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Sorghum ergot can develop without local *Claviceps africana* inoculum from nearby infected plants

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Batches of glasshouse-grown flowering sorghum plants were placed in circular plots for 24 h at two field sites in southeast Queensland, Australia on 38 occasions in 2003 and 2004, to trap aerial inoculum of *Claviceps africana*. Plants were located 20–200 m from the centre of the plots. Batches of sorghum plants with secondary conidia of *C. africana* on inoculated spikelets were placed at the centre of each plot on some dates as a local point source of inoculum. Plants exposed to field inoculum were returned to a glasshouse, incubated at near-100% relative humidity for 48 h and then at ambient relative humidity for another week before counting infected spikelets to estimate pathogen dispersal. Three times as many spikelets became infected when inoculum was present within 200 m of trap plants, but infected spikelets did not decline with increasing distance from local source within the 200 m. Spikelets also became infected on all 10 dates when plants were exposed without a local source of infected plants, indicating that infection can occur from conidia surviving in the atmosphere. In 2005, when trap plants were placed at 14 locations along a 280 km route, infected spikelets diminished with increasing distance from sorghum paddocks and infection was sporadic for distances over 1 km. Multiple regression analysis showed significant influence of moisture related weather variables on inoculum dispersal. Results suggest that sanitation measures can help reduce ergot severity at the local level, but sustainable management will require better understanding of long-distance dispersal of *C. africana* inoculum.

Keywords: aerial spore dispersal, long-distance spore dispersal, secondary conidia, *Sorghum bicolor*

Introduction

Of the three pathogens that cause ergot of grain sorghum (*Sorghum bicolor*), *Claviceps africana*, *C. sorghi* and *C. sorghicola*, only *C. africana* has been recorded in Australia (Komolong *et al*., 2002) since the disease was first discovered in Queensland in 1996 (Ryley *et al*., 1996). Sorghum ergot has been a major impediment to commercial seed production in Australia, causing between 30 and 100% loss in nurseries and parent seed production blocks. The seed industry is now reliant on regular use of the fungicide triadimenol together with other practices to effectively manage sorghum ergot. The economic impact of sorghum ergot stems from reductions in grain yield, increased cost of ergot management and potential risk of animal toxicity from ergot alkaloids. Honeydew-covered panicles of grain sorghum can clog up harvesters and other machinery and expensive changes to planting and harvesting procedures are essential (Ryley & Henzell, 1999). Isolates of *C. africana* from Australia and elsewhere can all produce alkaloids (mainly dihydroergosine), although the level of production varies considerably *in planta* and *in vitro* (Blaney *et al*., 2006), and potential animal toxicity from alkaloids in ergot-infected grains has raised concerns for the livestock industry (Blaney *et al*., 2000). Consequently, strict limits on the level of ergot contamination allowed in grains have been imposed and the previous limit of 0·3% w/w sclerotia/sphacelia in grains for stockfeed has been reduced to 0·1% w/w for sorghum for feedlot cattle (http://www2.dpi.qld.gov.au/health/3568.html, accessed 5 December, 2007).

Since its discovery in India in 1915 (see Bandyopadhyay *et al*., 1998) sorghum ergot had been restricted to parts of Asia and Africa for nearly 80 years, before it was detected in Brazil in early 1995 (Reis *et al*., 1996). The teleomorph of the Indian pathogen, originally described by its anamorph *Sphacelia sorghi*, was identified as *Claviceps sorghi* in 1976 (Kulkarni *et al*., 1976) and it was assumed that this was the fungus causing sorghum ergot in Asia and Africa. However, the related *C. africana* has emerged as the dominant pathogen of sorghum ergot internationally, following a formal description of this taxon from Zimbabwe in 1991 (Frederickson *et al*., 1991). The ability of *C. africana* to produce secondary conidia that can become wind-borne

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is believed to have led to its rapid spread over vast geographical areas including throughout India, where this species has all but replaced the indigenous *C. sorghi* (Bandyopadhyay *et al.*, 2002). Rapid and widespread dissemination has meant that the same or closely-related *C. africana* genotypes have spread to geographically isolated regions such as Africa and the Americas (Tooley *et al.*, 2000; Pažoutová & Frederickson, 2005) and between Asia and Australia (Komolong *et al.*, 2002) within a short period of time.

Many hypotheses have been advanced to explain the rapid dissemination of ergot across large geographical distances, including as a seed contaminant, sub-clinical levels of endemic pathogen that may have remained undetected until an explosive epidemic, mechanical and/or anthropogenic spread via contaminated apparel or equipment, and the transport of secondary conidia in intercontinental air masses (Bandyopadhyay *et al.*, 1996), but the actual mode of dissemination and the frequency of such events occurring remain unclear. At the paddock level insect, wind and water splash have all been implicated in localized dispersal of the pathogen within and between fields (Frederickson *et al.*, 1993). When local sources of inoculum are available, weather and pollen-related factors influence ergot spread and severity. For instance, low temperatures during pre- and post-anthesis, and high relative humidity and rainfall at flowering can lead to severe ergot outbreaks (McLaren & Wehner, 1992; Frederickson *et al.*, 1993; McLaren & Flett, 1998; Ryley *et al.*, 2002).

