Effect of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on Survival and Reproduction of the Filth Fly Parasitoid, *Spalangia cameroni* (Hymenoptera: Pteromalidae)

C. NIELSEN,[1, 2, 3] H. SKOVGÅRD,[1] AND T. STEENBERG[1]

Danish Institute of Agricultural Sciences, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark

Environ. Entomol. 34(1): 133–139 (2005)

ABSTRACT Pupal parasitoids are released as biocontrol agents against house flies and stable flies in livestock production systems, and entomopathogenic fungi may be compatible with the parasitoids. However, little is known on the dynamic interactions between pupal parasitoids and entomopathogenic fungi when based on releases of both biological control agents. Therefore, the compatibility of the pupal parasitoid *Spalangia cameroni* Perkins and the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin was examined in laboratory experiments. As a first step in this study, the susceptibility of *S. cameroni* to fungus infection was examined in a dose–response experiment, which was followed by a study on the effect of *M. anisopliae* on female survival and reproduction. The female parasitoids were moderately susceptible to *M. anisopliae*, with an LC_{50} value 6 d after treatment of 2.97×10^7 conidia/ml. When previously infected *S. cameroni* were provided with surplus fly puparia, no overall effect of fungal treatment on reproduction was found, although treatments with 1×10^8 conidia/ml resulted in 50% infected individuals. The fungus infection significantly reduced the survival of the females, while the total fecundity (86.26 ± 8.63 progeny per female) was not statistically different from that of uninfected parasitoids (95.23 ± 6.88 progeny per female). As a result, the susceptibility of the parasitoids to fungal infection had no discernible effect on the intrinsic rate of natural increase (r_m). This strongly suggests that the isolate of *M. anisopliae* and *S. cameroni* could be compatible under field conditions. The results are discussed in relation to integrated fly control by combined use of *S. cameroni* and hyphomycetous fungi.

KEY WORDS *Spalangia cameroni*, *Metarhizium anisopliae*, biological control, population dynamics, house flies

HOUSE FLIES, *Musca domestica* L., and stable flies, *Stomoxys calcitrans* L., are the major insect pests on swine and cattle farms in Denmark where insecticides are still widely used for fly control. However, the ability of fly populations to develop insecticide resistance (Keiding 1999) and the increasing environmental awareness in the public and among farmers have promoted research on biocontrol methods as an alternative or supplement to chemical control.

The solitary pupal parasitoid *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) is the predominating species of fly parasitoids in Danish indoor livestock farms (Skovgård and Jespersen 1999, 2000). A significant supression of house fly populations was found in a release study with *S. cameroni*, whereas the effect on stable fly populations was less clear (Skovgård 2004, Skovgård and Nachmann 2004). The parasitoid attacks the pupal stage, and to increase the level of control, releases of other biocontrol agents that target adult and immature fly stages should be considered. Several studies have focused on the potential of hyphomycetous fungi to control adult and immature stages of house flies (e.g., Barson et al. 1994, Watson et al. 1996, Renn et al. 1999), while less attention has been given to stable flies (Watson et al. 1995). Infection and mortality of house fly larvae and adult stable flies are lower than for adult house flies (Steinkraus et al. 1990, Watson et al. 1995), which are highly susceptible to fungal infection under laboratory conditions (Barson et al. 1994, Geden et al. 1995). Carswell et al. (1998) showed that <25 conidia of *Metarhizium anisopliae* (Metschnikoff) Sorokin would kill adult house flies within 9 d, and Bywater et al. (1994) showed reduced fecundity in flies treated with this fungus. Entomopathogenic fungi therefore seem to have potential for fly control. However, entomopathogenic fungi often have wide host ranges

[1] Danish Institute of Agricultural Sciences, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark.
[2] Department of Terrestrial Ecology, University of Copenhagen, Universitetsparken 15, DK-2100 Copehagen Ø, Denmark.
[3] Corresponding author: Danish Institute of Agricultural Sciences, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark (e-mail: chn@kvl.dk).

0046-225X/05/0133–0139$04.00/0

(Goettel et al. 1990). Several studies have shown the susceptibility of parasitoids to fungal infection (Furlong and Pell 1996, Danfa and van der Valk 1999, de la Rosa et al. 2000, Lord 2001), and besides the reduced parasitoid survival, additional sublethal effects such as behavioral changes, reduced feeding, or decreased fecundity may occur (Madelin 1963, Tanada and Kaya 1993, Hajek and St. Leger 1994). Thus far, studies examining the combined effect of hyphomycetous fungi and pupal parasitoids to control stable and house flies is limited to the experiments of Geden et al. (1995), in which only the direct effect of the fungus *Beauveria bassiana* Balsamo (Vuillemin) to the parasitoid *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae) was evaluated.

