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Factors influencing the germination of macroconidia and secondary conidia of *Claviceps africana*

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Abstract. The influences of temperature, time, and moisture on the germination of macroconidia and secondary conidia of Australian isolates of *Claviceps africana* were studied *in vitro*. The optimum temperature for germination of both macroconidia and secondary conidia of *C. africana* was 20°C. Although germination of macroconidia ceased near 31°C, approximately 30% of secondary conidia germinated at 37°C after 48 and 72 h of incubation. Sorghum flower extract agar stimulated macroconidium and secondary conidium germination, irrespective of temperature. Germination of macroconidia and secondary conidia on water agar started after 4 h of incubation at 20°C, reaching a maximum after 16–24 h and 14 h, respectively. Maximum germination of both macroconidia and secondary conidia was at ≥−5 bars at 20°C. Germination of secondary conidia ceased at –35 bars, whereas macroconidia germinated at water potentials as low as –55 bars at 20°C.

Introduction

Sorghum ergot, caused by *Claviceps africana* Frederickson, Mantle & de Milliano, is an important disease of sorghum throughout the world (Bandyopadhyay *et al.* 1998). *C. africana* was confined to Africa until the mid 1990s, but in recent years it has been reported in North and South America (Reis *et al.* 1996; Isakeit *et al.* 1998), Australia (Ryley *et al.* 1996), and India (Bogo and Mantle 1999). In particular, it affects the production of F_1 hybrid seed, because male sterile (A) lines in the absence of pollen are very susceptible (Bandyopadhyay *et al.* 1998). *C. africana* colonises the ovaries of sorghum after conidia germinate on the stigmas, styles, or ovary wall, and hyphae grow into the ovary (Bandyopadhyay *et al.* 1998). Successful fertilisation of the ovary inhibits invasion by the pathogen. Approximately 7–8 days after colonisation of the ovary, clear, sticky drops of honeydew, which contain macroconidia, ooze from infected spikelets (Bandyopadhyay *et al.* 1998). Under high relative humidity, macroconidia germinate, producing sterigma bearing a single apical secondary conidium (Bandyopadhyay *et al.* 1998). Secondary sporulation occurs from honeydew on inflorescences, vegetative plant parts, and soil below ergot-infected inflorescence (Bandyopadhyay *et al.* 1991). It is believed that these airborne secondary conidia are responsible for the rapid spread of the pathogen (Frederickson *et al.* 1993), and are the major infective propagules (Bandyopadhyay *et al.* 1998).

The biology of *C. africana*, in particular the factors affecting the germination of secondary conidia, is poorly understood. Frederickson (1990) studied the influence of temperature on *in vitro* germination of macroconidia, and found that germination started after 12 h incubation at an optimum temperature of 19°C. Bandyopadhyay *et al.* (1998) reported that the macroconidia germinate on the stigmas of sorghum florets 8–24 h after inoculation. There are no reports on the influence of other factors, such as the effect of water potential on macroconidium germination at various temperature regimes, and on the factors influencing the germination of secondary conidia. Knowledge of the factors influencing macro- and secondary conidium germination will provide a better understanding of the biology and ecology of the pathogen. The objectives of research reported here were to study the effects of some of these factors on the *in vitro* germination of macroconidia and secondary conidia of Australian isolates of *C. africana*.

Materials and methods

Source of inoculum and general procedures

Unless stated otherwise, the procedures described here were used in all experiments. Fresh honeydew was collected from ergot-infected panicles on plants of *Sorghum bicolor* (L.) Moench growing at Gatton (approx. 27° 55′ S, 152° 33′ E), Queensland, Australia, and diluted with sterile deionised water. The macroconidium concentration in the honeydew suspension was determined with the aid of a haemocytometer and adjusted to 1.5×10^6 conidia/mL.

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The honeydew suspension was streaked with a sterile cotton bud or with a sterile loop onto 4 separate areas on the surface of agar medium in unsealed 9-cm Petri dishes and incubated in the dark. Preliminary trials had shown that good germination could be obtained on unsealed plates using 1.5% water agar (WA) (Agar, 750 Gel, Chem-Supply, Australia), without the addition of antibiotics. Sealing plates with plastic film (Parafilm 'M', American National Can, Chicago) decreased the germination of both macroconidia and secondary conidia. After incubation for 24 h, the germination of 100 conidia was counted on each of 2 randomly selected areas under a compound microscope at 100×. A conidium was considered to have germinated when it produced a secondary or tertiary conidium, or a germ tube of at least half the length of the spore.

