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Effect of fungal infection on reproductive potential and survival time of *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract The effect of fungal infection on the reproductive potential of two-spotted spider mite, *Tetranychus urticae*, was evaluated as part of the full biocontrol potential of three entomopathogenic fungi by modeling of fecundity probability. Female mites (≤2-day-old) on leaves were exposed to the sprays of *Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Metarhizium anisopliae* at the concentrations of 1.13×10^3, 1.55×10^3 and 0.95×10^3 deposited conidia mm^{-2} and then individually reared at 25°C and 12:12 L:D for oviposition. Mite mortalities 10 days after spraying were 73.1, 75.4 and 67.9% in the fungal treatments versus 15.5% in control. On average, females infected by the three fungal species survived 5.8, 6.2 and 6.3 days, and laid 3.1, 4.0 and 4.0 eggs per capita, respectively. These were 3–4 fold lower than the control fecundity at 12.3. The cumulative probabilities $[P(m \leq N)]$ for the counts of infected and non-infected (control) females laying m eggs per capita ($m \leq N$) during 10 days fit very well the equation $P(m \leq N) = 1/[1 + \exp(a + bm)]$ ($r^2 \geq 0.98$), yielding a solution to the probability for the female mites to achieve a specific fecundity $\{P(m \leq N) - P[m \leq (N - 1)]\}$. Consequently, the infected mites had 71–78% chance to lay ≤5 eggs per capita but only 5–8% to deposit >10 eggs despite some variation among the tested fungi. In contrast, the chances for the non-infected mites to achieve the low and high fecundities were 23 and 55%. The fitted probabilities provide a full coverage of the fecundity potential of infected versus non-infected mites and are more informative than the mean fecundities.

Keywords Spider mites · *Beauveria bassiana* · *Paecilomyces fumosoroseus* · *Metarhizium anisopliae* · *Tetranychus urticae* · Mortality · Fecundity · Longevity

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Introduction

Spider mites are pests infesting a large number of crops worldwide (Hazan et al. 1974; Hill 1983; Ho et al. 1997). As long-term reliance of spider mite control on chemical acaricides has caused resistance and public concerns of residues in agroproducts (Herron et al. 1998; Guo et al. 1998; Meng et al. 2000; Zhao et al. 2001; He et al. 2003), new solutions have been sought. One of the suggested strategies is microbial control of spider mites by fungal agents (Chandler et al. 2000; Van der Geest et al. 2000; Maniania et al. 2008).

Beauveria bassiana (Balsamo) Vuellemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Paecilomyces fumosoroseus* (Wize) Brown & Smith are fungal biocontrol agents that have been widely applied for insect pest control (Feng et al. 1994; Faria and Wraight 2001; Roberts and St Leger 2004; De Faria and Wraight 2007). Some selected isolates of the insect pathogens have also proven potential for spider mite control. Sprays of *B. bassiana* and *M. anisopliae* formulations have resulted in significant control of spider mites on eggplants (Batta 2003), citrus (Shi and Feng 2006) and cotton (Shi et al. 2008a). The conspicuous efficacies in the field trials are partially supported by the results from laboratory bioassays. For instance, some of the tested isolates of *B. bassiana, M. anisopliae* or *P. fumosoroseus* are highly infective to the eggs and females of *Tetranychus cinnabarinus* (Boisduval) (Shi and Feng 2004; Shi et al. 2008b, c) and the active stages of *T. urticae* Koch (Alves et al. 2002) and *T. evansi* Baker & Pritchard (Wekesa et al. 2005, 2006). *T. cinnabarinus* is a synonym of *T. urticae* (Ros and Breeuwer 2007).

The effects of the fungal biocontrol agents against target pests are usually estimated as median lethal concentrations (LC_{50} s) and times (LT_{50} s) through multiple-dose bioassays on certain stages or instars. The virulence indices are useful for determining desired candidates from the tested isolates but do not reveal any effect of fungal infection on the reproductive potential of the pests. Such an effect, however, is part of the full potential of a fungal candidate and could be more implicative of the persistency of pest control in the field. Reduced fecundity caused by the infection of *B. bassiana* and *M. anisopliae* was observed in insects (Fargues et al. 1991; Ekesi and Maniania 2000), spider mites (Wekesa et al. 2006) and livestock ticks (Kaaya et al. 1996; Samish et al. 2001). However, the effects of the fungal pathogens on the reproductive potential of the pests have not been demonstrated more than by simple comparisons of their mean fecundities.

