Effect of Storage Temperature and Sphaelium Age on *Claviceps africana* Conidia Survival

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Abstract: Sorghum ergot, caused by *Claviceps africana* Frederickson, Mantle and de Milliano, is a disease that poses a serious threat to sorghum, especially in hybrid seed production. The initial sign of the disease is called sphaelium that contains macroconidia that could play a role in the survival of the pathogen. Sorghum A-line ATx623 was planted in the greenhouse during 2001, 2002 and 2003 at College Station, Texas. Flowering panicles were inoculated until runoff with a suspension of 1.5 x 10^6 *C. africana* conidia mL^-1. Sphaelium were collected at several stages depending on their maturity. Petri dish plates containing sphaelium were arranged in a factorial experiment with 16 treatments out of the combination of sphaelium maturity and temperature. Every month a conidia germination test was made. Conidia located on the sphaelium surface had greater germination than the conidia located inside the sphaelium. This may be due to the developmental maturity of the conidia located on the outside. Warmer storage temperatures (21°C) significantly reduced conidia viability compared with freezing or cool temperatures. Dry and cool temperatures are required to preserve conidia viability and newly-formed sphaelium have the highest conidial viability especially if conidia are located on the sphaelium surface. However, they show a greater viability reduction through time compared with conidia from older sphaelium, showing that conidial maturity can play a role on the survival of the conidia.

Key words: Sorghum, ergot, macroconidia, survival, sphaelium

INTRODUCTION

Losses due to sorghum ergot caused by *Claviceps africana* Frederickson, Mantle and de Milliano in seed production fields can be high. In India, losses up to 80% have been reported in seed production fields whereas in Zimbabwe the annual losses are between 12 and 25% and sometimes up to 100% (Bandyopadhyay et al., 1998). In 1997, nearly 45% of the hybrid seed production fields in the Texas Panhandle had ergot with varying degrees of severity (Workneh and Rush, 2003). Losses from import rejection can occur. For example, in 1999, the Nicaraguan Inspection and Certification Department intercepted seeds with honeydew and sphaelium tissues mixed with seed in a shipment from the USA. This shipment was quarantined, resulting in losses of millions of dollars to seed companies.

Usually, this disease is not important in hybrid grain sorghum fields. Losses of seed quality can be an issue, because of honeydew contamination of healthy sorghum grain, increasing colonization by saprophytic fungi. McLaren (1992) found such seed had reduced germination. In addition, honeydew stickiness can interfere with harvest.

Many pathogenic ascomycetes that produce resting structures generate ascospores following aecidial germination. There are differences among the maturity of such structures and their capability to survive. Within a crop, *C. africana* produces sphaelium, or perhaps also sclerotia. At harvest, sphaelium differ in age or have different degrees of sclerotial tissue development. Survival of the pathogen may be affected by the level of fungal development or by environmental conditions. Bhuyan et al. (2002b) showed that *C. africana* macroconidia present in sorghum panicles that were held above soil surface survived for more than eight months over winter, suggesting that local survival can provide inoculum for future epidemics in Australia. Storage of sphaelium at high temperature (>32°C) resulted in a rapid decrease in viability of *C. africana* macroconidia with no spores viable after two weeks of storage. Conidia germinated after 17 weeks storage at 20°C.

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cool temperatures (6°C) were evaluated by Odvody et al. (1999), who observed that conidia of newly-formed sphaecelia maintained viability at its maximum up to 12 weeks and then decreased 50% at 22 weeks of storage. In another study, Prom et al. (2005) showed that conidia located on sphaecelia that were held above soil surface for a year survived and infected sorghum florets on male-sterile line ATx623.

The problem of honeydew on the surface of sorghum seeds had been addressed by Dahlberg et al. (1999), who found that contact fungicides captan (Captan 400SH) and thiram (42-S ThiramSH) were effective in inhibiting conidiophores and secondary conidia formation without drastically reducing the viability of the sorghum seed (1-4%). Frederickson and Odvody (2003) observed that conidia viability of newly intact sphaecelia treated with captan (Captan 400SH) was significantly reduced (63%) and cores from treated sphaecelia did not show a major reducton compared with the control. They suggested that this could be due to the slight penetration ability of captan within the sphaecelia or desiccation of the sphaecelia.