Effect of temperature on conidium germination: macroconidia

A standard macroconidium suspension was spread over the surfaces of 60 WA plates using sterile cotton buds. Six plates were incubated at each of 8, 10, 13, 18, 20, 24, 26, 29, 31, and 33°C in a multi-range temperature incubator. The temperature in each chamber was monitored every 30 min with a sensor that was connected to a data logger. Each sensor had been calibrated with a standard mercury thermometer. After 24 h, 3 plates were removed from each temperature chamber and the germination of macroconidia was determined. The same procedures were followed with the remaining plates after 48 h incubation.

Effect of temperature on conidium germination: secondary conidia

Fresh honeydew was diluted with sterile deionised water to 5×10^7 conidia/mL. The suspension was streaked with a sterile cotton bud onto WA media in 5 Petri dishes and incubated at 20°C. After 24 h, when secondary conidia had developed, 5 mL sterile deionised water containing one drop of Tween-80 was poured into each plate. A sterile camel hair brush was used to dislodge conidia from the agar, and the suspensions from the 5 plates were poured into a 25-mL beaker and stirred with a magnetic stirrer for 1 h to assist in the detachment of the secondary conidia from sterigmata. The conidium suspensions were centrifuged for 5 min at 20°C and 2500 rpm (r_{av} 8 cm, Roto-Uni II, BHG, Germany). After discarding the supernatant, the conidia were resuspended with sterile deionised water and adjusted to 1×10^6 conidia/mL. The suspension, containing germinated and ungerminated macroconidia and ungerminated secondary conidia, was streaked onto WA in Petri dishes. Nine Petri dishes were incubated in the dark at each of 10 temperatures (7, 11, 15, 18, 21, 24, 27, 30, 33, and 37°C) in a multi-range temperature incubator for 24, 48, and 72 h. The germination of the distinctive pear-shaped secondary conidia was assessed on 3 plates at each temperature–time combination.

Germination of conidia on 2 media at various temperatures

The germination of macroconidia and secondary conidia on sorghum flower extract agar (SFEA) and WA over a range of temperatures was compared. The former medium was prepared by boiling 50 g of sorghum spikelets, collected from healthy sorghum panicles of a male sterile line (AQL 23188) at Gatton, Queensland, in 250 mL of deionised water into a 500-mL flask for 20 min. After cooling, the supernatant was sieved through 4 layers of cheesecloth and collected in a 250-mL bottle. Two hundred mL of boiled extract was mixed with 800 mL of 1.5% water agar, autoclaved, and poured into 9-cm Petri dishes.

To assess macroconidium germination, diluted honeydew suspension was streaked onto WA and SFEA using sterile cotton buds. The agar plates were incubated at 10, 15, 20, 25, and 30°C for 24 h in the dark, and germination was assessed on 2 plates for each temperature by medium combination. A suspension of secondary conidia was streaked onto plates of both media with a sterile loop. Plates of each medium were incubated at 10, 15, 20, 25, and 30°C in the dark for 48 h, when germination of secondary conidia was determined.

Germination of macroconidia on slides and free water at 100% RH at various temperatures

A macroconidium suspension of *C. africana* was streaked on 24 grease-free, sterile, dry microscope slides with the aid of a sterile cotton bud and dried for 1 h in a laminar flow cabinet before incubation. One drop of the conidium suspension was also placed on another 24 slides immediately before incubation. All slides were placed separately on glass rods in glass Petri dishes (10 cm diameter) containing 3 layers of moistened sterile blotting paper to maintain 100% RH. Four slides of each type were incubated at 14, 18, 20, 23, 27, and 29°C in the dark in a multi-range temperature incubator. Four plates of water agar were used as controls in each treatment.

Germination of conidia over time

The course of macroconidium germination over time was determined on WA or SFEA media. The SFEA media were prepared by either boiling (as was described above) or blending sorghum spikelets. For the latter, 50 g of spikelets was blended in 250 mL of water for 20 min and sieved, and the extract was collected as described previously. Two hundred mL of blended extract was mixed with 800 mL of 1.5% water agar, autoclaved, and poured in 9-cm Petri dishes. A standard honeydew suspension was streaked onto each of 36 plates of SFEA and WA using a sterile cotton bud. All plates were incubated at 20°C in the dark. Every 2 h, 3 plates of each medium were removed for conidium germination determination. The germination of secondary conidia over time was also determined using the method described above, but only using boiled SFEA medium.