In this study, a probability model that was developed to assess postflight colonization potential of alate aphids infected preflight by Entomophthorales (Chen and Feng 2006; Feng et al. 2007) was introduced to evaluate the effects of selected isolates of *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* on the fecundity potential of spider mites. The two-spotted spider mite, *T. urticae*, was chosen as a representative species of the pest group because it infests a wide range of crops and horticultural plants (Hazan et al. 1974; Ho et al. 1997). The modeling method would reveal the biocontrol potential of the fungal agents more than routine virulence bioassays and provide deeper insights into the long-term effects of applied formulations on spider mite populations.

Materials and methods

Fungal isolates

The isolates of *B. bassiana* (Bb2860; derived from an infected aphid) and *M. anisopliae* (Ma759; host unknown) were provided by the Plant Protection Research Unit (US Plant,

Soil and Nutrition Laboratory, Ithaca, NY) and an isolate of *P. fumosoroseus* (Pfr116; derived from an infected whitefly) was obtained from the Beneficial Insects Research Unit (USDA-ARS Subtropical Agricultural Research Center, Weslaco, TX). These isolates were selected for their virulence against *T. urticae* eggs (LC_{50} s for Bb2860, Pfr116 and Ma759: 5.48×10^2, 8.48×10^2 and 17.17×10^2 conidia mm^{-2} leaf surface) and females (LC_{50} s for the three isolates: 1.77×10^2, 5.87×10^2 and 5.97×10^2 conidia mm^{-2}) (Shi and Feng 2004; Shi et al. 2008b).

Production of conidia and *Tetranychus urticae* females

Conidia of all three isolates were produced on steamed rice, harvested through a vibrating sieve and dried to a water content of ca. 5% at ambient temperature on a vacuum drier as described by Shi et al. (2008b). Dried conidia were sealed in glass tubes and stored immediately at 4°C for use within 5 months. Conidial viability exceeded 92% at the time of use.

A *T. urticae* culture was maintained on caged fava bean (*Vicia faba* L.) plants in a walk-in growth room at 23 ± 2°C and 12:12 L:D. Quiescent deutonymphs taken from the plants were gently transferred onto detached fava bean leaves on 90-mm-diameter petri dishes with 1.5% agar containing a rhizocaline at 25°C and 12:12 L:D for development into adults. Each of the leaves with hairy roots growing from the petiole into agar supported the mites for normal development (Shi et al. 2008b). Newly emerged females (≤2 days after last ecdysis) were then ready for fungal sprays after males were removed from the leaves.

Fecundity assays

Dry conidia of the isolates were suspended in 0.02% Tween-80 in water and diluted to the concentration of 1×10^8 conidia ml^{-1}. Each petri dish with leaves bearing ca. 25 females was placed on the specimen dish (11 cm diameter) of an Automatic Potter Spray Tower (Burkhard Scientific Ltd., Uxbridge, Middx, UK) and exposed to a spray of a 2-ml spore suspension or 0.02% Tween-80 as control from the top nozzle of the spray tower at a working pressure of 0.7 kg cm^{-2}. After exposure, females were individually transferred onto fresh leaves with hairy roots in 60-mm-diameter petri dishes (one female per leaf dish) and placed in an incubator at 25°C and 12:12 L:D for oviposition. The relative humidity inside each dish was merely sourced from the agar plate, likely exceeding 90% in the first 2 or 3 days but rarely having visible dew on the lid. Eggs laid by each female were counted daily under stereomicroscope at 25× magnification until it died from mycosis or laid no more eggs (usually by day 10). Six batches of females in petri dishes (ca. 25 females per dish) were sprayed with the spore suspensions of the tested isolates plus control within 5 months, including a total number of 156 females for Bb2860, 155 for Pfr116, 137 for Ma759 and 155 for the control. The mean (±SD) concentration of conidia deposited onto the leaves was 1.13 (±0.14) $\times 10^3$, 1.55 (±.22) $\times 10^3$ and 0.95 (±0.26) $\times 10^3$ conidia mm^{-2} for the three isolates, respectively. These estimates were based on five microscopic counts from each of glass cover slips (20 × 20 mm) that had been placed beside the leaf dish to collect deposited conidia during spraying (Shi et al. 2008c).

Data analysis

Female mites were categorized in the two groups of being mycosed or dead within 10 days after spraying and of surviving more than 10 days. The effect of treatment on fecundity

(no. eggs per female) and time length to death (no. days after treatment) was analyzed using one-factor analysis of variance.