In the sorghum hybrid seed production industry, the main goal is to obtain and maintain sorghum seed with high viability. Nevertheless, viability is highly influenced by storage conditions. The most critical conditions for seed in storage are low seed moisture and low temperature. In addition to these factors, seeds need to be free from inert material such as plant debris that could contain pathogens and insects.

Pathogens that are carried in seed lots may be either internally seed-borne or present on the seed surface, in plant debris, or infected weed seeds. These pathogens can survive long periods of storage along with dried seeds in a dormant stage and usually do not resume activity until seeds germinate (Gerard, 1984; Gilbert et al., 1997). Bhuian et al. (2002b) demonstrated that macroconidia of C. africana can survive on honeydew-coated seed for more than 12 months at 4°C (42-100% RH), suggesting that international seed exchange was a possible route for the accidental introduction of this pathogen to Australia (Komolong et al., 2002). Since ergot was detected during 1997 in the Texas seed production area, it is possible that pathogen structures could be spread with shipments exported overseas, possibly to sorghum producing areas that are free of the pathogen. Ellas (1984) reports that temperature has a dramatic effect on seed longevity and concluded that each 5°C reduction in seed temperature doubles the life of seeds. However, low temperatures can maintain pathogen viability. The objectives of this study were to determine the effect of storage temperature on the viability of C. africana macroconidia located on the surface and within the sphaecelum and to observe the effect of sphaecelum age on the macroconidial survival.

**MATERIALS AND METHODS**

Sorghum A-line ATx623 was planted in the greenhouse during 2001, 2002 and 2003 at College Station, Texas. Conidia were collected from a local C. africana isolate that was fresh maintained under greenhouse conditions in College Station. The greenhouse conditions to increase the inoculum were above 80% relative humidity and 30°C. Flowering panicles of ATx623 were tagged and inoculated with the local isolate by hand atomizer until runoff with the suspension of 1·6·10^6 C. africana conidia mL⁻¹. Several panicles were selected according to their sphaecelial development 7 to 10 days after inoculation. The greenhouse conditions during the development of the sphaecelum were 50% relative humidity and 30°C. Sphaecelia were collected at several stages depending on their maturity. Sphaecelial structures were grouped into four maturity classes based on sphaecelum development:

**Class 1:** Newly-formed sphaecelia showing slightly transparent honeydew ooze.

**Class 2:** One-week-old sphaecelia showing high quantities of transparent honeydew ooze.

**Class 3:** Two-week-old sphaecelia with dark-brown dried honeydew.

**Class 4:** Three-week-old sphaecelia showing hardness on the sphaecelia surface and honeydew crust.

Sphaecelia, attached to the panicle rachis were placed in petri dishes containing color silica gel (as a desiccant). Every time that the silica gel showed changes in color, it was changed for a new one (this was done to ensure that the relative humidity inside the dish plate was low). Dishes were sealed with parafilm. The incubation temperatures of sphaecelia were fluctuating sub-freezing (0 to -3°C), 7, 14 and 21°C. Dishes were arranged in a factorial experiment with 16 treatments out of the combination of sphaecelum maturity (four levels) and temperature (four levels); each plot was replicated four times in a randomized complete design. The model used was:

\[ Y_{ik} = \mu + \alpha_i + \beta_j + \epsilon_{ik} \]
Where $\mu$ is the overall mean conidia germination, $\alpha_i$ is the effect of the $i$th level of temperature, $\beta_j$ is the effect of the $j$th level of sphaecium age and $\alpha\beta_{ij}$ is the interaction effect of the $i$th level of temperature with the $j$th level of sphaecium age. Conidia survival was measured as the proportion of germinated macroconidia showing conidiophore formation with secondary conidia at their tips and was measured almost every month by sampling macroconidia located on the sphaecium surface and within the sphaecium interior. A random sample of 20 sphaecia was taken from each one of the treatments and after removing plant tissue, was placed into vials containing 20 mL distilled water. Vials were stirred for one minute and a portion of the suspension (1 mL) was placed onto water-agar plates. Four replications per treatment were made and incubated overnight at room temperature (21°C). The germination observed in this sample was named germination on the sphaecium surface. After rinsing the remaining sphaecia with a jet of water for 30 sec, they were macerated using a mortar, suspended in 10 mL distilled water, stirred for 30 sec and placed onto water-agar plates. The germination percentage obtained here was named within the sphaecium. Original data was transformed using the arcsine of the square root of each value to comply with normality distribution assumptions. To determine significant differences between means, Tukey’s mean separation was used at $p<0.01$. A Chi square test was performed to see if the variances between years were homogeneous.

