Effect of temperature, humidity and photoperiod on mortality of *Mononychellus tanajoa* (Acari: Tetranychidae) infected by *Neozygites* cf. *floridana* (Zygomycetes: Entomophthorales)

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ABSTRACT

The effect of temperature, humidity and photoperiod on the development of *Neozygites* cf. *floridana* (Weiser and Muma) in the cassava green mite, *Mononychellus tanajoa* (Bondar) was studied in the laboratory. Dead infected mites began to appear 2.5 days after inoculation. At 33 and 28°C peak mortalities were higher and occurred earlier (after 2.5 days), than at 23 and 18°C. Mean LT_{50} (time for half the infected mites to die) decreased with increasing temperature as follows: 3.9, 3.0, 2.9 and 2.5 days at 18, 23, 28 and 33°C, respectively. When placed under conditions of high relative humidity for a period of 24 h, the percentage of dead infected mites from which the fungus sporulated was highest at 28°C (51.4%) and lowest at 33°C (6.5%). The development of the fungus inside the mite was not significantly affected by ambient humidity or photoperiod. No significant interactions between tested factors were found.

Key words: *Neozygites* cf. *floridana*, *Mononychellus tanajoa*, mortality, hyphal body, capilliconidia, temperature, humidity, saturation deficit, photoperiod.

INTRODUCTION

The cassava green mite, *Mononychellus tanajoa* (Bondar), is an important pest of cassava in the tropics and humid subtropics of the neotropics and in Africa. A fungal pathogen, *Neozygites* sp. has been reported to cause epizootics in this pest in South America (Agudelo-Silva, 1986; Delalibera *et al.*, 1992). Since *Entomophthora* (= *Triplosporium* = *Neozygites*) spp. was

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reported to attack *Eutetranychus banksi* (Weiser and Muma, 1966), its association with various tetranychid mites has been the subject of widespread research (Selhime and Muma, 1966; Carner and Canerday, 1968; Kenneth *et al.*, 1972; Nemoto and Aoki, 1975; Carner, 1976; Brandenburg and Kennedy, 1982; Smitley *et al.*, 1986; Klubertanz *et al.*, 1991; Mietkiewski *et al.*, 1993). Epizootics of this fungus have been observed to occur during periods of high atmospheric moisture (> 90% RH) and temperatures below 30° C (Carner and Canerday, 1968; Humber *et al.*, 1981; Brandenburg and Kennedy, 1982; Smitley *et al.*, 1986). These observations suggest that prevailing atmospheric conditions affect the development of the fungal pathogen.

Information on the response of *Neozygites* sp. to key environmental factors may lead to a better understanding of the requirements for the development of this fastidious fungus as a biological control agent. Since successful biological control usually requires knowledge of the rearing of the natural enemy, such information could be useful. Although the role of temperature and humidity on the development of *Neozygites* sp. within other tetranychid hosts has been studied (Smitley *et al.*, 1986), the simultaneous effects of temperature, humidity and photoperiod on the incubation period of this fungus in *M. tanajoa* have not been studied. This paper reports the results of such a study.

MATERIALS AND METHODS

Mononychellus tanajoa killed and mummified by *Neozygites* cf. *floridana* (Weiser and Muma) were collected in January 1993 from cassava fields in Piritiba, state of Bahia, north-eastern Brazil. These mummies were brushed onto cotton wool which was placed on another piece of cotton wool partially soaked in 10 ml of glycerol in 3 cm diameter by 5 cm high plastic tubes with tight fitting lids. The mummies were stored in the dark at 4° C in a refrigerator for a period not exceeding 5 months before being used in the experiment.

Three mummies were left to sporulate for 2 days on cassava leaf discs (each 2.0 cm in diameter) placed onto moist cotton wool in tightly closed plastic containers (19 × 15 × 5 cm) maintained at 23° C. Conditions of high humidity were achieved in the containers allowing the formation of a halo of infective conidia (capilliconidia) around each mummy. Fifteen young (less than 2 days old) adult female *M. tanajoa* were then placed near the mummies on each disc and the container covered for 24 h. This allowed germination of the capilliconidia and penetration of the germ

tubes into the mite's body to occur under identical conditions. The treatments were meant to influence only the development of this fungus in its host. Fifteen additional mites were similarly placed on discs treated in the same way, but without the fungus as controls. Twelve mites from the exposed group and 12 from the control group were then chosen at random and placed in cages for evaluation.

