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Occurrence of airborne spores of fungi causing grain mould over a sorghum crop

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Airborne spores of *Fusarium*, *Curvularia* and *Alternaria* species which cause sorghum grain mould were monitored over rainy season crops of the grain-mould susceptible sorghum hybrid CSH 1 using a Hirst spore trap. Spore trapping began at the flowering stage (GS 61) and was continued beyond grain maturity (GS 92). Spores of all three fungal genera were present during the post-flowering stages. However, more spores were trapped after the hard dough stage (GS 87) than at earlier growth stages. Spore content in the air increased after grain maturity (GS 92) under moist or humid conditions. *Fusarium* spores were most prevalent before dawn, whereas most spores of *Alternaria* and *Curvularia* were trapped during the day. The frequency of *Fusarium* and *Alternaria* spores in the two years differed while that of *Curvularia* was similar in both years. The predominant species isolated from surface-sterilized moulded grain on malt-streptomycin agar were *A. tenuissima*, *F. moniliforme*, *C. lunata* and *Phoma sorghina*. These results prove that spores of mould causal fungi were naturally available in the air and initiated grain mould epidemics under suitable weather conditions.

Grain mould is one of the major diseases of sorghum [*Sorghum bicolor* (L.) Moench]. It occurs when grain develops under warm and humid conditions and increases in severity if harvesting is delayed after grain maturity (Siddiqui & Khan, 1973; Williams & Rao, 1981). Among more than 40 species of fungi associated with moulded sorghum grain, *Fusarium moniliforme* Sheld., *F. pallidoroseum* (Cooke) Sacc., *Curvularia lunata* (Wakker) Boedijn, and *Phoma sorghina* (Sacc.) Boerema *et al.* are considered as the major mould causal fungi because they are frequently isolated from moulded grain (Williams & Rao, 1981). Incidence of grain mould requires the presence of the causal fungi when favourable moisture and temperature coincide with grain development. Appearance of the disease whenever mould-conducive environmental conditions prevailed (Bandyopadhyay & Mughogho, 1988) suggested that inocula of the causal fungi were readily available. To confirm this hypothesis, the occurrence and quantity of spores of the grain mould causal fungi in the air over a mould-susceptible sorghum crop was monitored during grain development, and at maturity, the fungi that colonized the grain were isolated.

MATERIALS AND METHODS

Agronomy

Experiments were conducted during 1985 and 1986 rainy seasons in a Vertisol at the ICRISAT farm, Patancheru, India. Each season, 0·12 ha (40 m × 30 m) plot of a grain-mould susceptible sorghum hybrid CSH 1 was established in the same area of a field. The plot was 30 m from a farmer's field planted with sorghum on one side, and the other three sides were planted with experimental sorghum lines. Seeds were machine-drilled in ridges, spaced 0·75 m apart, on 6 June 1985 and 18 June 1986. Seedlings emerged 3–4 d after planting and were thinned 14 d after emergence to maintain 8–10 plants m^{-1} row. The field received 56 kg N ha^{-1} and 28 kg P ha^{-1} (as diammonium phosphate) as basal fertilizer before sowing, and 40 kg N ha^{-1} (as urea) as top dressing 21–30 d after sowing. Carbofuran granules (20–40 kg ha^{-1}) were drilled into the seed zone at planting to control shoot fly (*Atherigona soccata* Rondani). Weeds were controlled with the pre-emergence herbicide Atrazine at the rate of 1 kg ha^{-1}, and by manual weeding. The crop was raised with normal rainfall.

