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Production and Germination of Primary Conidia of *Neozygites floridana* (Zygomycetes: Entomophthorales) under Constant Temperatures, Humidities, and Photoperiods

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The production and germination of primary conidia of *Neozygites floridana* as affected by temperature, humidity, and photoperiod was studied in the laboratory. All tested factors significantly affected the two processes studied. Production increased with increasing temperature between 13 and 23°C, with means of 35.5 and 55.4 conidia, respectively, discharged from each mummified mite cadaver. No conidia were produced at 28 or 33°C. Conidial production dropped significantly from 96.1/mummy in a moisture-saturated environment [saturation deficit (SD) 0] to 33.9/mummy at SD 0.2. Very few conidia were produced at SD 0.7 (0.6/mummy) and none at SD 1.2. Significantly fewer conidia were produced under continuous light (11.2/mummy) than under continuous darkness (40.1/mummy) or 12L:12D (46.7/mummy) photoperiods. Between 82 and 100% of the conidia produced under 12L:12D photoperiod were released in the dark phase. Germination of primary conidia started within 2 hr and increased with decreasing temperature between 13 and 28°C. Percentage germination of 20.1% at 13°C and 17.6% at 18°C was significantly higher than 11.2% at 28°C. There was no germination at 33°C. High humidities (>95%) were necessary to effect germination. Germination at SD 0 (27.2%) and 0.2 (23.4%) was significantly higher than at SD 0.7 (0.4%), where germination began after 6 hr and was observed only at 13, 18, and 23°C. No germination was observed at SD 1.2. There was no germination among conidia maintained under continuous light. © 1996 Academic Press, Inc.

KEY WORDS: ***Neozygites floridana;*** **primary conidia; sporulation; germination; mummy; temperature; humidity; saturation deficit; photoperiod.**

INTRODUCTION

Population declines of several tetranychid mites due to epizootics caused by the fungal pathogen *Neozygites* (= *Entomophthora* = *Triplosporium*) spp. have been reported (Weiser and Muma, 1966; Carner and Canerday, 1968; Ramaseshiah, 1971; Kenneth *et al.,* 1972; Nemoto and Aoki, 1975; Humber *et al.,* 1981; Brandenburg and Kennedy, 1982). The initiation and duration of such epizootics are favored by certain environmental conditions. Brandenburg and Kennedy (1982) reported that temperatures lower than 29°C and relative humidities higher than 90% should prevail for about 40 hr before an increase in the incidence of *Neozygites* infections on *Tetranychus urticae* could be noted. These conditions, encountered mainly during rainy periods and at night, have also been reported to be ideal for the production of primary conidia by other entomophthoralean fungi (Glare *et al.,* 1986; Hajek *et al.,* 1990; Hajek and Soper, 1992; Smitley *et al.,* 1986; Steinkraus and Slaymaker, 1994; Wilding, 1969). Once produced, primary conidia of fungi *Neozygites* may germinate in one of three ways: by forming thick germ tubes, by producing conidiophores from which secondary conidia are forcibly ejected, or by forming long slender capillary tubes on which passively detached capilliconidia are borne (Carner, 1976; Ben Ze'ev and Kenneth, 1981). The presence of only capilliconidia attached to infected mites exposed to *Neozygites* sp. has led to the conclusion that the capilliconidia are the infective stage of this fungus (Selhime and Muma, 1966; Carner and Canerday, 1968; Nemoto and Aoki, 1975). The germination of primary conidia to produce capilliconidia is, therefore, a critical process in the life cycle of this fungus.

Neozygites floridana-caused epizootics in populations of the cassava green mite *Mononychellus tanajoa* show similarities with epizootics of *Neozygites fresenii* in populations of *Aphis gossypii* in cotton. Effect of tem-

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perature and humidity on conidia of the latter fungus has comprehensively been studied by Steinkraus and Slaymaker (1994). Epizootics in *A. gossypii* populations often occur in relatively dry regions. Cassava green mite populations also become frequently infected by *N. floridana* in areas with moderate rainfall (from 800 mm a year), while the fungus is also able to survive periods with extremely low humidities which are common in the states Bahia and Pernambuco in northeastern Brazil. These observations gave the impetus to the present study.

Primary conidial discharge and germination to produce capilliconidia are important processes which increase the pathogen load in the environment and subsequent chances of a fungal entomopathogen coming into contact with its host. A knowledge of how temperature, saturation deficit, and photoperiod individually or interactively influence these processes may improve the understanding of how fungal epizootics are initiated and maintained. Other aspects of the performance of the fungus under different climatic conditions have been reported in (Oduor *et al.,* 1995a, b, 1996).

MATERIALS AND METHODS

Mummified adult female cassava green mite, *M. tanajoa,* killed by *N. floridana* were collected in March 1992 during an epizootic in a cassava field in Piritiba, State of Bahia, in northeastern Brazil. The mummies, which were collected prior to sporulation of the fungus, were brushed onto a piece of dry cotton wool maintained a few centimeters from another piece of cotton wool partly soaked in 95% glycerol in plastic tubes (3 cm diameter × 5 cm high) with tight-fitting lids. The mummies were stored in the dark in a refrigerator at 4°C for less than 7 months before being used.

Production of Primary Conidia

Mummies were attached individually, using a double-sided sticky tape, to the inside bottom of cages (2.5 cm diameter × 0.5 cm high) cut from transparent plastic tubes. Two cages were turned open end down and attached on either end of a microscope slide (75 × 25 mm). The mummies were positioned with their dorsal sides facing down onto the slides. These slides were then placed on a plastic platform (15 × 9 × 4 cm) which maintained them over sulfuric acid solutions in a clear plastic lunch box (19 × 15 × 8 cm) that served as the humidity chamber. Four slides were placed in each chamber which contained 400 ml of sulfuric acid solutions at different concentrations to provide different levels of humidity (Stevens, 1916; Solomon, 1951). Pure distilled water was used to attain a saturated environment. The acid solutions were allowed to stabilize for 24 hr before mummies were added. Saturation deficit, the difference between the actual amount of water