Most pathogen–parasitoid interactions are detrimental to the parasitoids (Brooks 1993), and the effect of a fungal pathogen on fly hosts or parasitoid individuals may have a severe impact on the population dynamics of the parasitoids. Such impacts may be both direct (parasitoid infection) and indirect in the form of shared competition for host resources (Petersen 1986, Rosenheim et al. 1995)

The impact of the pathogen on a parasitoid population can be evaluated using the intrinsic rate of natural increase, r_m because it includes information on both age-specific fecundity and age-specific longevity for a stable population (Birch 1948, Begon et al. 1996).

Therefore, the objectives of this study were to examine the susceptibility of female *S. cameroni* to an isolate of *M. anisopliae* and to evaluate the effect of the fungus on reproduction and survival of female parasitoids because these parameters may be affected by application of fungal propagules against adult or immature flies in the field. These parameters were used to calculate generation time, net reproductive rate, and intrinsic rate of natural increase. The results are discussed in relation to the compatibility of *S. cameroni* and hyphomycetous fungi for fly control.

Materials and Methods

Parasitoids. Adult *S. cameroni* were obtained from a laboratory colony initiated in 2000 from Danish field collected puparia and reared on living house fly puparia at 25°C, 55 ± 5% RH, and a photoperiod of 12:12 (L:D). The parasitized house fly puparia were incubated in 0.5-liter plastic containers capped with mesh screen lids for 3–4 wk to allow for *S. cameroni* development.

The colony of house flies was collected on a Danish farm near Copenhagen in 1989 and has since been bred for >190 generations. The adult house flies were offered sugar and powdered milk and provided with a cup of crumpled filter paper soaked in milk for egg laying. Eggs of house flies are generally laid in clumps and can therefore easily be collected. Eggs were transferred to fresh larval medium consisting of wheat bran (24.6%), alfalfa (12.3%), yeast (0.6%), malt (0.9%), and water (61.6%). After pupation. the fly puparia were separated from the diet by water flotation. The generation time of this strain of house flies is 17–18 d at 27°C and ≈65–70% RH. The size of house fly puparia used in the experiments was 5.7 ± 0.3 (SE) mm in length and 2.3 ± 0.1 mm in width.

Fungal Isolates. The Met84 isolate of *M. anisopliae* was isolated from a beetle larva and subcultured on Sabouraud dextrose agar supplemented with yeast extract (SDAY) up to three times in 9.0-cm petri dishes and kept at 25°C and 60 ± 5% RH. (The isolate is deposited in the Collection of Entomopathogenic Fungal Cultures of the USDA–ARS Plant Protection Research Unit as ARSEF strain 5858.) Conidia from 10- to 20-d-old cultures were harvested with deionized water containing 0.02% Tween 80 and sieved through filter paper into sterile vials. Conidia were counted in a compound microscope using a hemocytometer (0.0625 mm^2; Fuchs-Rosenthal Merck Eurolab) to calibrate a suspension of 1×10^8 conidia/ml. Lower concentrations from 1×10^4 to 1×10^7 conidia/ml were prepared by dilution series.

Spore viability was determined by spreading 2 ml of a 1×10^6 ml^{-1} conidia suspension on SDAY medium and estimating the number of germinated propagules after the conidia had been incubated for 24 h at room temperature. Propagules were considered viable if germ tube lengths were corresponding to the width of the *M. anisopliae* conidia. The viability of conidia was assessed immediately before each experiment was started, and percentage germination was estimated to >95% for all experiments.

Dose–Response Experiments. Five concentrations of Met84, 1×10^8, 1×10^7, 1×10^6, 1×10^5, and 1×10^4 conidia/ml, were prepared as described above. For each concentration, there were five replicates of 20 female parasitoids. The parasitoids were anesthetized with CO_2 and placed in 30-ml plastic cups where they were swirled for 5 s in 5 ml spore suspension and poured through a strainer, and the retained parasitoids were gently moved to a filter paper, where excessive conidia suspension was absorbed. The parasitoids were transferred in groups of 20- to 30-ml plastic cups capped with mesh screen lids and provided with 20 house fly puparia to avoid initial starvation. Controls were treated with 0.02% Tween 80. The parasitoids were incubated at 25°C, 60 ± 5% RH, and a photoperiod of 12:12 (L:D), and the mortality of the parasitoids was recorded on day 2, 6, and 10. The experiment was conducted twice. The parasitoids were of different age at the onset of the experiment and mortality increased markedly because of aging after day 6 for all treatments. Therefore, mortalities recorded on day 6 for the dose–response experiment were subjected to probit analysis (SAS Institute 1998) after correction for control mortality (Abbott 1925).

