Influence of Soil Moisture Stress and *Macrophomina phaseolina* in Charcoal Rot Development in Grain Sorghum

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Abstract

The effects of *Macrophomina phaseolina,* soil moisture stress (SMS), and their interaction on charcoal rot development in sorghum were studied in field experiments. Individual effects of the pathogen and that of SMS were studied by eliminating SMS by furrow irrigation, and the pathogen by soil fumigation with methyl bromide, respectively. Significant effects of the pathogen, SMS, and pathogen x SMS interaction were found on charcoal rot development (percentage lodging) in sorghum hybrid CSH 6. Soil fumigation drastically reduced the population of the pathogen. Lodging was 3.18% in no-SMS plots as against 100% in the SMS plots. Under SMS, lodging was 6.27% in the fumigated plots compared with 100% in the non-fumigated plots, indicating that moisture stress alone cannot cause significant lodging in the absence of the pathogen. There was no colonization of stalks by the pathogen in the fumigated SMS plots compared with very high colonization in the non-fumigated SMS plots. Grain yield reduction due to charcoal rot was estimated between 20 and 33%.

मेक्रोफोमिना फेजियोलीना, मृदा आर्द्रता प्रतिबल (एस एन एस) और उनके पारस्परिक क्रियाओं का ज्वार के चारकोल विगलन के विकास पर पड़ने वाले प्रभावों का क्षेत्र प्रयोगों में अध्ययन किया गया। रोग जनक और एस एम एस के पृथक प्रभावों का भी अध्ययन किया गया, खूड़ सिंचाई विधि से एस एम एस को प्रभाव हीन किया गया और मिथाइल ब्रोमाइट द्वारा मृदा धूमन कर रोग जनक को निष्क्रिय किया गया। ज्वार संकर सी एस एच-6 में चारकोल विगलन विकास (पतन प्रतिशतता) की प्रक्रिया में रोग जनक, एस एम एस और रोग जनक X एस एम एस पारस्परिक क्रिया के सार्थक प्रभाव मिले। मृदा धूमन से रोग जनक की संख्या यकायक घट गई। एस एम एस रहित प्लाटों में पतन 3.18% जबकि एस एम एस मय प्लाटों में 100% था। एस एम एस के अन्तर्गत किए गए प्रयोगों में तुलनात्मक रूप में धूमित प्लाटों में पतन 6.27% जबकि अधूमित प्लाटों में 100% था, जिससे यह संकेत मिलता है कि एकमात्र आर्द्रता प्रतिबल, रोगजनक की अनुपस्थिति में सार्थक पतन का कारण नहीं हो सकता। यह भी देखा गया कि धूमित एस एम एस प्लाटों की तुलना में अधूमित एस एम एस प्लाटों में रोग जनक द्वारा वृंत का अत्याधिक कॉलोनीकरण हुआ। चारकोल विगलन द्वारा अनाज की ऊपज में अनुमानित कमी 20 से 33% तक आंकी गई।

Charcoal rot of sorghum [*Sorghum bicolor* (L.) Moench], caused by *Macrophomina phaseolina* (Tassi.) Goid., is a disease of economic significance in areas where sorghum is grown during Lhe post-rainy season under receding soil moisture. The pathogen infects plants through the roots and advances into the crown and stem where it causes rotting. Infected plants lodge, when subjected to soil moisture stress (SMS) during grain development (Pande *et al*., 1989). Significant grain yield losses have been reported by several workers (Anahosur and Patil, 1983; Pande & Karunakar, 1992). Henzell and Gillicron (1973) and Chamberlin (1978) considered lodging as a mere physiological problem, and they suggested that SMS reduces assimilate supply to the lower parts of the plant resulting in senescence, disintegration of pith cells and subsequent lodging. While Dodd (1980) proposed that lodging occurred due to interaction between pathogen and SMS.

The objective of this research was to study the effects of the pathogen and SMS separeteiy, and in combination on the charcoal rot development in Sorghum.

