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Survival of *Neozygites* cf. *floridana* (Zygomycetes: Entomophthorales) in mummified cassava green mites and the viability of its primary conidia

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ABSTRACT

The survival of *Neozygites* cf. *floridana* (Weiser and Muma) as dry hyphal bodies in mummified cassava green mites, *Mononychellus tanajoa* (Bondar), at 5.0% RH in the dark was affected by storage temperature. Survival of the fungus in mummies kept at 24 ± 1.0°C could be demonstrated for 6–7 months. When stored at 4°C, the fungus sporulated from 90% of the mummies liberating an average of 186.9 primary conidia per mummy even after a storage period of 16 months, when the experiment was terminated. The temperature, humidity and light condition significantly affected the viability of primary conidia. The percent viability across all factors dropped from 98.4% after 0 h (beginning of the experiment) to 23.4% after a 1 h exposure to the conditions tested. Lower temperatures maintained higher viabilities with 86.3% of the conidia surviving after 18 h at 18°C, whereas almost all conidia died after 12 h at 33°C. Conidia survived less than 1 h when exposed to SDs (saturation deficit) of 2.0 mm Hg or higher at any tested temperature.

Key words: *Neozygites* cf. *floridana, Mononychellus tanajoa,* mummy, survival, primary conidia, germination, viability, temperature, humidity, saturation deficit, light condition.

INTRODUCTION

The transmission of a fungal pathogen to healthy hosts is one of the most critical processes in the life cycle of the pathogen. The rate of transmission, which is important in the initiation and duration of epizootics, is determined mainly by three factors: the host population, the pathogen population and the environment (Benz, 1987; Tanada and Watanabe,

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1987; Fuxa, 1987). Entomopathogenic fungi generally pass the period before epizootics either at low prevalence (enzootic) levels in the living hosts in the stage of hyphal bodies or as resting spores in dead hosts. The latter strategy aids the fungus in surviving through unfavourable seasons (Sawyer, 1931; Kenneth *et al.*, 1972; Wilding, 1973; Nemoto and Aoki, 1975; Carner, 1976). Some fungi form conidia in young immature stages of their hosts and resting spores in older ones, which may be an attempt to increase survival (Wilding and Lauckner, 1974; Newman and Carner, 1975; Shimazu, 1979; Shimazu and Soper, 1986). Under favourable conditions, in particular a high relative humidity, hyphal bodies or resting spores may liberate primary conidia into the surrounding environment. Some time may invariably elapse before these conidia germinate. Primary conidia that do not germinate soon after being produced may have their viabilities reduced by the prevailing environmental conditions. Clerk and Madelin (1965) reported that the conidia of *Beauveria bassiana* (Balsomo) Vuillemin, *Paecilomyces farinosus* (Holm) Brown and Smith and *Metarhizium anisopliae* (Metchnikoff) Sorokin remained viable for longer periods when maintained at low temperatures, low relative humidities and in the dark. How environmental conditions affect the survival of *Neozygites* cf. *floridana* as hyphal bodies in mummified *Mononychellus tanajoa* and as primary conidia in the open environment is the subject of this study.

MATERIALS AND METHODS

Mummified adult female cassava green mites, *M. tanajoa* killed by *N. floridana* were collected in March 1992 during an epizootic in a cassava field in Piritiba, State of Bahia in north-eastern Brazil. The mummies were brushed onto a piece of dry cotton wool maintained a few centimetres from another piece of cotton wool partly soaked in 95% glycerol in plastic tubes (3 cm diameter × 5 cm high) with tightly fitting lids. The mummies were thus stored in a refrigerator (4°C) in the dark at approximately 5.0% relative humidity (RH).

Survival of N. floridana

To test the effect of storage temperature on the survival of the fungus in these mummies, one tube was placed in a refrigerator maintained at 4°C and another placed on a bench at ambient room conditions of 24 ± 1.0°C. At 1 month intervals, ten mummies were retrieved from each tube and placed separately on microscope slides which were maintained at 23°C in the dark for 24 h on a water-soaked foam pad placed on the bottom of a

$19 \times 15 \times 5$ cm plastic box with a tightly fitting lid. After this period, the number of mummies from which the fungus had sporulated and the number of conidia in an area of 3×3 mm around each mummy were counted under a compound microscope. The fungus was considered non-viable when no conidia were observed on three consecutive samples.