Germination of conidia and various temperature × water potential combinations

Various amounts of potassium chloride (KCl) and sucrose were added to 1.5% WA to produce a range of water potentials. The formulae used to adjust water potential of water agar were:

$$\Psi(\text{KCl}) = -2RTm$$
$$\Psi(\text{sucrose}) = -RTm$$

where Ψ is the water potential (bars or atms), R is the gas constant (0.083 L.bar/K.mol), T is the absolute temperature (°K), and m is molality of KCl or sucrose (mole/L) solution.

Each KCl or sucrose agar solution was autoclaved at 121°C for 15 min. Approximately 15 mL of media was poured into 9-cm plastic Petri dishes. The water potentials, adjusted for each temperature, were –5, –15, –25, –35, –45, and –55 bars. The water potential of the basal medium (WA) was calculated by measuring the a_w value using a water activity measuring device (Thermoconstantner TH200, Novasina, Switzerland). The a_w values were converted into water potentials by the formula (Manandhar and Bruehl 1973):

$$\Psi = \frac{RT}{V}\log_{10} a_w = 10.6T\log_{10} a_w$$

Standard suspensions of either macroconidia or secondary conidia were streaked onto each medium using the procedures outlined above. Plates were incubated at 10, 15, 20, 25, and 30°C in the dark for 24 h for macroconidia, and 48 h for secondary conidia. The germination of 100 conidia was determined on each of 4 replicate plates.

Statistical analysis

Analyses of the germination of macroconidia and secondary conidia were performed on logit-transformed (Bartlett 1947) data. As there

were zero values in some data series, we first calculated a modified proportion as:

P = (number of germinated conidia + 0.5)/(total number counted +1)

The logit-transformed data were then derived by the formula:

$$Y = \log_e \frac{P}{1-P}$$

All experiments were initially analysed using the SAS (SAS Institute, version 6.12. Cary, NC, USA) general linear model (GLM) procedure. Response functions were then fitted to the mean data either by linear or non-linear regressions.

Conidium germination showed a quadratic type response to temperature in all cases. For macroconidia, a simple quadratic was found to be adequate:

$$Y = \alpha_1 + \alpha_2 t + \alpha_3 t^2$$

where t is the temperature (°C).

Germination behaviour of secondary conidia was more complex, requiring an inverse quadratic model:

$$Y = \alpha_1 + \frac{\alpha_2 + \alpha_3 t}{1 + \alpha_4 t + \alpha_5 t^2}$$

Optimum temperatures were derived by differentiating the response functions to obtain the maximum.

A logistic model (Campbell and Madden 1990) was used in describing the effect of time on macro- and secondary conidium germination as:

$$P = \frac{M}{1 + e^{-k(h-HM)}}$$

where M is estimated maximum germination; k is estimated rate of germination; HM is estimated median germination time, i.e. when germination = $M/2$; and h is germination time (h).

Both Gompertz (Ratkowsky 1983) and Logistic models were effective in describing the relationship between germination and water potential. We used a Logistic model combined with a quadratic response to temperature to describe estimated maximum germination (G_{max}):

$$P\,(\text{germination}) = \frac{G_{max}}{1 + \exp(\theta_0 + \theta_1 \Psi)}$$

where Ψ is the water potential (bars), and $G_{max} = \alpha + \beta t + \gamma t^2$ where t is temperature (°C).

Results

Germination of macroconidia and secondary conidia at various temperatures

Macroconidia germinated at between 8 and 31°C, with an optimum at 20°C after 24 h (70%) and 48 h (68%) (Fig. 1*a*). The influence of temperature on macroconidium germination after incubation for 24 and 48 h was described by the following equation:

$$Y\,(\text{logit germination}) = -16.3983 + 1.683t - 4.18E - 02t^2 \quad R^2 = 0.96$$

