

THE EFFECT OF NITROGEN NUTRITION ON SEEDLING BLIGHT OF SORGHUM  
INCITED BY FUSARIUM MONILIFORME SHELDT.

by

ILESANMI DELE ERINLE

B.Sc., Ahmadu Bello University, Zaria, Nigeria, 1968

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**THIS BOOK  
CONTAINS  
NUMEROUS PAGES  
WITH DIAGRAMS  
THAT ARE CROOKED  
COMPARED TO THE  
REST OF THE  
INFORMATION ON  
THE PAGE.**

**THIS IS AS  
RECEIVED FROM  
CUSTOMER.**

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## INTRODUCTION

Fusarium moniliforme Sheldon, the conidial stage of Gibberella fujikuroi (Saw.) Wr., is the causal organism in certain cases of seedling blight and stalk and root rots of sorghum, occurring singly or in complexes with other soil-inhabiting plant pathogens. A similar syndrome occurs in corn.

In the past, losses due to F. moniliforme were not considered great enough to warrant concerted attention. However, the advent of certain cultural practices such as high nitrogen fertilization, high plant populations, minimum tillage and continuous cropping has seemingly accentuated the problem, especially on irrigated lands where soil temperatures are high.

Recommended levels of nitrogen vary from 30-80 lbs. actual N/acre for dryland sorghum in northeastern Kansas, to 80-120 lbs. actual N/acre for irrigated sorghum in southwestern Kansas.

This study was designed to determine, under controlled conditions, if level of nitrogen in the growth medium affects seedling blight severity. In addition, it was designed to examine the effect of specific forms of nitrogen on pathogen populations in the growth medium before planting of seeds and what effect the final populations and/or the nitrogen forms might have on subsequent seedling blight severity.

## REVIEW OF LITERATURE

F. moniliforme as a Pathogen of Corn and Sorghum

Fusarium moniliforme as a gramineous root and stalk pathogen was first reported on corn (14, 29, 40, 49). However, there is lack of agreement among these early reports. Many workers (13, 29, 14, 49) claimed that the organism was definitely pathogenic. Others (26, 50) were convinced it was only a weak pathogen, and some (6, 42) claimed that it was non-pathogenic. Leonian (27) examined the potential of variability in this organism. From a single-spore culture of a vigorously-growing isolate, 50 segregants were isolated within a year. These isolates showed considerable variability in morphology, physiology and pathogenicity.

The first report of a sorghum disease caused by this organism came from Pammel et al. (40) in connection with studies on stalk rots in Iowa. Valleeau (49) found while experimenting with root and stalk rots of corn that F. moniliforme could cause damping-off of sorghum seedlings. He was, however, unable to reproduce the experiment. Tullis (48) reported a stalk rot of sorghum caused by this fungus. He also noted that F. moniliforme rarely formed macroconidia in culture.

Leukel and Martin (28) showed in greenhouse tests that the organism caused a reduction in seedling emergence that was most severe at low temperatures. It also caused damping-off of young seedlings which increased with rise in temperature. Seed-treatment was more effective against seed-borne inoculum than against soil-borne inoculum. Futrell and Webster (11) found that the incidence of F. moniliforme was high in sorghum heads covered with pollination bags. They consistently isolated this organism from scabbed seeds. Johnson (23) noted that F. moniliforme caused a reduction in root elongation.

Limber (29) indicated that the death or survival of infected plants depends on the speed with which the fungus destroys the secondary root system before permanent roots are initiated. Futrell and Kilgore (10) implicated a toxin in the reduction of corn root growth after infection by F. moniliforme.

#### Nitrogen Nutrition and Plant Diseases

The relationship between plant nutrition and disease development has been studied extensively; an early review was given by Wingard (54). In particular, nitrogen nutrition has gained considerable attention. Many investigators (5, 8, 36, 44) have found that excess nitrogen predisposes plants to disease. Some of the crops in which the role of nitrogen nutrition and Fusarium diseases has been studied include cotton (38, 55), tomato (5, 8, 52), red clover (4), bean (33, 46, 53) and corn (9, 39, 45).

For seedling blight of corn caused by Gibberella fujikuroi, Edwards (7) found that the level of N-P-K in sand culture had no significant effects on disease severity. Omission of any of these elements did not affect seedling growth during the initial 2-3 weeks following emergence. He concluded that mineral reserves in the seed were sufficient to compensate for any deficiencies in the growth medium.

The determination of disease severity was explained in many of the above cases on the basis of optimum or sub-optimum levels of nitrogen for the host, pathogen, or host/pathogen interaction in a given environment. With beans, the ratio of carbon to nitrogen (C/N) was held by some authors (34, 35, 41, 43) to be a critical factor, the hypothesis being that increased disease incidence was favored by a narrow C/N ratio. Other reports (16, 21, 11), however, dispelled C/N ratio as the crucial factor. For example, these reports indicated that many residues with a low C/N ratio provided as good control of bean root rot as did others with a high C/N ratio.

According to Huber (16), soil-borne diseases often increase or decrease according to the specific form of nitrogen maintained in the soil. Ammonium-nitrogen applied in the spring increased the severity of foot rot of wheat but nitrate-nitrogen had no effect. A fall application of ammonium-nitrogen had no effect, presumably because nitrification of the ammonium-nitrogen had occurred by spring. According to the same author, ammonium-nitrogen increases the incidence of some diseases incited by species of Fusarium, Aphanomyces, and Rhizoctonia such as stalk rot of corn, root rot of beans, and root rot and foot rot of wheat. Nitrate-nitrogen has the opposite effect. On the other hand, nitrate-nitrogen aggravates certain diseases caused by species of Verticillium and Streptomyces, such as early dying of potatoes and potato scab. These diseases are reduced by the ammonium form of nitrogen.

#### MATERIALS AND METHODS

In all experiments, the growth medium (soil or sand) and containers were autoclaved at 15 psi for 2 hours. Inoculum was obtained from F. moniliforme cultures on potato dextrose agar (PDA). Spores or spores and mycelia were harvested and suspended in sterile distilled water. Sorghum seeds were surface-sterilized with 1% sodium hypochlorite for 15 minutes, then washed several times with sterile distilled water and dried.

##### Establishing Pathogenicity of F. moniliforme Isolates

Four isolates were identified as F. moniliforme by using Snyder and Hansen's system of classification, outlined by Toussoun and Nelson (47). However, macroconidial characteristics could not be used because none of the isolates formed macroconidia in culture. In addition, two confirmed isolates

of F. moniliforme were obtained from R. A. Frederikson.<sup>1</sup> Sources of the isolates are given with results in Table 1 (p. 11).

For pathogenicity tests of all 6 isolates, equal volumes of inoculum were added to labeled 13 cm pots which contained a potting mix. The mix was composed of a sandy loam soil containing 1% organic matter, 5 ppm available phosphorus, 169 ppm exchangeable potassium and less than 25 ppm of available nitrogen. It had a pH of 7.5. Two hundred ml of tap water was added initially to each pot and the mix was then held at moderate moisture conditions for 5 days for the inoculum to become saprophytically established in the mix.

Pots were separated into 2 equal groups. After planting 50 seeds in each pot, a group was placed at each of two temperature regimes, one in a controlled environment chamber (Sherer Model No. Cel. 255-6) set at  $24 \pm 2^{\circ}\text{C}$  and the other in a greenhouse where the temperature fluctuated between 26 and 38C during the course of the experiment. Growth chamber illumination was maintained at 1000 ft-c for 12 continuous hours per day. Relatively high moisture levels were maintained by daily additions of tap water. A record of seedling emergence and mortality was kept.

#### Effect of Sources of Nitrogen on the Population and Pathogenicity of F. moniliforme

The growth medium was the same greenhouse potting mix referred to in pathogenicity tests. Autoclaved 23.8 x 13.7 cm metal trays were filled with 1.6 kg of soil (oven-dry basis). Sorghum residues to the equivalent of about 4% (W/W) were added to each tray. The residues consisted of mature stalks and leaves which had been oven-dried at 105C for 12 hours each on 2 consecutive days and then ground in a Wiley mill fitted with a 20-mesh screen. For each

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<sup>1</sup>Department of Plant Sciences, Texas A & M University

of 3 replications, a pre-weighed amount of ammonium sulfate, ammonium nitrate, sodium nitrate, or urea was added and mixed with the soil. Nitrogen in ammonium sulfate is available as ammonium ions while that in sodium nitrate is present in nitrate ions. Ammonium nitrate yields both ions. Only residues were added to the soil in checks. The initial amount of each of these nitrogen carriers chosen for soil amendment was equivalent to 0.4 gm of total nitrogen per kg of medium.

Inoculum was prepared by passing a spore/mycelium suspension through a double layer of sterile cheesecloth to eliminate most hyphal fragments. The crude filtrate was mechanically stirred for 30 seconds. Then the number of microconidia per unit volume was determined by using a hemacytometer.

Soil was infested by mixing 10 ml of spore suspension per tray; this amount supplied approximately  $2.5 \times 10^4$  conidia per gram of soil (oven-dry basis). The moisture level in the trays was raised with the addition of 200 ml distilled water. The trays then were transferred to an ISCO E-2 environmental chamber and incubated for 8 weeks at a program of 12 hours each of light (1200 ft c.) at 24C and darkness at 20C. Relative humidity was kept above 50%. The soil was kept moist by daily additions of distilled water.

At 2, 4, 6 and 8 weeks after infestation, five 2-cm core samples of the soil in each tray were removed with a glass tube and the density of inoculum in core sample composites for 3 replicates per treatment estimated by the dilution-plate technique. In brief, the soil composite from each of the five treatments was separately mixed in a plastic bag; then 1 g of soil from each bag was added to 99 ml of 0.1% sterile water agar in a 250 ml volumetric flask. The flask contents were hand-shaken vigorously and 1 ml of the soil suspension was transferred to the next dilution flask. Then 1 ml of the final dilution was pipetted onto Nash-Snyder medium (37) in petri dishes after the medium had

been incubated in the dark for 5 days. The incubation period allowed the medium to dry somewhat and thus be capable of rapidly absorbing the water in the soil suspension. The dishes were then rotated and tilted several times until the agar surface became completely covered with the 1 ml of diluted soil-suspension. To retard growth of associated bacteria, this medium was fortified with streptomycin sulfate and neomycin sulfate as outlined by Toussoun and Nelson (47). Petri dishes were incubated in the laboratory 5-7 days before Fusarium colonies were identified and counted. Data for each plate were converted by square root transformation prior to analysis of variance.

Six weeks after initiation of the experiment, a second addition (in 50 ml water) of each of the selected nitrogen compounds was added to its respective tray. The check was given a like amount of distilled water.

At the end of the 8-week incubation period and immediately before planting, the pH of the soil in each group of flats was determined. Freshly-removed soil was suspended in distilled water (1:2 soil/water suspension). The suspension was then allowed to stand for 2 hours except for occasional stirring and, finally, readings on a Photovolt pH meter were taken.

Bacterial populations representing each treatment were determined by soil dilution-plate on nutrient media.

Subsequently, surface-sterilized Spur Feterita seeds were planted in the trays at the rate of 50 seeds per tray. After planting, air temperature was elevated to 28C during the light hours and 20C in the dark. The length of photoperiod was 14 hours. A record of emergence and growth of the seedlings was kept.

Three weeks after emergence, the plants were removed, the roots were washed free of soil and samples were taken to record (a) the height of top



growth, (b) fresh weight of the same, and (c) condition of the roots as indicated by the number of adventitious roots and the size of lesions on the primary roots.

#### Effect of Levels of Nitrogen on Disease Severity

Equal volumes of inoculum were added to one lot of potted sand. Another lot (check) received no inoculum. In order to reduce contamination, this lot was compartmentalized in a plastic cage with an opening on one side. Pots were frequently irrigated with distilled water and were covered with a single layer of cardboard to retard drying. Seed of 3 sorghum genotypes, Redlan (1966 seed), RS 610 (1968 seed) and Spur Feterita (1964 seed) was prepared for planting. Seeds were hand-picked; broken, shrunken or scabbed seeds were discarded and the remaining seeds were surface-sterilized.

Three days after sand had been inoculated, RS 610 and Redlan were sown at the rate of 25 seeds per pot. The rate was 40 seeds per pot for Spur Feterita because of its lower viability. The seeds were lightly covered first with moist sand, then with a 1-cm layer of vermiculite. Greenhouse temperature was set at 20C. This temperature was not optimum for prompt germination, but it lessened possible problems of desiccation.

A split-plot design was used. Varieties (sub-plots) were randomized within one level of nitrogen, but the different levels were arranged so as to lie in strips (main plots) across the greenhouse bench.

Stand counts were taken 10 days after planting. At the same time, plants were thinned to 15 per pot. The greenhouse temperature was raised to 28C. Occasionally the temperature rose to 32C.

The basal nutrient composition was adapted from McNew and Spencer (36); each liter contained 0.213 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.875 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.429 g of  $\text{KH}_2\text{PO}_4$ . Iron was supplied as 5 ml of fertile EDTA per liter. In addition,



enough zinc sulfate was added to the stock solution to give 5 ppm of zinc. Different levels of nitrogen were made up by dissolving predetermined weights of ammonium nitrate in distilled water to give 0, 50, 100, 200 and 400 ppm nitrogen in the resulting stock solutions. Starting 2 weeks after seedling emergence, 50 ml of these nutrient solutions were added to sand medium, and this was followed as necessary by supplemental additions of distilled water. Records of growth patterns were kept in both inoculated and check series. The plants were harvested five weeks after planting. The composite of root systems in each pot was recovered from the sand medium by washing it in running tap water. No attempt was made to separate individual plants because the roots were in a dense tangle. Rather, the tops were severed at the soil line and a random sample of 5 tops per pot was selected for determination of height, and fresh and dry weights. The composite of root systems in each pot was given a Disease Severity Index based on the preponderance of lesions and the size of the root system itself:

- 0 = no lesions; a well-developed root system.
- 1 = a small necrotic area on the primary root.
- 2 = a small necrotic area on the primary root, plus a few lesions on the adventitious roots.
- 3 = extensive lesions on the adventitious roots.
- 4 = elongated dark brown necrosis on primary root; stubby adventitious roots.
- 5 = no root system; extensive necrosis on primary root and usually, mortality occurred.

Oven-dry weights of both stems and roots were determined after heating samples in aluminum cans at 105°C for 8 hours.

