Pakistan J. Zool., vol. 41(4), pp. 289-295, 2009.

Influence of the Entomopathogenic Fungus, *Verticillium lecanii* on the Whitefly Predator, *Axinoscymnus cardilobus* (Coleoptera: Coccinellidae) Under Laboratory Conditions

Di Xu, Shaukat Ali, Zhen Huang, Fu Cai Zhou, Muhammad Afzal and Muhammad Hamid Bashir

Engineering Research Centre of Biological Control, Ministry of Education, College of Natural Resources and Environment, South China Agricultural University, P.R. China, 510642 (DX, ZH, SA), School of Horticulture and Plant Protection, Yangzhou University, P.R. China, 225009 (FCZ), University College of Agriculture, University of Sargodha, Sargodha (MA), and Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan (MHB)

Abstract.- The effects of entomopathogenic fungus *Verticillium lecanii* on the biological characteristics and life table of the whitefly predator, *Axinoscymnus cardilobus* Pang and Ren (Coleoptera: Coccinellidae) were studied by using five different conidial concentrations under laboratory conditions. The total developmental period (from egg to adult) among the treatments did not differ between fungus treatments and control. The longest total development period for *A. cardilobus* was observed when treated with 1×10^7 spore/ml. No Significant difference was found for *V. lecanii* on the percent survival of all immature stages of *A. cardilobus*. The treatment with *V. lecanii* did not elicit any significant effect on mean generation time, intrinsic rate, the finite rate of increase and longevity of *A. cardilobus* when compared with control treatment. It can be concluded that control strategies tested are compatible to a greater extent and incorporation of these have promising prospect for control of whitefly.

Key words: Entomopathogenic Fungus, whitefly predator, A*xinoscymnus cardilobus, Verticillium lecanii*

INTRODUCTION

Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) is a very severe pest in all tropical and subtropical regions of the world (Brown, 1994; Oliveira *et al*. 2001). Isolates of *Zoophthora radicans* (Brefeld) Batko (Pell *et al*., 1993), *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Altre *et al*., 1999), and *Beauveria bassiana* (Balsamo) Vuillemin can infect whitefly under screenhouse or field conditions.

Of these fungal species, *Verticillium lecanii* (Zimm) Viegas is one of the most promising fungal species for control of whiteflies, and other insect pests. Wang *et al.* (2005) studied virulence of six strains of *V. lecanii* against *Bemisia tabaci*. Their results indicated that strains V16063, V3450 and Vp28 were the most virulent with LC_{50} values of 2.57×10^5, 6.03×10^5 and 6.05×10^5 conidia/mL, respectively.

* Corresponding author: hamid_uaf@yahoo.com, hzsau@scau.edu.cn

0030-9923/2009/0004-0289 $ 8.00/0

Whiteflies are also attacked by a variety of predatory coccinellids (Nordlund and Legaspi, 1996; Gerling *et al*., 2001; Ren *et al*., 2004). Earlier studies have indicated that the coccinellid predators belonging to the genus *Axinoscymnus* (Coleoptera: Coccinellidae) are consistently considered the most suitable whitefly predators under field as well as laboratory conditions (Ren and Pang, 1992; Huang *et al*., 2003, 2006a, b, 2008). *A. cardiolobus* feeds upon all stages of whitefly and had shown a great potential as an effective biocontrol agent against *B. tabaci* (Huang *et al*., 2003, 2006a, b). Mycoses in nature have been observed in a number of predatory insects (Goettel *et al*., 2000); however little is known about their epizootology and resultant effects on predators particularly the non target species. Many species of predatory insects seems refractory to fungal infection when challenged under laboratory conditions (Porawaski *et al*., 1998).

The infectivity and pathogenicity of entomopathogenic fungi have been screened on coccinellids under laboratory conditions besides developing a standard bioassay protocol (James and Lighthart, 1992; Todorova *et al*., 1994). However, effects of insect control agents (entomopathogens

and chemical pesticides) other than direct kill may also inhibit the beneficial capacity of non-target natural enemies of pests. Although, many reports are available on short term detrimental effects of entomopathogenic fungi on non target organisms (Goettel *et al*., 1990), little is known about their indirect effects. This study therefore, aimed at evaluating the direct impacts of *V. lecanii* on the survival and reproductive biology of *A. cardilobus* by using life table analysis for quantitative evaluations of natural enemies in terms of detailed description of age-specific mortality of individuals in the whitefly population.

