The mycoflora of sorghum malt

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A quantitative and qualitative study of the mycoflora of both commercial and industrial sorghum malt was undertaken. Samples were collected from three industrial (22 samples) and 10 commercial (65 samples) maltsters over a period of three years. Fungal propagule counts were made of surface sterilized kernels on four different media (potato dextrose agar, acidified Czapeks-Dox medium, malt-salt agar and pentachloronitrobenzene medium) using both the dilution plate and the whole kernel method.

Counts were unacceptably high in many samples, especially in certain commercial malts. Counts differed between malts from different industrial malt producers, some consistently showing higher contamination levels than others. Differences were found between the dominant fungi occurring in the two malt types and the two evaluation methods also gave different results regarding certain taxa.

In all, 61 different species occurring in 29 genera were found to occur in sorghum malt. Fungi most frequently encountered were: Yeasts, Rhizopus rhizopodiformis (Cohn) Zopf, R. oryzae Went & Prinsen Geerligs, Aspergillus clavatus Desmazières, A. flavus Link, Fusarium moniliforme Sheldon, F. moniliforme Sheldon var. subglutinans Wollenw. & Reinking, F. chlamydosporum Wollenw. & Reinking and Phoma sorgina (Sacc.) Boerema, Dorenbosch & Van Kesteren.

S. Afr. J. Bot. 1984, 3: 251–255

Introduction

Sorghum (Sorghum caffrorum Beauv.) is grown extensively in many countries for food and feed and is also used on a large scale for the brewing of beer throughout southern, central, west and east Africa. Malting conditions (wet grain kept at 25°–28°C for 4–6 days) are ideal for the development of fungi and the formation of mycotoxins. In South Africa, sorghum malt is produced either indoors in a Saladin-type malting plant (industrial malt), or outdoors on concrete floors (commercial or floor malt, Novellie 1968). Both types of malt may sporadically be heavily infested with fungi. The fungi found in malt can originate either as contaminants that develop in the malting plant during the malting process, or, are brought in on contaminated grain. The latter are mainly plant pathogenic fungi that develop on plants in the field or storage fungi that develop under suboptimal storage conditions.

The object of the present study was to make a quantitative and qualitative assessment of those fungal species that occur regularly and in dominant numbers in both commercial and industrial malt. This information is an essential prerequisite for the determination of the possible presence of mycotoxins in sorghum malt.

Mycotoxins such as aflatoxin B1 and zearalenone have been shown to be present in home-brewed sorghum beer (Alozie et al. 1980; Lovelace & Nyathi 1977; Martin & Keen 1978), while the mycotoxins aflatoxin B1, alternariol, alternariol monomethyl ether, ochratoxin A, sterigmatocystin, T-2 and zearalenone have been found in sorghum grain (Bhat & Rukmini 1980; Elegbede et al. 1982; Schroeder & Hein 1975; Uraih & Ogbadu 1980; Shotwell et al. 1980; Tripathi 1973; Rukmini & Bhat 1978; Sauer et al. 1978; Seitz et al. 1975; Diener et al. 1981). These toxigenic fungi which can produce unknown toxic metabolites also occur in sorghum grain.

The mycoflora of sorghum seed has been extensively investigated in different parts of the world. Not only do the pre-harvest pathogenic fungi differ from one area to another, but also post-harvest conditions may affect the kind and concentration of fungi (Petit & Taber 1978). In India, Rai & Gupta (1977) found Alternaria tenuis, Curvularia lunata, C. lunata var. aerea, Chaetomium globosum, C. bostrychodes and Pestalotia spp. to be the most common in stored grain. Bhale & Khare (1980) also found C. lunata most commonly associated with sorghum seeds. Mathur et al. (1975) found...
*Fusarium moniliforme* and *C. lunata* to predominate, followed by *F. semitectum*, *Drechslera sorghicola*, *D. rostrata* and *Phoma* spp. Verma & Khan (1965) listed 23 different species isolated from sorghum seed.

In the United States of America, Swarup et al. (1962) reported that *Alternaria* spp. are the most important fungal species found on sorghum seed and this was confirmed by Castor & Frederiksen (1980) who also identified *F. semitectum*, *C. lunata*, *C. protuberata*, *F. moniliforme* and *Helminthosporium* spp. Diener et al. (1981) found *Curvularia*, *Penicillium*, *Mucor* and *Aspergillus* to be the dominant genera in sorghum grain in Alabama. Burroughs & Sauer (1971) found that *F. moniliforme* was the best competitor in sorghum seed kept at 23% moisture while Lopez & Christensen (1963) showed that only species of the *Aspergillus glaucus* group developed at moisture contents of 15–16%.