Cages used in the experiment consisted of bottomless plastic tubes (2.5 cm diameter × 1.0 cm high) with four holes on the sides sealed with fine mite-proof gauze. The bases of these cages were then attached to cassava leaflets with the cut ends of their mid-ribs dipped in water in small vials, the tops of which were tightly sealed with Parafilm®. The roof of each cage consisted of removable pieces (3.5 × 3.5 cm) cut from overhead transparencies. Clear plastic containers (19 × 15 × 8 cm) served as the humidity chambers. Smaller 15 × 9 × 4 cm plastic blocks formed platforms on which four cages rested above the surface of 400 ml of either pure distilled water or different concentrations of sulphuric acid which served to maintain different humidities (Stevens, 1916; Solomon, 1951). Solutions were allowed to stabilize for 24 h before use. The saturation deficit, the difference between the actual amount of water vapour 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). Three levels of humidity (saturation deficit (SD) = 0, 2 and 10) were used. Since saturation deficit is a function of both the temperature and humidity, the experimental SDs mentioned above were attained at different relative humidities as shown in Table 1. The temperatures studied were 18, 23, 28 and 33° C and were achieved by placing the humidity chambers in incubators. Light was provided by two 15 W daylight fluorescent tubes giving a light intensity of 10 μE m^{-2} s^{-1} at the position of the cages. Light was controlled by covering the humidity chambers with light excluding cloth bags to give photoperiods of 6, 10 and 14 h per day in a constantly lit incubator. Each humidity chamber contained two control and two treatment cages. The experiment was performed three times.

Observations were made every 12 h. All dead mites were removed and counted. The presence of *N. floridana* was confirmed by placing dead mites on microscope slides under 100% relative humidity in the dark at 23° C for a period of 12 h to induce sporulation. These cadavers were then stained with Amman's lactophenol-cotton blue and observed under a compound microscope. Individuals with sporulated fungus or with hyphal

TABLE 1

Temperatures, saturation deficits and corresponding relative humidities used to study their effects on the incubation of *N. floridana* in *M. tanajoa*.

Temperature (° C)	Saturation deficits in mm Hg corresponding % RH		
	0	2	10
18	100	87.4	35.0
23	100	90.9	50.0
28	100	93.2	65.5
33	100	94.8	72.5

bodies or germinated capilliconidia were considered to have died of mycosis due to the fungus. Only these mites were included in the analyses.

Treatment mortality was corrected for control mortality using the method of Abbott (1925). For each combination of treatments, probits of the cumulative mortalities were regressed to the logarithms of the time to death using probit analysis via the SAS PROBIT procedure (SAS Institute, 1988) which computed the LT_{50}s. These LT_{50}s were transformed by square root to ensure homogeneity of variance, then analysed using a three-way factorial ANOVA (SAS Institute, 1988) with the temperature, humidity and photoperiod as the main factors. The Student–Newman–Keuls test was used to compare means at a significance level of 0.05. Untransformed values are presented in Tables 2 and 3.

RESULTS

The exposure of healthy *M. tanajoa* to capilliconidia on leaf discs led to infection levels of between 54.0 and 89.1% depending on the temperature (Table 3). At all temperatures tested, dead mites were observed. The first dead mites were observed 2.5 days after exposure to the fungus. At this time 77.9% of the infected mites maintained at 33° C had died of mycosis (Fig. 1). Peak mortalities at 28 (35.7%) and 23° C (36.1%) were lower than at 33° C and occurred after 3.0 days. The mortality rate was lowest at 18° C, with the highest percentage of mites (29.9%) dying after 3.5 days.

