Alkaloid production by isolates of the sorghum ergot pathogen (*Claviceps africana*) from Australia and other countries

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Abstract. Isolates of *Claviceps africana* from Australia, Africa, Asia, and America were tested for the production of dihydroergosine (DHES), and its biogenic precursors dihydroelymoclavine (DHEL) and festuclavine (FEST), in culture. Several growth media were evaluated to optimise alkaloid production with little success. The best of these involved 2-stage culturing on high-sucrose substrate. Australian *C. africana* isolates varied widely and inconsistently in alkaloid production, with DHES concentrations in mycelium ranging from: <0.1 to 9 mg DHES/kg; <0.1 to 1.6 mg DHEL/kg; and <0.1 to 0.4 mg FEST/kg. In a separate experiment using similar culturing techniques, DHES was produced by 2 of 3 Australian isolates, 1 of 3 USA isolates, 1 of 4 Indian isolates, the sole Puerto Rican isolate, the sole Japanese isolate, but not the sole South African isolate. In this experiment, DHES concentrations detected in mycelium of Australian isolates (0.1–1.0 mg DHES/kg) were of similar magnitude to isolates from other countries (0.2–1.8 mg DHES/kg). Three *C. africana* isolates, including one that produced only traces of alkaloid in culture after 8 weeks, were inoculated onto panicles of sterile male sorghum plants. After 8 weeks, all 3 isolates produced 10–19 mg DHES/kg in the panicles, demonstrating that the growing plant favoured more consistent alkaloid production than culture medium.

Additional keywords: mycotoxin, fungus, sclerotium, sphacelium.

Introduction

Sorghum ergot disease has long been known in Asia and Africa, but only recently detected in Australia and in South and North America. After infection, the ovaries of sorghum plants are replaced by a sporulating fungal stroma, the sphacelium, from which conidia-containing honeydew serves as the source of secondary infection. Subsequently, further tissue differentiation occurs in the sphacelium to eventually produce a hard sclerotium, a dormant structure able to withstand degradation in the environment. The sorghum ergot pathogen (anamorph, *Sphacelia sorghi*) was first observed in India (McRae 1917), and some years later in Africa. Kulkarni *et al*. (1976) recognised the perfect stage in India as *Claviceps sorghi*. Subsequently, the perfect stage of sorghum ergot in Africa was recognised as a distinct species and called *Claviceps africana* by Frederickson *et al*. (1991). A third species, *Claviceps sorghicola* has recently been described in Japan (Tsukiboshi *et al*. 1999). According to Frederickson *et al*. (1991), a primary distinguishing feature of *C. africana* is production of the alkaloid dihydroergosine (DHES; Mantle and Wright 1968), since they found other species causing sorghum ergot to produce little or none of this alkaloid. Tsukiboshi *et al*. (1999) reported that *C. sorghicola* produced

low concentrations (2 mg/kg in sclerotia) of a different alkaloid similar to paliclavine. Bogo and Mantle (2000) reported that the major alkaloid produced by *C. sorghicola* is caffeine, and that *C. sorghi* also produces caffeine.

Since its explosive worldwide spread, molecular markers have been used to identify specific strains of *C. africana* and their movement around the globe. Different populations and lineages have been reported from different parts of the world and from within a country (Pazoutova *et al*. 2000). For instance, a number of polymorphisms exist in the United States, indicating that the recently introduced population contains multiple genotypes (Tooley *et al*. 2000). Australian isolates have been delineated into 2 different clusters, but both appear to have originated on the Indian subcontinent (Komolong *et al*. 2002) rather than Africa. Isolates from Australia have been identified as *C. africana*, based on morphology (Ryley and Henzell 1997), molecular analysis (Komolong *et al*. 2002), and production of DHES (Blaney *et al*. 2003).