Viability of primary conidia

Primary conidia were collected on 12 glass coverslips placed below 12 mummies in each of several Petri dishes as described above. The total number of conidia collected was counted from two coverslips from each Petri dish. To assess the initial germination capacity, two coverslips were removed from each of the same Petri dishes immediately after the 1 h collection period and incubated in a Petri dish with disks of moist filter paper for 24 h at 23°C in the dark. Each coverslip was then inverted over a drop of Amman's Lactophenol-cotton Blue on a microscope slide and examined using a compound microscope to determine the percentage germinated. Any conidium with a capilliconidiophore at least the length of its diameter was considered to have germinated.

Eight coverslips, each with a halo of primary conidia, were placed on a platform in each of the humidity chambers described above and maintained at different combinations of temperature (18, 23, 28 and 33°C), humidity (saturation deficits 0, 2, 6 and 10 mm Hg) and light conditions (continuous darkness or continuous light). Two coverslips, each representing a replicate, were removed from each chamber after 1, 6, 12 and 18 h and the viabilities of the primary conidia were assessed based on germination tests as described above.

Statistical analyses

The mean number of conidia produced by the ten mummies maintained at each temperature was calculated for each observation and subjected to square-root transformation. Data were analysed by a two-way ANOVA (SAS Institute Inc., 1988) with temperature and storage time as the main effects. In the experiment on the viability of primary conidia, the mean 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 analysed with a four way factorial ANOVA (SAS Institute Inc., 1988) with time, temperature, humidity and photoperiod as the main effects. To study the effect of the different treatments at each observation, the percent 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 (SAS Institute Inc., 1988) were then done for each time interval using temperature, humidity and light condition as the grouping variable. A Student–Newmann–Keuls test was used to test for the difference between the means at a significance level of 5%.

RESULTS

Survival of N. floridana

Neozygites floridana survived significantly longer in mummies at 4°C than at 24°C ($F = 147.33$, df = 1, 15, $p < 0.05$) (Fig. 1). Although the rate of sporulation of the fungus from mummies maintained at 24°C was approximately equal for the first 3 months, it declined rapidly thereafter with a total loss of viability occurring between 6 and 7 months. Rates of survival of *Neozygites* sp. declined more gradually at 4°C and were still high after 16 months when the fungus sporulated from 90% of the mummies, liberating an average of 186.9 conidia per mummy. In addition, the length

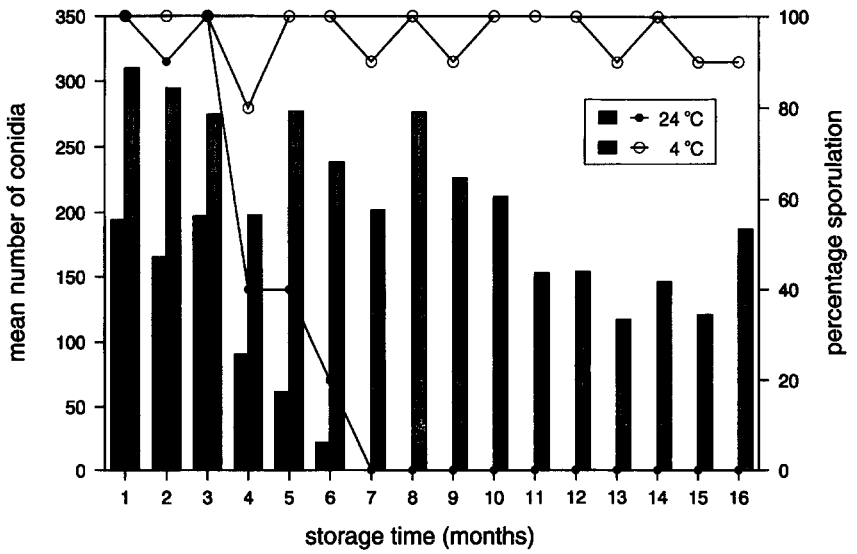

Fig. 1. Survival of *N. floridana* in mummies of *M. tanajoa* after storage in the dark at 4 and 14°C. The left axis shows the mean number of primary conidia liberated from each mummy after different periods of storage, the right axis shows the percentage of mummies from which conidia were liberated.

of the storage period significantly affected the survival of the fungus ($F = 5.33$, df = 1, 1.15, $p < 0.05$).

