J. Phytopathology 153, 1–4 (2005)
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ISSN 0931-1785

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Inoculated Host Range and Effect of Host on Morphology and Size of Macroconidia Produced by *Claviceps africana* and *Claviceps sorghi*

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Received April 15, 2004; accepted October 30, 2004

Keywords: *Sphacelia sorghi*, ergot, sorghum, sugary disease

Abstract

Twenty graminaceous plant species were evaluated for their susceptibility to the two sorghum ergot pathogens *Claviceps sorghi* and *Claviceps africana*. Five species viz., *Sorghum arundinaceum*, *Sorghum halepense*, *Sorghum versicolor*, *Sorghum virgatum* and *Pennisetum glaucum* were found to become infected by both pathogens via inoculation with 10^6 conidia/ml. Species which did not become infected under these conditions included *Pennisetum pedicellatum*, *Zea mays*, and species of *Panicum*, *Brachiaria*, *Cenchrus*, *Andropogon*, *Dichanthium*, *Chrysopogon*, *Iseilema*, *Bothriochloa* and *Chloris*. Honeydew secretions were observed from infected flowers of susceptible plant species. There was marked variation in size of macroconidia of both *C. sorghi* and *C. africana* on different hosts on which the pathogens were able to establish symptoms. Dimorphism was observed for macroconidia produced on *P. glaucum*, as elliptical and spindle shaped macroconidia were observed. Based on inoculation under greenhouse conditions, we conclude that *C. sorghi* and *C. africana* may have similar host ranges.

Introduction

Ergot is a serious constraint in sorghum [*Sorghum bicolor* (L.) Moench] hybrid seed production as the disease curtails seed set, which may render seed production uneconomical. The most obvious sign of the disease is the appearance of a spore-laden sticky fluid called honeydew, which exudes from sphacelia that replace infected ovaries. In India, ergot is caused by two different pathogens, *Claviceps sorghi* Kulkarni, Seshadri and Hegde, the native pathogen of India and *Claviceps africana* Frederickson, Mantle and de Milliano, an exotic pathogen first reported from Africa (Bandyopadhyay et al., 2002). Splash-dispersed macroconidia and airborne secondary conidia play a vital role in pathogen dissemination (Bandyopadhyay et al., 1998). Collateral hosts (Futrell and Webster, 1966; Chinnadurai and Govindaswamy, 1971; Bandyopadhyay et al., 1991), sclerotia (Mantle, 1968) and ergot-infected seed lots (Bandyopadhyay et al., 1998) may act as sources of inoculum for new growing seasons. Sclerotial germination is low, therefore collateral hosts may serve as significant inoculum reservoirs for initiation and perpetuation of disease (Bandyopadhyay et al., 1998).

The host range of *C. sorghi* (Reddy et al., 1968; Chinnadurai and Govindaswamy, 1971; Sundaram, 1974; Sangitrao and Moghe, 1995) is variable and contrasting (Bandyopadhyay et al., 1998). No research has been conducted either on the host range of *C. africana* in India (Bandyopadhyay et al., 2002) or on host range comparisons between the two pathogens. Our goal was to identify and evaluate potential collateral hosts of *C. sorghi* and *C. africana* by performing greenhouse inoculations under controlled environmental conditions.

Materials and Methods

These studies were performed at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India. Eight representative isolates of *C. africana* and two isolates of *C. sorghi* were selected from a group of 89 isolates collected during disease surveys in India (Bandyopadhyay et al., 2002). Selected isolates of *C. africana* (and the names of geopolitical states of origin in parenthesis) included NI2 (Uttar Pradesh), NI5 (Uttaranchal), NI12 (Rajasthan), Guj6 (Gujarat), MH71 (Maharashtra), SK-20-24 (Karnataka), AP17 (Andhra Pradesh) and TN13 (Tamil Nadu), while those of *C. sorghi* were NAP7 (Andhra Pradesh) and MH74 (Maharashtra).

