Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbst20

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Available online: 21 Apr 2010

To cite this article: Fucai Zhou, Shaukat Ali & Zhen Huang (2010): Influence of the entomopathogenic fungus *Isaria fumosorosea* on *Axinoscymnus cardilobus* (Coleoptera: Coccinellidae) under laboratory conditions, Biocontrol Science and Technology, 20:7, 709-722

To link to this article: http://dx.doi.org/10.1080/09583151003714083

Biocontrol Science and Technology,
Vol. 20, No. 7, 2010, 709–722

Taylor & Francis
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RESEARCH ARTICLE

Influence of the entomopathogenic fungus *Isaria fumosorosea* on *Axinoscymnus cardilobus* (Coleoptera: Coccinellidae) under laboratory conditions

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(*Received 7 July 2009; returned 6 August 2009; accepted 18 February 2010*)

Effects of entomopathogenic fungus *Isaria fumosorosea* on biological characteristics and life table parameters of *Axinoscymnus cardilobus*, a predator of whiteflies, were studied using five different conidial concentrations under laboratory conditions. Non-significant differences were observed among different fungal concentrations on the percent survival of all immature stages. The survival of *A. cardilobus* on different host plants did not differ significantly. The prey consumption of third instar *A. cardilobus* on *Bemisia tabaci* treated with different fungal concentrations differed significantly at different time intervals. The developmental periods for all immature stages (from eggs, first, second, third, fourth instar nymph and pupae up to emergence) among the treatments were not statistically significant when compared to that of control. In the present study, fecundity, longevity, egg viability and life table parameters of females were not statistically significant over the different concentrations. It can be concluded that control strategies tested are compatible to a greater extent and incorporation of these have promising prospects for control of whitefly.

Keywords: *Isaria fumosorosea*; *Axinoscymnus cardilobus*; biological characteristics; life table

Introduction

Bemisia tabaci (Homoptera: Aleyrodidae) is a serious pest in tropical and subtropical regions worldwide (Brown 1994; Oliveira, Henneberry, and Anderson 2001), and can be infected by several entomopathogenic fungi (Lacey, Fransen, and Carruthers 1996; Faria and Wraight 2001). Of these fungal species known to infect whiteflies, *Isaria fumosorosea* (*Paecilomyces fumosoroseus* designated as *Isaria fumosorosea*, Zimmermann 2008) is the most promising entomopathogen (Vidal, Lacey, and Fargues 1997). This fungas has been used as a mycopesticide for whitefly management in the United States, Europe and China both in greenhouse as well as in the open environment (Horowitz and Ishaaya 1996; Sterk, Bolckmans, and Eyal 1996; Vidal et al. 1997; Wraight, Carruthers, Bradley, Garza, and Galani-Wraight 2000; Faria and Wraight 2001; Huang and Ren 2004; Huang, Ren, and Wu 2006c, 2007).

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ISSN 0958-3157 print/ISSN 1360-0478 online

DOI: 10.1080/09583151003714083
http://www.informaworld.com

Whiteflies are also attacked by a variety of predatory arthropods (Nordlund and Legaspi 1996; Gerling, Alomar, and Arno 2001). In China, many coccinellid predators have been used for biological control of whitefly (Ren, Huang, and Yao 2004). Studies on the morphology, life habits, life history and predation of *A. cardilobus*, a coccinellid predator belonging to tribe *Axinoscymnus* (Coleoptera: Coccinellidae), indicate that it is the most effective predator in the field as well as under laboratory conditions (Ren and Pang 1992; Huang, Ren, and Yao 2003, 2006a,b; Huang, Ren, and Musa 2008). *A. cardilobus* larvae can consume 44–195 whitefly eggs or 2–147 nymphs in 24 h depending on the larval stage (Huang et al. 2003, 2006a, b). This predator is a voracious feeder of all stages of whitefly and has a great potential as an effective biological agent against *B. tabaci*.