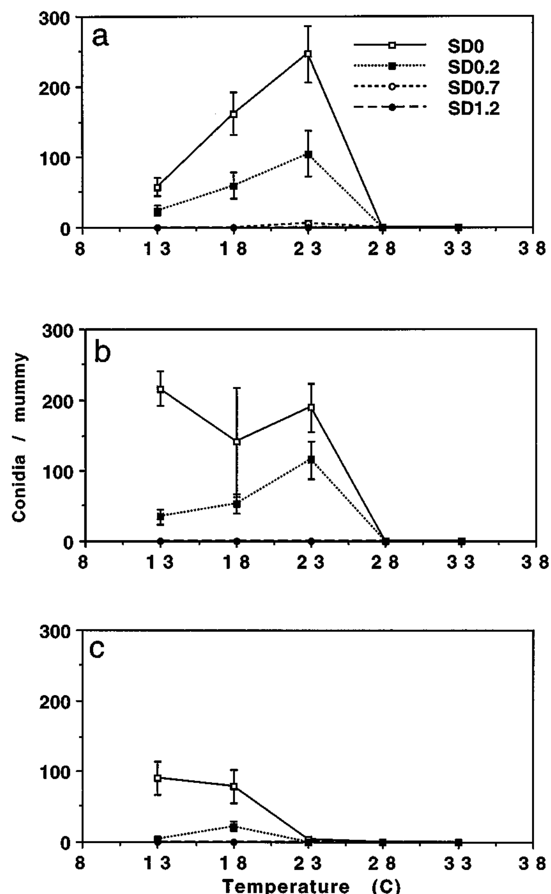

FIG. 1. Mean number of primary conidia of *N. floridana* released from mummified cassava green mites, *M. tanajoa,* at different temperatures, SDs and (a) 0L:24D, (b) 12L:12D, and (c) 24L:0D photoperiods. Each point represents the average value for observations from 24 mummies.

vapor present and the amount present at saturation point at the same temperature, is an indication of the "drying power" of the air and is therefore a more biologically relevant measure of atmospheric moisture than is relative humidity (Anderson, 1936; Ferro and Chapman, 1979).

Four saturation deficits (0, 0.2, 0.7, and 1.2 mm Hg) were used, as shown in Table 1. Humidity was checked

TABLE 1

Temperature and Saturation Deficit Combinations Used to Study Their Effect on the Production and Germination of Primary Conidia of *N. floridana* from Cadavers of *M. tanajoa*

Temperature (°C)	Saturation deficit in mm Hg (corresponding % RH)			
13	0 (100)	0.20 (98.2)	0.69 (93.9)	1.13 (89.9)
18	0 (100)	0.20 (98.7)	0.68 (95.6)	1.19 (92.3)
23	0 (100)	0.19 (99.1)	0.65 (96.9)	1.10 (94.8)
28	0 (100)	0.14 (99.5)	0.71 (97.5)	1.25 (95.6)
33	0 (100)	0.19 (99.5)	0.68 (98.2)	1.17 (96.9)

by inserting the probe of a thermohygrograph (Model Hygroskop GT-L, Rotronic, Zurich, Switzerland) through a resealable hole bored through one wall of the chambers. The temperatures tested were 13, 18, 23, 28, and 33°C. Temperature was controlled by placing the chambers in a continuously lit incubator which provided a precision of ±1.0°C. Light was provided by two 15-W daylight fluorescent tubes, giving a light intensity of 10 μEm^{-2} sec^{-1}. Light intensity was measured with a quantum/radiometer/photometer Model Li 185A (Li-Cor, Inc., Lincoln, NE). Photoperiod was controlled by placing some chambers into light-excluding black cloth bags without altering the ambient temperature conditions. The photoperiods used were 24L:0D (continuous light), 12L:12D (alternating 12 hr light and 12 hr dark), and 0L:24D (continuous darkness). The 12L:12D photoperiod treatment started with the light phase.

Slides in the containers were replaced at 3-hr intervals until no conidia were collected for two consecutive periods. Conidia deposited on the slides were stained with Amman's lactophenol-cotton blue and counted under a compound microscope at 63× magnification. Grid lines forming 3-mm squares drawn on a transparency and placed beneath the microscope slide aided in counting the conidia. Mummies from which the fungus had not produced spores after 54 hr were maintained on slides in the dark at 23°C in a saturated environment for 24 hr to stimulate sporulation. The experiment was done three times, giving a total of 24 mummies for every given combination of temperature, saturation deficit, and photoperiod.

Germination of Primary Conidia

Ten mummies were stuck in an upright position to the underside of a glass petri dish lid (14 cm diameter) using double-sided sticky tape. Four pieces of filter paper (13.5 cm diameter) were stacked on top of each other on the bottom of each petri dish and soaked with distilled water. The petri dishes were closed and left for a period of 4 hr in the dark at 23°C for initiation of the sporulation process. The lids were then lifted and five clean microscope slides (7.6 × 2.6 cm) were placed on the moist filter papers. The petri dish lids with the sporulating fungus were then placed on the petri dish bottoms so that two mummies were positioned directly above each microscope slide onto which primary conidia were deposited. The mummies were left in this position for 1 hr.

Five microscope slides each with two halos of primary conidia were then transferred onto a platform in each humidity chamber as described above to study sporulation. The environmental conditions studied were also similar except photoperiod, which was either continuous darkness or continuous light.

The germination of primary conidia was assessed 2, 6, 10, 14, and 18 hr after the collection of the conidia. A slide was removed from each chamber, representing a combination of three tested factors, at each sampling period. A drop of Amman's lactophenol–cotton blue was placed on each halo of conidia followed by a coverslip. The conidia were observed under a compound microscope at 100× magnification to determine the number of primary conidia that did not germinate the number that germinated to form capilliconidiophores only, and the number that germinated to form capilliconidiophores with distinct capilliconidia. In this study, only primary conidia that produced capilliconidia were considered germinated. Each slide represented a replicate and the experiment was conducted four times.

