Proc. Indian Acad. Sci. (Plant Sci.), Vol. 98, No. 6, December 1988, pp. 501–505.

Wind-tunnel estimation of fungi colonizing sorghum seed from field to storage

P SHANKARA and A RAMALINGAM*
Department of Post-graduate Studies and Research in Botany, University of Mysore, Manasagangotri, Mysore 570 006, India
*Present address: Department of Studies in Botany, Karnatak University, Dharwad 580 003, India

MS received 30 May 1988; revised 15 October 1988

Abstract. Microbial loads of sorghum seeds were estimated using a wind tunnel at 3 successive stages; mid ripe, threshing and storage. Very high concentration of field fungi viz., *Curvularia*, *Drechslera*, *Fusarium*, *Phoma* and *Sphacelotheca sorghi* were recorded from seeds at threshing stage, whereas their populations decreased during storage. Storage fungi such as *Penicillium* and *Aspergillus* increased considerably after storage. High concentration of microbes were released even with least agitation.

Keywords. Seed microflora of sorghum; wind tunnel; Andersen sampler.

1. Introduction

Seeds harbour a variety of microorganisms which are responsible for biodeterioration (Thomas 1977), loss of viability (Tripathi 1974; Rati and Ramalingam 1974), mycotoxicosis (Sreenivasamurthy 1977), allergic diseases (Clarke and Madelin 1987) and infection (Gustavo and Mendes 1987).

Seeds receive microbes from air-spora, from other parts of plant, soil, storage structures and infestation by insects (Lacey *et al* 1980). Some may germinate and colonize the growing plant, others may be carried into storage. Which of them develop before or after harvest depends upon the environment. Some fungi may occur only in small numbers, close to the limits of detection before harvest, but the crop may become further contaminated during harvesting, threshing and storage. The microbial load of sorghum seed collected at 3 successive stages; i.e. from the field before harvest when the crop was in mid-ripe stage, from the threshing floor and two months after storage was estimated by wind-tunnel method.

2. Materials and methods

A wind-tunnel similar to the design given by Gregory (1973) was used to estimate the spores released from surface of sorghum seed subjected to varied intensities of agitation. Spores released into wind-tunnel from perforated rotating drum are sampled by an Andersen sampler (Andersen 1958) following Lacey and Dutkiewicz (1976) and rotary drum miniature spore trap (RDMST, Ramalingam 1978). The wind tunnel is used on the basis of a previously planned and tested programme (table 1). A known quantity of sorghum seeds were agitated by rotating the drum approximately at the rate of 0·5 to 2 rotations/s. The air flow was maintained at a constant speed of 1·3 m/s. Samples were taken at different intervals by using Andersen sampler with potato dextrose agar and Czapek Dox agar plates. The

Table 1. A model programme of operation of wind-tunnel used for sampling microflora of sorghum seed.

Interval (min)	Operation	RDMST sampling	Andersen sampling
0–13	No. grain in the drum lab. air sampled	Operated continuously	3–6 min I/CDA 9–12 min II/PDA
13–15	Adding seed to drum	–do–	—
15–20	Seed drum rotation 1 rotation/2 s	–do–	16–17 min III/CDA 19–20 min IV/PDA
20–32	1 rotation/s	–do–	25–36 min V/CDA 30–31 min VI/PDA
32–40	2 rotations/s	–do–	—
40–60	2 rotations/s	–do–	42–44 min VII/CDA 48–50 min VIII/PDA

CDA, Czapek Dox agar; PDA, potato dextrose agar.

sampling period ranged from 0·5 to 3 min. However, air was sampled continuously for 60 min duration using RDMST. Total number of microbes released into the wind-tunnel during the 1 h operation (agitation) was estimated and the effect of agitation on the release of fungi from seed surface was determined. Concentrations of microbes estimated by the Andersen sampler and RDMST are multiplied by the volume of air passed through the wind-tunnel during different episodes and then totalled to arrive at the cumulative total release.

3. Results

Data on the total number of microbes released from 1 kg of sorghum seed at different stages viz., field, threshing and storage, estimated with the help of Andersen sampler are presented in table 2. The data collected with the help of RDMST are given in table 3.

Very high concentrations of microbes were recorded from seeds collected from the threshing floor where *Sphacelotheca sorghi* spores predominate. *Cladosporium*, *Alternaria* and species of *Aspergillus* and *Penicillium* showed continuous increase from field to storage. Fungi such as *Curvularia*, *Drechslera*, *Fusarium*, *Phoma* and *S. sorghi* occurred in high concentrations during threshing stage, whereas they decreased during storage.