Materials and Methods

Effect of fumigation. During the 1985-86 post-rainy season, a field experiment involving two treatments (fumigation and non-fumigation) was conducted at ICRISAT Asia Center (IAC), Patancheru and at the University of Agricultural Sciences (UAS), Dharward, about 500 km south-west of IAC. The experiment was planned in a randomized block design with three replications. The plot was deep ploughed, thoroughly rotavated, and covered with a transparent polythelene tarp, the edges of which were buried on all sides of the plot and securely sealed before fumigation.

Three of the six randomly selected plots were fumigated with methyl bromide (Dowfume-MC-2) at the rate of 500g a.i. 5m^2 per plot (about 13 cans of 500g for 8 x 8m plot in 1985-86, and 30 cans for 10 x 15m plot in 1986-87). The fumigant was released in the gaseous form under the raised polythelene tarp (tarp was raised using 100kg capacity gunny bags stuffed with dry grass) through plastic tubes from the pressurized cans with the help of an applicator (Fig. 1). The polythelene tarp was removed 4 days later both from the fumigated and non-fumigated plots. Seeds of a charcoal rot susceptible sorghum hybrid CSH 6 were sown 2 days later. Plants were spaced at 10cm in a row and at 75cm between rows. The crop was raised either on intermidient rains or under receding soil moisture. The other cultural practices and fertilization were followed as the general recommendation for the crop.

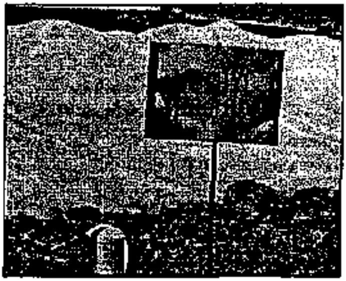

Figure 1. A plot fumigated with methyl bromide. The fumigant was released in the gaseous form under raised polythelene tarp through plastic lubes.

The sowing was done on 17 September 1985 at IAC and on 10 September at Dharwad. The rainfall during the early part of crop growth at IAC was 170mm and 18.8mm at Dharwad. Soil moisture content before fumigation as estimated by the gravimetric method, was 17% at IAC and 2356 at Dharwad.

Effect of fumigation and SMS. The experiment conducted during the 1986-87 post-rainy season at IAC, involved four treatments [fumigation, non-fumigation, soil moisture stress (SMS), and no soil moisture stress (NSMS)]. The experiment was planted in a split-plot design with fumigation and non-fumigation as main plots, and SMS and NSMS as the sub-plots with three replications. The plot size was 10m x 15m with 6m gap between plots. Plots were fumigated with methyl bromide and other operations were similar to that described in the previous experiment. SMS was induced by withholding furrow irrigation from the boot leaf stage till crop maturity. Seeds of CSH-6 were sown on 19 September 1986. About 99mm rainfall was recorded in the early stages of crop growth, and the soil moisture content was 16% before fumigation.

Quantitative estimation of soil fungi. Soil samples were collected at different depths (0-5, 5-10, and 10-15cm) from three plots which were fumigated, 1 day before and 3 days after fumigation. Composite samples were made of the soil from each plot separately, the soil was air-dried for 15 h, grinded and sieved through 0.1mm mesh, and plated on modified Czapek dox agar medium for isolating *Fusarium* species (Sharma and Singh, 1973), Chloroneb-mercuric chloride-rose bengal agar medium for isolating *M.phaseolina* (Meyer, *el al.,* 1973). Sieved soil (200mg) was sprinkled on the solidified media, in 20 petriplates for each of the treatment and incubated at 30 C with 12 h photoperiod for 7 days. Plates were examined for the presence of various fungal colonies, and their quantitative estimations were made as the number of colonies per gram of soil.

Disease assessment. At the physiological maturity of the crop, plants which were previously tagged at the boot leaf stage, in a net marked plots of 4m x 3m in 1985, and 9m x 3m in 1986-87, were recorded for percentage lodging, and stalks colonized with *M. phaseolina* (observed visually after split-opening the stalks).