## EXPERIMENTAL RESULTS

### Establishing Pathogenicity of F. moniliforme Isolates

At the two temperature regimes, inoculation caused only a slight reduction in emergence (Table 1).

In the growth chamber, damping-off was extremely severe within just a few days after emergence. In seven days, virtually every plant died in all treatments except the uninoculated check. Eventually slight mortality also occurred in the check pots; evidently, inoculum had spread from adjacent inoculated pots. Actively-sporulating Fusarium colonies appeared on the soil surfaces in many of the inoculated pots. Before falling over, plants showed a necrotic zone in the crown region.

In the greenhouse where the temperature was higher and soil moisture lower, less mortality occurred. Here too, many of the plants had a necrotic crown, but instead of falling over and dying, they commonly recovered. Some plants exhibited rapid regeneration of new crown roots. These plants, however, fell over and were very susceptible to soil-moisture stress. There was no obvious stunting of any of the infected seedlings. As occurred in the growth chamber, some check plants also became infected from cross-transfer of inoculum from adjacent inoculated pots.

F. moniliforme was re-isolated from the margins of root and crown lesions plated on acidified PDA.

From these results, all 6 isolates were considered distinctly pathogenic and were maintained on PDA slants. The cultures were transferred to new slants at monthly intervals.

Table 1.--The effect <sup>a/</sup> of soil inoculation with 6 isolates of F. moniliforme on seedlings <sup>b/</sup> of Redlan sorghum grown at 2 temperature regimes, one in the growth chamber, the other in the greenhouse

Isolate <sup>c/</sup>	Source	Growth Chamber at 24C		Greenhouse at 26-38C	
		Emergence %	Mortality <sup>d/</sup> %	Emergence %	Mortality <sup>e/</sup> %
I	Sorghum stalks	78.0	96.5	83.3	26.4
II	Sorghum stalks	87.3	95.5	86.0	12.4
III	Blighted sorghum seedling	80.0	98.5	83.3	13.6
IV	Soil dilution plate	79.3	98.4	86.0	20.9
V	Acme dwarf broomcorn seed	78.0	96.6	87.3	34.4
VI	Dwarf yellow milo seed	75.3	93.0	84.0	10.3
Check	————	82.7	2.4	89.3	2.2

<sup>a/</sup> Effect as recorded 1 week (growth chamber) and 3 weeks (greenhouse) after emergence.

<sup>b/</sup> There were 50 seeds per pot.

<sup>c/</sup> Isolates I and II were obtained from R. A. Frederiksen, Department of Plant Sciences, Texas A & M University.

<sup>d/</sup> Mortalities resulted from damping-off.

<sup>e/</sup> Mortalities resulted from rot of primary roots.

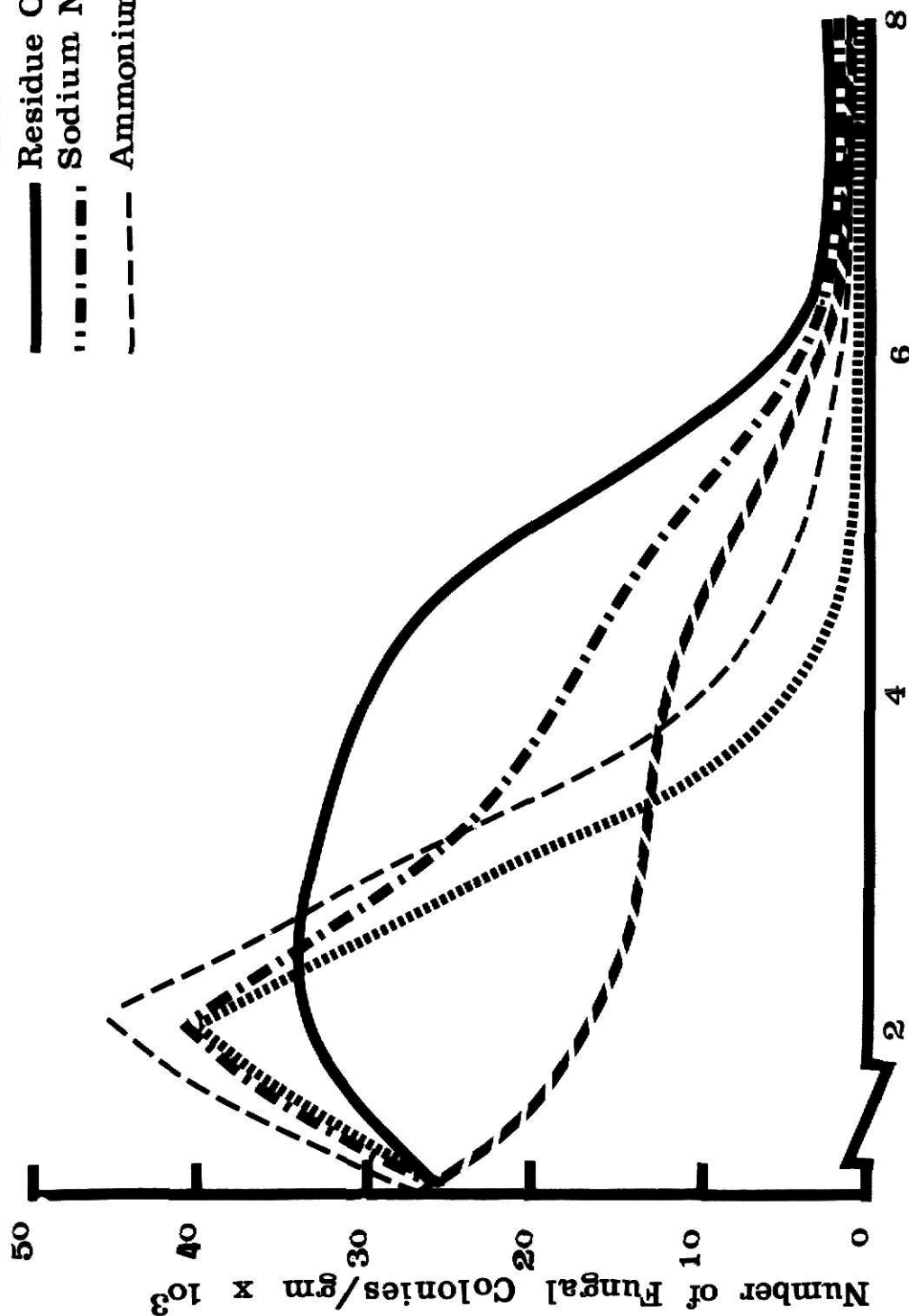
Effect of Sources of Nitrogen on the Population and  
Pathogenicity of F. moniliforme

About a week following infestation of soil, visible, extensive white mycelia were present on soil surfaces in the ammonium sulfate and urea treatments, and to some extent in the trays that received ammonium nitrate treatment. Very low initial populations were noted in the ammonium nitrate treatment. Soil surfaces in the ammonium sulfate treatment often became covered with actively-sporulating Ostracoderma pezizae, a brown saprophyte common on sterilized soils.

There was a consistent decline in the inoculum density with increasing length of the incubation period (Table 2, Fig. 1). This trend was not affected by the second application of the nitrogen compounds. Data in Table 3 show that the range in pH among treatments was only 1.5. The check had a very high bacterial population compared to the other treatments.

Emergence was most rapid and complete in the residue-only treatment (Table 4). The only direct correlation between nitrogen source and disease severity occurred early in the sodium nitrate treatment. For surviving plants, there was no significant difference among the nitrogen sources.

// Ammonium Nitrate  
 ..... Urea  
 — Residue Only  
 - - - Sodium Nitrate  
 --- Ammonium Sulfate



Incubation Period in Weeks

Fig. 1. Influence of various sources of nitrogen on rate of decline in population of *F. moniliforme* as determined by the dilution plate technique.

Table 2.--Influence of various sources of nitrogen on rate of decline in population <sup>a/</sup> of F. moniliforme as determined by the dilution plate technique

Treatments	Incubation period in weeks <sup>b/</sup>			
	2	4	6	8
Ammonium sulfate	40.4cd	7.9cd	1.6c	1.2c
Urea	39.2cd	5.8c	1.7c	1.6c
Ammonium nitrate	14.2*	13.7cde	3.0cd	1.9cd
Sodium nitrate	40.0cd	18.3def	3.6de	3.5e
Residue only (check)	32.7c	30.0fg	4.4def	3.4e

<sup>a/</sup> Data x  $10^3$  gives actual numbers of fungal colonies/g of soil, uncorrected for analysis of variance.

<sup>b/</sup> Means for a given column followed by same letter do not differ significantly at the 5% level (Duncan's multiple range test) when plate to plate data were analyzed after square root transformation ( $\sqrt{x+1}$ ) to correct for normal distribution.

\* Very low probably due to sampling error.

Table 3.--Soil pH and bacterial (+Actinomycetes) population <sup>a/</sup> as affected by the addition of various sources of nitrogen

Treatments	pH	Bacterial colonies (x 10 <sup>5</sup> )
Ammonium sulfate	6.3	36.6
Urea	6.7	7.4
Ammonium nitrate	6.6	4.9
Sodium nitrate	7.7	86.5
Residue only (check)	7.8	252.0

<sup>a/</sup> Determined after 8 weeks of incubation as colonies per gram of soil.

Table 4.--Influence of various sources of nitrogen on the pathogenicity of F. moniliforme after both had been incubated for 8 weeks before planting with Spur Pterita

Treatments <sup>a/</sup>	Emergence %	Mortality %	Height of top growth (cm)	Fresh wt. of top growth (g) <sup>c/</sup>	No. of adventitious roots <sup>d/</sup>
Ammonium sulfate	56.7*	7.4*	28.7	3.02	8
Urea	56.0*	6.0*	27.3	2.39	8
Ammonium nitrate	58.0*	5.7*	28.3	2.37	4
Sodium nitrate	37.4	24.2	21.9	1.96	5
Residue only (check)	67.4	0.9	36.6	5.00	25
LSD.05	13.9	6.3	5.9	1.22	3.9

a/ Residue supplemented with the given nitrogen compound except for last treatment which had no nitrogen.

b/ Mean for 30 plants per treatment.

c/ Sum of 10 plants/replicate, mean of 3 replicates.

d/ The total number of adventitious roots for 10 plants per replication.

\* Values for sources of nitrogen which are significantly different from those of sodium nitrate.



The length of lesions on the primary roots (Table 5) was directly related to severity of seedling blight (Table 4). Out of a sample of 30 plants in the residue-only treatment, only 1 had a primary root lesion which was greater in length than 1.4 cm. For ammonium nitrate, sodium nitrate, urea and ammonium sulfate treatments, 21, 19, 15 and 7 of 30 plants, respectively, had lesions 1.5 cm or greater in length. Number of adventitious roots (Table 4) was inversely related to the size of root lesions (Table 5). The check had the greatest number of roots per replicate, while ammonium nitrate and sodium nitrate treatments had the least. Root numbers for urea and ammonium sulfate treatments were intermediate.

#### Effect of Levels of Nitrogen on Disease Severity

Observations before nutrient applications---Stand counts showed that

F. moniliforme significantly reduced germination of Spur Feterita and Redlan seeds but not those of RS 610 (Table 6).

Table 5.--Influence of various sources of nitrogen on the pathogenicity of F. moniliforme as evidenced by lesion development on primary roots of Spur Feterita

Treatments	Frequency distribution <sup>a/</sup> of length (cm) of lesion on the primary root							
	0- 0.4	0.5- 0.9	1.0- 1.4	1.5- 1.9	2.0- 2.4	2.5- 2.9	3.0- 3.4	3.5- 3.9
Ammonium sulfate	2	10	11	4	3	0	0	0
Urea	1	10	4	9	5	1	0	0
Ammonium nitrate	0	4	5	16	2	2	0	1
Sodium nitrate	0	3	8	10	7	1	1	0
Residue only (check)	<u>4</u>	<u>16</u>	<u>9</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>
Totals	7	43	37	39	18	4	1	1

<sup>a/</sup> Based on a sample of 30 plants per treatment.

Table 6.--Effect of sand infestation with F. moniliforme on seedling emergence of 3 sorghum genotypes at greenhouse temperature of 20C

Rep. No. <sup>b/</sup>	Number of emerged plants <sup>a/</sup> of:					
	<u>Spur Feterita</u>		<u>RS 610</u>		<u>Redlan</u>	
	Inoculated	Check	Inoculated	Check	Inoculated	Check
1	25	26	23	22	13	20
2	18	23	22	23	20	21
3	21	23	21	23	15	21
4	12	24	22	21	18	18
5	20	29	24	20	10	18
6	22	27	24	23	20	19
7	16	26	22	20	13	20
8	23	24	19	20	14	22
9	15	22	23	24	16	20
10	21	21	20	23	23	19
11	25	29	21	20	16	22
12	15	25	24	24	17	24
13	20	27	19	22	17	25
14	18	26	17	23	13	22
15	14	28	18	23	14	21
16	21	23	18	20	23	22
17	18	26	23	24	19	22
18	22	24	21	20	19	20
19	22	25	22	23	23	23
20	24	24	24	21	19	19
Mean	19.6	25.1	21.4	22.0	17.6	20.9
	H.S.		N.S.		H.S.	

<sup>a/</sup> 25 seeds of Redlan and RS 610 were sown per pot; 40 seeds of Spur Feterita were sown per pot.

<sup>b/</sup> There was one inoculated and one uninoculated pot in each replication.

H.S. = Significant difference at 1%

N.S. = Non-significant at 5%

In the inoculated pots, many of the plants removed in thinning were covered with a mass of Fusarium mycelium. This was uncommon among seedlings thinned from the check, but a few showed the presence of other fungi such as Alternaria sp. Within 4 days after emergence, inoculated plants were distinctly shorter than uninoculated plants, a feature which had been observed in earlier experiments. Again, the differences became less apparent as plants grew older. In the inoculated pots, many Spur Feterita plants exhibited a purplish streaking in lower leaves; they also were unthrifty. Check plants of the same variety had stout stems and broad, undiscolored leaves. Foliage of the other 2 varieties had no obvious disease symptoms.

Observations after nutrient applications---Among checks, best growth was noted at 200 ppm for Spur Feterita but for the other two genotypes, there were no significant growth differences between 100, 200 and 400 ppm nitrogen. At 400 ppm, some symptoms of excessive nitrogen were noted for Spur Feterita, but the other two seemed to tolerate this nitrogen level quite well. Growth was distinctly inferior at 0 and 50 ppm (Table 7, Figs. 2-4).