Spore trapping

A 24-h Hirst spore trap (Hirst, 1952) was used to monitor fungal spores over the sorghum plot. The trap was placed 0·2 m above the crop canopy in the centre of the field. The suction pump of the trap operated on a 12 V DC battery that was charged every 24 h prior to sampling. Air was drawn into the trap through the orifice at the rate of 10 l min^{-1} and impinged on a polyvinyl alcohol-coated sticky slide on which spores and other particulate matter were deposited in a 14 mm wide band. A clock mechanism in the trap moved the slide 2 mm h^{-1} across the orifice. The 48 mm-long band on the slide, representing the 24 h sampling period, was marked into 24 sections, each 2 mm wide, that had spores collected in each hour of the day. In each of the 24 sections, two 490 µm-wide

traverses across the 14 mm width of the slide were scanned, the numbers of spores counted, and the mean hourly concentration of spores m^{-3} (H) calculated. The 24 values of spores m^{-3} in a day were averaged to give the daily concentration of spores m^{-3} (D). The number of spores of the fungal genera *Fusarium* (macroconidia), *Curvularia*, and *Alternaria* was recorded without identifying the species, because of the difficulty of doing so on the basis of spore characteristics as seen on the trapping slides.

In 1985, spore sampling began on 2 Aug., when spikelets at the tip of the majority of the panicles flowered [growth stage (GS) 61; Tottman & Broad, 1987], and ended on 10 Oct. In 1986, panicles flowered on 17 Aug., and spores were sampled from 26 Aug. (milk growth stage; GS 75) to 25 Oct. The spore trap could not be operated every day during the sampling period. It was operated for 50 days in 1985 and 39 days in 1986 out of the 69 days covered by the sampling period in each year. Seasonal periodicity of each fungal genus was plotted as a function of daily concn of spores m^{-3} against the sampling days in each year. Diurnal periodicity of each fungus was determined using the mean normalized hourly concn of spores calculated as follows:

$$NH_i = 1/n\left[\sum_{j=1}^{n} H_{ij}/D_j\right],$$

where NH_i = mean normalized hourly concn for *i*th hour of all the sampling days, H_{ij} = mean hourly concn for the *i*th hour on the *j*th day, D_j = daily concn for the *j*th day, i = hour of the day (1 ... 24) and n = total number of sampling days. Data on mean hourly concn for each day were normalized primarily to avoid disproportionate effect of days with large daily spore concn while plotting diurnal periodicity. Hourly concn of rainy days were omitted while calculating normalized hourly concn. Also excluded were days with spores encountered only in a single hour, to avoid unduly large normalized concn for that hour.

Isolation of fungi from grain

About 30 panicles were harvested two weeks after physiological maturity (28 Sep. in 1985 and 10 Oct. in 1986) and threshed. Physiological maturity of sorghum grain was identified by the formation of a black layer at the hilar end (Eastin, Hultquist & Sullivan, 1973) and was considered similar to GS 92 of the decimal code of growth stages described by Tottman & Broad (1987). Grain was analysed for the predominant fungi and the frequency of occurrence. One hundred seeds were surface-sterilized in a 0·1% mercuric chloride solution for 3 minutes, rinsed thoroughly in sterile distilled water, and transferred aseptically to Petri dishes (2 seeds per dish) containing 2% malt agar and 50 μg l^{-1} streptomycin sulphate. The plates were incubated at 25 °C for 7–14 days. The fungi growing from the grains were subcultured and identified.

Environment

Temperature, relative humidity, and rainfall data were obtained from a meteorological station situated about 200 m away from the experimental plot.

RESULTS

Environment

The pattern of rainfall during 1985 and 1986 rainy seasons differed considerably. In 1985, rainfall was well distributed during the post-flowering stages, and the post-maturity period was particularly wet and humid. In 1986, more rain fell in the pre-flowering than in the post-flowering period and rainfall ceased one day before GS 92 (Fig. 1; Table 1). Relative humidity during grain development, and after GS 92, was generally higher in 1985 than in 1986. Temperatures between 2 Aug. and 15 Oct. were similar in 1985 (min. 20°, max 33·8°) and 1986 (min 19°, max. 35°), except that minimum temperatures fell to 13° between 16 and 25 Oct. 1986.

Mould fungi in the air during post-flowering growth stages

Spores of species of *Fusarium*, *Curvularia* and *Alternaria*, were present in the air during all grain development stages. However, they were 2 to 32 times more abundant after GS 92 than before GS 92 (Table 1). In general, spore concentration of the three fungal genera increased substantially after the hard dough stage (GS 87). Spore concn continued to increase after GS 92 in the wetter 1985 season, but declined in the drier 1986 season (Fig. 1).