Survival and Reproduction of Inoculated Female Parasitoids. Two concentrations of Met84 (1×10^8 and 1×10^6 conidia/ml) were prepared, and newly emerged females (<24 h old) were inoculated as described above for the dose–response experiment. For each of the two concentrations, 10 female parasitoids were treated individually. Parasitoids for control were swirled in 0.02% Tween 80. Each female was transferred to a 30-ml plastic cup with a screened lid, and

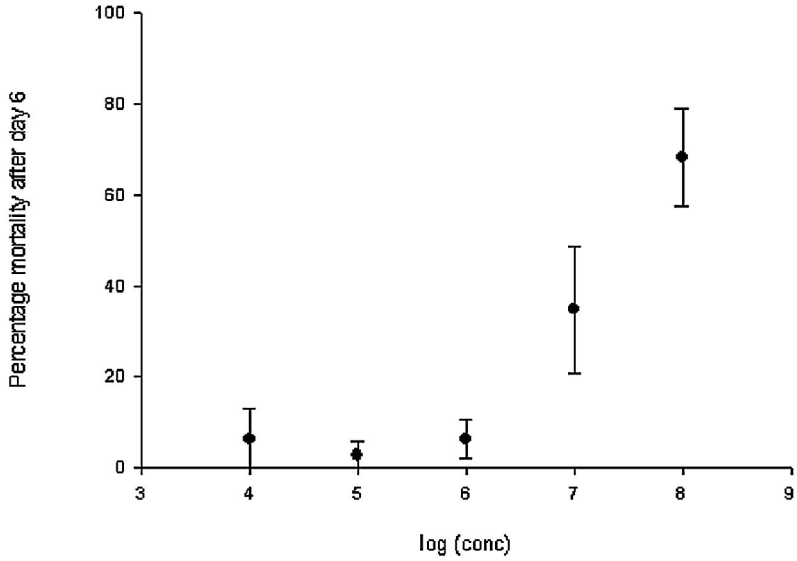

Fig. 1. Mean percentage mortality (±SE) of *S. cameroni* after exposure to conidia suspensions of the Met84 isolate of *M. anisopliae* in the dose–response experiment. LC_{50} value: 2.97×10^7 conidia/ml (95% CL: 2.19×10^7–4.16×10^7 conidia/ml). Mean percentage mortality was corrected for 8% control mortality (Abbott 1925).

30 house fly pupae (24–48 h old) were provided daily until death of the female. To ensure that the female was mated at the onset of the experiment, two male *S. cameroni* were placed with the female for 24 h. Parasitized puparia were maintained in the laboratory at 25°C, 60 ± 5% RH, and a photoperiod of 12:12 (L:D). Survival of the females was recorded daily for up to 30 d, and the reproduction was subsequently calculated as the summarized number of hatched progeny. Dead females were placed in petri dishes lined with moist filter paper and sealed with parafilm to confirm death from infection by *M. anisopliae* as verified by sporulating cadavers. After 35 d, fly puparia without parasitoid emergence holes were dissected to determine host feeding and the number and sex of unhatched progeny. *S. cameroni* feeds on the exudate of pupae by probing the posterior spiracles of the pupal case. Therefore, fly pupae killed by parasitic feeding could be determined by a dark necrotic area in their end spiracles. The experiment was conducted three times. To test for differences in survival of female *S. cameroni*, PROC LIFEREG in SAS was applied to data with days of female survival (t) as a continuous driving variable and treatment ($0, 1 \times 10^6$ and 1×10^8 spores) as a class variable (SAS Institute 1998). The response variable number of surviving females at day t was tested on a $\log_{10}$ transformation against the Weibull distribution. To test for significant differences in reproduction, the total numbers of progeny produced per female parasitoid were subjected to a one-way analysis of variance (ANOVA) using treatment as the explanatory variable (SAS Institute 1998). Students *t*-test was conducted to test for significant differences in total number of progeny produced per female between uninfected females and females killed by fungal infection (verified by sporulating cadavers). The ANOVA was based on type III sum of squares, and *P* values <0.05 were considered statistically significant.

Life Table. To estimate the possible effect of the fungal treatment at population level, data of immature survival, adult survival, and female reproduction were used to make a cohort life table and fecundity schedule according to Birch (1948) and Begon et al. (1996). However, the comparison of net reproductive rates (R_0) may be misleading unless the generation times (T_c) are the same. Because the parasitoids in the two fungal treatments may differ in their mean length of generation time, the intrinsic rate of natural increase (r_m) was also computed.

