Production and Germination of Conidia of *Trichoderma stromaticum*, a Mycoparasite of *Crinipellis perniciosa* on Cacao

S. Sanogo, A. Pomella, P. K. Hebbar, B. Bailey, J. C. B. Costa, G. J. Samuels, and R. D. Lumsden

First author: Wye Research and Education Center, University of Maryland, Queenstown 21658; second author: Almirante Cacau, Itabuna, Bahia, Brazil; third author: Mars Inc., Elizabethtown, PA; first, third, fourth, and seventh authors: Alternate Crops and Systems Laboratory; fifth author: CEPLAC, Itabuna, Bahia, Brazil; and sixth author: Systematic Botany and Mycology Laboratory, USDA-ARS, BARC-West, Beltsville, MD 20705.

Current address of S. Sanogo: Entomology, Plant Pathology and Weed Science Department, New Mexico State University, P.O. Box 30003, MSC 3BE, Las Cruces 88003.

Accepted for publication 5 June 2002.

ABSTRACT

Sanogo, S., Pomella, A., Hebbar, P. K., Bailey, B., Costa, J. C. B., Samuels, G. J., and Lumsden, R. D. 2002. Production and germination of conidia of *Trichoderma stromaticum*, a mycoparasite of *Crinipellis perniciosa* on cacao. Phytopathology 92:1032-1037.

Growth characteristics of the fungus *Trichoderma stromaticum*, a mycoparasite on the mycelium and fruiting bodies of *Crinipellis perniciosa*, the causal agent of witches'-broom disease of cacao, were evaluated under controlled environmental conditions. The ability of *T. stromaticum* to produce conidia and germinate on dry brooms was evaluated at three constant temperatures (20, 25, and 30°C) and two constant relative humidities (75 and 100%). *T. stromaticum* produced abundant conidia on brooms at 100% relative humidity and incubation temperatures of 20 and 25°C, but none at 30°C. Sporulation of *T. stromaticum* was not observed at 75% relative humidity at any temperature. At 100% relative humidity and either at 20 or 25°C, treatment of brooms with *T. stromaticum* suppressed *C. perniciosa* within 7 days. In contrast, at 30°C, treatment with *T. stromaticum* had no effect on the pathogen in brooms maintained at either 75 or 100% relative humidity. Mycelium of *C. perniciosa* grew from brooms at all temperatures at 100% relative humidity. Conidial germination on broom tissue approximated 80% at temperatures from 20 to 30°C. Results suggest that applying *T. stromaticum* under high-moisture conditions when the air temperature is below 30°C may enhance the establishment of this mycoparasite in cacao plantations.

Additional keyword: *Theobroma cacao.*

Witches'-broom disease, caused by the basidiomycete *Crinipellis perniciosa*, is a major constraint on the production of cacao (*Theobroma cacao* L.) in Central and South America (6,14,17). Basidiospores of *C. perniciosa* infect young and actively growing meristematic tissues, causing various physiological and hormonal changes (11) leading to tissue swelling and the formation of numerous succulent vegetative branches called brooms. Damage inflicted by this disease amounts to approximately 21% of the global crop loss due to major cacao diseases (13).

There are four main strategies for minimizing the impact of the disease: phytosanitation, chemical control, genetic resistance, and biological control. Phytosanitation, which involves the removal and destruction of infested plant materials, reduces pod loss and delays disease epidemics. However, this strategy is tedious, and in one study removal of 95% of brooms was required to achieve a 50% reduction in pod loss (15). Chemical control with contact and systemic fungicides is feasible but is not a routine practice in cacao production, because of high costs, possible contamination of cacao seed, and the risks to environmental health. The development of genetically resistant cacao cultivars is an ongoing endeavor in many countries affected by witches'-broom, and it is expected that the use of these cultivars will reduce the incidence of the disease (14).

The prospects of management of witches'-broom by biological control have been investigated for several years, and this research has led to the isolation and identification of a new species of *Trichoderma*, *T. stromaticum* (16), which was reported in earlier literature as *T. viride* or *T. polysporum* (2,5). *T. stromaticum* is a parasite of the mycelium and basidiocarps of *C. perniciosa* (3). In Brazil, two methods are employed to apply *T. stromaticum* as an aqueous conidial preparation to manage witches'-broom. In the first method, brooms are pruned down to the plantation litter and sprayed with a conidial preparation. This method has been shown to reduce basidiocarp production and hasten broom decomposition (3,5), but it is very tedious and costly, because it requires pruning prior to spraying. In the second method, a conidial preparation is directly applied to the plant canopy, without pruning. However, this method has not been widely adopted by cacao farmers, because of low and inconsistent performance of *T. stromaticum* in reducing basidiocarp production and enhancing broom decomposition (3,5).

