Susceptibility to ergot in Zimbabwe of sorghums that remained uninfected in their native climates in Ethiopia and Rwanda

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Forty-four local Ethiopian and Rwandan sorghums (Sorghum bicolor) were observed to remain free of ergot, or had only low incidence, in their natural equatorial latitudes and were potentially of interest, in the design of male-sterile lines for F1 hybrid breeding, if they possessed a physiologically based resistance mechanism. These sorghums were therefore also investigated under natural and artificial disease pressures in Zimbabwe where unadapted development and inappropriate long daylength prevented flowering in 18 accessions. Of the remaining 16 Ethiopian and 10 Rwandan accessions which flowered, only one from each country remained free of ergot. The susceptibility expressed was ascribed to observed asynchrony of stigma exsertion with anthesis. In the Rwandan accession that persistently remained free of ergot in Zimbabwe, histology of ovules showed pollination before floret gaping, so that a general principle of disease escape due to efficient pollination is proposed for the Ethiopian and Rwandan sorghums in their native climates. The findings emphasize that cleistogamy is a desirable character for avoiding ergot infection in self-fertile sorghums and suggest that the Ethiopian and Rwandan sorghums may not generally be useful for breeding ergot-resistant male-sterile female lines. However, a few accessions deserve more detailed study as a potential genetic resource, before a firm conclusion that all apparent resistance is disease escape owing to efficient pollination.

INTRODUCTION

Ergot disease of sorghum (Sorghum bicolor) was first reported in southern Africa in Tanganyika by Mason (1926). Although the pathogen has been generally known by its imperfect designation (Sphacelia sorghi McRae), and assumed to be included within Claviceps sorghi Kulkarni, Shedadi and Hegde (Bandyopadhyay, 1992), the disease is caused in Africa by Claviceps africana Frederickson, Mantle and De Milliano (Frederickson et al., 1991). Ergot disease of sorghum is an important constraint on F1 hybrid seed production in southern Africa (Anon., 1992) where sclerotia of the fungus are formed at many locations (De Milliano, 1992). Exploitation of male-sterile female parents (A-lines) used in the sorghum seed production process is seriously threatened by annual outbreaks of ergot and the consequent reduction in both seed yield and quality to below economic thresholds. C. africana is widely endemic in Africa and is distinctly different, in several mycological and biochemical characteristics, from C. sorghi which is apparently confined to the Indian subcontinent (Frederickson et al., 1991). The pathogen in Thailand (Boon-Long, 1992), which has seriously constrained F1 hybrid sorghum breeding, seems to be most like C. africana (Frederickson et al., 1991), but in Japan (Kimigafukuro, 1992) appears, from examination of the pathogen (and personal communication with T. Tsukiboshi), to be quite different from either C. sorghi or C. africana.

Control of ergot disease of sorghum through pollen management is envisaged, utilising efficient pollination of the A-line ovary to prevent infection. However, in Africa some sorghum landraces of Ethiopian and Rwandan origin were observed to remain free from ergot (presumed to be C. africana) or to have significantly lower infection incidence of spikelets, after artificial inoculation in their natural environment, even after careful inoculations (Bandyopadhyay, 1992). They therefore appeared in prospect to offer alternative control through sorghum breed-
ing (Hulluka, 1982; ICRISAT, 1990) if an as yet unrecognized physiological mechanism was operating in this pool of local sorghums. Consequently, arising from preliminary screening by the International Crops Research Institute for the Semi-Arid Tropics (Bandyopadhyay, 1992), 44 selected sorghum accessions were grown in Zimbabwe in the 1990/91 season to identify those that would flower in this environment. Twenty-six of the accessions that flowered in Zimbabwe were then subjected to natural and/or artificial disease pressures. The plantings provided an opportunity to observe susceptibility to the local pathogen (C. africana) and further, by electron microscopy, to explore persistent escape from disease. The 1991/92 drought in southern Africa and the reduced prioritization as a result of the present findings, because of only long-term economical prospects and the high risk nature of the research, has precluded replication and extension of the findings.

