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Host species, age and sex as factors affecting the susceptibility of the African Tephritid fruit fly species, *Ceratitis capitata, C. cosyra* and *C. fasciventris* to infection by *Metarhizium anisopliae*

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Abstract

The effect of host age and sex on the susceptibility of 3 tephritid fruit fly species, *Ceratitis capitata* (Wiedemann), *C. cosyra* (Walker) and *C. fasciventris* (Bezzi) to the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin was studied in the laboratory. Three adult host ages, 0 (<1 day-old), 7-day-old and 14-day-old, were used. All 3 species were susceptible to fungal infection, although there were differences in the levels of susceptibility among the hosts. Age accounted for the largest variability in mortality followed by species, while sex had the lowest variability. Of the 3 host ages tested, the 0- and 7-day-old flies were more susceptible to fungal infection than the 14-day-old flies. Male and female *C. fasciventris* flies generally succumbed earlier to fungal infection than the other two species. Female flies of *C. cosyra* and *C. fasciventris* were also generally more susceptible to fungal infection than the males, although differences were apparent at 3 and 4 days after treatment but not 5 days after treatment. Age accounted for the largest variability in lethal time mortality values (LTs). Mean LT-values generally indicated that the speed of kill was faster among younger flies than the older flies. LT_{95} ranged between 3.9–4.9 days in the 0-day-old flies, 4.3–6.1 days in the 7-day-old flies and 4.6–6.1 days in 14-day-old flies in the different species and sexes. The implication of this study for the management of fruit flies is discussed.

1 Introduction

Tephritid fruit flies are among the major pests of fruits and vegetables worldwide and represent the most economically important group of phytophagous Diptera (Allwood, 1997; De la Rosa et al., 2002). In Africa, their control has basically depended on the use of chemical pesticides as aerial sprays for control of the adults and soil drenches for control of the immature stages (Peña et al., 1998). However, due to the adverse effects of chemical pesticides on the environment, considerable progress has been made in recent years to develop environmentally benign pest control methods in Integrated Pest Management (IPM) programs (Vänninen et al., 2000; Ayyappath et al., 2000). Thus, several entomopathogens including fungi are being developed as biological control agents for various insect species including tephritid fruit flies (Navrozidis et al., 2000; Lezama-Gutierrez et al., 2000, Castillo et al., 2001; Ekesi et al., 2002). Recently at the International Centre of Insect Physiology and Ecology (ICIPE), steps have been undertaken to develop entomopathogenic fungi as biological control agents for the management of African fruit fly species. Several isolates of the entomopathogenic fungi *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin were screened against adult and immature stages of Tephritid fruit fly species, *Ceratitis capitata* (Wiedemann), *C. cosyra* (Walker), *C. fasciventris* (Bezzi), *C. anonae* (Graham) and *C. rosa* (Karsch). Six isolates of *M. anisopliae* were selected for their pathogenicity to the 5 species of fruit flies (Ekesi et al., 2002; Dimbi et al., 2003).

Insect susceptibility to fungal infection has been reported to be affected by a number of factors, such the properties of the pathogen population, the host population as well as environmental conditions (Benz, 1987; Fuxa and Tanada, 1987; Inglis et al., 2001). Among the host factors, host species, host age, the developmental stage and sex have been reported to affect insect susceptibility to entomopathogenic fungi (Ferron, 1985; Feng et al., 1985; Maniania and Odulaja, 1998). According to Butt et al. (2001), an understanding of host properties that influence susceptibility to infection is important in the development of management tactics and will enable the optimization of the impact of biological control agents. No studies have been undertaken so far to investigate the differential susceptibility of African tephritid fruit flies to fungal infection. This study, therefore, investigates the effects of species, age and sex of adults of *C. capitata, C. cosyra* and *C. fasciventris* to their susceptibility to *M. anisopliae* and the implications for biological control of these pests.