Currently, there is an incomplete understanding of potential effects of microbial insecticides on non-target invertebrates in natural systems. Predicting the ecological host range (as opposed to physiological host range) is particularly more difficult with self perpetuating organisms that function at the tertiary trophic level, such as entomopathogens and predatory insects. Mycoses in nature have been observed in a number of predatory insects (Goettel, Inglis, and Wraight 2000); however little is known about their epizootiology and resultant effects on predators and other non-target species. Many species of predatory insects seem refractory to fungal infection when challenged under laboratory conditions (Poprawski, Legaspi, and Parker 1998).

The effects of entomopathogenic fungi on coccinellids have been studied under laboratory conditions primarily aimed at mortality and development and at developing a standardized bioassay protocol (Lacey 1997). However, other detrimental effects may also inhibit the beneficial capacity of non-target natural enemies of pests. Although, many reports are available on the short-term detrimental effects of entomopathogenic fungi on non-target organisms (Goettel, Poprawski, Vandenberg, Li, and Roberts 1990), reports on indirect effects are very few.

The goals of this study were to evaluate the impact of *I. fumosorosea* on the survival of immatures, fecundity, reproduction and egg viability of newly emerged adults of *A. cardilobus* in order to determine their compatibility in management of whitefly populations.

Materials and methods

Bemisia tabaci, Trialeurodes vaporariorum *and* Axinoscymnus cardilobus

Bemisia tabaci, *Trialeurodes vaporariorum* Westwood and *A. cardilobus* were obtained from stock colonies kept in a greenhouse at the Engineering Research Center of Biological Control, South China Agricultural University (SCAU) on *Euphorbia pulcherrima* Willd (poinsettia). Plants of *Solanum melongena* L., *E. pulcherrima* and *Codiaeum variegatum* L. were grown in plastic pots (15 cm diameter). Sufficient slow release fertilizer (N:P:K = 13:7:15) was added as required to maintain normal plant growth. Intact plants were maintained in another greenhouse. In these studies *A. cardilobus*, *T. vaporariorum* and *B. tabaci* used were moved into a room maintained at $26 \pm 2^{\circ}$C, RH 80 ~ 90%, 14 h L:10 h D, after being maintained on host plants for several generations. A large number of either *B. tabaci* or *T. vaporariorum* adults were placed into the plastic bags with small holes caused by needle punctures. The leaves of poinsettia were fixed in plastic bags. Whitefly adults

were taken out after 6 h of egg laying. Nymphs of whiteflies were kept on host plants in an air-conditioned room as previously described. When nymphs of whiteflies entered into the second instar, 100 nymphs per leaf were reserved for treatment.

Isaria fumosorosea

Strain PF01-N4 of *I. fumosorosea* was used in all assays. It was originally isolated from a *B. tabaci* nymph (Huang and Ren 2004), maintained in tubes containing Sabouraud Dextrose Agar (SDA) and deposited at the Engineering Research Center of Biological Control, South China Agricultural University. It was cultured on potato dextrose agar (PDA) and incubated at $26 \pm 2^{\circ}$C for 10 days. Conidia were harvested with deionized water containing 0.02% Tween 80 (Weiga Chemicals, Guangzhou, China) and sieved through filter paper into sterile vials. Conidia were counted using a compound microscope and a hemocytometer (0.0625 mm^2; Fuchs-Rosenthal Merch Eurolab) and calibrated to the highest concentration of 1×10^8 conidia/mL of *I. fumosorosea.* Lower concentrations of 1×10^7 to 1×10^4 and 0 conidia/mL were prepared by serial dilutions, and adjusted by counting as described above.

Spore viability was determined before suspension preparation by spreading 0.2 mL of 1×10^4 conidia/mL on PDA and estimating the number of germinated propagules after 24 h at room temperature. Propagules were considered viable when the germ tube lengths corresponded to the width. The viability of conidia was assessed immediately before each experiment and percentage germination was estimated at $>95\%$ for all experiments.

LC_{50} of Isaria fumosorosea *on* Bemisia tabaci *immatures*

Six different concentrations of *I. fumosorosea* (0, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia/mL) were prepared as described above. The newly molted second instar nymphs of *B. tabaci* were treated by dipping infested leaves (not excised leaves) into each of the six concentrations of *I. fumosorosea* (new suspensions) for 15 s and removing to air dry before transferring to the cages ($60 \times 60 \times 60$ cm) along with the plant. Water was added to the bottom of the cage daily to maintain high relative humidity. Each treatment (each concentration) was repeated three times with a new batch of insects and fresh conidial suspensions. For each repetition there were four leaves with 100 whitefly nymphs per leaf. All treatments were done at one time, using randomized groups of insects from a single batch.