To establish initial inoculum of each isolate, sclerotia and sclerotia–sphacelia were removed from infected

panicles collected during field surveys, macerated in a mortar and pestle, and suspended in water to release macroconidia produced in the sphacelial cavities. The resultant inoculum suspension was sprayed onto panicles of male-sterile sorghum genotype 296A at the 50% flowering stage that had been bagged after emergence from boot leaves to avoid contamination with external sources of inoculum. The bags were briefly removed prior to inoculation and replaced immediately after inoculation, and the plants were incubated in a greenhouse at 25°C. Bags were finally removed from the panicles when the first sign of honeydew exudation was noticed. Removal of bags and exposure of panicles to less than 85% relative humidity suppressed secondary conidiation (Bandyopadhyay et al., 1990) that could otherwise contaminate plants. Plants infected with each isolate were maintained in separate greenhouses to further avoid cross-contamination. All inoculation studies for host range and cross-inoculation studies were performed using the bagging and incubation conditions.

Infected rachis containing honeydew were immersed in sterile water for approximately 1 min to allow dispersal of conidia. The suspension was filtered through two layers of cheesecloth and diluted to produce a suspension of 10^6 macroconidia/ml (Puranik and Mathre, 1971; Frederickson et al., 1989). When stigmas of the top 50% spikelets had emerged, plants of sorghum male-sterile line 296A were sprayed to runoff with the conidial suspension (Puranik and Mathre, 1971; Frederickson et al., 1989). Inoculated panicles were covered with paper bags to maintain high relative humidity and avoid external contamination. Inoculated plants were placed in dew chambers at 25°C and 100% relative humidity for 24 h to allow infection. Following dew chamber incubation, the plants were transferred to a greenhouse at 25°C and greater than 80% relative humidity, with paper bags enclosing the panicles. Observation of honeydew formation was made on the eighth day after inoculation by removing the paper bags. Upon commencement of honeydew secretion the infected panicles were kept uncovered to avoid secondary conidiation, which may contaminate the purity of the isolates within the greenhouse. The infected plants were used for further studies pertaining to inoculated host range. Honeydew less than 5-day-old was used as the inoculum source for host range studies.

Twenty graminaceous plant species, including four wild sorghum species were evaluated as potential hosts of *C. africana* and *C. sorghi*. The plant species included: *Sorghum arundinaceum* (Desv.) Stapf, *Sorghum halepense* (L.) Pers., *Sorghum versicolor* (Steud.) Stapf, *Sorghum virgatum* (Hack.) Stapf, *Pennisetum glaucum* (L.) R. Br., *Pennisetum pedicellatum* Trin., *Zea mays* L., *Panicum maximum* Jacq., (cv. Guinea grass), *Panicum maximum* Jacq. (cv. Giant guinea), *Panicum antidotale* Retz., *Brachiaria mutica* (Forsk) Stapf., *Brachiaria decumbens* (Forsk) Stapf, *Cenchrus ciliaris* L., *Cenchrus setigerus* Vahl., *Andropogon gayanus* Kunth, *Dichanthium annulatum* (Forsk.) Stapf, *Chrysopogon fulvus* (Spring.) Chiov., *Iseilema laxum* Hack., *Bothriochloa pertusa* (L.) A. Camus and *Chloris gayana* Kunth.

Test plants were grown in 25-cm diameter plastic pots. Two plants were maintained in each pot and 10 panicles of each test plant species were inoculated with each pathogen isolate following the same bagging and incubation conditions. When stigmas emerged from the first few florets at the tip of the panicles, wild sorghum genotypes and pearl millet were sprayed to runoff with a honeydew suspension containing 10^6 conidia/ml (Puranik and Mathre, 1971; Frederickson et al., 1989), while maize was inoculated by dipping the silk in conidial suspension prior to pollen shed. The other grass hosts were dip-inoculated when the stigmas emerged from the spikelets but before the anthers shed pollen.

To determine the infectivity of conidia produced on grass hosts, honeydew was collected from each of the infected host panicles and cross-inoculated to sorghum male-sterile line 296A when flowering reached 50%. Further, to corroborate the initial observed pathogenicity on test plants, honeydew from cross-inoculated 296A sorghum was again used as inoculum to infect respective test plants. The male-sterile genotype 296A was included as a control in all tests to determine if external sources of inoculum interfered with the experiments. Non-inoculated control plants were also maintained for all species.

The experiments were repeated three times using new sets of maize and pearl millet plants in each experiment. For grass hosts and wild sorghum species, inoculated tillers were removed from the pots after each experiment and a new set of panicles emerging from tillers in the same pot were inoculated in subsequent experiments.