2 Materials and methods

2.1 *Fungal culture*

The fungus used was an isolate of *M. anisopliae* (ICIPE 62) obtained from the ICIPE culture collection, isolated from the soil sample from Kinshasa, Democratic Republic of Congo in 1990 using the '*Galleria* bait method' (Zimmermann, 1986). The virulence of the strain was maintained by regular passage through an insect host (Schaerffenberg, 1964). Its virulence against *Ceratitis* spp. was reported in an earlier work (Dimbi et al., 2003). The fungus was maintained on Sabouraud dextrose agar (SDA plates at room temperature (25 ± 4 °C). Conidia were harvested from the surface culture by scraping. Viability tests were carried out using the technique described by

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Goettel and Inglis (1997). Conidial suspension (0.1 ml) titrated to 3×10^6 conidia ml^{-1} was spread-plated on 9-cm petri dishes containing SDA medium. Percentage germination was determined by counting the number of germinated conidia/100 conidia in 4 separate areas per plate at ×200 magnification after incubation at 25 ± 2 °C for 20 h. Four replicate plates were used.

2.2 *Insects*

Adult flies of all 3 species (*C. capitata*, *C. cosyra* and *C. fasciventris*) were obtained from mass-rearing stock maintained at ICIPE since 1997. The initial colonies of *C. capitata* and *C. fasciventris* originated from coffee, *Coffea arabica*, collected from farms in the Central Highlands of Kenya at Ruiru (1°5.72'S; 36°54.22'E; 1609 m above sea level) and Rurima (0°38.39'S; 37°29'E; 1228 m above sea level). *Ceratitis cosyra* colony was derived from collections obtained from mango, *Mangifera indica* Linnaeus, and marula, *Sclerocarya birrea*, at Nguruman, Kenya (1°47'S; 36°05'E; 700 m above sea level). The larvae of the 3 species were fed on carrot-sugar-based artificial diet, which is a modification of that previously developed by Hooper (1987). Adult flies were reared in ventilated Plexiglas cages (60 × 35 × 70 cm) at temperatures between 24–28 °C and photoperiod 12:12 L:D, on a 4:1 mixture of sugar (Mumias Sugar Co, Kenya) and yeast hydrolysate (Yeast hydrolysate enzymatic, USB, Corporation, Ohio) based artificial diet. Flies of both sexes and of 3 ages, 0 (<1-day-old), 7 and 14 days old, were used.

2.3 *Application of* M. anisopliae

Test insects were contaminated through a velvet cloth covering the inner side of a cylindrical plastic tube. Flies of the same age from each species were transferred to the cylindrical tube in groups of 20 males or females and allowed to walk on the fungus-contaminated cloth for 3 minutes, after which they were transferred to clean ventilated Plexiglas cages (150 × 150 × 200 mm). The insects were provided with sugar and yeast hydrolysate diet; a petri dish (50 mm diam.) with cotton wool soaked in water was also provided as a water source. Controls consisted of groups of males or females of the different species and age groups that had been exposed to fungus-free velvet. Flies were maintained as described above. Each treatment consisted of 20 insects, and the experiment was repeated 4 times. Mortality was recorded daily for 10 days. 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 fungal growth on the surface of the cadaver. Only dead flies that showed sporulation on the surfaces were considered for analysis.

2.4 *Statistical analysis of the data*

Percentage mortality data were arcsine-transformed and subjected to three-way analysis of variance (ANOVA) (Proc, ANOVA, SAS Institute, 1999–2001) to determine the effects of species, sex and age groups. Log day probit (LT_{25}, LT_{50}, LT_{75} and LT_{90}) were determined for each replicate using the probit analysis method for correlation of data (Throne et al., 1995). The LT values were compared among species and sexes using ANOVA. Means were separated using the Student-Newman-Keuls (SNK) method when the F-ratios were significant ($P \leq 0.05$).

3 Results

In viability tests, 92.5 % of conidia germinated after 20 h. Mortality in the controls was less than 1.3 % in male and female *C. fasciventris*, 10.0 % in male *C. cosyra*, 13.7 % in female *C. cosyra*, 8.8 % in male *C. capitata* and 7.5 % in female *C. capitata*.

Species-by-sex and sex-by-age interactions were significant on all days, while species-by-age interactions were significant on day 3 and 4 but not on day 5 (table 1). Fly age had the most significant effect on mortality followed by species. Sex had a significant effect on mortality only on day 3 post-infection (tables 1 and 2). Species by age by sex interactions were not significant on any of the days. Significant differences in mean mortalities between age groups for all combinations of species and sexes were noticeable on all days, except at day 4 and day 5 post-treatment in *C. cosyra* males and at day 5 post-infection in *C. capitata* males (table 3). The general trend was that the younger flies (0-day-old) succumbed earlier to infection than older flies, but this was not consistent and varied with fly species and days. For example on day 3, mortality was significantly higher in the 0-day-old group than flies of the14 day-old group in all sexes and species (table 3). However, no significant differences in mortality were observed between the 0- and 7-day-old flies in females of all 3 species and between 7- and 14-day-old males of *C. capitata* and *C. cosyra* (table 3). Four days after

