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Ergot resistance in sorghum in relation to flowering, inoculation technique and disease development

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Ergot is an important disease of sorghum (*Sorghum bicolor*) in parts of Africa and Asia. Studies were conducted to determine the relationship between flowering biology and ergot infection, and to develop an artificial field-screening technique to identify ergot resistance in sorghum. Spikelets resisted infection after anthesis, but each day's delay in anthesis after inoculation supported 8·3% more ergot. The screening technique consisted of three components: trimming of panicles to remove pollinated spikelets before inoculation, a single inoculation of trimmed panicles, and panicle bagging for 7–10 days. Inoculated panicles were evaluated by a qualitative visual rating method (on a 1–5 scale) and a quantitative spikelet counting method. Selected accessions from the world collection of sorghum germplasm were screened at Karama Research Station, Rwanda, for two seasons and 12 ergot-resistant lines were identified. These were also resistant at ICRISAT Centre, India.

INTRODUCTION

Ergot of sorghum occurs only in Asia and Africa. It is caused by the fungus *Claviceps africana* in parts of southern Africa (Frederickson *et al.*, 1991) and by *C. sorghi* in India (Kulkarni *et al.*, 1976). The anamorph of both teleomorphic *Claviceps* species is *Sphacelia sorghi*. The major difference between the two species appears to be the alkaloids found in the sclerotia. Conidia of *S. sorghi* infect the stigma at flowering and replace the ovary with a soft fungal stroma, which is later transformed into a hard sclerotium in place of grain. The stroma produces numerous conidia in a sugary, sticky fluid called honeydew, which smears healthy grain. Under humid conditions, honeydew-smeared grains may be covered with saprophytic growth of several fungi, which reduces the quality of grains (Bandyopadhyay *et al.*, 1990).

Ergot causes widespread damage to traditional and improved cultivars in farmers' fields (Molefe, 1975; de Milliano *et al.*, 1991), but it is most damaging in hybrid seed production plots where use is made of male sterility (Futrell & Webster, 1965; Bandyopadhyay, 1992). Host resistance is a practical and economical method of sorghum ergot control, but its use requires the availability of locally adapted ergot-resistant cultivars. Identification of resistant lines requires screening of a large number of sorghum genotypes using a reliable and biologically sound resistance-screening technique. Several techniques have been proposed (Chinnadurai *et al.*, 1970; Sundaram, 1970, 1971; Khadke *et al.*, 1978), but each technique differs from the others with respect to the growth stage at inoculation, number of inoculations, and evaluation methods. Most of these techniques have apparently not taken into consideration the effect of pollination on ergot resistance, as demonstrated by Futrell & Webster (1965) in sorghum and also by Thakur & Williams (1980) in pearl millet (*Pennisetum glaucum*). Screening results obtained using these techniques are therefore not likely to be reliable or repeatable.

The purpose of this study was to quantify the effects of timing of pollination and inoculation on ergot development in order to develop a reliable field-screening technique for ergot resistance.

MATERIALS AND METHODS

Conidial inoculum for all experiments was prepared by washing honeydew from infected panicles in water. The suspension was strained

through a sieve or 2–3 layers of muslin cloth, and was diluted to approximately 1×10^6 conidia/ml using a haemocytometer.

Relationship between flowering and ergot development

A trial was conducted at Rubona (altitude 1706 m), Rwanda using the self-fertile cultivar Ikinyaruka in which anthers and stigmas emerge together from gaping glumes at the time of anthesis (the terms anthesis and flowering are used interchangeably).

Four panicles (each panicle representing a replicate) were selected in which anthesis began on the same day (day 1). In the afternoon of day 3, glumes of all spikelets that had flowered on days 1–3 were marked with red ink. The panicles were dip-inoculated and bagged in the afternoon of day 4, after spikelets that had flowered 8 h before inoculation had been marked with blue ink. Bags were briefly removed in the afternoons of subsequent days to mark spikelets that flowered on days 5 and 6 with black and green ink, respectively. Spikelets that flowered on days 7–11 (completion of flowering) were not marked. Colour-coded markings on glumes facilitated subsequent identification of flowering day in relation to inoculation day. Bags were removed when honeydew exuded from infected spikelets 10 days after inoculation. In each panicle, 514–886 spikelets were observed, 20 days after inoculation, for the number of spikelets bearing stroma (infected) and grain (healthy) within each group of marked spikelets. The percentage infected spikelets was calculated and subjected to statistical analyses using analysis of variance and simple linear regression.