Temperature showed a significant effect ($F = 105.73$, df = 3, 72, $p < 0.05$) on LT_{50} (time for half the infected mites to die). Increasing the temperature from 18 to 33° C reduced the LT_{50} from 3.9 to 2.5 days, respectively (Table 2). The incubation periods at 18 and 33° C were significantly different from other temperatures (Table 2). The mortality of
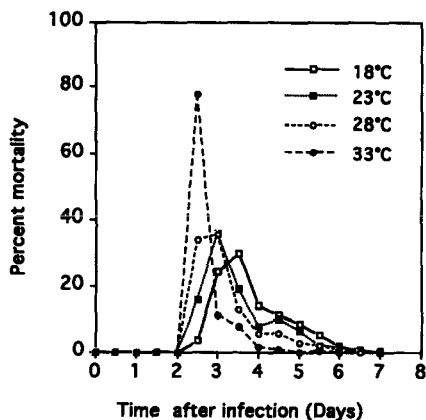
Fig. 1. Percentage of *M. tanajoa* infected with *N. floridana* dying at 12 h intervals at different constant temperatures.

mites in the control after 7 days also varied with temperature. The 72.1% control mortality recorded at 33° C was significantly higher than the 49.1, 41.7 and 28.8% recorded at 28, 23 and 18° C, respectively (Table 2).

The proportion of mites which became infected by the fungus increased with increasing temperature between 18 and 28° C, but dropped at 33° C (Table 3). Table 3 also shows the percentages of infected mites harbouring the different developmental stages of the fungus in the four temperature treatments. At 18 and 23° C, the percentages of infected mites with germinated capilliconidia and those with sporulated hyphal bodies were low and approximately equal. At both these temperatures, the percentages of mites with non-sporulated hyphal bodies were higher than those with other developmental stages of the fungus. At 28° C, only a few of the infected mites had germinated capilliconidia (6.0%), more had hyphal bodies (42.6%) and even more had sporulated hyphal bodies (51.4%). Of the infected mites at 33° C, few had germinated capilliconidia (14.4%), most harboured hyphal bodies (79.1%) and only a few had produced conidia (6.5%). At the two extreme temperatures of 18 and 33° C, there was a high proportion of mites with the early developmental stages of the fungus (germinated capilliconidia and hyphal bodies), but in only a few mites was the fungus able to develop further and produce conidia. The trend was similar at 23° C, although the fungus developed to the sporulating stage in a larger proportion of mites. However, at 28° C, unlike at other temperatures, very few mites had only germinated capilliconidia and an increasing proportion of the mites harboured the fungus at its later stages of development. At this temperature, the fungus produced spores in

TABLE 2

Mean percent mortality of healthy *M. tanajoa* and LT_{50} (in days) of adult female mites maintained at different temperatures, humidities and photoperiods after infection with *N. floridana* (SE standard error).

Factor	Level	Percent mortality in control (SE)[a]	Mean LT_{50} in days for infected mites (SE)[a]
Temperature (° C)	18	28.8 (3.44)[a]	3.9 (0.06)[a]
	23	41.5 (5.97) ab	3.0 (0.06) b
	28	49.1 (4.06) b	2.9 (0.06) b
	33	72.1 (4.37) c	2.5 (0.06) c
Humidity (saturation deficit)	0	38.3 (4.31) a	3.1 (0.05) a
	2	39.6 (3.86) a	3.1 (0.05) a
	10	65.9 (4.50) b	3.0 (0.05) a
Photoperiod (h of light per day)	6	47.6 (4.40) a	3.1 (0.05) a
	10	48.4 (4.90) a	3.1 (0.05) a
	14	47.9 (4.91) a	3.0 (0.05) a

[a] 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).

more than half of the infected mites. At 33° C, although most of the mites had hyphal bodies in them, very few produced conidia. Humidity and photoperiod did not significantly affect the development of the fungus ($F = 1.11$, df = 2, 72, $p > 0.05$ and $F = 0.95$, df = 2, 72, $p > 0.05$, respectively). No interaction effects between either temperature, humidity, or photoperiod were observed.