#### EXPLANATION OF PLATE I

PLATE I. Four-week old Spur Feterita seedlings raised in sand-culture fertilized with 200 ppm nitrogen in form of ammonium nitrate: seeding rate was the same in both cases.

PLATE I

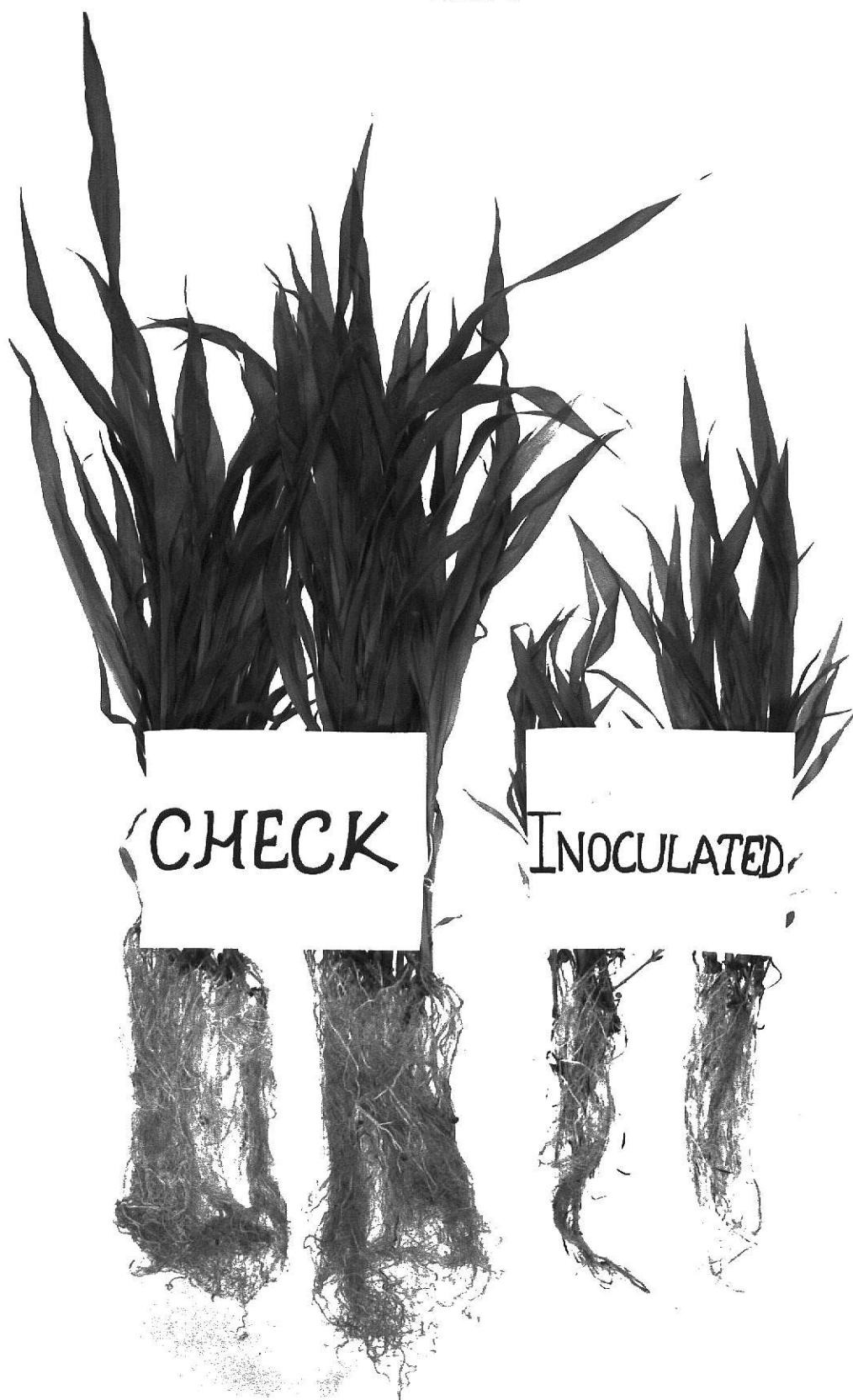


Table 7.--Effect of 5 levels of nitrogen on the development of 3 sorghum genotypes in sand culture with or without F. moniliforme in greenhouse tests at 20-32C, as indicated by the height of top growth of 30-day old plants

ppm N	Mean <sup>a</sup> / height (cm) for:											
	Spur F e t e r i t a				R S 6 1 0				R e d l a n			
	Inoculated	Check	reduction %	Growth	Inoculated	Check	reduction %	Growth	Inoculated	Check	reduction %	Growth
0	18.1	24.3	25.5		23.6	25.0	5.6		19.3	22.6	14.6	
50	21.6	23.8	9.3		29.1	30.0	3.0		22.7	26.7	15.0	
100	22.8	29.4	22.5		34.9	37.4	6.7		27.9	37.1	24.8	
200	22.3	38.0	41.3		31.9	39.7	19.6		32.6	36.5	10.7	
400	25.6	35.9	28.7		37.7	41.9	10.0		29.2	38.9	24.9	

a/ Mean of 5 plants/sample x 4 replicates.

L.S.D. (.05) of %growth reduction between 2 levels of N in same variety = 12.5

L.S.D. (.05) of growth reduction between 2 variety means of the same level of nitrogen = 13.4



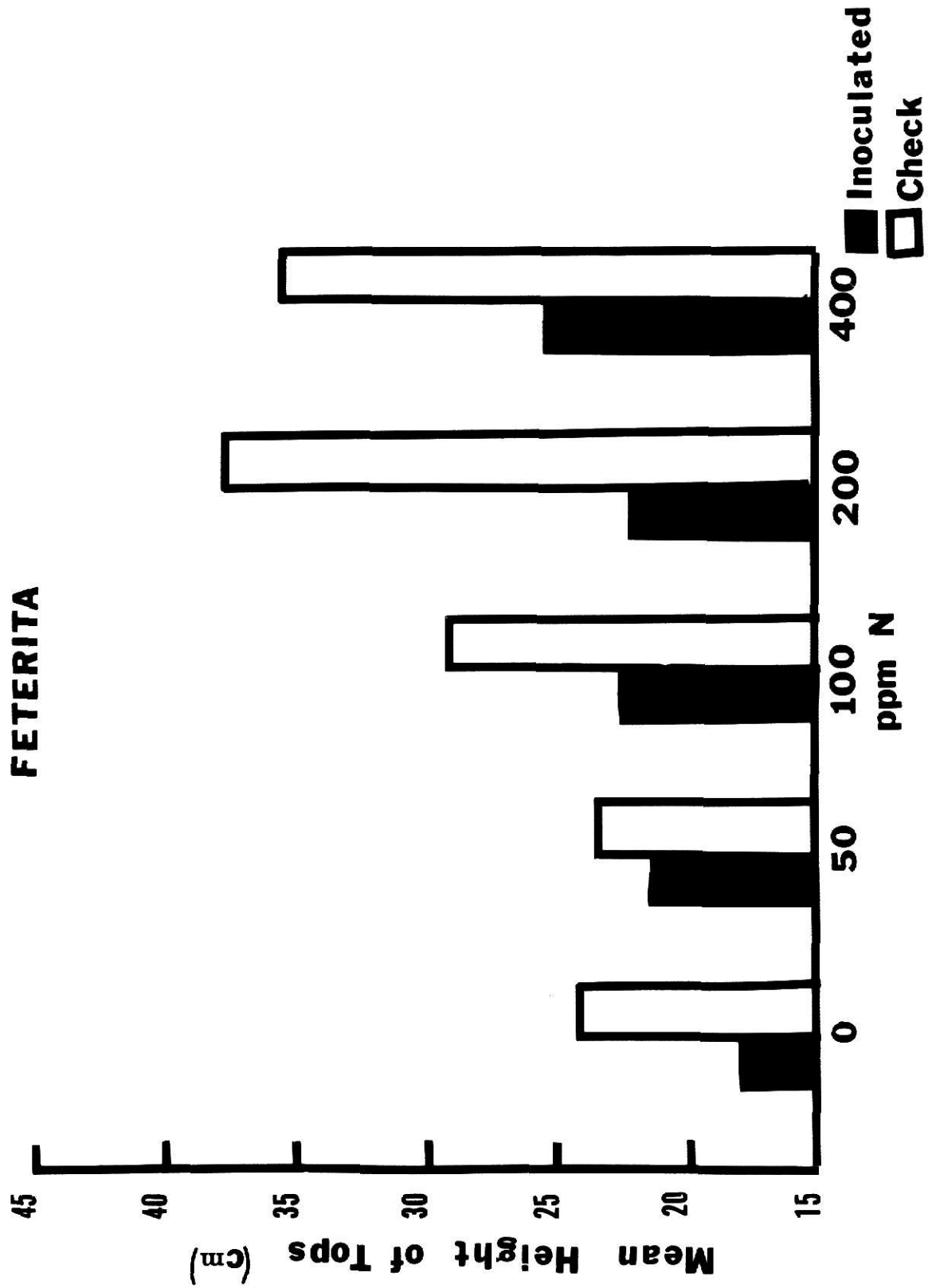


Fig. 2. Effect of 5 levels of nitrogen on the development of Spur Feterita in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the height of top growth of 30-day old plants.

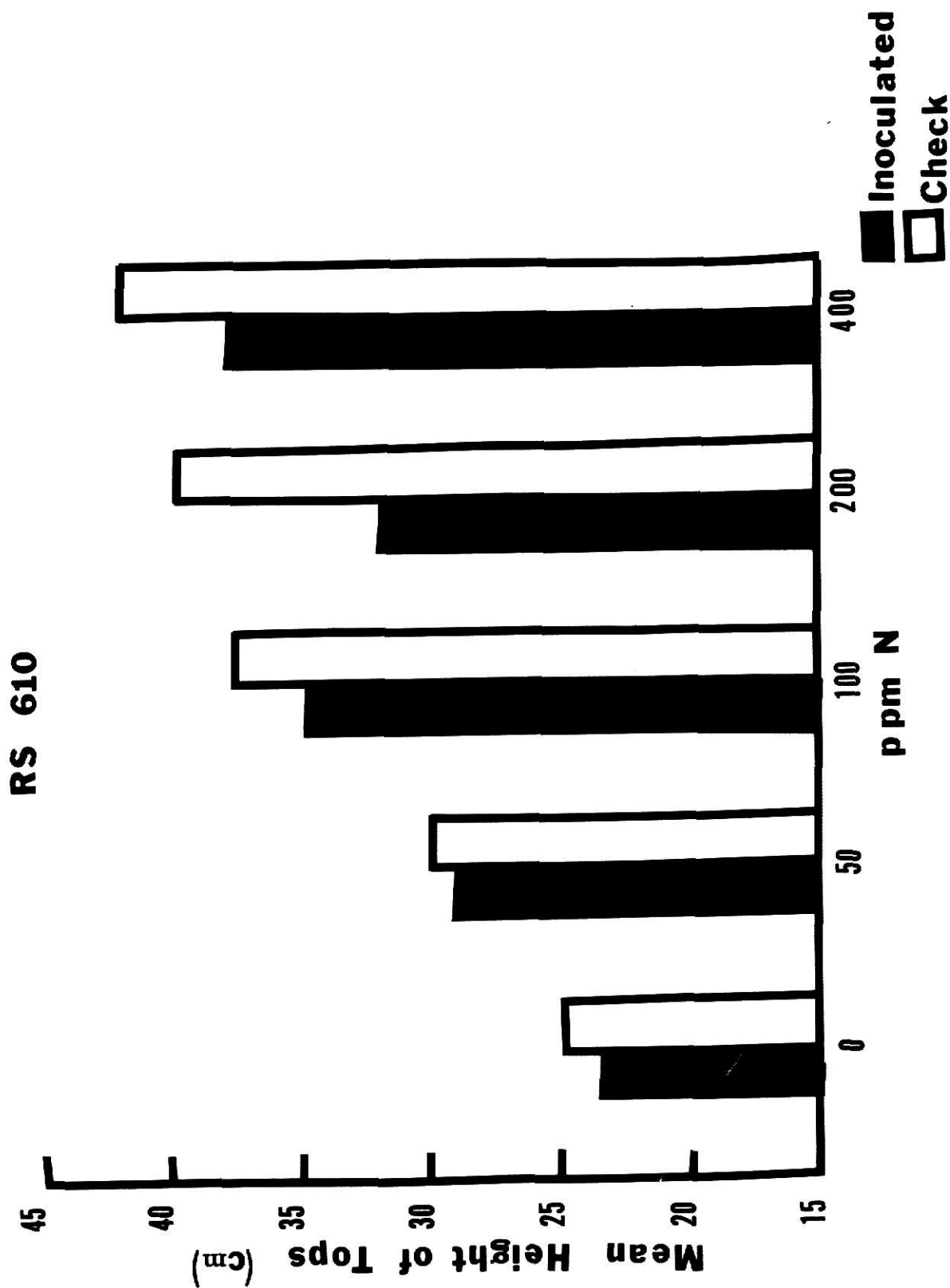


Fig. 3. Effect of 5 levels of nitrogen on the development of RS 610 in dandelion culture with or without *F. moniliforme* in greenhouse tests at 20-32°C, as indicated by the height of top growth of 30-day old plants.