Grain yield loss assessment. Grain yield loss was estimated in the above tagged plants in the net plots by the following method.

Grain yield loss = (A-B)/A .

where A = Yield in fumigated SMS plot, and B = Yield in non-fumigated SMS plot.

Statistical analysis. Data were analysed using Genstat program for analysis of variance for percent lodging, colonization of stalks by *M. phaseolina,* and grain yield loss, and LSDs were computed to find significant effects of different treatments.

Results and Discussion

Effect of fumigation on soil fungi. The initial fungal population was dominated by the *Fusarium* species, which were more than 50% of the total fungi present in the soil before fumigation (Table 1). Populations of all fungi reduced drastically after fumigation at both locations in two years. *M. phaseolina* population which was 23-35% of the total fungal populations in the soil before fumigation got reduced to zero after fumigation. Populations of other fungi were also reduced by 95% after fumigation. Methyl bromide proved a very effective fungicide for most of the soil fungi.

Table 1. Fungal species isolated from soil, before and after fumigation with methyl bromide at ICRISAT Asia Center (IAC) and Dharwad during post rainy seasons 1985-86 and 1986-97

Location and season	Fungal spp.	Fungal colonies (number)		Reduction in colonies (%)
		Before	After	
IAC 1985-86	*Fusurium* spp	-11330	270	97.6
	M. phaseolina	8825	0	100.0
	Other fungi	4675	222	95.2
SE±			0.12	
Dharwad 1985-86	Fusarium spp	13985	225	98.1
	M.phaseolina	6125	0	100.0
	Other fungi	4785	90	98.1
SE±			0.07	
IAC 1986-87	*Fusarium* spp	15235	180	98.8
	M. phaseolina	5075	1	99.9
	Other fungi	1820	20	98.9
SE±			1.89	

Figures are the mean of three replications.

The effectiveness of methyl bromide in reducing the *M. phaseolina* populations in soil and controlling diseases has been reported for black root rot of slash pine (Rowan, 1971) and soybean root rot (Gray, 1978; Pearson *et al.,* 1984).

Effect of fumigation and SMS on charcoal rot development. Under receding soil moisture, lodging was 6.5% at IAC and 24% at Dharwad in 1985-86 (Table 2) and 27% at IAC in 1986-87 post-rainy season in the SMS fumigated treatment (Table 3). This is in contrast to 100% lodging in the SMS non-fumigated situation. The split-opened stalks of lodged plants (Fig. 2) in the SMS fumigated plots had less *M. phaseolina* colonization than that in the SMS non-fumigated plots. Of the 24% lodged plants at Dharwad, none of the plants showed stalk colonization by *M. phaseolina.* Similarly, of 6.5% and 27.4% lodged plants at IAC during 1985-86 and 1986-87 only 2.7% and 11% plants showed colonization, respectively (Tables 2 and 3). In contrast, about 97% stalks of lodged plants showed colonization in the SMS non-

Table 2. Mean lodging (%),*Macrophomina phaseolina* incidence, and grain yield in sorghum hybrid CSH 6 in fumigated and non-fumigated plots under receding Soil moisture at ICRISAT Asia Center (IAC) and Dharwad during the postrainy season 1985-86

Treatment	Location	Lodging (%)	*M.phaseolina* incidence (%)	Grain Yield/ (Kg/plot[a])
Fumigation	IAC	6.5 (14.0)	2.7 (6.8)	2.1
	Dharwad	24.0 (29.1)	0.0 (2.4)	3.6
Non-Fumigation	IAC	99.7 (88.0)	97.3 (82.4)	1.4
	Dharwad	100.0 (90.0)	98.0 (86.3)	2.9
LSD(P< 0.05)	IAC	21.2	3.2	0.2
	Dharwad	(13.3)	(20.4)	0.1
Grain yield loss (%)	IAC			33.3
	Dharwad			19.5

[a]plot size- 4m x 3m.
Figures are mean of three replications (Plot size 4m x 4 rows in each replication).
Figures in parenthesis are angular transomed values.