The insects were placed in a temperature controlled room as previously described and monitored daily until adult emergence. Mortalities of *B. tabaci* were recorded after 6 and 12 days of treatment. To determine the infective mortalities, the cadavers were taken out and separately incubated at $26 \pm 2^{\circ}$C and $>95\%$ RH to encourage sporulation. If sporulation of *I. fumosorosea* was observed on a cadaver, the cadaver was considered as having been infected by *I. fumosorosea.*

Influence of Isaria fumosorosea *on the survival of immatures of* Axinoscymnus cardilobus

The different life stages of *A. cardilobus* (eggs, first, second, third, and fourth instars, and pupae) with poinsettia leaves having eggs and immatures of *B. tabaci* and

T. vaporariorum as food, were directly dipped into the prepared suspensions (0, 1×10^4, 1×10^8 conidia/mL) for 15 s and then placed on filter paper. To maintain nearly saturated humidity, the lids of the Petri dishes were closed with parafilm and incubated for 24 h at 26 ± 2°C and 14 h L:10 h D. Following the initial 24 h, the *A. cardilobus* were transferred to new Petri dishes with insect infested poinsettia leaves as food and kept in a temperature controlled room as previously described. The Petri dishes were covered with plastic film with small holes for aeration. Similar experiments were carried out with *A. cardilobus* on *Solanum melongena* L., *Euphorbia pulcherrima* Willd and *Codiaeum variegatum* L. leaves with eggs and immatures of *B. tabaci* as food.

Mortality of beetles was recorded at 24-h intervals until adult emergence. Dead larvae were sterilized with 2% sodium hypochlorite for 1 min and were placed on filter paper. After air drying, dead insects were placed on PDA media and incubated in a temperature controlled room. Beetles from which mycelia and conidia of *I. fumosorosea* were observed were considered to have died of mycosis. For each conidial concentration, 40 individuals of every beetle life stage were used and the entire experiment was repeated 5 times.

Prey selection of Axinoscymnus cardilobus *larvae on* Bemisia tabaci *nymph treated with* Isaria fumosorosea

Leaves with 800 eggs or 200 third instar nymphs of well dispersed *B. tabaci* were directly dipped into the fungal suspensions (0 and 1×10^8 conidia/mL) for 15 s and then placed on filter paper. To maintain nearly saturated humidity, the lids of the Petri dishes were sealed with parafilm and were incubated for 24 h at 26 ± 2°C and 14 h L:10 h D. After 0, 1, 2 and 3 days, the treated *B. tabaci* nymphs were supplied as food to third instar nymphs of *A. cardilobus*, kept in a temperature controlled room as previously described. No food was supplied to third instar larvae of *A. cardilobus* within 12 h before the treatments. The Petri dishes were covered with plastic film with small holes for aeration. After 8 h, the number of prey, eggs and third instars nymphs of *B. tabaci* preyed were recorded. Preyed eggs were identified by presence of the egg pedicel. Preyed nymphs were identified by the presence of integument or punctured cadavers. Prey ratio was calculated as the number of nymphs preyed divided by the number of total nymphs. For each conidial concentration, 40 beetles were used and the entire experiment was repeated 5 times.

Influence of Isaria fumosorosea *on the development of immatures of* Axinoscymnus cardilobus

The different life stages of *A. cardilobus* (eggs, first, second, third, fourth instar larvae and pupae) with poinsettia leaves having eggs and immatures of *B. tabaci* as food were directly dipped into the prepared suspensions (0, 1×10^4, 1×10^8 conidia/mL) for 15 s and then were taken out by placing on filter paper. To maintain near saturated humidity, the lids of the Petri dishes were sealed with strip of parafilm and were incubated for 24 h at 26 ± 2°C and 14 h L:10 h D. Following the initial 24 h in humidity, the individuals of *A. cardilobus* were removed to new Petri dishes with poinsettia leaves having eggs and immatures of *B. tabaci* as food and kept in a temperature controlled room as previously described. The Petri dishes were covered

with plastic with small holes for aeration. The egg hatchability and developmental time of each stage until the next molt was also recorded. For each conidial concentration, 40 individuals of every life stage of the beetles were used and the entire experiment was repeated 5 times.

