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Inhibition of germination and sporulation of *Claviceps africana* from honeydew encrusted sorghum with seed treatment fungicides

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Abstract

The inhibitory effects of six fungicides on macrospore germination and production of secondary conidia of *Claviceps africana* were studied. Effects of these fungicides on germination of sorghum seed also were determined. Panicles from male-sterile sorghums with honeydew exudate were harvested from the USDA-ARS Research Farm, Isabela, Puerto Rico in the Spring of 1997. Honeydew exudate-infested panicles were used as inoculum to infest ergot-free sorghum seed. Seeds were placed on 1 and 2% water agar containing 100 mg/l each of streptomycin sulfate and ampicillin and were also plated on soil. They were incubated at 21–22°C, 16 h of light. At 24 and 48 h, samples were examined under a microscope, equipped with 50× and 100× objectives, for germination of macroconidia and the formation of conidiophores and secondary conidia on both seed, soil, and agar surfaces. Samples also were evaluated for seed germination. Abundant conidiophores with conidia were observed over the entire seed surface of (control) honeydew-infested seed while none were observed on untreated control seed. Fungicidal effects and seed germination results showed that captan (Captan 400®) and thiram (42-S Thiram®) effectively inhibited conidiophore production and secondary conidia formation without severely reducing viability of the sorghum seed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Sorghum; Macrospore; Conidiophores; Honeydew; Secondary conidia; Ergot

1. Introduction

In mid-1995, sorghum [*Sorghum bicolor* (L.) Moench] ergot, caused by *Claviceps africana* Frederickson, Mantle and de Milliano, was first reported in the Americas in Brazil (Reis et al., 1996). Ergot disseminated approximately 8000 km north and consequently has been reported in the Caribbean, Mexico, Texas, Georgia, Nebraska, Mississippi, New Mexico and Kansas within the last two years. Spores of *C. africana* colonize and infect the stigmas of sorghum flowers and convert the ovaries into a sphacelium, which exudes copious honeydew, containing vast numbers of macroconidia and microconidia. Under humid conditions, macroconidia germinate within a few hours to form aerial secondary conidia that can be spread and rapidly disseminated by wind (Bandyopadhyay et al., 1996). The ability of the macroconidia to produce secondary conidia is considered an epidemiologically significant parameter to determine the viability of the macroconidia present in the honeydew.

In the continental US, sorghum is grown in the field only during the summer months. To provide additional generations per year for breeding programs, both the public and private US sorghum industry utilizes major winter nurseries in tropical locations. Major winter nursery sites utilized in Puerto Rico, Jamaica, Dominican Republic, and Mexico reported occurrences of sorghum ergot in the Spring of 1997. The economic impact of this disease was felt immediately. Several research programs lost a full breeding cycle due to a primarily self-imposed ban on importing seed from those countries where ergot was confirmed. Consequently, it was estimated that ergot cost the US sorghum seed industry an estimated $2.5 million in 1997 (T. Lust, personnel communication).

Self-imposed bans were instituted to prevent the spread of *C. africana*. There was a concern by scientists and breeders that seed, encrusted by honeydew produced by *C. africana* might contain viable inoculum that would

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enhance the dissemination of the pathogen (Bandyopadhyay et al., 1996). A method that can rid the seed of viable inoculum would allow for a continued importation of seed from winter nurseries from outside the US and prevent the spread of isolates from different geographical origins. Research was conducted to evaluate the possibility that macrospores in encrusted honeydew on seed would germinate and produce secondary conidia, and if so, what control methods might reduce or prevent macrospore germination.

2. Materials and methods

Panicles from male-sterile sorghums with characteristic ergot symptoms of honeydew exudate were harvested from the USDA-ARS Isabela Farm, Isabela, Puerto Rico in the spring of 1997. Harvested panicles were incubated in a plastic chamber containing a Kaz (model # 3300) evaporative humidifier for 24 h to ensure continued output of honeydew. Ergot-free seed from "PR_5BR", a sorghum population developed in Puerto Rico, were infested with the secreted exudate from the colonized panicles to provide a uniform coating on the seeds. This method was used to simulate field conditions in which infected florets producing exudate will infest developing seed on the panicle. Honeydew was allowed to air-dry on the coated seed for 24 h before treatments began.

