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Ovary colonization by *Claviceps africana* is related to ergot resistance in male-sterile sorghum lines

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Ergot, caused by *Claviceps africana*, has emerged as a serious threat to sorghum hybrid seed production worldwide. In the absence of gene-for-gene-based qualitative resistance in commercial cultivars, varieties with high pollen production that can escape ergot infection are preferred. Recent demonstration of differences in ergot susceptibility among male-sterile lines has indicated the presence of partial resistance. Using chitin-specific fluorescin-isothiocyanate-conjugated wheat germ agglutin and callose-specific aniline blue, this study investigated the process of sorghum ovary colonization by *C. africana*. Conidia germinated within 24 h after inoculation (a.i.); the pathogen was established in the ovary by 79 h a.i., and at least half of the ovary was converted into sphacelial tissue by 120 h a.i. Changes in fungal cell wall chitin content and strategic callose deposition in the host tissue were associated with penetration and invasion of the ovary. The rate of ovary colonization differed in three male-sterile lines that also differed in ergot susceptibility. This work demonstrates a possible histological basis for partial resistance in male-sterile sorghum lines that could lay the foundation for variety improvement through further breeding and selection.

Keywords: histopathology, host–pathogen interaction, inoculum concentration

Introduction

In recent years, ergot of sorghum (*Sorghum bicolor*), caused by the fungus *Claviceps africana*, has emerged as a serious threat to sorghum production worldwide (Bandyopadhyay *et al*., 1998). *Claviceps* spp. are organ-specific pathogens, infecting flowers and replacing the host ovary with a reproductive structure, the sphacelium. A sweet, sticky fluid, honeydew, containing masses of macroconidia, is secreted from infected florets (Luttrell, 1981). Only unfertilized ovaries are infected, and there is a high correlation between nonpollinated spikelets and ergot infection. Consequently, male-sterile sorghum lines are highly susceptible, which has severely affected commercial hybrid seed production (Bandyopadhyay *et al*., 1996). The cost of production of hybrid seed has increased as a result of the need for regular fungicide applications and an increase in the male : female ratio in crossing blocks, and seed sanitation (Bandyopadhyay *et al*., 1998).

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In Australia, losses between 30 and 100% were experienced in 1996 in nurseries and parent seed-production blocks (Ryley & Henzel, 1999). In addition, the presence of alkaloids in sclerotia of *C. africana* is causing major concern in the commercial sorghum grain industry (Ryley *et al*., 2001), as small amounts in feed rations can adversely affect livestock (Blaney *et al*., 2001).

There is an international effort to develop resistance to ergot, and sorghum lines with partial resistance have been identified in the USA and South Africa (Frederickson *et al*., 1994; Musabyimana *et al*., 1995; McLaren, 2000). The underlying mechanism in these lines is a form of disease escape through efficient pollination, which is not effective under environmental conditions that affect pollen viability (Wang *et al*., 2000). Hence clearly defined resistance with a physiological, anatomical or biochemical basis that affects pathogenesis mechanisms is a more desirable option for ergot management. Recent greenhouse (Komolong, 2003) and field studies (Dahlberg *et al*., 2001; Reed *et al*., 2002) have shown that some male-sterile sorghum lines vary in their susceptibility to ergot. In the absence of pollen-mediated resistance, the differences in susceptibility between male-sterile lines may represent traits that confer a type of partial resistance.

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Histological changes associated with host–pathogen interactions can point to potential resistance mechanisms. The path of infection, parasitic differentiation and fungal mechanisms involved in host colonization have been studied in detail for some *Claviceps* spp. using light, transmission and scanning electron microscopy (Shaw & Mantle, 1980; Frederickson & Mantle, 1988; Tudzynski *et al*., 1995). However, there has been no comparative histological analysis of male-sterile sorghum lines with different levels of partial resistance.

In this work the infection and colonization of sorghum ovaries by *C. africana* were studied using bright-field and optical epifluorescence microscopy with appropriate staining to detect changes in host and fungal cell walls. In addition, the fluorescent staining technique was applied to determine whether the extent of ovary colonization of the three male-sterile sorghum lines was related to their ergot susceptibilities.