MATERIALS AND METHODS

Insects

Bemisia tabaci and *Axinoscymnus cardilobus* were collected from the stock colony kept in greenhouse of the Engineering Research Center of Biological Control, South China Agricultural University (SCAU) on poinsettia. Plants were grown in plastic pots having a diameter of 15-cm. 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. *A. cardilobus* used in the experiments were moved into an air-conditioned room at 26 ± 2 ^{o}C, RH 80~90%, L : D = 14 : 10, after maintaining on host plants for several generations. A large number of *B. tabaci* adults were put into the plastic bags with small holes for ventilation. The leaves of poinsettia were fixed in plastic bags. Six hours later, *B. tabaci* adults were removed . Nymphs of *B. tabaci* were kept on host plants in an air-conditioned room, when nymphs of whiteflies entered into second instar, 100 second instar nymphs per leaf were marked in a circle with colour pen for treatment.

Fungi preparing

V. lecanii used for bioassays was isolated from *Traileurodes* sp, maintained in tubes containing Sabouraud Dextrose Agar (SDA) and kept in the Engineering Research Center of Biological Control, SCAU, was cultured on potato dextrose agar (PDA) and incubated at 26 ± 2^{o}C for 10 days. Conidia 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 (0.0625mm^2; Fuchs-Rosenthal Merch Eurolab) to calibrate a suspension of 1×10^7 conidia/ml of *V. lecanii.* Lower concentrations of 1×10^6 to 1×10^3 conidia/ml were prepared by serial dilutions.

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

Influence of V. lecanii *on immature stages of* A. cardilobus

The different life stages of *A. cardilobus* (eggs, 1st instars, 2nd instars, 3rd instars, 4th instars and pupae) on poinsettia leaves having eggs and immatures of *B. tabaci* as food were directly dipped into the prepared fungal suspensions (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 conidia/ml) for 15 seconds and then dried up on filter paper. Each filter paper with treated stages was placed into a Petri dish. To maintain nearly saturated humidity, the lids of the Petri dishes were closed with strip of parafilm and incubated for 24 hours at 26 ± 2°C and 14:10 (L:D). Twenty four hours later, treated stages of *A. cardilobus* were removed to new Petri dish with poinsettia leaves having mixed population of *B. tabaci* eggs and nymphs as food and kept at 60 – 70% RH under similar conditions of temperature and light. Petri dishes were covered with plastic sheet having small holes for aeration.

The mortality of beetles was recorded at 24 h intervals until adult emergence. The dead larvae were sterilized with 2% sodium hypochlorite for 1 min and were dried by using filter paper (Fazal, 2004). After drying by aeration, dead insects were cultured on PDA media. Beetles showing mycelia and conidia of *V. lecanii* on the cadavers were considered dead from infection of the fungus. The

Petri dishes were incubated at 26±2°C and 80%±5% RH. The egg hatchability and developmental time of each stage until the next molt was also recorded. As a control, 0.03% Tween 80 (Whiga Chemicals, Guangzhou, China) was used (Fazal, 2004). For each conidial concentration, 40 individuals of every life stage of *A. cardilobus* were used for each fungal concentration and the entire experiment was repeated 5 times.

Influence of V. lecanii *on* A. cardilobus *females*

Pairs of sexually mature beetles (4 days old) collected from the stock culture were dipped into five different conidial concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7conidia/ml) of *V. lecanii* for 15 seconds while adults of *A. cardilobus* were treated with water mixed with 0.1% Tween 80 served as a control. The beetles were then transferred to plastic Petri dishes with poinsettia plant leaves. The Perti dishes were incubated at 26 ± 2°C, 80% ± 5% for 24 h. The leaves were changed every day and the numbers of eggs laid by each pair were recorded until adult died. For each conidial concentration six pairs of the beetles were used against each conidial concentration and the entire experiment was repeated 5 times.