The first report of disease in livestock caused by sorghum ergot was of impaired milk production and feed refusal in sows and cows, which occurred in Queensland in 1997 (Blaney *et al*. 2000). More recently in 2003,

cattle in several Queensland feedlots suffered severe losses from hyperthermia due to sorghum ergot (Blaney *et al.*, unpublished data). Feeding trials have demonstrated that sorghum infected with *C. africana* can produce many of the same toxic effects as rye ergot (*C. purpurea*) in pigs and cattle (Blaney *et al.* 2001). In contrast, reports from overseas (Frederickson *et al.* 1991) indicated very low toxicity to mice of *C. africana* sclerotia compared with those of *C. purpurea*. This raised questions of whether Australian isolates of *C. africana* are more toxigenic than those from other continents. The profile of alkaloids in collections of Australian sphacelia/sclerotia was predominantly DHES (Blaney *et al.* 2003), which is consistent with some African isolates (Frederickson *et al.* 1991). However, the concentrations found in Australian sclerotia varied widely, which could account for variable toxicity. The differences found in sclerotia might arise from inherent differences in the toxigenic potential of the isolates, or else from different environmental factors. We wished to establish standard culturing procedures to compare *in vitro* alkaloid production of isolates from different Australian regions with some isolates from other countries.

A process for inducing alkaloid production *in vitro* would also be useful for distinguishing strains of *Claviceps* species, although large numbers of isolates would need to be examined to see how consistently this matches other aspects of taxonomy. However, there are several challenges in the production of mycotoxins in culture. Firstly, there is the issue of achieving optimal mycelial growth; secondly, genetically identical isolates express secondary metabolism under different environmental conditions, often under conditions of specific nutrient limitation. *Claviceps* species are known to be particularly fickle in regard to expression of alkaloid production in culture: the lengthy investigations by pharmaceutical companies seeking to facilitate this process have been reviewed by Minghetti and Crespi-Perellino (1999). In many studies, despite luxurious growth in culture, only traces of alkaloid were detected. Selection of *C. purpurea* strains for industrial production was finally achieved by screening many thousands of mycelial fragments, and re-screening subfragments of the most productive fragments. Mantle and Tonolo (1968) compared the morphology of alkaloid-productive and non-productive strains of *C. purpurea* and found that an alkaloid-productive strain was distinguished by development of sclerotium-like tissue in culture.

Mantle (1973) took a similar approach of lengthy selection processes to identify a strain of sorghum ergot (described only as *Sphacelia sorghi*) from Nigeria that produced sclerotial-like tissue and high concentrations of DHES in culture, with lesser amounts of dihydroelymoclavine (DHEL) and festuclavine (FEST), the latter alkaloids being biogenic precursors of DHES (Barrow *et al.* 1974). However, he considered it possible that sphacelial hyphae could also synthesise alkaloids, and that the correlation between alkaloid production and sclerotial tissue was not as absolute as in *C. purpurea*. This offered the possibility that alkaloid production *in vitro* by sorghum ergot isolates could be an indication of subsequent production within sclerotia, both for chemo-taxonomic purposes and as a guide to potential toxicity to livestock.

Once a strain of *C. purpurea* producing high alkaloid concentrations was identified, a very high sucrose concentration (30%) in culture maximised alkaloid production (Amici *et al.* 1967), and a similar result was obtained by Mantle (1973) for *C. africana*. Phosphate has a key role in alkaloid production (Kren 1999) and the optimum level ranges from 1 to 4 mM in high-alkaloid-producing strains of *C. purpurea*. A drop in intracellular phosphate coincides with cessation of mycelial growth and the onset of alkaloid production (Arcamone *et al.* 1970; Pazoutova and Rehacek 1984), but high levels of phosphate induce alkaloid-degrading enzymes, lowering alkaloid yields (Robbers *et al.* 1978). Consequently, we explored the role of phosphate for optimising alkaloid production *in vitro*. We also considered that DHES production in inoculated sorghum ovaries might provide a guide to the effectiveness of our *in vitro* techniques.

The objectives of this investigation were as follows: to standardise conditions for fungal growth and alkaloid production *in vitro*; to compare *in vitro* alkaloid production by isolates of *C. africana* from different Australian regions, and to compare this with the production of alkaloids by isolates of *C. africana*, *C. sorghicola*, and *C. sorghi* from the USA, India, Japan, Puerto Rico, and South Africa; and to examine alkaloid production in inoculated sorghum ovaries by Australian strains having different *in vitro* alkaloid production ability.

Materials and methods

Fungal isolates

Australian isolates of *C. africana* were obtained from single conidia or single sphacelia and sclerotia obtained from different regions. Ten exotic isolates of *C. africana*, from the USA (3), India (4), Puerto Rico, Japan, and South Africa, were obtained as pure cultures and treated under normal quarantine restrictions. One isolate of *C. sorghicola* was obtained from Japan, and one of *C. sorghi* from India. Details are given by Komolong *et al.* (2002). Isolates were grown on potato dextrose agar (PDA), and maintained on agar discs in 15% glycerol in liquid nitrogen.