Materials and methods

Host lines and pathogen isolates

Three male-sterile lines, A296 (an Indian line used in the hybrid CSH13R); A_3IS8525 (a putative ergot-resistant line); and AKS4 (a Kafir type, one of the first sorghum hybrid parents), were obtained from Dr David Jordan of the Hermitage Research Station, Queensland Department of Primary Industries (QDPI). Of these, A296 is the most susceptible, while A_3IS8525 and AKS4 are equally resistant to ergot infection. In a bioassay using 16 *C. africana* isolates in a controlled-environment facility (CEF), A296 showed significantly higher disease severity, with >8% spikelets infected compared to <5% for either A_3IS8525 or AKS4 (Komolong, 2003). The susceptible A296 also produced significantly larger amounts of honeydew and more than four times as many conidia per spikelet than the more resistant lines. Plants were raised in the CEF at the CSIRO Plant Industry research laboratories in Brisbane. Growth conditions in the CEF were 28°C day/25°C night, 65% relative humidity day/95% night, a 12 h photoperiod and 500 μmol m^{-2} s^{-1} photosynthetic photon flux density.

Five monoconidial isolates of *C. africana*: CQ15, SE43, NQ71, NT32 and NSW33A, were selected from different geographic regions in Australia to represent the two dominant genetic lineages in Australia (Komolong *et al*., 2002). These represented members of four of the five clusters determined by a numerical analysis of morphological attributes, including: mycelium density; colony elevation, margin and pigmentation; area under colony growth curve; and macroconidial length and length : breadth ratio (Komolong, 2003). As isolates usually do not sporulate in culture, honeydew was produced by separately inoculating mycelium fragments of each isolate onto a male-sterile sorghum line, B700002V (QDPI), in a greenhouse. Honeydew containing conidia from mycelium inoculations was collected on sterilized cotton buds and stored in 5% glycerol at −70°C to serve as inoculum for all future inoculations.

Infection and colonization of ovaries

The process of infection and colonization of the ovary in the male-sterile sorghum line A296 was studied using the *C. africana* isolate NSW33A, originally isolated from grain sorghum (Komolong *et al*., 2002). At flowering, individual spikelets of A296 were marked and one of the two stigmas was inoculated with a 1 μL drop of a 10^6 conidia mL^{-1} suspension of NSW33A in an inoculum buffer (15% w/v sucrose, 1·5% w/v fructose and 0·5% w/v glucose, 0·1% Tween 20). Plants were incubated in near-saturated relative humidity in the CEF. Twenty replicate spikelets were sampled at 24, 48, 65, 79, 96, 103 and 120 h and 6 and 8 days after inoculation (a.i.), and the ovule was dissected out to avoid difficulties in sectioning the indurated lemma and palea of the spikelet.

Extent and rate of ovary colonization

The three sorghum male-sterile lines differing in susceptibility to *C. africana* infection (Komolong, 2003) were raised in the CEF. Flowering panicles were separately inoculated with each of the five *C. africana* isolates. Inoculum was prepared and applied to 20 marked spikelets for each isolate × line combination, as described above. Two spore concentrations, 10^6 and 10^5 conidia mL^{-1}, were used to examine the influence of inoculum concentration on the expression of partial resistance. At least 10 inoculated spikelets from each line–isolate combination for each inoculum concentration were collected for examination 103 and 120 h a.i.

Histological techniques

Stigmas and ovaries were fixed in FAA (containing per 100 mL: 90 mL 70% ethanol, 5 mL acetic acid and 5 mL formaldehyde) for at least 12 h or stored in FAA at 4°C until further use. Ovaries were dehydrated in an ethanol/tertiary-butyl-alcohol series and embedded in paraffin wax (56–58°C melting temperature). Embedded ovules were sectioned longitudinally at thicknesses of 5 μm (for the infection process) or 18 μm (for colonization rate) using a rotary microtome (Minot 1212, Leica, Wetzlar, Germany). Serial sections were transferred to microscope slides and sections from the area between the two stigmas were selected for all observations.