Table 1. ANOVA mean squares for differential susceptibility between fruit fly species, sex and age to *Metarhizium anisopliae.*

Source	df	3 dat	4 dat	5 dat
Age	2	0.21***	0.77***	0.80***
Species	2	0.19***	0.40***	0.07^{ns}
Sex	1	0.22***	0.04^{ns}	0.01^{ns}
Species*Age	4	0.03**	0.20***	0.01^{ns}
Species*Sex	2	0.04*	0.10**	0.01*
Age*Sex	2	0.03*	0.08**	0.10*
Species*Age*Sex	4	0.01^{ns}	0.02^{ns}	0.07^{ns}

^{ns}not significant; *significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.0001$; df.: degrees of freedom; dat: days after treatment.

Table 2. Effect of species, age and sex on the susceptibility of three species of fruit flies to *Metarhizium anisopliae.*

	Days after treatment		
	3 dat	4 dat	5 dat
Age			
0-day-old	30.80 ± 6.8a	77.07 ± 5.6a	98.34 ± 1.7a
7-day-old	24.92 ± 7.6b	68.34 ± 7.8b	93.90 ± 2.9b
14 day-old	12.46 ± 3.8c	51.98 ± 5.7c	89.30 ± 4.0c
Species			
C. capitata	22.91 ± 5.3b	58.48 ± 7.5b	94.06 ± 3.4a
C. cosyra	15.66 ± 5.5c	63.58 ± 4.6b	92.68 ± 4.0a
C. fasciventris	32.30 ± 8.5a	75.33 ± 9.6a	94.81 ± 2.7a
Sex			
♂♂	18.64 ± 5.6b	64.91 ± 6.4a	93.55 ± 4.2a
♀♀	28.47 ± 7.8a	66.69 ± 8.5a	95.10 ± 2.5a

Within column means followed by the same letter are not significantly different by Student-Newman-Keuls (SNK) at $P < 0.05$; dat: days after treatment.

Table 3. Mean percentage mortality (±se) of 3 fruit fly species at different days after treatment (dat) with *Metarhizium anisopliae.*

Age (days)	3 dat	4 dat	5 dat
C. capitata Females			
0	21.85 ± 1.3a	72.36 ± 3.3a	97.36 ± 2.4a
7	23.50 ± 1.7a	53.04 ± 7.2b	97.50 ± 1.4a
14	12.98 ± 0.7b	35.42 ± 6.3c	79.75 ± 0.1b
C. capitata Males			
0	33.31 ± 4.1a	73.36 ± 3.3a	98.75 ± 1.1a
7	13.92 ± 0.1b	60.74 ± 3.8ab	96.20 ± 2.4a
14	15.75 ± 4.5b	56.96 ± 5.2b	96.19 ± 2.4a
C. cosyra Females			
0	28.18 ± 5.5a	75.0 ± 2.5a	100 ± 0.0a
7	27.30 ± 5.0a	72.2 ± 2.4a	89.6 ± 2.8b
14	10.21 ± 1.5b	58.6 ± 4.3b	85.5 ± 2.3b
C. cosyra Males			
0	14.18 ± 2.3a	60.5 ± 3.3a	96.05 ± 2.5a
7	10.28 ± 1.4ab	56.8 ± 2.7a	93.75 ± 2.3a
14	3.84 ± 2.2b	58.3 ± 3.3a	90.90 ± 1.1a
C. fasciventris Females			
0	50.62 ± 6.9a	92.4 ± 1.4a	100 ± 0.0a
7	51.00 ± 7.0a	92.5 ± 1.4a	97.5 ± 1.4a
14	19.50 ± 3.5b	50.0 ± 4.5b	90.9 ± 1.0b
C. fasciventris Males			
0	36.68 ± 2.1a	89.9 ± 2.8a	98.5 ± 1.0a
7	23.50 ± 2.2b	74.8 ± 5.4b	89.4 ± 4.1b
14	12.50 ± 1.4c	52.5 ± 1.4c	92.5 ± 1.4ab

Within column means (by species and sex) followed by the same letter are not significantly different ($P \leq 0.05$); dat: days after treatment.