Components of resistance screening

Three components of a screening technique for resistance were tested using two genotypes (Ikinyaruka and Amasugi) at Rubona; because pollinated spikelets resist infection, the first component was removal of all spikelets that had completed anthesis (spikelet trimming). This ensured inoculation of only non-pollinated, potentially susceptible spikelets. The second component was inoculation to ensure pathogen presence, and the third component was panicle bagging to ensure the high humidity favourable for infection. Combinations of spikelet trimming (no trimming or trimming of spikelets that completed anthesis prior to first inoculation), panicle inoculation (none, one or two spray inoculations until run-off at 2-day intervals), and panicle bagging (no bagging or bagging immediately after first inoculation) were compared in a $2 \times 3 \times 2$ factorial design. For each genotype, 60 panicles were selected in which 25% or more spikelets had completed flowering. For 30 panicles in each genotype, all spikelets that had completed anthesis were trimmed, whereas the remaining 30 panicles were left intact. Within each trimming treatment, 10 panicles were inoculated once, 10 twice, and the remainder were not inoculated. Five panicles in each of the inoculation treatments were bagged for 7–10 days and the remaining five were not bagged.

The role of bagging in enhancing ergot severity was also studied at Rubona in a separate experiment using four genotypes (CSH 1, Ikinyaruka, WS 1297, and ICSH 2265). Eight panicles of each genotype were trimmed and spray inoculated twice, at 2-day intervals. Four of the eight panicles were bagged for 7–10 days after the first inoculation and the remaining four were not bagged.

In both experiments, spikelets were observed 20 days after inoculation to record infected, unfilled, and healthy spikelets, and the percentage infected spikelets was calculated. Each panicle was considered a replicate. Angular transformation of the percentage infected spikelets was used to analyse each genotype separately using analysis of variance. The data were analysed with ($2 \times 3 \times 2$ factorial) and without ($2 \times 2 \times 2$ factorial) the non-inoculated control treatments. There was less than 1% disease development in the control treatments. Bagging treatments in the second experiment were compared within each genotype using Student's *t*-test.

Effectiveness of screening

A selection was made of 246 accessions from the Genetic Resources Unit of ICRISAT collected from high-altitude areas of different countries and likely to be adapted in Rwanda. The countries represented (with the number of accessions in parenthesis) were Burundi (20), Ethiopia (92), Lesotho (50), Rwanda (47), and Yemen Arabic Republic (37). Eighty-two locally adapted genotypes collected and maintained by the Rwanda national programme were also screened.

Trials were sown at Karama (altitude 1350 m) in January to February 1988 and 1989, such that test lines flowered during frequent rains in

mid-May and early June. Initial screening was carried out in unreplicated 4-m-row plots. Panicles were spray-inoculated, and accessions with coloured grain and up to 20% infected spikelets were selected for advanced screening trials. The latter were sown in two 4-m-row plots in a completely randomized design. Lines selected from advanced screening trials in Rwanda were also evaluated at ICRISAT Centre in the 1989/90 post-rainy season in overhead sprinkler-irrigated plots (planted in September and flowered in December) using a randomized block design with two replications. In Rwanda, five to seven trimmed panicles in each plot were spray-inoculated, twice, with a conidial suspension. At ICRISAT Centre, 10 trimmed panicles in each plot were dip-inoculated once. Panicles were bagged for 7–10 days after inoculation.

Inoculated panicles were rated visually for infected spikelets at grain maturity on a 1–5 rating scale where 1 = no ergot, 2 = 1–10%, 3 = 11–25%, 4 = 26–50%, and 5 = more than 50% spikelets infected by ergot. Panicles with ergot ratings of 4–5 were rejected as susceptible, and the remainder were harvested. To confirm the ratings of individual harvested panicles, one panicle branch was removed from each node of the rachis to make a composite sample in which the number of infected and healthy spikelets were counted, and the percentage of infected spikelets calculated.

