BioControl **49:** 83–94, 2004.

Effect of constant temperatures on germination, radial growth and virulence of *Metarhizium anisopliae* to three species of African tephritid fruit flies

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Received 14 February 2002; accepted in revised form 27 November 2002

Abstract. The effect of temperature on conidial germination, mycelial growth, and susceptibility of adults of three tephritid fruit flies, *Ceratitis capitata* (Wiedemann), *C. fasciventris* (Bezzi) and *C. cosyra* (Walker) to six isolates of *Metarhizium anisopliae* were studied in the laboratory. There were significant differences among the isolates in the effect of temperature on both germination and growth. Over 80% of conidia germinated at 20, 25 and 30 °C, while between 26 and 67% conidia germinated at 35 °C and less than 10% at 15 °C within 24 hours. Radial growth was slow at 15 °C and 35 °C with all of the isolates. The optimum temperature for germination and mycelial growth was 25 °C. Mortality caused by the six fungal isolates against the three fruit fly species varied with temperature, isolate, and fruit fly species. Fungal isolates were more effective at 25, 30 and 35 °C than at 20 °C. The LT_{90} values decreased with increasing temperature up to the optimum temperature of 30 °C. There were significant differences in susceptibility between fly species to fungal infection at all the temperatures tested.

Key words: *Ceratitis capitata*, *Ceratitis cosyra*, *Ceratitis fasciventris*, germination, *Metarhizium anisopliae*, temperature

Introduction

The African fruit flies, *Ceratitis capitata* (Wiedemann), *C. cosyra* (Walker) and *C. fasciventris* (Bezzi), formerly *C. rosa* var. *fasciventris* (De Meyer, 2001) are among the most economically important pests of fruit and vegetables in tropical and subtropical regions of the world (Allwood, 1997). In Africa, quality fruit and vegetable production is curtailed by severe infestation with fruit flies. Mango, for instance, suffers losses of 20–40% on average, rising in some areas to 70% (Lux et al., 1997). In addition to these direct losses, producer countries often lose potential markets due to stringent quarantine regulations imposed by importing countries to avoid entry and

establishment of unwanted fruit flies. Development of effective non chemical control methods is needed because of the deleterious effects of chemical pesticides used for the suppression of these pests.

Entomopathogenic fungi are being developed as alternatives to chemical pesticides for the control of fruit flies (García et al., 1984; Castillo et al., 2000; Lezama-Gutierrez et al., 2000; Ekesi et al., 2002). However, entomopathogenic fungi are subject to a number of biotic and abiotic factors that influence their survival and ability to cause diseases (Hall and Papierok, 1982; Benz, 1987; Carruthers and Soper, 1987; Inglis et al., 2001). To better predict efficacy under field conditions, the effects of environmental constraints must be determined. Of the various environmental parameters that affect insect fungal pathogens, temperature, humidity and solar radiation are probably the most severe (Inglis et al., 2001). Temperature is an abiotic limiting factor that affects rate of germination, growth, sporulation and survival of entomopathogenic fungi (Roberts and Campbell, 1977; Benz, 1987). It also influences the host, and host-pathogen interaction (Fargues et al., 1992; Maniania and Fargues, 1992; Blanford and Thomas, 2000). Optimal temperatures for entomopathogenic fungal growth, sporulation and infection often range between 20–30 °C, but variation in temperature tolerance within a strain also can be significant (Hall and Bell, 1960, 1961; Stimmann, 1968; Walstad et al., 1970; Roberts and Campbell, 1977; Carruthers and Haynes, 1986; Fargues et al., 1992, 1997). Recently, we screened 16 isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin against adult *C. capitata*, *C. cosyra* and *C. fasciventris*, and selected 6 strains of *M. anisopliae* which were pathogenic (Dimbi et al., 2003). This paper presents the effects of constant temperature on conidial germination, mycelial growth, and susceptibility of the three fruit fly species to the six isolates of *M. anisopliae.* The goal was to select isolate(s) efficacious against all the three species over a wide range of temperature.