Fecundity, adult longevity and egg viability of newly emerged females

Newly emerged adults from the immature treatment, fed on *B. tabaci* as prey and treated with either of three different conidial concentrations (0, 1×10^4 and 1×10^8 conidia/mL) were separated in a small cage and allowed to mate for about 5 h, 4 days later. The mating adult pairs of beetles from each concentration were dipped into either of three different conidial concentrations (0, 1×10^4 and 1×10^8 conidia/mL) of *I. fumosorosea* for 15 s. The adults were treated with the same spore concentration as in the treatment from which they emerged. After being maintained in nearly saturated humidity for 24 h as previously described, the beetles were then transferred to plastic Petri dishes with poinsettia plant leaves. The Petri dishes were kept in a temperature controlled room as previously described. The leaves were changed daily and the numbers of eggs laid by each pair were recorded until adult death. For each conidial concentration, six pairs of beetles were used against each conidial concentration and the entire experiment was repeated 5 times.

Eggs laid by newly emerged adults from the immature treatment, fed on *B. tabaci* as prey and treated with either of three different conidial concentrations (0, 1×10^4 and 1×10^8 conidia/mL) in less than 12 h were taken out. Once the egg batches were obtained, 100 eggs from each treatment were selected at random. The eggs were placed in a temperature controlled room as previously described. The number of eggs enclosed and first instar larvae emerged were counted after 5 days and comparisons were made between treatment groups and their respective controls. The viability of beetle eggs descended from *I. fumosorosea* treated insects was calculated as the percentage emergence, i.e., the number of first instar larvae emerging divided by total number of eggs.

Life table analysis

A cohort of X eggs laid within 24 h were taken out and monitored until the appearance of adults in a temperature controlled room as described above. According to the survival rate of beetles treated with three different conidial concentrations (0, 1×10^4 and 1×10^8 conidia/mL) from the experiment on *B. tabaci* as prey, the number of X eggs in each concentration was 100 eggs for beetle, providing the information for age-specific survivorship of the larvae. All newly emerged adults were then monitored daily for age-specific fecundity and survival. The sex ratios of all newly emerged adults from the cohort of X eggs were determined after the death of the adults. The life table parameters were computed according to Birch (1948):

$$R_0 = \sum l_x m_x,$$

$$T = 1/_{R0} \sum \mathrm{X} l_x m_x,$$

$$r_m = 1/_T \ln R_0,$$
$$\lambda = \exp(r_m),$$

X is the beetle age in days, L_x is the survivorship at the corresponding time, M_x is the number of female eggs laid according to sex ratio laid per female per day. R_0 is the net productive rate, r_m is the intrinsic rate of increase, T is the mean generation time, λ is the finite rate of increase.

Data analysis

The curves of LC-p were calculated and tested by chi-square test, LC_{50} and their confidence intervals were calculated by probit analysis using SPSS (Statistical Package for Social Science in personal computers) 8.0 for windows (SPSS 1997).

The duration of developmental period, the percentage of immature survival and egg viability were subjected to square root arcsin transformation prior to computation. The duration of oviposition, longevity and fecundity of the beetles treated with fungal conidial suspensions were compared using Analysis of Variance (ANOVA). The difference between the means among the different concentrations were compared using Tukey's Studentized range test (HSDT $P = 0.05$). All analyses were done using SAS program (SAS 2000).