Life table analysis

Life and fertility tables were calculated from the cohort of eggs according to the method of Fatiha *et al.* (2008). The death and survival rates were recorded daily for all the immature stages. The probability of surviving from birth (cohort eggs) to age *x* for every immature stage (*lx*) was also calculated. The intrinsic rate of population increase (r_m) was calculated using the Fatiha *et al.* (2008):

$$R_0 = \sum l_x m_{x,}$$
$$T = 1/R_0 \sum l_x m_{x,}$$
$$r_m = \ln R_0/T,$$
$$\lambda = \exp (r_m),$$

Where 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. The net productive rate R_0 is the mean number of female progeny produced by a single female during its mean life span.

This parameter expresses the generation growth rate of the population and is related to discrete daily growth rate and the finite rate of increase (λ).

Data analysis

The developmental period, percentage of survival, duration of oviposition, longevity and fecundity of the beetles treated with fungal suspension of different concentrations were compared using Analysis of Variance (ANOVA). The difference between the means among the different concentrations were compared using Duncan's Multiple Range Test (DMRT $P = 0.05$). All the analyses were done using SAS program (SAS, 2000).

RESULTS

Influence of V. lecanii *on the survival and development of* A. cardilobus

The percent survival of each stage (eggs, 1st, 2nd, 3rd, 4th instar nymphs, and pupae) treated with different concentrations up to emergence was not significantly different when compared with that of the control. The increasing concentrations of conidia also did not have any significant effect on survival (Table I).

The total developmental periods for all immature stages (from eggs to adult emergence) at different concentrations were not significantly different from the control (Table II). The total development period was longest for the colony treated with conidial concentrations of 1×10^7 spore/ml, and the shortest development period for different life stages was observed in the control colony. The pre-imaginal developmental time was longest for eggs and 1st instar larvae and pupae at different concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 conidia/ml), while it was shortest for fourth instar larvae and pupae (Table II).

Fecundity

Data indicated that the application of the pathogenic fungus *V. lecanii* had no significant effect on the fecundity of *A. cardilobus*. The lowest number of eggs was observed when the predator was treated with 1×10^6 conidia/ml with an average

Table I.- Percentage survival (Mean ± SE) of *A. cardilobus* immature stages treated with different concentrations of *V. lecanii.*

Treatments (conidia/ml)	Egg	1st instar	2nd instar	3rd instar	4th instar	Pupa
Control	0.91±0.011 a	0.92 ±0.025a	1.00 ±0.000 a	1.00 ±0.000a	1.00 ±0.000 a	0.89 ±0.023 a
1×10^3	0.90±0.014 a	0.91 ±0.074 a	1.00 ±0.000 a	1.00 ±0.000a	1.00 ±0.000 a	0.89 ±0.028 a
1×10^4	0.88±0.074 a	0.90 ±0.012 a	0.98 ±0.011 a	1.00 ±0.000a	1.00 ±0.000 a	0.88 ±0.020 a
1×10^5	0.88 ±0.074a	0.90 ±0.013 a	0.97 ±0.022 a	1.00 ±0.000a	1.00 ±0.000 a	0.86 ±0.025 a
1×10^6	0.87±0.030 a	0.89 ±0.022 a	0.96 ±0.034 a	0.98 ±0.000a	0.98 ±0.021 a	0.86 ±0.031 a
1×10^7	0.86 ±0.031a	0.88 ±0.041 a	0.96 ±0.023 a	0.98±0.000 a	0.98 ±0.017 a	0.86 ±0.042 a
df, *F*, *P*	5,1.03, 0.3957	5,0.94, 0.52	5, 1.05, 0.50	5,0.83, 0.62	5, 0.49, 0.60	5, 1.05, 0.34

Means compared by one way ANOVA, number within the same column followed by the same letters are not significantly different (DMRT, $P> 0.05$).