One reason for the differential performance of *T. stromaticum* may be the influence of environmental conditions on the establishment of the mycoparasite in cacao plantations. Little is known about the effect of environmental variables, such as temperature and moisture, on *T. stromaticum*, and the available information pertains only to the shelf life of conidial and mycelial propagules after storage at different temperatures (4).

To optimize the use of *T. stromaticum* as a biocontrol agent, there is a need for further understanding of the relationship of the physical environment to the survival, growth, and reproduction of this mycoparasitic fungus. The objectives of this study were to determine the effects of temperature and relative humidity on the phenology of *T. stromaticum* and on conidial production and germination on brooms in controlled environments.

Corresponding author: S. Sanogo; E-mail address: ssanogo@nmsu.edu

Publication no. P-2002-0816-01R

MATERIALS AND METHODS

Maintenance of culture and inoculum production. An isolate of *T. stromaticum*, GJS-97-183 (16), which is currently used in Brazil, was maintained on 0.5- to 1-cm^2 portions of sterile filter paper (Whatman No. 1) in glass tubes with screw caps. To establish a culture of this strain, a filter paper portion was placed on acidified potato dextrose agar (PDA) in petri plates and incubated for 3 days at 25°C in the dark. To produce conidia for controlled environment studies, disks of mycelium (1 cm in diameter) were transferred from the edge of the culture to PDA (Difco Laboratories, Detroit, MI) medium in petri plates. The PDA plates were incubated at 25°C for 7 to 10 days, at which time conidial production was observed. Conidia were harvested by scraping the surface of the agar with a plastic spatula into a 50-ml plastic test tube containing 25 ml of sterile distilled water. The test tube was agitated on a vortex mixer, and the resulting suspension was passed through six layers of sterile cheesecloth to separate mycelium and agar fragments from conidia. The concentration of conidia was determined with a hemacytometer.

Maintenance of brooms and evaluation of broom infestation by *C. perniciosa*. Dry brooms from cacao plants infected with *C. perniciosa* (with stems only or both stems and leaves) were obtained from Almirante Cacau, Itabuna, Bahia, Brazil, and were maintained in paper bags at room temperature (22 to 23°C) for up to 6 months before use. The moisture content of the brooms was approximately 10 to 12% on a dry-weight basis. Broom segments 0.7 to 1 cm in diameter were used in the study. To test whether the broom segments in a given batch were uniformly infected with *C. perniciosa*, 20 to 30 segments were soaked in water for 15 min and incubated in a moisture chamber at 25°C ± 0.5°C for 3 to 4 days. White mycelium emerging from the cut ends indicated infection. The emerged mycelium was transferred to PDA, and the culture was identified as *C. perniciosa* by (i) morphological characteristics, in comparison to cultures established from basidiospores obtained from basidiocarps produced on brooms; (ii) the production of basidiocarps by the isolated mycelium on bran-vermiculite medium (8); and (iii) a random amplified polymorphic DNA (RAPD) procedure, described by Anderbrhan and Furtek (1).

Determination of broom moisture content in a controlled environment and in the field. Broom segments used in controlled environment studies and three whole brooms collected from a cacao plantation at the Almirante Cacau farm on each of 10 dates were examined to determine their moisture content. The fresh weight (FW) of broom samples was recorded, the samples were placed in an oven at 65 to 70°C, and their dry weight (DW) was recorded after 48 h. Moisture content (MC) was computed on a dry-weight basis as MC (%) = [(FW – DW)/DW] × 100. Under field conditions, brooms were collected at arbitrary times, and weather variables during the 24-h period prior to broom collection were recorded. Measured weather variables were rainfall (in millimeters), maximum and minimum temperatures (in degrees Celsius), and average relative humidity (percent).

Effect of temperature on conidial germination, mycelial growth, and sporulation of *T. stromaticum* on growth medium. The effect of five temperatures (15, 20, 25, 30, and 35°C ± 0.5°C) on conidial germination, mycelial growth, and sporulation on PDA was examined.

To assess germination, a 10-µl aliquot of a suspension of conidia of *T. stromaticum* (1×10^4 conidia/ml) was placed at each of nine spots on petri plates (9 cm in diameter) containing PDA. The location of each spot was marked with a circle on the bottom of the plate, and the drops were allowed to dry. The plates were then sealed with Parafilm and incubated at the five selected temperatures in the dark for 24 h. Five spots were arbitrarily selected from the nine spots marked on each plate, and the first 20 conidia were observed on each selected spot. Thus, a total of 100 conidia were observed on each plate. A conidium was considered germinated if the germ tube was longer than the conidium. The experiment was performed three times and was conducted in a completely randomized design, with four plates constituting an experimental unit.