To quantify the effect of the tested fungal isolates on the fecundity of the mite females, the number of the infected females (n_m) laying m eggs ($m = 0, 1, 2, \ldots, N$) during the survival period of j days after spraying ($j = 0, 1, 2, \ldots, 10$) was summed based on their egg deposits. The cumulative probability for their laying m eggs predeath was estimated as $P(m \leq N) = \sum_0^N p_m$ where $p_m = n_m/n$, $n = \sum_0^N n_m$. Those surviving the fungal treatments for more than 10 days were not included in the counts. For comparison, the counts of the control females producing m eggs per capita during the 10-day survival were also made for the estimates of $P(m \leq N)$. The relationship between m and $P(m \leq N)$ was then fitted to the logistic equation $P(m \leq N) = K/[1 + \exp(a + bm]$, where $K = 1$ due to $P(m \leq N) \leq 1$, a is an intercept for the fitted curve and b depicts a variability in fecundity among the concerned females (Chen and Feng 2006; Feng et al. 2007). The fitted equation for each of the fungal treatments or the control gave a solution to a specific probability $\left(\hat{P}_m\right)$ for the infected or non-infected females to lay m eggs after spraying, i.e., $\hat{p}_m = \hat{P}(m \leq N) - \hat{P}[m \leq (N-1)]$. The homogeneity between the counts of the females (n_m) observed to have laid m eggs and those computed $\left(\hat{n}_m = n\hat{P}_m\right)$ from the fitted equation was examined by a likelihood-ratio G test. An updated version of DPS software (Tang and Feng 2007) was used in all the analyses.

Results

Survival and fecundity of fungus-infected and non-infected females

Days to death and fecundity of *T. urticae* females are summarized in Table 1. The mite mortality observed was 73.1, 75.4 and 67.9% for Bb2860, Pfr116 and Ma759, respectively, whereas the control mortality was 15.5%. The control females that died within 10 days laid significantly more eggs prior to death ($F_{3,344} = 5.9$, $P < 0.01$) than those treated with fungi due to significantly longer surviving ($F_{3,344} = 6.82$, $P < 0.01$). The fecundity of females surviving more than 10 days after fungal treatment was also significantly lower than what was observed in the control ($F_{3,251} = 13.79$, $P < 0.01$). Neither days to death nor predeath fecundities differed significantly among the three fungal treatments (Tukey's HSD, $P > 0.05$). However, female mites not killed by mycosis on day 10 laid significantly

Table 1 Days to death and fecundity (no. eggs per capita) of *Tetranychus urticae* females after exposure to *Beauveria bassiana* (Bb2860), *Paecilomyces fumosoroseus* (Pfr116), *Metarhizium anisoplae* (Ma759) and blank control

Fungal isolates	Mean ± S.E.M.*				
	Females living ≤10 days			Females living >10 days	
	n	Days to death	Fecundity	n	Fecundity
Control	24	7.71 ± 0.32 a	7.38 ± 1.45 a	131	12.31 ± 0.74 a
Bb2860	114	5.82 ± 0.17 b	3.09 ± 0.35 b	42	6.33 ± 0.98 b
Pfr116	117	6.16 ± 0.17 b	3.97 ± 0.41 b	38	5.08 ± 0.76 b
Ma759	93	6.29 ± 0.21 b	4.02 ± 0.50 b	44	8.75 ± 1.35 b

* Means with different lowercase letters in each column differed significantly (Tukey's HSD, $P < 0.05$)

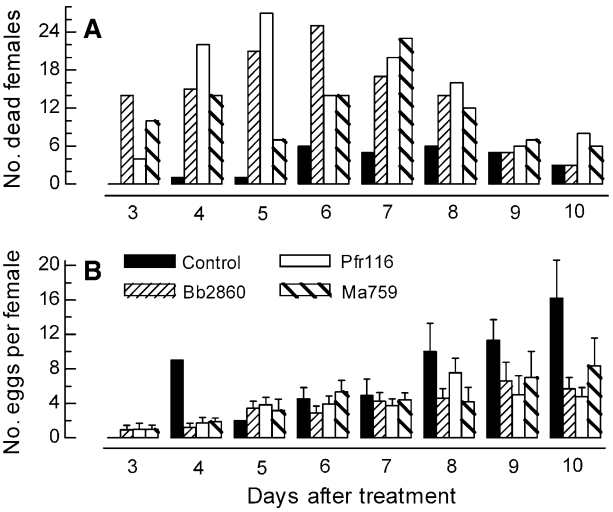

Fig. 1 Counts (n) and predeath fecundities (no. eggs per capita) of *Tetranychus urticae* females killed at different days after exposure to *Beauveria bassiana* (Bb2860), *Metarhizium anisopliae* (Ma759), *Paecilomyces fumosoroseus* (Pfr116) and control. *Error bars*: M.S.E

more eggs than those killed by the fungal treatments ($P < 0.01$ in Student's t tests) except Pfr116 ($t_{153} = 1.32$, $P = 0.19$).