Table II.- Developmental periods (Mean±SEM days) of immature stages of *A. cardilobus* treated with different concentrations of *V. lecanii*

Treatments (conidia/ml)	Egg-Adult	1st instar-Adult	2nd instar-Adult	3rd instar-Adult	4th instar-Adult	Pupa-Adult
Control	18.5±1.69a	14.6 ±1.25a	13.1±1.14 a	11.3 ±1.26a	10.1±1.61 a	7.5 ±1.05a
1×10^3	18.5 ±1.21a	14.6 ±1.02a	13.2 ±1.37a	11.4 ±1.19a	10.1±1.48 a	7.5 ±0.88 a
1×10^4	18.5 ±1.55 a	14.7 ±1.36a	13.2 ±1.05a	11.4 ±1.05a	10.1 ±1.02a	7.5 ±1.00 a
1×10^5	18.6 ± 1.29a	14.7±1.11 a	13.4 ±1.77a	11.4 ±1.04a	10.1±0.95 a	7.5 ±0.95 a
1×10^6	18.8 ± 1.42a	14.8 ±1.41a	13.6 ±1.01a	11.5 ±1.41a	10.3 ±1.06a	7.6 ±1.00 a
1×10^7	18.8 ±1.16a	15.0 ±1.06a	13.7±1.23a	11.5 ±1.29a	10.3 ±1.25a	7.6 ±1.02 a
df, *F*, *P*	5,0.61, 0.5643	5,1.02, 0.5022	5,0.68, 0.6017	5,0.97, 0.3979	5,0.79, 0.5052	5,0.58, 0.4942

Means compared by one way ANOVA, number within the same column followed by the same letters are not significantly different (DMRT, $P> 0.05$).

of 126.2±34.85 eggs/female, whereas maximum number of eggs (133.2±32.18 eggs) was recorded in control beetles (Table III).

Table III.- Fecundity (Mean±SE) preoviposition and longevity of *A. cardilobus* female treated with different concentrations of *V. lecanii.*

Treatments (Conidia/ml)	Fecundity	Preoviposition (days)	Longevity (days)
CK	133.2±29.9 a	7.8± 1.17 a	71.7±8.5 a
1×10^3	130.2±39.5 a	7.9±1.36 a	70.2±7.5 a
1×10^4	128.5±36.8 a	7.9±1.41a	69.8±6.9 a
1×10^5	127.8±32.3 a	8.0±1.01 a	68.2±7.2 a
1×10^6	126.2±34.9 a	8.1±1.53 a	66.8±5.5 a
1×10^7	126.7±30.9 a	8.3±1.61 a	64.7±6.8 a
df, *F*, *P*	5, 1.09, 0.6146	5, 0.81, 0.5875	5, 0.98, 0.6023

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (DMRT, $P> 0.05$)

Pre-oviposition period

The duration of the pre-oviposition period of *A. cardilobus* showed no significant difference among the treatments compared to the control (Table III). The longest lowest pre-oviposition period (8.3±1.61 days) was recorded in 1×10^7 conidia/ml while the shortest one was observed in the control with a mean value of 7.8±1.97 days.

Adult longevity

Longevity of adult females treated with different concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 conidia/ml) did not differ significantly as compared to the control, while, the longest longevity of 71.7±8.45 days was observed in control, the shortest longevity of 64.7±6.81 days was recorded in 1×10^7 conidia/ml (Table III).

Life table parameters

The observed values of the net reproduction rate were not significantly different from among

Table IV.- Life table parameters (Mean ± SE) of *A. cardilobus* treated with different concentrations of *V. lecanii*

Treatments (Conidia/ml)	R_0 (Progeny/female)	r_m (Progeny/female)	T (days)	λ (Finite rate of increase)
CK	49.8 ± 9.01 a	0.0571 ± 0.06 a	67.9 ± 12.47 a	1.061 ± 0.43 a
1×10^3	48.0 ± 8.24 a	0.0576 ± 0.10 a	67.4 ± 20.26 a	1.059 ± 0.32 a
1×10^4	47.8 ± 7.73 a	0.0574 ± 0.10a	66.7 ± 13.89 a	1.059 ± 0.51 a
1×10^5	46.1 ± 8.13 a	0.0580 ± 0.23 a	65.6 ± 13.31 a	1.060 ± 0.38 a
1×10^6	45.2 ± 8.56 a	0.0584 ± 0.21 a	65.1 ± 11.45 a	1.060 ± 0.29 a
1×10^7	44.8 ± 6.21 a	0.0587 ± 0.09 a	64.8 ± 10.09 a	1.060 ± 0.48 a
df, *F*, *P*	5, 0.75, 0.5816	5, 0.52, 0.5821	5, 1.06, 0.5083	5, 0.64, 0.6002