MATERIALS AND METHODS

Nine of the Ethiopian accessions that flowered in Zimbabwe had been planted at two ecologically similar experimental stations (Henderson and Panmure, 30 and 100 km north of Harare, respectively) where Claviceps africana epiphytotics have occurred in recent years (Mtisi, 1992). Another seven accessions were planted only at Panmure. Ten Rwandan accessions were planted only at Henderson. The two locations were chosen also for their more predictable rainfall pattern, enabling early planting and minimizing mid-season water stresses. Planting was on 14 and 17 November 1990 for Ethiopian material at Henderson and Panmure, respectively, and 27 November 1990 for the Rwandan material.

Ethiopian sorghums were seeded in two to six 5 m rows, whereas Rwandan accessions, limited by availability of seed, were sown in plots of two 5 m rows.

The experiment at Panmure relied entirely on natural disease pressure which was expected to be mainly due to airborne secondary conidia as inoculum (Frederickson et al., 1993). Before experimental sorghums flowered, ergot was abundant in a large area of other sorghum A-lines immediately adjacent. Since honeydew exudation was profuse and secondary conidiation (Frederickson et al., 1989) clearly evident, the A-lines were regarded as a source of inoculum for the Ethiopian sorghums, which commenced flowering only in late March 1991. At harvest, in late June 1991, symptoms of ergot (sclerotia, and honeydew) were often masked by saprophytic Cerebella sp., which made it difficult to make more than a conservative estimate of the occurrence of ergot in each row of each accession.

At Henderson all experiments were under artificial disease pressure imposed by spraying suspensions of macroconidia (Frederickson et al., 1989). Flowering was delayed in those Ethiopian and Rwandan sorghums which eventually flowered until late March or early April 1991, most panicles having been in boot for a protracted period of about 4 weeks. As soon as individual panicles of each accession commenced anthesis, the first anthesing florets at the tip of the inflorescence were excised and the panicle was bagged. The following morning bagged individuals (usually 5, but up to 16, per row) were inspected, and representative freshly anthesing florets, and as yet unopened adjacent florets, were excised and fixed in 2.5% glutaraldehyde in cacodylate buffer prior to preparation for electron microscopy (Frederickson & Mantle, 1988). The glumes of other freshly anthesing florets were marked with ink. General floral characteristics and flowering behaviour were also noted. Finally, the panicle was inoculated by spraying until run-off with a fresh opaque aqueous suspension of ergot conidia prepared from diluted honeydew. The infectivity of the inoculum was tested in a similar manner at anthesis on five other very late-planted ICRISAT sorghum varieties which were known to contract the disease.

Marked florets were sampled 24 h post-inoculation for microscopy. Plants were scored for ergot disease 25 days post-inoculation, counting the proportion of infected panicles (disease incidence) and scoring the marked florets which were infected in each panicle (disease severity).

Histological preparation of gynoecia involved direct squashes in 1% neutral red in 45% acetic acid to detect pollen tubes, or in 0.1% cotton blue in lactophenol to detect hyphae of the pathogen. Subsequent transmission electron microscopy (Frederickson & Mantle, 1988) sought specific evidence of pollen tubes in ovaries and particularly in the micropylar region.

RESULTS

All 26 of the Ethiopian and Rwandan sorghums grown in Zimbabwe, and which eventually flowered, exhibited photoperiod-sensitivity and flowering only after a protracted period of vegetative growth, resulting in very tall (> 5 m) phenotypes with long internodes.
Sorghum susceptibility to ergot

Table 1. Ergot disease and floret fertility in Ethiopian and Rwandan sorghums subjected to artificial disease pressure in Zimbabwe

<table>
<thead>
<tr>
<th>Sorghum accession</th>
<th>Artificial disease pressure (Henderson)</th>
<th>Floret fertility (percentage pollination)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>disease incidence (%)</td>
<td>mean disease severity (%)</td>
</tr>
<tr>
<td>Ethiopian^a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETS 3252</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ETS 3251-1</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>ETS 4145</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>ETS 4457</td>
<td>47</td>
<td>34</td>
</tr>
<tr>
<td>ETS 4927</td>
<td>0</td>
<td>0</td>
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<td>8</td>
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<td>ETS 3912</td>
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<td>ETS 1446</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rwandan</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>50</td>
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</tr>
<tr>
<td>IS 25576</td>
<td>100</td>
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<td>12192</td>
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<tr>
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<td>66</td>
<td>1</td>
</tr>
<tr>
<td>IS 25485</td>
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<td>0</td>
</tr>
</tbody>
</table>

^a Accession which became ergotized under natural disease pressure at Panmure.