Sorghum stigmas were prepared for fluorescence microscopy using a modification of the aniline blue staining technique of Hood & Shew (1996). Stigmas were incubated in 1 m KOH for 2 h at room temperature, mounted in 0·05% aniline blue in PBS (containing per 1000 mL: 8 g NaCl, 0·2 g KCl, 1·44 g Na_2HPO_4 and 0·24 g KH_2PO_4, pH 7·3) and examined using epifluorescence microscopy (Olympus BX60, Australia) under UV light excitation (excitation filter 340–380 nm, barrier filter LP425 nm).

Infection and colonization of the ovary

Changes in plant and fungal cell walls during infection were monitored using the chitin-specific lectin wheat

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germ agglutin conjugated with fluorescein isothiocyanate (FITC-WGA) (O'Connell, 1991; Latunde-Dada *et al*., 1999), and the callose-specific dye aniline blue. Ovary sections were dewaxed for 10 min in xylene, rinsed briefly in 100% ethanol and air-dried. Sections were placed in aqueous 0·1% toluidine blue (Sigma, Balcatta, Australia) for 3 min, rinsed thoroughly (Feder & O'Brien, 1968; Shaw & Mantle, 1980), mounted in XAM mounting medium (Gurr, London, UK), and examined under bright-field illumination (Olympus BX60). Other sections were first stained with aqueous 4% safranin O and subsequently in 0·05% aniline blue mixed with 0·01% FITC-WGA in PBS. Sections were vacuum-infiltrated for 10 min and rinsed in three changes of PBS for 1 h (Latunde-Dada *et al*., 1999). All sections were mounted in PBS and examined under UV light excitation for aniline blue and blue light (excitation filter 450–490 nm, barrier filter LP515 nm) for FITC-WGA. Photomicrographs were recorded with a digital camera (Olympus DP50) and processed using the software programs VIEWFINDER LITE and STUDIO LITE (Olympus).

Ovary colonization rate in male-sterile lines

Sections were stained with safranin O, aniline blue and FITC-WGA, mounted, examined and photographed at 40× magnification as above. Two photos taken per section to cover the entire ovary were combined using PHOTOSHOP (Adobe Systems Incorporated, San Jose, USA), and colonized and total areas were estimated using SCIONIMAGE (Rasband, 2000). The colonization rate was determined from two measurements of the percentage of ovarian tissue colonized taken at 103 and 120 h a.i. In addition at least 12 disease-free control ovaries per line were examined from sections taken between the two stigmas; these ovaries were photographed and their cross-sectional areas were determined. Analysis of variance was used to determine the effects of line, isolate, inoculum concentration and their interactions using the GLM procedure in STATISTICA (StatSoft Corporation, Tulsa, USA).

Results

Macroscopically, the first evidence of successful infection of gynoecia by *C. africana* was wilting and discoloration of the stigmas. However, this can be ambiguous as discoloration may not always be caused by infection. A more reliable visual indicator of infection was the enlargement of the ovary, which began 65 h a.i. After this time the ovaries continued to enlarge and at 103 h a.i. the base turned white and hyphae were visible on the outside (Fig. 1). The diameters of infected ovaries were 1·5–2 times larger than those of ergot-free controls.

Ovary colonization

The first signs of a successful infection were visible 22 h a.i., when fine strands of hyphae were visible in the stigmatic hairs. Although many conidia germinated, penetrated and invaded the stigmatic hairs, the number of germ tubes that grew through the style to invade the ovary could not be determined as it was not possible to track individual hyphae growing through the host tissue.

