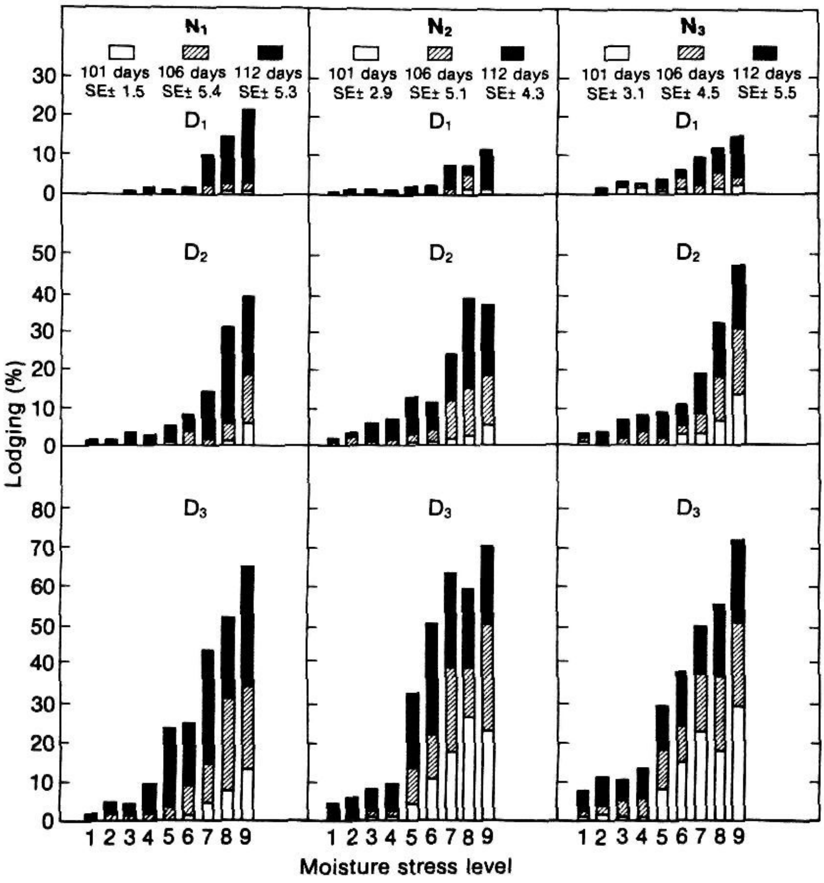

Fig. 2. Periodical lodging (%) at three nitrogen levels (N_1 = 20, N_2 = 60 and N_3 = 120 kg N ha^{-1}), three plant densities (D_1 = 66,675, D_2 = 133,350 and D_3 = 266,700 plants ha^{-1}), and nine moisture stress levels 1 (S_1) = nearest to LS and 9 (S_9) farthest from LS at ICRISAT Center, during 1981—82 postrainy season

Effect of nitrogen

Analysis of variance did not show any significant difference ($P < 0.05$) in charcoal rot incidence (% lodging) among the three levels of nitrogen fertilization.

Effect of plant density

Since the differences among nitrogen treatments were not significant ($P < 0.05$), data for the three nitrogen levels at each density were averaged to explain the effect of plant density on charcoal rot incidence. Significant differences ($P < 0.001$) in lodging were recorded among the three plant densities. The highest number of lodged plants (29.9 %) across MSL was obtained in D_3 and the lowest (4.8 %) in D_1 (Table 1). The trend in lodging was in accordance with the moisture stress, with least lodging near the LS and most lodged plants in the plant densities farthest from the LS (Figs 2 and 3B).

Table 1

Percent lodging at harvest (112 days after emergence) in sorghum cultivar CSH 6 at three plant densities subjected to nine moisture stress levels with line source sprinkler irrigation in 1981—82 postrainy season at ICRISAT Center, India

Moisture stress[a] level	Plant density[b] and percent lodging Density-1	Density-2	Density-3	Mean
Stress — 1 (S_1)	0.2	1.8	4.6	2.2
Stress — 2	0.6	2.3	7.2	3.4
Stress — 3	1.2	5.2	7.7	4.7
Stress — 4	1.4	5.5	10.5	5.8
Stress — 5	1.8	8.4	26.1	12.1
Stress — 6	2.8	9.6	37.4	16.6
Stress — 7	8.6	18.4	51.9	26.3
Stress — 8	10.8	32.9	55.1	32.9
Stress — 9 (S_9)	15.8	39.8	68.9	41.6
Mean	4.79	13.76	29.93	16.16

SE(m) for stress ± 1.66

SE(m) for density ± 1.54

SE(m) for comparing density at same level of stress ± 2.89

SE(m) for comparing stress at same or different levels of density ± 3.12

[a] Stress — 1 = nearest to line source (lowest moisture stress level), Stress — 9 = farthest from line source (highest moisture stress level).