Table 3. Mean lodging (%). *Macrophomina phaseolina* incidence, and grain yield in sorghum hybrid CSH 6 under different combinations of soil fumigation and soil moisture stress at ICRISAT Asia Center during postrainy season 1986-87

	Loding (%)		*M. phaseolina* incidence (%)		Grain yield (kg/plot')	
Treatment	SMS[b]	NSMS[c]	SMS	NSMS	SMS	NSMS
Fumigation	27.4 (31.4)	2.9 (5.7)	11.0 (16.8)	12 (3.6)	11.6	14.6
Non-fumigation	100,0 (90.0)	17.6 (24.5)	96.9 (84.1)	12.2 (17.6)	7.8	14.1
LSD	(25.8)		(33.1)		2.7	
Grain yield loss (%)					32.8	

[a]Net plot site = 9mx3m
[b]SMS = Soil moisture stress.
[c]NSMS = No soil moisture stress.
Figures are mean of three replicalions (Plot size of 9m x 4 rows in each replication).
Figures in paremiters are angular transformed values.

fumigated plots. Our results clearly contradict the findings of Henzell and Gillieron (1973); Chamberlin (1978); and Henzell *el al.* (1984) who

Figure 2. Split-open stalk from non-fumigated and soil moisture stressed plot showing-severe colonization by *Macrophomina phaseolina* compared with a healthy stalk from fumigated and soil moisture stressed plot.

hypothesized that lodging and charcoal rot development occurs due to physiological stress caused by low source/sink ratio at the grain-filling stage and involvement of pathogen is either negligible or secondary. Our results provide experimental evidence supportive of Dodd's hypothesis (1980) that the interaction among the pathogen, SMS, and host cultivar can alone lead to the charcoal rot development.

Effect of SMS on charcoal rot development. Under SMS, fumigated plots had the mean lodging of 27% compared with 100% in the non-fumigated plots (Table 3, Fig. 3). Under NSMS however, the fumigated plots had mean lodging of 3% compared with 18% in the iron-fumigated *plots.* In the absence of SMS, lodging was significantly reduced. These results support the earlier findings (Edmunds, 1964; Odvody and Dunkle, 1979; Pande *et al.,* 1989; Pande & Karunakar, 1992) on the importance of SMS in the charcoal rot development.

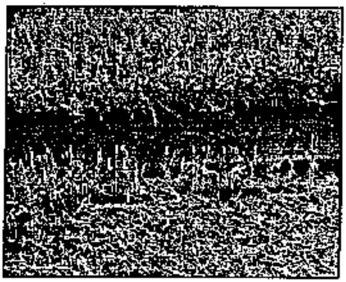

Figure 3. Severe lodging in the non-fumigated and soil moisture stressed plot compared with fumigated and soil moisture stressed plot in the background.

Grain yield loss. Grain yield loss was estimated to be 19.5% and 33.3% at Dharwad and IAC, respectively, in 1985-86, and 32.8% at IAC in 1986-87 under SMS non-fumigated condition (Table 2 and 3). Although SMS alone could contribute to some yield reduction, the combined effects of SMS and the pathogen significantly increased the level of yield loss. There are quite a few reports about grain yield loss due to charcoal rot (Chaudhari and Tikholkar, 1987). However, 15-55% and 23-64% loss in the grain weight was reported by Anahosur and Patil (1983), and Pande and Karunakar (1982), respectively.

Soil fumigation with methyl bromide had significant (P=0.05 to < 0.001) effects on lodging, 'occurrence of *M. phaseolina* in the stalks, and on the grain yield loss, in both the years at IAC and Dharwad (Table 4). Highly significant (*P* = <0.001) effects of SMS and SMS x fumigation interaction for the above three parameters (except for grain yield loss during 1986-87 at IAC where the significance level was *P* = 0.05), were observed.