Claviceps africana required at least 48 h from inoculation on the stigmas to reach the ovary, with signs of infection becoming apparent between 48 and 65 h a.i. By 65 h a.i., hyphae had invaded all parts of the ovary wall (Fig. 2a–e), and were clearly visible with 0·1% toluidine blue staining (Fig. 2b). Hyphae mostly grew intercellularly, and plant cells did not show any sign of invasion or disruption (Fig. 2e). Fluorescent microscopy with FITC-WGA staining did not detect clear signs of hyphae, as only small dots of green fluorescence (Fig. 2c) were visible, possibly because of the low chitin content of the cell walls of invading hyphae. Aniline blue staining did not detect any callose deposits at this stage.

An uneven distribution of fungal mycelium was visible in the ovarian tissue by 79 h a.i. Hyphae were found mostly around the chalazal zone on the abaxial side, fluorescing intensely as a result of lectin labelling. Staining with aniline

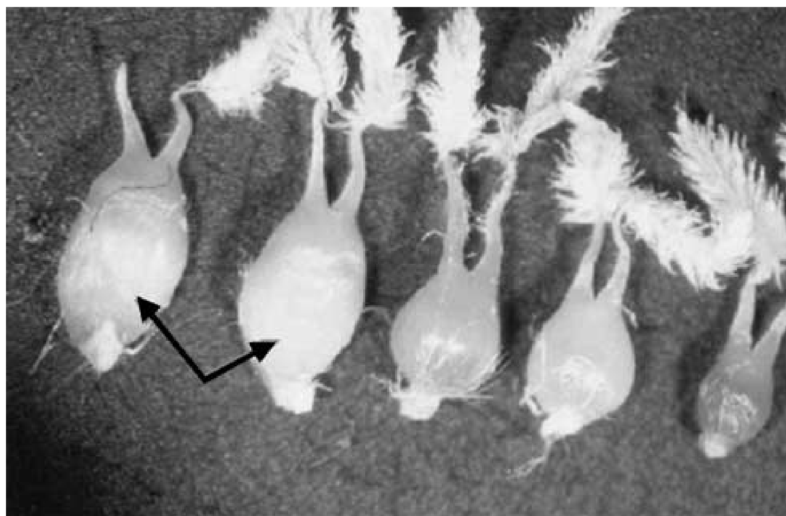

Figure 1 Sorghum ovaries showing macroscopic signs of infection following inoculation with *Claviceps africana*. Right to left: uninfected, and enlarged ovary at 65, 72, 103 and 120 h after inoculation, with the base turning white and hyphae becoming visible on the surface (arrows) in the latter stages.