Alkaloid assays

Mycelial samples were either extracted directly, or after freeze-drying. If freeze-dried, 4 g of mycelium was reconstituted by blending for 1 min with 20 mL of water before extraction. Wet mycelium was blended with 10 mL of water. The extracts were made alkaline with aqueous ammonia (pH 8.5–9), and extracted twice with 40 mL of methylene chloride. The extracts were combined and filtered through a phase-separating paper with 5 g of anhydrous sodium sulfate. Culture filtrates (20 mL) were adjusted to pH 8.5–9, and alkaloids were partitioned with blending into a single extract of 40 mL of methylene chloride, using centrifugation (10 min at 3400 *G*) to resolve emulsions. Methylene chloride extracts were reduced to dryness under an air stream, dissolved

in 1 mL of methanol, and filtered into a sealed vial. These were assayed using a Shimadzu LC-10 liquid chromatograph equipped with UV (SPD-M10A diode array) and fluorescence (RF-10A XL) detectors, as described by Blaney *et al*. (2003). Separation was on a Novopac (Waters) C18 column, operated isocratically at 40°C, using acetonitrile : methanol : 0.1% ammonium acetate (31 : 20 : 50). Results are expressed on the basis of wet, drained mycelium weight. Infected sorghum spikelets were comminuted in a high-speed blender, subsampled, and assayed by similar methods. Average ($\pm$ s.e.) recoveries of alkaloids spiked into culture broths at concentrations of either 1 or 5 mg/L were $93 \pm 0.90\%$ for DHES, $79 \pm 0.84\%$ for DHEL, and $88 \pm 0.95\%$ for FEST.

Experimental procedures

Preliminary studies indicated that sorghum grain broth, V8 vegetable juice broth, and the medium of Bacon *et al*. (1979) were unsuitable for *Claviceps* spp., but satisfactory growth was obtained with the medium of Mantle (1973). Mantle's medium is composed of: sucrose 150 g; L-asparagine 15 g; KH_2PO_4 0.25 g; $MgSO_4.7H_2O$ 0.25 g; $FeSO_4.7H_2O$ 0.033 g; $ZnSO_4.7H_2O$ 0.027 g; distilled water 1 L; and adjusted to pH 5.5.

Alkaloid production on Mantle's medium and the effect of phosphate depletion

This experiment compared mycelium growth and production of DHES by 6 *C. africana* isolates from north-eastern, central-eastern, and south-eastern Queensland in the medium of Mantle (1973). For each culture, 100 mL of medium was placed in a 500-mL conical flask, covered with a cotton-wool plug, and autoclaved at 106°C for 20 min. It was then inoculated with 2 disks (5-mm diam.) cut from a fungal culture grown on PDA, and the mycelium was teased out using sterile needles to encourage the growth of floating rafts of the fungus. The cultures were then incubated for 3 weeks at 27°C in the dark. Mycelial mats were carefully removed by decantation with the aid of a spatula, freeze-dried, and weighed, before storing at −20°C. Broth was also stored at −20°C until assay.

The effect of phosphate depletion on alkaloid production was examined in 2 *C. africana* isolates from central-eastern (CQ15) and south-eastern Queensland (SE58A). The isolates were first grown in Mantle's medium as before for 3 weeks, and then the spent broth was decanted and replaced with a new batch of Mantle's medium, modified to contain various phosphate concentrations: 2 mM Mantle's standard concentration; 1 mM P; 0.5 mM P; 0.25 mM P; and zero P. It was incubated for a further 3 weeks. At the end of the second incubation, mycelium and broth were separated by decanting, weight of mycelium was recorded, and mycelium and broths from both incubations were stored at −20°C until assayed for alkaloids. Cultures were duplicated, results were analysed using analysis of variance, and means were separated using Duncan's multiple range test.

Alkaloid production by Australian and exotic isolates

This experiment compared alkaloid production by 7 *C. africana* isolates from Queensland after 2-stage fermentation on Mantle's medium as above, but without phosphate depletion. The first incubation was for 3 weeks and the second incubation for 5 weeks. After replacing spent broth with fresh medium, the flask was shaken vigorously by hand until the submerged culture broke into floating fragments. At the end of the second incubation the mycelial mat was removed by decantation, rinsed with distilled water, and stored at −20°C until assay. Broths from both incubations were also stored at −20°C.