Honeydew collected from inoculated host plants infected with a representative isolate of *C. africana* (NI2) and *C. sorghi* (NAP7) were used to determine the dimensions of macroconidia and microconidia. Honeydew of 3–4-day-old from the test plants was collected for observing macroconidial dimensions, while honeydew of 15–20-day-old was collected for observing microconidial dimensions. In each experiment, shape, size and colour of 50 macroconidia and 50 microconidia from each host infected by isolates of *C. africana* and *C. sorghi* were compared. Conidial dimensions determined from three replicate experiments were analysed using SAS software (SAS Institute, 1999) in a completely randomized design.

Results

Of the 20 plant species tested, only *S. arundinaceum*, *S. halepense*, *S. versicolor*, *S. virgatum* and *P. glaucum* were infected by all isolates of both pathogen species in the three replicate experiments. All infected plants showed typical honeydew symptoms. On all wild sorghum genotypes, honeydew exudation was observed 7 days after inoculation, while on *P. glaucum*, honeydew exudation was observed 10 days after inoculation

Table 1
Conidial measurements of *Claviceps africana* (NI2) and *Claviceps sorghi* (NAP 7) isolates on different hosts

Host	Macroconidia				Microconidia			
	Size[a] (μm)		Shape		Size[a] (μm)		Shape	
	NI2	NAP7	NI2	NAP7	NI2	NAP7	NI2	NAP7
Sorghum arundinaceum	13.60 × 6.97	9.53 × 5.78	Cylindrical	Cylindrical	2.57	2.57	Spherical	Spherical
Sorghum halepense	14.73 × 7.56	9.18 × 4.52	Cylindrical	Cylindrical	2.59	2.72	Spherical	Spherical
Sorghum versicolor	14.66 × 6.72	10.07 × 6.65	Cylindrical	Cylindrical	2.57	2.57	Spherical	Spherical
Sorghum virgatum	17.04 × 7.32	9.48 × 5.91	Cylindrical	Cylindrical	2.57	3.00	Spherical	Spherical
Pennisetum glaucum	11.24 × 7.48	7.45 × 4.88	Elliptical or spindle	Elliptical or spindle	3.00	2.57	Spherical	Spherical
Sorghum bicolor	15.14 × 7.92	13.06 × 6.25	Oblong to ellipsoidal	Spindle or cylindrical	2.78	2.59	Spherical	Spherical
± SE[b]	1.81 × 0.13	0.99 × 0.52			0.04	0.03		
LSD[c]	5.05 × 0.36	3.98 × 0.41			0.12	0.27		
Coefficient of variation (%)	14.5 × 12.7	16.3 × 14.7			11.9	12.4		

[a]Mean of 50 conidia per repetition; three replicates were conducted;
[b]Standard error of the mean based on measurements in three repeated tests;
[c]Least significant difference (LSD) value; P = 0.05.

with all isolates of the two pathogen species. There was no observed difference in incubation period between the two pathogen species on a given host. Profuse exudation of honeydew was observed on wild sorghum species, but not on *P. glaucum*. The consistency of honeydew was very thick in all infected plants tested. Sclerotial formation was not observed on any of the five infected hosts and on the sorghum male-sterile line 296A. Non-inoculated control plants of all species were disease-free as the experiments were conducted during the dry (postrainy) season when natural dispersal of the pathogen through secondary conidia and macroconidia does not occur.

Size of macroconidia of the *C. africana* isolate NI2 was similar on all wild sorghum hosts and cultivated sorghum *S. bicolor* (Table 1). On all hosts, macroconidia of *C. sorghi* isolate NAP7 were smaller than those of *C. africana*. The shape of macroconidia of *C. sorghi* and *C. africana* varied on *S. bicolor* but was similar on all four wild sorghum hosts. For both isolates, the shape of macroconidia differed on cultivated sorghum compared to wild sorghum hosts, but there was no variation observed with respect to the size and shape of the microconidia. For both *Claviceps* species, macroconidia were smaller on *P. glaucum* than on the five *Sorghum* species tested. Dimorphism was observed within macroconidia on *P. glaucum*; some macroconidia were elliptical while others were elongated or spindle shaped. However, no variation was observed with respect to the size and shape of microconidia produced on *P. glaucum*.