Niles (1976) isolated 51 fungal species from sorghum grain stored in pits in Ethiopia while El-Shafie (1982) found that *M. rouxii* to be the most common species in sorghum seed. This was confirmed by Webster (1981) who also isolated from sorghum seed.

### Methods

Sorghum malt samples (65 commercial malts and 22 industrial malts) were collected from ten different commercial maltsters and three industrial maltsters over a period of three years. Samples (1–2 kg each) were dried at 45°C for 24 h on the day of collection and stored at ±1°C in linen bags until used. In some cases samples were randomly collected to reflect the average situation in a given production batch, whilst in other cases biased samples were taken of malt showing fungal infestation.

After thorough rinsing in distilled water the malted kernels were surface sterilized (80:20 ethanol:water) for 3 min, rinsed three times in sterile water and plated out. Enumeration of fungi was done using both the dilution plate method as well as the direct or whole kernel method. Both methods were used because of the known problems concerning enumeration of fungi (Mislivec & Bruce 1977; Lacey et al. 1980; Flannigan 1973). Direct plating was done using five surface sterilized kernels per petri dish (five plates per treatment). For dilution plates, comminution was done for 2 min. Fifty grams of dried, surface sterilized malt kernels were milled to a fine flour in a coffee mill and 1 g material suspended in 10 ml sterile water. Plates were incubated in the dark at 25°C for 4–8 days and the resultant colonies of the dominant species were counted individually where possible. In many instances individual colonies of the dominant species were isolated in pure culture for identification.

All samples were plated out (both dilution plates and whole kernels) on four different media: (i) Potato dextrose agar (Difco); (ii) Malt extract salt agar; (iii) Acidified Czapeks-Dox agar; (iv) Pentachloronitrobenzene agar (Nash & Snyder 1962).

It was impossible to make counts of individual species of yeasts and all yeast species are grouped under yeasts. The same holds true for certain other taxa such as *Fusarium* and *Rhizopus*.

### Table 1 Fungi isolated from sorghum malt

| Fungi isolated from sorghum malt |
|---------------------------------|
| *Alternaria alternata* (Fr.) Keissler |
| *Aspergillus candidus* Link |
| *Aspergillus clavatus* Desmazières |
| *Aspergillus flavus* Link |
| *Aspergillus flavus* Link var. *colunnaris* Raper et Fennell |
| *Aspergillus fumigatus* Fresenius |
| *Aspergillus giganteus* Wehmer |
| *Aspergillus glaucus* Link |
| *Aspergillus nidulans* (Eidam) Wint. |
| *Aspergillus niger* Van Tieghem |
| *Aspergillus terreus* Thom |
| *Aspergillus usus* (Bain.) Thom & Church |
| *Aspergillus versicolor* (Vuill.) Tiraboschi |
| *Aspergillus wentii* Wehmer |
| *Aureobasidium pullulans* |
| *Aureobasidium pullulans* (de Bary) Arnaud |
| *Candida albicans* (Robin) Berkhout |
| *Candida chodatii* (Nechitch) Berkhout |
| *Candida krasei* (Cast.) Berkhout |
| *Candida macedoniensis* (Cast. et Chalmers) Berkhout |
| *Chaetomium* spp. |
| *Curvularia lunata* (Wakker) Boedijn |
| *Cylindrocarpon* sp. |
| *Dictyosporium discoidetum* Raper |
| *Drechslera* spp. |
| *Fusarium anavenaceum* (Fr.) Sacc. |
| *Fusarium chlamydosporum* Wollenw. & Reinking var. *fuscum* Gerlach |
| *Fusarium concolor* Wollenw. & Reinking |
| *Fusarium equiseti* (Corda) Sacc. |
| *Fusarium chlamydosporum* Wollenw. & Reinking |
| *Fusarium merismoides* Corda |
| *Fusarium moniliforme* Sheldon |
| *Fusarium moniliforme* Sheldon var. *subglutinans* Wollenw. & Reinking |
| *Fusarium oxysporum* Schlecht. |
| *Fusarium sambucinum* Fuckel |
| *Fusarium scripi* Lambotte & Fautr |
| *Fusarium scripi* Lambotte & Fautr. var *compactum* Wollenw. |
| *Fusarium semitectum* Berk. & Rav. |
| *Fusarium solani* (Mart.) Sacc. |
| *Geotrichum* spp. |
| *Gonatobotrys* spp. |
| *Hansenula anomala* (Hansen) H. et P. Sydow |
| *Helminthosporium* spp. |
| *Kluveromyces marxianus* (Hansen) v.d. Walt |
| *Monodictys* sp. |
| *Mucor circinelloides* Van Tieghem |
| *Mucor indicus* Lindner |
| *Oidiodendron* spp. |
| *Penicillium* spp. |
| *Penicillium islandicum* Sopp |
| *Phoma sorghina* (Sacc.) Boerema, Dorenbosch & Van Kesteren |
| *Phoma* spp. |
| *Piptocephalis tieghemiana* Matruchot |
| *Rhizopus oryzae* Went & Prinsen Geerligs |
| *Rhizopus rhizopodiformis* (Cohn) Zopf |
| *Saccharomycopsis fibuligera* (Lindner) Klockner |
| *Sclerotium rolfsii* Sacc. |
| *Streptomyces* spp. |
| *Syncephalis cornu* Van Tieghem & Le Monnier |
| *Trichoderma viride* Pers. ex Gray |
| *Trichothecium roseum* (Pers.) Link ex Gray |