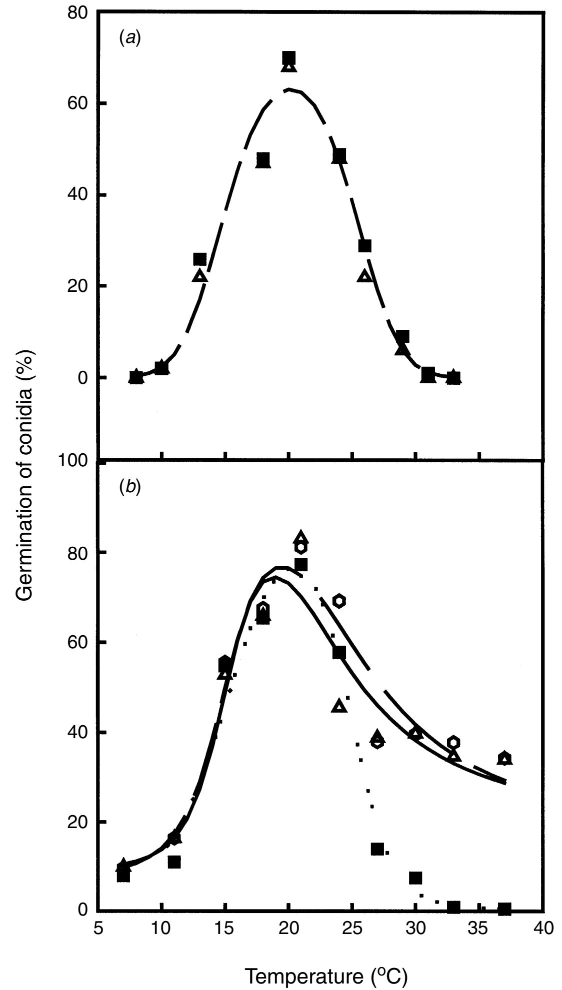

Fig. 1. Effects of temperature on germination of (*a*) macroconidia of *C. africana* after 24 h (■) and 48 h (△) of incubation, and (*b*) secondary conidia of *C. africana* after 24 h (■, …), 48 h (△, —), and 72 h (○, – –) of incubation.

The estimated optimum temperature from this equation was 20.1°C.

There were highly significant ($P < 0.001$) time, temperature, and time × temperature effects on secondary conidium germination. After 24 h, the temperature response of secondary conidia germination was very similar to that of macroconidia (Fig. 1*b*). However, after 48 and 72 h there was relatively high germination (>33%) above 30°C. The effects of temperature on germination of secondary conidia can be described by the following equations:

$$Y\,(24\text{ h}) = -6.25 + (2.362 - 0.0515t)/(1 - 0.0738t + 0.001639t^2) \quad R^2 = 0.95$$

$$Y\,(48\text{ h}) = -1.719 + (-0.499 + 0.0472t)/(1 - 0.108t + 0.0033t^2) \quad R^2 = 0.80$$

Table 1. Germination (% ± s.e.m.) of macroconidia and secondary conidia of *C. africana* on water agar (WA) and sorghum flower extract agar (SFEA) at various temperatures

Temperature	Macroconidia			Secondary conidia		
(°C)	WA	SFEA	Sign.	WA	SFEA	Sign.
10	6.8 ± 1.7	10.8 ± 2.5	**	2.8 ± 0.9	3.0 ± 1.3	n.s.
15	69.3 ± 2.8	82.5 ± 1.9	**	72.8 ± 1.3	80.3 ± 1.4	**
20	79.3 ± 1.8	90.0 ± 0.9	**	70.8 ± 2.3	91.3 ± 0.9	**
25	54.5 ± 0.9	80.8 ± 1.3	**	31.3 ± 1.1	80.8 ± 1.1	**
30	14.3 ± 0.5	36.5 ± 1.3	**	9.3 ± 0.5	25.3 ± 0.8	**
s.e.d.	± 2.4			± 1.7		

**$P < 0.01$ for comparison between WA and SFEA; n.s., not significant.

$$Y\,(72\text{ h}) = -1.928 + (-0.50 + 0.0536t)/(1 - 0.1021t + 0.003t^2) \quad R^2 = 0.88$$

The optimum temperatures for secondary conidium germination estimated from these equations were:

24 h = 20.4°C, 48 h = 18.9°C, 72 h = 19.5°C

Germination of conidia on 2 media at different temperatures

There were highly significant ($P < 0.001$) differences in germination of both macroconidia and secondary conidia at different temperatures, on the 2 media and between different media × temperature combinations. Significantly higher conidium germination occurred on SFEA medium compared with that on WA (Table 1) at all temperatures, except for secondary conidium germination at 10°C.

Macroconidium germination at 100% RH at various temperatures

Germination of macroconidia in water droplets, or at 100% RH on slides, was significantly ($P < 0.001$) lower (1–10%) at all temperatures than on WA (2–58%). Most macroconidia germinated myceliogenically under these conditions.

Germination of conidia over time

At 20°C, macroconidium germination started after 2 h (0.2%) on both WA and SFEA and increased rapidly over time (Fig. 2*a*). Sterigmata developed in the first 6 h of incubation followed by secondary conidia development. In this experiment the relationships between macroconidium germination and time on SFEA and WA media were described by the following equations:

$$P\,(\text{blended SFEA}) = 91.40/(1 + e^{-1.30(h-5.90)}) \quad R^2 = 0.98$$

$$P\,(\text{boiled SFEA}) = 91.73/(1 + e^{-1.15(h-6.46)}) \quad R^2 = 0.99$$

$$P\,(\text{WA}) = 76.34/(1 + e^{-1.03(h-7.05)}) \quad R^2 = 0.99$$

where P is % of germination and h is hour after incubation.