Predicting the outbreak of ergot in previously un-infested areas has remained elusive. The source and timing of arrival of *C. africana* inoculum to start the initial focus of infection has obvious implications for on-farm management to restrict ergot severity and/or spread. The effectiveness of management options such as burial of infected sorghum residues, removal of alternative crop and weed hosts and planting of non-host barrier crops, depends on the relative importance of local and long-distance inoculum sources. What is the minimum distance from an existing *C. africana* inoculum source that would allow ergot-free sorghum production is a pertinent question for growers and seed producers. The aim of this field-based research over three successive sorghum growing seasons was to study the importance of local and long-distance inoculum in initiating sorghum ergot infection. In this work ergot infected sorghum or other grass hosts within a distance of 200 m was considered a local source. The influence of local inoculum was considered in two different ways; first, by positioning trap plants at various distances from ergot-infected sorghum plants serving as a local source, and secondly, by exposing trap plants in the absence of a local source. An additional objective was to determine how local weather conditions influenced ergot severity in the presence or absence of local sources of inoculum.

Materials and methods

Disease-free flowering sorghum plants were exposed to *C. africana* inoculum for 24 h at field sites with or without a local inoculum source of artificially inoculated sorghum plants. Exposed plants were assessed for ergot severity following incubation in a near-saturated environment.

Raising plants and inoculation

A male sterile *S. bicolor* line A23171 obtained from Dr David Jordan of the Hermitage Research Station, Queensland Department of Primary Industries & Fisheries, was used in all experiments. Male sterile sorghum lines are highly susceptible to ergot infection. Batches of sorghum plants to be used to trap inoculum were raised during February–July of 2003, 2004 and 2005 in plastic pots (7·5 cm diameter) containing pasteurized commercial potting mixture in a naturally illuminated glasshouse with 12 ± 2 h photoperiod, 28 ± 3°C average day, and 23 ± 3°C average night temperatures. Washed fine sand and peat (1:1) was applied to the soil surface to conserve moisture. Three seeds were sown per pot and thinned to one plant per pot after 2 weeks. Plants were fertilized weekly with half strength nutrient solution containing 27% nitrogen, 5·5% phosphorus, 9% potassium and micronutrients ('Thrive', Arthur Yates and Co.). No ergot-infected plant was allowed in this glasshouse.

Batches of plants to serve as local inoculum sources were raised in a separate glasshouse with similar growing conditions. These plants were artificially inoculated. The sowing and inoculation dates were adjusted so that sporulation from secondary conidiation of inoculated spikelets coincided with 50% anthesis in a given batch of trap plants. Local inoculum sources were laid out and removed from the field together with the trap plants.

A monoconidial isolate of *C. africana*, SE86S obtained from South East Queensland (Komolong *et al.*, 2002), was used to inoculate sorghum flowers of plants that served as local inoculum source. As isolates of *C. africana* usually do not sporulate easily in culture, honeydew was produced by firstly inoculating flowering panicles of A23171 grown in a glasshouse with fragments of mycelia. Mycelia were grown in darkness at 27°C in a broth containing sucrose 150 g; l-asparagine 15 g; KH_2PO_4 0·25 g; $MgSO_4 \cdot 7H_2O$ 0·25 g; $FeSO_4 \cdot 7H_2O$ 0·033 g; $ZnSO_4 \cdot 7H_2O$ 0·027 g; distilled water 1 L; and pH adjusted to 5·5 (Mantle, 1973). The mycelial mat was homogenized and filtered through cheesecloth before spraying onto flowering sorghum panicles. Honeydew containing conidia appearing 7–9 days after mycelium inoculation were collected on sterile cotton-buds and stored in 5% glycerol at –80°C for all future inoculations. Inoculum was prepared by suspending conidia from sterile cotton-buds in sterile distilled water, concentration was adjusted to 10^5 conidia mL^{-1} using a haemocytometer and flowering panicles were sprayed using a pressurized atomiser (Preval Sprayers). Plants were incubated in near-saturated relative humidity for 48 h and then returned to the glasshouse for a further period until a white cottony layer of secondary conidia was produced on the honeydew.

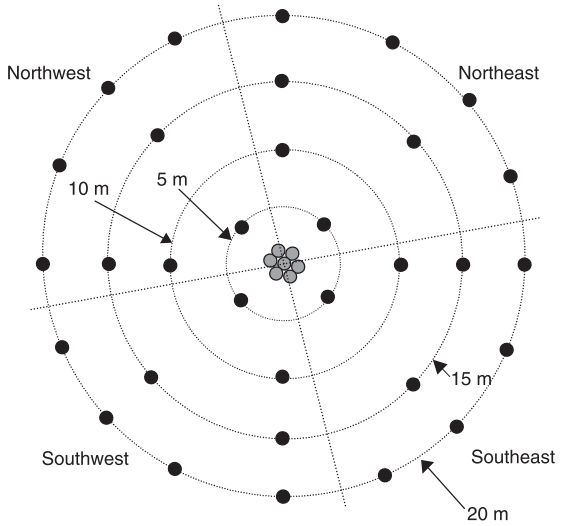

Figure 1 Layout of circular plots used to study the dispersal of *Claviceps africana* inoculum at Kingsthorpe and Gatton in 2003 with and without a local point source of inoculum at the centre.

Field experiments

The Gatton Research Station (27°05′S, 152°33′E) and Kingsthorpe site (27°51′S, 151°78′E) operated by the Queensland Department of Primary Industries & Fisheries were used in 2003 and 2004. Both sites are in a mixed cereal and vegetable producing region. Each year an area covering a 5 km radius surrounding each site was surveyed to estimate the proximity of nearest infected sorghum crop and/or large stands of Johnson grass (*S. halepense*). Each site (*ca*. 1 ha) was cultivated and regularly sprayed with broad-spectrum herbicide so that no weed or volunteer plant was present during the experiment.