Materials and methods

Fungal cultures

The six isolates of *M. anisopliae* were obtained from the International Centre of Insect Physiology and Ecology (ICIPE) culture collection and most of them were isolated from soil using "*Galleria* bait method" (Zimmermann, 1986), except isolate ICIPE 32, which was isolated from *Amblyomma variegatum* (Fabricius). The virulence of the strains was maintained by regular passage through an insect host (Schaeffenberg, 1964). Conidia were obtained by scraping the surface of three week-old cultures maintained on Sabouraud

dextrose agar (SDA). Conidia were then suspended in sterile distilled water containing 0.05% Triton-X-100 and viability was determined by spread-plating 0.1 ml of the suspension (3×10^6 conidia ml^{-1}) on SDA plates. Sterile microscope cover slips were placed on each plate. Plates (n = 4 per isolate) were then incubated at 24–28 °C and examined after 24 hours. Percentage germination was determined by counting approximately 100 conidia for each plate at 400× magnification.

Insects

Colonies of the three species, *C. cosyra*, *C. capitata* and *C. fasciventris* were mass reared at the International Centre of Insect Physiology and Ecology (ICIPE). The initial colonies of *C. capitata* and *C. fasciventris* were derived from coffee, *Coffea arabica* Linnaeus collected from farms in the Central Highlands of Kenya at Ruiru (1° 5.72′S; 36° 54.22′E; 1609 m above sea level). *Ceratitis cosyra* colonies were derived from collections obtained from mango, *Mangifera indica* Linnaeus and marula, *Sclerocarya birrea* (A. Richards) at Nguruman, Kenya (1° 47′S; 36° 05′E; 700 m above sea level). The larvae of the three species were fed on carrot-sugar based artificial diet, modified from Hooper (1987). Adult flies were reared in perspex cages (60 cm × 35 cm × 70 cm), at 27–28 °C and photoperiod 12:12 L:D and were provided with tap water and a diet of yeast hydrolysate (yeast hydrolysate enzymatic, USB, Corporation, Ohio, USA) and sugar (Mumias Sugar Co, Kenya) at a ratio 4:1. In all experiments, 5 to 10-day-old adult flies were used.

Effect of temperature on germination

Conidial suspension (0.1 ml of 3×10^6 conidia ml^{-1}) was spread on SDA plates (Ekesi et al., 1999). Sterile microscope cover slips were placed on each plate and inoculated plates were sealed with Parafilm Membrane and incubated at 15, 20, 25, 30 and 35 °C in complete darkness. At 24 hours post inoculation germination was halted by transferring 1 ml of formaldehyde (0.5%) onto each plate, and percentage germination was determined by counting 100 conidia for each plate at 400× magnification. Four plates (replicates) were used per treatment and each plate served as a replicate.

Effect of temperature on radial growth

A conidial suspension (0.1 ml of 1×10^7 conidia ml^{-1}) was spread plated on SDA plates. Plates were then incubated at 25 °C for three days in order to obtain mycelial mats. The unsporulated mycelial mats were then cut from

the culture plates into round agar plugs using an 8mm-diameter cork borer. Each agar plug was then transferred singly onto the center of a fresh SDA agar plate of 90 mm diameter. Four replicate Petri dishes were sealed with Parafilm and incubated upside down in complete darkness at 15, 20, 25, 30 and 35 °C. Radial growth was recorded daily for 12 days using two cardinal diameters, through two orthogonal axes previously drawn on the bottom of each petri dish to serve as a reference.

Effect of temperature on virulence

Test insects were contaminated through contact with a velvet cloth covering the inner side of a cylindrical plastic tube (9.5 cm × 4.8 cm) that had the bottom removed and replaced with a white nylon netting. Dry conidia (0.3 g) were spread evenly onto the velvet. Twenty flies were transferred and confined to the cylindrical tube. Flies were allowed to walk on the velvet for 3 minutes after which they were transferred to a clean ventilated Plexiglas cage (15 cm × 15 cm × 20 cm). The cages were incubated at 15, 20, 25, 30 or 35 °C. The insects were fed on a 4:1 mixture of sugar and yeast hydrolysate and a Petri dish (50 mm diam.) with cotton wool soaked in water was also provided. Control insects were exposed to fungus-free velvet before being transferred to the same ventilated Plexiglas cages. Each treatment consisted of four replicates of 20 insects each and was repeated twice.

Mortality was recorded daily for ten days or until all insects died. Dead insects were surface sterilized in 70% alcohol followed by 3 rinses in sterile distilled water and transferred to Petri dishes lined with damp sterilized filter paper to allow for growth of fungus on the surface of the cadavers. Mortality due to the fungus was confirmed by microscopic examination of hyphae and spores on the surface of the cadaver.