Results

LC_{50} of Isaria fumosorosea *on* Bemisia tabaci *immatures*

The mean mortalities for second instars of *B. tabaci* treated with the five conidial concentrations were 12, 31, 41, 55, 62 and 74% after 6 days and 19, 58, 70, 80, 93 and 95% after 12 days, for the 0, 10^4, 10^5, 10^6, 10^7 and 10^8 conidial treatments, respectively. Based on the above initial mortality data, the concentration–mortality response regression analysis for *I. fumosorosea* was calculated by assaying six concentrations against newly molted *B. tabaci* nymphs or larvae (Table 1). The LC_{50} values of *I. fumosorosea* against *B. tabaci* at different time intervals are presented in Table 1. There was a decrease in LC_{50} values with the passage of time after the fungal treatment.

Influence of Isaria fumosorosea *on the survival of* Axinoscymnus cardilobus

The percent survival of each stage (eggs, first, second, third, fourth instar larvae, and pupae) to adult emergence was not significantly different between the two insect species treated with the different concentrations of fungi (Table 2). Similarly the differences in survival on the different host plants treated with two different concentrations were also

Table 1. Regression analysis of probit mortality and log-concentration data of bioassay with *Isaria fumosorosea* against *Bemisia tabaci*.

Insect	Days	Slope (M ± SE)	x^2	LC_{50} value (95%FL)
Bemisia tabaci	6	0.33 ± 0.03	0.81	2.16×10^6 (9.4×10^5–5.0×10^6)
	12	0.42 ± 0.10	0.10	1.44×10^4 (4.5×10^3–4.6×10^4)

Table 2. Percentage survival (Mean ± SE) of *Axinoscymnus cardilobus* immature stages on different insects treated with three concentrations of *Isaria fumosorosea.*

Prey	Treatments (conidia/mL)	Egg	1st instar	2nd instar	3rd instar	4th instar	Pupa
B. tabaci	0	72.5 A (±0.02)	73.6 A (±3.85)	90.0 A (±0.00)	90.0 A (±0.00)	90.0 A (±0.00)	70.6 A (±3.03)
	1×10^4	71.6 A (±5.74)	72.5 A (±4.02)	84.3 A (±3.03)	90.0 A (±0.00)	90.0 A (±0.00)	70.6 A (±2.02)
	1×10^8	71.2 A (±4.85)	70.1 A (±2.98)	81.3 A (±2.49)	84.0 A (±0.00)	83.7 A (±3.80)	70.6 A (±1.83)
T. vaporariorum	0	71.6 A (±1.84)	73.0 A (±2.36)	84.3 A (±2.03)	90.0 A (±0.00)	90.0 A (±0.00)	69.7 A (±2.15)
	1×10^4	71.6 A (±1.78)	72.5 A (±2.72)	81.7 A (±1.93)	90.0 A (±0.00)	90.0 A (±0.00)	69.7 A (±2.54)
	1×10^8	71.6 A (±3.55)	71.6 A (±2.31)	80.0 A (±2.16)	84.3 A (±2.09)	84.3 A (±2.18)	69.7 A (±1.88)
	df, F, P	5, 2.74, 0.5345	5, 3.51, 0.6324	5, 3.17, 0.5292	5, 2.36, 0.4024	5, 2.01, 0.6091	5, 3.45, 0.5051

Data on means (±SE) percentage survival were subjected to square root arcsin transformation prior to computation and compared by one way ANOVA, numbers within the same column followed by the same letter are not significantly different (Tukey's Studentized range test, $P = 0.05$).

not significant except the percent survival of third and fourth larval instar to emergence (Table 3) and a possible reason for this difference can be the nature of host plants used rather than the use of different fungal concentration.

Prey selection of Axinoscymnus cardilobus *larvae on* Bemisia tabaci *nymph treated with* Isaria fumosorosea

There were no significant differences of consumption by third instars of *A. cardilobus* preying on eggs of *B. tabaci* treated by fungi (Table 4). Beetle consumption differed significantly on third instar nymphs of *B. tabaci* with the passage of time after fungal treatment. There was no prey selection by third instars of *A. cardilobus* preying on eggs of *B. tabaci*, but it was reversed when preying on third instars of *B. tabaci*. Punctures were found in only five cadavers with the body contents untouched. However, 31 empty integuments were found in 0-day treatment. We conclude that beetle larva avoid preying on *B. tabaci* nymphs that have been infected by the fungus.