Statistical Analyses

The average number of conidia produced by each mummy maintained under the different treatments was calculated, transformed to square root ($x + 0.5$), and subjected to ANOVA using a SPSS-X statistical software package (SPSS, 1988) using the 60 values resulting from the factorial design with temperature (5) × humidity (4) × photoperiod (3). In the germination experiment, the average percentage germination at each observation was calculated for all combinations of factors. These data were then normalized by arcsin square-root transformation (Zar, 1984) and separately analyzed with a four-way factorial ANOVA (SPSS, 1988) with time, temperature, humidity, and photoperiod as the main effects. For ANOVAs, photoperiod was considered a "class" variable, while temperature and humidity were analyzed as continuous variables. To study the effect of the different treatments after each time interval, the percentage germination of primary conidia from each replicate in each experiment was calculated at each exposure time and normalized by arcsin square-root transformation (Zar, 1984). Separate three-way factorial ANOVAs (SPSS, 1988) were then done for each time interval using temperature, humidity, and light condition as the grouping variables. Student–Newman–Keuls test was used to determine the differences between means at a significance level of 5%.

RESULTS

Production of Primary Conidia

The average number of primary conidia produced from each mummy at different combinations of temperatures, humidities, and photoperiods was 32.7. The highest number of primary conidia produced from a single mummy was 510 in the saturated environment (SD 0) at 23°C in the dark. Temperature ($F = 24.1$, saturation deficit ($F = 51.8$, df = 3, 108, $P < 0.05$), and photoperiod ($F = 9.7$, df = 2, 108, $P < 0.05$) significantly influenced the production of primary conidia.

TABLE 2

Mean Numbers of Primary Conidia of *N. floridana* Produced from Each Mummified *M. tanajoa* at Different Temperatures, Humidities, and Photoperiods (SE, Standard Error)

Factor	Level	Mean conidia per mummy (SE)[a]
Temperature (°C)	13	35.5 (12.1) a*
	18	43.2 (14.4) a
	23	55.4 (17.4) a
	28	0.0 b
	33	0.0 b
Humidity (saturation deficit)	0	96.1 (19.5) a
	0.2	33.9 (11.5) b
	0.7	0.6 (0.4) c
	1.2	0.0 c
Photoperiod	0L:24D	40.1 (12.6) a
	12L:12D	46.7 (13.4) a
	24L:0D	11.3 (5.9) b

[a] Values low because are averages pooled over *all* tested conditions.
* Means Means corresponding to each factor followed by the same letter are not significantly different at 5% significance level (Student–Newman–Keuls test).

Production was observed between 13 and 23°C, but was suppressed at 28 and 33°C (Fig. 1). Mummies maintained at 28 and 33°C dried up, appeared shrivelled, and did not liberate conidia even after being maintained for 24 hr at 23°C, SD 0, in the dark.

Production of conidia decreased significantly with increasing SDs between SD 0.7 and 0 (Table 2). Production at SD 0.7 was observed only at 23°C in the dark. Conidia were not produced at SD 1.2. However, 85.8 and 88.9% of the mummies previously unable to liberate conidia at SDs 0.7 and 1.2, respectively, at different temperatures, readily did so when incubated for 24 hr at SD 0 in the dark at their respective temperatures.

Significantly more conidia were produced under continuous darkness and 12L:12D photoperiods than under continuous light (Table 2). Continuous light reduced the number of conidia liberated and inhibited spore production from mummies at 23°C. However, an average of 75% of the mummies previously maintained in the light at this temperature readily liberated conidia when transferred to SD 0 in the dark (data not shown).

There was a significant interaction between temperature and SD ($F = 10.1$, df = 12, 108, $P < 0.05$) because the effect of SD was not the same at all temperatures, the differences being greatest at 23°C with 0L:24D photoperiod and at 13°C with 12L:12D and 24L:0D photoperiods (Fig. 2). Temperature had less effect on sporulation at higher SDs where only a few conidia were released. In the saturated environment (SD 0) between 13 and 23°C, increasing temperature led to increasing sporulation at 0L:24D photoperiod, little

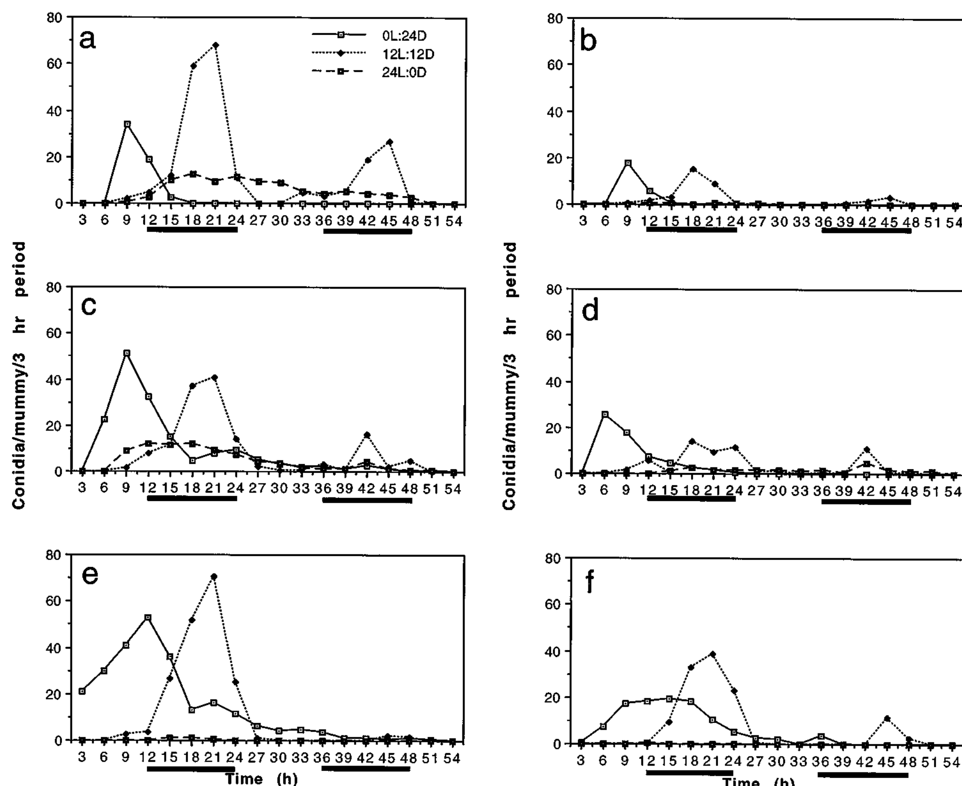

FIG. 2. Primary conidia production at different photoperiods, saturation deficits of (a) 0 and (b) 0.2, at 13°C; (c) 0 and (d) 0.2, at 18°C, and (e) 0 and (f) 0.2, at 23°C. The bars at the bottom indicate the dark phase of the 12L:12D photoperiod.

difference in sporulation at 12L:12D, and decreasing sporulation at 24L:0D photoperiod (Fig. 2). This partly explains the significant interaction between temperature and photoperiod ($F = 3.9$, df = 8, 108, $P < 0.05$). Whereas sporulation at SD 0, between 13 and 23°C, was high with large differences in spore production between photoperiods, this difference was lower at the higher SDs, partly explaining the significant interaction between SD and photoperiod ($F = 3.4$, df = 6, 108, $P < 0.05$).