Most of the infected females died from mycosis on days 3–8 (Fig. 1a). Mortality in the control females was not observed until day 4. Higher fecundities prior to death (Fig. 1b) were significantly correlated to a longer survival time (Bb2860: $r^2 = 0.88$, $F_{1,6} = 46.0$, $P < 0.01$; Pfr116: $r^2 = 0.56$, $F_{1,6} = 7.6$, $P = 0.03$; Ma759: $r^2 = 0.82$, $F_{1,6} = 28.2$, $P < 0.01$; control: $r^2 = 0.91$, $F_{1,6} = 42.5$, $P < 0.01$) based on the analysis of linear correlation weighted with the counts of dead mites on a given day.

Fecundity probability for infected and non-infected females

The cumulative probability [$P(m \leq N)$] of the infected or non-infected females laying $\leq m$ eggs per capita is illustrated in Fig. 2. The observations of m and $P(m \leq N)$ were fitted very well to the logistic equation (Fig. 2) with high coefficients of determination for all the treatments ($r^2 \geq 0.98$). In the likelihood-ratio G test for the goodness of fit (Fig. 3), no significant heterogeneity was found for either the infected females ($P \geq 0.35$) or the infection-free mites ($P = 0.18$).

Based on the fitted equations, the infection-free females were highly capable of laying more eggs than those infected. The fitted (vs. observed) probability for the fecundity of >10 eggs per capita within 10 days was 0.5501 (0.5496) for the control, 0.0484 (0.0702) for Bb2860, 0.0767 (0.1026) for Pfr116, and 0.0647 (0.0968) for Ma759. The probabilities for the fecundity of 6–10 eggs per capita were estimated as 0.2160 (0.2290), 0.1714 (0.1491), 0.2177 (0.1709) and 0.2157 (0.1505) for the control and the isolates Bb2860, Pfr116 and Ma759, respectively. In contrast, the females infected by Bb2860, Pfr116 and Ma759 had the high probabilities of 0.7802 (0.7807), 0.7056 (0.7265) and 0.7196 (0.7527) to lay ≤5 eggs per capita. These greatly exceeded the probability of 0.2317 (0.2214) for the same low fecundity among the infection-free females. In fact, the probability observed to

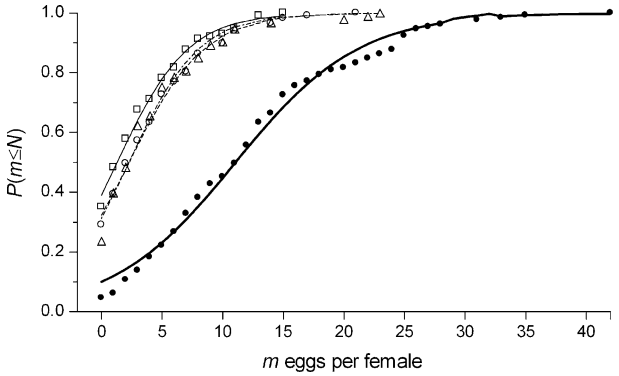

Fig. 2 The distribution of cumulative probability [$P(m \leq N)$] for a specific fecundity of *Tetranychus urticae* females (*m* eggs per capita) within 10 days after fungal infection. □, infected by *Beauveria bassiana* (Bb2860) and fitted to the *solid curve* $P(m \leq N) = 1/[1 + \exp(0.4447–0.3423\ m)]$ ($r^2 = 0.991$, $F_{1,12} = 1340$, $P < 0.0001$). △, infected by *Metarhizium anisopliae* (Ma759) and fitted to the *dot-dash curve* $P(m \leq N) = 1/[1 + \exp(0.7860–0.3457\ m)]$ ($r^2 = 0.981$, $F_{1,14} = 738$, $P < 0.0001$). ◯, infected by *Paecilomyces fumosoroseus* (Pfr116) and fitted to the *dash curve* $P(m \leq N) = 1/[1 + \exp(0.7403 - 0.3229\ m)]$ ($r^2 = 0.996$, $F_{1,13} = 2{,}965$, $P < 0.0001$). ●, non-infected (control) and fitted to the *bold solid curve* $P(m \leq N) = 1/[1 + \exp(2.1872–0.1977\ m)]$ ($r^2 = 0.991$, $F_{1,31} = 3{,}560$, $P < 0.0001$)

produce no eggs was considerably high among the females infected by Bb2860 (0.3509), Pfr116 (0.2906) or Ma759 (0.2366) but very low in the control (0.0458).