Six fungicides, Baytan® 30[1] (triadimenol, β-(4-chlorophenoxy)-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) applied at three rates (see Table 1), Dividend® 3MG[2] (difenoconazole, 1-{2-[4-(chlorophenoxy)-2-chlorophenyl-(4-methyl-1,2-dioxolan-2-yl)-methyl]}-1H-1,2,3-triazole) and Maxim® 4FS[2] (4-(2,2-difluoro-1,3-benxodioxol-4-yl)-1H-pyrrole-3-carbonitrile) both applied at two rates, and Captan® 400[1] (captan, N-[(trichloromethyl)thio]-4-cyclo-hexene-1,2-dicarboximide), 42-S Thiram®[1] (thiram, Tetramethylthiuram disulfide) and RTU®-Baytan®-Thiram®[1] (triadimenol and thiram) applied at one rate, were used on 113.5 g samples of both infested and healthy seed. Samples were then divided into two portions, with one shipped to the USDA-ARS Foreign Disease-Weed Science Research Lab at Frederick, MD for evaluation of spore germination and the other kept in Mayagüez, Puerto Rico for seed germination testing. Experimental design was a completely randomized design with two factors, fungicide treatments and time of observation. In each of the experiments there were three replications for each treatment and each experiment was repeated twice.

Table 1
Efficacy of fungicides in preventing germination and secondary conidia formation from macroconidia of *Claviceps africana* on the surface of sorghum seed infested with ergot honeydew

Treatment	Rate (g a.i. 100 kg^{-1})	Sporulation compared to control[a]
RTU®-Baytan®-Thiram®	32, 93	Absent
Baytan® 30	16	Abundant
Baytan® 30	31	Abundant
Baytan® 30	62	Abundant
42-S Thiram®	109	Absent
Captan® 400	55[b]	Absent
Captan® 400	94[b]	Absent
Captan® 400	140	Absent
Dividend® 3MG	12	Abundant
Dividend® 3MG	24	Abundant
Maxim® 4FS	2.5	Trace[c]
Maxim® 4FS	5.0	Absent[c]
Infested control	—	Abundant
Non-infested control	—	Absent

[a] Sporulation in comparison to that produced on the control seed coated with ergot honeydew. Seeds were incubated at 100% RH (on water agar and soil) for 24 h at 21 to 22°C. Sporulation is defined as macroconidium within the honeydew germinating to produce an aerial conidiophore that bears a single secondary conidium at its tip. Non-infested control seed had no visible sporulation.
[b] Plated on 2% agar only.
[c] Trace = 50–100 conidia per seed. Both treatments had some apparent macroconidial germination with production of sparse segmented mycelia.

Approximately 25 sorghum seeds from non-infested seed and from ergot infested seed, both treated with fungicides (factor 1), were placed onto 1 and 2% (experiments 1 and 2, respectively) water agar containing 100 mg l^{-1} each of streptomycin sulfate and ampicillin. Seeds were incubated at 21 to 22°C with 16 h of light. At 24 and 48 h (factor 2), samples were examined under a microscope, equipped with 50× and 100× objectives, for germination of macroconidia and formation of secondary conidia on both seed and agar surfaces. The above procedure was repeated with approximately 50 seed from each of the treatments and the presence or absence of conidiophores and secondary conidia observed at 24, 48, and 72 h after plating.

Seed from each treatment also were plated onto wetted, non-sterile field (experiment 3) soil to determine the potential for conidiophore and conidia production that might exist in a more natural, nonsterile, field-type

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environment. Soil was placed in 24 petri plates, wetted, and then allowed to equilibrate for 4 h until no free water was observed in any of the plates. Approximately 50 seed were distributed over the soil surface of each plate, with two replicate plates for each treatment. The plates, with soil and seed, were incubated at 21 to 23°C with 16 hours of light. Seeds were examined for conidiophore formation at 24 and 48 h.

Three hundred seeds from each treatment, except Captan 400® at the 94 and 140 rates, were placed on germination paper, wetted with distilled water, placed in a germination oven (Seedburo Model 548 RLDS) at 29.4°C and percent germination was recorded for each treatment after 3, 5 and 7 days. Germination tests were replicated (3 reps) over time. Data were analyzed statistically using PROC GLM and contrasts were performed.