The net reproductive rate, R_0, was calculated as the sum of the number of female progeny produced per original female parasitoid each day:

$$R_0 = \sum l_x \times m_x,$$

where l_x = the proportion of the original cohort being alive at age x, and m_x = progeny produced per original female during each day.

The true intrinsic rate of natural increase, r_m, was found by iteration of the equation:

$$\sum e^{-r_m x} \times l_x \times m_x = 1$$

and the true cohort generation time, T_c, was calculated as

$$T_{c\,\mathrm{true}} = R_0 / r_{m\,\mathrm{true}}$$

Results

Dose–Response Experiments. Female *S. cameroni* were susceptible to infection by *M. anisopliae*, which caused 68.19 ± 10.81% mortality at the highest con-

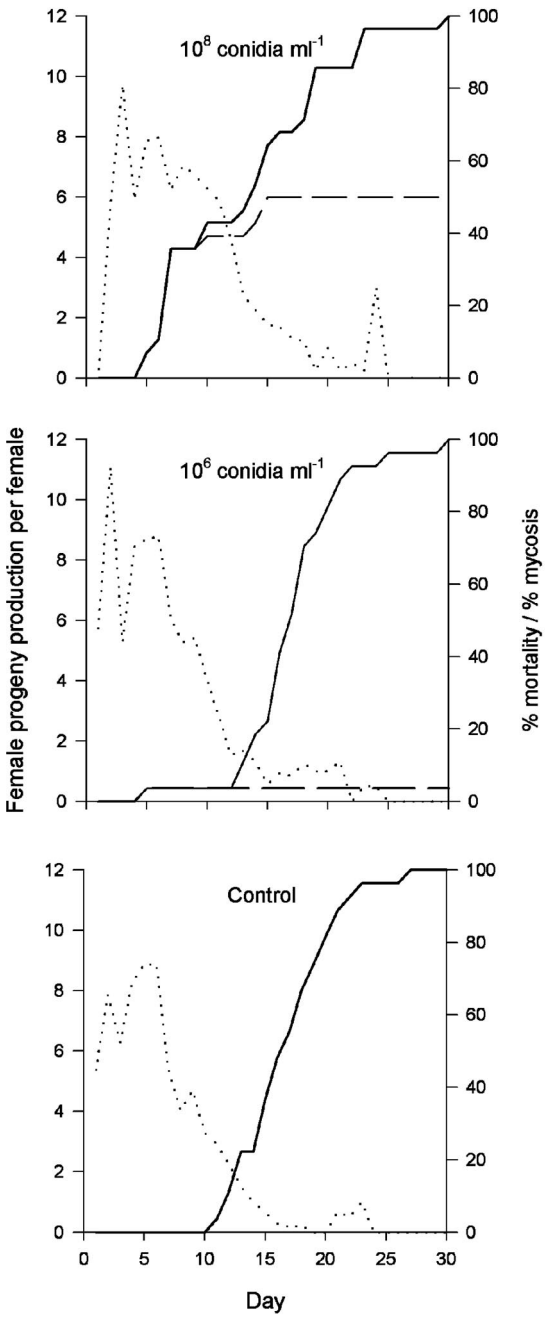

Fig. 2. Progeny production (·····), cumulated mortality (——), and cumulated mycosis (– – –) of female *S. cameroni* exposed to aqueous conidia suspensions of *M. anisopliae* or control treatment of 0.02% Tween 80 solution. The progeny production is depicted as age-specific female progeny production per surviving female. Day 1 on the x-axis corresponds to the day of adult parasitoid emergence and inoculation. Mortality of the parasitoids treated with 1×10^8 condia/ml differed significantly from the control and 1×10^6 condia concentration ($\chi^2 = 15.32$, $N = 82$, $P < 0.001$).

centration of 1×10^8 conidia/ml (Fig. 1). Although the lower concentrations had only a minor effect on mortality, there was a distinct greater response of the inoculated parasitoids at the highest concentrations (Fig. 1). The probit analysis of the overall moderate response (slope: 0.99 ± 0.09; $\chi^2 = 3.3958$, $P = 0.3345$) resulted in a LC_{50} value of 2.97×10^7 conidia/ml (95% CL: 2.19×10^7–4.16×10^7 conidia/ml).