In 2003 two replicate circular plots, 40 m in diameter, were laid out at each site, with at least 60 m separating the two circles. Four concentric circles 5, 10, 15 and 20 m from the centre were marked and 4, 4, 8 and 16 flowering trap plants, respectively, were positioned at the four distances in northeast, southeast, southwest and northwest directions (Fig. 1). At the same time a group of five ergot-infected sorghum plants with the panicle covered in honeydew with secondary conidia was placed in the centre of each circular plot to serve as a point source of local inoculum. At Gatton a fresh batch of trap plants from the glasshouse was exposed to a fresh batch of local inoculum source on 12, 14, 19, 21 and 26 May, and 2, 4 and 9 June; and without a local source on 20 and 27 May and 3, 10 and 13 June. Dates of exposure at Kingsthorpe were 21, 23, 28 and 30 April and 5 May with local source; and 22 and 29 April without a local source. At each exposure, trap plants were left in the field for 24 h, returned to a separate glasshouse for incubation in near-saturated relative humidity (using trays lined with a thin layer of water inside a tent) for 48 h and maintained for another week before disease and other assessments were made. Each panicle was covered with a plastic bag and sealed with adhesive tape during transport between glasshouse and field sites to avoid contamination. Plants that served as the local inoculum source were removed and discarded at the end of each 24 h exposure.

In 2004 two replicate plots were used at Kingsthorpe to expose trap plants at 20, 30 and 50 m from a local source but only one plot could be accommodated at Gatton to expose plants at 30, 50, 100 and 200 m from a local source. At Gatton trap plants were only exposed in the southeast and southwest directions due to the large plot size. At Kingsthorpe trap plants were exposed on 5, 7, 12, 14, 19 and 21 April with local inoculum source; and on 6, 13 and 20 April without a local inoculum source. Because of a large sorghum-ergot infected paddock nearby, plants at Gatton could only be exposed in the presence of local inoculum on 26 and 28 April and 3, 4, 5, 10, 11, 12 and 17 May.

In 2005 groups of six replicate trap plants were exposed for 48 h on 24 March and 7 and 21 April at 12 locations along a 280 km route in Southeast Queensland (Table 1) and at two sites within Brisbane City limits, to assess infection from commercial sorghum crops located at different distances from the trap plants. The sites were from several hundred metres to over 80 km from large commercial sorghum paddocks. As before, flowering panicles were sealed in individual plastic bags during transportation between field and glasshouse and trap plants were similarly incubated before severity assessment.

Data collection and analysis

The number of infected and total spikelets in panicles of all trap plants was counted within 7–9 days after exposure to avoid infection from secondary conidia between and within infected panicles. As the latent period of *C. africana* is less than a week (Komolong, 2003), this ensured that only infections from field exposure were counted in all assessments. With the assumption that a diseased spikelet resulted from a single infection, the number of infected spikelets can be used as an estimate of inoculum abundance in the field during exposure. Data on infected spikelets were $ln(x + 1)$ transformed to stabilise variance and the effect of site, distance from local inoculum source, presence of local inoculum and the date of exposure was determined from analysis of variance using general linear models in SAS.

In 2003 and 2004 hourly measurements of air temperature, relative humidity, wind speed, rainfall and leaf wetness were recorded using Tinytag™ dataloggers (Gemini Data Loggers Ltd.) located within 30 m of the field plots. In addition to maximum, minimum and mean values, hours of relative humidity over 90% and hours of temperature less than 12°C were computed. Data on infected spikelets were summarized, plotted and Pearson's correlation coefficients determined for each weather variable both during and 24 h prior to exposure. The influence of weather on the number of infected spikelets was determined by multiple linear regression analysis using SAS. Data for each site and year were analysed separately and a stepwise procedure was used with the condition for entry and retention of a variable set at $P \leq 0{\cdot}05$.

Table 1 Location of the 14 field sites on a 280 km route in southeast Queensland including two sites in Brisbane city, where flowering sorghum trap plants were exposed for 48 h on 24 March and 7 and 21 April in 2005, and the mean number of spikelets infected by infected following exposure

Site	Latitude and longitude	Nearest sorghum crop	Infected spikelets
Ma Ma Creek	27°72′S 152°12′E	3–4 km	0·0 (0·0)[a]
West Haldon	27°82′S 152°10′E	3 km	0·05 (± 0·05)
Neds Gully	27°99′S 151°99′E	> 300 m	0·06 (± 0·06)
Thanes Creek	28°16′S 151°70′E	> 10 km	0·0 (0·0)
Rocky Creek Road	27°99′S 151°34′E	200 m	0·0 (0·0)
Back Creek	27°86′S 151°30′E	250 m	0·05 (± 0·05)
Brookstead	27°76′S 151°44′E	1 km	0·19 (± 0·14)
Southbrook	27°66′S 151°72′E	300 m	1·06 (± 0·26)
Wyreema Road	27°61′S 151°89′E	500 m	2·33 (± 0·64)
Lockyer Valley	27°55′S 152°01′E	Not determined	0·05 (± 0·05)
Gatton bypass	27°55′S 152°18′E	> 300 m	0·83 (± 0·32)
Gatton station	27°53′S 152°34′E	1 km	0·11 (± 0·11)
Queensland Bioscience Precinct (rooftop)	27°29′S 153°00′E	> 80 km	0·0 (0·0)
Long Pocket Laboratory (outside lawn)	27°30′S 152°59′E	> 80 km	0·0 (0·0)

[a]Standard error of mean in parenthesis.