Yeasts
Results

Sixty-one species of seed-borne fungi representing 29 genera were isolated from sorghum malt (Table 1). The frequency of occurrence of the dominant taxa in commercial and industrial malt is shown in Tables 2 & 3. The dominant fungal taxa common to both commercial and industrial malt were: *Saccharomyces* spp., *Rhizopus/Mucor* spp., *Fusarium* spp., *Aspergillus clavatus*, *A. flavus* and *Phoma sorghina*. The *Rhizopus* species most commonly encountered were *R. oryzae* and *R. rhizopodiformis* whilst *F. moniliforme*, *F. moniliforme var. subglutinans*, *F. chlamydosporum* and *F. oxysporum* were the *Fusarium* species most often encountered.

Fungal propagule counts, using the dilution plate method (Table 3), showed that many malt samples were highly contaminated (counts above 50 000 propagules/g). Both malt types showed high yeast counts, while commercial malt on average had a higher infestation of *Rhizopus* spp. than industrial malt. On the other hand, industrial malt on average had a higher infestation of *A. clavatus* than did commercial malt.

The whole kernel method in some instances gave slightly different results from the dilution plate method (Tables 2 & 3). With both methods yeasts dominated but *Rhizopus/Mucor* species showed relatively higher counts in industrial malt using the dilution plate method. The incidence of *Fusarium* species was approximately the same in both malt types using the whole kernel method whereas results of the dilution plate method showed a higher incidence in industrial than in commercial malt. Both methods showed a high incidence of *A. clavatus* in industrial malt. The whole kernel method showed a relatively higher frequency of occurrence of *Phoma sorghina* in industrial than in commercial malt, whereas with *Alternaria alternata* the highest frequency was found in commercial malt using this method. A larger variety of fungi could be detected in commercial than in industrial malt and some of these could only be detected using the whole kernel method.

The fungal infestation of malt from three different maltsters is shown in Table 4. The malt from one producer (B) was comparatively mould-free most of the time, whilst that of maltster A was regularly heavily infested by *Aspergillus clavatus*. The malt produced by maltster C showed that it was frequently possible to produce malt free even from *Rhizopus/Mucor* species, although the malt of this producer was regularly heavily contaminated with field fungi such as *Phoma sorghina* and *Fusarium* species. Species of the *Aspergillus glaucus* group (storage fungi) were found in a low percentage of commercial malt samples.

Discussion

Total fungal propagule counts using the dilution plate method showed that many malt samples were highly contaminated by fungi. Many of the dominant fungal species (yeasts, *Rhizopus/Mucor* spp., *A. clavatus* and probably *A. flavus*) are fungi that develop mainly during the malting process. The source of primary inoculum is most probably to be found in poor house-keeping practices. Species of *Fusarium*, *Phoma sorghina* and *Alternaria alternata* are plant pathogens.

### Table 2 Percentage commercial and industrial sorghum malt samples infested by the dominant fungal species (whole kernels)

| Isolates                 | Commercial malt<sup>a</sup> | Industrial malt<sup>b</sup> |
|--------------------------|-----------------------------|-----------------------------|
| Yeasts                   | 86                          | 100                         |
| *Rhizopus/Mucor* spp.   | 82                          | 73                          |
| *Fusarium* spp.         | 52                          | 64                          |
| *Aspergillus clavatus*  | 9                           | 45                          |
| *Aspergillus flavus*    | 29                          | 23                          |
| *Phoma sorghina*        | 8                           | 36                          |
| *Alternaria alternata*  | 26                          | 14                          |
| *Oidiodendron* spp.     | 8                           | 18                          |
| *Aspergillus fumigatus* | 6                           | 5                           |
| *Aspergillus niger*     | 6                           | 0                           |
| *Aspergillus glaucus*   | 5                           | 0                           |
| *Piptocephalis* spp.<sup>c</sup> | 3          | 0                           |
| *Sclerotium rolfsi*     | 2                           | 0                           |
| *Streptomyces* spp.     | 2                           | 0                           |
| *Trichoderma viride*    | 2                           | 0                           |
| *Gonatobotrys* spp.     | 2                           | 0                           |

<sup>a</sup> 65 samples  <sup>b</sup> 22 samples  <sup>c</sup> Parasitic on *Rhizopus* spp.