The sorghum varieties used at Henderson to determine the pathogenicity of the artificial inoculum all became infected and showed a mean disease incidence of 64% and mean disease severity of 16%, confirming a potentially infective inoculum.

Under natural disease pressure at Panmure, only one of the 16 Ethiopian accessions (ETS 1446, five rows) failed to become infected with ergot. In the other 15 accessions, 8 expressed the disease in all rows (ETS 2518, 3215, 3251-1, 3252, 4145, 4145-1, 4145-2, 4145-3) and, in the other 7, inflorescences in one to five rows out of two to six were diseased (ETS 2448, 2465, 3125, 3912, 4145-4, 4457, 4927).

Artificial inoculation at Henderson caused the disease on 12 of the 19 Ethiopian and Rwandan sorghums tested (Table 1). Whereas the incidence was often quite high, sometimes higher than in the varieties used for a quality test on the inoculum, severity was generally low. However, five of the six Ethiopian sorghums which were free from ergot disease in the experiment at Henderson research station contracted the disease from natural sources at Panmure. Therefore, of the 26 sorghums in the experiment, only two, one each from Ethiopia (ETS 1446) and Rwanda (IS 25485), remained ergot-free in Zimbabwe.

Staining of gynoecia with cotton blue, 24 h post-inoculation, verified the presence of conidia on stigmas of all Ethiopian and Rwandan sorghums at Henderson. Some conidia were already germinating. However, Rwandan IS 25485 had additionally an abundance of germinated pollen on stigmatic hairs at this time, pollen tubes having penetrated the stigma and residual pollen grains appearing slightly collapsed (Fig. 1). Examination of florets collected at the same time, though developmentally still about 1 day pre-gaping, showed that cleistogamous pollination had already occurred in 44% of IS 25485 florets. The floral cavities were full of pollen, anthers having...
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Fig. 1. A stigmatic hair of Rwandan sorghum accession IS 25485 penetrated by germinated pollen grain 24 hours after floret gaping. (White bars at top of figure, 10 μm.)

Fig. 2. Transmission electron micrograph of a section of the upper ovary wall of Rwandan sorghum accession IS 25485 before floret gaping, showing a pollen tube in transverse section located in the transmission tract (× 2500).

Fig. 3. Transmission electron micrograph of a near median longitudinal section through the proximal end of an ovule of Rwandan sorghum accession IS 25485 24 hours after floret gaping. The egg apparatus (centre) is bounded to the right and below by nucellar cells. The space at top left is the central cell. A pollen tube adjacent to the proximal aspect of the egg apparatus is shown magnified in Fig. 4 (× 1900).

already dehisced, and pollen tubes were progressing down the styles. Longitudinal sections revealed intercellular pollen tubes as far as the top of the ovary (Fig 2), some interposed between the innermost tissues of the ovary wall and the ovule. When IS 25485 florets gaped, stigma exsertion could be expected, but it did not happen. All these florets were already self-pollinated. At 24 h post-inoculation, when spores had only just germinated, a pollen tube was recognized, by electron microscopy, in the micropylar region of an ovary and within the micropylar cuticle reaching a position immediately exterior to the embryosac in the matrix-filled space adjacent to the egg cell (Figs 3 and 4). The proximity of the pollen tube indicated that fertilization was imminent in IS 25485 if it had not already occurred.

A similar floral biology was observed in the Rwandan TURA; 50% of the florets were cleistogamous, and, following further self-pollination at anthesis, a large proportion of florets had been pollinated before ergot inoculum could germinate on stigmas. Although a high incidence (66%) of ergot resulted, the 99% seed set ensured that the severity was low. Fertile pedicelled spikelets may also have increased the pollen pressure.