Influence of Isaria fumosorosea *on the development of* Axinoscymnus cardilobus

The developmental periods of egg to adult emergence at different conidial concentrations (0, 1×10^4, 1×10^8 conidia/mL) were not statistically significant, with developmental periods ranging from 18.5 ± 1.79 days in the 0 treatment to 19.1 ± 1.78 days in the 1×10^8 conidia/mL treatment ($F = 0.34$; df = 5; $P = 0.9812$). The differences in developmental periods for the larval stages at the different concentrations were not statistically significant ($F = 0.51$; df = 5; $P = 0.8794$ for first larval instar; $F = 0.48$; df = 5; $P = 0.9163$ for second instar; $F = 0.72$; df = 5; $P = 0.9051$ for third instar and $F = 0.37$; df = 5; $P = 0.9051$ for fourth instar). The longest developmental periods for different larval instars (15.3, 14.1, 12.1 and 7.9 days for first, second, third and fourth instar, respectively) were observed in the 1×10^8 conidia/mL treatment, whereas the shortest developmental period for all the larval instars were observed in the 0 treatment with mean values of 14.6, 13.1, 11.3 and 10.1 days for first, second, third and fourth instars, respectively. Differences in developmental periods of pupa to adult emergence at the different concentrations were not statistically significant, with the longest developmental period of 7.9 ± 1.10 days for the 1×10^8 conidia/mL treatment and 7.5 ± 1.14 days in the 0 treatment ($F = 0.46$; df = 5; $P = 0.8986$).

Fecundity, adult longevity and egg viability of newly emerged female

There were no significant differences in the fecundity of females among the treatments. The number of eggs ranged from 133.2 ± 32.18 laid by the 0 treatment beetles, to 121.6 ± 30.41 eggs/female in the 1×10^8 conidia/mL treatment ($F = 1.73$; df = 2; $P = 0.8974$). Longevity of adult females treated with different conidial concentrations (1×10^4, 1×10^8 conidia/mL) did not vary significantly with the shortest longevity of 62.4 ± 6.83 days for the 1×10^8 conidia/mL treatment and 71.7 ± 8.45 days in the 0 treatment ($F = 2.05$; df = 2; $P = 0.2102$). Differences in the egg viabilities of the newly emerged adults were not statistically significant among the different treatments, with viabilities ranging from 7.8 ± 1.31 in the 1×10^8 conidia/mL treatment to 8.0 ± 1.64 in the 0 treatment ($F = 2.05$; df = 2; $P = 0.2102$).

Table 3. Percentage survival (Mean ± SE) of *Axinoscymnus cardilobus* immature stages on different plants having *Bemisia tabaci* treated with *Isaria fumosorosea.*

Host plant	Treatments (conidia/mL)	Egg	1st instar	2nd instar	3rd instar	4th instar	Pupa
Solanum melongena L.	1×10^8	66.0 A (±0.13)	68.0 A (±2.18)	73.6 A (±1.02)	69.7 B (±2.03)	64.2 B (±1.59)	63.4 A (±2.11)
	0	67.2 A (±3.14)	68.9 A (±2.72)	75.8 A (±1.73)	70.6 B (±2.04)	69.3 B (±1.15)	64.2 A (±1.82)
Codiaeum variegatum L.	1×10^8	71.6 A (±2.32)	71.6 A (±1.82)	80.0 A (±2.05)	84.2 A (±1.83)	90.0 A (±2.14)	67.7 A (±2.01)
	0	71.6 A (±2.14)	72.5 A (±1.41)	81.9 A (±2.05)	90.0 A (±0.00)	90.0 A (±0.00)	68.9 A (±2.42)
Euphorbia pulcherrima Willd	1×10^8	71.2 A (±4.85)	70.1 A (±2.98)	81.3 A (±2.49)	84.0 A (±0.00)	83.7 A (±3.80)	70.6 A (±1.83)
	0	72.5 A (±0.02)	73.6 A (±3.85)	90.0 A (±0.00)	90.0 A (±0.00)	90.0 A (±0.00)	70.6 A (±3.03)
	df, *F*, *P*	5, 3.01, 0. 4015	5, 1.91, 0.3247	5, 2.89, 0.0529	5, 32.17, 0.0008	5, 48.16, <0.0001	5, 2.12, 0.6356

Data on means (±SE) percentage survival were subjected to square root arcsin transformation prior to computation and compared by one way ANOVA, numbers within the same column followed by the same letter are not significantly different (Tukey's Studentized range test, $P = 0.05$).