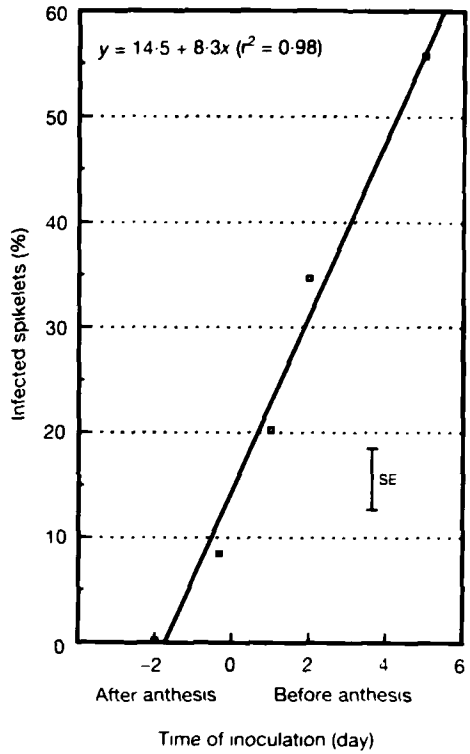

Fig. 1. Relationship between percentage of ergot-infected spikelets in the sorghum cultivar Ikinyaruka and inoculation of panicles with *Sphacelia sorghi* on various days, before or after anthesis.

RESULTS

Relationship between flowering and ergot development

Spikelets inoculated 1–3 days after anthesis had negligible ergot infection (0·2%) which increased progressively from 8·5% in spikelets inoculated 8 h after anthesis to 55·8% in spikelets inoculated 3–7 days before anthesis (Fig. 1). Inoculation of spikelets 8 h to 3 days after anthesis produced statistically ($P < 0·01$) similar infection levels and had significantly less disease than those inoculated before anthesis. Progressive delay in anthesis after inoculation promoted susceptibility as illustrated by an increase in disease incidence at a rate of 8·3% per day (Fig. 1).

Components of resistance screening

Ergot severity in trimmed panicles was significantly higher than in untrimmed panicles (Table 1). Similarly, bagged panicles had significantly more ergot than those not bagged (Tables 1 and 2). Fewer than 1% spikelets were infected in the non-inoculated control panicles. Inoculation significantly increased ergot severity compared with the control, but the number of inoculations did not influence ergot severity. All two- or three-way interactions among the three treatments were not significant ($P < 0·05$). The treatment combinations that produced optimum ergot were spikelets trimming, one inoculation and bagging of panicles.

Effectiveness of screening

Twelve of the 246 sorghum genotypes screened were resistant to ergot (⩽ 10% severity) in repeated screening for up to 2 years in Rwanda (Table 3). Resistance in these 12 accessions was also confirmed at ICRISAT Centre. Ergot pressure was high in all the years as shown by the ergot severity data for susceptible controls

Table 1. Effect of panicle trimming, bagging, and inoculation on severity of ergot in sorghum genotypes Amasugi and Ikinyaruka

Treatment			Amasugi		Ikinyaruka	
trimming[a]	bagging	inoculation (number)	spikelets observed (number)	spikelets infected (%)	spikelets observed (number)	spikelets infected (%)
Yes	Yes	2	6541	78·3 (62·3)[b]	2007	55·3 (48·0)
Yes	Yes	1	4333	74·7 (59·9)	1509	54·2 (47·4)
Yes	Yes	0	2506	0·0	2570	0·8
Yes	No	2	4009	43·1 (41·0)	2618	40·1 (39·3)
Yes	No	1	3867	39·2 (38·7)	1656	37·5 (37·7)
Yes	No	0	1521	0·1	1211	0·4
No	Yes	2	7018	26·6 (30·3)	6096	31·5 (33·0)
No	Yes	1	4653	21·9 (27·4)	4260	28·7 (31·1)
No	Yes	0	6798	0·0	3302	0·1
No	No	2	5505	9·8 (17·3)	3816	9·4 (16·7)
No	No	1	8280	11·1 (18·6)	2440	3·3 (10·5)
No	No	0	6154	0·1	2199	0·4
SE[c] to compare any two treatment levels				±(2·70)		±(3·70)

[a] Panicle trimming indicates that all flowering spikelets were pruned to retain and inoculate only nonpollinated spikelets. In the treatment without trimming, panicles with 25% or more flowering spikelets were selected randomly for inoculation.