Survival and Reproduction of Inoculated Female Parasitoids. As expected, there was an overall significant effect on survival with age of the female parasitoids ($\chi^2 = 222.42$, $N = 82$, $P < 0.001$), whereas only females exposed to 1×10^8 conidia concentration were found to have a significant lower expected survival ($\chi^2 = 15.32$, $N = 82$, $P < 0.001$) compared with the control and the 1×10^6 conidia/ml treatment (Fig. 2). Females exposed to conidia of *M. anisopliae* died from day 5 onward, whereas no control mortality was observed until day 11. All the parasitoids that died within the first 9 d had clear signs of mycosis, which provided a good indication of infection by *M. anisopliae.* In total, 50% of the females that had been treated with the high conidia concentration had clear signs of *M. anisopliae* infection, whereas only 4% produced sporulating cadavers at the low concentration (Fig. 2).

Female reproduction at both fungal treatments and the controls peaked on the second or third day of the experimental period and remained constantly high in the fourth to seventh day, although some fluctuations occurred between 7.5 and 12.0 progeny per female per day (Fig. 2). From day 7 onward, a pronounced decline in reproduction was recorded, especially for the low conidia treatment (1×10^6 conidia/ml) and the controls, whereas the number of progeny of females treated with 1×10^8 conidia/ml seemed to stabilize for a further 3 d at around six to seven individuals per day before it declined markedly as well (Fig. 2). For all treatments, the host feeding fluctuated between 2 and 10%, independent of the number of puparia parasitized (data not shown).

Despite the reduced survival of females exposed to a high concentration of conidia, the total reproduction per female was not affected by the fungal exposure ($F_{2,79} = 0.563$, $P = 0.572$, Fig. 3). Females treated with the high concentration of conidia consisted of 50% infected and 50% apparently uninfected individuals (Fig. 2), and the reproduction during the first 6 d of infected females (60.60 ± 6.68) was not significantly different from that of the uninfected females (63.79 ± 6.69; $t_{26} = -0.337$, $P = 0.739$, Fig. 4). Neither the total reproduction (up to day 25) of infected (69.74 ± 22.82) and uninfected females (102.79 ± 26.95) differed significantly when progeny production was compared ($t_{26} = -2.021$, $P = 0.054$, Fig. 4). Progeny production of infected females during the first 6 d made up 87% of the total progeny production, and reduction in reproduction from day 6 onward obviously was related with the high mortality 3–7 d after fungal exposure (Fig. 2).

Life Table. The life table parameters of reproduction rate, cohort generation time, and intrinsic rate of natural increase proved to be very similar for both

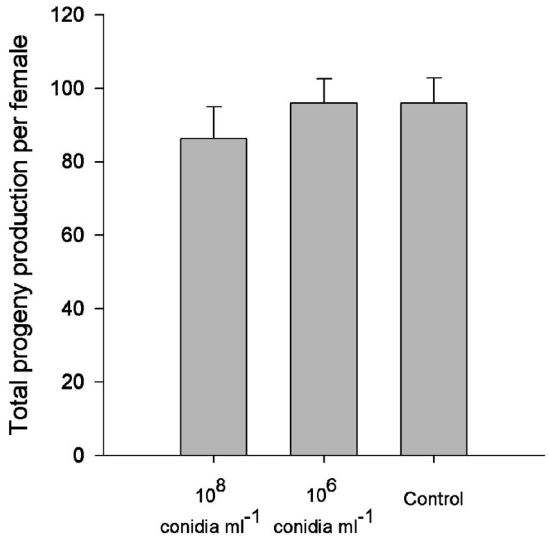

Fig. 3. Reproduction of *S. cameroni* females after exposure to *M. anisopliae*. Bars indicate the total number of progeny produced per female ($n = 82$). The total reproduction of the fungus treated females and control was not significantly different ($F_{2,79} = 0.563$, $P = 0.572$).

treatments and control (Table 1). The net reproductive rate (R_0) of the females treated with the highest conidia concentration ($R_0 = 65.72$) differed slightly from the values in the low conidia treatment ($R_0 = 75.66$) and control ($R_0 = 69.73$) because of the nonsignificantly lower total progeny production. The reduced survival at the high conidia concentration had little impact on the cohort generation time (T_c), because the age-specific progeny production during the first 10 d of the female parasitoid life constituted the largest contribution to the value of T_c. Similar values were found for the intrinsic rate of natural increase (r_m values: 0.1350, 0.1389, and 0.1364 of 1×10^8, 1×10^6 conidia/ml, and control, respectively), which strongly indicates that treatment with *M. anisopliae* has an insignificant effect on the population dynamics of *S. cameroni* in this study.