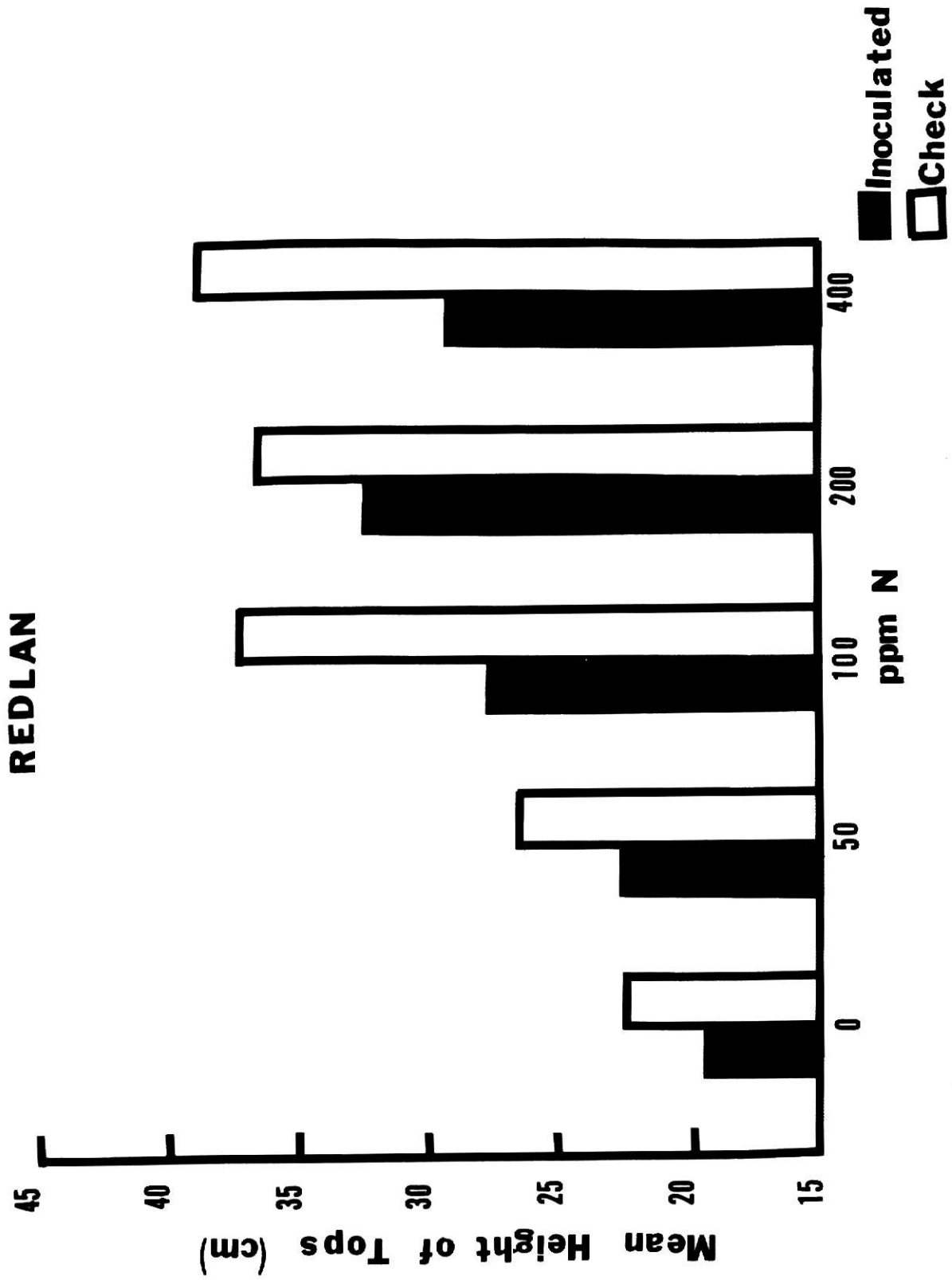


Fig. 4. Effect of 5 levels of nitrogen on the development of Redlan in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the height of top growth of 30-day old plants.

There was no linear relationship between levels of nitrogen and seedling blight severity. Greatest stunting of plant growth occurred in inoculated plants of Spur Feterita and RS 610 at 200 ppm (Table 7 and Plates I and II). Spur Feterita, the most susceptible variety, also had the greatest plant weight reductions at 200 ppm (Tables 8 and 9). Weight data (Figs. 5-13) indicated that all varieties had considerable Fusarium-induced weight reductions at 0 ppm, nitrogen.

As judged by combined height, plant weights and mortality, least disease development occurred at 50 and 100 ppm. At 400 ppm disease severity was greater than at 50 and 100 ppm, but it was less than at 200 ppm, especially in the case of Spur Feterita.

Root lesions were concentrated mostly in the upper part of the root system, although the inoculum was well-distributed throughout the sand in which the plants were growing. Although the differences were small, Mean Disease Severity Index increased with increasing level of nitrogen (Table 10 and Figs. 14-16). Indices for checks were not zero because seed- and air-borne fungi, including Fusarium spp., usually gave rise to some root lesions.

Table 8.--Effect of 5 levels of nitrogen on the development of 3 sorghum genotypes in sand culture with or without F. moniliforme in greenhouse tests at 20-32C, as indicated by the fresh weight of tops of 30-day old plants

ppm N	Mean $\frac{a}{weight}$ (g)																	
	S p u r F e t e r i t a						R S 6 1 0						R e d l a n					
	Inoculated	Check reduction %	Weight	Inoculated	Check reduction %	Weight	Inoculated	Check reduction %	Weight	Inoculated	Check reduction %	Weight	Inoculated	Check reduction %	Weight			
0	1.24	2.74	54.6	0.85	1.71	50.5	0.83	1.34	38.0									
50	3.93	4.37	10.1	3.77	3.72	0	2.58	3.13	17.6									
100	3.98	6.87	42.1	6.31	6.52	3.3	4.19	6.11	31.4									
200	4.11	9.83	58.2	4.60	7.20	36.2	5.33	5.57	4.2									
400	5.20	9.89	47.4	7.10	9.39	24.4	4.67	6.71	30.4									

a/ Mean of 5 plants/sample x 4 replicates.

## FETERITA

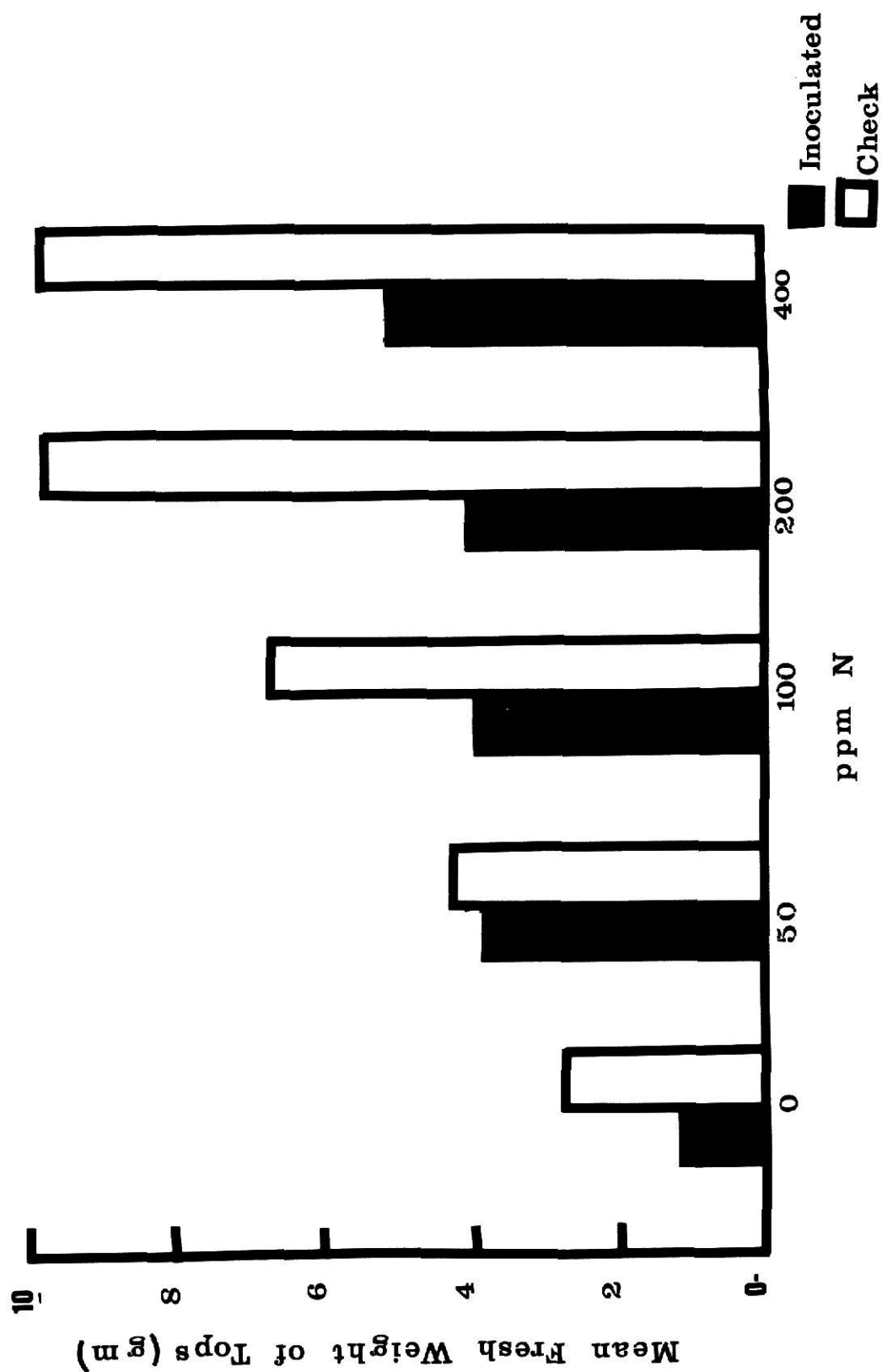


Fig. 5. Effect of 5 levels of nitrogen on the development of Spur Feterita in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32°C, as indicated by the fresh weight of top growth of 30-day old plants.

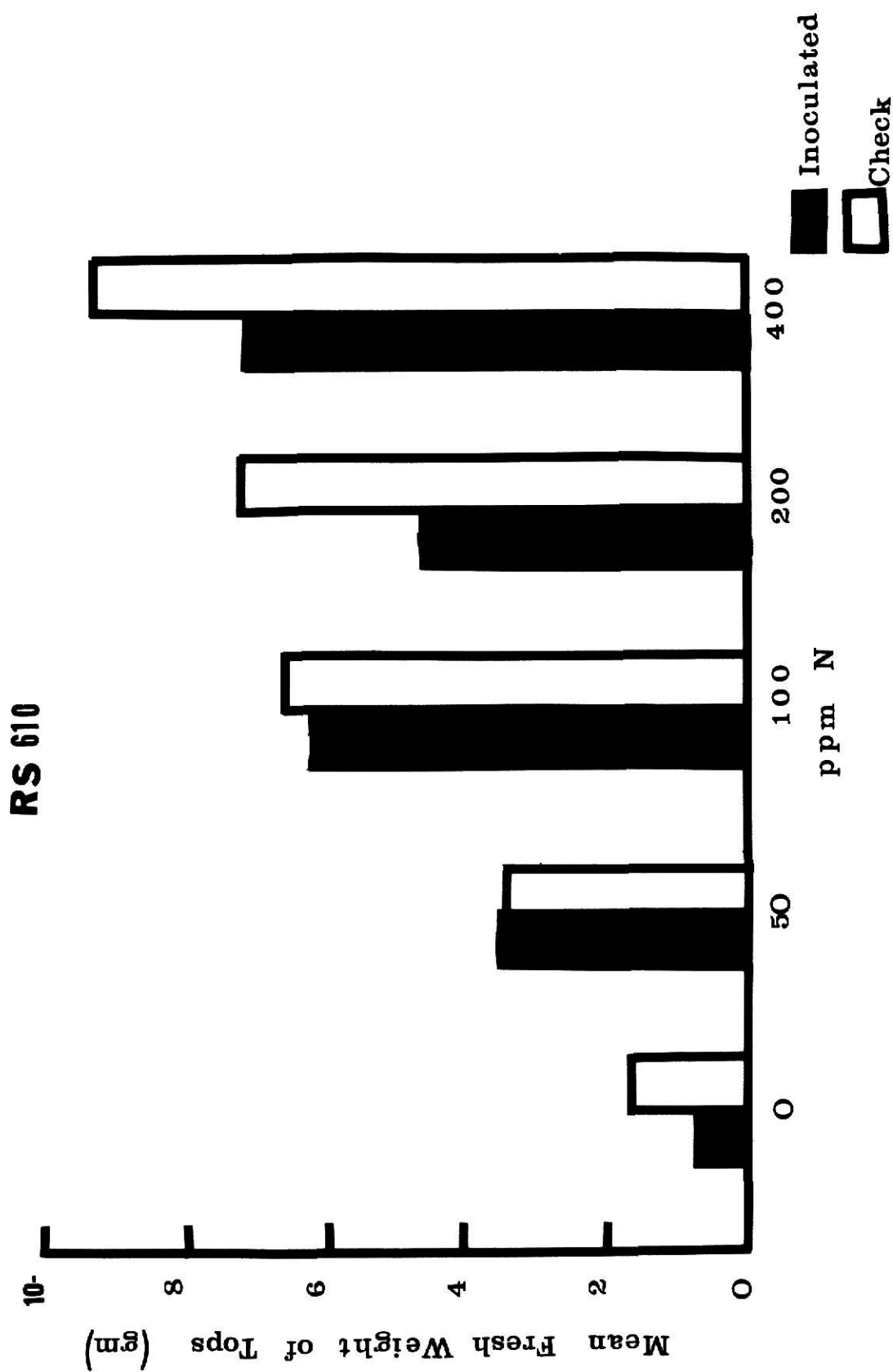


Fig. 6. Effect of 5 levels of nitrogen on the development of RS 610 in sand culture with or without F. moniliforme in greenhouse tests at 20-32C, as indicated by the fresh weight of top growth of 30-day old plants.