The time to reach 95% of estimated maximum germination, calculated from the above equations, is 8.2 h, 9 h, and 9.9 h for blended SFEA, boiled SFEA, and WA, respectively.

Secondary conidia started to germinate after 4 h of incubation (0.3–0.5%) on SFEA and WA media (Fig. 2*b*). The relationship between secondary conidium germination and time on SFEA and WA media can be described by the following equations:

$$P\,(\text{SFEA}) = 88.84/(1+e^{-0.88(h-8.80)}) \quad R^2 = 0.99$$

$$P\,(\text{WA}) = 59.81/(1+e^{-0.61(h-11.46)}) \quad R^2 = 0.99$$

From the equations above, the time to reach 95% of germination of secondary conidia was estimated as 12.1 h and 16.3 h for SFEA and WA, respectively. A comparison of the estimated values of maximum (*M*), rate (*k*), and median (*HM*) germination of macroconidia (Table 2), using a simple *t*-test, revealed that there was no significant difference in estimated maximum germination (*M*) on blended and boiled sorghum flower extracts, although both were significantly higher ($P < 0.001$) than the control (WA). Similarly, there were no significant differences between the rates (*k*) of macroconidium germination on blended, boiled, and WA media. On the other hand, the median (*HM*) germination time was significantly ($P < 0.001$) shorter on blended than on boiled sorghum extract medium, which was in turn significantly ($P < 0.001$) shorter than the control. Secondary conidia showed significantly ($P < 0.001$) higher maximum germination (*M*), higher germination rate (*k*), and shorter median (*HM*) time on SFEA than on WA media (Table 2).

Germination of conidia and various temperature × water potential combinations

In the sucrose-amended media, macroconidium germination was highest (approx. 85%) at 20°C and –5 bars (Fig. 3), decreasing as water potential dropped, reaching

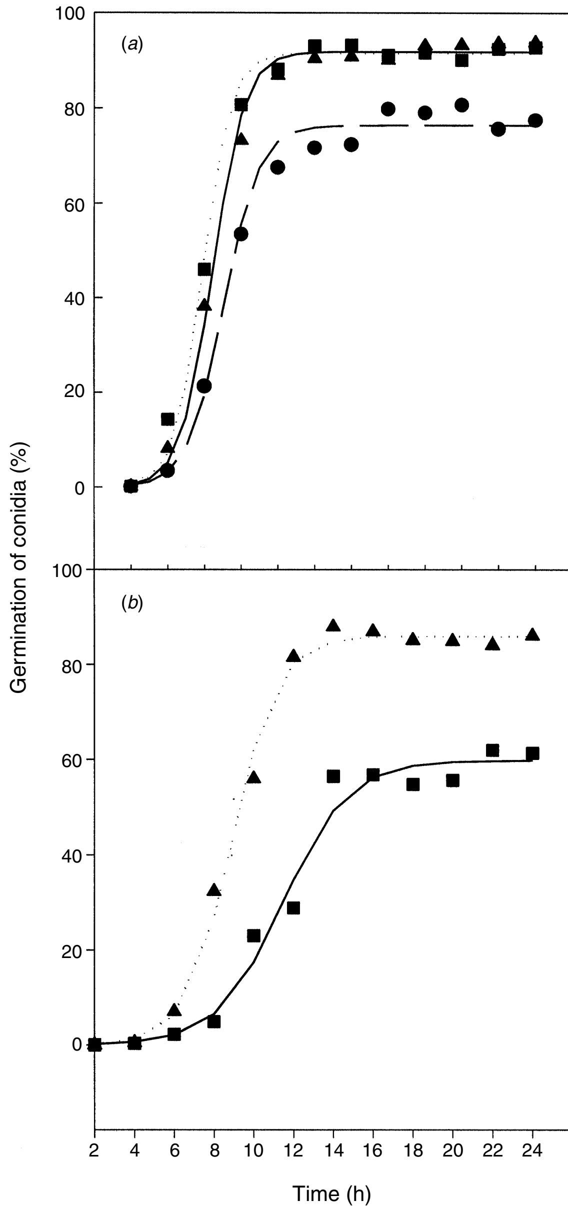

Fig. 2. Germination of (*a*) macroconidia on boiled (▲, —) and blended (■, ···) sorghum flower extract agar (SFEA), and water agar (WA) (●, ––), and (*b*) secondary conidia on SFEA (boiled) (▲) and WA (■) of *C. africana* over time at 20ºC.