Table 4. Prey consumption of third instar *Axinoscymnus cardilobus* on *Bemisia tabaci* treated with different concentrations of *Isaria fumosorosea* at different time intervals.

Prey	Concentration (conidia/mL)	0 day	1 day	2 days	3 days	df, *F, P*
Egg	1×10^8	136 ± 12 aA	134 ± 14 aA	137 ± 11 aA	133 ± 13 aA	3, 2.45, 0.8265
	0	140 ± 14 aA	137 ± 13 aA	138 ± 12 aA	135 ± 11 aA	3, 1.96, 0.7307
3 instar	1×10^8	31 ± 4 aB	17 ± 5 bC	8 ± 3 cC	5 ± 2 cC	3, 30.58, < 0.0001
	0	30 ± 5 aB	28 ± 6 aB	31 ± 4 aB	29 ± 7 aB	3, 2.08, 0.5719
	dF, F, P	3, 126.23, < 0.0001	3, 129.17, < 0.0001	3, 183.46, < 0.0001	3, 194.15, < 0.0001	

Data on means (±SE) percentage survival were compared by one way ANOVA, numbers within the same column followed by the same uppercase letter or the same line followed by the same lowercase letter are not significantly different (Tukey's Studentized range test, $P = 0.05$).

Life table characteristics

The value of the net reproduction rate (R_0) was highest in 0 treatment with a mean value of 49.7 progeny/female while the lowest net reproductive rate (45.6 progeny/female) was observed for 1×10^8 conidia/mL (Table 5). The values of r_m, the mean generation time (T) and the finite rate of increase (λ) were also significantly similar among the different treatments (0, 1×10^4, 1×10^8 conidia/mL).

Discussion

The use of different biological control agents is important for integrated pest management of *B. tabaci*. To use both *I. fumosorosea* and *A. cardilobus* it is important to select the optimum concentration of *I. fumosorosea* which is effective against *B. tabaci* and at the same time is compatible with *A. cardilobus.* The highest concentration of fungus used in this experiment was 1×10^8 conidia/mL and the use of this concentration was based on the LC_{50} values obtained from the current as well as from our previous research in greenhouses against *B. tabaci* (Huang et al. 2007). Vidal, Osborne, Lacey, and Fargues (1998) used 8×10^6 conidia/mL for host plant of cabbage and 3.33×10^6 conidia/mL for tomato and cucumber against *Bemisia argentifolii* in greenhouses.

To evaluate the effect of *I. fumosorosea* for whitefly control, we determined how a direct application of conidia to a predatory coccinellid, *A. cardilobus* affects its survival. The findings of current research work clearly suggest that *I. fumosorosea* had a very low pathogenic effect against immatures of *A. cardilobus.* Our results are in accordance with Poprawski et al. (1998), who reported only 2.2% corrected mortality of second instar of another coccinellid predator, *Serangium pacesetosum* (Coleoptera: Coccinellidae) up to adult emergence when treated with low, medium and high dosages of *P. fumosoroseus.* They further reported that neither *Beauveria bassiana* nor *P. fumosoroseus* had sublethal effects on *S. parcesetosum*. Our results are not in line with the findings of James and Lighthart (1994) who dipped first instar of *Hippodomia convergens* (Coleoptera: Coocinellidae) in five concentrations of four entomopathogenic fungi. *B. bassiana* caused 75–95% mortality, *M. anisopliae* caused up to 97% and *P. fumosoroseus* caused up to 56% mortality while *Nomuraea rileyi* did not kill the larvae. They concluded that *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* have the potential to infect *H. convergens* but also acknowledged that direct effects could also be different in the field. In order to identify the possible reasons of non-significant effects of fungal application on survival of *A. cardilobus*,

Table 5. Life table parameters (Mean ± SE) of *Axinoscymnus cardilobus* treated with different concentrations of *Isaria fumosorosea*.