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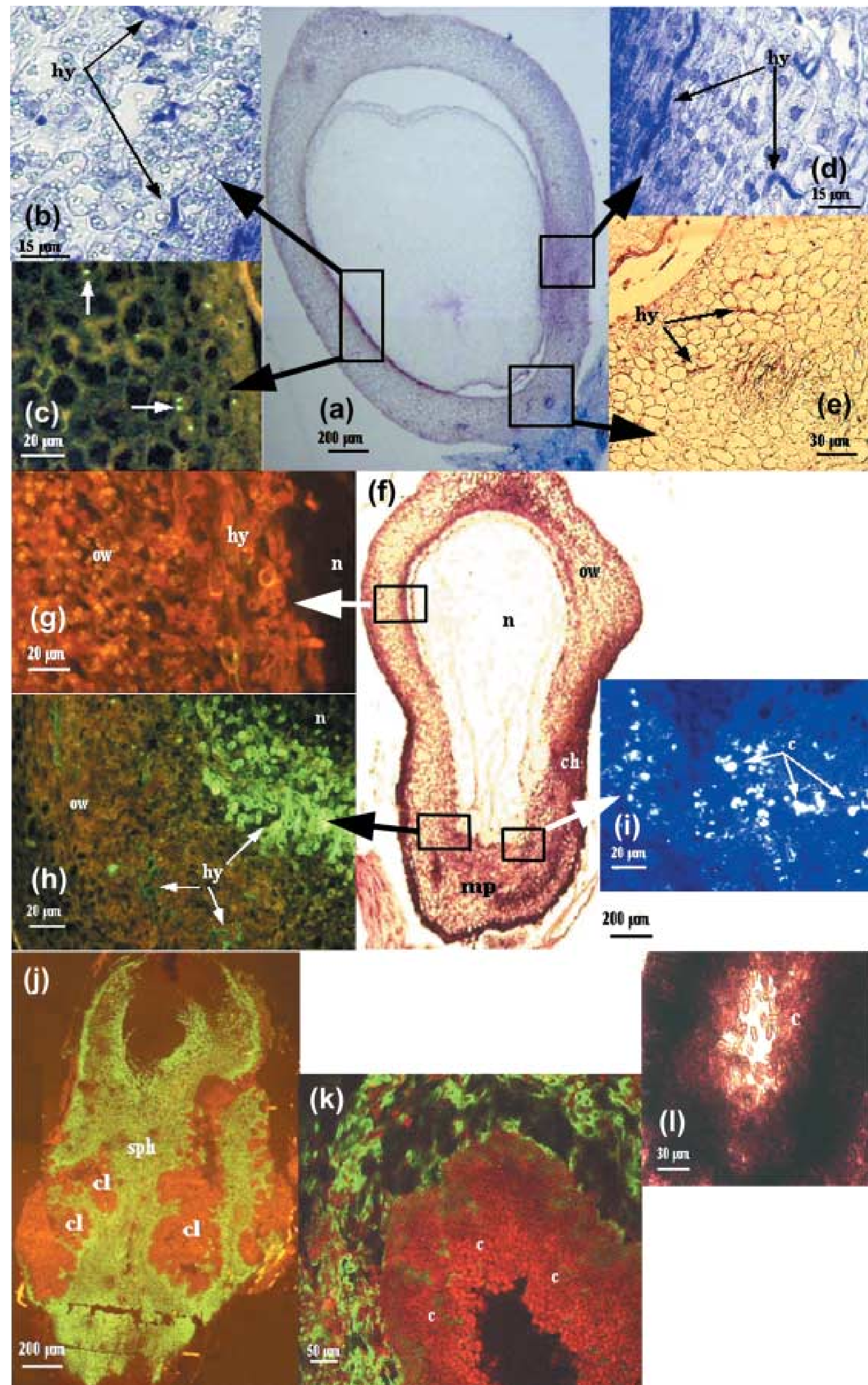

Figure 2 Longitudinal sections of sorghum ovaries infected with *Claviceps africana* at various stages after inoculation showing the extent of fungal (hy) penetration. (a–e) 65 h after inoculation: (a) overview of ovary stained with toluidine blue; (b) toluidine blue-stained intercellular hyphae in the ovary wall on the adaxial side; (c) FITC-WGA staining of the same region as in (b) with fluorescing dots indicating the presence of hyphae (arrow); (d) toluidine blue-stained hyphae in the outer ovary wall on the abaxial side; (e) toluidine blue-stained hyphae at the base of the ovary. (f–i) 96 h after inoculation: (f) ovary showing chalazal zone (ch), micropyle (mp), ovary wall (ow) and nucellus (n) with hyphae in the nucellus; (g) safranin O-stained upper ovary wall, integuments replaced by fungal hyphae showing a brownish fluorescence, only faint FITC-WGA labelling, cells in the ovary wall still intact but difficult to distinguish from hyphae; (h) FITC-WGA stained micropyle region with hyphae (hy) almost replacing the plant tissue, but intense fluorescence only near inner ovary wall; (i) aniline blue-stained micropyle region with extensive callose (c) deposits. (j–l) 6 days after inoculation: (j) FITC-WGA and safranin O-stained ovary almost completely replaced by the sphacelium (sph), with conidiogenous locules (cl) present throughout; (k) FITC-WGA- and safranin O-stained locule filled with conidia (c); (l) a single toluidine blue-stained locule showing conidia (c).

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blue indicated the presence of callose, especially in the inner ovary wall and integuments, possibly produced by the ovary as a response to *C. africana* infection. The integument and ovary remained largely intact during this period.