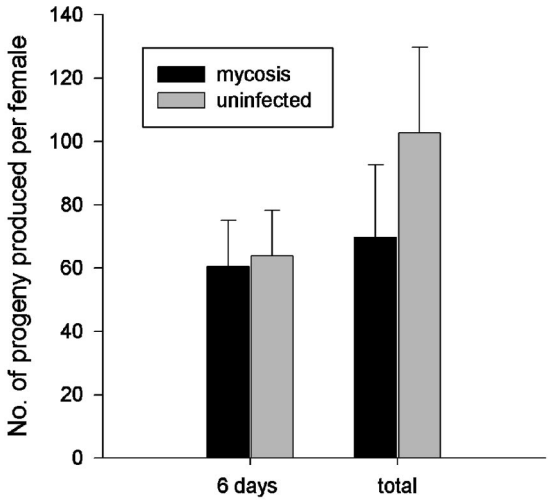

Fig. 4. Comparison of total progeny production by infected and uninfected *S. cameroni* females ($n = 28$) after exposure to the high concentration of conidia (1×10^8 condia/ml). The reproduction of the two groups was not significantly different either when compared on day 6 ($t_{26} = -0.337$, $P = 0.739$) or in total ($t_{26} = -2.021$, $P = 0.054$).

Discussion

The dose–response experiment showed that although *S. cameroni* was susceptible to infection by *M. anisopliae*, even the highest concentration of 1×10^8 conidia/ml, which approximates the maximum challenge, caused a mortality of only 68.19 ± 10.81% (Fig. 1). When the susceptibility of hymenopteran parasitoids to fungal infection has been studied in the laboratory, the parasitoids are often infected as readily as their hosts (Geden et al. 1995, Danfa and van der Valk 1999, de la Rosa et al. 2000). The isolate of *M. anisopliae* used in this study was previously tested against two hosts of *S. cameroni*. Larvae of the stable fly (*S. calcitrans*) were more susceptible than the parasitoid, whereas house fly larvae were less affected by the treatment (C. N., unpublished data). The moderate susceptibility of *S. cameroni* to fungal infection is in marked contrast with the results of Carswell et al. (1998), who found that adult house flies were highly susceptible to infection by an isolate of *M. anisopliae*.

In addition to mortality as a direct effect of fungal infection, decreased fecundity of the parasitoids may occur as a sublethal effect, but in this study, the overall progeny production was not affected, even though a significantly reduced survival was observed (Fig. 2). At the highest condia concentration, the total progeny production of infected and uninfected females was not significantly different, but the high reproductive rate of infected females may represent a sublethal effect of infection to compensate for their significantly reduced lifespan. Similarly, progeny production of fungus-infected females compared with those of the control group were also found in a study on *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) (Lacey et al. 1997). Females treated with the hyphomycetes *Paecilomyces fumosoroseus* (Wize) Brown and Smith showed no decrease in daily mummy production, but the mortality caused by the fungus resulted in more aphids parasitized by the untreated control females (Lacey et al. 1997). In contrast, decreased fecundity of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) females was observed within the first day of *Zoophthora radicans* (Zygomycetes: Entomophthorales) infection, although the parasitoids survived for 4 d (Furlong and Pell 2000). Sublethal effects on reproduction may be observed even before females start to produce progeny. In contrast to the absence of adverse effects of fungus infection concerning the fecundity of *S. cameroni*, house flies have been shown to produce fewer offspring when treated with *M. anisopliae* (Bywater et al. 1994). This, of course,

Table 1. Lifetable parameters of *S. cameroni* exposed to fungal treatment with *M. anisopliae*

Parameters	*M. anisopliae* (1×10^8 conidia/ml)	*M. anisopliae* (1×10^6 conidia/ml)	Control
Total no. progeny	2,345	2,500	2,486
No. female progeny	1,862	2,067	1,905
R_0 (females/female)	65.7180	75.6553	69.7258
$T_{c\ true}$ (days)	30.9936	31.1573	31.1192
$r_{m\ true}$ (day^{-1})	0.1350	0.1389	0.1364

Lifetable parameters refer to the net reproductive rate (R_0), cohort generation time (T_c), and the intrinsic rate of natural increase (r_m).

increases the potential of *M. anisopliae* for fly control.

Based on the life table analysis in this study, *S. cameroni* does not seem to have been affected by infection of *M. anisopliae* because the intrinsic rates of natural increase (r_m; 0.1350, 0.1389, and 0.1364 d^{-1}, Table 1) were virtually similar irrespective of the treatment. Although r_m is a theoretical value that expresses the population increase when the age distribution is stable and resources are unlimited (Birch 1948, Begon et al. 1996), it is probably the best parameter when the effects of different treatments of a species are to be compared. Changes in reproduction and survival of parental females caused by fungal infection may affect the value of r_m negatively, but the time of the changes is important as well. This was confirmed by testing the sensitivity of r_m, where the infection rate of the fungus treated females (1×10^8 conidia/ml) was increased from 50 to 100%. A pronounced decline in survival and reproduction from day 7 onward had no impact on the value of r_m, which was changed from 0.1350 to 0.1321 d^{-1}. However, if the death of the 100% infected females had occurred 3 d earlier, the value of r_m would have been reduced to 0.1137 d^{-1}. Thus, the reduced survival caused by a fungal infection only seems to be an important factor if the fecundity during the first days of female life is affected.