Discussion

As shown above, the infection of *B. bassiana*, *P. fumosoroseus* and *M. anisopliae* not only killed *T. urticae* females but greatly reduced their fecundity. The fitted probabilities indicate that the infected females had 71–78% chance to lay ≤5 eggs per capita but only 5–8% to achieve a fecundity of more than 10 eggs per capita despite some variation among the tested fungal agents. In contrast, the chances for the infection-free females to lay ≤5 and >10 eggs per capita are 23 and 55%, respectively.

Noticeably, small portions of the female mites exposed to fungi were not mycosed during the 10-day period of observation. However, their fecundity was significantly reduced, compared to the control, but higher than those observed from the females mycosed within 10 days. Those mites might have been subjected to sublethal infections, which perhaps attributed to a variation in random deposition of the conidia sprayed in the tower. This is well in accordance with the significant effect of a sublethal concentration of *B. bassiana* and *M. anisopliae* on the reproductive potential of *T. evansi* females (Wekesa et al. 2006).

The effect of fungal infection on the fecundity of pests is a solid contribution to the full potential of fungal biocontrol agents and is often revealed by fecundity differences between infected and non-infected insects (Fargues et al. 1991), mites (Wekesa et al. 2006) and ticks (Kaaya et al. 1996; Samish et al. 2001). Comparison of the differences, such as the results in Table 1, is straightforward but not sufficient to take an overview to the reproductive potential of the infected females because of a large variation in their survival duration and fecundity, as illustrated in Fig. 1. The fitted probabilities for the infected versus non-infected female mites to achieve specific fecundities provide precisely a full

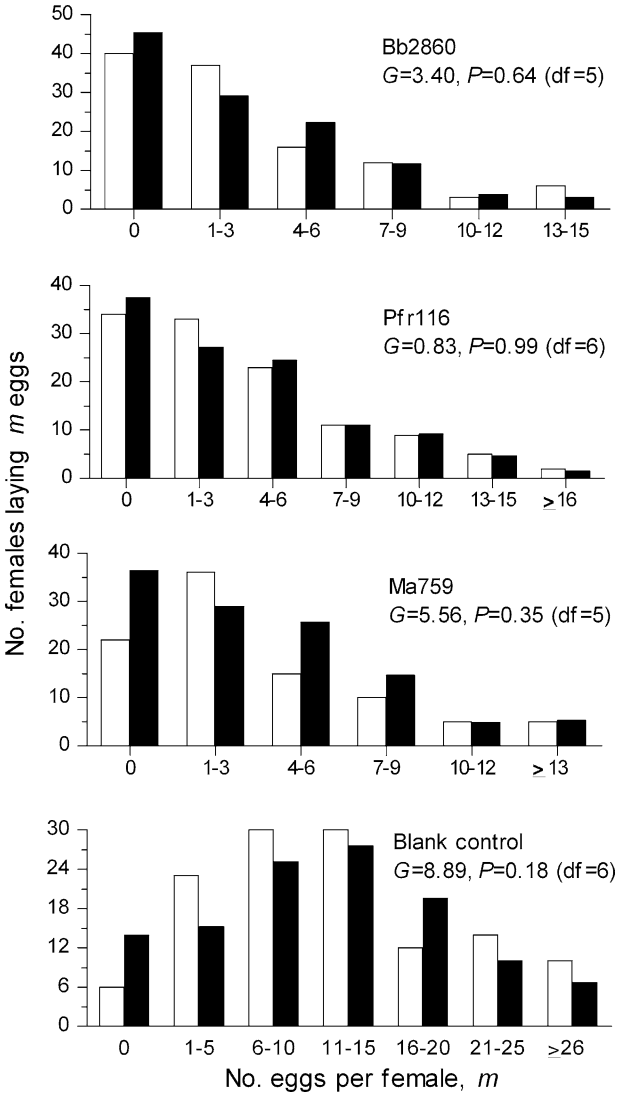

Fig. 3 The likelihood-ratio G test for the homogeneity between the observed (*white bars*: n_m) and fitted (*black bars*: $\hat{n}_m$) counts of *Tetranychus urticae* females achieving a specific fecundity (m eggs per capita) within 10 days after infection by *Beauveria bassiana* (Bb2860), *Metarhizium anisoplae* (Ma759) and *Paecilomyces fumosoroseus* (Pfr116). Control: non-infected

coverage of their fecundity potential and are more informative than the mean fecundities. Thus, the modeling analysis of the fecundity probability might be an interesting method for evaluating the effect of the fungal infection on pest fecundity.

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