By 96 h a.i., hyphae were concentrated mainly on the abaxial side of the ovary around the chalazal zone, but had largely replaced the ovary tissue and had started advancing into the nucellus (Fig. 2f–i). Hyphae had also broken through the outer ovary wall and were visible on the outside. Vascular tracts at the base of the ovary were surrounded by hyphae. Further colonization proceeded in all directions, but hyphae appeared to advance first along the ovary integuments, separating the nucellus from the ovary wall. Hyphae had replaced integuments in the upper part of the ovary on the adaxial side (Fig. 2g). More hyphae showed bright fluorescence as a result of lectin labelling, indicating a gradual increase in chitin levels in fungal cell walls coinciding with infection and colonization of the ovary (Fig. 2h). Invading hyphae advancing along the integuments showed only very faint staining (Fig. 2g), while well established hyphae in the inner ovary wall at the micropyle were strongly stained with FITC-WGA (Fig. 2h). These changes in cell walls may mark a transition from invading hyphae to sphacelial hyphae. Callose deposition continued to increase and appeared to be correlated with the extent of fungal colonization (Fig. 2i).

As colonization of the ovary progressed from the chalazal zone, plant tissue was increasingly disrupted and replaced by fungal tissue. At 103 h a.i., sphacelial tissue had advanced along the inner ovary wall and the integuments, predominantly on the abaxial side, where it had reached the upper parts of the ovary wall. In the following hours, invasion of plant tissues progressed rapidly and by 120 h a.i. the lower half and parts of the upper half of the ovary wall on the abaxial side and half of the ovule had been replaced by sphacelial tissue. By 6 days a.i. almost the entire ovary had been converted and remnants of the ovary wall were only found in the apical region (Fig. 2j). By this time honeydew had started to exude from the sphacelium, and conidiogenous locules, containing large numbers of conidia, occupied much of the sphacelium (Fig. 2k,l). At this stage only a little callose was detected at the base of the sphacelium, and it disappeared completely by day 8.

Extent and rate of ovary colonization

The FITC-WGA staining technique developed in the first part of this study was used to investigate variation in the host–pathogen interaction using five *C. africana* isolates on three sorghum male-sterile lines. Lectin labelling of hyphae was not detected in a number of sections. If callose deposits were found under UV light excitation, the colonized area was recorded as the minimum value of 0·01 mm^2 measurable with the SCIONIMAGE software. Sections that did not show any callose staining were recorded as uninfected.

For all *C. africana* isolates the colonization of all host lines and lectin binding to the cell walls proceeded in a manner similar to that described above. This suggests a similar mechanism of invasion of these lines by the five isolates. Colonization rates of all isolates were significantly ($P < 0{\cdot}01$) influenced by inoculum concentration (Table 1).

Generally, inoculation with 10^6 conidia mL^{-1} resulted in higher colonization rates than with 10^5 conidia mL^{-1}. A significant difference ($P < 0{\cdot}01$) in mean colonization rate between lines was found only with the lower inoculum concentration; A296 was the most susceptible and AKS4 the least susceptible line. However, there were only minor differences in colonization rates among isolates on the same line at both conidial concentrations. All three lines showed the same level of susceptibility to the isolates at a concentration of 10^6 conidia mL^{-1}. NSW33A had the highest and NT32 the lowest mean colonization rates. There was no significant difference in ovary cross-sectional area among the three sorghum lines. This suggests that the colonization rates reflect the level of partial resistance in these lines.

Discussion

During penetration and invasion of the sorghum ovary by *C. africana*, physiological changes were detected in the fungal cell wall using chitin-specific FITC-WGA staining, and strategic callose deposition was demonstrated in the host tissue using aniline blue staining. This fluorescence microscopy study offers new insights into the host–pathogen interaction and confirms previous findings on the path of events during infection, established using light and electron microscopy (Shaw & Mantle, 1980; Frederickson & Mantle, 1988; Tudzynski *et al*., 1995). In addition, this is the first report to demonstrate a putative histological basis for the partial resistance to *C. africana* observed in three male-sterile sorghum lines under field (Dahlberg *et al*., 2001; Reed *et al*., 2002) and controlled (Komolong, 2003) environments. Being independent of pollen-mediated resistance, differences in ovary colonization rate may be useful in selecting and improving the level of ergot resistance in breeding lines.