Figure 2 shows the pattern of conidial release from mummies maintained at SDs 0 and 0.2 at different temperatures and photoperiods over a 54-hr period. Whereas there were time lags of 6–9 and 3–6 hr before conidial release could begin at 13 and 18°C, respectively, conidiation began within 3 hr at 23°C.

At 13°C and SD 0, constant darkness led to a peak conidial production rate of 33.8 conidia/mummy/3-hr period 9 hr after the beginning of the experiment (Fig. 2a). Few conidia were released thereafter. At a 12L:12D photoperiod, few conidia were released during the first 12-hr light phase, but this was followed by a steep increase beginning with the dark phase to a maximum of 68.1 conidia 9 hr later. After a decrease in the rate of conidiation during the intervening light phase, there was another increase to a peak release of 26.8 conidia another 9 hr after the onset of the second dark phase. There was no clear peak conidial production when the fungus was maintained under constant light. At SD 0.2, total darkness led to the highest conidial production rate of 18.0 conidia/mummy/3-hr period after 9 hr (Fig. 2b). The 12L:12D photoperiod led to lower peaks of 15.2 and 3.3 conidia in the two dark phases, separated by a low production period during the intervening light phase. Under continuous light, the fungus produced an average of 0.2 conidia/mummy/3-hr period without a distinct peak.

At 18°C and SD 0, the highest number of conidia released under continuous darkness (51.1) and continuous light (11.8) occurred 9 and 12 hr, respectively, after the test began (Fig. 2c). From initial low numbers, the conidial release from the fungus at 12L:12D photoperiod rose to a maximum of 40.6 conidia, 9 hr after the onset of the first dark phase. At SD 0.2, maximum conidial release occurred after 6 hr in total darkness (25.4 conidia) and during the first dark phase among mummies maintained at 12L:12D photoperiod (13.9 conidia). An average of 1.3 conidia/mummy/3-hr period were released under continuous light (Fig. 2d).

Conidial production at 23°C began after 3 hr among mummies maintained in total darkness at SDs 0 and 0.2 (Fig. 2e). Total darkness led to an early peak production of 53.0 conidia. At 12L:12D photoperiod, few conidia were released during the first 12 hr, under light. However, after the onset of the dark phase the rate increased to a maximum of 70.3 conidia 9 hr later. This was followed by a steep decline as the second light phase commenced. Constant light barely led to the release of conidia. At SD 0.2, conidiation under constant darkness began after 3 hr and increased gradually to a maximum of 19.2 conidia after 15 hr (Fig. 2f). Very few conidia were released from mummies maintained at 12L:12D photoperiod until the onset of the dark phase when a maximum of 38.9 conidia were released. An average of 0.03 conidia/mummy/3-hr period were released under constant light.

Few conidia were released at SD 0.7, regardless of temperature or photoperiod, with the highest rate (2.6 conidia/mummy/3-hr period) occurring after 9 hr at the 0L:24D photoperiod.

Among mummies exposed to 12L:12D photoperiod, 82–100% of the conidia collected were released during the dark phase. No consistent difference was noted in the duration of conidiation from mummies maintained at different conditions of temperature, humidity, and photoperiod.

Germination of Primary Conidia

The germination of primary conidia was significantly affected by exposure time ($F = 160.5$, df = 4, 600, $P < 0.05$). Germination began within 2 hr at SD 0 and 0.2 and within 6 hr at SD 0.7. Temperature significantly affected germination which appeared to increase with decreasing temperatures between 28 and 13°C ($F = 279.9$, df = 4, 600, $P < 0.05$). There was no germination at 33°C.

The effect of saturation deficit on germination was also significant ($F = 1201.0$, df = 4, 600, $P < 0.05$) with germination at SDs 0 (27.2%) and 0.2 (23.4%) being significantly higher than that at higher SDs (Table 3).

Germination at SD 0 in the dark increased with time at all temperatures except 33°C (Table 4). After only 2 hr, germination was already high at 13°C (68.6%), but had not begun at 28°C. The germinated conidia at this time had only the protruding capilliconidiophores without any capilliconidia. After 6 hr, the percentages of primary conidia that had formed capilliconidia were 57.7, 42.5, 39.1, and 0% at 13, 18, 23, and 28°C, respectively (Table 4). The germination was above 85% at all temperatures after 18 hr.

Germination at SD 0.2 in the dark followed a similar trend although at a slower rate (Table 4). After 6 hr, the percentages of primary conidia with capilliconidia were 14.3, 13.1, 1.6, and 0% at 13, 18, 23, and 28°C, respectively. Germination at 28°C was first observed (42.1%) 10 hr after the deposition of the primary conidia.

Germination was low at SD 0.7 with capilliconidia being formed (less than 1%) only at 18°C (Table 4). Germination reached a maximum of 5.6, 2.6, and 1.8% at 13, 18, and 23°C, respectively. There was no germination at 28°C at this SD.

TABLE 3

Mean Percentage Germination of Primary Conidia of *N. floridana,* Pooled across All Factors, after Different Periods of Time under Different Environmental Conditions (SE = Standard error)

Factor	Level	Mean percentage germination (SE)
Exposure time (hr)	2	3.0 (1.8) a*
	6	10.2 (3.9) b
	10	15.8 (5.1) b
	14	17.4 (5.6) b
	18	17.3 (5.5) b
Temperature (°C)	13	20.1 (5.7) a
	18	17.6 (5.4) a
	23	14.9 (4.8) ab
	28	11.2 (4.4) b
	33	0.0 c
Humidity (saturation deficit)	0	27.2 (5.7) a
	0.2	23.4 (5.0) a
	0.7	0.4 (0.1) b
	1.2	0.0 b
Light condition	Dark	25.5 (3.8) a
	Light	0.0 b

* Values within columns, corresponding to each factor, followed by the same letter are not significantly different at 5% significance level (Student–Newman–Keuls test).