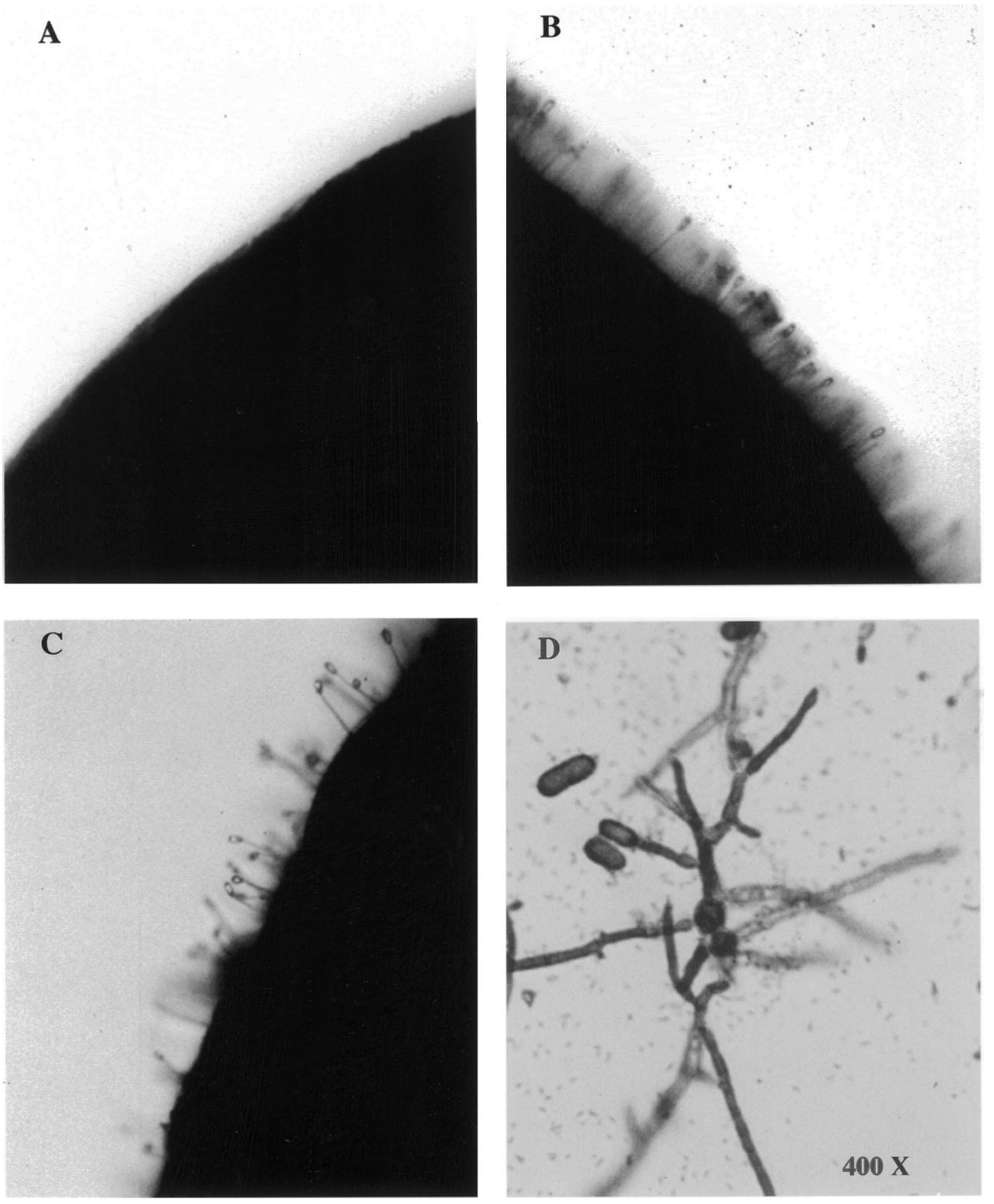

Fig. 1. Production of secondary conidia of *Claviceps africana* on sorghum seed after incubation on water agar for 24 h viewed at 400×. (A) Seed dipped in water, but not inoculated with (non-infested control) pathogen. No conidiophore formation was observed on the seed or agar surface within the various agar or soil plates. (B) Seed coated with honeydew contains macroconidia (infested control). Abundant conidiophores with secondary conidia observed over the entire seed surface. (C) Honeydew coated seed treated with Maxim® 4FS at the rate of 2.5 g a.i. 100 kg^{-1}. Sparse conidiophore formation was observed, estimated at only 50 to 100 conidiophores per seed. On the agar surface (not shown) some germination appeared to have occurred, forming sparse, septate mycelia, but no conidiophores. (D) Honeydew coated seed treated with Maxim® 4FS at the rate of 5.0 g a.i. 100 kg^{-1}. No conidiophore formation was observed on the seed or agar surface, however, on the agar surface some germination appeared to have occurred, forming sparse, septate mycelia, but no conidiophores.