Statistical analysis

Analysis of variance (ANOVA procedure of SAS) was used to analyze percentage germination, growth and mortality data (SAS institute, 1990) after arcsine transformation to normalize the data. Percentage mortality (at 4 day post-treatment) was also adjusted for natural mortality in controls using Abbott (1925) formula before analysis and was then analyzed using three-way analysis of variance for a completely randomized design. The LT_{90} values were determined for each replicate using the probit analysis method for correlation data (Throne et al., 1995) and compared among themselves using ANOVA followed by mean separation using Student-Newman-Keuls (SNK) test ($P = 0.05$).

Table 1. Effect of temperature on germination (%) of 6 isolates of *Metarhizium anisopliae*

	Temperature				
	15 °C	20 °C	25 °C	30 °C	35 °C
ICIPE 18	3.3 ± 0.2bcD	70.0 ± 1.2aB	93.4 ± 1.8bA	91.1 ± 1.1aA	26.3 ± 0.8cC
ICIPE 20	2.8 ± 0.3bcD	64.6 ± 1.6aB	90.2 ± 1.8bA	90.1 ± 1.5aA	27.8 ± 1.7cC
ICIPE 32	3.4 ± 0.3bcE	60.0 ± 0.7aC	94.5 ± 1.3bA	85.7 ± 0.8aB	49.8 ± 3.8bD
ICIPE 40	2.6 ± 0.2cD	62.8 ± 0.2aC	91.6 ± 1.7abA	86.0 ± 1.7aB	67.2 ± 1.2aC
ICIPE 41	4.0 ± 0.4bD	66.3 ± 5.1aB	86.6 ± 0.6cA	86.6 ± 1.6aA	37.1 ± 7.7bcC
ICIPE 62	5.3 ± 0.5aE	65.7 ± 2.3aC	94.9 ± 0.7aA	86.6 ± 1.0aA	48.0 ± 2.1bE

Means (± S.E.) within-column followed by the same lower case letter and within row bearing the same upper case letter are not significantly different by Student-Newman-Keuls' test ($P < 0.05$).

Results

Effect of temperature on germination

There were significant effects of temperature on germination of conidia at 24 h post-inoculation (F = 1653.80; df = 4,87; $P = 0.0001$). A significant isolate by temperature interaction was also observed (F = 12.09; df = 20,87; $P = 0.0001$). Germination at 15 °C was less than 5% in all the isolates. The maximum germination was observed at 25 °C and varied between 86.6–94.9% (Table 1). There were also significant differences between the reaction of the fungal isolates to temperature (F = 4.62; df = 5,87; $P = 0.0009$), except at 20 and 30 °C (Table 1).

Effect of temperature on growth

The rate of mycelial growth was significantly affected by temperature (F = 172.88; df = 4,87; $P = 0.0001$) and isolate (F = 9.94; df = 5,87; $P = 0.0001$) with a significant temperature by isolate interaction (F = 2.89; df = 25,87; $P = 0.0003$). While growth occurred at all temperatures, it was slower at 15 and 35 °C as compared to 20, 25 and 30 °C. The optimal temperature for growth for all isolates was 25 °C (Table 2).

Effect of temperature on virulence

Mortality in the controls was low and did not exceed 10% at any of the temperatures. Fruit fly mortality was significantly affected by temperature (F = 3044.43; df = 4,267; $P = 0.0001$), isolate (F = 21.05; df = 5,267; $P = 0.0001$) and host species (F = 4.75; df = 2,267; $P = 0.0001$). A significant

Table 2. Influence of temperature on growth rate day^{-1} of six isolates of *Metarhizium anisopliae* cultured on SDA media

M. anisopliae/ isolate	Rate of growth (mm/day)				
	15 °C	20 °C	25 °C	30 °C	35 °C
ICIPE 18	1.18 ± 0.02aC	1.72 ± 0.10bB	2.30 ± 0.03aA	2.12 ± 0.10bA	1.33 ± 0.03abC
ICIPE 20	1.33 ± 0.03aC	2.27 ± 0.58aB	2.90 ± 0.10aA	2.37 ± 0.04abB	1.19 ± 0.05dC
ICIPE 32	1.20 ± 0.04aC	2.17 ± 0.11aB	2.75 ± 0.10abA	2.45 ± 0.06abB	1.45 ± 0.08aC
ICIPE 40	1.10 ± 0.10aC	2.09 ± 0.11aB	2.52 ± 0.11abA	2.33 ± 0.10abAB	1.35 ± 0.04abC
ICIPE 41	1.30 ± 0.07aB	2.16 ± 0.03aA	2.33 ± 0.12bA	2.30 ± 0.04abA	1.31 ± 0.03abB
ICIPE 62	1.35 ± 0.03aD	2.23 ± 0.16aC	3.22 ± 0.11aA	2.55 ± 0.12aB	1.22 ± 0.06bD