There was no germination at SD 1.2 at any temperature.

Germination was also significantly affected by light condition and no germination was observed among primary conidia maintained under continuous light ($F = 3720$, df = 1, 600, $P < 0.05$).

The effects of temperature, humidity, and photoperiod on germination at each sampling period are shown in Table 5. The effects of temperature and SD were not significant after a 2-hr exposure time, but became significant after 6 or more hr. This explains the significant interaction between exposure time and temperature ($F = 19.7$, df = 16, 600, $P < 0.05$) and between exposure time and SD ($F = 49.8$, df = 12, 600, $P < 0.05$). Whereas germination was not observed in the light, it increased with exposure time and decreased with increasing temperatures and SDs in the dark. This explains the significant interactions between light condition and exposure time ($F = 150.5$, df = 4, 600, $P < 0.05$), light condition and temperature ($F = 49.8$, df = 12, 600, $P < 0.05$), and light condition and SD ($F = 11667$, df = 3, 600, $P < 0.05$). The significant interaction between temperature and SD ($F = 84.7$, df = 12, 600, $P < 0.05$) is partly due to the large differences in germination at different temperatures at low SDs being suppressed at higher SDs, where there was little or no germination.

DISCUSSION

Entomophthoralean fungi have been reported to sporulate over a wide range of temperatures. *Zoophthora phalloides* sporulates within a range of 4 to 25°C (Glare *et al.,* 1986), *Entomophthora* spp. sporulates between 5 and 25°C (Wilding, 1971; Milner, 1981), *Zoophthora radicans* sporulates between 15 and 30°C (Milner and Lutton, 1983), *Entomophaga maimaiga* sporulates between 2 and 25°C (Hajek *et al.,* 1990), *Entomophthora gammae* sporulates between 10 and 26.7°C, and *Neozygites floridana* sporulates between 10 and 26.7°C (Smitley *et al.,* 1986). In all these cases, higher temperatures inhibited conidial release. Similar results were recorded in this study whereby sporulation was possible at 23°C but was suppressed at 28 and 33°C. The minimum temperature that allows sporula-

TABLE 4

Mean Percentage Germination of Primary Conidia of *N. floridana* after Different Exposure Times at Different Temperatures and Humidities under Continuous Darkness

Temperature (°C)	Humidity (SD)	Germination state[a]	Exposure time (hr) 2	6	10	14	18
13	0	NG	31.4	15.0	4.0	7.7	4.9
		CPH	68.6	27.3	12.7	6.5	2.3
		CAP	0	57.7	83.4	85.8	92.8
	0.2	NG	59.6	23.3	24.4	11.2	8.8
		CPH	40.4	62.5	49.0	50.1	29.5
		CAP	0	14.3	26.6	38.6	61.8
	0.7	NG	100.0	94.6	98.6	98.5	98.3
		CPH	0	5.6	1.4	1.5	1.7
		CAP	0	0	0	0	0
18	0	NG	85.7	32.6	9.1	8.9	7.5
		CPH	14.4	25.0	17.7	9.9	2.3
		CAP	0	42.5	73.2	81.3	90.2
	0.2	NG	98.6	33.4	13.6	10.9	14.5
		CPH	1.4	53.5	69.4	48.1	29.3
		CAP	0	13.1	17.0	41.0	55.9
	0.7	NG	100.0	100.0	97.7	97.1	98.8
		CPH	0	0	2.3	2.6	1.2
		CAP	0	0	0	0.4	0
23	0	NG	97.7	36.8	22.0	7.3	12.5
		CPH	2.3	24.1	20.8	16.7	4.9
		CAP	0	39.1	57.2	76.0	82.7
	0.2	NG	100.0	59.7	24.8	22.3	24.3
		CPH	0	38.7	59.7	44.2	19.0
		CAP	0	1.6	15.5	33.5	56.6
	0.7	NG	100.0	100.0	98.2	98.7	98.7
		CPH	0	0	1.8	1.3	1.3
		CAP	0	0	0	0	0
28	0	NG	100.0	99.0	18.3	6.9	14.6
		CPH	0	1.0	42.3	49.4	19.0
		CAP	0	0	39.4	43.6	66.4
	0.2	NG	100.0	100.0	57.9	32.3	24.8
		CPH	0	0	36.7	42.3	38.1
		CAP	0	0	5.4	25.5	37.1
	0.7	NG	100.0	100.0	100.0	100.0	100.0
		CPH	0	0	0	0	0
		CAP	0	0	0	0	0

Note. There was no germination at 33°C and not at SD 1.2, irrespective of the conditions.

[a] NG, Nongerminated conidia; CPH, germinated conidia with only capilliconidiophore; CAP, germinated conidia with capilliconidia.

TABLE 5

ANOVA of the Germination of Primary Conidia of *N. floridana* after Different Time Intervals Given the Effect of Temperature, Humidity, and Light Condition

	Exposure time (hr)				
Factor[a]	2	6	10	14	18
Temperature					
F ratio	2.9	3.6	3.3	3.5	3.5
df	4, 120	4, 120	4, 120	4, 120	4, 120
P value	0.067	0.039	0.048	0.043	0.030
Saturation deficit					
F ratio	2.6	6.3	14.5	15.4	15.7
df	3, 120	3, 120	3, 120	3, 120	3, 120
P value	0.104	0.008	0.000	0.000	0.000
Light condition					
F ratio	7.2	21.5	49.5	53.6	54.9
df	1, 120	1, 120	1, 120	1, 120	1, 120
P value	0.020	0.001	0.000	0.000	0.000

[a] The levels of factors tested were temperature 13, 18, 23, 28, and 33°C, saturation deficit 0, 0.2, 0.7, and 1.2 mm Hg and light conditions continuous darkness and continuous light.

tion by *N. floridana* was not determined in this study, but it is certainly below 13°C, the lowest temperature tested. Kenneth *et al.* (1972) reported that a species of *Neozygites,* closely related to the one in this study, sporulated even at 4°C. The optimum temperature for spore release of 18–23°C reported in this study approaches the 16–21°C obtained by Smitley *et al.* (1986) in the United States for *N. floridana* but differs from 27–30°C reported by Kenneth *et al.* (1972) in Israel for the same species.