Andersen sampler data revealed the existence of very high concentrations of bacteria, which increased with time of storage, whereas actinomycetes showed a decline. RDMST data revealed the presence of very high count of starch grains and fragments of hyphae in addition to spores, where the former predominated in seeds collected from standing crop in the field and the latter in the stored seeds. *Trichothecium roseum* was not recorded in the seed samples collected from the field either by Andersen sampler or by RDMST.

A moderate liberation of microbes from the seed surface was observed even at the least agitation employed in the study (adding the seed to the drum) and a rapid increase with only moderate agitation (1 rotation of seed drum/2 s). The number declined even at higher rates of agitation extending for longer periods. The data on the observed concentrations and estimated number of spores released from the agitated seed at each episode of agitation are plotted against time in figure 1.

Table 2. Total number of microbes released from 1 kg of sorghum seed into wind-tunnel under agitation as estimated by Andersen sampler.

	Seeds collected from		
Colony types	Field	Threshing floor	Storage
Bacteria	2,677·43	5,098·56	6,189·33
Actinomycetes	216·06	59·00	51·72
Alternaria	132·40	166·71	239·59
Aspergillus candidus	—	5·86	11·09
A. flavus	—	—	39·98
A. niger	10·61	13·89	67·70
A. ochraceus	—	—	31·60
A. oryzae	—	—	13·89
Other *Aspergillus* spp.	23·77	109·91	310·43
Cladosporium	871·36	1,516·06	2,239·11
Curvularia	0·71	38·52	20·46
Drechslera	14·38	20·46	13·89
Fusarium	10·27	108·48	28·45
Nigrospora	14·38	—	—
Penicillium	27·31	62·09	662·50
Phoma	57·03	196·09	176·40
Syncephalastrum	—	2·89	6·66
Trichothecium roseum	—	509·71	567·91
Unidentified	120·00	36·04	21·43
Total	4,175·71	7,945·13	10,692·14

Values in thousands.

Table 3. Total number of microbes released from 1 kg of sorghum seed into wind tunnel under agitation as estimated by RDMST.

	Seeds collected from		
Spore types	Field	Threshing floor	Storage
Alternaria	5·55	68·61	70·18
Aspergilli	5·41	16·35	16·80
Cladosporium	228·82	989·91	1,444·63
Curvularia	1·50	16·35	12·27
Drechslera	0·81	21·12	12·27
Epicoccum	—	0·68	2·05
Fusarium	2·50	21·80	8·29
Nigrospora	—	2·73	3·41
Periconia	2·58	0·68	3·41
Puccinia purpurea	1·49	—	—
Sphacelotheca sorghi	25·39	1,730·50	812·02
Trichoconis	4·87	2·73	—
Trichothecium roseum	—	610·47	783·49
Hyphal fragments	16·26	620·05	703·06
Pollen of Poaceae	—	2·04	—
Starch grains	2,043·31	874·84	836·59
Unidentified	3·46	19·76	11·59
Total	2,341·95	4,998·62	4,720·06

Values in lakhs.

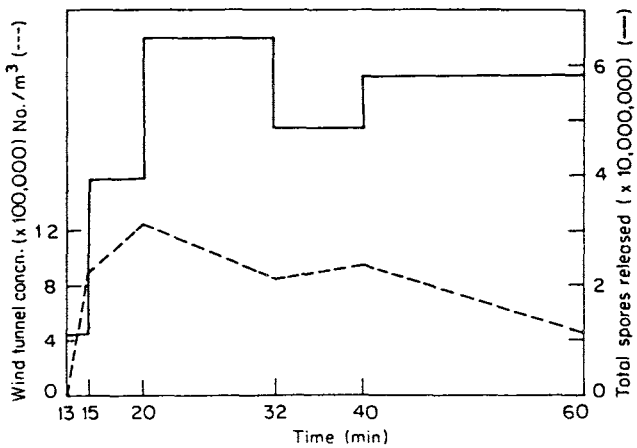

Figure 1. Estimates of fungus spores released from sorghum seed collected at field stage into the wind-tunnel at each episode of agitation.

4. Discussion

Sorghum crop in heading stages often develop head molds which are greater when the earheads are compact and infested by insects (Reddy and Nusrath 1985). In our experiment, ear heads of standing crop developed head molds after the collection of the first batch of seed samples because of their compactness and exposure to long periods of moist rainy weather at the time of harvest. Seeds sampled at threshing stage revealed very high concentrations of *T. roseum*, which however was not recorded on seed collected from field. Probably, spore traps are not as efficient as earheads in receiving inoculum or the fungus might have been received by an insect vector. Very high concentrations of *S. sorghi* during the threshing stage might be due to the rupturing of sori during threshing operation thereby further contaminating the seeds.