[b] Density — 1 = 66,675 plants ha^{-1}, Density — 2 = 133,350 plants ha^{-1}, and Density — 3 = 266,700 plants ha^{-1}.

Effect of moisture stress

The total amount of water received through the LS during grain filling in the first two observational units S_1 and S_2 nearest to the LS was 142 mm. Thereafter it decreased linearly and continuously so that the last unit S_9 received less than 10 mm (Fig. 3A). The pattern of water application resulted in a linear decrease in crop transpiration and this was confirmed by measuring leaf-air temperature differentials (Fig. 3A).

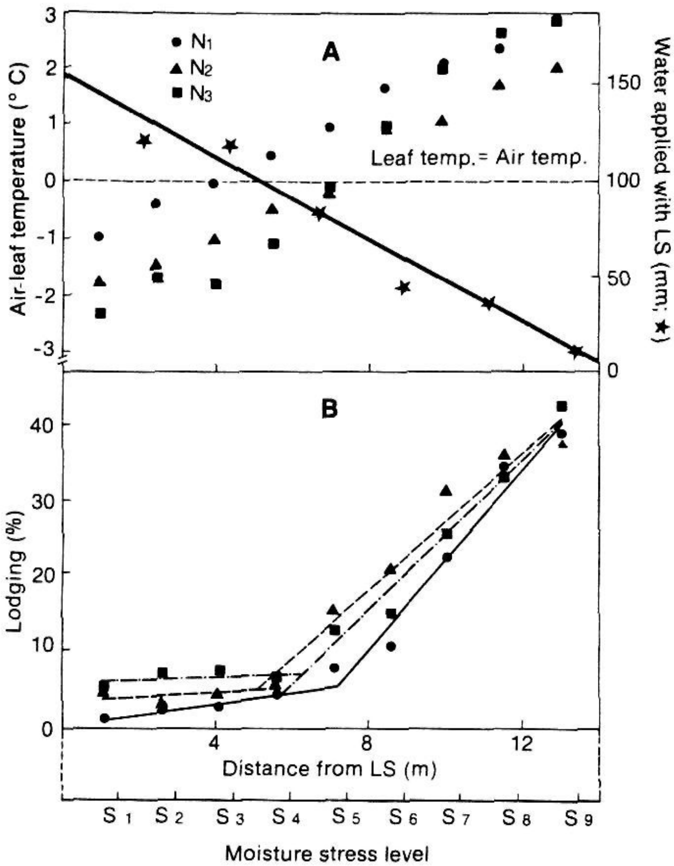

Fig. 3 A. Relationships between moisture stress levels, and LS irrigation water applied (solid line), or air-leaf temperature differential in nitrogen main plots
B. Relationship between moisture stress levels and percent lodging in nitrogen main plots

As the experiment was conducted during the milder postrainy season (Fig. 1), crop growth and grain filling were not affected, but yields decreased slightly after the fourth unit (up to about 6.5 m from the LS). The effect of moisture stress on disease incidence, as expressed by lodging, was apparent in the whole range of water supply (Table 1).

Leaves in the four observational units S_1 to S_4 nearest to the LS were cooler than the air indicating high transpiration (Fig. 3 A), and lodging was low in these units (Fig. 3 B). Farther from the LS leaves were hotter than air and lodging increased linearly with distance from the LS, i.e., with increasing moisture stress (Table 1).

Effect of inoculation

There were no significant differences in lodging ($P < 0.05$) among inoculation treatments (I_0, I_1 and I_2) within plant densities, moisture stress and nitrogen levels. Data were averaged for all combinations of moisture stress and plant

Table 2

Percent lodging in sorghum cultivar CSH 6 at three nitrogen levels with respect to three artificial inoculation treatments in *1981—82* postrainy season at *ICRISAT* Center, India

Inoculation treatment	Nitrogen level[a] N_1	N_2	N_3	Mean
I_0[b]	17.4	19.5	20.6	19.1
I_1	16.0	18.9	19.5	18.2
I_2	17.5	20.8	21.2	19.8
Mean	17.0	19.7	20.4	19.0
SE(m) for inoculation	± 0.89	± 1.02	± 0.94	

[a] N_1 = 20 kg nitrogen ha^{-1}, N_2 = 60 kg nitrogen ha^{-1}, N_3 = 120 kg nitrogen ha^{-1}.
[b] I_0 = natural infection, I_1 = inoculation with sterilized toothpick, I_2 = inoculation with *M. phaseolina* infested toothpick.

densities for each nitrogen level. Lodging did not vary more than 2 % among inoculation treatments in all three nitrogen levels (Table 2).