Female *S. cameroni* spend most of their time foraging for fly puparia in the manure matter of livestock installations, and it is difficult to asses the risk of parasitoid fungal infection by inundative releases of *M. anisopliae* conidia against immature stages or adult house flies and stable flies. Entomopathogenic fungi with broad host ranges in the laboratory are frequently far more specific under field conditions, where many natural enemies of the hosts seem refractory to fungal infection (Goettel et al. 1990, Brooks 1993). It seems unlikely that a significant proportion of the adult parasitoid population will become infected under field conditions when considering the moderate susceptibility of *S. cameroni*, notably if adult flies are the target. The nontarget effect of *B. bassiana* application on the parasitoid *M. raptor* was evaluated in a semifield experiment (Geden et al. 1995), and the susceptibility of the pupal parasitoid was found comparable with that of adult house flies. However, because the parasitoids spend relatively little time on indoor building surfaces where house flies aggregate (Geden et al. 1995), the risk of parasitoid infection by microbial control of adult flies seems even lower than when larvae in bedding material or manure are considered treated.

In conclusion, *M. anisopliae* was moderately virulent at high conidia concentrations to the parasitoid *S. cameroni*. When *S. cameroni* was provided with surplus number of fly puparia, no overall effect of fungal treatment on reproduction was found. The increased reproductive rate of fungus infected females may compensate for the reduced survival and thus represent a sublethal effect of fungus infection. Furthermore, the physiological susceptibility of the parasitoids to fungal treatment was not expressed in the life table parameter r_m, which seems to indicate that the isolate of *M. anisopliae* and *S. cameroni* are compatible under field conditions. In this study, the progeny production data were taken from a setting in which no search for hosts was involved, and perhaps infection would alter search ability. The experimental setup was simplified by optimum laboratory conditions and did not reflect the complexities of the multidimensional environment of a field situation, but inoculation on the day of emergence maximized the possible impact of infection on lifetime fecundity. In the field, the parasitoids may not acquire infections until later in their reproductive life and thus might be less affected by fungal exposure than these laboratory results would indicate.

Acknowledgments

The authors thank B. Nielsen and C. Dahl for technical assistance in the laboratory, L. Peschel and G. Jensen for rearing house fly puparia, and P. Holter (University of Copenhagen) and K.-M. Vagn-Jensen (Danish Pest Infestation Laboratory) for advice on statistical procedures.

References Cited

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

Barson, G., N. Renn, and A. F. Bywater. 1994. Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica*), a pest of intensive animal units. J. Invertebr. Pathol. 64: 107–113.

Begon, M., J. L. Harper, and C. R. Townsend. 1996. Ecology. Individuals, populations and communities. Blackwell, Oxford, UK.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15–26.

Brooks, W. M. 1993. Host-parasitoid-pathogeni, pp. 231–272. *In* N. E. Beckage, S. N. Thompson, and B. A. Federici (eds.), Parasites and pathogens of insects. Academic, San Diego, CA.

Bywater, A. F., G. Barson, and N. Renn. 1994. The potential of oil-based suspensions of *Metarhizium anisopliae*

conidia for the control of the housefly (*Musca domestica*), a pest of intensive animal units, pp. 1097–1102. *In* Proceedings of the Brighton Crop Protection Conference. British Crop Protection Council, Farnham, UK.

Carswell, I., R. Spooner-Hart, and R. J. Milner. 1998. Laboratory susceptibility of *Musca domestica* L. (Diptera: Muscidae) and *Bactrocera tyroni* (Frogatt) (Diptera: Tephritidae) to an isolate of *Metarhizium anisopliae* (Metsch.) Sorokin. Austr. J. Entomol. 37: 281–284.

Danfa, A., and H.C.H.G. van der Valk. 1999. Laboratory testing of *Metarhizium* spp. and *Beauveria bassiana* on Sahelian non-target arthropods. Biocontr. Sci. Technol. 9: 187–198.

de la Rosa, W., H. R. Segura, J. F. Barrrera, and T. Williams. 2000. Laboratory evaluation of the impact of entomopathogenic fungi on *Prorops nasuta* (Hymenoptera: Bethylidae), a parasitoid of the coffee berry borer. Environ. Entomol. 29: 126–131.