Table 2. Estimated maximum (*M*), rate (*k*), and median (*HM*) germination of macroconidia and secondary conidia of *C. africana* on sorghum flower extract agar (SFEA) media

Values in parentheses are the standard errors of estimated parameters. Within conidium type, values followed by the same letters are not significantly ($P > 0.05$) different using a *t*-test

Media	*M*	*k*	*HM*
	Macroconidia		
Blended	91.4a (±1.03)	1.30a (±0.12)	5.90a (±0.23)
Boiled	91.7a (±0.92)	1.15a (±0.09)	6.46b (±0.22)
WA (control)	76.4b (±1.34)	1.03a (±0.05)	7.05c (±0.18)
	Secondary conidia		
SFEA (boiled)	85.8a (±1.16)	0.88a (±0.04)	8.80a (±0.20)
WA	59.8b (±3.04)	0.61b (±0.04)	11.46b (±0.18)

approximately 3–10% at –55 bars. At 15°C, the maximum germination (70%) occurred at approximately –1 bar, decreasing sharply to 0% at –35 to –45 bars. At other temperatures, the maximum germination was close to or less than 20%, at all water potentials. There were highly significant ($P < 0.001$) effects of temperature, water potential, and their interactions on macroconidium germination.

There were similar interactions for germination of secondary conidia on sucrose-amended agar. Maximum

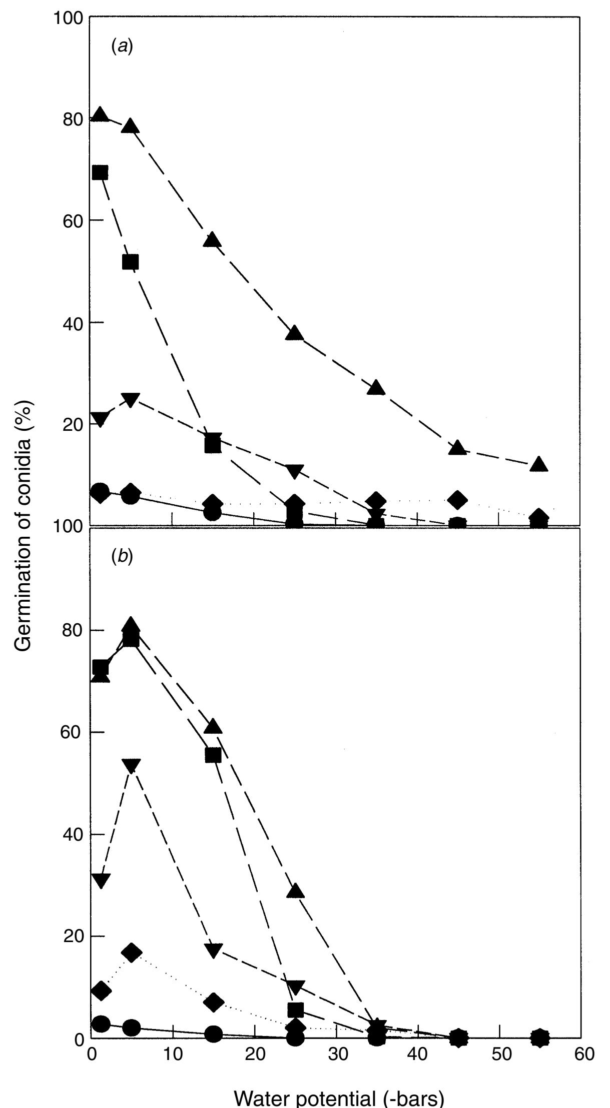

Fig. 3. Germination of (*a*) macroconidia and (*b*) secondary conidia of *C. africana* with sucrose at 10°C (●), 15°C (■), 20°C (▲), 25°C (▼), and 30°C (◆) and water potential regimes.