[b] Figures in parentheses are arc sine transformed values of the percentage of infected spikelets.

[c] Standard error of mean calculated using arc sine transformed values of percentage infected spikelets. Non-inoculated control treatment was not used in the analysis.

Table 2. Percentage ergot-infected spikelets in bagged and nonbagged panicles of four sorghum genotypes inoculated twice with *Sphacelia sorghi*

Genotype	Number of spikelets observed	Panicle bagging[a]	
		yes	no
CSH 1	5931	33·2 x	17·3 y
Ikinyaruka	4140	42·0 x	20·6 y
WS 1297	6039	42·2 x	19·9 y
ICSH 2265	6529	84·3 x	62·6 y

[a] Numbers in the same row followed by the same letter are not significantly different as determined by Student's *t*-test ($P < 0·01$).

(>70% infection). Except for one (IS 25480), all resistant accessions originated in Rwanda. All the resistant accessions were caudatum-based and had red and brown grain, a key determinant for farmer preference in Rwanda.

DISCUSSION

This paper describes the development of a screening technique for ergot resistance in sorghum, based on studies to establish the role of pollination, microclimate (bagging), and inoculation on ergot development. Because pollinated spikelets resist ergot infection, the essential requirements of the technique were at least one artificial inoculation of non-pollinated spikelets, and bagging of panicles. Studies on the

Table 3. Pedigree, origin, race, grain characteristics and percentage infected spikelets of *Sorghum bicolor* accessions resistant to ergot in field screening at Karama, Rwanda and ICRISAT Centre (IC), India

IS Number	Pedigree	Origin[b]	Race[c]	Grain characteristics		Ergot-infected spikelets (%)[a]		
				colour[d]	testa[e]	Karama 1988	Karama 1989	IC 1989
Resistant accesions								
25480	PB-16	Bur	C	RB	A	9·6	7·5	4·4
25527	Amamena	Rwa	C	B	P	8·8	5·3	7·4
25530	Tura	Rwa	C	RB	A	8·2	4·2	3·5
25531	PS-14	Rwa	C	RB	A	6·3	5·6	0·9
25533	PS-19	Rwa	C	RB	A	10·0	5·3	3·0
25537	PS-28	Rwa	C	RB	A	5·4	10·0	4·3
25551	Mugabo	Rwa	C	RB	A	7·6	7·6	–[f]
25554	Nyirarumogo	Rwa	C	RB	A	6·5	10·0	3·7
25555	Kigufi	Rwa	DC	RB	A	7·2	8·8	6·3
25570	PS-96	Rwa	C	RB	A	3·8	8·0	–
25576	Gikonda	Rwa	C	RB	A	8·0	6·5	1·4
25583	Mabernyingdma	Rwa	C	RB	A	9·5	4·7	1·8
Susceptible controls								
ET 2421		Eth	–	–		85·8	>50·0[g]	–
CSH 11		Ind	C	W	A		>50·0[g]	70·8
SE[h]						±2·9	±2·7	±2·0

[a] Based on counts of infected and healthy spikelets in each panicle. The values are means of up to 20 panicles in two replicates.
[b] Bur, Burundi; Eth, Ethiopia; Ind, India; Rwa, Rwanda.
[c] C, caudatum; DC, durra-caudatum. According to the classification of Harlan & de Wet (1972).
[d] B, brown; RB, reddish brown; W, white.
[e] A, testa absent; P, testa present.
[f] Data not available.
[g] Data on percentage infected spikelets of susceptible controls not available, but visual ergot rating (based on a 1–5 rating scale where 1 = no ergot and 5 = more than 50% spikelets infected by ergot) was 5·0.
[h] Standard error of mean ($P < 0·01$).