RESULTS

Out of the three years of study, two variances were homogeneous. By this result the data for the three year study was combined. There was a highly significant effect of the main factor temperature across the six month study on germination of *Claviceps africana* conidia located on the sphaecium surface, while sphaecium age had a highly significant effect up to the fifth month, same as the interaction between these two factors (Table 1). Almost the same results were obtained in the ANOVA table for germination of conidia located within the sphaecium, that was affected by both factors up to the fifth month (Table 2). Warmer storage temperatures (21°C) significantly reduced germination across the 6-month period in conidia located on the sphaecium surface. The reduction ranged from 42 to 100% compared with frozen temperatures and from 26 to 100% compared with cool temperatures (7°C). At the end of the 6-month study, frozen temperatures show the highest significant conidia germination, with a reduction of 59% in that period. Conidia on younger sphaecium showed significantly more germination (47-65%) compared with older sphaecium conidia in the first 3-month period. However, this situation was reversed in the second 3-month period where newly-formed sphaecia gave the lowest conidia germination. At the end of the 6-month trial, all sphaecial ages gave statistically the same conidia germination on the sphaecium surface (Table 3). Identical situations were observed in conidia located within the sphaecium, where the warmer treatment reduced conidia viability from 46 to 100% compared with frozen temperatures and newly formed sphaecia showed 42 to 73% more conidia germination than older sphaecia in the first 3-month period. After that, conidia germination was statistically similar in both sphaecial ages (Table 4).

Comparing the average germination across years and dates in both conidia locations, conidia from the sphaecium surface had more germination at all levels of storage temperature and sphaecium age (Table 5) than

Table 1: Observed mean squares and test of significance of main factors on germination of *C. africana* conidia located on the sphaecium surface

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (A)</td>
<td>3</td>
<td>0.444**</td>
<td>0.794**</td>
<td>2.202**</td>
<td>0.664**</td>
<td>0.604**</td>
<td>0.923**</td>
</tr>
<tr>
<td>Age (B)</td>
<td>3</td>
<td>0.908**</td>
<td>0.719**</td>
<td>0.308**</td>
<td>0.069**</td>
<td>0.054**</td>
<td>0.055ns</td>
</tr>
<tr>
<td>A x B</td>
<td>9</td>
<td>0.140**</td>
<td>0.089**</td>
<td>0.097**</td>
<td>0.147**</td>
<td>0.063**</td>
<td>0.029ns</td>
</tr>
<tr>
<td>Error</td>
<td>176</td>
<td>0.055</td>
<td>0.032</td>
<td>0.032</td>
<td>0.032</td>
<td>0.001</td>
<td>0.026</td>
</tr>
</tbody>
</table>

** = Highly significant effect at $p<0.01$; * = Significant effect at $p<0.05$; ns = Not significant

Table 2: Observed mean squares and test of significance of main factors on germination of *C. africana* conidia located within the sphaecium

<table>
<thead>
<tr>
<th>Source</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (A)</td>
<td>3</td>
<td>0.614**</td>
<td>0.544**</td>
<td>1.199**</td>
<td>0.556**</td>
<td>0.431**</td>
<td>1.009**</td>
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<tr>
<td>Age (B)</td>
<td>3</td>
<td>0.648**</td>
<td>0.377**</td>
<td>0.078**</td>
<td>0.058**</td>
<td>0.033**</td>
<td>0.004ns</td>
</tr>
<tr>
<td>A x B</td>
<td>9</td>
<td>0.090**</td>
<td>0.162**</td>
<td>0.062**</td>
<td>0.108**</td>
<td>0.053**</td>
<td>0.032**</td>
</tr>
<tr>
<td>Error</td>
<td>176</td>
<td>0.035</td>
<td>0.033</td>
<td>0.021</td>
<td>0.003</td>
<td>0.0013</td>
<td>0.026</td>
</tr>
</tbody>
</table>

** = Highly significant effect at $p<0.01$; ns = Not significant
interior conidia. Conidia from within the sphaecium and the sphaecium surface stored at warmer conditions showed a significant reduction of 75 and 73.5%, respectively compared with frozen temperatures in the same location. Also, conidia from younger sphaecium showed 50 to 53% more germination than conidia from older sphaecium in both conidia locations. The combined analysis showed significant differences among storage temperatures at each sphaecium age. At all sphaecia ages, conidial germination decreased as temperature increased from 0 to 21°C (Table 6). This suggests that temperatures at locations with summer-fall planting dates could promote survival of conidia outside and inside the sphaecia, creating a viable source of inoculum for the next crop season.