Means (± S.E.) within-column followed by the same lower case letter and within row bearing the same upper case letter are not significantly different by Student-Newman-Keuls' test ($P < 0.05$).

temperature by isolate, temperature by species, species by isolate and temperature by isolate by species interaction was also observed (F = 8.56; df = 20,267; $P = 0.0001$); (F = 5.76; 8,267; $P = 0.0001$); (F = 4.56; df = 10,267; $P = 0.0001$) and (F = 1.98; df = 40,267; $P = 0.0008$), respectively. Fungal isolates were more effective at 25, 30 and 35 °C than at 20 °C (Table 3). At 20 °C, isolate ICIPE 18 caused higher mortality than isolates ICIPE 20, 32 and 40, while mortality rates for ICIPE 62 and 41 were similar. At 25 °C, isolates ICIPE 32 and 62 were more active than the other isolates (Table 3). At 30 °C, mortality by isolates ICIPE 18, 40 and 32 was significantly higher than by the other isolates. At 35 °C, isolate ICIPE 62 caused significant mortality followed by ICIPE 41 and 18 whereas isolate ICIPE 20 was the least active (Table 3).

There were differences in susceptibility to fungal infection between fly species at all the temperatures (Table 3). For example, *C. fasciventris* was less susceptible to isolate ICIPE 20 at 20 and 25 °C, and to isolates ICIPE 41 and 62 at 30 °C (Table 3). *Ceratitis cosyra* was also less susceptible to isolate ICIPE 32 at 25 °C and to isolates ICIPE 32, 41 and 62 at 35 °C, while *C. capitata* was less susceptible to isolates ICIPE 32 and 41 at 35 °C.

Time to death was also significantly affected by Temperature (F = 716.72; df =3,213; P =0.0001) and isolate (F = 9.96; df = 5,213; $P = 0.0001$), with significant species by isolate interactions (F = 7.83; df = 10,213; $P = 0.0001$). The LT_{90} values decreased with increasing temperature up to the optimum temperature of 30 °C. LT_{90s} ranged from 5.8 to 8.4 days at 20 °C, 3.4 to 4.8 days at 25 °C, 2.8 to 3.9 days at 30 °C, and from 3.0 to 5.2 days at 35 °C (Table 4). No significant differences in LT_{90} values among fungal isolates were observed at 20 °C. There were however significant differences in LT_{90} values at 25, 30 and 35 °C. The speed with which the three species of fruit flies

Table 3. Percent mortality *Ceratitis capitata, C. cosyra* and *C. fasciventris* by different isolates of *M. anisopliae* 4 days after exposure to 4 temperature levels

Species	ICIPE 18	ICIPE 20	ICIPE 32	ICIPE 40	ICIPE 41	ICIPE 62
	20 °C*					
C. capitata	26.4 ± 6.4a	15.0 ± 2.1a	14.2 ± 3.8a	6.3 ± 1.5a	17.5 ± 3.1a	21.7 ± 2.8a
C. cosyra	10.0 ± 2.1a	7.5 ± 1.4a	16.3 ± 1.7a	5.0 ± 0.0a	11.3 ± 1.2a	15.2 ± 2.1a
C. fasciventris	21.3 ± 1.2a	5.0 ± 2.1b	8.8 ± 2.3a	5.0 ± 2.6a	20.2 ± 3.6a	16.1 ± 2.4a
	25 °C*					
C. capitata	77.5 ± 3.2a	77.5 ± 7.2a	83.3 ± 4.2a	71.2 ± 7.1a	78.8 ± 5.1a	91.3 ± 5.9a
C. cosyra	58.8 ± 6.8a	75.1 ± 2.0a	72.5 ± 3.2b	56.6 ± 3.8a	62.5 ± 4.7a	81.3 ± 3.1a
C. fasciventris	77.5 ± 4.3a	48.8 ± 4.2b	90.0 ± 2.1a	75.0 ± 5.4a	72.5 ± 4.3a	88.8 ± 2.3a
	30 °C*					
C. capitata	100a	100a	100a	100a	100a	100a
C. cosyra	100a	100a	100a	100a	100a	100a
C. fasciventris	100a	95 ± 2.8a	100a	100a	89.3 ± 3.5b	86.0 ± 9.4b
	35 °C*					
C. capitata	90.0 ± 5.6a	67.5 ± 4.3a	88.6 ± 4.1b	91.5 ± 3.6a	93.7 ± 6.2b	100a
C. cosyra	83.2 ± 7.2a	68.8 ± 2.4a	85.1 ± 4.5b	87.0 ± 5.8b	81.2 ± 3.1b	88.8 ± 4.2b
C. fasciventris	97.3 ± 1.5a	65.0 ± 3.5a	91.7 ± 4.6a	90.7 ± 3.3a	100a	100a