Table *4.* Analysis of variance for lodging, occurrence of *M. phaseolina,* and grain yield in sorghum hybrid CSH 6 under different fumigation and soil moisture stress treatments at Dharwad and ICRISAT Asia Center (IAC)

Source of variation	df	Mean sum of squares Lodging(%)	 *M. phaseolina* (ft)	 Grain yield (kg/plot)
Darwad 1985-86				
Fumigation	1	8816.70*	1-1401.10***	0.7.1.1***
trior	2	213.10	6.10	0.001
IAC 1985-86				
Fumigation	1	13028.43***	13419.96***	0.56"
Error	2	12.01	2.21	0.00.1
IAC 1986-87				
Fumigation (F)	1	5715.97**	7047.10***	13.215*
Main plot error	2	87.10	14.60	0.21
Soil moisture stress (SMS)	1	8581.40***	6702.40***	65.10***
SMS x F	1	25I4..10"'	42JJ.30***	8.31*
Sub-ploterror	4	16.73	114.10	0.99

*significant =P = 0.05 **significant at *P* = 0.01
***significant at P = 0.001

This study clearly shows that SMS alone cannot cause significant lodging in the absence of the pathogen. SMS predisposes the plants to invasion by *M. phaseolina* resulting in charcoal rot development, increased lodging of plants and grain yield reduction.

References

Anahosur, K.H. and S.H. Patil. 1983. Assessment in losses in sorghum seed weight due to charcoal rot. *Indian Phytopath.*36:85-88

Chaudhari, S.D. and A. G. Tikholkar. 1987. Effects of charcoal rot on the physiological parameters responsible for grain yield in kharif sorghum. *J. Maharashtra Agri. Univ.* 12:286-287.

Chamberlin, R.J. 1978. The physiology of lodging of grain sorghum [*Sorghum bicolor* (L.) Moench], Queensland: University of Queensland, Ph.D. thesis.

Dodd, J.L. 1980. The photosynthetic stress translocation balance concept of sorghum stalk rots. In: R.J. Williams, R. A. Frederiksen, L.K.

Mughogho and G.D. Bengston (eds.) *Proceedings of the Internationald Workshop on Sorghum Diseases.* Patancheru, A.P. 502 324, India: ICRISAT.pp 300-305.

Edmunds, L.K. 1964. Combined relation of plant maturity, temperature, and soil moisture to charcoal stalk rot development in grain sorghum. *Phytopathology* 54:514-17.

Gray, L.E. 1978. Effects of soil fumigation on soybean disease and plant yield. *Plant Dis.* Repter. 62:613-615.

Henzell, R.G., R.L. Dodman, A.A. Done, R.L. Brengman and D.E. Meyers. 1984. Lodging, stalk rot, and root rot in sorghum in Australia. *In:Mughogho,* L.K., Rosenberg, G, (*eds.*) *Sorghum Root and Stalk Rots - A Critical Review. Proceedings of the Consultative Group Discussion on Research Needs and Strategies for Control of Sorghum Root and Stalk Rot Diseases.* 1983. Bellagio, Italy: ICRISAT. pp 225-236.

Henzell, R.G. and W. Gillieron. 1973. Effect of partial and complete panicle removal on the rate of death of some *Sorghum bicolor* genotypes under moisture stress. *Queensland Journal of Agriculture and Animal Sciences* 30:291-299.

Meyer, W.A., J.B. Sinclair and M.N. Khare. 1973. Biology of *Macrophomina phaseoli* in soil studied with selective media. *Phytopathology* 63:613-620.

Odvody, G.N. and L.D. Dunkle. 1979. Charcoal stalk rot of sorghum: effect of environment in host-parasite relations. *Phytopathology* 69:250-254.

Pande, S., L.K. Mughogho, N.S. Seetharama and R.I. Karunakar. 1989. Effects of nitrogen, plant density, moisture stress and artificial inoculation with *Macrophomina phaseolina* on charcoal rot incidence in grain sorghum. *J. Phytopathology* 12:343-352.