Germination means were compared by the LSD procedure using SAS (SAS Institute Inc., 1985).

3. Results and discussion

Abundant conidiophores with secondary conidia were observed over the entire seed surface of the (control) honeydew-infested seed while none were observed on the non-infested control seed (Figs. 1A and B). No apparent significant increase in the number of conidiophores was observed between 24 and 48 h on seed of any treatment and no differences were observed between the use of 1% or 2% water agar or soil. Conidiophores formed from germinating macroconidia present on the agar surface and from within honeydew on the seed surface. Germination of macroconidia and the production of secondary conidia are shown in Table 1 and expressed in comparison to the (control) honeydew-infested seed as the standard.

Microscopic observations of every seed within treatments showed that RTU®-Baytan®-Thiram®, 42-S Thiram®, and Captan® 400 completely prevented conidial germination. Due to the absence of aerial secondary conidia, seed treated with these three fungicides appeared similar to the non-infested seed after incubation. Neither of the triazoles (triadimenol [Baytan] or difenoconzaole [Dividend]) at any rate tested had any apparent effect in reducing either the germination of macroconidia or production of secondary conidia when compared to the honeydew-coated seed. The inactivity of triadimenol indicated that the prevention of germination and sporulation by RTU®-Baytan®-Thiram® was probably due to the presence of 42-S Thiram®. Maxim® 4FS, at the 2.5 g a.i. 100 kg^{-1} rate, allowed low levels of macroconidia germination with production of conidiophores and secondary conidia (50–100 secondary conidia $seed^{-1}$), however, at the 5.0 g a.i. 100 kg^{-1} rate, low levels of germination with production of only sparsely segmented mycelia and no conidiophores were observed (Fig. 1C and D).

Analyses of the seed germination tests revealed significant differences between treatment levels on germination (Table 2). No significant interaction between time and treatments were found. The fungicide Baytan® 30 at 16 g a.i. 100 kg^{-1} seemed to have the greatest inhibitory effect on germination of sorghum seed compared to the other fungicides used in the study, while Maxim® 4FS had the least effect on seed germination. Both Captan® 400 and 42-S Thiram® showed small decreases in germination (1–4%) when compared to the controls (Table 2). Contrasts were found to be significant among fungicides, with Maxim having significantly higher germination rates than the other fungicides. There were also significant contrasts between fungicide treatments and controls, with both controls having significantly higher germination rates than all of the fungicide treatments except for Maxim.

Table 2
Germination of ergot-infested seeds treated with various fungicides at different rates three days after incubation. Means of the treatment levels and their effect on germination

Fungicide	Rate (g a.i. 100 kg^{-1})	% Germination [a]
Infested control seed	0	92.00 [a]
Maxim® 4FS	5.0	92.00 [a]
Non-infested control seed	0	91.50 [a]
Maxim® 4FS	2.5	91.17 [a]
42-S Thiram®	109	90.83 [ab]
Dividend® 3MG	12	87.50 [abc]
Captan 400	140	87.17 [abc]
Baytan® 30	62	84.33 [bcd]
Dividend® 3MG	24	84.33 [bcd]
Baytan® 30	31	82.00 [cd]
RTU®-Baytan®-Thiram®	32.93	80.500 [d]
Baytan® 30	16	78.67 [d]

[a] Means followed by different letters indicate significant differences using LSD ($p = 0.05$)

To continue unrestricted movement of sorghum within the Americas, an effective fungicide treatment should be in place to prevent the spread of *C. africana* on honeydew infested sorghum seed. Fungicide and seed germination results presented herein show that both captan (Captan® 400) and thiram (42-S Thiram®) would safely eliminate the potential of macroconidia as an infective propagule without severely reducing viability of the sorghum seed.

Disclaimer

Mention of a trademark or proprietary name does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of the products that may also be suitable. This article reports the results of research only. Baytan® 30, Captan 400®, 42-S Thiram®, and RTU®-Baytan®-Thiram® were supplied by Gustafson. Dividend® 3MG and Maxim® were supplied by Novartis.

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