Treatments (Conidia/mL)	Number of adult pairs	R_0 (Progeny per female)	r_m (Progeny per female)	T (days)	λ
0	37	49.7	0.0574	68.1	1.0590
1×10^4	36	48.9	0.0582	66.8	1.0600
1×10^8	37	45.6	0.0598	63.9	1.0616

the effects of host plants and different prey stages were also observed. The results regarding these aspects also showed non-significant effects on survival of *A. cardilobus.* On the basis of these findings, only two concentrations of *I. fumosorosea* were used in subsequent experiments.

Little information is available on sublethal and chronic effects of entomopathogenic fungi (when applied directly to the insects) on development time of coccinellid predators. Development time of *S. japonicum* (Coleoptera: Coccinellidae) reported by Yao (2003) for larvae feeding on eggs and first instar of *B. tabaci* was 14–15 days at 26°C. In the present work, the development time of each immature stage was within 7–19 days and remained unaffected by the fungi. Similarly, the larval development time of *P. versicolor* from first instar to adult stage was decreased by 6.8% (4 days) when *B. bassiana* was applied to the immature stages of the beetles (Traugott, Weissteiner, and Strasser 2005). A possible reason for this could be that the entomopathogens are not host specific, as many unusual hosts can be infected in the laboratory. The different ecological host ranges of different entomopathogens (e.g., coevolution between hosts and pathogens) could partially explain the difference in susceptibility found in our study and previous studies.

We also studied the impact of infected prey on the survival as well as development of the predator by exposing *A. cardilobus* to whiteflies treated with different conidial concentrations of *I. fumosorosea.* Our experiment revealed no significant differences in survival and developmental time when the beetles were fed on whiteflies infected with different fungal concentrations (Tables 2 and 3) which is similar to the findings of Traugott et al. (2005) who also revealed no significant differences in mortality rates and developmental time when *P. versicolor* larvae were exposed to *B. brongniartii*-infected prey. However, the infection of one *P. versicolor* larva by *B. brongniartii* showed that this pathogen was weakly pathogenic to *P. versicolor* larvae under certain conditions.

In the present work, differences in fecundity, longevity, egg viability and life table parameters of newly emerged females were not statistically significant over the different concentrations. It was reported that *Delphastus catalinae* suffered no significant effects on fecundity and longevity, when exposed to *V. lecanii*, a well known pathogen of whiteflies with a host range almost similar to *I. fumosorosea* (Wang, Huang, You, and Liu 2005). In this study, the net reproduction rate in the control was more than that observed for different concentrations, the mean generation time (T) was not significant, and the r_m values were similar for different concentrations (Wang et al. 2005). These results are also in-line with the findings of Fatiha, Huang, Ren, and Ali (2008), who studied the effect of *V. lecanii* characteristics and life tables of *S. japonicum.* They showed no significant effects of *V. lecanii* on mean generation time, intrinsic rate, the finite rate of increase and longevity of *S. japonicum*. Sewify and El Arnaouty (1998) studied the effect of infection of *Chrysoperla carnea* larvae with the fungus *V. lecanii* (Zimm.) Viegas in the laboratory with two fungal isolates under relative humidities of 65 and 95%. One isolate was highly pathogenic to third instar larvae, impaired their feeding and searching capacity, and decreased emergence of adults. Feeding of the larvae with infected aphids had similar effects, and also decreased fecundity.

It can be concluded that control strategies tested are compatible to a greater extent and incorporation of these are promising for control of whitefly. The predatory species (*A. cardilobus*) was not highly susceptible to *I. fumosorosea* when

spores were applied directly to the predator and thus beneficial capacity of the predator was not affected dramatically but the aspects such as the crop, the type of pest or pests to control, the method and timing of fungal application are also important and should be kept in mind. Therefore, further knowledge is needed about the method of fungal application in the field as well as the timing adjustments for various releases of both biological control agents to obtain maximum additive effectiveness.

Acknowledgements

This research was funded by grants from the National Basic Research Program (called 973 Program) (No. 2006CB102005), South China Agricultural President Foundation (No. 2006K06349).

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