Table 2 Significant terms in separate analysis of variance with degrees of freedom (DF) and the probability of a greater F ratio ($P > F$) for infected spikelets at each site and year when flowering sorghum plants were exposed to a local point source of *Claviceps africana* inoculum

Site	Year	Source	DF	Type III sums of squares	$P > F$
Gatton	2003	Date of exposure	7	454·28	< 0·001
		Date*distance*direction[a]	63	106·66	0·008
	2004	Date of exposure	7	198·01	< 0·001
		Date*direction	7	17·02	< 0·001
Kingsthorpe	2003	Date of exposure	4	538·18	< 0·001
	2004	Date of exposure	5	349·61	< 0·001
		Date*distance*direction	30	13·58	0·041

[a]Plants were exposed in the northeast, southeast, southwest and northwest directions at various distances from local point source; in 2003: 5, 10, 15 and 20 m at both sites; in 2004: 20, 30 and 50 m in all four directions at Kingsthorpe and 30, 50, 100 and 200 m only in the southeast and southwest directions at Gatton.

Results

Dispersal of *C. africana*

Aerial inoculum of *C. africana* was present at Gatton and Kingsthorpe on all 38 occasions in 2003 and 2004. On each occasion, flowering sorghum plants trapped *C. africana* inoculum and these plants developed ergot after incubation at high relative humidity following exposure in the field, with or without infected sorghum plants serving as a local source of inoculum. The number of consecutive days without ergot-infected plants as the local inoculum source ranged from 2 to 7 days. On these occasions infection on trap plants were caused by conidia from local sources surviving in the atmosphere for up to 7 days along with conidia arriving from external sources. The average number of infected spikelets ranged from 0·25 to 198 per panicle in 2003, and from 0·65 to 45 per panicle in 2004. The maximum number of infected spikelets in a panicle was 464 at Kingsthorpe in 2003. In 2005 only a small number of spikelets became infected when flowering plants were exposed at 12 different field sites in southeast Queensland and two sites in Brisbane.

In 2003 a summary analysis of variance of infected spikelets (log transformed) from the two sites showed a significant effect of site and local inoculum but the distance or direction from the local source were not significant (output of analysis not shown). With an average 71·6 ± 4·7 standard error, Kingsthorpe had significantly ($P < 0·05$) more infected spikelets per panicle than Gatton (11·8 ± 1·2). Overall, 36·7 ± 2·4 spikelets became infected in the presence of ergot-infected plants as a source of local inoculum and this was significantly higher than 14·7 ± 2·8 infected spikelets in the absence of a local source. To explore the effect of date of exposure and distance and direction from local source further, data for each site were analysed separately for dates when a local inoculum source was present. This showed a significant ($P < 0·05$) effect for date of exposure at each site but the distance and direction from the source were not significant (Table 2). The significant three-way date*distance*direction interaction at Gatton is most likely due to shifts in wind direction.

Box and whisker plots were generated using SAS to display mean, median, quartiles, and minimum and maximum number of infected spikelets for each distance to summarize data for each site better. No concentration

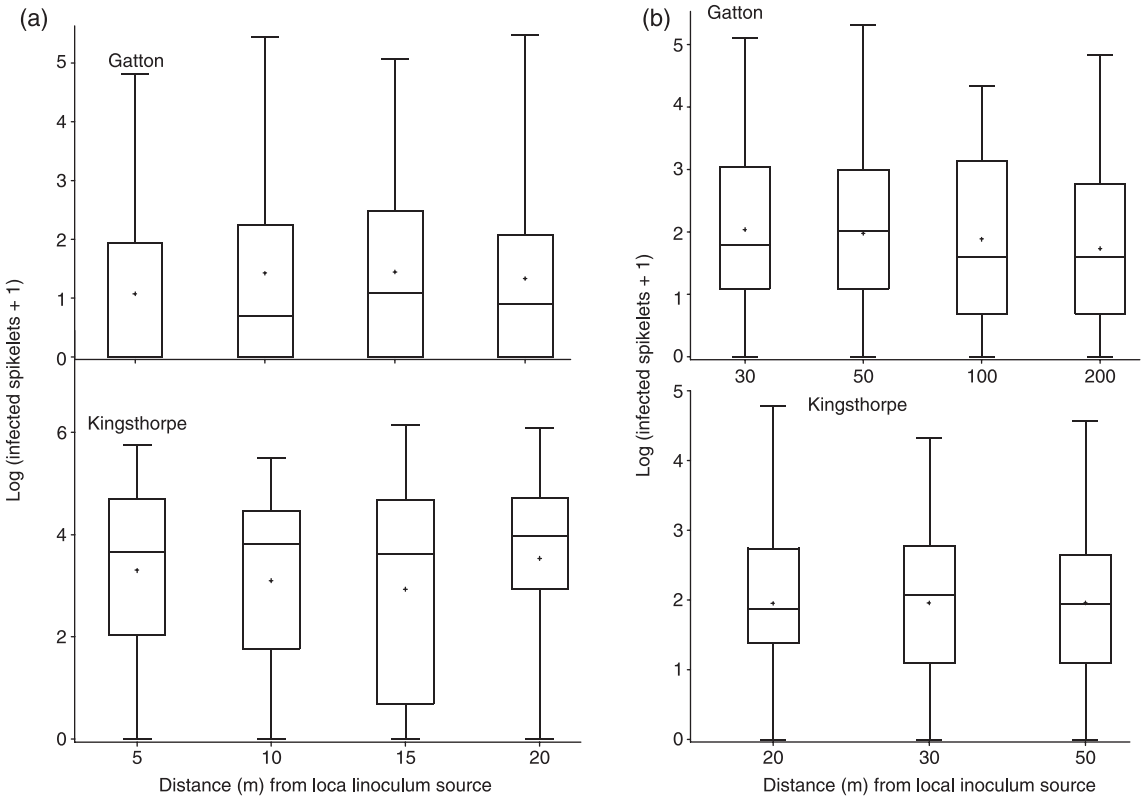

Figure 2 Box and whisker plots of *Claviceps africana* inoculum dispersal to various distances from a local source at Gatton and Kingsthorpe in 2003 (a) and 2004 (b). The '+' symbol represents overall mean, horizontal line within box is the median, upper and lower boundaries of the box represent 75th and 25th percentiles, respectively, and the whiskers represent maximum and minimum number of infected spikelets.