Pande, S. and R.I. Karunakar. 1992. Stalk rots. In:de Milliano WAJ, Frederiksen RA, Bengston GD,(eds.), *Sorghum and Millet Disease:* a second world review: Patancheru, A.P. 502 324, India: ICRISAT, pp 219-234.

Pearson, C.A.S., F.W. Schwenk, F.J. Crone and K. Kelley. 1984. Colonization of soybean roots by *Macrophomina phaseotina. Plant Dis.* 68:1086-1088.

Rowan, S.J. 1971. Soil fertilization, fumigation and temperature affects severity of black root rot of slash pine. *Phytopathology* 61:184-187.

Sharma, R.D. and R.S. Singh. 1973. A technique for selective isolation of *Fusarium moniliforme* from soil and plant tissues. *Indian J, Mycol.Plant Path.*3:67-70.

Induction of Autopoly ploidy in *Agaricus bisporus*

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Abstract

Basidiospores, fragments of vegetative mycelium and that of protoplast regenerants of *Agaricus bisporus* were treated with colchicine to induce polyploidy. Only the protoplast regenerants appeared to respond this treatment. Four putative polyploids were isolated and characterized for the cultural characteristics, the DNA content and the extracellular protein content. The colchicine treated cultures showed a multifold increase in the DNA content while growth rote and protein content were lower than that of the parent strain.

एगैरिकस बाइस्पोरस के बेसिडियम बीजाणुओं, कायिक माइसीलियम के खंडों और प्रोटोप्लास्ट पुनर्जनकों को बहुगुणिता प्रेरित करने के लिए कोल्चिसीन से उपचारित किया गया। केवल प्रोटोप्लास्ट पुनर्जनकों ने ही इस उपचार के प्रति रुझान दर्शाया। चार तथाकल्पित बहुगुणित, डी एन ए मात्रा और कोशिका बाह्य प्रोटीन के लिए पृथक्कृत तथा अभिलक्षणित किए गए थे। कोल्चिसीन से उपचारित संवर्धनों में जनक अभेद की तुलना में डी एन ए की मात्रा में अत्यधिक वृद्धि देखी गई जबकि वर्धन गति और प्रोटीन मात्रा निम्न स्तर की थी।

The cultivation technology of *Agaricus bisporus* is well established through agronomic practices but the genetic basis of the improved strains is poorly defined (Thakur, 1995). A *bisporus* is a secondary homothallic inbred fungus: The mating type system in this fungus is similar to unifactorial homothallism (bipolar incompatibility) but the two post-rneiotic nuclei migrate into each spore which germinate to behave as a homothallic dikaryotic mycelium capable of forming fruit bodies (Elliott, 1985). Although appropriate physical, nutritional and environmental factors (Quimio *et al.,* 1995) alongwith certain chemical substances (reviewed by Handa, 1994) have been reported to induce fruiting in heterokaryotic mycelium yet control of fruiting is known to have a genetic base (Thakur, 1995). In *Schizophyllum commune,* the fruiting competence is reported to be inherited genetically and the genetic control of fruiting is polygenic (Raper and Krongelb, 1958). The genetic improvement of A *bisporus* strain is restricted due to the coenocytic nature of hyphal cells and the absence of a true hapioid stage in its life cycle as well as the lack of an established gene transformation system (Elliott, 1985; Sodhi, 1992). An alternate route for strain improvement at genomic level could be expected on changing ploidy level of the culture. Various chemicals namely, benlate, benomyl, colchicine, coumarin, p-fluorophenyl-D-alanine and trypan blue have been used to induce ploidy in filamentous fungi (Hastie, 1970; Lhaos, 1961; Toyama and Toyama, 1990; Upshall *et al.,* 1977). Since fruit body like structures were generated from colchicine treated autopolyploid *Trichoderma reesei* strains (Toyama and Toyama, 1990), colchicine has been used during the present study to induce autopolyploidy in A *bisporus.*