Combined effect of nitrogen, moisture stress and plant density

The overall interactions among these three factors were not significant ($P < 0.05$). However, lodging began earlier and was highest in $D_3 \times S_9 \times N_3$ treatment combination (Fig. 2). The increase in lodging in different treatment combinations of these factors was linear.

Correlation among disease parameters

Charcoal rot assessed as soft stalk, number of nodes crossed, root infection and plant senescence was highly correlated ($P < 0.05$) with lodging (Table 3). This high positive correlation of lodging with other parameters of charcoal rot disease confirmed the validity of using lodging as an appropriate parameter for disease measurement.

Table 3

Correlation coefficients among scores of sorghum charcoal rot disease parameters in 1981—82 postrainy season at ICRISAT Center, India

Disease parameter	Percent soft stalk	Mean No. of nodes crossed	Mean score for root infection	Plant senescence
Percent lodging	0.69**	0.64**	0.57**	0.63**
Percent soft stalk		0.87**	0.69**	0.84**
Mean No. of nodes crossed			0.61**	0.72**
Mean score for root infection				0.83**

Correlation coefficient at $P < 0.05$ = 0.088 at $P < 0.01$ = 0.115 (** Significance at $P < 0.01$).

Discussion

Our results confirm the work of others (EDMUNDS 1964, ODVODY and DUNKLE 1979) that moisture stress at grain filling is an important factor that influences the incidence of charcoal rot in grain sorghum. The gradient of soil moisture stress created through the LS sprinkler irrigation resulted in increased charcoal rot incidence with increasing distance from the LS. This linear increase in the disease is a clear demonstration that, under field conditions, charcoal rot develops only in moisture-stressed plants (EDMUNDS 1964, EDMUNDS *et al.* 1964, HSI 1961).

Our results also provide evidence that charcoal rot incidence increases at higher plant densities, as previously observed by PATIL *et al.* (1982) and WADSWORTH and SIEGLINGER (1950). However, this effect was not independent of moisture stress. It appears that high plant densities increased the inter-plant competition for available soil moisture, and that this competition increased linearly with the gradient of water supply, and hence the linear increase in disease incidence.

Reports that high levels of nitrogen fertilization increase charcoal rot incidence (ANAHOSUR *et al.* 1977, AVADHANI *et al.* 1979, MOTE and RAMSHE 1980) were not confirmed by the results of this investigation. However, we observed more vigorously growing plants at the highest nitrogen level (N_3). Lodging started earlier and initially more lodged plants were recorded in the N_3 treatment. Later, when the effect of moisture stress became pronounced, we did not find any significant difference in lodging among the three nitrogen levels. This observation suggests that the effect of nitrogen is perhaps relatively less evident as it was masked by the effect of moisture stress. It appears that higher levels of nitrogen increased charcoal rot incidence probably by their indirect effect on the ratio of root-shoot growth. Nitrogen promotes luxuriant shoot growth relative to root development (AYERS 1978). Under moisture stress the lack of a sufficient root system reduces the ability of a plant to obtain moisture, while at the same time its water requirement is increased by the luxuriant shoot growth (AYERS 1978).

Several inoculation methods have been used to induce charcoal rot in moisture-stressed plants (EDMUNDS 1964, HSI 1961, RAO *et al.* 1980). Our results on artificial inoculation with *M. phaseolina* infested toothpicks did not show any significant increase in charcoal rot incidence over natural soilborne infection. This study suggests that if plants are properly moisture-stressed in the presence of sufficiently available natural soilborne inoculum of *M. phaseolina,* there is no need for artificial inoculation. In any case the pathogen does not normally enter through the stem, as in toothpick inoculation, but through the roots of moisture-stressed plants. This is clearly demonstrated by the higher correlations between root infections and other disease parameters.

ROSENOW (1984) reported significant correlations between plant nonsenescence, lodging and charcoal rot resistance. DUNCAN (1984) and ROSENOW (1984) described and utilized the nonsenescence trait in selection for charcoal rot resistance. Our data also agree with their findings and suggest that moisture stress enhances plant senescence and is directly correlated with charcoal rot incidence.

The data presented in this paper provide evidence that effective field screening for resistance to charcoal rot in sorghum is possible provided plants are subjected to soil moisture stress at an appropriate growth stage. Artificial inoculation is unnecessary in soils with *M. phaseolina* propagules. Lodging and plant senescence would be important parameters for selecting charcoal rot resistant sorghum lines.

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