gradient was apparent for either median or mean number of infected spikelets in 2003 at either site (Fig. 2a). The non-significant effect of distance and direction and no clear concentration gradient indicates a lack of attenuation of the local source with increasing distance and/or contribution from inoculum sources other than the ergot-infected plants at the centre of each plot. A survey of adjoining areas surrounding the sites showed that the nearest external inoculum source was 90 m at Gatton and 40 m at Kingsthorpe from the edge of the circular plots at the beginning of the trial. These distances increased with time as crops in the adjoining areas matured and were harvested.

In 2004 there was no significant ($P < 0{\cdot}05$) difference in infected spikelets between Gatton ($13{\cdot}8 \pm 1{\cdot}2$) and Kingsthorpe ($11{\cdot}1 \pm 0{\cdot}7$) sites and at Kingsthorpe significantly more spikelets ($13{\cdot}2 \pm 0{\cdot}7$) were infected in the presence of local inoculum source than without ($4{\cdot}9 \pm 0{\cdot}5$) (output not shown). Data for the two sites were analysed separately to account for difference in plot size and distance from local source. As in 2003, date of exposure was significant ($P < 0{\cdot}05$) at each site but the distance and direction from the local source were not significant, although the two or three-way interaction between date, distance and direction was significant (Table 2). No apparent concentration gradient was observed at Kingsthorpe despite increasing the maximum distance between local point source and trap plants from 20 to 50 m (Fig. 2b). At Gatton there was a general reduction in the number of infected spikelets once the maximum distance increased beyond 100 m (Fig. 2b). However, the concentration gradient represented by the slope of linear regression was not significantly different from zero (output not shown) and other commonly used models such as negative exponential or inverse power law models have not been fitted, and the distance at which the concentration decreases by half (half-distance) has not been computed. At the start of 2004 trial the nearest external source of ergot inoculum was 20 m from the local source at Gatton and over 200 m at Kingsthorpe. This meant that at Gatton, exposed plants on the outer most circle of the plot were 220 m away from the external inoculum source. As in 2003, distance to external inoculum source increased with the harvesting of nearby crops.

In 2005 low levels of ergot infection developed on plants exposed at 9 of 14 sites (Table 1). Of these, trap plants developed ergot on all three dates only at Southbrook and Wyreema Road sites, which are both within 300–500 m from large commercial sorghum paddocks. However, infection was either very low or absent at Rocky Creek Road and Back Creek sites, both within 250 m of large sorghum paddocks. Low level of ergot infection occurred at Brookstead and Gatton bypass sites on two

Table 3 Pearson's product moment correlation coefficient for number of infected spikelets and weather during exposure and for the previous 24 h, when flowering sorghum trap plants were exposed at two field sites in 2003 and 2004 with or without local source of *Claviceps africana* inoculum

	Weather during exposure		Previous 24 h weather	
Weather variable	Without local inoculum source	With local inoculum source	Without local inoculum source	With local inoculum source
Maximum temperature	–0·11 (0·11)[a]	–0·05 (0·047)	–0·19 (0·003)	–0·22 (< 0·001)
Minimum temperature	–0·29 (< 0·001)	0·08 (0·003)	0·21 (0·001)	0·28 (< 0·001)
Wind speed	–0·22 (< 0·001)	–0·04 (0·11)	0·12 (0·079)	0·29 (< 0·001)
Rainfall	nd[b]	0·22 (< 0·001)	nd	0·06 (0·02)
Relative humidity (Rh)	0·27 (< 0·001)	0·05 (0·061)	0·50 (< 0·001)	0·29 (< 0·001)
Average temperature	–0·23 (< 0·001)	–0·03 (0·32)	–0·04 (0·527)	0·07 (0·016)
Rh > 90% (Hr)	0·48 (< 0·001)	0·01 (0·743)	0·34 (< 0·001)	0·26 (< 0·001)
Temperature < 12°C (Hr)	0·13 (0·061)	–0·09 (< 0·001)	–0·05 (0·461)	–0·35 (< 0·001)
Leaf wetness duration (Hr)	0·40 (< 0·001)	0·15 (< 0·001)	0·38 (< 0·001)	0·22 (< 0·001)

[a]In parenthesis: Prob > |r| under H0: Rho = 0.
[b]nd = not determined due to lack of rain.