Viability of primary conidia

A mean of 195 ± 83 ($n = 160$) primary conidia were collected on each coverslip. The mean germination rate of primary conidia before exposure to the different environmental conditions was 98.4%.

Exposure time, temperature, humidity and light conditions had a significant effect on the viability of primary conidia of *N. floridana* (Table 1). The interactions, except between the temperature × light condition and temperature × humidity × light condition were also significantly different (Table 1).

The viability of primary conidia, pooled across all tested conditions, declined significantly with time from 98.4% after 0 h to 23.4% after 1 h and to 7.9% after 18 h (Table 2).

The loss in the rate of viability was lowest at lower temperatures (Table 2). At saturation deficit (SD) 0, there was no significant effect of temperature ($p = 0.813$) after exposure for 1 h with the mean viability ranging between 87.1 and 98.5% (Table 3, Fig. 2). However, there was a significant

TABLE 1

Analysis of variance for mean percent viability (arcsin transformed) of the primary conidia of *N. floridana* at different exposure times (time), temperatures (temp), humidities (hum) and light conditions (Photo)

Source of variation	df	SS	*F* ratio	*p* value
Time	3	5.69	184.0	0.0001
Temp	3	5.04	163.1	0.0001
Hum	3	78.78	2548.9	0.0001
Photo	1	0.05	5.1	0.0250
Time × temp	9	1.59	17.2	0.0001
Time × hum	9	17.04	183.8	0.0001
Time × photo	3	0.20	6.6	0.0001
Temp × hum	9	15.28	164.7	0.0001
Temp × photo	3	0.02	0.0	0.5290
Hum × photo	3	0.14	4.5	0.0040
Time × temp × hum	27	4.85	17.5	0.0001
Time × temp × photo	9	0.27	2.7	0.0030
Time × hum × photo	9	0.64	6.9	0.0001
Temp × hum × photo	9	0.80	0.9	0.5600
Time × temp × hum × photo	27	0.81	2.9	0.0001
Residual	640	6.58		

TABLE 2

Mean viability expressed as percent germination of primary conidia of *N. floridana* pooled across all factors, after being exposed for different periods to different temperatures, humidities and light conditions

Factor	Level	Percent germination (SD)
Exposure time (h)[1]	1	23.4 (7.3) a*
	6	15.5 (5.7) b
	12	11.1 (4.6) b
	18	7.9 (3.9) b
Temperature (°C)	18	22.7 (7.1) a
	23	16.3 (5.7) b
	28	12.4 (4.7) bc
	33	6.6 (4.1) c
Humidity (saturation deficit)	0	58.0 (6.9) a
	2	0.0 b
	6	0.0 b
	10	0.0 b
Light condition	Dark	15.3 (4.0) a
	Light	13.7 (3.9) a

[a] Germination after 0 h was 98.4%.

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

effect of temperature after 6 h (Table 3). The loss in the rate of viability at 18°C was gradual. After 18 h, the viability was 86.3 and 72.4% in the dark and light, respectively (Fig. 2). At 23 and 28°C, there was a steep decline in viability, in particular for conidia in the light. After 18 h at this photoperiod only approximately 5.0% of the conidia were viable at both temperatures. In the dark after 18 h, 57.7% of the conidia were viable at 23°C and 27.8% at 28°C. Primary conidia were least tolerant to 33°C with all conidia dead within 6 h in the light and 18 h in the dark.

The germination of primary conidia was sensitive to low humidities and was significantly affected by humidity after each exposure time (Table 3). Although germination was high before exposure to different treatments, no conidia survived even 1 h exposure to SDs 2 or higher, at any temperature. Instead of germinating to form capilliconidiophores (on which the infective capilliconidia are formed), primary conidia at these lower humidities produced the morphologically similar secondary conidia.

Light conditions did not seem to affect the viability of the primary conidia as there was no significant difference viability in the dark and light (Tables 1 and 2).