DISCUSSION

The need to study individual and possible interactive effects of more than one environmental factor in an attempt to explain why and how epizootics occur has been emphasized by Benz (1987). Although different levels of three factors were included in this study, the effect of humidity and photoperiod on the development of *N. floridana* in *M. tanajoa* was not found to be significant. After the successful germination of the capilliconidia and penetration of the hosts' cuticle by the germ tube, further development of *N. floridana* does not appear to be influenced by the ambient humidity or photoperiod. Similarly, the photoperiod was reported to have no significant effect on the pathology of *Entomophthora muscae* (Cohn) Fres. in carrot flies (Eilenberg, 1987). Further, Tillotson *et al.*

TABLE 3

Percentage of infected *M. tanajoa* adult females at four constant temperatures and the proportion of infected mites, harbouring different stages of *N. floridana*.

Temperature (° C)	Numbers exposed	Total infected (%)	Germinated capilliconidia (%)	Hyphal bodies	
				Non-sporulated (%)	Sporulated (%)
18	648	54.0	15.9	69.6	14.5
23	648	63.8	19.1	61.6	19.3
28	648	89.1	6.0	42.6	51.4
33	641	79.7	14.4	79.1	6.5

(1990) found that neither the photoperiod nor its interaction with the temperature had an effect on the pathogenesis of *Entomophaga grylli* (Fres.) Batko pathotype 2 in the differential grasshopper, *Melanoplus differentialis* (Thomas). Despite high humidity being necessary for the release of conidia and their subsequent germination, the development of *Entomophthora* sp. in *Tetranychus urticae* Koch was reported to be independent of the ambient humidity (Carner, 1976).

Among arthropod hosts, the rate of mortality caused by fungal diseases increases with increasing temperature (Stimmann, 1968; Wilding, 1970; Milner and Bourne, 1983; Milner and Lutton, 1983; Eilenberg, 1987). However, this rate may decline once an optimum temperature is attained. Incidences of heat therapy, whereby arthropod hosts minimize the effects of or eliminate fungal pathogens by increasing their body temperatures or by occupying habitats with high temperatures which the pathogens cannot tolerate, have been reported (Carruthers *et al.*, 1992; Watson *et al.*, 1993). This may partly explain the drop in infection among mites maintained at 33° C in this study. Since the time from infection by the fungus to the death of the mite host may be used as a measure of the rate of development of the pathogen, it can be concluded that increases in temperature from 18 to 33° C lead to an increase in the rate of the development of *N. floridana*. The maximum mortality at 33° C occurred 2.5 days after exposure, whereas it occurred after 3.0 days at 28 and 23° C and 3.5 days at 18° C (Fig. 1). However, although 77.9% of the infected mites died after 2.5 days at 33° C, few mites died thereafter. This sudden decline in mortality may indicate the lack of development of the fungus at this temperature. Increasing the temperature between 18 and 28° C led to an increasing proportion of mites being infected by the fungus. Sporulation of the fungus and its subsequent infection of *M. tanajoa* were

performed under uniform conditions; therefore, this difference in infection at different temperatures cannot be clearly accounted for. A possible explanation may be that the different temperatures influenced the further development of the capilliconidia attached to mites just before the end of the exposure period. At 28° C, 89.1% of the exposed mites became infected and the fungus was also able to complete the cycle from infection to sporulation in the highest percentage of infected mites. At other temperatures, in particular at 33° C, although the fungus was able to develop to the stage where hyphal bodies were formed, further development appeared to be hampered. At this temperature, the infected mites may have succumbed to the high temperature before pathogenesis due to the fungus could be completed. This, together with the high mortality (72.1%) among the controls at 33°C, suggests that the ideal temperature for the development of this fungus is near 28° C. This temperature compares well to the 25° C reported by Milner and Lutton (1983) for *Zoophthora radicans* (Brefeld) Batko and the range of 25–32° C obtained by Smitley *et al.* (1986) for *N. floridana* (Weiser and Muma) Remaudiere and Keller.

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