Most *Fusarium* spores were detected when the crop passed GS 92 in 1985, and in GS 87 in 1986. In 1985, the concn of *Fusarium* spores in the air tended to increase as more rain fell and humidity increased; in 1986 spore concn peaked at GS 92 after a wet spell and then declined in the absence of rainfall and high relative humidity. In spite of these differences, more *Fusarium* spores were encountered in 1986 than in 1985 (Fig. 1).

Curvularia spores were encountered almost daily from GS 61 onwards in 1985, and from GS 75 when observations began in 1986. The concn of *Curvularia* spores in the air increased 3 days after GS 92 in 1985, and at GS 87 in 1986, and showed two periods with more spores in the air, interspersed with period(s) of less spores (Fig. 1). However, the total number of *Curvularia* spores in the air was more or less similar in both years.

Unlike *Fusarium*, more *Alternaria* spores were encountered in 1985 than in 1986. Prior to GS 92 in 1985, there was one major peak on 27 Aug. (256 spores m^{-3}) among days with few or no spores. In 1986, *Alternaria* spores were found on all but one day when it rained continuously. During the late stages of grain development, *Alternaria* concn changed similar to those of *Curvularia* (Fig. 1).

Diurnal periodicity

Diurnal periodicity of *Alternaria* and *Curvularia* were almost similar but different from *Fusarium* (Figs 2–4). The normalized hourly concn of *Alternaria* and *Curvularia* spores were less than 1 during the night, increased steeply at 09.00 h, continued to remain > 1 during the day, and then declined to < 1 after sunset. Hourly concn of *Fusarium* spores from 08.00 h to 17.00 h were usually less than half the mean daily concn