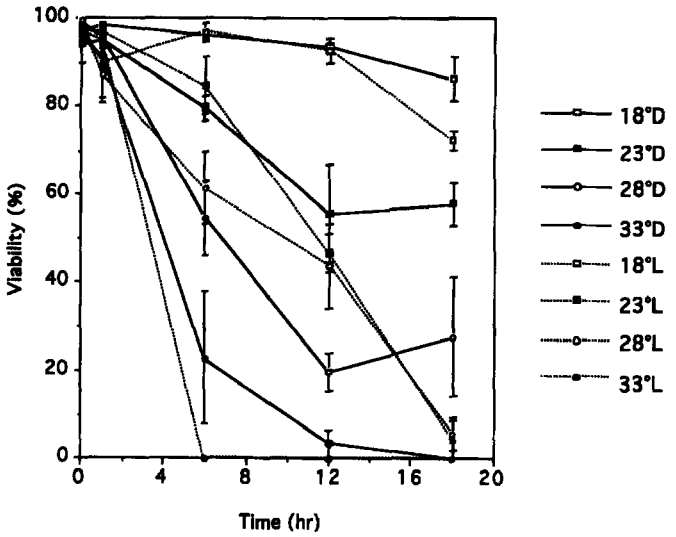

Fig. 2. Percent viability (+SE) of primary conidia of *N. floridana* maintained at different temperatures and light conditions (D = dark, L = light) at saturation deficit 0. Each point represents a mean of six values.

Interrelationships between the effect of some tested factors were observed. The significant interaction between exposure time × temperature was due to the temperature having a minimal effect on the viability

TABLE 3

ANOVA of the viability of the primary conidia of *N. floridana* after different time intervals given the effect of temperature, humidity and light condition

Factor	Exposure time (h)			
	1	6	12	18
Temperature				
F ratio	0.3	12.6	27.3	10.9
df	3,9	3,9	3,9	3,9
p value	0.813	0.001	0.000	0.003
Humidity				
F ratio	1344.9	174.7	208.7	54.3
df	3,9	3,9	3,9	3,9
p value	0.000	0.000	0.000	0.000
Light condition				
F ratio	0.7	0.4	0.0	4.3
df	1,9	1,9	1,9	1,9
p value	0.440	0.549	0.934	0.069

after 1 h, but having a significant effect after 6 and subsequent hours of exposure to different treatments (Table 3). The interaction between exposure time × humidity was also significant because germination was high at all humidities at the beginning of the experiment, but dropped subsequently to zero at all humidities except at SD 0. There was a significant temperature × humidity interaction indicating that the viability of primary conidia at each temperature was differentially affected by humidity. Although lower temperatures maintained viability better than higher temperatures at high humidities, all capilliconidia at all temperatures died when maintained at lower humidities. Whereas viability was maintained better in the dark than in the light at SD 0, there was no difference between the two light conditions at higher SDs where all the conidia died. The differences in viability between conidia maintained in the dark and light increased with time. These observations explain the significant interactions between light condition and humidity and also light condition and exposure time.

DISCUSSION

Seasons when the environmental conditions are not favourable for the active development of entomopathogenic fungi or when host populations are low necessitate that these fungi persist in a stage that will ensure survival. In this study *N. floridana* in the form of hyphal bodies maintained at 5% RH in the dark, survived for 6 months at 24°C and much longer at 4°C. The survival of this fungus is longer than that reported for *Entomophaga aulicae* (Reichard) Sorokin which, at 0% RH, maintained its viability for 4 weeks at 21°C and for 12 weeks at 4°C (Tyrrell, 1988). Whereas the decline in viability of the fungus in this study was more rapid at 24°C, survival of the fungus inside the mummy persisted longer at 4°C where there was still sporulation after 16 months. The importance of storage at low temperatures is emphasized by Pell and Wilding (1992) who showed that at 4°C, there was no decline in the viability of *Zoophthora radicans* (Brefeld) Batko in mummified larvae of the diamondback moth, even after storage for 34 weeks.

The results of our study present a practical method by which *N. floridana* can be stored for future use or during transportation. It is not yet known how this fungus passes the dry hot conditions between the rainy seasons, but it could be doing so by persisting in the dry hyphal body stage in mummies hidden in some unknown locations on cassava or alternative plants. This is a more likely survival strategy as resting spores as these are encountered very rarely.

These results may have practical uses in biological control. Preparations of whole or macerated mummies, formulated with suitable adjuvants (stickers, wetting agents, spreaders and emulsifiers), may be tested as biopesticides. It seems advisable to apply such preparations to the underside of the cassava leaves, while timing will also be of importance (e.g. early in the morning when the relative humidity is at its maximum).