The sporulation by *N. floridana* in this study was limited to SDs 0 and 0.2 at temperatures between 13 and 23°C, except at SD 0.7 where few conidia were produced at 23°C. This represents relative humidities higher than 95%. Smitley *et al.* (1986) showed that *N. floridana* could not sporulate at humidities of less than 98% between 10.0 and 32.2°C (i.e., SD > 0.7). The need for such high humidities may not be as restrictive as it appears since a period of only 3 to 9 hr under these conditions is needed for substantial numbers of conidia to be formed and released. Similar results were reported by Millstein *et al.* (1982) who found that a minimum of 3 hr at relative humidity higher than 92% was needed to induce conidial release in *Erynia* sp. in the field. Whereas Smitley *et al.* (1986) reported that a two-spotted spider mite infected with *N. floridana* could produce up to 3141 primary conidia, our study recovered a maximum of 510 conidia from a mummy. An explanation could be that, although primary conidia in our study were allowed to fall down from a mummy, some conidia could have remained attached to the mummy or cages to which they were stuck. Second, whereas conidia in our study were counted within 3 hr of their discharge, thereby excluding the morphologically similar secondary conidia, Smitley *et al.* (1986) counted conidia 5 days after the mummies were exposed to the experimental conditions during which several secondary, tertiary, etc., conidia could have been formed.

Light suppressed conidial production and almost all of the conidia liberated at 12L:12D photoperiod were discharged during the dark phase. This shows that the onset of darkness in nature stimulates sporulation, since the dark phases in the present study were accompanied by increased discharge of conidia. That the fungus was capable of resuming spore production after the intervening light phase shows that it can commence sporulation in one evening, reduce or suspend it during daytime, and resume the liberation of spores the following evening. Studies on the effect of light on sporulation by different entomopathogenic fungi have produced conflicting results. Although light has been reported to stimulate sporulation in *Conidiobolus coronatus* and *Basidiobolus ranarum* (Callaghan, 1969, 1978) and *Conidiobolus obscurus* (Wilding, 1971), the results of Glare *et al.* (1986) indicate that the onset of the dark phase stimulates primary spore release in *Zoophthora phalloides.* Similarly, Kenneth *et al.* (1972) found that sporulation was observed in 75% of *Tetranychus* spp. infected by *N. floridana* maintained under constant darkness, as opposed to 33% of those that received 13 hr of light per day.

Primary conidia of *Neozygites* sp. were able to germinate and produce capilliconidia over a temperature range of 13 to 28°C. This range compares well with 10 to 29°C for *N. floridana* (Smitley *et al.,* 1986), 0 to 36°C for *Z. radicans* (Roermund *et al.,* 1984), and 10 to 20°C for *Z. phalloides* (Glare *et al.,* 1986). The primary conidia of the species of *Neozygites* used in this study may germinate at temperatures below 13°C. The broad temperature range over which germination occurs may partly explain the wide distribution of *Neozygites* sp. in both the temperate and the tropical regions of the world (Brandenburg and Kennedy, 1982; Keller, 1991; Nemoto and Aoki, 1975; Ramaseshiah, 1971; Milner and Holdom, 1986; Wilding *et al.,* 1986). The ability of this fungus to germinate over a wide range of temperatures could also increase its chances of establishing if it is to be introduced into new areas. This enables capilliconidia to be produced over a long period of time in the night and allows a higher density of infective propagules that will in turn increase the chances of transmission.

The production of capilliconidia by *N. floridana* was possible at SD 0 and 0.2 and only scarcely at SD 0.7. Glare *et al.* (1986) also found that whereas a high percentage of primary conidia of *Z. phalloides* germinated to form capilliconidia at 100% RH (SD 0) and 98% RH (SD 0.20), there was no germination at either 94% RH (SD 0.77) or 91% RH (SD 1.15). They reported

further that germination occurred over all the temperatures tested between 10 and 20°C. These results suggest that the germination process of the primary conidia of *Neozygites* sp. was more fastidious with respect to humidity than to temperature. The same pattern has been reported for *Entomophaga maimaiga, Erynia neoaphidis, Entomophthora gammae,* and *Entomophthora delphacis* (Hajek *et al.,* 1990; Milner and Bourne, 1983; Newman and Carner, 1975; Shimazu, 1977). Free water or near-saturated environment has been reported to be necessary for the germination of the primary conidia of various entomophthoralean fungi (Yendol, 1968; Newman and Carner, 1975; Shimazu, 1977; Carruthers and Haynes, 1986; Glare *et al.,* 1986; Hajek *et al.,* 1990). Although the need for such high humidities was shown in this study, these conditions need not prevail for long. For example, 57 and 85% of the primary conidia had already germinated after 2 and 6 hr, respectively, when the saturation deficit was 0 and the temperature was 13°C. At SD 0.2, under similar conditions, the respective germination rates were 41 and 76%. Further, Steinkraus and Slaymaker (1994) reported that 93% of the primary conidia of *N. fresenii* held at 25°C and 100% R.H. (SD 0) germinated to form capilliconidia within only 6 hr.

Exposure to direct light is detrimental to the viability of the conidia of some entomopathogenic fungi (Brobyn *et al.,* 1985; Carruthers and Haynes, 1986; Carruthers *et al.,* 1988). Some of the reasons advanced for this lethal effect include photodeactivation of nucleic acids (Ignoffo *et al.,* 1977) and/or mere overheating (Leach, 1977). The effect of light on primary conidia observed in this study at all levels of temperature and SD indicates that in nature, germination occurs at night. The onset of darkness has been shown to stimulate the production of primary conidia by some capilliconidia producing entomophthoralean fungi like *Z. phalloides* (Glare *et al.,* 1986) and also in this study. That both the production and the germination of the primary conidia need similar light conditions may enable the accelerated development of these fungi and shorten the time when it is exposed to deleterious environmental conditions, especially sunlight, desiccation, and high temperatures. That almost all primary conidia germinated to form the infective capilliconidium stage, rather than other secondary conidia, may further shorten this exposure period. Even if the capilliconidia do not infect their host, it is advantageous for the fungus to be in this developmental stage, since they have a thicker cell wall and are more resistant to adverse environmental conditions than the primary conidia, as is the case in *Er. radicans, Z. phalloides* and *N. fresenii* (Uziel and Kenneth, 1991; Uziel and Shtienberg, 1993).