In contrast to IS 25485 and TURA, ergot in IS 25570 reached 100% incidence. Interestingly,
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Fig. 4. Transmission electron micrograph of a section of the proximal region of the ovule illustrated in Fig. 3. and orientated similarly, showing the lateral edge of the egg apparatus (left) and pollen tube with dense cytoplasm (right) immediately exterior to the embryo sac. Imminent fertilization is implied (× 7100).

although pollination and seed set were low, ergot severity was also low. Conidia were, however, viable, observed germinating on stigmas 24 h post-inoculation. Often, 48 h post-inoculation, styles of IS 25570 exhibited a necrotic spotting reaction at the mid-point, progressing to a uniformly necrotic region a day later so that some stigmas fractured. This necrosis may have served to limit invasion of the fungus down the style of a fertilized ovary, although it could also likewise hinder passage of the pollen tube when an ovary is infected by C. africana. Other accessions were intermediate in nature, exhibiting lesser degrees either of cleistogamy or of the necrotic reaction (ETS 3251-1; ETS 4457).

DISCUSSION

Integration of the results of natural and artificial inoculation of Ethiopian and Rwandan sorghums, reputed to be resistant to ergot at least in their country of origin, has shown that, in all except two, gynoecia could become parasitized by C. africana under the conditions of this experiment. Even in this somewhat unnatural environment, in which their growth habit was altered, flowering behaviour of the apparently resistant accessions still ensured sufficient self-pollination to exclude the ergot pathogen from most of the florets. Indeed, the Rwandan IS 25485 was so efficient by selfing before gaping that it completely escaped infection. It is concluded, from consideration also of the histological evidence and that of the observations on flowering behaviour, that at least most of the accessions tested had no special gynoecial physiology which prevented ergot parasitism. The resistance expressed in their natural environment will result from efficient self-pollination, allowing insufficient time for colonization of the ovary by the slower pathogen. However, the local necrotic reactions in stigmas of three accessions in particular may be somewhat analogous to that in pearl millet (Willingale et al., 1986), although necrosis would still not exclude the pathogen if the fungus had entered the ovary first.

The simple circumstances of the experiment cannot sustain too much comparison of the results of natural and artificial disease pressures. Nevertheless, the occurrence of ergot in the Ethiopian sorghums under natural disease pressure at Panmure illustrates how readily disease spreads naturally. It also raises the possibility, for example, that first gaping florets at night, giving access to a few airborne secondary conidia as inoculum, may have become infected before pollination, whereas spraying a much more concentrated inoculum into gaping florets later the next day, as happened in experiments at Henderson, failed. However, there is still the possibility that accession ETS 1446 (provided by the Ethiopian Sorghum Improvement Programme) may be of interest. Although it hardly set seed, it still failed to become infected. Possible explanations could be that gynoecial formation was abnormal in the changed environment or that gynoecia were unreceptive in this environment similarly both to pollen and pathogen as stigma-invading filaments. However, the finding concerning this accession might justify a more concerted effort to explore pre-fertilization gynoecial susceptibility to C. africana in Ethiopia before being sure it has no value.

It appears that some fundamental structural or physiological gynoecial character, not directly involved in pollination and fertilization, that would exclude the ergot fungus and could be incorporated into elite A-lines, does not exist in the accessions which flowered in the present study, as is apparent concerning all other ergot host interactions. The only character that effects freedom from ergot in a particular graminaceous host concerns efficient pollination fertilization and therefore constitutes a disease escape mechanism.
However, further search for intrinsic gynoecial resistance to ergot in sorghums is warranted before excluding such as a potentially exploitable heritable character. Chemically induced male sterility may aid the exploration of gynoecial susceptibility in sorghums which naturally escape ergot when in their native latitude or when induced to flower in more convenient experimental environments. Nevertheless, the consistently low ergot severity despite artificial inoculation of the accessions described here, combined with some instances of high seed set, is an encouraging finding. However, if potentially useful resistance to ergot disease is found in sorghums, the diversity of pathogens in Africa and Asia will require multilocalional testing of potential cultivars.

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