To assess mycelial growth, a mycelial plug (4 mm in diameter) of *T. stromaticum*, grown on 2% water agar for 4 days, was placed at the edge of a PDA plate, approximately 4 cm from the center of the plate. Plates were incubated at the temperatures stated above, and colony radius was measured after 48 h.

To evaluate conidial production, the plates used to assess mycelial growth were incubated for an additional 12 days. From each plate, conidia were harvested by scraping the surface of agar with a plastic spatula into a 50-ml plastic test tube containing 20 ml sterile distilled water. The test tube was agitated on a vortex mixer, and the resulting suspension was passed through six layers of sterile cheesecloth to separate mycelium and agar fragments from conidia. The resulting suspension was serially diluted, and three 10-µl drops from each dilution were separately spread over three equidistant 2-cm^2 areas on a plate containing TME, a semiselective medium for *Trichoderma* (12). The plates were incubated at 25°C ± 0.5°C for 7 to 10 days to assess the number of colony-forming units (CFU) per milliliter. The experiment was conducted as a completely randomized design, with an experimental unit consisting of four plates. The experiment was performed three times.

Effects of temperature and relative humidity on the production and germination of conidia of *T. stromaticum* on brooms. Two experiments were conducted to assess the ability of *T. stromaticum* to germinate and sporulate on dry brooms (stem and leaves) of cacao.

In the first experiment, the effects of three constant temperatures (20, 25, and 30°C ± 0.5°C) on conidial germination and sporulation were determined. Stem segments (4 cm long) and leaf disks (5 mm in diameter) of broom were wrapped separately in cheesecloth and immersed in sterile distilled water in beakers for 20 to 30 min to raise the moisture content of the brooms to approximately 40%. To assess conidial germination, a 10-µl aliquot of a suspension of conidia of *T. stromaticum* (1×10^4 conidia/ml) was placed on each leaf disk and allowed to dry, and the moistened stem segments were sprayed with conidial suspension at the rate of 1 ml of suspension per centimeter of broom tissue. The stem segments and leaf disks were then placed in deep petri plates (100 mm in diameter and 80 mm in depth) containing 250 g of sterilized builder's sand overlaid with two layers of sterile filter paper and moistened with 50 ml of sterile distilled water. There were eight segments and six leaf disks per plate. The plates were sealed with Parafilm and maintained in incubators set at the desired temperatures. The experiment, consisting of six treatment combinations (treated with *T. stromaticum* and untreated, each at three temperatures), was conducted as a factorial in a completely randomized design, with one experimental unit (six petri plates) per treatment combination. The experiment was performed four times.

The second experiment was conducted to assess the effects of three constant temperatures (20, 25, and 30°C ± 0.5°C) and two relative humidities (75 and 100%) on the sporulation of *T. stromaticum* on dry brooms of cacao. Broom stem segments (4 cm long) and leaf disks were prepared as described above, placed separately in uncovered 5-cm-diameter petri plates (four segments or six leaf disks per plate), and inserted in humidity chambers containing water or saturated sodium chloride (NaCl) on the bottom. Water and saturated NaCl solution were used in the chambers to generate high (100%) and moderate (75%) relative humidity, respectively (7,18). The chambers were then maintained in incubators set at the desired temperatures. The experiment, consisting of 12 treatment combinations (treated with *T. stromaticum* and untreated, each at three temperatures and at two relative humidities), was conducted in a split-plot design, with temperature as the whole-plot factor and the combination of relative humidity and