Discussion

Although *C. sorghi* and *C. africana* differ with respect to their cultural characteristics such as morphology of sexual and asexual spores, secondary conidia production, rate of disease spread and amount of dihydroergosine in sclerotia (Bandyopadhyay et al., 2002), we failed to detect variation in susceptibility of graminaceous plants to these two pathogen species. Of the 20 graminaceous hosts tested, only five viz., *P. glaucum*, *S. arundinacium*, *S. halepense*, *S. versicolor* and *S. virgatum* were infected by both *Claviceps* species. Ramakrishnan (1947); Tarr (1962); Loveless (1964); Sundaram (1970, 1974; Boon-Long (1992) and Alderman et al. (1999) also reported infection of these hosts by *Sphacelia sorghi*. In contrast to the reports of Futrell and Webster (1966) and Chinnadurai and Govindaswamy (1971), *Z. mays*, *C. ciliaris* and *C. setigerus* were non-hosts of the sorghum ergot pathogens in our studies. Also in our studies, *P. maximum*, *P. antidotale* and *D. annulatum* were found to be non-hosts of *C. sorghi*, which is in agreement with the work of Chinnadurai and Govindaswamy (1971) and Sundaram (1970), but contrasts with that of Futrell and Webster (1966); Molefe (1975); Boon-Long (1992) and Sangitrao and Moghe (1995). In our study, we did not specifically look for natural infection of ergot on any of the test plants that showed infection following inoculation. However, several earlier reports suggest natural infection of *Sp. sorghi* can occur on several collateral hosts such as *P. glaucum* (Sundaram, 1970), *Dichanthium caricosum*, *D. annulatum*, *Ischaemum pilosum* and *Sehima nervosum* (Sangitrao and Moghe, 1995).

Reed et al. (2002) reported infection by *C. africana* on certain accessions of wild sorghum genotypes such as *S. bicolor* ssp. *verticilliflorum* (Steud.) Piper., *S. bicolor* ssp. *arundinaceum* (Steud.), *S. bicolor* ssp. *drummondi* (Steud.) and *S. halepense* ssp. *almum*. However, in their study the tests plants such as *P. glaucum* (GenBank accession no. NPM-1), and some accessions of *S. bicolor* ssp. *arundinaceum* (GenBank accession nos IS14257, IS14357, IS14301, PI185574) and *S. bicolor* ssp. *drummondii* (GenBank accession no. IS14131), *Panicum miliaceum*, *P. virgatum*, *Andropogon gerardii*, *Andropogon scoparius* were found to be highly resistant to inoculation with *C. africana*. The non-occurrence of disease in their study on *P. glaucum* (GenBank accession no. NPM-1) may be because of its genetic make-up expressing high level of resistance to the pathogen. The probable role of *Pennisetum* sp. as a collateral

host for *S. sorghi* was reported earlier by Reddy et al. (1968); Sundaram (1970); Molefe (1975); Bandyopadhyay et al. (1992) and Alderman et al. (1999). However, Alderman et al. (1999) reported that the disease on *P. glaucum* did not occur under field conditions. Thus, reports describing collateral hosts of the sorghum ergot pathogens appear inconsistent.

Conidial size and shape of *C. sorghi* were altered when passed through different hosts. The shape of the macroconidia on wild sorghum genotypes showed limited variation except that conidia were elongated and thinner than those on sorghum. Macroconidia on *P. glaucum* showed dimorphism as some macroconidia were elliptical while others were elongated or spindle shaped. This finding was reported earlier by Chinnadurai and Govindaswamy (1971) in describing macroconida of *Sp. sorghi* on maize. Macroconidia of the pearl millet ergot fungus *Claviceps fusiformis* are longer and more slender (15.9×3.9 μm) (Thakur et al., 1984) than *C. sorghi* and *C. africana* suggesting that ergot infection in our experiments were not due to *C. fusiformis*.

As inoculum from sorghum could infect wild sorghum species and pearl millet and similarly, inoculum from these hosts can infect sorghum without loss of pathogenicity, these collateral hosts could act as important potential sources of inoculum which could initiate and perpetuate disease both within a single growing season and between different growing seasons. Thus, our studies with these potential collateral hosts reinforce suggestions that removal of known collateral hosts could represent a key component in managing sorghum ergot.

Acknowledgements

This publication is based upon work supported in part by the US Department of Agriculture, under special agreement no. 58-1920-9-F110. Any opinions, findings, conclusions or recommendations are those of the authors and do not necessarily reflect the view of the US Department of Agriculture.

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