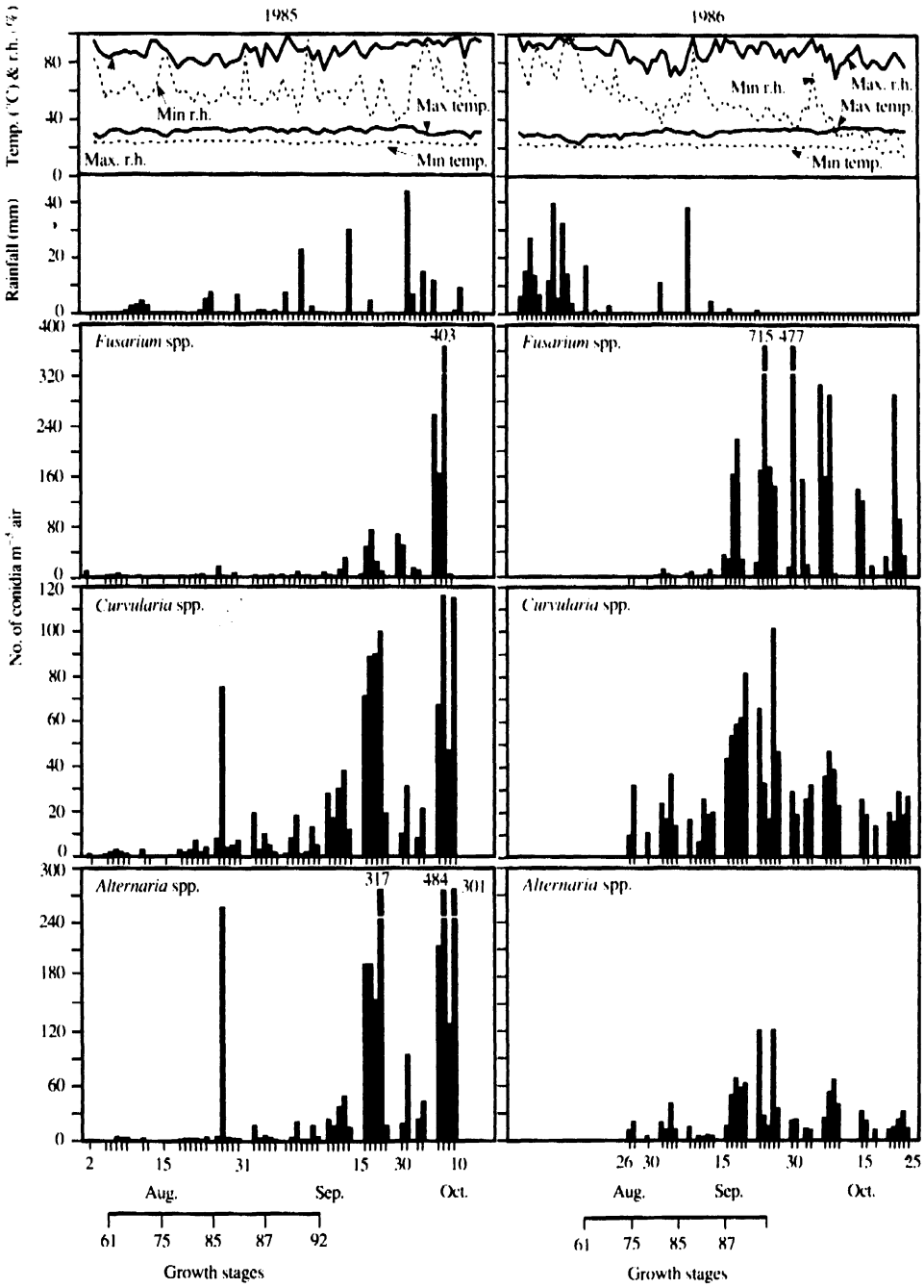


Fig. 1. Temperature, relative humidity (r.h.), rainfall, and daily concn of spores of *Fusarium*, *Curvularia* and *Alternaria*, in the air over a sorghum crop from flowering (GS 61) to post-grain maturity (beyond GS 92) during 1985 and 1986 rainy seasons at Patancheru. The different growth stages were flowering (GS 61), milk (GS 75), soft dough (GS 85), hard dough (GS 87), and physiological grain maturity (GS 92). Ticks in *x*-axis show the dates when spores were trapped in the air.

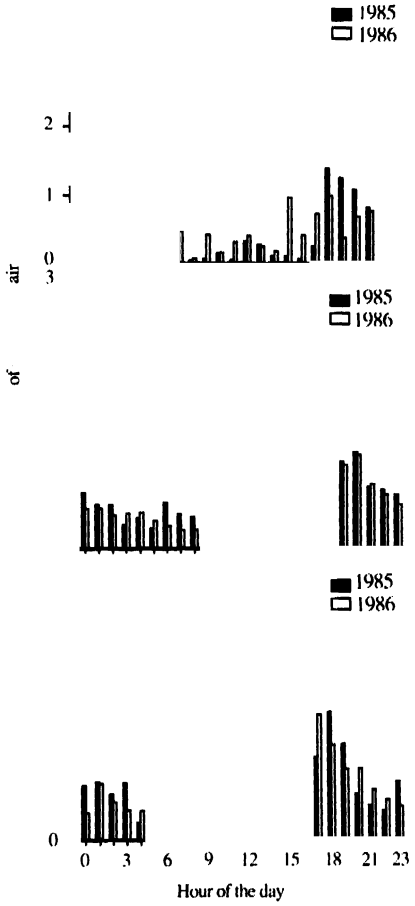
(except at 15.00 h and 17.00 h in 1986), whereas it was almost 2–3 times from 02.00 h during the night.

Rainfall considerably altered the diurnal periodicity of *Fusarium* spores (Fig. 5). In 1985, 24–29 Sep. were dry days and spores of *Fusarium* had typical noctural pattern of diurnal periodicity on each of these sampling days. On 30 Sep., the dry spell was broken at 12·30 h when 4 mm rain fell as a result of which spores were encountered in the air until 17.00 h. However, no spores were found in the air from 18.00 h to 21.00 h because of a severe rainstorm (12 mm; maximum intensity 8·5 mm in 15 min) at 17·05 h which may have washed spores from the atmosphere. Spores were again trapped in the night air from 22.00 h of 30 Sep. until 05.00 h of 1 Oct.