Alkaloid production by 40 Australian and 12 exotic isolates of *C. africana*, plus reference isolates of *C. sorghi* and *C. sorghicola*, was studied in a separate experiment using the same procedure. All cultures were in triplicate, results were subjected to analysis of variance, and means were separated using Duncan's multiple range test.

Alkaloid production in infected sorghum ovaries

This experiment measured production of alkaloids in sorghum panicles, following artificial inoculation by each of 3 Australian isolates (NT32, CQ15, SEGRSO) that were relatively low, moderate, and high alkaloid producers, respectively, across the various *in-vitro* tests. The monoconidial culture of a single isolate was grown on PDA, blended into fragments, and inoculated onto flowering male-sterile sorghum panicles. When infection was established, honeydew was collected and stored in 15% glycerol at −80°C. A standardised inoculum concentration of 10^6 conidia/mL was then obtained by mixing the stored honeydew with the necessary volume of distilled water. The panicles on 18 male-sterile sorghum plants (6 plants per isolate; cv. Wheatland) were inoculated upon flowering in a controlled environment cabinet (27°C day, 25°C night, and a 12-h photoperiod). The inoculum was sprayed on using Preval pressure pack sprayers (Precision Valve Corp., Yonkers, New York) until run-off. The inoculated plants were incubated in a dew chamber for 48 h and then moved to a naturally illuminated glasshouse at ambient temperature. The honeydew was rinsed from the panicles to maintain clean spikelets free of contaminating fungi. Infected panicles were collected at 3 and 8 weeks after inoculation, to mirror the incubation times used in the *in-vitro* studies of the Australian isolates. Panicles from each plant were assayed separately to form 3 replicates for each isolate and sampling time. Statistical treatment was as in previous experiments.

Results

Growth and alkaloid production on Mantle's medium

The results in Table 1 give average DHES production data for 6 Australian isolates. Although mycelium growth of all isolates was comparable across replicates, their DHES production was generally low and variable. The isolate SEGRSO yielded relatively higher amounts of DHES, which was present in both mycelium and broth.

Two-stage fermentation with phosphate depletion

Compared with a single fermentation in Mantle's medium, mycelial production was increased by the 2-stage fermentation (Table 2). Isolate CQ15 produced moderate concentrations of DHES for all treatments regardless of the amount of phosphate in the replacement media. Although the

Table 1. Mycelium growth and dihydroergosine (DHES) production by isolates of *C. africana* from 3 regions of Queensland in the medium of Mantle (1973)

Results are the mean of duplicate cultures

Region and isolate	Wt mycelium (g/L medium)	DHES (mg/kg mycelium)	DHES (mg/L broth)
South-eastern			
SE4	8	0.06	<0.1
SE58A	15	0.3	<0.1
SE84B	7	0.1	<0.1
SEGRSO	15	6.4	0.7
Central-eastern			
CQ15	9	0.09	<0.1
North-eastern			
NQ58	8	0.05	<0.1

Table 2. Effects of phosphate-depletion in the second stage of a 2-stage fermentation on dihydroergosine (DHES) production by an isolate of *C. africana* (CQ15)

Results are the mean of duplicate cultures. Within columns, differences are not significant ($P < 0.05$)

Phosphate conc. in stage 2[A]	Wt mycelium (g/L medium)	DHES (mg/kg mycelium)	DHES (mg/L broth)	Total DHES (μg) in: Mycelium	Broth
2 mM P (control)	23	8.8	3.7	85	370
1 mM P	25	2.1	1.1	27	110
0.5 mM P	22	2.5	0.9	29	85
0.25 mM P	18	0.7	0.2	9	23
Zero P	34	4.0	1.3	47	130

[A]The medium was that of Mantle (1973) in the first stage (containing 2 mM phosphate), and the resultant mycelium was re-incubated in a second batch of Mantle's medium in which the phosphate content was varied.

effect of phosphate was not statistically significant, highest concentrations were produced without phosphate depletion. In contrast, a second isolate, SE58A, produced no detectable alkaloid in any of the treatments, although mycelial growth was satisfactory (data not given).