The way in which temperature, humidity, and light influence the release and germination of primary conidia is complex, as indicated by the various significant interactions. For example, the increase in release and germination of primary conidia at decreasing SDs was not uniform at each temperature, as evidenced by the significant humidity and temperature interactions. Whereas these processes were stimulated at SDs 0 and 0.2 at 13, 18, and 23°C, they were suppressed or inhibited at 28 and 33°C at the same SDs. Specific levels of various environmental factors have to prevail simultaneously to enable the fungus to develop. These results partly explain the sudden appearance and disappearance of epizootics caused by *N. floridana* among populations of *M. tanajoa.*

The results of these studies indicate that the time of the day when production and germination of primary conidia are stimulated is after sunset when temperatures and saturation deficits are low. These processes are very sensitive to the ambient humidity. The humidities near the leaf surfaces (where the conidia are generally located), especially on calm nights, may be near the saturation point and quite different from the ambient air humidity (Nobel, 1974; Ferro and Southwick, 1984). Although processes other than production and germination of primary conidia are also critical in the infection process, these results partly explain why epizootics caused by this fungus occur during or just after the rainy season, as has been observed in Piritiba in the state of Bahia, Brazil (G. J. Moraes, unpublished data). The broad temperature range within which the discharge and the germination of the primary conidia of this fungus occur, together with the capacity of these processes and the existence of a higher humidity in the microhabitat occupied by the cassava green mite, makes this fungus a good candidate for the biological control of this pest.

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REFERENCES

Anderson, D. B. 1938. Relative humidity or vapour pressure deficit. *Ecology* **17,** 277–282.

Ben-Ze'ev, I., and Kenneth, R. G. 1982. Features-criteria of taxonomic value in the Entomophthorales: 1. A revision of the Batkoan Classification. *Mycotaxon* **14,** 393–455.

Brandenburg, R. L., and Kennedy, G. G. 1982. Relationships of *Neozygites floridana* (Entomophthorales: Entomophthoraceae) to two-spotted spider mite (Acari: Tetranychidae) populations in field corn. *J. Econ. Entomol.* **75,** 691–694.

Brobyn, P. J., Wilding, N., and Clark, S. J. 1985. The persistence of infectivity of conidia of the aphid pathogen *Erynia neoaphidis* on leaves in the field. *Ann. Appl. Biol.* **107,** 365–376.

Callaghan, A. A. 1969. Light and spore discharge in Entomophthorales. *Trans. Br. Mycol. Soc.* **53,** 87–97.

Callaghan, A. A. 1978. Influence of nutrient level, pH and light on conidial germination in entomophthoraceous fungi. *Trans. Br. Mycol. Soc.* **70,** 271–275.

Carner, G. R. 1976. A description of the life cycle of *Entomphthora* sp. in the two-spotted spider mite. *J. Invertebr. Pathol.* **28,** 245–254.

Carner, G. R., and Canerday, T. D. 1968. Field and laboratory investigations with *Entomophthora fresinii,* a pathogen of *Tetranychus* spp. *J. Econ. Entomol.* **61,** 956–959.

Carruthers, R. I., and Haynes, D. L. 1986. Temperature, moisture and habitat effects on *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) conidial germination and survival in the onion agroecosystem. *Environ. Entomol.* **15,** 1156–1160.

Carruthers, R. I., Feng, Z., Ramos, M. E., and Soper, R. S. 1988. The effect of solar radiation on the survival of *Entomophaga grylli* (Entomophthorales: Entomophthoraceae) conidia. *J. Invertebr. Pathol.* **52,** 154–162.

Ferro, D. N., and Southwick, E. E. 1979. Effect of different constant humidities and temperatures on the two-spotted spider mite egg hatch. *Environ. Entomol.* **8,** 701–705.

Ferro, D. N., and Southwick, E. E. 1984. Micro-climates of small arthropods: Estimating humidity within the leaf boundary layer. *Environ. Entomol.* **13,** 926–929.

Glare, T. R., Milner, R. J., and Chilvers, G. A. 1986. The effect of environmental factors on the production, discharge and germination of primary conidia of *Zoophthora phalloides* Batko. *J. Invertebr. Pathol.* **48,** 275–283.

Hajek, A. E., Carruthers, R. I., and Soper, R. S. 1990. Temperature and moisture relations of sporulation and germination by *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), a fungal pathogen of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environ. Entomol.* **19,** 85–90.

Hajek, A. E., and Soper, R. S. 1992. Temporal dynamics of *Entomophaga maimaiga* after death of Gypsy moth (Lepidoptera: Lymantriidae) larval hosts. *Environ. Entomol.* **21,** 129–135.

Humber, R. A., Moraes, G. J., and dos Santos, J. M. 1981. Natural infection of *Tetranychus evansi* (Acari: Tetranychidae) by *Triplosporium* sp. (Zygomycetes: Entomophthorales) in northeastern Brazil. *Entomophaga* **26,** 421–425.

Ignoffo, C. M., Hostetter, D. L., Sikorowski, P. P., Sutter, G., and Brooks, W. M. 1977. Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus and protozoan by an ultraviolet light source. *Environ. Entomol.* **6,** 411–415.

Keller, S. 1991. Arthropod-pathogenic Entomophthorales of Switzerland. II. *Erynia, Eryniopsis, Neozygites, Zoophthora* and *Tarichium. Sydowia* **43,** 39–122.

Kenneth, R. G., Wallis, G., Gerson, U., and Plaut, H. N. 1972. Observations and experiments on *Triplosporium floridanum* attacking spider mites in Israel. *J. Invertebr. Pathol.* **19,** 366–369.

Leach, C. M. 1971. A practical guide to the effects of visible and ultraviolet light on fungi. *In* "Methods in Microbiology" (C. Booth, Ed.), Vol. 4, pp. 609–664. Academic Press, New York.

Millstein, J. A., Brown, G. C., and Nordin, G. L. 1982. Microclimatic humidity influence on conidial discharge in *Erynia* sp. (Entomophthorales: Entomophthoraceae), an entomopathogenic fungus of the alfalfa weevil (Coleoptera: Curculionidae). *Environ. Entomol.* **11,** 1166–1169.

