Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 6 (4): 312-317, 2007
ISSN 1812-5387

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

[1]Noé Montes-García, [2]Thomas Isakeit, [3]Gary N. Odvody, [4]Louis K. Prom and [2]William L. Ronney
[1]INIFAP-CERIB, Apdo. Postal 172, Río Bravo, Tamaulipas, CP 88900, México
[2]2132 TAMU, College Station, Texas 77845
[3]TAMU Agricultural Research and Ext. Center, Rt 2, Box 589, Corpus Christi, TX, 78406
[4]USDA-ARS, 2765 F and B Road, College Station, TX 77845

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 sphacelium 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 1.6×10^6 *C. africana* conidia mL^{-1}. Sphacelia were collected at several stages depending on their maturity. Petri dish plates containing sphacelia were arranged in a factorial experiment with 16 treatments out of the combination of sphacelia maturity and temperature. Every month a conidia germination test was made. Conidia located on the sphacelium surface had greater germination than the conidia located inside the sphacelium. 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 sphacelia have the highest conidial viability especially if conidia are located on the sphacelium surface. However, they show a greater viability reduction through time compared with conidia from older sphacelia, showing that conidial maturity can play a role on the survival of the conidia.

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

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 sphacelial 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 carpogenic germination. There are differences among the maturity of such structures and their capability to survive. Within a crop, *C. africana* produces sphacelia, or perhaps also sclerotia. At harvest, sphacelia 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. Bhuiyan *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 sphacelia 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. The effect of

Corresponding Author: Noe Montes-García, INIFAP-CERIB, Apdo. Postal 172, Río Bravo, Tamaulipas, CP 88900, México
Tel: (956) 279-6072

cool temperatures (6°C) were evaluated by Odvody *et al.* (1999), who observed that conidia of newly-formed sphacelia maintained viability at its maximum up to 12 weeks and then decreased 50% at 22 weeks of storage. In other study, Prom *et al.* (2005) showed that conidia located on sphacelia 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 400®) and thiram (42-S Thiram®) were effective in inhibiting conidiophore 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 sphacelia treated with captan (Captan 400®) was significantly reduced (63%) and cores from treated sphacelia did not show a major reduction compared with the control. They suggested that this could be due to the slight penetration ability of captan within the sphacelia or desiccation of the sphacelia.

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). Bhuiyan *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. Ellis (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 sphacelium and to observe the effect of sphacelium 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 sphacelial development 7 to 10 days after inoculation. The greenhouse conditions during the development of the sphacelia were 50% relative humidity and 30°C. Sphacelia were collected at several stages depending on their maturity. Sphacelial structures were grouped into four maturity classes based on sphacelium development:

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

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

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

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

Sphacelia, attached to the panicle rachis were placed in petri dishes containing color silica gel (as a dessicant). 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 sphacelia 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 sphacelia maturity (four levels) and temperature (four levels); each plot was replicated four times in a randomized complete design. The model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Where μ is the overall mean conidia germination, α_i is the effect of the ith level of temperature, β_j is the effect of the jth level of sphacelia age and $\alpha\beta_{ij}$ is the interaction effect of the ith level of temperature with the jth level of sphacelia 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 sphacelium surface and within the sphacelium interior. A random sample of 20 sphacelia 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 sphacelium surface. After rinsing the remaining sphacelia 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 sphacelium. 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 sphacelium surface, while sphacelium 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 sphacelium, 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 sphacelium 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 sphacelium showed significantly more germination (47-65%) compared with older sphacelium conidia in the first 3-month period. However, this situation was reversed in the second 3-month period where newly-formed sphacelium gave the lowest conidia germination. At the end of the 6-month trial, all sphacelial ages gave statistically the same conidia germination on the sphacelium surface (Table 3). Identical situations were observed in conidia located within the sphacelium, where the warmer treatment reduced conidia viability from 46 to 100% compared with frozen temperatures and newly formed sphacelia showed 42 to 73% more conidia germination than older sphacelia in the first 3-month period. After that, conidia germination was statistically similar in both sphacelial ages (Table 4).