role of flowering on ergot development showed that spikelets remained susceptible until anthesis (pollination), after which the pathogen failed to infect the pistils (Fig. 1). Similar results were reported by Futrell & Webster (1965). Understanding the influence of flowering biology and pathogen biology, and their interactions on disease development is therefore critical in the creation of artificial epiphytotics for ergot resistance screening. The sorghum inflorescence is a raceme in which spikelets flower basipetally in descending waves for about 7–14 days. The flowering (anthesis or pollen shedding or pollination) process of individual spikelets usually begins in the morning, and requires about 30 min to complete (ICRISAT, 1988). A logical inference from the flowering process is that the number of pollinated spikelets that become resistant to ergot increase with each successive day. Thus, increased delay in inoculation after initiation of flowering in panicles would result in decreased ergot severity. Two panicles flowering on the same day but inoculated on different days will therefore have a variable number of pollinated spikelets and yield variable ergot severity. Panicle trimming to remove pollinated spikelets before inoculation offers a practical method to reduce variability in ergot severity ensuring that only non-pollinated, innately susceptible spikelets are inoculated (Table 1).

At least one artificial inoculation was essential to ensure infection by making available adequate inoculum to susceptible host tissues. Single inoculation also produced sufficient disease

pressure in the screening trials conducted by Chinnadurai *et al.* (1970), Sundaram (1970) and McLaren (1992). After inoculation, enclosing panicles in paper bags enhanced ergot severity by providing the high humidity favourable for ergot infection (Molefe, 1975). Bagging also ensured that each panicle was tested without interference from external sources of pollen that could interfere with disease development. Furthermore, it facilitated production of selfed seed, allowing direct selection of ergot-resistant plants. Sundaram (1970, 1971) bagged panicles after inoculation, while Chinnadurai *et al.* (1970), Khadke *et al.* (1978) and McLaren (1992) did not. The inoculation technique (one inoculation at flower gaping followed by bagging) used by Sundaram (1970) was similar to the most effective inoculation technique according to our results.

Ergot resistance in the 12 accessions reported here has been confirmed in repeated tests unlike other accounts of ergot resistance based on a single evaluation (Chinnadurai *et al.*, 1970; Sundaram 1970, 1971; Khadke *et al.*, 1978). McLaren (1992) used regression techniques to analyse data from multiple resistance-screening trials having variable disease pressure, to quantify resistance in several lines in South Africa under warmer climatic conditions (24·5–27·1°C) than those in our study. Mean temperature during the preflowering stage ranged from 18·1 to 19·8°C at Karama, and 18·8 to 20·2°C at ICRISAT Centre. The minimum night temperatures during this period were 10·8°C at Karama, and 9·2°C at ICRISAT. Expression of resistance in sorghum ergot has been shown to be temperature-dependent; the lower the temperature, the more severe the disease (McLaren & Wehner, 1990). Ovule abortion and pollen sterility can occur owing to abnormal megasporogenesis and microsporogenesis at 5–21°C (Brooking, 1979). McLaren & Wehner's (1992) and our results suggest that genotypic variation to ergot susceptibility is possibly mediated through low-temperature-induced pollen sterility. Despite the reported taxonomic differences between *C. sorghi* in India and *C. africana* in Africa (Frederickson *et al.*, 1991), the genotypes resistant in Rwanda were also resistant in India, with no evidence for differential pathogenicity in the two *Claviceps* species.

Among the high-altitude sorghums in the world collection, resistance was identified in accessions from Rwanda and neighbouring Burundi, indicating the importance of local adaptation for ergot resistance. Races and grain colour of all the resistant lines were similar to those of the predominant sorghums grown by farmers in Rwanda. Further evaluation of yield potential and grain quality characteristics of these accessions is needed to determine their suitability for direct introduction in farmers' fields in Rwanda. These lines could also be used as sources of resistance and to study the genetics and mechanism of resistance to ergot. It should, however be noted that the resistant lines described here are photoperiod-sensitive and may not be well adapted to other parts of the world.

A limited quantity of seed of the ergot-resistant lines reported in this paper is available from ICRISAT's Genetic Resources Unit in India.

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