Milner, R. J. 1981. Pattern of primary spore discharge of *Entomophthora* spp. from blue green aphid, *Acyrthosiphon kondoi. J. Invertebr. Pathol.* **38,** 419–425.

Milner, R. J., and Bourne, J. 1983. Influence of temperature and leaf wetness on infection of *Acyrthosiphon kondoi* with *Erynia neoaphidis. Ann. Appl. Biol.* **102,** 19–27.

Milner, R. J., and Lutton, G. G. 1983. Effect of temperature on *Zoophthora radicans* (Brefeld) Batko: An introduced microbial control agent of the spotted alfalfa aphid *Therioaphis trifolii* (Monell) f. *maculata. J. Aust. Entomol. Soc.* **22,** 167–173.

Milner, R. J., and Holdom, D. J. 1986. First record of *Neozygites fresenii* (Nowakowski) Batko, a fungal pathogen of aphids in Australia. *J. Aust. Ent. Soc.* **25,** 85–86.

Nemoto, H., and Aoki, J. 1975. *Entomophthora floridana* (Entomophthorales: Entomophthoraceae) attacking the sugi spider mite, *Oligonychus hondoensis* (Acari: Tetranychidae) in Japan. *Appl. Entomol. Zool.* **10,** 90–95.

Newman, G. G., and Carner, G. R. 1975. Environmental factors affecting conidial sporulation and germination of *Entomophthora gammae. Environ. Entomol.* **4,** 615–618.

Nobel, P. S. 1974. Introduction to biophysical plant ecology. W. H. Freeman, San Francisco.

Oduor, G. I., Yaninek, J. S., van der Geest, L. P. S., and de Moraes, G. J. 1995a. Survival of Neozygites cf. floridana (Zygomycetes: Entomophthorales) in mummified cassava green mites and the viability of its primary conidia. *Exp. Appl. Acarol.* **19,** 479–488.

Oduor, G. I., de Moraes, G. J., Yaninek, J. S., and van der Geest, L. P. S. 1995b. Effect of temperature, humidity and photoperiod on mortality of *Mononychellus tanajoa* (Acari: Tetranychidae) Infected by *Neozygites* cf. *floridana* (Zygomycetes: Entomophthorales). *Exp. Appl. Acarol.* **19,** 571–579.

Oduor, G. I., Yaninek, J. S., van der Geest, L. P. S., and de Moraes, G. J. 1996. Germination and viability of capilliconidia of *Neozygites floridana* (zygomycetes: Entomophthorales) under constant temperature, humidity and light conditions). *J. Invertebr. Pathol.,* **67,** 267–278.

Ramaseshiah, G. 1971. Occurrence of an *Entomophthora* sp. on tetranychid mites in India. *J. Invertebr. Pathol.* **18,** 421–424.

Roermund, H. J. W., van Perry, D. F., and Tyrrell, D. 1984. Influence of temperature, light, nutrients and pH an determination of the mode of conidial germination in *Zoophthora radicans. Trans. Br. Mycol. Soc.* **82,** 31–38.

Selhime, A. G., and Muma, M. H. 1966. Biology of *Entomophthora floridana* attacking *Eutetranychus banksi. Fla. Entomol.* **49,** 161–168.

Shimazu, M. 1977. Factors affecting conidial germination of *Entomophthora delphacis* Hori (Entomophthorales: Entomophthoraceae). *Appl. Ent. Zool.* **12,** 260–264.

Smitley, D. R., Kennedy, S. G., and Brooks, M. W. 1986. Environmental effects on production of primary and secondary conidia, infection and pathogenesis of *Neozygites floridana,* a pathogen of the two-spotted spider mite, *Tetranychus urticae. J. Invertebr. Pathol.* **47,** 325–332.

Slatyer, R. O. 1967. Plant water relationships. Academic Press, New York.

Solomon, M. E. 1951. Control of humidity with potassium hydroxide, sulphuric acid or other solutions. *Bull. Entomol. Res.* **42,** 543–553.

SPSS, 1988. "SPSS-X User's Guide," 3rd ed., SPSS, Chicago, IL.

Steinkraus, D. C., and Slaymaker, P. H. 1994. Effect of temperature and humidity on formation, germination, and infectivity of conidia of *Neozygites fresenii* (Zygomycetes: Neozygitaceae) from *Aphis* gossypii (Homoptera: Aphididae). *J. Invertebr. Pathol.* **64,** 130–137.

Stevens, N. E. 1916. A method of studying humidity relations of fungi in culture. *Phytopathology* **16,** 428–432.

Uziel, A., and Kenneth, R. G. 1991. Survival of primary conidia and capilliconidia at different humidities in *Erynia* (sub genus *Zoophthora*) spp. and in *Neozygites fresenii* (Zygomycotina: Entomophthorales), with special emphasis on *Erynia radicans. J. Invertebr. Pathol.* **58,** 118–126.

Uziel, A., and Shtienberg, D. 1993. Effects of meteorological variates on persistence of primary conidia and capilliconidia of *Erynia radicans* (Zygomycotina: Entomophthorales) under natural conditions. *Ann. Appl. Biol.* **122,** 441–450.

Weiser, J., and Muma, M. H. 1966. *Entomophthora floridana* n. sp. (Phycomycetes: Entomophthoraceae), a parasite of the Texas citrus mite *Eutetranychus banksi. Fla. Entomol.* **49,** 155–159.

Wilding, G. N. 1969. Effect of humidity on sporulation of *Entomophthora aphidis* and *E. thaxteriana. Trans. Br. Mycol. Soc.* **53,** 126–130.

Wilding, G. N. 1971. Discharge of conidia of *Entomophthora thaxteriana* from pea aphids, *A. pisum* Harris. *J. Gen. Microbiol.* **69,** 417–422.

Wilding, N., Mardell, S. K., and Brobyn, P. J. 1986. Introducing *Erynia neoaphidis* into a field of *Aphis fabae:* Form of the inoculum and effect of irrigation. *Ann. Appl. Biol.* **108,** 373–385.

Yendol, W. G. 1968. Factors affecting the germination of *Entomophthora* conidia. *J. Invertebr. Pathol.* **10,** 116–121.

Zar, J. H. 1984. “Biostatistical Analysis.” Prentice Hall, New Jersey.