Comparing the average germination across years and dates in both conidia locations, conidia from the sphacelium surface had more germination at all levels of storage temperature and sphacelium 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 sphacelium surface

		Months					
Source	df	1	2	3	4	5	6
Temp (A)	3	0.444**	0.794**	2.202**	0.664**	0.604**	0.923**
Age (B)	3	0.968**	0.719**	0.308**	0.069**	0.054*	0.055ns
A×B	9	0.140**	0.089**	0.097**	0.147**	0.063**	0.029ns
Error	176	0.055	0.032	0.032	0.032	0.001	0.026

** = 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 sphacelium

		Months					
Source	df	1	2	3	4	5	6
Temp (A)	3	0.614**	0.544**	1.199**	0.550**	0.431**	1.009**
Age (B)	3	0.648**	0.377**	0.078**	0.058**	0.033**	0.004ns
A×B	9	0.090**	0.162**	0.062**	0.108**	0.053**	0.032**
Error	176	0.035	0.033	0.021	0.003	0.0013	0.026

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

Table 3: Effect of main factors on the average *C. africana* conidia germination located on the sphacelium surface

	Months					
Factor	1	2	3	4	5	6
Storage temperature (°C)						
0	40.50a*	32.19a	31.96a	20.06a	18.94a	16.53a
7	31.75a	28.94a	23.29b	19.75a	18.06a	9.31b
14	31.39a	26.16a	10.79c	12.06b	9.19b	2.56c
21	23.35b	7.16b	2.98d	0.13c	0.25c	0.00c
Sphacelium age (days)						
0	47.87a	37.84a	22.50a	7.63c	6.06b	5.13a
7	30.75b	22.94b	17.89b	17.75a	12.44a	11.22a
14	28.35bc	20.75b	16.81b	15.44ab	14.31a	7.19a
21	20.02c	12.91c	11.81b	11.19b	13.63a	4.88a

* = Treatments with the same letter(s) in each category are statistically similar according to Tukey ($p<0.01$)

Table 4: Effect of main factors on the average *C. africana* conidia germination (%) located within the sphacelium

	Months					
Factor	1	2	3	4	5	6
Storage temperature (°C)						
0	24.52a*	25.19a	17.02a	13.06c	15.19a	11.03a
7	28.31a	18.53a	17.02a	26.00a	12.75b	12.59a
14	30.06a	18.94a	10.91b	17.06b	3.00c	2.03b
21	13.21b	6.09b	1.52c	2.19d	0.50d	0.00c
Sphacelium age (days)						
0	36.19a	20.06a	13.33a	11.56c	11.25a	5.97a
7	25.44b	23.63a	12.75ab	17.88a	8.75ab	6.53a
14	21.58b	19.56a	12.67ab	16.19b	5.56c	6.63a
21	12.89c	5.50b	7.73b	12.69bc	5.88b	6.63a

* = Treatments with the same letter(s) in each category are statistically similar according to Tukey ($p<0.01$)

Table 5: Effect of storage temperature and sphacelium age on germination of *C. africana* conidia located on the surface and within the sphacelial tissue

Factor	Sphacelium surface	Within sphacelium
Storage temperature (°C)		
0	29.48a*	19.75a
7	23.29b	18.77ab
14	17.10c	15.45b
21	7.81d	4.92c
Sphacelium age (days)		
0	25.89a	18.62a
7	20.37b	16.79ab
14	18.43b	14.74b
21	12.99c	8.71c

* = Treatments with the same letter(s) in each category are statistically similar according to Tukey ($p<0.01$)

Table 6: Effect of the interaction between sphacelium age and storage temperature on germination of *C. africana* conidia

Temperature (°C)	Age	Germination (%)
0	0	28.65
0	7	26.37
0	14	28.07
0	21	13.44
7	0	29.73
7	7	14.89
7	14	22.04
7	21	19.40
14	0	21.66
14	7	24.17
14	14	12.48
14	21	6.73
21	0	9.00
21	7	8.90
21	14	3.74
21	21	3.83

interior conidia. Conidia from within the sphacelium and the sphacelium 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 sphacelium showed 50 to 53% more germination than conidia from older sphacelium in both conidia locations. The combined analysis showed significant differences among storage temperatures at each sphacelium age. At all sphacelia 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 sphacelia, creating a viable source of inoculum for the next crop season.