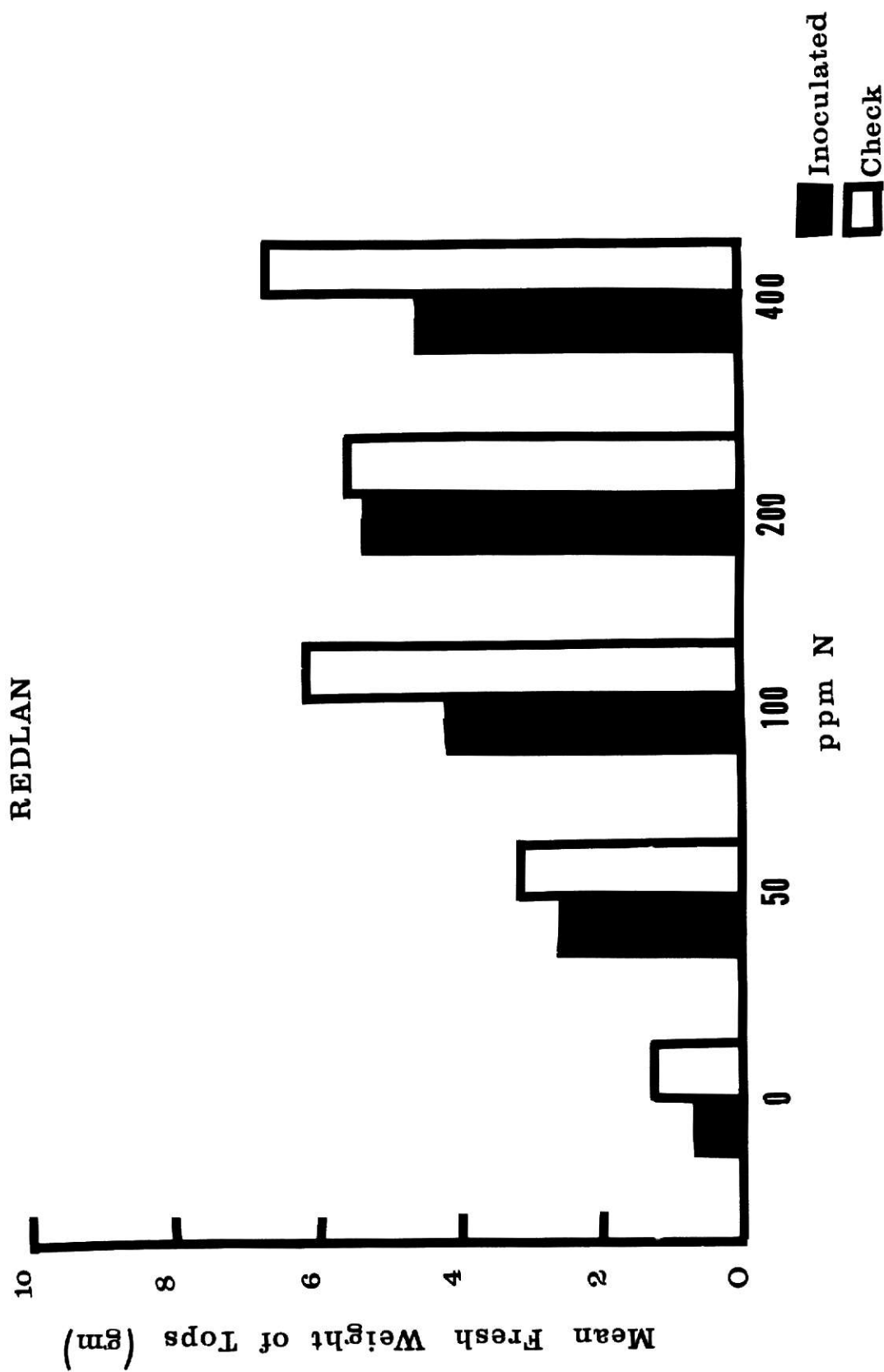


Fig. 7. Effect of 5 levels of nitrogen on the development of Redlan in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the fresh weight of top growth of 30-day old plants.



Table 9.--Effect of 5 levels of nitrogen on the development of 3 sorghum genotypes in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the oven-dry weights of the tops and roots of 30-day old plants

ppm N	Treatment	Mean <sup>a/</sup> weight (g)					
		Feterita		RS 610		Redlan	
		Tops	Roots	Tops	Roots	Tops	Roots
0	Inoculated	0.33	1.27	0.33	1.15	0.26	1.01
	Check	0.56	1.82	0.48	1.49	0.42	1.44
	Wt. reduction %	41.5	30.3	31.3	22.8	38.9	29.9
50	Inoculated	0.40	1.07	0.47	1.81	0.31	0.96
	Check	0.50	1.72	0.53	1.84	0.40	1.36
	Wt. reduction %	20.0	38.1	11.6	1.6	24.3	29.5
100	Inoculated	0.43	0.78	0.76	1.08	0.50	0.91
	Check	0.78	1.87	0.81	1.75	0.78	1.14
	Wt. reduction %	45.7	58.5	7.0	38.7	36.4	20.1
200	Inoculated	0.40	0.63	0.50	1.17	0.59	0.55
	Check	1.08	1.53	0.81	1.50	0.63	0.94
	Wt. reduction %	63.0	58.5	38.5	22.0	6.3	41.4
400	Inoculated	0.50	1.17	0.81	1.35	0.53	0.75
	Check	0.93	1.63	1.03	1.64	0.74	0.95
	Wt. reduction %	46.1	28.4	21.4	17.5	28.3	28.3

<sup>a/</sup> Mean of 5 plants/sample x 4 replicates; for roots, mean of entire roots in 4 replicates.

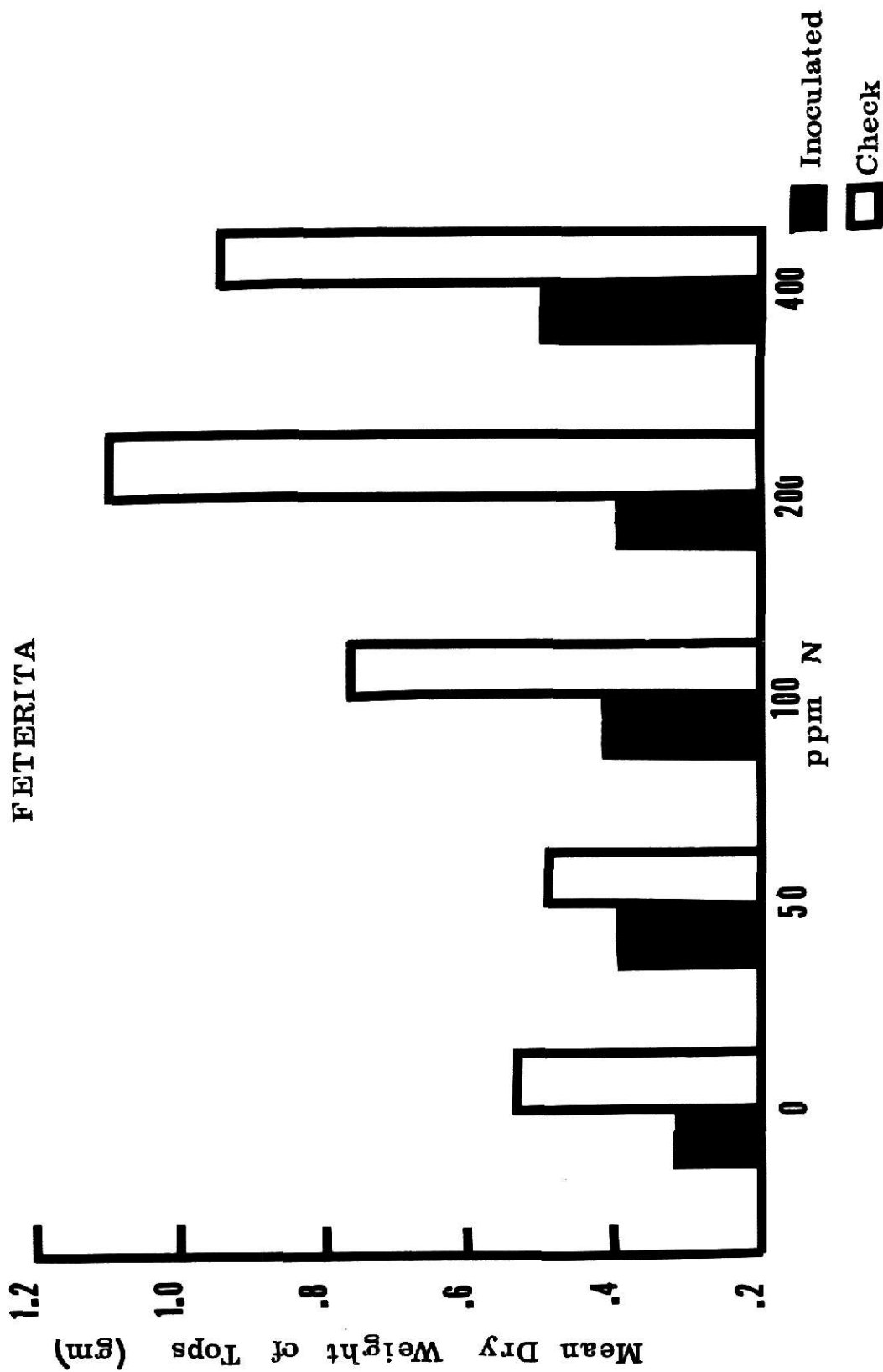


Fig. 8. Effect of 5 levels of nitrogen on the development of Spur Feterita in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the dry weight of top growth of 30-day old plants.

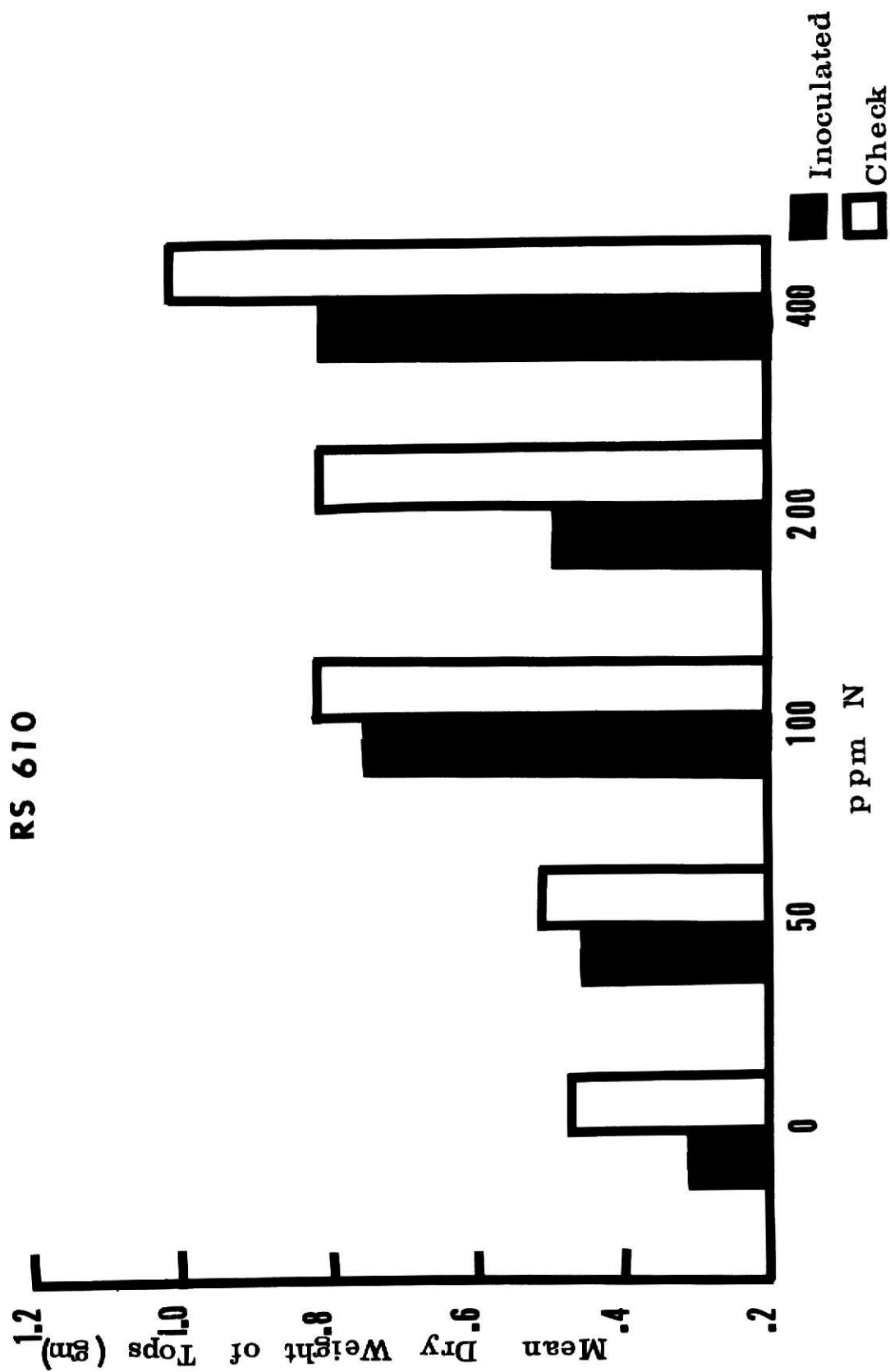


Fig. 9. Effect of 5 levels of nitrogen on the development of RS 610 in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the dry weight of top growth of 30-day old plants.

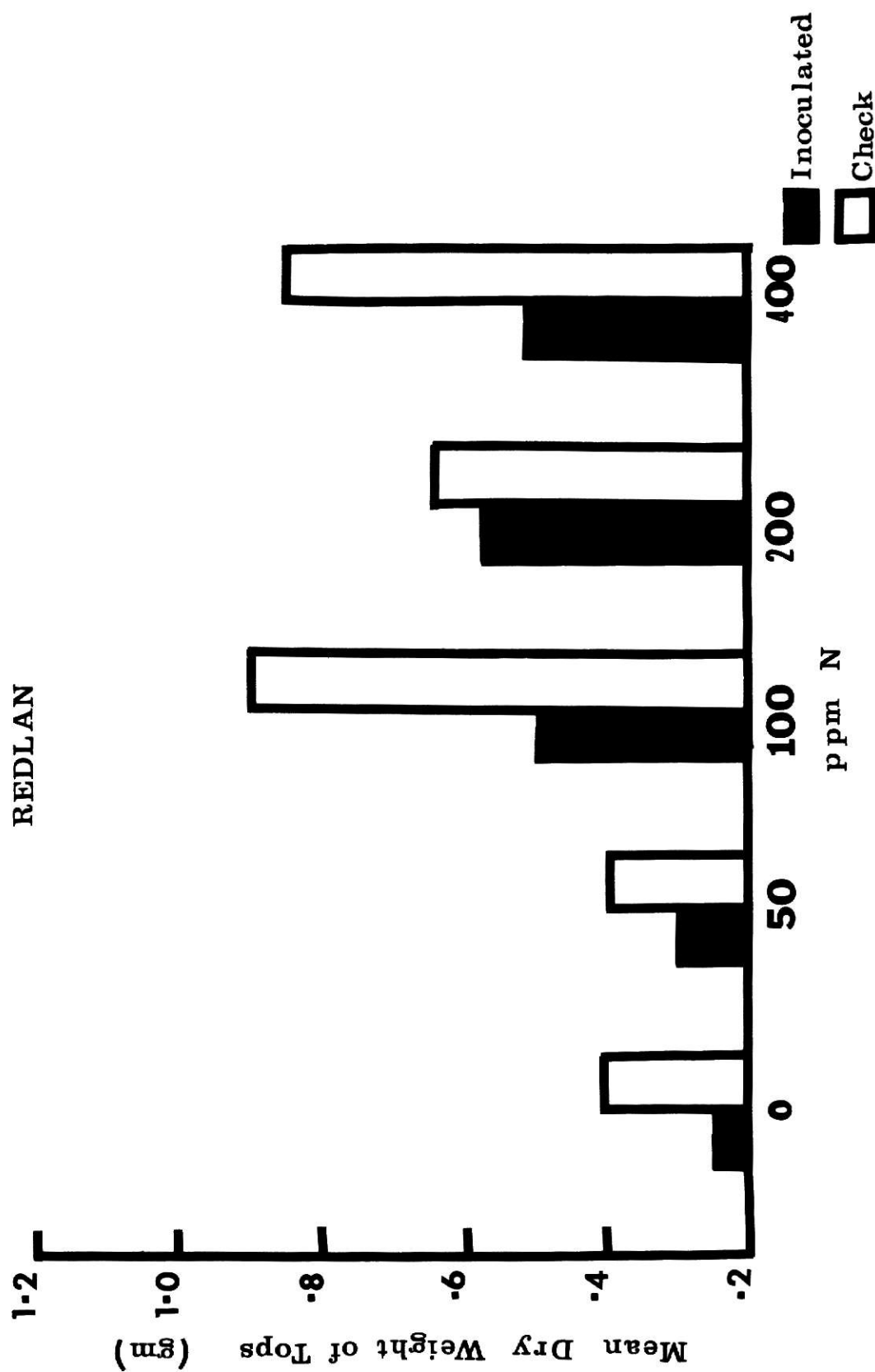


Fig. 10. Effect of 5 levels of nitrogen on the development of Redlan in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the dry weight of top growth of 30-day old plants.