Water activity in the stored grain, the intergranular gas composition and temperature of storage are stated to be important factors in the development of microflora in stored grain (Hill and Lacey 1983). Field fungi like *Drechslera*, *Curvularia*, *S. sorghi*, *Phoma*, *Trichoconis*, *Puccinia purpurea* and *Nigrospora* were reduced during storage, whereas storage fungi like species of *Penicillium* and *Aspergillus* were increased during storage as stated by Christensen and Kaufmann (1965).

A large proportion of the microbes on the seed are probably dead as shown by the differences between spore and colony estimates (figure 2). Broken grains and grain dust are stated to predispose seed to microbial invasion and fast deterioration (Lacey *et al* 1980). In such cases the period of safe storage may be reduced. Procedures to reduce such materials before storage may decrease microbial build up in grain and increase storage period. High concentration of starch grains recorded in field collected seeds is probably due to their collection in premature stages and further breakage of grain during separation from ear heads. Seeds collected from threshing floor and storage had a comparatively lesser concentration of starch grains which might be due to their utilization by microbes and insects.

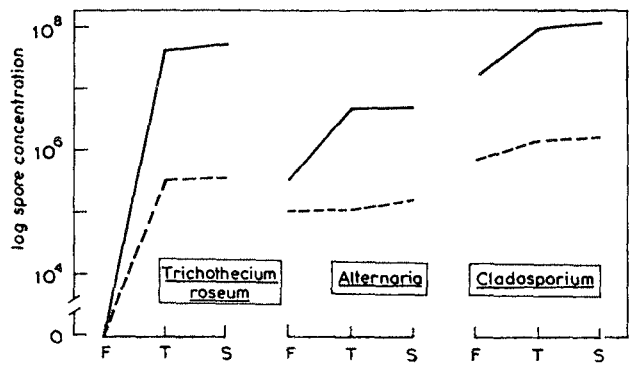

Figure 2. Spore (——) and colony (– – –) estimates of 3 fungi/kg of sorghum seed at field (F), threshing (T) and storage (S) stages.

Our studies indicate that in addition to grain dust, the starch grains in seed should also be recognised as an important factor in the process of biodeterioration.

Acknowledgement

One of the authors (PS) is grateful to the University Grants Commission, New Delhi for financial assistance.

References

Andersen A A 1958 New sampler for the collection, sizing and enumeration of viable air-borne particles; *J. Bacteriol.* **76** 471–484

Christensen C M and Kaufmann H H 1965 Deterioration of stored grains by fungi; *Annu. Rev. Phytopathol.* **3** 69–84

Clarke A F and Madelin T 1987 Technique for assessing respiratory health hazards from hay and other source materials; *Equine Vet. J.* **19** 442–447

Gregory P H 1973 *Microbiology of the atmosphere* 2nd edition (London: Leonard Hill)

Gustavo N and Mendes 1987 Pathogenic potential of fungi in the genus *Aspergillus*; *Arg. Inst. Bacteriol. Cam. Penstana* **14** 27–40

Hill R A and Lacey J 1983 Factors determining the microflora of stored barley grain; *Ann. Appl. Biol.* **102** 467–483

Lacey J and Dutkiewicz 1976 Methods for examining the microflora of moulding hay; *J. Appl. Bacteriol.* **41** 13–27

Lacey J, Hill S T and Edwards M A 1980 Micro-organisms in stored grains: their enumeration and significance; *Trop. Stored Prod. Infect.* **39** 19–32

Ramalingam A 1978 A rotary drum miniature spore trap; *Sci. Cult.* **44** 366–367

Rati E and Ramalingam A 1974 Effect of *Aspergillus flavus* on the germinating seeds of some tropical crop plants; *Indian Phytopathol.* **27** 579–582

Reddy B N and Nusrath M 1985 Mycoflora in relation to earhead and grain in sorghum; *Indian Phytopathol.* **38** 751–753

Sreenivasamurthy V 1977 Mycotoxins—A public health problem; *Arogya J. Health Sci.* **3** 4–13

Thomas A R 1977 The genus *Aspergillus* and biodeterioration; in *Genetics and physiology of Aspergillus* (eds) J R Smith and J A Pateman (New York: Academic Press) pp 453–479

Tripathi R K 1974 Head fungi, phytotoxins and their effects on seed germination; *Indian Phytopathol.* **27** 499–501