Alkaloid production by Australian isolates

Seven of the 40 isolates screened using the 2-stage fermentation in Mantle's medium produced moderate amounts of alkaloids. Five of these 7 were collected in central-eastern Queensland and 2 in south-eastern Queensland. An additional 8 isolates from central-eastern Queensland, 18 from south-eastern Queensland, 1 from north-eastern Queensland, 3 from the Northern Territory, and 2 from New South Wales did not produce detectable alkaloids (<0.1 mg/kg) in mycelium under these conditions. Although all positive isolates were from central-eastern and south-eastern Queensland, there were too few isolates from other locations for a valid comparison of toxigenic potential between regions.

Results are given only for the 7 positive isolates in Table 3. There were significant differences in alkaloid

Table 3. Production of dihydroergosine (DHES), dihydro-elymoclavine (DHEL), and festuclavine (FEST) by isolates of *C. africana* from central-eastern (CQ) and south-eastern (SE) Queensland after a 2-stage fermentation in the medium of Mantle (1973)

Results are the mean of 3 replicates. Within columns, means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test

	Mycelium (mg/kg)			Broth (mg/L)		
	DHES	DHEL	FEST	DHES	DHEL	FEST
CQ6C	4.9ab	1.5a	0.3	0.13b	0.10	0.01
CQ10	1.3b	0.5b	<0.1	0.10b	0.17	0.03
CQ20	7.3a	1.3a	0.4	0.20b	0.09	0.02
CQ21	0.4b	0.3b	0.2	0.12b	0.09	0.09
CQ22	0.5b	0.2b	0.4	0.63a	0.13	0.30
SE10763	9.4a	1.6a	0.5	0.27ab	0.16	0.21
SEGRSO	8.4a	1.3a	0.1	0.33ab	0.23	0.04

production among these isolates. The effect of broth/mycelium and isolate, as well as the isolate*broth/mycelium interaction was significant for DHES and DHEL but none was significant for FEST. The production of all 3 alkaloids was always higher in mycelia than in broth, and there was a clear correspondence between alkaloids in mycelium and broth. The general pattern of alkaloid production observed in sorghum ergot sclerotia from Queensland (Blaney *et al.* 2003) was also observed in these cultures: DHES predominated, with smaller amounts of DHEL and FEST.

Alkaloids were not detected in the spent broths from the first incubation in Mantle's medium (<0.01 mg/L), whereas they were present in broth after the second incubation as shown in Table 3. This indicated that alkaloid production had increased as a result of the dual fermentation of the 7 isolates that produced alkaloids in this experiment, even though the process failed to induce alkaloid production in the other isolates.

Alkaloid production by Australian v. exotic isolates

The results of this experiment are in Table 4. Of the 10 exotic *C. africana* isolates, 1 of 3 from the USA, 1 of 4 from India, and the sole isolates from Puerto Rico and Japan produced detectable amounts of alkaloids at levels comparable with 2 of 3 Australian isolates used for comparison. The analysis of variance tested the effect of country, and Australian *v.* non-Australian isolates, and both factors were not significant ($P < 0.05$).

Alkaloid production in infected sorghum ovaries

The results are in Table 5. After 8 weeks, the ovaries of inoculated spikelets were replaced with a fungal mass or sphacelia, but no fully formed sclerotia were evident. Levels of DHES produced by all 3 isolates after 8 weeks were more than 20-fold higher than in culture medium. DHES production by all 3 isolates was of a similar order, although SEGRSO produced the largest amounts in both experiments.

Table 4. Production of dihydroergosine (DHES), dihydroelymoclavine (DHEL), and festuclavine (FEST) by isolates of *C. africana*, *C. sorghicola*, and *C. sorghi* from several countries after a 2-stage fermentation on the medium of Mantle (1973)
Results are the mean of 3 replicate cultures