Frederickson & Mantle (1988) determined that infection of the sorghum ovary is primarily initiated through the stigmas. Although early infection events – germination of conidia, penetration of the stigma and growth through the style – could not be documented well in this study using fluorescence or bright-field microscopy because of stigma anatomy, it was found that a number of conidia germinated and produced hyphae that grew towards the style. Frederickson & Mantle (1988) report that 'spent' hyphae in the transmission tracts rapidly become lysed, suggesting that only some hyphal strands reach the ovary. Although it could not be ascertained whether all the hyphae that grew through the stigmatic hair actually reached the style to invade the ovary, colonization rate increased significantly with increasing inoculum concentration, suggesting that hyphae from various infection points did grow

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Table 1 Mean rate of ovary colonization between 103 and 120 h after inoculation of three male-sterile sorghum lines with 10^5 and 10^6 conidia mL^{-1} of five *Claviceps africana* isolates

Line	Isolate NSW33A	SE43	CQ15	NQ71	NT32	Line mean
Mean colonization rate (% h^{-1}) with 10^5 conidia mL^{-1}						
A296	3·62	3·32	1·30	3·11	2·68	2·81 A[a]
A_3IS8525	2·00	1·74	2·20	0·53	1·43	1·58 B
AKS4	1·40	0·40	0·44	0·59	0·12	0·54 C
Isolate mean	2·34 A[b]	1·82 AB	1·31 B	1·41 B	1·41 B	
Mean colonization rate (% h^{-1}) with 10^6 conidia mL^{-1}						
A296	2·37	3·00	1·50	3·50	2·52	2·58 A
A_3IS8525	4·38	4·44	3·06	2·61	0·90	3·08 A
ASK4	2·89	1·89	4·40	2·53	2·66	2·85 A
Isolate mean	3·21 A	3·11 A	2·98 A	2·88 A	2·03 B	
Overall mean	2·78 A	2·46 AB	2·15 AB	2·14 AB	1·68 B	

ANOVA

Source	df	SS	$P > F$
Isolate	4	20·16	0·0126
Concentration	1	54·30	<0·0001
Line	2	24·79	0·0005
Isolate × concentration	4	4·92	0·5187
Isolate × line	8	49·19	0·0003
Concentration × line	2	42·62	<0·0001
Isolate × concentration × line	8	27·84	0·0248

[a]For each inoculum concentration means within a column followed by the same letter do not differ significantly ($P \leq 0·05$) according to Tukey's HSD test.

[b]Means within a row followed by the same letter do not differ significantly ($P \leq 0·05$) according to Tukey's HSD test.

and colonize the ovary. This is in contrast with the pollination process, where multiple pollen tubes are reduced at various points in the pollen tube pathway and only one tube enters the micropyle for fertilization (Heslop Harrison *et al*., 1985).

Intercellular invasion of the ovary and further colonization from the chalazal area are findings similar to those of previous studies of *Claviceps* spp. on several hosts (Luttrell, 1977; Shaw & Mantle, 1980; Frederickson & Mantle, 1988). The present study offers new insights into physiological aspects of the colonization process by *C. africana*. Callose deposits were detected at different stages in the infection process and in areas (mostly along the inner ovary wall and in the chalazal and micropyle regions of the ovary) congruent with accumulations of fungal hyphae. However, sorghum lines in which callose deposition occurs early in the infection process may offer enhanced ergot resistance, and future screening needs to use histological tools in addition to conventional severity ratings. The lack of callose in the later stages of colonization (more than 6 days a.i.) is probably a result of its digestion by fungal enzymes, as suggested by Tenberge (1999). Production of callose had previously been demonstrated in rye ovaries during infection by the related *C. purpurea* (Hambrock *et al*., 1992).