Column means by temperature (± S.E.) bearing the same letter are not significantly different by Student-Newman-Keuls' ($P < 0.0001$) test.
*Mean separation is at each temperature.

succumbed to infection by the six isolates varied with different temperatures (Table 4). For example at 20 °C, *C. capitata* had the shortest LT_{90} among all the isolates tested (Table 4). At 25 °C, *C. cosyra* had the shortest LT_{90s} with isolates ICIPE 41 and 62, while *C. fasciventris* had the shortest LT_{90} with isolate ICIPE 32. At 30 °C, *C. capitata* succumbed faster to infection by isolates ICIPE 40 and 62, while *C. cosyra* had the shortest LT_{90} with isolate ICIPE 41. LT_{90} in *C. fasciventris* was not significantly different from *C. capitata* with isolate ICIPE 40 (Table 4). At 35 °C, both *C. capitata* and *C. fasciventris* had the shortest LT_{90s} for isolates ICIPE 18, 32, 40, 41 and 62 (Table 4).

Discussion

There were differences among the isolates in the effect of temperature on conidial germination, mycelial growth, and fruit fly susceptibility to fungal infection. Spore germination and mycelial growth occurred at all the temper-

Table 4. Mean (± S.e) lethal time mortality ($LT_{90)}$ for *C. capitata, C. cosyra* and *C. fasciventris* treated with isolates of *M. anisopliae* at 4 temperature levels

Species	ICIPE 18	ICIPE 20	ICIPE 32	ICIPE 40	ICIPE 41	ICIPE 62
	20 °C*					
C. capitata	5.8 ± 0.1b	7.4 ± 0.1a	6.1 ± 0.2b	6.5 ± 0.2b	5.8 ± 0.1b	6.3 ± 0.1a
C. cosyra	7.5 ± 0.1a	7.4 ± 0.3a	7.5 ± 0.1a	6.7 ± 0.4ab	7.2 ± 0.1a	8.4 ± 0.2a
C. fasciventris	6.9 ± 0.0a	7.4 ± 0.1a	6.9 ± 0.1ab	7.6 ± 0.1a	7.0 ± 0.0a	7.3 ± 0.0a
	25 °C*					
C. capitata	4.2 ± 0.1b	4.2 ± 0.1a	4.4 ± 0.1b	4.6 ± 0.1a	4.4 ± 0.1b	4.0 ± 0.1a
C. cosyra	4.7 ± 0.1a	4.6 ± 0.1a	4.7 ± 0.1a	4.3 ± 0.0a	5.2 ± 0.1a	3.5 ± 0.0b
C. fasciventris	4.3 ± 0.1b	4.8 ± 0.3a	4.0 ± 0.1c	4.5 ± 0.1a	4.5 ± 0.1b	4.1 ± 0.1a
	30 °C*					
C. capitata	3.2 ± 0.2a	3.4 ± 0.4a	3.0 ± 0.1a	3.1 ± 0.2b	3.2 ± 0.2b	3.1 ± 0.2b
C. cosyra	3.6 ± 0.09a	3.4 ± 0.1a	3.1 ± 0.1a	3.7 ± 0.1a	2.8 ± 0.1c	3.8 ± 0.1a
C. fasciventris	3.3 ± 0.1a	3.5 ± 0.3a	3.0 ± 0.1a	3.4 ± 0.1b	3.9 ± 0.1a	3.8 ± 0.1a
	35 °C*					
C. capitata	3.5 ± 0.1b	5.0 ± 0.1a	3.6 ± 0.1b	3.7 ± 0.1b	3.3 ± 0.1b	3.0 ± 0.1b
C. cosyra	4.7 ± 0.1a	4.7 ± 0.0a	4.8 ± 0.1a	5.2 ± 0.0a	5.2 ± 0.0a	4.2 ± 0.0a
C. fasciventris	3.7 ± 0.1b	5.0 ± 0.1a	3.4 ± 0.1b	3.4 ± 0.1b	3.6 ± 0.0b	3.3 ± 0.1b