Table 4 Multiple regression equations that best describe quantitative relationships between ergot infected spikelets and weather variables at Gatton and Kingsthorpe in 2003 and 2004 seasons with local source of *Claviceps africana* inoculum

Site	Year	Equation[a]	R^2
Gatton	2003	$y = -1{\cdot}02(\pm 0{\cdot}14) - 0{\cdot}19(\pm 0{\cdot}02)rain + 0{\cdot}21(\pm 0{\cdot}01)lwp24$	0·47
	2004	$y = 14{\cdot}49(\pm 2{\cdot}93) - 0{\cdot}07(\pm 0{\cdot}01)wind + 0{\cdot}16(\pm 0{\cdot}02)rh90 + 0{\cdot}25(\pm 0{\cdot}05)tles - 0{\cdot}58(\pm 0{\cdot}05)lwp + 1{\cdot}08(\pm 0{\cdot}08)tmn24 - 1{\cdot}12(\pm 0{\cdot}19)rain24 - 1{\cdot}09(\pm 0{\cdot}11)temp24 + 0{\cdot}22(\pm 0{\cdot}05)tles24$	0·63
Kingsthorpe	2003	$y = 9{\cdot}28(\pm 2{\cdot}36) - 0{\cdot}39(\pm 0{\cdot}13)tmx - 0{\cdot}32(\pm 0{\cdot}08)tmx24 + 0{\cdot}37(\pm 0{\cdot}07)wind24 + 0{\cdot}52(\pm 0{\cdot}12)temp24$	0·82
	2004	$y = -5{\cdot}41(\pm 1{\cdot}05) - 0{\cdot}09(\pm 0{\cdot}02)rain + 0{\cdot}13(\pm 0{\cdot}01)rh90 - 0{\cdot}12(\pm 0{\cdot}04)tmn24 + 0{\cdot}11(\pm 0{\cdot}02)rh24$	0·78

[a]*rain*, average daily rainfall; *lwp*, duration of leaf wetness; *wind*, average daily wind speed; *rh90*, hours of relative humidity over 90%; *tmn*, minimum temperature; *tmx*, maximum temperature; *temp*, mean daily temperature; *tles*, hours of temperature less than 12°C; *rh*, mean daily relative humidity. A '24' suffix indicates weather variable for the 24 h period prior to exposure.

dates and at West Haldon on one occasion. These sites were at least 1–3 km from nearest sorghum paddocks. No ergot developed at sites that were further removed from sorghum paddocks.

Weather and *C. africana* dispersal

Daily average, maximum and minimum temperature, hours of temperature less than 12°C, relative humidity (RH) and hours of RH over 90%, average rainfall, wind speed and duration of leaf wetness were calculated for the 24 h period when trap plants were exposed to inoculum and for the preceding 24 h. Relationship between infected spikelets and weather variables were initially examined using Pearson's correlation coefficient (Table 3). Overall, moisture related variables including hours of RH over 90%, duration of leaf wetness and average RH were highly correlated with infected spikelets in trap plants. The correlation coefficients were higher for the vast majority of weather variables for the preceding 24 h than those during exposure of trap plants. As ergot-infected plants were only present during exposure, this finding leads to two important conclusions. First, key weather variables had accelerated the production and dispersal of inoculum on ergot-infected plants in adjoining areas of the trial plots and secondly, inoculum from external sources contributed to infection on trap plants. The correlation coefficients for moisture related variables and infected spikelets on trap plants were also high in the absence of local inoculum source. However, only limited data were available from trap plants exposed without local inoculum.

Quantitative relationships between infected spikelets with local *Claviceps africana* inoculum and weather were developed separately for each site and year. At least half of the significant terms in these multiple regression models included weather for the preceding 24 h, and in the 2003 model for Kingsthorpe only one of four significant variables was related to weather during exposure (Table 4). Models were not developed for dates when no local inoculum source was present due to limited data.

Discussion

This work has examined the role of ergot-infected sorghum plants as a source of local inoculum by exposing flowering sorghum plants to trap *C. africana* inoculum for 24 h at

two field sites over 2 years. This was achieved first, by positioning trap plants at various distances from the local inoculum source, and secondly, by exposing trap plants in the absence of a local source. *Claviceps africana* inoculum was consistently trapped by flowering sorghum plants at both field sites in southeast Queensland on 38 different occasions between April and June in 2003 and 2004 and on all three dates in 2005 at many of the 14 sites. Significantly more spikelets became infected as plants trapped additional *C. africana* inoculum when ergot-infected sorghum plants were used a point source within 200 m of trap plants. Despite a strong influence of local inoculum, there was no significant difference in the number of infected spikelets on trap plants placed at various distances up to 200 m and/or directions from local point source in the 2 years of this study. Consequently, there was no obvious concentration gradient within at least 200 m of the local point source. Infection of spikelets also occurred on all 10 occasions when trap plants were exposed without ergot-infected plants. Inoculum on these occasions consisted of conidia produced on the local source used on a previous occasion and/or conidia from other distant sources, which survived and dispersed through the atmosphere.

In 2005 trap plants were placed at 14 different locations along a 280 km route in Southeast Queensland and the number of infected spikelets was generally reduced with increasing distance between inoculum source from large sorghum paddocks and the trap plants, and infection was sporadic when the distance increased to more than 1 km. With such large distances it was not possible to map all sorghum and other host plants, and ergot-infected alternative host plants such as Johnson grass may have also acted as sources of inoculum. Results clearly show that infection does not solely depend on local inoculum sources, although local sources increase infection levels. Moisture related weather variables influenced the number of infected spikelets. Extended hours of RH over 90%, duration of leaf wetness and higher average RH often increased the abundance and/or dispersal of inoculum. Correlation coefficients between infection on trap plants and most weather variables for the day before exposure were higher than those during exposure. This suggests that a proportion of spikelets were infected by inoculum coming from sorghum and other host plants as and when weather conditions became favourable for the production and dispersal of *C. africana* conidia.