Table 1. Concn of spores of *Fusarium* spp., *Curvularia* spp., and *Alternaria* spp. in the air above a crop of a grain mould-susceptible sorghum hybrid CSH 1 from flowering (GS 61) through hard dough (GS 87) to post-grain-maturity (beyond GS 92) development stages in 1985 and 1986 rainy seasons at Patancheru

Year	Grain development stage*	Sampling days (no.)	Rain fall (mm)	Number of spores m^{-3} air		
				Fusarium spp.	*Curvularia* spp.	*Alternaria* spp.
1985	GS 61 to GS 87	20	34·8	2·4†	6·7	14·4
	GS 87 to beyond GS 92	30	149·9	39·4	33·3	79·4
1986	GS 61 to GS 87	14	75·2	4·6	18·9	11·5
	GS 87 to beyond GS 92	25	3·2	151·2	36·7	38·1

* The number of days covered within the grain development stages were 30 from GS 61 to GS 87, and 39 from GS 87 to beyond GS 92.
† The values are mean daily concn of spores for the sampling days within the grain development stages.



Figs 2–4. Diurnal periodicity of spores of *Fusarium* (**Fig. 2**), *Curvularia* (**Fig. 3**), and *Alternaria* (**Fig. 4**) over a sorghum field during 1985 and 1986 rainy seasons. Spores were sampled in the air from flowering growth stage (GS 61) to post-grain maturity (beyond GS 92) of the sorghum crop. Diurnal periodicity was plotted using the mean normalized hourly concn of spores m^{-3} calculated by dividing the hourly concn of spores of each dry day with the respective daily concn, and then computing the mean of the hourly ratios of all sampling days without rain.

Fungi isolated from grain

Severe grain mould (score of 5 on a rating scale of 1 to 5 where 1 = no mould, and 5 = > 50% grain surface moulded) was recorded, and the grain was internally colonized by several fungi. The predominant species was *A. tenuissima*, *F. moniliforme*, *C. lunata* and *P. sorghina*. The frequencies of grains colonized by *F. moniliforme* was almost similar in both years (Fig. 6). *C. lunata* colonized approx. 7% more grains in 1986 compared to 1985. However, *A. tenuissima* grew out from more grains in 1985 (35%) compared to 1986 (19%).

DISCUSSION

The aeromycological data presented in this paper show that spores of the fungal genera *Fusarium*, *Curvularia* and *Alternaria* were present in the air over a sorghum field during grain development. They could also be isolated from the moulded grain at harvest. These fungi are also predominant constituents of the aerial mycoflora in cultivated and uncultivated areas in Nigeria (Dransfield, 1966) and India (Sreeramulu, 1959; Sreeramulu & Ramalingam, 1964; Reddi & Ramakrishna, 1978).

The concn of spores of different mould fungi in the air varied between seasons and also during the season. For example, spores of *Fusarium* were one-third less in 1985 than in 1986, whereas *Alternaria* spores were twice as numerous in 1985 compared to 1986. The general trend of increasing spore concn after GS 92 in the wetter 1985 season, and the decreasing spore concn in the drier 1986 season suggests that intermittent wetness and high humidity favoured spore production and dispersal. Rain assisted dispersal of *Fusarium* spores (Fig. 5). However, severe rain can scrub spores from the air which has been also shown earlier by Dransfield (1966). The diurnal periodicity of spores of *Fusarium*, *Curvularia* and *Alternaria* were uniform in both years and agrees in principle with published work (Sreeramulu, 1959; Sreeramulu & Seshavataram, 1962). However, diurnal periodicity can be altered by rainfall as shown in the case of *Fusarium* in this study.