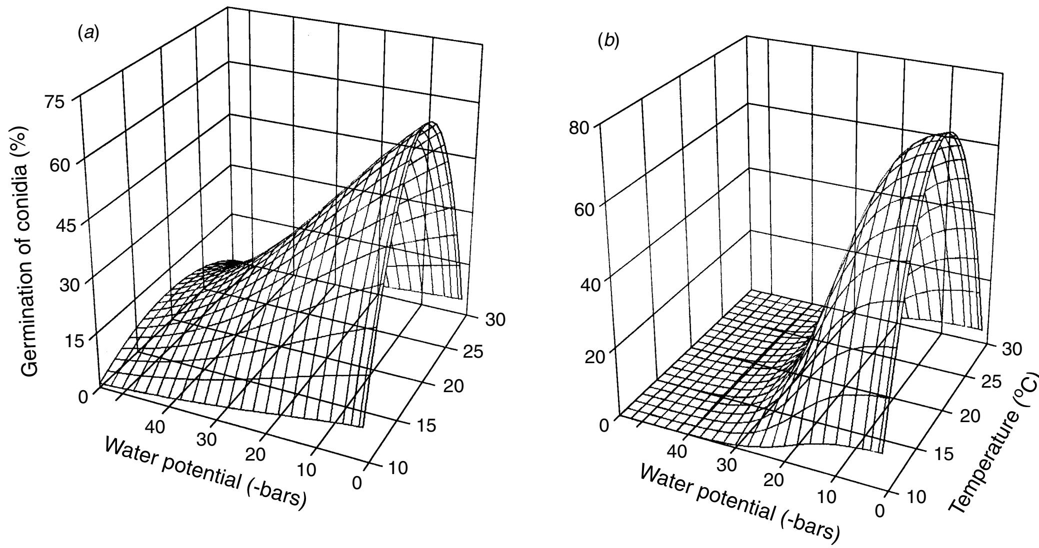

Fig. 4. Predicted influence of temperature and water potential on germination of (*a*) macroconidia and (*b*) secondary conidia of *C. africana* with sucrose. Estimated parameters are shown in Table 3.

germination (approx. 80%) occurred at –5 bars at 20 and 15°C (Fig. 3), with germination dropping to 0% at –35 to –45 bars. At 25°C, the maximum germination was approximately 50% at –5 bars, and germination decreased as the water potential decreased. At 10 and 30°C there was <20% germination at all water potential regimes.

Similar results were observed with KCl-amended media for both macroconidia and secondary conidia (data are not presented). The predicted temperature and water potential relationships for macroconidia and secondary conidia in sucrose are displayed in Fig. 4, and estimated parameters are shown in Table 3.

Discussion

The optimum temperature for macroconidium germination of Australian isolates of *C. africana* was 20°C, which is very similar to *C. africana* isolates from Zimbabwe (18–24°C) (Frederickson 1990) and South Africa (19.5°C) (McLaren 1999). Different optima for macroconidium germination have been reported for other sorghum ergot pathogens. The optimum temperature for germination of Indian isolates of *Sphacelia sorghi* has been reported to occur at 35°C (Frederickson 1990) and 20 ± 2°C (Bandyopadhyay *et al.* 1996). In both studies the isolates were referred to as being *Claviceps sorghi*, but their true identities, particularly those used in the second study (Bandyopadhyay *et al.* 1996), remain in doubt. The recent identification of *C. africana* in India (Bogo and Mantle 1999; Pazoutova *et al.* 2000), together with anecdotal evidence (Pazoutova *et al.* 2000), suggests that *C. africana* may have coexisted with *C. sorghi* in that country for many years. The optimum temperature for macroconidium germination of a Taiwanese isolate of *S. sorghi* occurred in the range 24–28°C (Chen *et al.* 1995). The optimum temperature for germination of macroconidia

Table 3. Estimated parameters and associated statistics for the model to describe the germination of macroconidia and secondary conidia of *C. africana* (with sucrose) at various temperatures and water potential regimes
ASE, Asymptotic standard error of the parameters estimates; R^2, 1–residual SS/regression SS

Parameter	Macroconidia		Secondary conidia	
	Estimate	ASE	Estimate	ASE
θ_0	–1.043	0.962	–4.161	0.686
θ_1	0.069	0.019	0.217	0.031
α	–236.666	70.108	–199.858	12.775
β	33.287	9.753	28.104	1.483
γ	–0.849	0.249	–0.711	0.037
R^2	0.795		0.891	
Residual d.f.	106		135	

of *Claviceps sorghicola*, the Japanese pathogen of sorghum ergot (Tsukiboshi *et al.* 1999), was reported to be near 27°C (Bandyopadhyay *et al.* 1996). There have been no reports on the effects of temperature alone on secondary conidium germination. Our results showed that secondary conidia germinated at temperatures up to 37°C, although the optimum was 20°C. Secondary conidia are believed to be the primary dispersal and infective propagule of *C. africana* (Frederickson *et al.* 1993) and our data suggest that secondary conidia are capable of germination at higher temperatures than honeydew-based macroconidia. This finding may have significance in the infection of sorghum spikelets by secondary conidia during warm weather.