The demonstration that *C. africana* conidia can survive in the atmosphere to allow dispersal and infection of sorghum crops in the absence of ergot-infected sorghum plants in the immediate vicinity is significant. Wind-dispersed secondary conidia of *C. africana* are believed to be a major factor behind the explosive global spread of this pathogen (Bandyopadhyay *et al*., 1996). Although dispersal of *C. africana* was not directly measured by counting trapped conidia, infected spikelets provided realistic estimates of aerial inoculum. First signs of ovary colonization by *C. africana* only become evident 65 h after inoculation (Komolong *et al*., 2003) and the incubation period lasts between 5 and 6 days depending on pathogen aggressiveness and host resistance (Komolong, 2003). Exposing flowering sorghum plants for 24–48 h has essentially sampled for conditions favouring inoculum dispersal rather than infection. Assessment of infected spikelets within 7–9 days of exposure further ensured that only infections from field inoculum trapped during an exposure were considered. However, secondary conidia of *C. africana* are often dispersed in clumps (Ryley & Chakraborty, 2008) and the assumption of a single infection resulting from a single conidium may have underestimated their abundance.

These results clearly show the importance of local source of inoculum. Nearly three times as many spikelets became infected when inoculum was present within 200 m of trap plants. The maximum distance (200 m) for local dispersal tested was based on the criterion that the length of dispersal gradient is > 99% of the diameter of a point source (Zadoks & Schein, 1979). However, in 2005, trap plants 500 m away from inoculum consistently developed high levels of spikelet infection, suggesting that dispersal within a 500 m radius may be considered 'local' for *C. africana*.

Previous research has shown that large numbers of secondary conidia are released after rainfall events and the peak concentrations in the late afternoon coincided with rising RH and decreasing temperatures (Frederickson *et al*., 1993). Although moisture related weather variables significantly influenced spikelet infection in this study, these were not associated with rainfall events and very little rain fell during the 2003 and 2004 exposure periods. A recent spore trapping study at two field sites over 3 years have shown that high levels of conidia were trapped during periods of higher median temperatures, wind speed and vapour pressure deficit (Ryley & Chakraborty, 2008). Spore release events were not directly related to rainfall. Highest numbers of conidia were trapped 1–3 days after rainfall events and prolonged high RH and suitable temperature are important for inducing profuse sporulation. In the current work the prominence of moisture related variables in correlation and regression analyses are in agreement with these findings.

With further studies, quantitative relationships between weather and spikelet infection may lead to the development of predictive models to determine the risk of ergot infection. One key factor not examined in the current study is pollen viability and low temperature induced predisposition of sorghum to ergot infection (McLaren & Wehner, 1992). Originally proposed from South African work, these relationships have been confirmed in Australian studies (Wang *et al*., 2000; Ryley *et al*., 2002).

Although there was no attenuation within 200 m, the abundance of aerial inoculum did decline when the closest likely inoculum source was more than 500 m away. Using spore trapping Ryley & Chakraborty (2008) failed to detect diurnal periodicity in the patterns of secondary conidia release in *C. africana* from nearby infected sorghum crops, although a previous study in Zimbabwe had reported a late afternoon peak (Frederickson *et al*., 1993). One likely explanation is that not all secondary

conidia trapped were from the local source and trapped conidia were released into the air from local and/or distant sources over a period of time. In the current study, an overall strong correlation between number of infections and weather in the absence of local inoculum and a lack of clear concentration gradients within 200 m from a point source of inoculum is further evidence that external inoculum sources had played a role in ergot infection.

A large number of weather variables for the previous 24 h period significantly influencing spikelet infection indicate the importance of survival and dispersal of inoculum produced on previous day(s). As the gap between two consecutive exposures with a local inoculum ranged from 2 to 7 days, conidia had to survive in the atmosphere for up to 7 days to infect trap plants along with conidia arriving from other sources. A lack of attenuation of inoculum concentration within 200 m further suggests that under suitable weather conditions, large numbers of conidia survive in the atmosphere and conidia from external sources may have travelled great distances to infect the trap plants. Although the effect of past weather beyond 24 h has not been examined, cumulative effect of weather on conidial survival may offer insights into conidia concentration in the atmosphere to help predict the risk of severe ergot outbreaks. Survival of *C. africana* inoculum in the atmosphere must be a prerequisite for its proposed mode of transport via intercontinental air masses (Bandyopadhyay *et al*., 1996) and weather would exert an obvious influence on their survival and spread. The long-distance transport of fungal spores including rust urediniospores (Nagarajan & Singh, 1990) via air currents is well established and forward trajectories of *Pseudoperonospora cubensis* and *Peronospora tabacina* are available to aid disease forecasting (Main *et al*., 2001). However, the strongly stochastic nature of long distance dispersal (Brown & Hovmøller, 2002) suggests that weather conditions that facilitate long-distance transport are likely to be different to those that influence local dispersal, and requires new research.

The planting season for sorghum in southeast Queensland extends from over several weeks to months depending on soil moisture availability, and with this the duration of flowering and ergot susceptibility. In all 3 years of this study exposure of trap plants coincided with flowering of commercial sorghum crops and aerial *C. africana* inoculum was present on all 41 occasions, although its abundance fluctuated with weather conditions. For on-farm ergot management, the importance of local inoculum strongly suggests that sanitation and other measures can help to reduce the risk of severe ergot development in commercial crops. For instance, early planting to avoid flowering later in the season when temperature is more conducive to infection, and strategic application of fungicides coinciding with predicted inoculum dispersal peaks, would be useful. Other measures such as burial of infected residues and removal of alternative crop and weed hosts would help reduce local inoculum. However, improved understanding of conditions favouring long-distance dispersal of *C. africana* inoculum will be necessary to predict ergot outbreak in previously uninfested areas.