## FETERITA

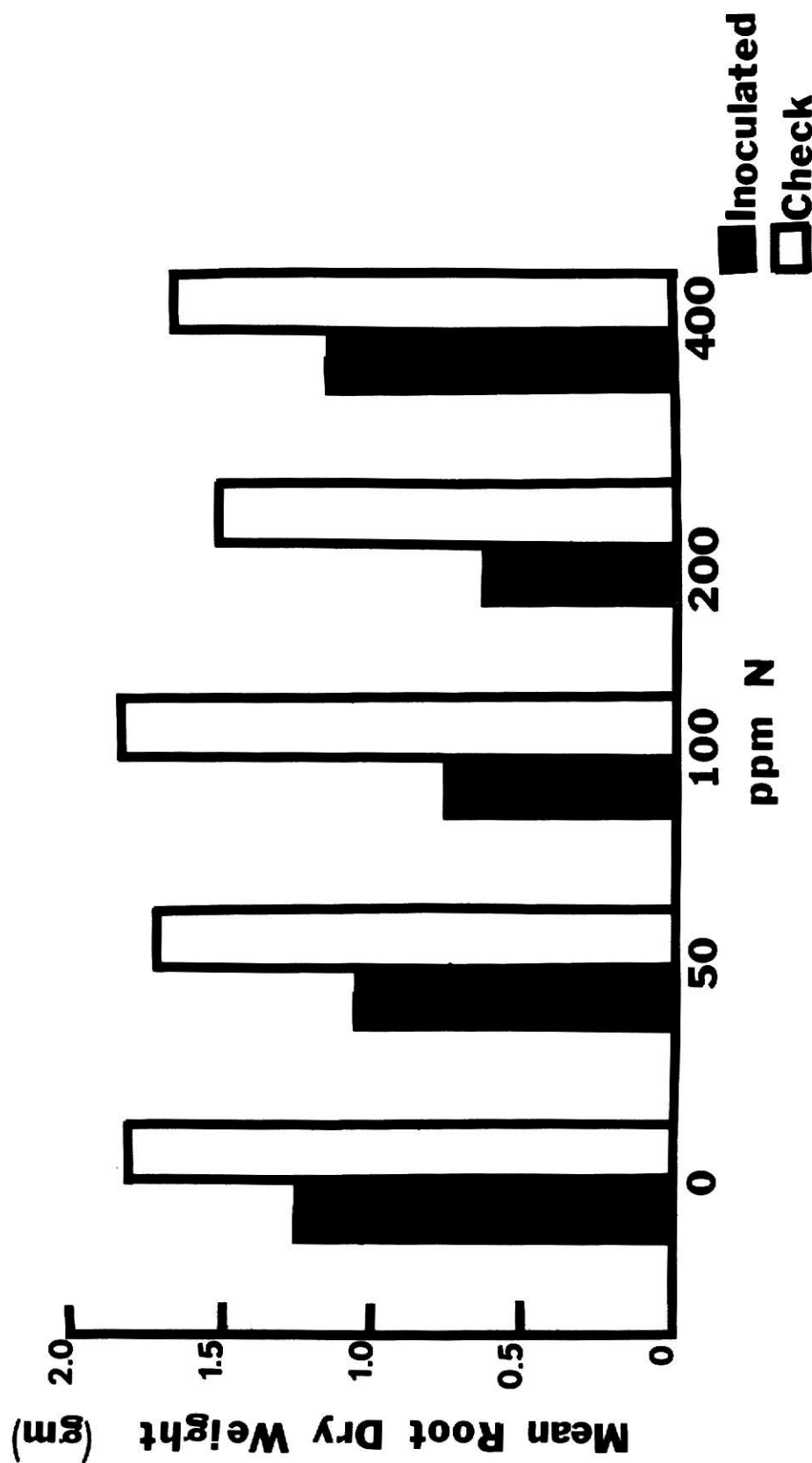


Fig. 11. Effects of 5 levels of nitrogen on the development of Spur Feterita in sand culture with or without F. moniliforme in greenhouse tests at 20-32C, as indicated by the dry weight of roots of 30-day old plants.

# RS 610

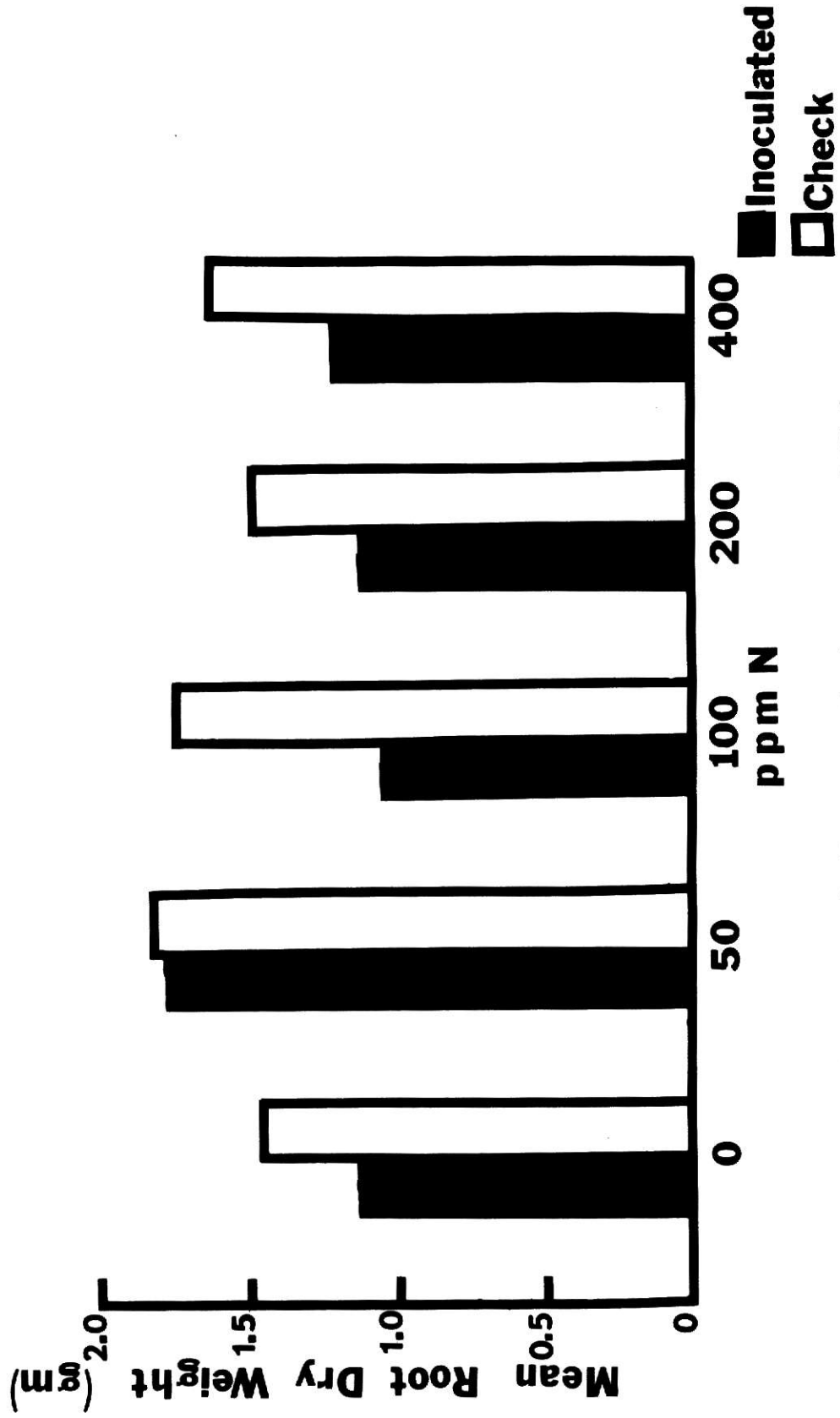


Fig. 12. Effect of 5 levels of nitrogen on the development of RS 610 in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the dry weight of roots of 30-day old plants.

## REDLAN

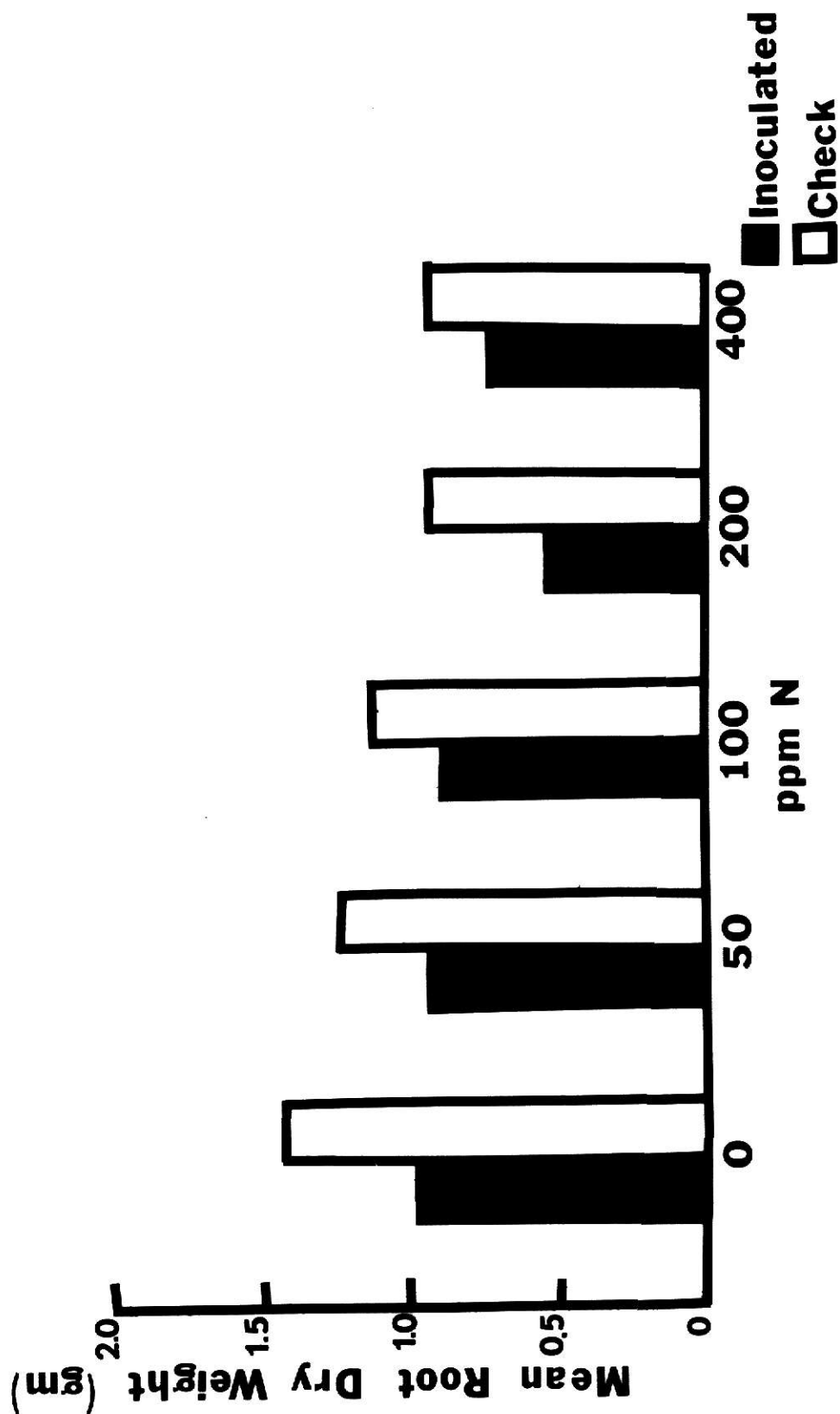


Fig. 13. Effect of 5 levels of nitrogen on the development of Redlan in sand culture with or without *F. moniliforme* in greenhouse tests at 20-32C, as indicated by the dry weight of roots of 30-day old plants.





#### EXPLANATION OF PLATE II

PLATE II. Root systems of individual plants: 3 plants on the left are healthy while the 3 on the right are diseased. The latter show a dark necrotic region on the primary root and varying degrees of reduction in number of secondary roots.