Column means by temperature (± S.E.) bearing the same letter are not significantly different by Student-Newman-Keuls' ($P < 0.0001$) test.
*Mean separation is at each temperature.

atures tested, but the optimum temperature for all the isolates was 25 °C. Similar results were reported by other workers on *M. anisopliae* isolates (Walstad et al., 1970; Roberts and Campell, 1977; Fargues et al., 1997; Ekesi et al., 1999). The mean growth rate day^{-1} was within the range of 1.1–1.5 mm at 15 °C and 35 °C, 1.7–2.3 mm at 20 °C and 2.1–3.2 mm at 25 °C and 30 °C. The fastest growing isolates were ICIPE 20, 32 and 62 with an optimum temperature for growth at 25 °C. While assessing the effect of temperature on vegetative growth of fungal isolates from different origins, Vidal et al. (1997) demonstrated that the potential for isolates to tolerate high or low temperatures is normally related to the climatic data of their geographic origin. A relationship between thermal tolerance and climate of origin has been shown for other isolates of entomopathogenic Hyphomycetes including *B. bassiana, Beauveria brongniartii* (Saccado) Petch, *Metarhizium anisopliae* (*flavoviride*) var. *acridum*, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Nomuraea rileyi* (Samson) (Fargues et al., 1992; Vidal et al., 1997). The isolates used in this study originated from tropical region. Our results therefore agree with the maximum growth rates of fungal isolates

from tropical or subtropical origins that have been reported to lie between 25–30 °C (Ferron, 1981; Fargues et al., 1992). According to Ferron (1981), the optimum growth temperature for *M. anisopliae* is near 27–28 °C. Fargues et al. (1992) reported an optimum growth temperature of 25 °C for some tropical *M. anisopliae* isolates. Ekesi et al. (1999), working on isolates from the ICIPE culture collection, also reported the same germination profiles for two *M. anisopliae* isolates, ICIPE 30 and 69 originally isolated from Kenya and the DR Congo, respectively.

Temperature not only regulates the physiology of the fungus and insect, but also the ability of the fungus to infect the host. The six fungal isolates caused mortalities in all the three fly species tested, but the onset of the disease and the total mortality differed with temperature. Fungal isolates were most effective at 30 °C, where mortality between 86–100% was achieved within 4 days in all the three species. At the lower temperature of 20 °C, the onset of the disease was delayed but did not affect total mortality of 100% which was achieved within 7 and 9 days. Our results, therefore, corroborate previous reports that the rate of disease development increases with temperature increase until an optimum level is reached. Ekesi et al. (1999) noted that there was a significant decrease in fungal infection of *Megalurothrips sjostedti* (Trybom) by some tropical *M. anisopliae* strains at 20 °C in comparison to 25 and 30 °C. Although exposure to constant low temperatures generally retarded disease development in the three tephritids in the laboratory, under field conditions flies will not be exposed to constant temperatures, but will be subject to the diurnal and seasonal fluctuations in temperatures. While assessing the effect of temperature on vegetative growth of fungal isolates of from different origins, Vidal et al. (1997) demonstrated that the potential for isolates of *Paecilomyces fumosoroseus* (Wize) Brown and Smith to tolerate high or low temperatures is normally related to the climatic data of their geographic origin. A relationship between thermal tolerance and climate origin has been shown for other isolates of entomopathogenic Hyphomycetes including *B. bassiana, Beauveria brongniartii* (Saccado) Petch, *Metarhizium anisopliae* (*flavoviride*) var. *acridum*, *P. fumosoroseus* and *Nomuraea rileyi* (Samson) (Fargues et al., 1992; Vidal et al., 1997). Thus while infection will be slower during the cooler periods it will be more rapid with higher day temperatures (Ekesi et al., 1999).