There is no information on the composition of the cell walls of conidia or infection structures of *C. africana*, but Schmauder & Gröger (1978) reported chitin in cell walls of *in vitro* cultures of *C. purpurea* and *C. fusiformis*. WGA-FITC staining of infected sorghum ovary sections did not detect any sign of chitin in the pathogen until 65 h a.i. At this time only small fluorescing dots were visible, which could represent parts of the hyphae that are labelled most strongly, such as the septa (Komolong, 2003). This observation was similar to findings from studies on the hemibiotrophic *Colletotrichum lindemuthianum* and biotrophic rust fungi, where infection structures contained only a little chitin when they first made contact with host cells (Chong *et al*., 1981; O'Connell & Ride, 1990; O'Connell, 1991). Lectin labelling started in *C. africana* hyphae around the chalazal zone and gradually spread to hyphae in other tissues. It has been suggested that *Claviceps* spp. essentially imitate the growth of pollen germ tubes during host invasion (Tenberge, 1999). As chitin can elicit lignification and other plant defence mechanisms (Barber *et al*., 1989), the absence or low levels of chitin in the cell walls of invading hyphae would aid the pathogen to avoid recognition and any host defence response until the pathogen has established itself in the invaded tissue. However, the presence of infection-induced (Hambrock *et al*., 1992) callose deposits in all lines shows that pathogen invasion does not proceed without a response from the host. Increased levels of chitin coincided with advanced stages in the colonization process, associated with the deterioration

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of plant tissue. This may represent the start of the sphacelial phase of the pathogen. Chitin is an important structural component in cell walls of many fungi (Wessels & Sietsma, 1981), and increases in the chitin levels may provide a better structural stability for the developing fungal stroma.

This is the first study to demonstrate a putative histological basis for the partial resistance observed in three male-sterile sorghum lines under field (Dahlberg *et al*., 2001) and controlled environments (Komolong, 2003). Although there was little difference in colonization rate between *C. africana* isolates, sorghum lines showed small but significant differences in their susceptibility to colonization. A296 was the most susceptible line, while colonization rates on both A_3IS8525 and AKS4 were consistently lower. This relative ranking of the three lines is consistent with the level of ergot susceptibility detected in a controlled environment against 16 *C. africana* isolates (Komolong, 2003). Ovary size of the three sorghum lines did not vary significantly, hence the differences in colonization rate probably reflect their levels of partial resistance. Further studies concentrating on the period between 65 and 79 h a.i. may reveal other differences at an early stage of pathogenesis that can be further exploited.

One of the major findings of this study was the significant effect of inoculum concentration on colonization rate. This concurs with the findings of Darlington *et al*. (1977), who established a linear relationship between inoculum density and the percentage of spikelets producing sclerotia following inoculation of several male-sterile lines of barley and wheat with *C. purpurea*. The present findings also demonstrated that the expression of partial resistance was dependant on inoculum concentration. Little attention has been paid to inoculum concentration in screening sorghum lines for ergot resistance. Commonly, screening for resistance has been done using either high (10^6 conidia mL^{-1}) inoculum concentrations in greenhouse assays (Frederickson *et al*., 1994; Tegegne *et al*., 1994; Reed *et al*., 2002) or variable concentrations in field infections (Dahlberg *et al*., 2001). Selection for partial resistance using high inoculum concentrations could lead to a 'cryptic error' in resistance testing, where lines that may have some resistance are rejected in high-disease environments (Zadoks & Schein, 1979).

In an epidemiological context, partial resistance comprises resistance to infection, colonization and reproduction (Parlevliet, 1979). The demonstration that variations in colonization rate match observed differences in partial resistance among selected male-sterile lines raises the possibility of improving resistance levels through breeding and selection. Small but consistent levels of resistance have been shown from repeated assays of these and other selected male-sterile lines under reproducible environmental conditions (Komolong, 2003). This is a significant development given the absence of strong genetic resistance in sorghum against *C. africana*. Whether the level of partial resistance can be increased through breeding and selection needs to be the focus of future research.

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