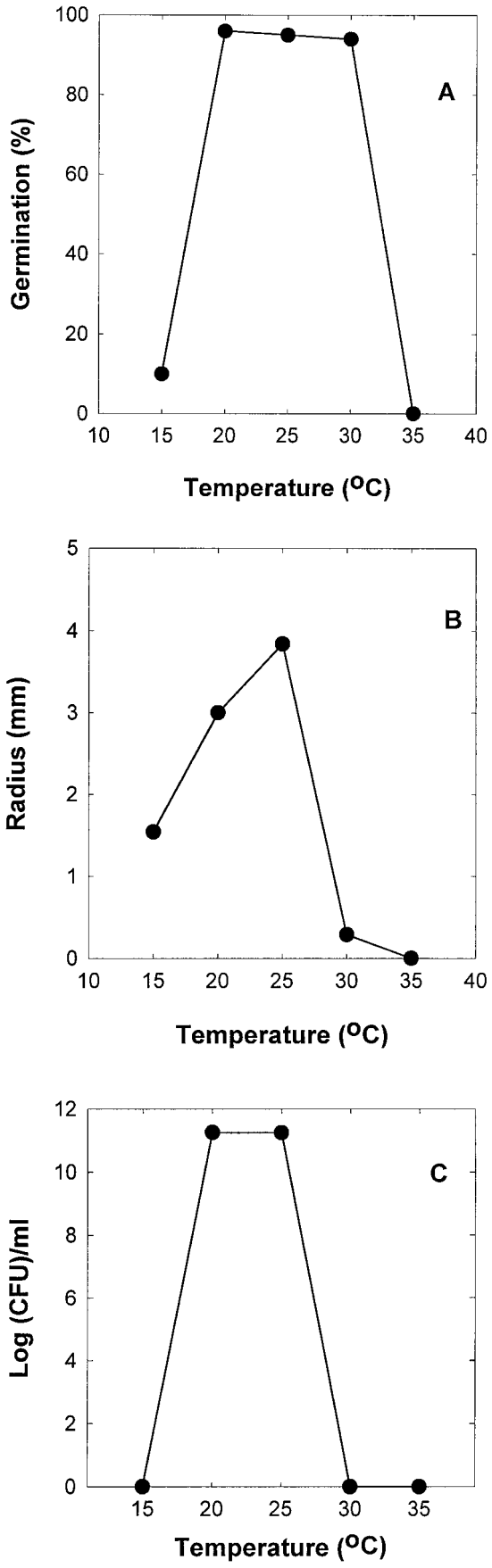

Fig. 1. Effect of temperature on **A,** conidial germination, **B,** mycelial growth, and **C,** sporulation of *Trichoderma stromaticum* after 24 h, 48 h, and 14 days of incubation, respectively, on potato-dextrose agar medium. Each data point is the mean of three experiments. For all data points, the length of a bar representing the standard error of the mean is less than the diameter of the data point.

treatment with *T. stromaticum* as the subplot factor. The experiment was performed four times.

In both experiments, after 24 h of incubation, two 4-mm disks of cortical tissue were removed from one broom stem segment from each of three plates. Leaf disks were also sampled. The stem and leaf disks were processed for fluorescence and scanning electron microscopy to evaluate conidial germination. For fluorescent microscopy, stem and leaf disks were mounted in a drop of 0.030% Calcofluor (Sigma Chemical Co., St. Louis), and samples were viewed with a light microscope equipped with an incident-light fluorescence illuminator (Zeiss Axioskop H/DK Stereo Microscope; Carl Zeiss, Inc., Thornwood, NY). Percentage of germination was assessed in the first 100 conidia observed in arbitrarily selected fields as described above. For scanning electron microscopy, stem and leaf disks were prepared as described by Klomparens et al. (10) and observed with a JSM-T300 microscope (JEOL, Ltd., Tokyo).

To assess sporulation, all broom stem segments were incubated for 14 days and then were removed and placed in flasks in which distilled water was added at the rate of 10 ml per broom segment. The flasks were agitated for 10 min. The resulting suspension was serially diluted, and three 10-µl drops from each dilution were separately plated on TME as described above. The plates were incubated at 25°C ± 0.5°C for 7 to 10 days to assess the number of CFU per broom segment.

To evaluate the survival of *T. stromaticum* and *C. perniciosa*, two broom stem segments treated with *T. stromaticum* were taken from each of six plates. The segments were cut into 1-cm sections (48 sections), which were divided into two samples of 24 sections each. The sections in the first sample were plated on a medium selective for *C. perniciosa* (9), and the sections in the second sample were plated on TME medium, as described above, to detect *T. stromaticum.* The percentage of broom stems yielding *C. perniciosa* or *T. stromaticum* was recorded after 10 to 14 days of incubation at 25°C ± 0.5°C. In addition to recovery on TME medium, production of conidia on broom segments treated with *T. stromaticum* was used as a gauge of the survival of the mycoparasite.

Data analysis. The variables measured in this study were (i) percentage of germinated conidia on growth medium and on brooms, (ii) colony radius on growth medium, and (iii) the number

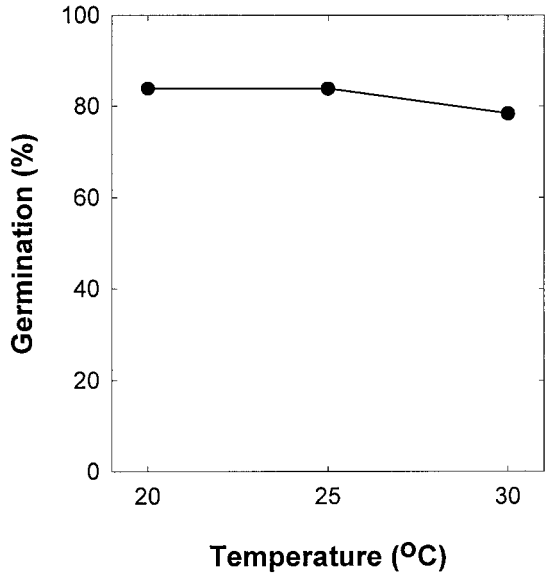

Fig. 2. Effect of temperature on conidial germination of *Trichoderma stromaticum* on cacao broom tissue after 24 h incubation. Each data point is the mean of results of four experiments. For all data points, the length of a bar representing the standard error of the mean is less than the diameter of the data point.