Despite slow mycelial growth at 35 °C, disease development was rapid with LT_{90} values between 3–5.1 days, compared to 3.5–4.7 days observed at 25 °C. Maniania and Fargues (1992) reported the lack of correlation between fungal growth and the infection of *Spodoptera littoralis* (Boisduval) larvae with isolates of *P. fumosoroseus*. According to Fargues et al. (1992), the optimum temperature for growth is not necessarily the same as that for

fungal infection of insects. Inglis et al. (1997) found a poor relationship between conidial germination, vegetative growth and mortality in *Melanoplus sanguinipes* (Fabricius) treated with a Brazilian isolate of *M. anisopliae* (*flavoviride*) var. *acridum*. Fargues et al. (1997) reported similar results with *M. anisopliae* (*flavoviride*) var. *acridum* against *Schistocerca gregaria* (Forskål). This confirms previous reports that factors other than germination and growth come into play (Wilding, 1981; Ferron, 1981; Fargues et al., 1997).

From the results of this study we have selected the isolate ICIPE 62 as a possible biological control agent for the three species of tephritid fruit flies. The isolate was identified as the superior one in terms of virulence to the target host insects and its activity over a broader range of temperatures. The optimal temperatures for development and maximum egg production for tropical tephritids are between 25–30 °C (Bateman, 1972; Allwood, 1997), which is in the range of the optimum temperature for growth and virulence of the selected isolate. This isolate can thus be further developed for biological control of the three species of fruit flies in areas where they co-exist and can be used within the framework of IPM programs.

Acknowledgements

The authors are grateful to Dr. A. Odulaja for statistical advice, Dr. N. Zenz and the AFFI fruit fly rearing team for providing the insects, Dr. S. Ekesi for reviewing the manuscript and to Messrs J.G. Kiarie and R. Rotich for technical assistance. This study was jointly supported by funds from the International Fund for Agriculture and Development (IFAD), the African Fruit Fly Initiative Project (AFFI), and German Academic Exchange Service (DAAD)/ICIPE through the African Regional Postgraduate Programme in Insect Science (ARPPIS).

References

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. *J. Econ. Entomol*. 18: 265–267.

Allwood, A.J., 1997. Control Strategies for fruit flies (Family: Tephritidae) in South Pacific. In: A.J. Allwood and R.A.I. Drew (eds), *Management of Fruit Flies in the Pacific*. A regional symposium, Nadi, Fiji 28–31 October 1996. ACIAR Proceedings No. 76. 267 pp.

Benz, G., 1987. Environment. In: J.R. Fuxa and Y. Tanada (eds), *Epizootiology of Insect Diseases*. Wiley and Sons, New York. pp. 177–214.

Bateman, M.A., 1972. The ecology of fruit flies. *Annu. Rev. Entomol.* 17: 493–518.

Blanford, S. and M.B. Thomas, 2000. Thermal behavior of two acridid species: Effects of habitat and season on body temperature and the potential impact on biocontrol with pathogens. *Environ. Entomol.* 29: 1060–1069.

Carruthers, R.I. and R.S. Soper, 1987. Fungal diseases. In: J.R. Fuxa and Y. Tanada (eds) *Epizootiology of Insect Diseases*. John Wiley and Sons, New York. pp. 357–416.

Carruthers, R.I. and D.L. Haynes, 1986. Temperature, moisture and habitat, Effects on *Entomophthora muscae* (Entomophthorales: Entomophthoracea) conidial germination and survival in the onion agroecosystem. *Environ. Entomol*. 15: 1154–1160.

Castillo, M.A., P. Moya and E. Primo-Yufera, 2000. Susceptibility of *Ceratitis capitata* Weidemann (Dipt: Tephritidae) to Entomopathogenic fungi and their extracts. *Biol. Control* 19: 274–282.

De Meyer, M., 2001. On the identity of the Natal fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Entomologie* 71: 55–62.

Dimbi, S., N.K. Maniania, S.A. Lux, S. Ekesi and J.M. Mueke, 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera : Tephritidae). *Mycopathologia*. In press.

Ekesi, S., N.K. Maniania and K. Ampong-Nyarko, 1999. Effect of temperature on germination, radial growth and virulence of *M. anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti. Biocontrol Sci. Technol*. 9: 177–185.

Ekesi, S., N.K. Maniania and S.A. Lux, 2002. Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana. Biocontrol Sci. Technol*. 12: 7–17.

Fargues, J., N.K. Maniania, J.C. Delmas and N. Smits, 1992. Influence de la température sur la croissance *in vitro* d'hyphomycètes entomopathogènes. *Agronomie* 12: 557–564.