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (DMRT, $P > 0.05$)

fungal treatments as compared to the control. The net reproductive rate was lowest in 1×10^7 conidia/ml with a mean value of 44.8 ± 6.21 progeny /female while the highest net reproductive (49.8 ± 9.01 progeny /female) was observed for the control (Table IV).

The values of r_m were significantly similar among different treatments (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 conidia/ml) and the control. The mean generation time (T) was also significantly similar among the treatments when compared with the control.

DISCUSSION

To evaluate the effect of *V. lecanii* used to control whiteflies, we looked how a direct application of conidia to a predatory coccinellid, *A. cardilobus* affects the survival. The findings of current research work clearly suggest that different conidial concentrations of *V. lecanii* had a very low pathogenic effect against immatures of *A. cardilobus* when compared with control. Our present results are in accordance with Poprawaski *et al*. (1998), who reported only 2.2% corrected mortality of 2nd instar of another coccinelid predator *Serangium parcesetosum* (Coleoptera: Coccinellidae) up to adult emergence when sprayed with low, medium and high dosages of *P. fumosoroseus*. They further reported that neither *Beauveria bassiana* nor *P. fumosoroseus* had sublethal effects on biology of *S. parcesetosum*. Also, James and Lighthart (1992) treated the 1st instar of *Hippodomia convergens* (Coleoptera: Coccinellidae) for 10 seconds in five concentrations of four entomopathogenic fungi. Two *B. bassiana* strains caused 75% to 95% mortality (Quesada-Moraga and Vey, 2004), *Metarhizium anisopliae* caused up to 56% mortality (Nielsen *et al*., 2005). They emphasize that further research is needed to determine how direct effects observed in laboratory play out in field environment.

Little information is available on sublethal and chronic effects of entomopathogenic fungi (when applied directly to the insects) on developmental time of *A. cardilobus*. Development time of *Serangium japonicum* (Coleoptera: Coccinellidae) reported by Yao (2003) for larvae feeding on eggs and 1st instar of *B. tabaci* was 14-15 days at 26°C. In the present work, the developmental time of each immature stage was within 7-19 days and was remained unaffected by the fungi. Similar to Poprawaski *et al*. (1998), eggs, larval and pupal developmental times were not significantly different for all application dosages with respect to control. Thus, it can be concluded that *P. fumosoroseus* had no sublethal effects on developmental biology of *S. japonicum* surviving the direct contamination by the entomopathogenic fungi.

In the present work, fertility, longevity and life table parameters of females were almost similar over the different concentrations (Tables III, IV). Our data are in agreement with those reported by Wang *et al.* (2005) who found that *Delphastus catalinae* suffered no significant effect on fecundity and longevity when exposed to *V. lecanii,* the net reproduction rate in the control was more than that observed for different concentrations. Also, the mean generation time (T) and the r_m values were

similar in the different concentrations. These results are also in-line with the findings of Fatiha *et al*. (2008), who studied the effect of *V. lecanii* biological characteristics and life table of *S. japonicum.* They showed non significant effects of *V. lecanii* on mean generation time, intrinsic rate, the finite rate of increase and longevity of *S. japonicum* among the treatments and control. Sewify and El Arnaouty (1998) studied the effect of infection of *Chrysoperla carnea* larvae with the fungus *Verticillium 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 have promising prospect for control of whitefly. The predatory species (*A. cardilobus*) was not highly susceptible to *V. lecanii* when spores were applied directly to the predator and thus beneficial capacity of predator was not affected dramatically. Further knowledge is needed about 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. 2006K0047).

REFERENCES

ALTRE, J.A., VANDENBERG, J.D. AND CANTONE, F.A., 1999. Pathogenicity of *Paecilomyces fumosoroseus* isolates to diamond back moth, *Pluttela xylostella* correlation with spore size, germination speed and attachment to cuticle. *J. Inverteb. Pathol*., **73**: 332-338.