Results from this study also show that the prevaling temperature and humidity determines how long primary conidia of *N. floridana* maintain their ability to germinate. Germination was higher among conidia maintained at lower temperatures, results which agree with those of Page *et al* (1947) who found that the lower the temperature the longer the conidia of *Helminthosporium oryzae* van Breda de Haan retained their viability at different relative humidities. Primary conidia died when held at high SDs. It was due to this sensitivity that data were taken after hours rather than days. The viability was maintained only at SD 0 and could have lasted well beyond 1 day at lower temperatures. Steinkraus and Slaymaker (1994) showed that exposure of primary conidia of *N. fresenii* (Nowakowski) Batko to 75% RH for 1 min resulted in a significant reduction in germination, as compared to those exposed to 100% RH for the same period of time. The primary conidia of the fungus used in our study are produced and germinate only at saturation deficits of 0.7 or less (G.I. Oduor, unpublished data) and their inability to germinate after exposure to the humidities tested in this study further emphasizes the sensitivity of these conidia to low humidities. The probable detrimental effect of this sensitivity may be minimal since the ideal humidity conditions for the production of primary conidia are the same as those for their germination to produce capilliconidia, which have a higher tolerance to lower humidities than do primary conidia (G.I. Oduor, unpublished data). Clerk and Madelin (1965) reported that the longevity of conidia of *B. bassiana, P. farinosus* and *M. anisopliae* was reduced by exposure to light. The lack of a significant effect of light on germination in this study may have been due to the short duration of the experiment, as the analysis of the effect of factors at each observation showed that the light had a near significant effect ($p = 0.069$) after storage for 18 h. The short period over which primary conidia retain their ability to germinate makes them a less preferred choice than, for example mummies, if *N. floridana* is to be formulated as a biopesticide.

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REFERENCES

Benz, G. 1987. Environment. In Epizootiology of insect diseases, J.R. Fuxa and Y. Tanada (eds), pp. 177–214. John Wiley and Sons, New York.

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

Clerk, G. C. and Madelin, M. F. 1965. The longevity of conidia of three insect-parasitising hyphomycetes. Trans. Br. Mycol. Soc. 48: 193–209.

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

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

Newmann, G.G. and Carner, G.R. 1975. Factors affecting the spore form of *Entomophthora gammae*. J. Invertebr. Pathol. 26: 29–34.

Page, R.M., Sherf, A.F. and Morgan, T.L. 1947. The effect of temperature and relative humidity on the longevity of the conidia of *Helminthosporium oryzae*. Mycologia 39: 158–164.

Pell, J.K. and Wilding, N. 1992. The survival of *Zoophthora radicans* (Zygomycetes: Entomophthorales) isolates as hyphal bodies in mummified larvae of *Plutella xylostella* (Lepidoptera: Yponomeutidae). Entomophaga 37: 649–654.

SAS Institute, Inc. 1988. SAS/STAT™ User's Guide. Release 6.03, ed. SAS Institute, Cary, N.C.

Sawyer, W.H. 1931. Studies on the morphology and development of an insect destroying fungus, *Entomophthora sphaerosperma*. Mycologia 23: 411–432.

Shimazu, M. 1979. Resting spore formation of *Entomophthora sphaerosperma* (Entomophthorales: Entomophthoraceae) in the brown planthopper, *Nilaparvata lugens* (Stahl) (Homoptera: Delphacidae). Appl. Entomol. Zool. 14: 383–388.

Shimazu, M. and Soper, R.S. 1986. Pathogenicity and sporulation of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) on larvae on the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). Appl. Entomol. Zool. 21: 589–596.

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.

Tanada, Y. and Fuxa, J.R. 1987. The pathogen population. In Epizootiology of insect diseases, J.R. Fuxa and Y. Tanada (eds), pp. 113–157. John Wiley and Sons, New York.

Tyrrell, D. 1988. Survival of *Entomophaga aulicae* in dried insect larvae. J. Invertebr. Pathol. 52: 187–188.

Watanabe, H. 1987. The host population. In Epizootiology of insect diseases, J.R. Fuxa and Y. Tanada (eds), pp. 71–112. John Wiley and Sons, New York.

Wilding, N. 1973. The survival of *Entomophthora* spp. in mummified aphids at different temperatures and humidities. J. Invertebr. Pathol. 21: 309–311.

Wilding, N. and Lauckner, F.B. 1974. *Entomophthora* infecting wheat bulb fly at Rothamstead, Hertfordshire, 1967–71. Ann. Appl. Biol. 76: 161–170.

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs NJ.