of CFU on growth medium and on brooms. The number of CFU was subjected to natural logarithm transformation ($\log_e$) before analysis. Other variables did not need transformation. For statistical analyses, data on each variable were pooled across repeated experiments to test the effect of temperature, relative humidity, and treatment with *T. stromaticum.* All analyses were performed using procedures in SAS version 6.12 (Statistical Analysis Systems Institute, Cary, NC).

RESULTS

Effect of temperature on germination of conidia, growth of mycelium, and sporulation of *T. stromaticum* on growth medium. On PDA, conidial germination was first observed after 13 h of incubation at 25°C. At this time, less than 5% of conidia had germinated. After 24 h of incubation, approximately 95% of conidia had germinated at temperatures from 20 to 30°C (Fig. 1A). Less than 15% of conidia germinated at 15°C, and no germination occurred at 35°C. The pattern of mycelial growth in relation to temperature is shown in Figure 1B. Radial growth of colonies was low at 15°C, increased by a factor of approximately 2 at 20°C, peaked at 25°C, and decreased at 30°C. No growth occurred at 35°C. Production of conidia was highest at 20 and 25°C, and no conidia were produced at 15, 30, and 35°C after 14 days of incubation (Fig. 1C). There was no significant difference in conidial production at 20 and 25°C according to a *t* test ($P = 0.8423$).

Effects of temperature and relative humidity on germination and production of conidia of *T. stromaticum* on brooms. Germination of conidia on stem and leaf tissue was similar on moistened sand and at 100% relative humidity, approximating 80% at temperatures from 20 to 30°C (Fig. 2). However, germ tubes, as revealed by scanning electron microscopy, were two to three times longer at 25 and 30°C than at 20°C. No conidia germinated on broom tissue at 75% relative humidity at any temperature tested.

No conidia were produced on broom segments at 75% relative humidity at any temperature tested. Production of conidia was comparable on brooms maintained on moistened sand (Fig. 3A)

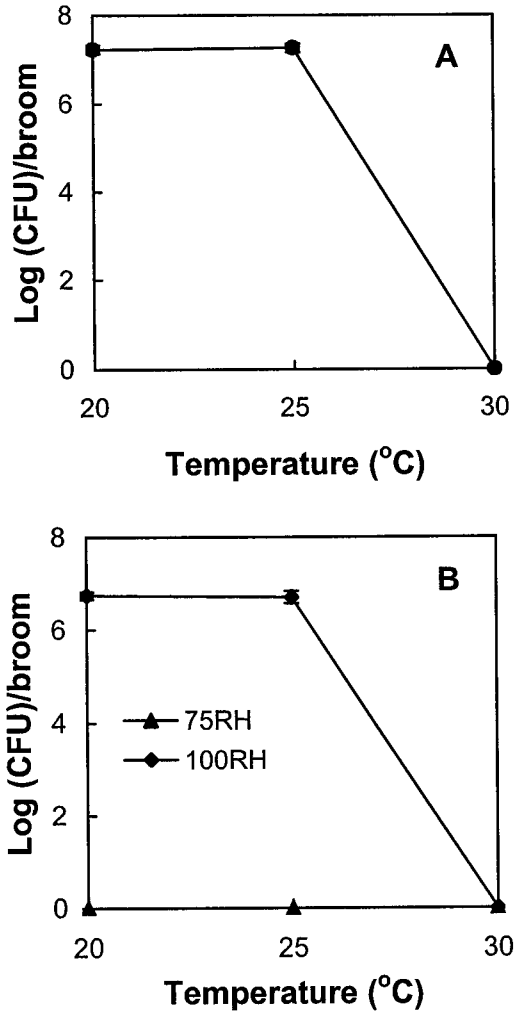

Fig. 3. Sporulation of *Trichoderma stromaticum* on cacao broom segments maintained on **A,** moistened sand and **B,** at a relative humidity of 75% (75RH) or 100% (100RH) at 20, 25, and 30°C. Each data point is the mean of results of four experiments. For all data points, the length of a bar representing the standard error of the mean is less than the diameter of the data point.