Furlong, M. J., and J. K. Pell. 1996. Interactions between the fungal entomopathogen *Zoophthora radicans* Brefeld (Entomophthorales) and two hymenopteran parasitoids attacking the diamondback moth, *Plutella xylostella* L. J. Invertebr. Pathol. 68: 15–21.

Furlong, M. J., and J. K. Pell. 2000. Conflicts between a fungal entomopathogen, *Zoophthora radicans*, and two larval parasitoids of the diamondback moth. J. Invertebr. Pathol. 76: 85–94.

Geden, C. J., D. A. Rutz, and D. C. Steinkraus. 1995. Virulence of different isolates and formulations of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor.* Biol. Control. 5: 615–621.

Goettel, M. S., J. D. Poprawski, J. D. Vandenberg, Z. Li, and D. W. Roberts. 1990. Safety to nontarget invertebrates of fungal biocontrol agents, pp. 209–231. *In* M. Laird, L. A. Lacey, and E. W. Davidson (eds.), Saftety of microbial insecticides. CRC, Boca Raton, FL.

Hajek, A. E., and R. J. St. Leger. 1994. Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39: 293–322.

Keiding, J. 1999. Review of the global status and recent development of insecticide resistance in field populations of the housefly, *Musca domestica* (Diptera: Muscidae). Bull. Entomol. Res. 89: S7–S67.

Lacey, L. A., A.L.M. Mesquita, G. Mercadier, R. Debire, D. J. Kazmer, and F. Leclant. 1997. Acute and sublethal activity of the entomopathogenic fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) on adult *Aphelinus asychis* (Hymenoptera: Aphelinidae). Environ. Entomol. 26: 1452–1460.

Lord, J. C. 2001. Response of the wasp *Cephalonomia tarsalis* (Hymenoptera: Bethylidae) to *Beauveria bassiana* (Hyphomycetes: Moniliales) as free conidia or infection in its host, the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Biol. Control. 21: 300–304.

Madelin, M. F. 1963. Diseases caused by hyphomycetous fungi, pp. 250–271. *In* E. A. Steinhaus (ed.), Insect pathology, vol. 2. Academic, New York.

Petersen, J. J. 1986. Evaluating the impact of pteromalid parasites on filth fly populations associated with confined livestock installations, pp. 52–56. *In* R. S. Patterson and D. A. Rutz (eds.), Biological control of muscoid flies, vol. 61. Entomological Society of America, Hyattsville, MD.

Renn, N., A. F. Bywater, and G. Barson. 1999. A bait formulated with *Metarhizium anisopliae* for the control of *Musca domestica* assessed in large-scale laboratory enclosures. J. Appl. Entomol. 123: 309–314.

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J. J. Marois, and B. A. Jaffee. 1995. Intraguild predation among biological-control agents: theory and evidence. Biol. Control. 5: 303–335.

SAS Institute. 1998. SAS/STAT user's guide. SAS Institute, Cary, NC.

Skovgård, H. 2004. Sustained releases of the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) for control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) in dairy cattle facilities. Biol. Control. 30: 288–297.

Skovgård, H., and J. B. Jespersen. 1999. Activity and relative abundance of hymenopterous parasitoids that attack puparia of *Musca domestica* and *Stomoxys calcitrans* on confined pig and cattle farms in Denmark. Bull. Entomol. Res. 89: 263–269.

Skovgård, H., and J. B. Jespersen. 2000. Seasonal and spatial activity of hymenopterous pupal parasitoids (Pteromalidae and Ichneumonidae) of the house fly on Danish pig and cattle farms. Environ. Entomol. 29: 630–637.

Skovgård, H., and G. Nachmann. 2004. Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative release of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 94: 555–567.

Steinkraus, D. C., C. J. Geden, D. A. Rutz, and J. P. Kramer. 1990. First report of the natural occurence of *Beauveria bassiana* (Moniliales: Moniliaceae) in *Musca domestica.* J. Med. Entomol. 27: 309–312.

Tanada, Y., and H. K. Kaya. 1993. Insect pathology. Academic, San Diego, CA.

Watson, D. W., C. J. Geden, S. J. Long, and D. A. Rutz. 1995. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). Biol. Control. 5: 405–411.

Watson, D. W., D. A. Rutz, and S. J. Long. 1996. *Beauveria bassiana* and sawdust bedding for the management of the house fly *Musca domestica* (Diptera: Muscidae) in calf hutches. Biol. Control. 7: 221–227.

Received 10 March 2004; accepted 21 October 2004.