Table 4. Sex differences in mortality rate in *Ceratitis capitata, C. cosyra* and *C. fasciventris* flies infected with *Metarhizium anisopliae.*

Species and sex of flies	Days after treatment		
	3 dat	4 dat	5 dat
Ceratitis capitata			
Male	21.0 ± 5.0a	63.8 ± 5.6a	97.5 ± 2.0a
Female	19.4 ± 2.5a	53.2 ± 9.1b	90.7 ± 7.4a
Ceratitis cosyra			
Male	9.4 ± 2.9b	58.6 ± 2.8b	93.6 ± 2.2a
Female	21.9 ± 5.8a	68.6 ± 4.7a	91.7 ± 3.7a
Ceratitis fasciventris			
Male	24.2 ± 5.5b	72.4 ± 2.1b	93.5 ± 3.2a
Female	40.4 ± 9.4a	78.3 ± 2.8a	96.2 ± 2.1a

Within column means (by dat and species) followed by the same letter are not significantly different by Student-Newman-Keuls test at $P < 0.05$; dat: days after treatment.

treatment, no significant differences were observed among the age groups in male *C. cosyra*, while younger flies of both sexes succumbed to infection earlier than the older in the other species (table 3). Five days after treatment, no differences between age groups were observed in *C. capitata* and *C. cosyra* males. However, 0-day-old *C. cosyra* females and *C. fasciventris* males had significantly higher mortality, while no significant differences were observed in mortality between the 0- and 7-day-old females of *C. capitata* and *C. fasciventris*.

Table 5. Mean (±se) lethal time-mortality values (LTs) of 3 species of fruit flies to *Metarhizium anisopliae.*

	LT_{25}	LT_{50}	LT_{75}	LT_{95}
C. capitata Females				
0	2.85 ± 0.2b	3.21 ± 0.4c	3.60 ± 0.2b	4.40 ± 0.2b
7	2.87 ± 0.1b	3.52 ± 0.2b	4.32 ± 0.6a	6.11 ± 0.8a
1	3.23 ± 0.5a	3.74 ± 0.6a	4.33 ± 0.1a	5.55 ± 0.3a
C. capitata Males				
0	2.85 ± 0.5b	3.21 ± 0.6b	3.61 ± 0.8b	4.41 ± 0.5b
7	3.14 ± 0.7a	3.54 ± 0.5a	3.97 ± 0.2a	4.84 ± 0.4a
14	3.11 ± 0.6a	3.57 ± 0.2a	4.09 ± 0.2a	5.15 ± 0.1a
C. cosyra Females				
0	2.87 ± 0.3b	3.16 ± 0.1b	3.47 ± 0.7b	4.08 ± 0.6b
7	2.87 ± 0.4b	3.20 ± 0.0b	3.57 ± 0.2b	4.30 ± 0.8b
14	3.22 ± 0.2a	3.67 ± 0.4a	4.18 ± 0.5a	5.22 ± 1.0a
C. cosyra Males				
0	3.14 ± 0.4b	3.53 ± 0.6b	3.98 ± 0.4a	4.86 ± 0.2a
7	3.26 ± 0.6b	3.64 ± 0.2a	4.08 ± 0.1a	4.93 ± 0.1a
14	3.43 ± 0.7a	3.72 ± 0.4a	4.03 ± 0.6a	4.60 ± 0.9a
C. fasciventris Females				
0	2.52 ± 0.8b	2.83 ± 0.3b	3.18 ± 0.8b	3.86 ± 0.8b
7	2.32 ± 0.6c	2.75 ± 0.5b	3.25 ± 0.2b	4.31 ± 0.6b
14	3.01 ± 0.1a	3.65 ± 0.1a	4.43 ± 0.1a	6.12 ± 1.2a
C. fasciventris Males				
0	2.62 ± 0.6c	3.07 ± 0.5b	3.59 ± 0.2b	4.67 ± 0.2b
7	2.93 ± 0.2b	3.28 ± 0.4b	3.66 ± 0.5b	4.41 ± 0.3b
14	3.22 ± 0.4a	3.69 ± 0.1a	4.22 ± 0.4a	5.31 ± 1.3a

Column means (by species and sex) followed by the same letter are not significantly different ($P \leq 0.05$).