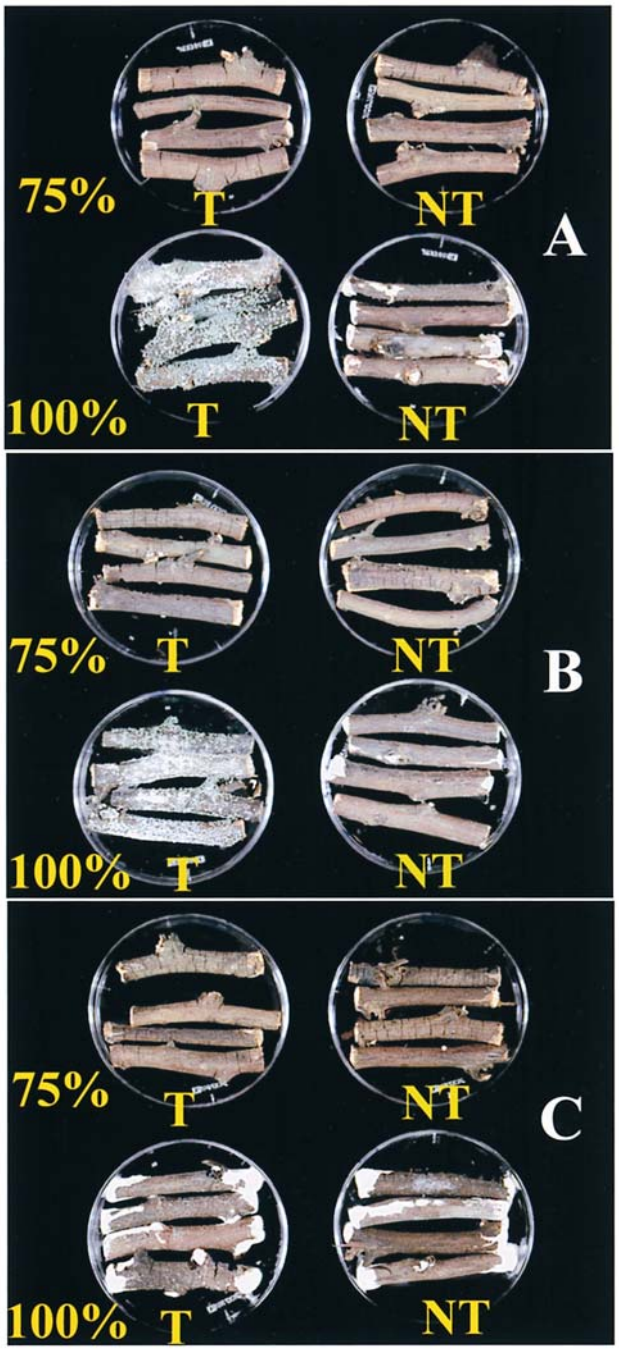

Fig. 4. Sporulation of *Trichoderma stromaticum* on broom segments maintained at 75 or 100% relative humidity at **A,** 20, **B,** 25, and **C,** 30°C. Broom segments were treated (T) or not treated (NT) with *T. stromaticum.*

and those at 100% relative humidity (Fig. 3B). Under both conditions, *T. stromaticum* produced abundant conidia at 20 and 25°C, but no conidia at 30°C. As indicated by *t* tests, there was no significant difference in conidial production on brooms maintained at 20 and 25°C over moistened sand ($P = 0.8340$) and on brooms maintained at 20 and 25°C at 100% relative humidity ($P = 0.8292$). At 20 and 25°C, conidial pustules were visible within 4 days of incubation and appeared as white stroma, which gradually became green (Fig. 4A and B). No mycelium of *C. perniciosa* was observed growing from brooms at 75% relative humidity, regardless of temperature. Mycelium of *C. perniciosa* grew from brooms maintained on moistened sand and at 100% relative humidity, but not from brooms treated with the mycoparasite and incubated at 20 and 25°C. Under high-moisture conditions (moistened sand and 100% relative humidity) and at 30°C, treating broom segments with *T. stromaticum* did not prevent mycelial growth of *C. perniciosa* (Fig. 4C).

T. stromaticum survived on brooms maintained at 20 and 25°C over moistened sand and at 100% relative humidity, whereas *C. perniciosa* was unrecoverable from these brooms within 7 days of incubation (Table 1). Conversely, at 30°C, *T. stromaticum* was not recovered from any broom segments, whereas *C. perniciosa* was recovered from all broom sections bioassayed. At 75% relative humidity, *T. stromaticum* survived at 20 and 25°C, but not at 30°C, whereas *C. perniciosa* was recovered from all broom sections at all temperatures.

Broom moisture content in a controlled environment and in the field. In brooms placed over moistened sand at all temperatures tested, broom moisture content rose from 40% to reach 60 to 65% within 4 days of incubation. In contrast, in brooms maintained at 75% relative humidity and at all temperatures tested, moisture content decreased to 11 to 14%. Under field conditions, broom moisture content varied between approximately 11 to 34% and was significantly correlated with the amount of rain ($r = 0.92$; $P = 0.0002$) and the daily maximum temperature ($r = –0.69$; $P = 0.0282$), but it was not significantly correlated with the average relative humidity ($r = 0.32$; $P = 0.360$) or with the daily minimum temperature ($r = 0.51$; $P = 0.1345$).