PLATE II

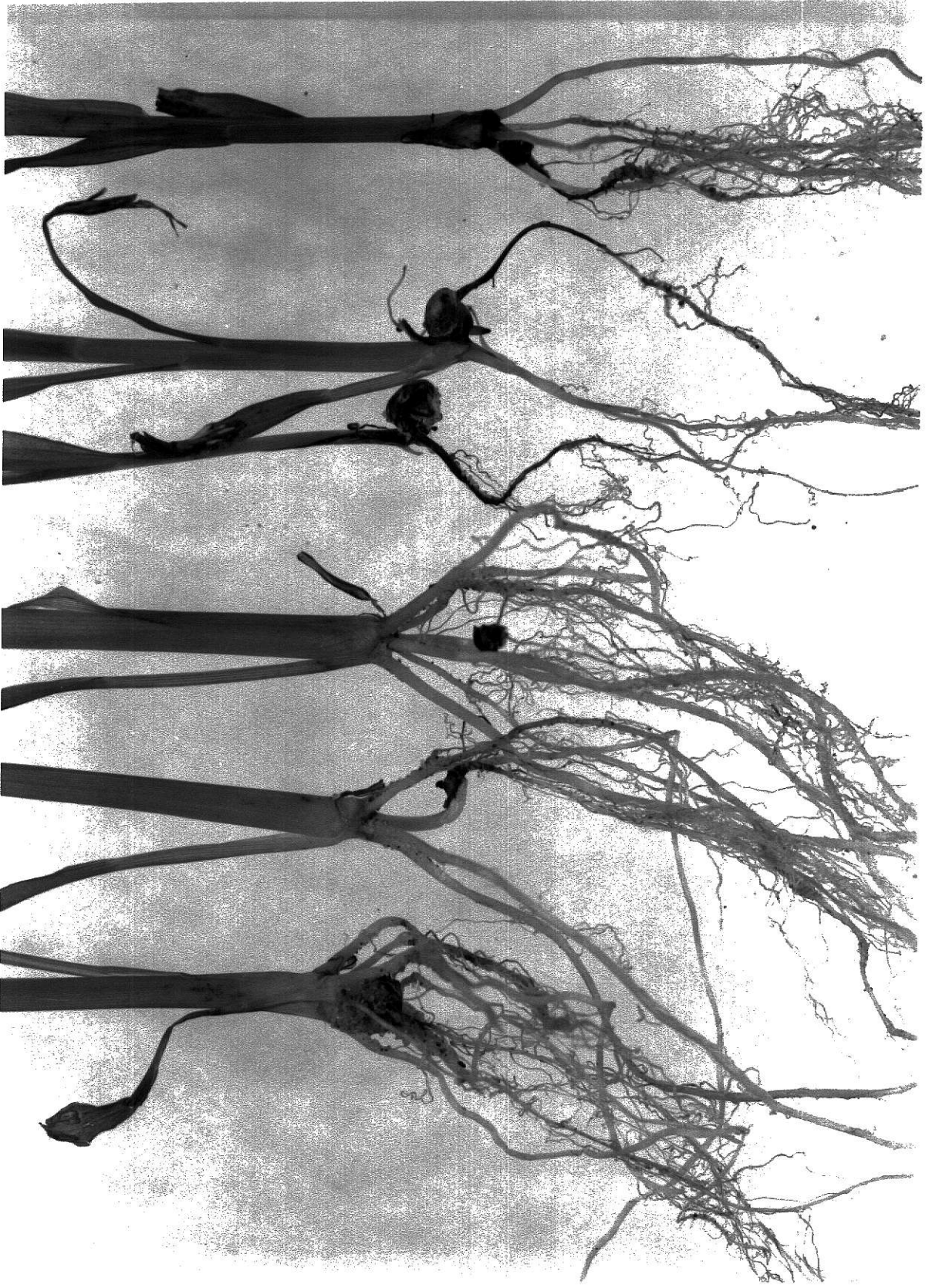


Table 10.--Effect of 5 levels of nitrogen on the severity of seedling blight in 3 sorghum genotypes

ppm N	Mean Disease Severity Index <sup>a/</sup>					
	<u>Spur Feterita</u>		<u>RS 610</u>		<u>Redlan</u>	
	Inoculated	Check	Inoculated	Check	Inoculated	Check
0	2.25	1.50	1.75	1.00	3.00	1.25
50	2.75	1.50	2.50	1.75	2.50	1.00
100	3.25	1.75	2.25	1.50	3.00	1.25
200	3.25	1.25	2.50	1.00	3.25	1.00
400	3.00	2.00	3.00	1.50	4.00	1.50

<sup>a/</sup> Based on a 0-5 scale for 4 pots per treatment; 0 = no disease symptoms and 5 = most severe symptoms.

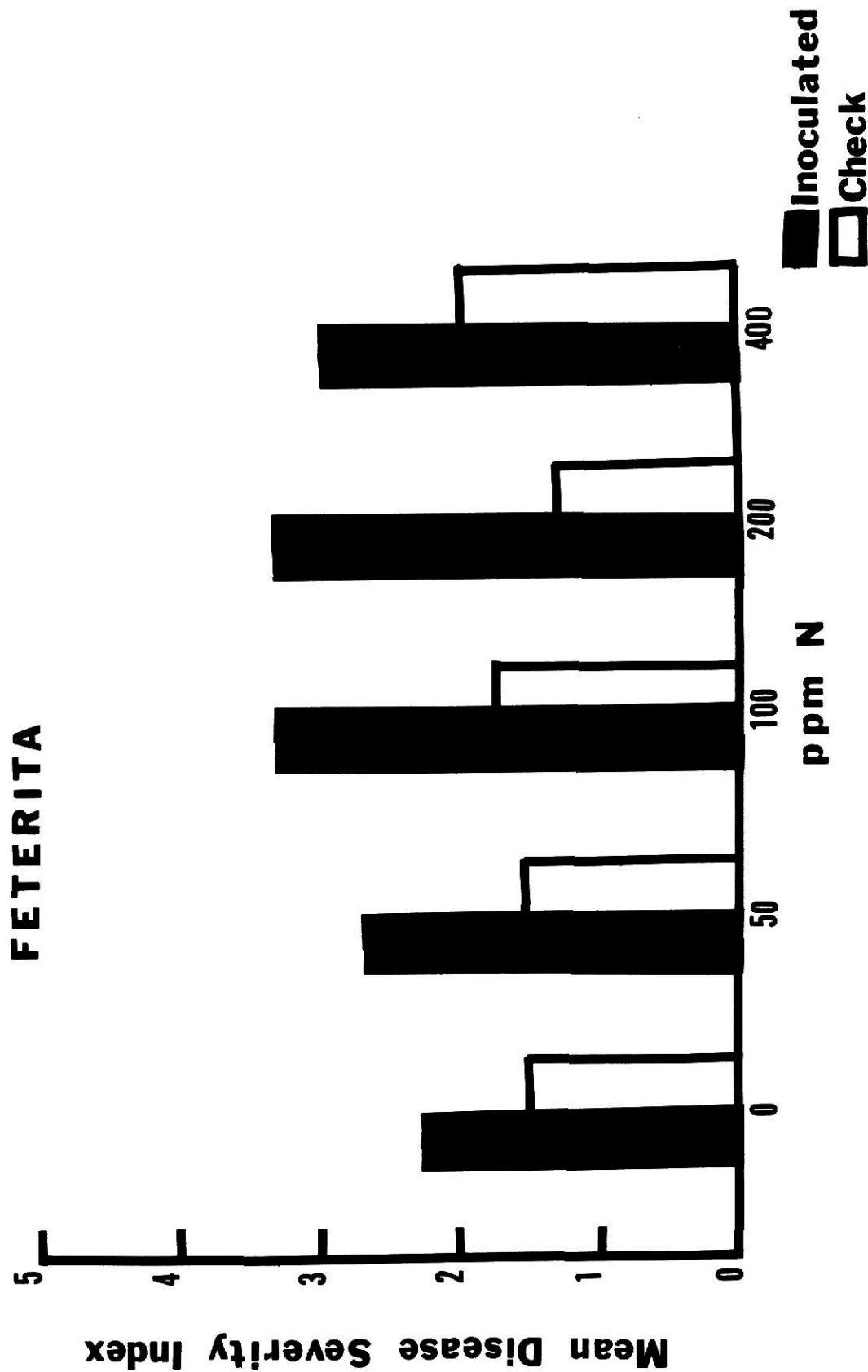


Fig. 14. Effect of 5 levels of nitrogen on the severity of seedling blight on Spur Feterita. Disease Severity Index was based on a 0-5 scale in which 5 represented most severe symptoms. A mean of 4 replicates was calculated for each treatment.

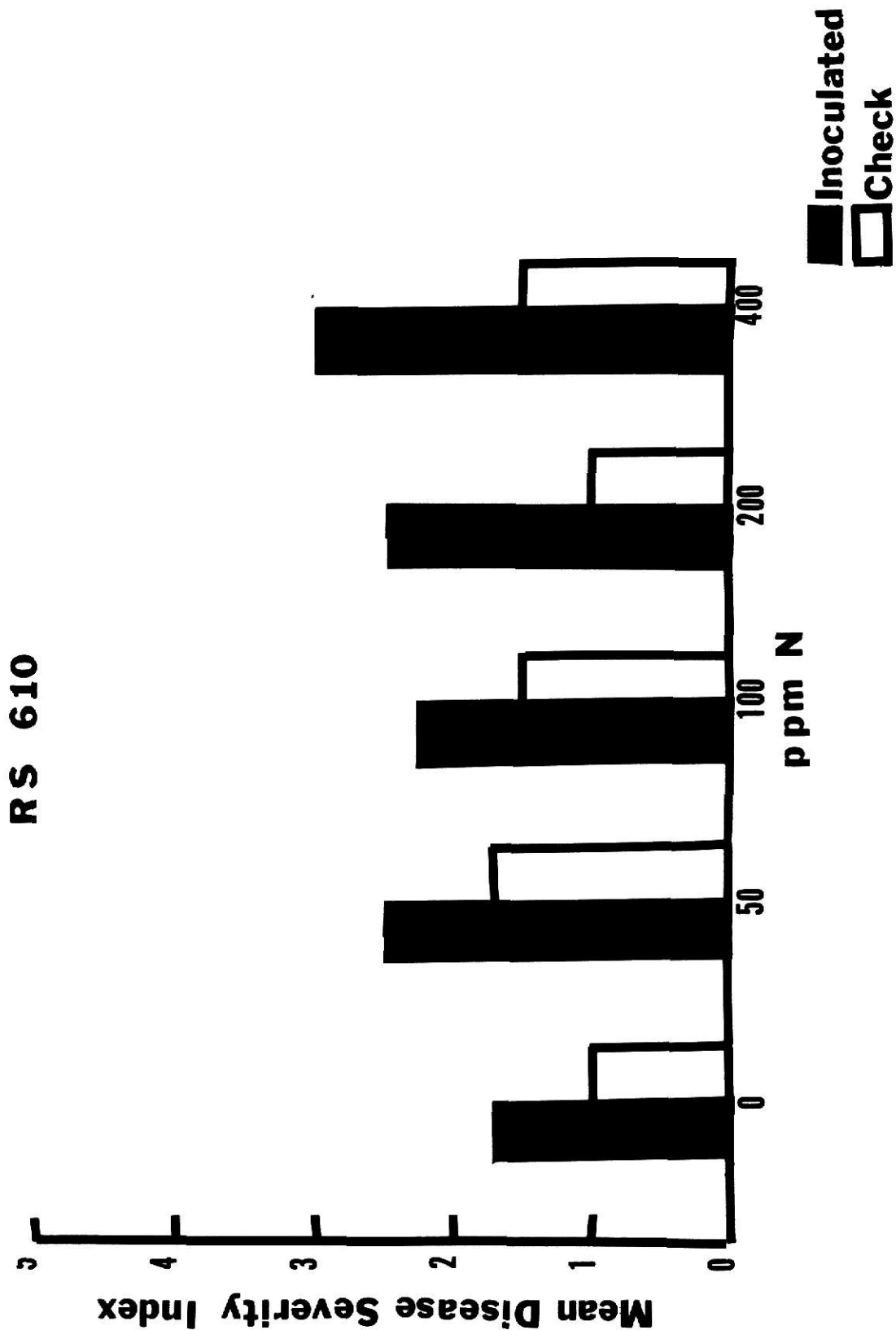


Fig. 15. Effect of 5 levels of nitrogen on the severity of seedling blight on RS 610. Disease Severity Index was based on a 0-5 scale in which 5 represented most severe symptoms. A mean of 4 replicates was calculated for each treatment.

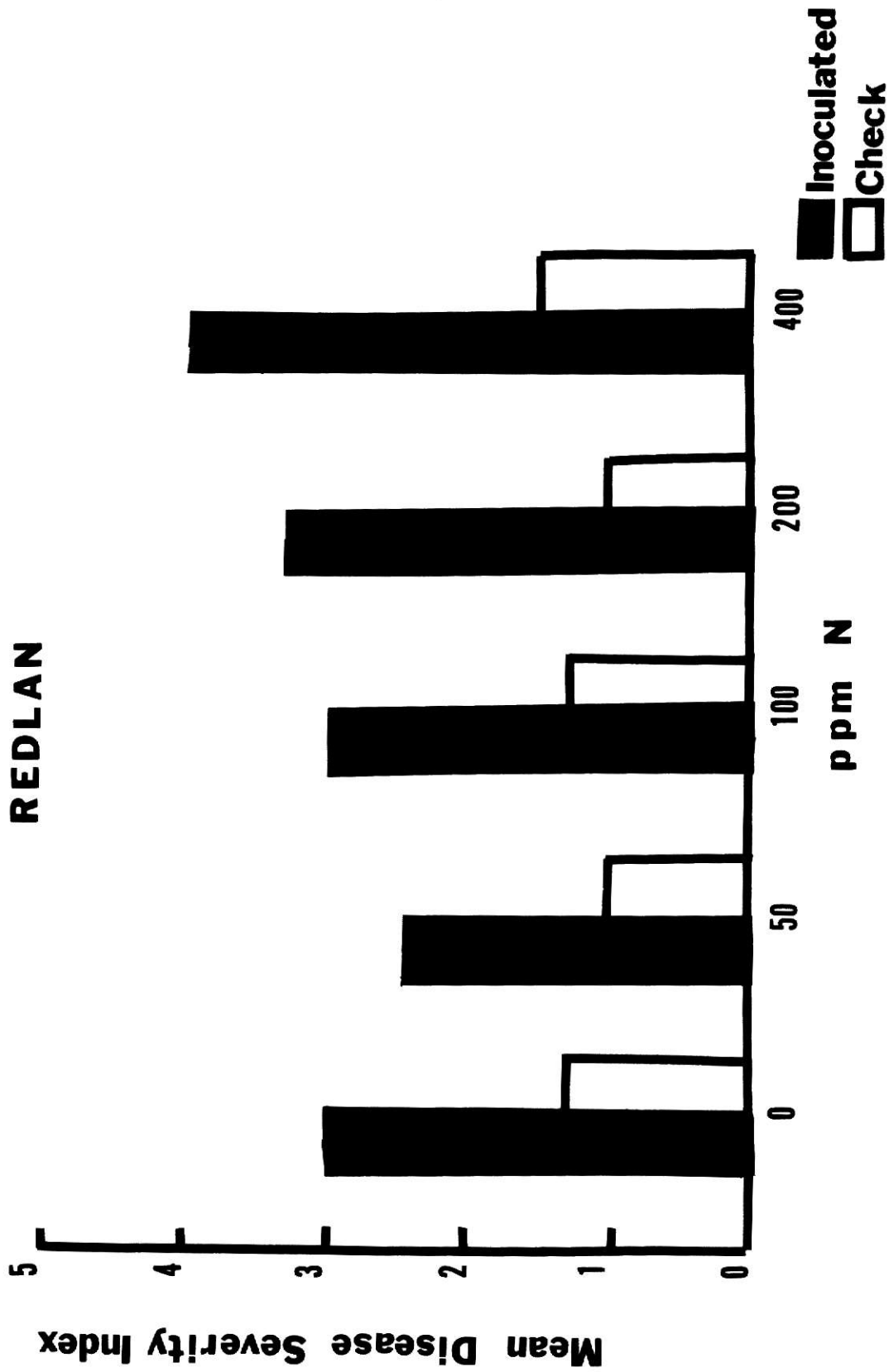


Fig. 16. Effect of 5 levels of nitrogen on the severity of seedling blight on Redlan. Disease Severity Index was based on a 0-5 scale in which 5 represented most severe symptoms. A mean of 4 replicates was calculated for each treatment.