In this study, macroconidia and secondary conidia of an Australian isolate started to germinate 2–4 h after incubation at 20°C, and 95% germination occurred after 8–10 h and 12–16 h, respectively. In contrast, Frederickson (1990) reported that macroconidia of Zimbabwean isolates of *C. africana* germinated after 12 h between 14°C and 35°C and that secondary conidia germinated after 15 h at 24°C. The apparent differential response in conidium germination over time between the isolates from Australia and Zimbabwe may be due to inherent genetic differences, or differences in media and/or methodology. RAPD analysis of *C. africana* isolates from throughout the world (Pazoutova *et al.* 2000) demonstrated that the isolates from Australia were more closely related to those from India than to those from Africa.

Wang *et al.* (2000*a*, 2000*b*) studied the relationships between weather variables and ergot severity, and analysed the regional and geographic distribution of the potential severity and number of ergot sorghum events in Australia. A rule-based method and a regression model were used to interrogate long-term historical weather data for localities from northern Queensland to southern New South Wales. An 'infection factor', based on a normal distribution of temperature about a maximum of 20°C (equivalent to an infection factor of 1), was incorporated into the regression model. This factor was designed to account for the effect of temperature on infection. The analyses showed that during the main flowering period for grain sorghum in Australia (December–March) the number of likely ergot events was low (<5 events/month) from southern Queensland to central New South Wales, but high (up to 20 events/month) in central Queensland. The relative severity in all regions over this period was <0.4 (scale 0–1). From April onwards, both the number and severity of events increased markedly. Another analysis of over 100 years of historical weather data (E. Wang, unpubl. data) showed that for 3 localities (Emerald, Dalby, and Moree) there was >60% probability in any year of there being 10 periods per month between December and March with 3 or more consecutive hours of 18–22°C. Both of these analyses, and the results reported in this paper, suggest that between December and March in the sorghum-growing areas of Australia there are frequent periods that are conducive to germination of both macroconidia and secondary conidia, which in turn contribute to outbreaks of ergot.

Our results would suggest that sorghum flower extract stimulates conidium germination irrespective of temperature, and that it does not lose effectiveness during boiling or autoclaving. High germination (96–100% between 15 and 30°C) of *C. sorghi* macroconidia was observed on agar amended with macerated stigmas (Bandyopadhyay *et al.* 1996), but germination was myceliogenic, whereas we observed mostly iterative (sporogenic) germination in our study. The method of preparation of SFEA (boiling or autoclaving) may influence the mode of germination. In another study, it was reported that stigmatic exudates of susceptible varieties induced high conidium germination, whereas exudates of resistant varieties reduced germination of conidia of *S. sorghi* (Chinnadurai *et al.* 1970).

It has been demonstrated that macroconidia are capable of germinating at lower water potentials than secondary conidia, reflecting their ability to survive and germinate inside honeydew, which is composed of an aqueous suspension of a range of sugars. Water potentials for optimum macroconidium germination were reported to be –10 bars for *S. sorghi* (Chen *et al.* 1995) and –3 to –30 bars for *C. purpurea* (Kaiser and Bruehl 1996). Although macroconidia of a Zimbabwean isolate of *C. africana* have been reported to germinate only at a sucrose content of 10% or lower at 18°C (Frederickson 1990) (which corresponds to a water potential of approx. –7 bars), macroconidia of Australian isolates can germinate at –55 bars (approx. 40% w/v of sucrose).

The findings reported in this paper have elucidated the effects of some parameters on the *in vitro* germination of macroconidia and secondary conidia of *C. africana*. To our knowledge there are no other reported studies on the influences of temperature, time, and water potential on the germination of secondary conidia, which are considered to be the main source of inoculum of *C. africana* (Bandyopadhyay *et al.* 1996). Apparent differences (present study, Frederickson 1990) in the germination response of macroconidia and secondary conidia of *C. africana* isolates from different countries over time, and to water potential, necessitate further comparative studies. In addition, interactions between temperature, time, relative humidity, and free water on the germination of secondary conidia on stigmas need to be studied.

The information reported in this paper has improved our understanding of some of the critical factors that lead to infection by *C. africana*. It also contributes to our ability to predict epidemics of sorghum ergot in Australia. Warnings of ergot epidemics will reduce the impact of the disease on grain growers, who can reduce contamination of harvested sorghum grain through agronomic practices, and on sorghum

seed producers who can minimise infection of commercial hybrid seed and breeder's lines through the timely application of fungicides (Ryley *et al.* 2001). Information from these studies, together with data from epidemiology studies conducted over the past 4 years in Australia (Ryley *et al.* 2001; MJ Ryley, unpubl. data) will be used to refine the ergot model developed by Wang *et al.* (2000*b*).

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