DISCUSSION

The purpose of this study was to gain some insight into aspects of the phenology, especially conidial germination and production, of the mycoparasitic fungus *T. stromaticum.* Conidial germination and production were used as a measure of the ability of *T. stromaticum* to establish itself in cacao. The experiments on growth media were designed to provide a baseline assessment of the behavior of *T. stromaticum* under optimum nutritional conditions, which may not be encountered in the field. The inclusion in the study of experiments conducted on brooms provided assessment of growth characteristics likely to be observed in the field. Our results on growth media closely paralleled those obtained on broom tissue and indicate that *T. stromaticum* is able to germinate optimally at temperatures from 20 to 30°C, with maximum mycelial growth at 25°C. This study also shows that *T. stromaticum* survives on broom tissue at temperatures below 30°C, with a high capacity to sporulate under high-moisture conditions.

The two experiments conducted on brooms were designed to represent two events in the application of *T. stromaticum* in cacao plantations. In the first scenario, brooms are pruned and left on the ground, where they are subsequently sprayed with a conidial suspension of *T. stromaticum.* In the second scenario, no pruning is performed, and brooms in the canopy are sprayed with *T. stromaticum.* Sporulation under these two conditions was comparable, especially at high relative humidity. However, in our field observations we routinely found that brooms sprayed and left on the ground were more extensively covered with conidia than those in the canopy. In instances where sporulation was observed in the tree canopy, it was associated with moistened apexes of the brooms. In this study, we maintained broom moisture content at 40%, because in a preliminary study no sporulation was observed at a moisture content lower than 30%. These observations imply that broom moisture content is an important factor for the production of conidia on brooms. It would appear that broom moisture content above 30% could occur in the field following heavy rainfall.

Our results indicate that *T. stromaticum* is not capable of surviving at 30°C, a characteristic that may explain the inconsistent performance of this mycoparasitic fungus. In cacao plantations, the daily maximum temperature may be well above 30°C, a level that poses a serious impediment to the survival of *T. stromaticum.* Bastos (2) reported that the survival of this antagonist decreased by 35% at 90 days after application. No reason was provided, but it is conceivable that temperature may have contributed to this decrease. This conclusion is corroborated by a study (4) of the shelf life of inoculum (powder of conidia and mycelial fragments), in which inoculum viability decreased with length of storage at various temperatures from 5 to 25°C, with no propagules surviving at 25°C after 12 weeks of storage. There are at least two possible explanations for the ability of *T. stromaticum* to germinate at 30°C and yet not be recovered from brooms. First, mycelium of *T. stromaticum* may be degenerated, resulting in limited colonization by the fungus. Second, the fungus may have shifted to the formation of short-lived resting propagules.

There are no previous reports on the effects of environmental factors, such as temperature and relative humidity, on conidial production by *T. stromaticum* on brooms. The selected levels of temperature and relative humidity are well within those commonly encountered in the cacao plantation environment. We have focused on conidial production by *T. stromaticum* for two principal reasons. First, conidial production would ensure that inoculum is readily available for dispersal throughout cacao plantations. Second, following wide dissemination of conidial inoculum, producers may not need multiple applications of *T. stromaticum* to reduce the impact of witches'-broom and thus could engender savings of resources.

This study has relevance to the optimal utilization of *T. stromaticum* as an agent for the biocontrol of witches'-broom, and our results may be used in two ways: (i) development of an integrated pest management schedule in which *T. stromaticum* will be applied during cooler periods of the year and (ii) formulation of *T. stromaticum* with humectants to increase broom moisture content.

TABLE 1. Effect of temperature and relative humidity on the survival and sporulation of *Trichoderma stromaticum* and the survival of *Crinipellis perniciosa* on cacao brooms treated with *T. stromaticum*

Temperature (°C)	Relative humidity (%)	Sporulation of *T. stromaticum*[a]	Survival of *T. stromaticum*[b]	Survival of *C. perniciosa*[c]
20	75	–	+	+
	100	+	+	–
25	75	–	+	+
	100	+	+	–
30	75	–	–	+
	100	–	–	+

[a] Assessment of sporulation was based on the presence (+) or absence (–) of conidial pustules on 24 broom segments (4 cm long) per treatment combination in each experiment.

[b] Assessment of survival was based on recovery (+) or lack of recovery (–) of *T. stromaticum* from 24 broom sections (1 cm long) per treatment combination in each experiment. Broom sections were plated on a semiselective medium and incubated at 25°C for 10 to 14 days.