The sphacelial age effect on conidia germination showed that conidia from the surface of newly formed sphacelium had statistically more germination than the other sphacelia ages during the first three months and then decreased after this time. During the 6-month period, newly-formed sphacelia had half the conidial germination of the older sphacelia for every unit increase of time. Viability of conidia located within newly-formed sphacelia was higher during the first two months. Older sphacelia had lower viability of conidia up to the fourth month. Conidia on newly-formed sphacelia decline in viability three times faster than the oldest sphacelia. 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 sphacelia. 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 sphacelia 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 sphacelia, 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 sphacelium surface had greater germination than the conidia located inside the sphacelium. 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 sphacelia have the highest conidial viability especially if conidia are located on the sphacelium surface. Averaged over all sphacelia 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 sphacelia 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 sphacelia had statistically more germination than the other sphacelial ages during the first three months. During the 6-month period, newly-formed sphacelia had a reduction in conidial viability of twice the value of the older sphacelia 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 sphacelia. 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 sphacelia stored under cool temperatures maintained conidial viability and newly-formed sphacelia located on the sphacelia surface had the highest conidial viability. However, they show a greater viability reduction through time compared with conidia from older sphacelia, 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.

REFERENCES

Bandyopadhyay, R., D.E. Frederickson, N.W. McLaren, G.N. Odvody and M.J. Ryley, 1998. Ergot: A new disease threat to sorghum in the Americas and Australia. Plant Dis., 82: 356-367.

Bhuiyan, S.A., V.J. Galea, M.J. Ryley, D. Tay and A.T. Lisle, 2002a. Factors influencing the germination of macroconidia and secondary conidia of *Claviceps africana*. Aust. J. Agric. Res., 53: 1087-1094.

Bhuiyan, S.A., M.J. Ryley, V.J. Galea, D. Tay and A.T. Lisle, 2002b. Survival of conidia of sorghum ergot (caused by *Claviceps africana*) on panicles, seed and soil in Australia. Australasian Plant Pathol., 31: 137-141.

Dahlberg, J.A., G.L. Peterson, G.N. Odvody and M. Bonde, 1999. Inhibition of germination and sporulation of *Claviceps africana* from honeydew encrusted sorghum with seed treatment fungicides. Crop Prot., 18: 235-238.

Ellis, R.H., 1984. The meaning of viability. In: Seed Management Techniques for Gene Banks. IBPGR/84/68, pp: 146-181.

Frederickson, D.E. and G.N. Odvody, 2003. Inhibition of germination of sphacelial conidia of *Claviceps africana* following treatment of seed-sphacelia admixtures with captan. Crop Prot., 22: 95-98.

Gerard, B.M., 1984. Improved monitoring tests for seed-borne pathogens and pests. In: Seed Management Techniques for Gene Banks. IBPGR/84/68, pp: 22-41.

Gilbert, J., A. Tekauz and S.M. Woods, 1997. Effect of storage on viability of fusarium head blight-affected spring wheat seed. Plant Dis., 81: 159-162.

Komolong, B., S. Chakraborty, M. Ryley and D. Yates, 2002. Identity and genetic diversity of the sorghum ergot pathogen in Australia. Agron. J. Agric. Res., 53: 621-628.

McLaren, N.W., 1992. Quantifying resistance of sorghum genotypes to the sugary disease pathogen (*Claviceps africana*). Plant Dis., 76: 986-988.

Odvody, G.N., D.E. Frederickson, T. Isakeit, J.A. Dahlberg and G.L. Peterson, 1999. The role of seedborne inoculum in sorghum ergot. In: Proc. 3rd International Seed Testing Assoc. Seed Health Symposium, Ames, IA., pp: 136-140.

Prom, L.K., T. Isakeit, G.N. Odvody, C.M. Rush, H.W. Kaufman and N. Montes, 2005. Survival of *C. africana* within sorghum panicles at several Texas locations. Plant Dis., 89: 39-43.

Workneh, F. and C.M. Rush, 2003. Status of sorghum ergot in the Texas Panhandle and efforts towards development of a risk forecasting model. In: Proceedings of the 23rd Biennial Sorghum Industry Conference, pp: 47.