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References

Bandyopadhyay R, Frederickson DE, McLaren NW, Odvody GN, 1996. Ergot – a global threat to sorghum. *International Sorghum and Millets Newsletter* **37**, 1–32.

Bandyopadhyay R, Frederickson DE, McLaren NW, Odvody GN, Ryley MJ, 1998. Ergot: a new disease threat to sorghum in the Americas and Australia. *Plant Disease* **82**, 356–67.

Bandyopadhyay R, Muthusubramanian V, Tooley PW, Chakraborty S, Pazoutová S, 2002. Distribution and diversity of the sorghum sugary disease pathogens in India. In: Leslie JF, ed. *Sorghum and Millets Diseases*. Ames, IA, USA: Iowa State Press, 75–8.

Blaney BJ, McKenzie RA, Walters JR *et al*., 2000. Sorghum ergot (*Claviceps africana*) associated with agalactia and feed refusal in pigs and dairy cattle. *Australian Veterinary Journal* **78**, 102–7.

Blaney BJ, Chakraborty S, Murray SA, 2006. Alkaloid production by isolates of the sorghum ergot pathogen (*Claviceps africana*) from Australia and other countries. *Australian Journal of Agricultural Research* **57**, 1023–8.

Brown JKM, Hovmøller MS, 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**, 537–41.

Frederickson DE, Mantle PG, DeMilliano WAJ, 1991. *Claviceps africana* sp. nov.; the distinctive ergot pathogen of sorghum in Africa. *Mycological Research* **95**, 1101–7.

Frederickson DE, Mantle PG, de Milliano WAJ, 1993. Windborne spread of ergot disease (*Claviceps africana*) in sorghum A-lines in Zimbabwe. *Plant Pathology* **42**, 368–77.

Komolong B, 2003. *The identity and diversity of* Claviceps *spp. causing sorghum ergot in Australia*. Brisbane, Australia: University of Queensland, PhD thesis.

Komolong B, Chakraborty S, Ryley M, Yates D, 2002. Identity and genetic diversity of the sorghum ergot pathogen in Australia. *Australian Journal of Agricultural Research* **53**, 621–8.

Komolong B, Chakraborty S, Ryley M, Yates D, 2003. Ovary colonization by *Claviceps africana* is related to ergot resistance in male-sterile sorghum lines. *Plant Pathology* **52**, 620–7.

Kulkarni BGP, Seshadri VS, Hedge RK, 1976. The perfect stage of *Sphacelia sorghi* McRae. *Mysore Journal of Agricultural Science* **10**, 286–9.

Main CE, Keever T, Holmes GJ, Davis JM, 2001. Forecasting long-range transport of downy mildew spores and plant disease epidemics. American Phytopathological Society Net

(APSnet), April–May 2001 [http://www.apsnet.org/online/feature/forecast/].
Mantle PG, 1973. Production of ergot alkaloids *in vitro* by *Sphacelia sorghi*. *Journal of General Microbiology* **75**, 275–81.
McLaren NW, Flett BC, 1998. Use of weather variables to quantify sorghum ergot potential in South Africa. *Plant Disease* **82**, 26–9.
McLaren NW, Wehner FC, 1992. Pre-flowering low temperature predisposition of sorghum to sugary disease (*Claviceps africana*). *Journal of Phytopathology* **135**, 328–34.
Nagarajan S, Singh DV, 1990. Long-distance dispersion of rust pathogens. *Annual Review of Phytopathology* **28**, 139–53.
Pažoutová S, Frederickson DE, 2005. Genetic diversity of *Claviceps africana* on sorghum and *Hyparrhenia*. *Plant Pathology* **54**, 749–63.
Reis EM, Mantle PG, Hassan HAG, 1996. First report in the Americas of Sorghum ergot disease, caused by a pathogen diagnosed as *Claviceps africana*. *Plant Disease* **80**, 463.
Ryley M, Henzell B, 1999. Sorghum ergot in Australia. In: Casela CR, Dahlberg JA, eds. *Proceedings of the Global Conference on Ergot of Sorghum, Sete Lagoas, Brazil*, 182–90.
Ryley MJ, Alcorn JL, Kochman JK, Kong GA, Thompson SM, 1996. Ergot on *Sorghum* spp. in Australia. *Australasian Plant Pathology* **25**, 214.
Ryley MJ, Chakraborty S, 2008. Patterns of release of the secondary conidia of *Claviceps africana*, the sorghum ergot pathogen in Australia. *Plant Pathology* **57**, 473–83.
Ryley MJ, Herde DJ, Bhuiyan SA, Henzell RG, Jordan DR, 2002. An overview of the biology of sorghum ergot. In: Leslie JF, ed. *Sorghum and Millets Diseases*. Ames, IA, USA: Iowa State University Press, 141–50.
Tooley PE, O'Neill NR, Goley ED, Carras MM, 2000. Assessment of diversity in *Claviceps africana* and other *Claviceps* spp. by RAM and AFLP analyses. *Phytopathology* **90**, 1126–30.
Wang E, Meinke H, Ryley MJ, Herde D, Henzell B, 2000. On the relation between weather variables and sorghum ergot infection. *Australian Journal of Agricultural Research* **51**, 313–24.
Zadoks JC, Schein RD, 1979. *Epidemiology and Plant Disease Management*. New York, USA: Oxford University Press.