### Table 3 Percentage commercial and industrial sorghum malt samples infested by different fungal taxa at two contamination levels (dilution plates)

| Species                  | Commercial malt<sup>a</sup> | Industrial malt<sup>b</sup> |
|--------------------------|-------------------------------|-----------------------------|
|                          | >5 000 propagules/g | >50 000 propagules/g | >5 000 propagules/g | >50 000 propagules/g |
| Yeasts                   | 72                          | 55                         | 91                          | 77                          |
| *Rhizopus/Mucor* spp.   | 55                          | 38                         | 18                          | 9                           |
| *Fusarium* spp.         | 17                          | 12                         | 32                          | 18                         |
| *Aspergillus clavatus*  | 3                           | 3                          | 27                          | 18                         |
| *Aspergillus flavus*    | 15                          | 9                          | 5                           | 5                           |
| *Phoma sorghina*        | 5                           | 2                          | 9                           | 0                           |
| *Aspergillus fumigatus* | 5                           | 3                          | 5                           | 5                           |
| *Geotrichum* spp.       | 11                          | 3                          | 0                           | 0                           |
| *Penicillium* spp.      | 6                           | 0                          | 5                           | 5                           |
| *Alternaria alternata*  | 6                           | 2                          | 0                           | 0                           |
| *Aspergillus glaucus*   | 3                           | 2                          | 0                           | 0                           |

<sup>a</sup> 65 samples;  <sup>b</sup> 22 samples
Table 4 Dominant fungus taxa in different industrial sorghum malt samples

| Malt producer | Sample number | Alternaria alternata | Aspergillus flavus | Aspergillus fumigatus | Fusarium spp. | Oidiodendron spp. | Phoma sorghina | Rhizopus/Mucor spp. | Yeasts |
|---------------|---------------|----------------------|-------------------|----------------------|---------------|------------------|----------------|-------------------|-------|
| SA 195        |               |                      |                   |                      |               |                  |                |                   |       |
| SA 196        |               |                      |                   |                      |               |                  |                |                   |       |
| SA 198        |               |                      |                   |                      |               |                  |                |                   |       |
| CA 199        |               |                      |                   |                      |               |                  |                |                   |       |
| CA 200        |               |                      |                   |                      |               |                  |                |                   |       |
| A 201         |               |                      |                   |                      |               |                  |                |                   |       |
| A 202         |               |                      |                   |                      |               |                  |                |                   |       |
| B 203         |               |                      |                   |                      |               |                  |                |                   |       |
| B 204         |               |                      |                   |                      |               |                  |                |                   |       |
| B 205         |               |                      |                   |                      |               |                  |                |                   |       |
| C 206         |               |                      |                   |                      |               |                  |                |                   |       |
| C 207         |               |                      |                   |                      |               |                  |                |                   |       |
| C 208         |               |                      |                   |                      |               |                  |                |                   |       |
| C 209         |               |                      |                   |                      |               |                  |                |                   |       |
| C 210         |               |                      |                   |                      |               |                  |                |                   |       |
| C 211         |               |                      |                   |                      |               |                  |                |                   |       |

* >75% kernels infected

and are brought into the malting house on infected grain. It is not certain to what extent these fungi can develop during the malting process. It seems as if certain Fusarium species, e.g. F. moniliforme can be good competitors during the malting process. Aspergillus flavus may to a certain extent already be present on grain brought into the malt house.

Commercial malt is infested by a larger variety of fungal species than industrial malt. Ordinarily one would not expect to find fungi such as Sclerotium rolfsii, Streptomyces sp., etc., in high concentrations on germinating seed. These species develop in commercial malt owing to poor housekeeping procedures. Storage fungi, that is fungi that develop at relatively low water activities such as those belonging to the Aspergillus glaucus group, were found infrequently.

A comparison of the dominant field fungi found on sorghum grain in other countries with those found locally in sorghum malt, shows that species belonging to the genus Drechslera was never found locally while Curvularia species occurred only infrequently. The frequent occurrence locally of F. moniliforme, Alternaria alternata and Phoma sorghina corresponds to results obtained in India and the USA.

The toxicity of the species that occurred most frequently is being investigated.

Acknowledgements

We gratefully acknowledge the assistance of Dr W.F.O. Marasas of the South African Medical Research Council, Dr J.P. van der Walt of the CSIR and Dr M.A.A. Schipper of the Centraalbureau voor Schimmelcultures for their help with the identification of Fusarium spp., yeasts and Mucorales, respectively.

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