Country	Isolate	Mycelium (mg/kg)			Broth (mg/L)		
		DHES	DHEL	FEST	DHES	DHEL	FEST
		C. africana					
Australia	CQ15	<0.01	0.07	<0.01	<0.01	<0.01	<0.01
Australia	NT32	0.11	0.11	<0.01	0.06	0.08	0.38
Australia	SEGRSO	1.03	0.26	0.02	0.93	0.51	0.01
USA	Cla5	0.20	0.12	0.02	0.17	0.38	0.03
USA	Cla10	<0.01	<0.01	<0.01	<0.01	0.03	<0.01
USA	K6(KG)	<0.01	<0.01	<0.01	<0.01	0.03	<0.01
Puerto Rico	Cla14	0.19	0.04	0.01	0.08	0.35	0.02
South Africa	Cla58	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Japan	Cla83	1.75	0.17	0.06	0.87	0.22	0.01
India	KA61B	0.25	0.06	<0.01	0.33	0.24	0.02
India	NI6	<0.01	<0.01	0.01	<0.01	<0.01	0.01
India	SK7	<0.01	0.02	<0.01	<0.01	0.01	<0.01
India	NI3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		C. sorghicola					
Japan	Cjap31	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		C. sorghi					
India	MH74	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 5. Dihydroergosine (mg/kg) production after 3 and 8 weeks, in sorghum panicles inoculated with isolates of *C. africana* from central-eastern Queensland (CQ), south-eastern Queensland (SE), and the Northern Territory (NT)
Results are the mean (range) of 3 replicates

Isolate	3 Weeks	8 Weeks
CQ15	<0.01 (<0.01–0.01)	12.7 (6–16)
NT32	0.22 (0.2–0.5)	10.2 (7–15)
SEGRSO	0.04 (0.02–0.07)	19.3 (16–21)

Discussion

This work has for the first time compared the *in vitro* alkaloid production of Australian isolates of *C. africana* with those from other continents. Some isolates from the USA, India, Puerto Rico, and Japan produced DHES in similar (albeit low) concentrations to those produced by Australian isolates. A single South African isolate did not produce alkaloids, but little significance can be attached to this, since DHES production by *C. africana* was first identified in that continent. DHES, DHEL, and FEST were not detected in single isolate, confirmed as *C. sorghi*, collected in 2000 in India, nor in a single isolate of *C. sorghicola* from Japan. However, larger collections of sclerotia from these 3 species should be examined to confirm if production of these alkaloids is unique to *C. africana*.

In common with previous workers, we were unable to induce field isolates to produce high concentrations of alkaloid in culture. Despite stringently following a rigorous schedule that produced a consistent amount of mycelial growth, alkaloid production was highly variable between experiments and between replicates within experiments. The cause of this is apparently variation in alkaloid production potential within the mycelium itself (Mantle 1973), but we rejected the approach of extensive selection of mycelial fragments in order to find high-alkaloid-producing strains, as this could not be evenly applied to all isolates. We were able to increase alkaloid production within individual cultures by a dual fermentation, but this did not overcome the primary limitation of mycelial variation. In comparison, some endophytic species of *Balansia*, a genus in the same Family (F. Clavicipitales) as *Claviceps*, are not so fickle in ergot alkaloid production (Bacon *et al*. 1979).

Low *in vitro* alkaloid production has been partly attributed to a failure to produce sphacelial/sclerotial tissues that support alkaloid production in culture (Mantle 1973). However, alkaloids are present in honeydew (or spores contained in honeydew) at concentrations up to 10 mg DHES/kg (Blaney *et al*. 2003) from the early stages of infection and long before sclerotial tissue develops. Ergot bodies composed mainly of sphacelial tissues tend to contain 10–200 mg DHES/kg, but concentrations in fully developed sclerotia can rise to 10 000 mg DHES/kg.

Alkaloid production per unit infected tissue increased many-fold in 3 Australian isolates artificially inoculated onto sorghum panicles, compared with *in vitro*, and results were less variable between replicates. However, quarantine restrictions did not allow a comparison with exotic isolates under similar conditions. The factor that promotes

development of alkaloid-producing tissues *in vivo* but not *in vitro* by *Claviceps* species has not been identified, but given the long co-evolutionary relationship involved in the lifecycle of plant and fungus, the fungus might be responding to factors in the living plant that determine grain filling and maturation, rather than to simple nutritional changes.

Conclusions

These results do not provide evidence to suggest that Australian isolates of *C. africana* are more toxigenic than the isolates from other continents. Unless methods are found to induce isolates to produce sclerotial tissue in culture, any future investigations should concentrate on comparisons of alkaloid production in sorghum panicles under controlled conditions. Such studies might be extended to investigate the influence of climatic factors on the initiation and development of sclerotia since, apart from infection, this appears the most critical factor associated with livestock toxicity.

Acknowledgments

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