The difference in the susceptibility of different fly sexes to fungal infection varied between species. Generally, females of *C. cosyra* and *C. fasciventris* were more susceptible than male flies at 3 and 4 days after treatment, while no differences were observed on day 5 after treatment (table 4). However in *C. capitata*, no significant differences in mortality between sexes were observed at 3 and at 5 days after treatment, although male mortality was higher at 4 days after treatment (table 4).

Age accounted for the largest variability in mortality and was, therefore, the most important factor determining mortality. The lethal time mortality (LT) values were subsequently compared between age groups for each combination of species and sex. Mean lethal-time mortality values were generally shorter among younger flies than the older flies (table 5). LT_{95} ranged between 3.9–4.9 days in the 0-day-old flies, 4.3–6.1 days in the 7-day-old flies and 4.6–6.1 days in 14-day-old flies, with the different species and sexes (table 5).

4 Discussion

The success in the use of entomopathogenic fungi as microbial control agents depends not only on the careful selection of the most efficacious isolates in terms of pathogenic activity but also on a thorough understanding of the host/pathogen interactions. BUTT et al. (2001)

stressed the need to study the relationship between entomogenous fungi and their hosts in order to identify vulnerable stages of the target. Moreover, understanding the interactions between host and pathogen is important since they are the key factors involved in disease initiation and development (Tanada and Fuxa, 1987).

Results of this study indicate that all 3 species of tephritid fruit flies tested were susceptible to *M. anisopliae* infection, but there were differences in levels of susceptibility within the different species, sex and ages. Maniania and Odulaja (1998) reported differences in the susceptibility of *Glossina morsitans morsitans* Westwood and *G. m. centralis* Machado to *M. anisopliae.*

Generally, females succumbed earlier to fungal infection than male flies. This concurs with the observations by Maniania and Odulaja (1998) on *G. m. morsitans* and *G. m. centralis* with *M. anisopliae*. Brefeld (1871) also reported similar results in female *Musca domestica* flies infected with *Entomophthora muscae* (Cohn) Fresenius. The author attributed the difference to the fact that females are larger and thus possess more body fat, the site for vegetative replication of the fungal pathogen (Mullens, 1985). On the other hand, Kaaya (1989) reported that *G. m. morsitans* male flies were more susceptible to *M. anisopliae* and *B. bassiana* infection than the females. Similar results were reported by Mullens (1985) with *M. domestica* flies infected with *E. musca*. In both cases, the susceptibility of males was attributed to their smaller sizes compared to the female flies.

The host age was found to have a significant overall effect on mortality, with the younger flies succumbing earlier to infection than older flies. These results agree with Maniania and Odulaja (1998), who also reported that younger *M. anisopliae*-infected tsetse flies died earlier than the older ones. Mullens (1985) also reported that young *M. domestica* were more susceptible to *E. muscae* infection than the older flies. However, Rizzo (1977) demonstrated that the different ages of adults of 3 dipteran flies, the housefly *M. domestica*, the black blowfly, *Phormia regina* Meigen, and the onion fly, *Delia antiqua* Meigen, were equally susceptible to infection with either *M. anisopliae* or *B. bassiana*.

The observed pathogenicity of *M. anisopliae* towards the 3 fruit flies in this study can be attributed to the wide host range of this fungal species which has been reported on more than 200 insect species from 7 orders, including the order Diptera (Zimmermann, 1993). The host range observed with this fungal isolate is more desirable than strict specificity, given the fact that all 3 species are normally found occupying the same ecological niche.

The results of this study demonstrate the uniqueness of each host/pathogen system and, therefore, underline the need to understand host/pathogen relationships in order to improve the efficacy of entomopathogenic fungi as biological control agents. Our study has shown that female fruit flies aged between 0 and 7 days are more susceptible to infection than males or older flies. The implication of these results is that fungus-contaminated young females are not likely to live longer; thus reducing damage caused by young fecund females when ovipositing in mango fruit. An autoinoculative device for field application of fungal spores for fruit fly control has recently been developed (Dimbi et al. in preparation). This technique requires attraction of flies to the autoinoculative device (bait station where fungal spores have been incorporated) where they are contaminated by fungal conidia. Since food baits that have to be used in the autoinoculative device are highly attractive to young sexually immature females in search of a protein diet for egg maturation (Allwood, 1997), a control strategy that targets females in this age group would probably be successful in controlling this pest.

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