## DISCUSSION

Nitrogen, more than any other element, has been shown to have a pronounced effect on individual soil fungi (24). It has been suggested that the ability of a fungus to utilize inorganic nitrogen, especially under sub-optimum conditions, contributes to its competitive saprophytic ability (3). In warm climates, F. moniliforme exists in the soil mainly as a vegetative mycelium and also in form of microconidia. Many fungal spores are known to require exogenous nutrients for germination and subsequent establishment, and the lack of these nutrients has been correlated with soil fungistasis (1, 25). The rapid decline in F. moniliforme populations with time apparently was not due to the exhaustion of the nitrogen supply. More likely, it was a result of some combination of antagonisms, competitions, and antibioses from other organisms in the re-infested soil. Research in related fields indicates that organic compounds produced during the microbial degradation of plant residues could result directly or indirectly in a reduction in pathogen population, especially in the absence of a susceptible host (30). The competitive potential of F. moniliforme under field conditions was not evaluated, but it did not appear to be very high in nitrogen-supplemented treatments of this study.

Source of nitrogen also appeared to influence activities of F. moniliforme. Possibly the pathogen did not utilize ammonium nitrogen as effectively as it used the nitrate form; or the former, by its slower rate of nitrification, may have attracted a greater saprophytic population which caused decline of the pathogen population. According to Huber et al. (20), ammonium sulfate is rapidly utilized by competitive or antagonistic soil microorganisms. The association of a profuse growth of Ostracoderma pezizae (a competitor) with the ammonium sulfate-treated soil seems to lend some credence to his

assertion. Perhaps this association was reflected in the relatively low pathogen population in final counts for this treatment.

That the rate of decline in pathogen population was least in the soil unsupplemented with nitrogen may have been due to initially lower numbers and kinds of other organisms, the rationale being that the low nutritional level supported only those organisms capable of overcoming a common limiting factor, in this case nitrogen. Most of these organisms appeared to be bacteria. Final population counts for this treatment did indicate that decline of F. moniliforme had reached a level equal to lowest counts obtained in the sodium nitrate-supplemented soil.

Seedling phases of disease caused by F. moniliforme roughly fall into 2 categories which respectively coincide with plant development before and after formation of the permanent root system. The first category includes seedling mortalities caused by damage to endosperm or embryonic tissues of the germinating seed, early invasion and destruction of the seminal root, crown necrosis near time of emergence, or possibly, toxin damage to the young seedling. The second category includes seedlings which are not killed before formation of adventitious roots. Under ideal growing conditions, development of branch or new adventitious roots may completely overcome effects of root damage. Under less favorable conditions, surviving plants exhibit varying degrees of stunting, unthriftness, or general debilitation.

Soil moisture, temperature, soil structure, inoculum survival and build-up, and genotype of pathogen and suscept appear to be integral factors in occurrence of mortality or varying degrees of recovery. For example, a severely diseased young seedling would die at high temperatures, but if the latter are reduced and/or the soil moisture level is increased, recovery often occurs.



From looking at Table 1, it is obvious that the lower temperature regime resulted in greater seedling mortality than occurred at the higher temperature regime. However, the deaths in the former were due to damping-off while those in the latter were due to relatively slow destruction of primary roots.

There was no correlation between inoculum density as observed in these experiments and disease severity. Certain reports (15, 37) indicate that populations of some pathogenic species of Fusarium and Rhizoctonia which possess high competitive saprophytic activity are not necessarily related to the amount of disease in the field, especially after a threshold number of infective units is present. The present experimental findings convincingly show that seedling blight was suppressed when only residues were added to soil of low nitrogen content. Any beneficial effect of such residues, however, was negated by supplemental nitrogen, whether of nitrate or ammonium form. This observation agrees closely with results from bean root rot experiments in which disease was suppressed by soil incorporation of various mature plant residues (31, 32, 33, 34, 35, 41, 43). These reports implied that competition for nitrogen, resulting from the addition of large amounts of carbon to the soil, was an important factor in pathogenesis. Apparently, soil microorganisms utilized the soil nitrogen in decomposing the energy source added to the soil, and F. solani f. sp. phaseoli under these highly competitive conditions was unable to effect pathogenesis. However, if nitrogen was added with the straw, the pathogen was reactivated and caused severe root rot. In the current experiment, the highest number of bacteria (and actinomycetes) was isolated from the soil which had received only residues. Whether this had been true throughout the entire period of incubation, however, is uncertain. In the bean root-rot studies, specific soil bacteria intimately associated

with F. solani f. sp. phaseoli gave an inverse correlation with incidence and severity of bean root rot without affecting the over-all growth or population of this pathogen in soil (2, 17, 18). Other forms of bacteria-induced fungistasis are reported by Huber and McKay (19) and Venkat Ram (51). In the former report, specific bacteria associated with sclerotia of Typhula idahoensis reduced the latter's germination. Removal of these bacteria resulted in a high percent germination, but re-inoculation again inhibited germination. The latter author reported the occurrence of bacteria-induced chlamydospores of Fusarium solani grown on agar; this was attributed to metabolites produced by the bacteria because their culture filtrates induced a similar effect. F. moniliforme usually does not produce chlamydospores but the same mechanism for rendering a fungal unit inactive might well apply.

The highest disease severity occurred in the nitrate-supplemented soil. This is significant because under field conditions, all sources of nitrogen sooner or later exist in form of nitrates.

That blight severity in sand culture was greatest at high levels of nitrogen agrees with Garrett's finding that nitrogen increases the susceptibility of individual roots (12). An increase in the nitrogen supply might increase the intrinsic susceptibility of individual roots to infection, but under slightly modified conditions, it also might promote disease escape by increasing the rate at which adventitious roots are formed. Nitrogen-starved plants (0 ppm) comparatively were more blighted than those that were raised in a low nitrogen medium (50 ppm). Nitrogen is such an indispensable element in over-all metabolism that a nitrogen-deficient plant also may be predisposed to infection.

The dilution plate technique is a satisfactory method of evaluating the relative pathogen populations in this sort of experiment. The actual

interpretation of the figures is, however, less meaningful than in cases where the pathogen exists in the form of discrete propagules such as chlamydo-spores (cf. *F. solani* f. sp. *phaseoli*).

Sterile sand provides a better medium for testing pathogenicity of *F. moniliforme* than sterile soil. Inoculum can be more easily dispersed within the former and population of microbial re-contaminants is much less than in soil.

While at times satisfactory, evaluating disease severity by means of mortality, growth reduction, etc., often produces inconsistent results. This largely is due to the fact that plant recovery often beclouds initial growth retardation, especially as occurs among plants of a fairly resistant genotype. Also, competition for nutrients between plants within the same container may result in predisposition of some and escape others.

Under current field operations, crop residues such as sorghum stalks are incorporated in soil with various degrees of tillage. Soils are heavily fertilized for high yields. These are conditions which might favor *Fusarium* seedling blight, especially where successive cropping to sorghum is practiced. Field experiments with various fertilizer types and amounts, rotational patterns and plant spacings would be the next logical step in evaluating effect of high-yield cultural methods on incidence of root damage caused by *F. moniliforme*. The manner in which soil-incorporation of organic and inorganic materials affects the physical, chemical and biological composition of the soil is just beginning to be understood. A fuller understanding of the phenomena involved would be of tremendous significance in attempts to control soil-borne plant pathogens.

## SUMMARY

Fusarium moniliforme Sheld. under greenhouse and growth chamber conditions resulted in a significant reduction in emergence and growth of Sorghum vulgare Pers. Damping-off of young seedlings, other forms of mortality, stunting and anthocyanin in the leaves were observed in infected seedlings.

There was a difference in varietal susceptibility. Spur Feterita was the most susceptible while RS 610 was comparatively resistant. The principal mode of Fusarium damage in the seedling phase was mediated through a retardation of seedling establishment after the germinating seed had been invaded by mycelium of F. moniliforme. Subsequent reduction in size of the secondary root system resulted in permanently stunted plants. The occurrence of root necrosis alone rarely correlated with a large reduction in plant growth.

In a sand-culture nutrition experiment, F. moniliforme caused the greatest growth reductions at high levels of nitrogen, especially at 200 ppm. Infected, nitrogen-starved plants (0 ppm nitrogen) also had severe reductions in weight. These results agree with many previous findings on nitrogen nutrition and root rot diseases.

When ammonium sulfate, urea, sodium nitrate and ammonium nitrate were separately added to a soil to which had been incorporated mature sorghum residues, they caused a greater decline in population of F. moniliforme than was observed in the check (residues only). Populations of the pathogen declined from an initial  $25 \times 10^3$  micronidia per gram of soil to  $1.2 - 3.5 \times 10^3$  colonies/gram of soil over an 8-week incubation period. Nitrate-nitrogen maintained a final population similar to that of the check. Ammonium-nitrogen was associated with the greatest decline; this treatment was associated with a

high saprophyte population.

The final density of pathogen inoculum had no direct correlation with severity of seedling blight. Mature sorghum residues apparently suppressed seedling blight even though this treatment maintained a very high level of inoculum. It was concluded that fungistasis was largely responsible for the reduction in pathogenicity of F. moniliforme when mature sorghum residues were added to a soil of low intrinsic nitrogen content. The highest incidence of blight occurred in the sodium nitrate-treatment where seedling emergence was only 37% compared to 67% in check and mortality was 24.2% and 0.9%, respectively. Other forms of nitrogen had intermediate effects.

It was concluded that the primary mechanism by which supplemental nitrogen influenced pathogenesis was through a differential effect on soil microflora.

Under current field operations, sorghum residues are plowed into the soil and the latter is heavily fertilized with nitrogen. Based on the limited findings in this report, these practices are considered likely to increase the incidence of seedling blight of sorghum.

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THE EFFECT OF NITROGEN NUTRITION ON SEEDLING BLIGHT OF SORGHUM  
INCITED BY FUSARIUM MONILIFORME SHELDT.

by

ILESANMI DELE ERINLE

B.Sc., Ahmadu Bello University, Zaria, Nigeria, 1968

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## ABSTRACT

With the advent of certain cultural practices such as high nitrogen fertilization, minimum tillage, high plant populations and continuous cropping, an increased incidence of sorghum root rot has been noted in several areas, such as the irrigated fields of southwestern Kansas. This study was restricted to seedling phases of the disease, produced under greenhouse and growth chamber conditions.

The experiment to determine the effect of level of nitrogen on disease severity was carried out in a greenhouse at  $27 \pm 2^{\circ}\text{C}$ . Plants were grown in unwashed river sand in 13-cm clay pots. Half of the pots were inoculated with a spore-mycelium suspension of F. moniliforme and seeded with 3 sorghum genotypes, namely, Spur Feterita, RS 610 and Redlan. The other half was similarly treated except that the pots were not inoculated (check). Nitrogen nutrition consisted of 5 treatments, application of 0, 50, 100, 200, and 400 ppm nitrogen in the form of ammonium nitrate in the watering solution.

As evaluated in Spur Feterita, the most susceptible variety, most severe blight occurred at the 200 ppm nitrogen level. Severe blight also occurred in nitrogen-starved (0 ppm nitrogen) plants. Least disease development occurred at low and intermediate levels of nitrogen (50 and 100 ppm). Nitrogen at 400 ppm was excessive and resulted in root injury. Data from Redlan and RS 610 often were inconsistent between one disease parameter and another.

The effect of different sources of nitrogen on populations of F. moniliforme was studied in an environmental chamber programmed at  $24^{\circ}\text{C}$  for 12 hours of light and  $20^{\circ}\text{C}$  in darkness during an 8-week incubation period. The soil used was a sterilized greenhouse potting mix contained in 23.8 x

13.7 cm metal trays, to each of which was added a suspension of  $2.5 \times 10^4$  microconidia/g of soil, mature sorghum residues to the equivalent of 4% (W/W), and 4 different sources of nitrogen to the equivalent of 0.8 g total nitrogen/kg of soil (split applications with half added initially, and the other half after 6 weeks). The check treatment received residues and microconidia, but no nitrogen. After 2, 4, 6, and 8 weeks following inoculation, inoculum densities were estimated by soil dilution plates on Nash-Snyder medium. At the end of the eighth week, the trays were seeded at the rate of 50 surface-sterilized Spur Feterita seeds per tray. After 3 weeks of growth, the seedlings were removed; height and fresh weight of top growth, and vigor of root system were recorded.

There was a more rapid decline of pathogen population in the nitrogen supplemented treatments than in the check. Final populations were 1.2, 1.6, 1.9, 3.5, and  $3.4 \times 10^3$  colonies/g of soil in ammonium sulfate, urea, ammonium nitrate, sodium nitrate and check treatments, respectively. There was, however, no direct correlation between these relative levels of inoculum and subsequent disease severity. Plants grown in nitrogen-supplemented soil were significantly more diseased than those of check. The most severe incidence of blight was in the sodium nitrate treatment.

Nitrogen fertilization enhanced blight severity, possibly by directly making the roots more susceptible. But in addition, fungistasis probably accounted for the low disease incidence in soil unsupplemented with nitrogen.