Fargues, J., A. Ouedraogo, M. Goettel, and C. Lomer, 1997. Effects of temperature, humidity and inoculation method on susceptibility of *Schistocerca gregaria* to *Metarhizium flavoviridae*. *Biocontrol Sci. Technol.* 7: 346–356.

Ferron, P. 1981. Pest control by the fungi *Beauveria* and *Metarhizium*. In: H. D. Burges (ed), *Microbial Control of Pests and Plant Diseases 1970–1980*. Academic Press, London. pp. 465–476.

García, A.S., C.L. Messians, H.L.M. Souza-de and A.E. Piedrabuena, 1984. Patogenicidade de *Metarhizium* var. *anisopliae* a *Ceratitis capitata* (Wiedemann) (Diptera: Tephiritidae). *Rev. Bras. Entomol*. 28: 421–428.

Hall, R.A. and B. Papierok, 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. *Parasitology* 84: 205–240.

Hall, I.M. and J.V. Bell, 1960. The effects of temperature on some entomopathogenic fungi. *J. Insect Pathology* 2: 247–253.

Hall, I.M. and. J.V. Bell, 1961. Further studies of temperature on some entomopathogenic fungi. *J. Insect Pathology* 3: 289–296.

Hooper, G.H.S., 1987. Application of quality control procedures for large scale rearing of the Mediterranean fruit fly. *Ent. Exp. App*. 44: 161–167.

Inglis, G.D., M.S. Goettel, T.M. Butt and H. Strasser, 2001. Use of hyphomycetous fungi for managing insect pests. In: T.M. Butt, C. Jackson and N. Magan (eds), *Fungi as Biocontrol Agents: Progress, Problems and Potential*. CABI Wallingford, pp. 27–69.

Inglis, G.D., D.L. Jonhson, K.J. Cheng and M.S. Goettel, 1997. Use of pathogen combinations to overcome constrains of temperature on entomopathogenic Hyphomycetes against grasshoppers. *Biol. Control* 8: 143–152.

Lezama-Gutierrez, R., A. Trujillo-De La Luz, J. Molina-Ochoa, O. Rebolledo-Dominguez, A. R. Pescador, Lopez-Edwards and M. Aluja, 2000. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): Laboratory and field trials. *J. Econ. Entomol.* 93: 1080–1084.

Lux, S.A., W. Overholt and S. Kimani, 1998. Economic role and distribution of fruit flies. In: *ICIPE Annual Scientific Report 1995–1997.* ICIPE Science Press, Nairobi. pp. 29–30.

Maniania, N.K. and J. Fargues, 1992. Susceptibility of *Mamestra brassicae* (L.) and *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) to the hyphomycetes *Paecilomyces fumosoroseus* (Brown and Smith) and *Nomuraea rileyi* (Samson) at two temperatures. *J. Appl. Entomol.* 113: 518–524.

Roberts, D.W. and A.S. Campbell, 1977. Stability of entomopathogenic fungi. *Ent. Soc. Amer*. 10: 19–76.

SAS Institute, 1990. *SAS/STAT User's Guide*. Version 6. 4th edition, Vol. 2. SAS Institute, Cary, NC.

Schaeffenberg, B., 1964. Biological and environmental conditions for the development of mycoses caused by *Beauveria bassiana* and *Metarhizium anisopliae. J. Insect Pathology* 6: 8–20.

Stimmann, M.W., 1968. Effects of temperature on infection of the garden symphylan by *Entomophthora coronata*. *J. Econ. Entomol*. 61: 1558–1560.

Throne, J.E., D.K. Weaver, V. Chew and J.E. Baker, 1995. Probit analysis of correlated data: Multiple observations over time at one concentration. *J. Econ. Entomol.* 88: 1510–1512.

Vidal, C., J. Fargues and L.A. Lacey, 1997. Intraspecific variability of *Paecilomyces fumosoroseus*: Effect of temperature on vegetative growth. *J. Invertebr. Pathol.* 70: 18–26.

Walstad, J.D., R.F. Anderson and W.J. Stambauch, 1970. Effects of environmental conditions on two species of muscardine fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). *J. Invertebr. Pathol.* 16: 221–226.

Wilding, N., 1981. Pest control by Entomophthorales. In: H.D. Burges (ed), *Microbial Control of Pests and Plant Diseases 1970–1980.* Academic Press, London. pp. 538–553.

Zimmermann, G., 1986. The "Galleria bait method" for detection of entomopathogenic fungi in soil. *J. Appl. Entomol.* 102: 213–215.