The sphaecial age effect on conidia germination showed that conidia from the surface of newly formed sphaeciae had statistically more germination than the other sphaecia ages during the first three months and then decreased after this time. During the 6-month period, newly-formed sphaeciae had half the conidial germination of the older sphaecia for every unit increase of time. Viability of conidia located within newly-formed sphaeciae was higher during the first two months. Older sphaeciae had lower viability of conidia up to the fourth month. Conidia on newly-formed sphaeciae decline in viability three times faster than the oldest sphaeciae. Cooler
storage temperatures showed significantly highest viability values through the 6-month period with 42 to 99% higher than the warmest temperature. Conidial germination rate declined 5 times faster at the warmest storage temperature as compared with the coolest. Conidial viability at the 21°C treatment was nil at sixth month, while conidia viability was more than 10% at cooler storage after the sixth month. Similar trends were observed with conidia from within the sphaecelia. However, the viability was lower at all the storage temperatures. The warmest storage temperature showed 4.5 times more reduction in the conidial germination as compared with the coolest and twice compared with the 14°C.

In general, the interaction showed that conidia from younger sphaecelia maintained statistically a high viability if they were exposed to cool temperatures of 0 to 7°C (Table 6).

DISCUSSION

This study shows that survival of conidia of older sphaecelia, which are most common during harvest of commercial or seed production fields, is very sensitive to the warmer temperatures that would be present during the summer in spring-planted sorghum production areas. Where cooler temperatures prevail following a crop (e.g., with summer or fall-planted crops), conidia may survive longer, perhaps contributing to local survival of inoculum for the next crop season. Conidia located on the sphaecelium surface had greater germination than the conidia located inside the sphaecelium. This may be due to the developmental maturity of the conidia located on the outside. Warmer storage temperatures (21°C) significantly reduced conidia viability compared with freezing or cool temperatures (<21°C). Dry and cool temperatures are required to preserve conidia viability and newly-formed sphaecelia have the highest conidial viability especially if conidia are located on the sphaecelium surface. Averaged over all sphaecelia ages, conidial viability decreased as temperature increased from 0 to 21°C (r = -0.75 at p<0.0009). Similar results obtained by Odvody et al. (1999) showed that C. africana macroconidia maintained viability stored at 6°C, with a maximum up to 12 weeks and then decreased 50% at 22 weeks of storage, whereas Bhuiyan et al. (2002b) showed that storage of sphaecelia at high temperature (>32°C) resulted in a rapid decrease in viability of C. africana macroconidia, with no spores viable after two weeks of storage.

Conidia from the surface of newly-formed sphaecelia had statistically more germination than the other sphaecelial ages during the first three months. During the 6-month period, newly-formed sphaecelia had a reduction in conidial viability of twice the value of the older sphaecelia for every increase unit of time. Conidia viability at the 21°C treatment declined to zero at the sixth month, while conidial viability at cooler temperatures were more than 10% at the sixth month. Similar trends were observed with the conidia from within the sphaecelia. Conidial viability at the highest storage temperature was eliminated at the sixth month. These results are similar to those of Bhuiyan et al. (2002a), who found that C. africana conidia showed little germination after 17 weeks storage at 20°C. Also, conidia survived for more than eight months stored outside over the winter months. These results support those of Prom et al. (2005), who showed that conidia could be viable up to 12 months under field conditions in Texas. Therefore, we can conclude that environmental conditions affecting viability of sphaecelia stored under cool temperatures maintained conidial viability and newly-formed sphaecelia located on the sphaecelium surface had the highest conidial viability. However, they show a greater viability reduction through time compared with conidia from older sphaecelia, showing that conidial maturity can play a role on the survival of the conidia and perhaps in the new infections that will develop on the following crop season.

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