[c] Assessment of survival was based on recovery (+) or lack of recovery (–) of *C. perniciosa* from 24 broom sections (1 cm long) per treatment combination in each experiment. Broom sections were plated on a selective medium and incubated at 25°C for 10 to 14 days.

ACKNOWLEDGMENTS

We thank M. Edens, J. Plaskowitz, and D. Clark at the U.S. Department of Agriculture, Agricultural Research Service, in Beltsville, Maryland, and A. Guimaraes and A. Ramos at the Almirante Cacau in Itabuna, Brazil, for technical assistance. Salaries and research support to the first author were provided through Specific Cooperative Agreement 5812759060 between the U.S. Department of Agriculture and the University of Maryland. Mention of trade names or commercial/experimental products in this publication does not imply recommendation or endorsement by the U.S. Department of Agriculture.

LITERATURE CITED

1. Anderbrhan, T., and Furtek, D. B. 1994. Random amplified polymorphic DNA (RAPD) analysis of *Crinipellis perniciosa* isolates from different hosts. Plant Pathol. 43:1020-1027.
2. Bastos, C. N. 1988. Resultados preliminaries sobre a eficácia de *Trichoderma viride* no controle da vassoura-de-bruxa (*Crinipellis perniciosa*) do cacaueiro. Fitopatol. Bras. 13:340-342.
3. Bastos, C. N. 1996. Mycoparasitic nature of the antagonism between *Trichoderma viride* and *Crinipellis perniciosa.* Fitopatol. Bras. 21:50-54.
4. Bastos, C. N. 1996. Effect of water potential on growth and viability of conidia of *Trichoderma viride* after storage at different temperatures. Summa Phytopathol. 22:258-261.
5. Costa, J. C. B., Bezerra, J. L., and Cazorla, I. M. 1996. Controle biologico da vassoura-de-bruxa do cacueiro na Bahia com *Trichoderma polysporum.* Fitopatol. Bras. 21(Suppl.):397.
6. Fulton, R. H. 1989. The cacao disease trilogy: Black pod, Monilia pod rot, and witches'-broom. Plant Dis. 73:601-603.
7. Gracia-Garza, J. A., and Fravel, D. R. 1998. Effects of relative humidity on sporulation of *Fusarium oxysporum* in various formulations and effect of water on spore movement through soil. Phytopathology 88: 544-549.
8. Griffith, G. W., and Hedger, J. N. 1993. A novel method for producing basidiocarps of the cacao pathogen *Crinipellis perniciosa* using a bran-vermiculite medium. Neth. J. Plant Pathol. 99:227-230.
9. Griffith, G. W., and Hedger, J. N. 1994. The breeding biology of biotypes of the witches' broom pathogen of cocoa, *Crinipellis perniciosa.* Heredity 72:278-289.
10. Klomparens, K. L., Flegler, S. L., and Hooper, G. R. 1986. Procedures for Transmission and Scanning Electron Microscopy for Biological and Medical Science, A Laboratory Manual. 2nd ed. Ladd Research Industries, Burlington, VT.
11. Orchard, J., Collin, H. A., Hardwick, K., and Isaac, S. 1994. Changes in morphology and measurement of cytokinin levels during the development of witches' brooms on cocoa. Plant Pathol. 43:65-72.
12. Papavizas, G. C., and Lumsden, R. D. 1982. Improved medium for isolation of *Trichoderma* spp. from soil. Plant Dis. 66:1019-1020.
13. Pereira, J. L. 1999. Management of Witches' Broom Disease of Cocoa: A Contemporary Retrospective. Cocoa Producers' Alliance, Lagos, Nigeria.
14. Purdy, L. H., and Schmidt, R. A. 1996. Status of cacao witches' broom: Biology, epidemiology, and management. Annu. Rev. Phytopathol. 34:573-594.
15. Rudgard, S. A., and Butler, D. R. 1987. Witches' broom disease in Rondonia, Brazil: Pod infection in relation to pod susceptibility, wetness, inoculum, and phytosanitation. Plant Pathol. 36:515-522.
16. Samuels, G. J., Pardo-Schultheiss, R., Hebbar, K. P., Lumsden, R. D., Bastos, C. N., Costa, J. C., and Bezerra, J. L. 2000. *Trichoderma stromaticum*, sp. Nov., a parasite of the cacao witches broom pathogen. Mycol. Res. 104:760-764.
17. Wheeler, B. E. J., and Suárez, C. 1993. The pathosystem. Pages 9-19 in: Disease Management in Cocoa: Comparative Epidemiology of Witches' Broom. S. A. Rudgard, A. C. Maddison, and T. Andebrhan, eds. Chapman & Hall, London.
18. Winston, P. W., and Bates, P. W. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41:232-237.