The concn of spores of all three fungal genera were low during the early stages of grain development and were abundant after GS 87. This was possibly due to several factors. Being facultative parasites, the three mould fungi we studied are known to colonize and sporulate on dead and decaying

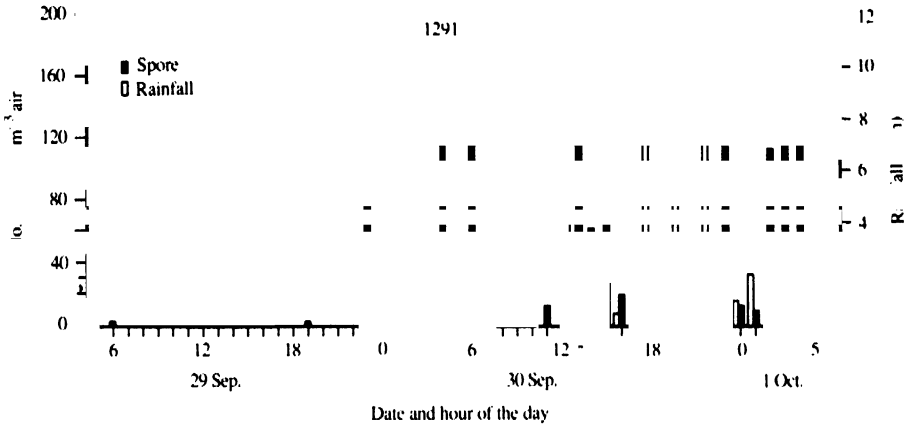


Fig. 5. Concn of *Fusarium* spores in the air and rainfall at hourly interval from 29 Sep. (06.00 h) to 1 Oct. (05.00 h) 1985. Note the change in hourly spore concn during the sunshine hours from nil on a dry day (29 Sep.) to abundant on a rainy day (30 Sep.).

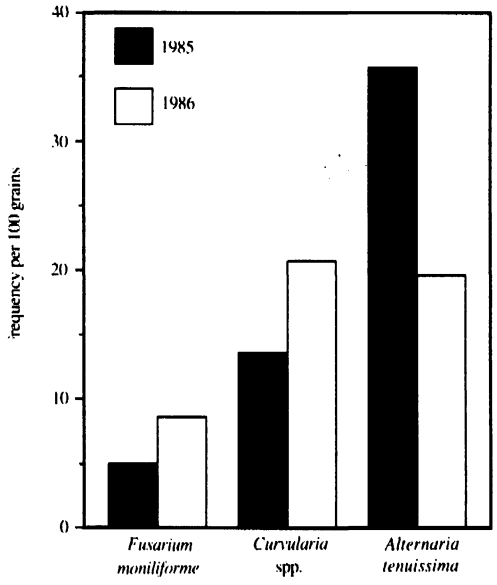


Fig. 6. Frequency of *Fusarium moniliforme*, *Curvularia* spp., and *Alternaria tenuissima* isolated from CSH 1 sorghum grain two weeks after maturity (GS 92).

plant debris (Domsch, Gams & Anderson, 1980; Burgess, 1981). Spores are then contributed to the aerial mycoflora by passive or active dispersal methods (Ingold, 1978). When sorghum plants approached maturity, lower leaves senesced and died. These moribund plant materials made available more substrate for fungal colonization and sporulation. As a result, spore content in the air increased as the crop reached GS 87. Another contributor of mould fungi in the air after grain maturity could be the moulded sorghum grains themselves. Mould fungi can infect sorghum ovaries and developing grains from GS 61 onwards, but they remain inside the developing grains until GS 87 when they sporulate on the grain surface (R. Bandyopadhyay, 1986 unpublished data). Shankara & Ramalingam (1988) also showed that mature, moulded sorghum grains, when agitated, released large numbers of *Fusarium*, *Alternaria*, and *Curvularia* spores in the air. Further studies are required to relate the concn of airborne spores of mould fungi at different growth stages to infection of, and sporulation on sorghum grain.

Fungi infect sorghum panicles and cause grain mould when grain development coincides with warm and humid weather. Natural availability of inoculum is assured but not the occurrence of mould-conducive wet environment. Therefore, the most critical factor favouring or limiting grain mould epidemics is the occurrence or non-occurrence of wet environment. These findings support the practice of providing a wet environment while screening for resistance to grain moulds, and it may be one of the reasons why the role of wet environment was justifiably more emphasized than that of the pathogen in grain mould incidence and resistance screening (Siddiqui & Khan, 1973; Bandyopadhyay & Mughogho, 1988; Bandyopadhyay, Mughogho & Prasada Rao, 1988).

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