BROWN, J.K., 1994. Current status of *Bemisia tabaci* as plant pest and virus vector in agroecosystem worldwide FAO. *Pl. Prot. Bull.,* **42**: 3-32.

FATIHA, L., HUANG, Z., REN, S.X. AND ALI, S., 2008. Effect of *Verticillium lecanii* on biological characteristics and life tables of *Serangium japonicum* (Coleoptera:Coccinellidae), a predator of whiteflies under laboratory conditions. *Insect Sci.,* **15**: 327-333.

FAZAL, S., 2004. *Biological control of* Bemisia tabaci *(Homoptera: Aleyrodidae) on poinsettia with* Eretmocerus sp. *(Hymenoptera: Aphelinidae),* Serangium japonicum *(Coleoptera: Coccinellidae) and* Paecilomyces fumosoroseus *(Deuteromycotina: Hyphomycetes)*. Ph.D. thesis, South China Agricultural University, Guangzhou, China

GERLING, D., OSCAR, A. AND JUDIT, A., 2001. Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Prot.,* **20**: 779-799.

GOETTEL, M.S., INGLIS, G.D. AND WRIAGHT, S.P., 2000. Fungi Hyphomycetes. In: *Manual of techniques in insect pathology.* Academic Press, the Netherlands, pp. 643–645.

GOETTEL, M.S., POPRAWSKI T.J., VANDENBERG, J.D., LI, Z. AND ROBERTS, D.W., 1990. Safety to non-target invertebrates of fungal biocontrol agents. In: *Safety of microbial insecticides* (eds. M. Larid, L.A. Lacey and E.W. Davidson). CRC, Boca Raton.

HUANG, Z., REN, S.X. AND MUSA, P.D., 2008. Effect of temperature on development, survival, longevity, and fecundity of the *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) predator, *Axinoscymus cardilobus* (Coleoptera:Coccinellidae). *Biol. Cont.,* **46**: 209-215.

HUANG, Z., REN, S.X. AND YAO, S.L., 2003. Morphological and life habit of *Axinoscymnus cardilobus*. *Ent. Know.*, **5**: 450-452 (in Chinese).

HUANG, Z., REN, S.X. AND YAO, S.L., 2006a. Life history of *Axinoscymnus cardilobus* (Col., Coccinellidae), a predator of *Bemisia tabaci* (Hom., Aleyrodidae). *J. appl. Ent.*, **130**: 437-441.

HUANG, Z., REN, S.X. AND YAO, S.L., 2006b. Predation of *Axinoscymnus cardilobus* to *Bemisia tabaci*. *Chinese J. appl. Ecol.,* **17**: 450-452 (in Chinese).

JAMES, R.R. AND LIGHTHART, B., 1992. Protocol for testing the effects of fungal pesticides on non target beetles using *Hippodamia convergens* (Coleoptera: Coccinellidae). *Nat. Tech. Serv. Publ*., **92**: 217-488, Springfield. VA.

NORDLUND, D.A. AND LEGASPI, J.C., 1996. Whitefly predators and their potential for use in biological control. In: *Bemisia 1995: Taxonomy, biology, damage, control and management* (eds. D. Gerling and R.T. Mayer). Intercept, Andover, UK. pp. 499-523.

NIELSEN, C., SKOVGARD, H. AND STEENBERG, T., 2005. Effect of *Metarhizium anisopilae* (Deuteromycotina: Hyphomycetes) on survival and reproduction of the filth fly parasitoid, *Spalangia cameroni* (Hymenoptera: Pteromalidae). *Environ. Ent.,* **34**: 133–139.

OLIVEIRA, M.R.V., HENNEBERRY, T.J. AND ANDERSON, P., 2001. History, current status, and collaborative

research projects for *Bemisia tabaci*. *Crop Prot.,* **20**: 709-723.

PELL, J.K., WILDIN, N., PLAYER, A.L. AND CLARK, S.T., 1993. Selection of an isolate of *Zoopthora radicans* (Zygomycetes; Entomopathorales) for biocontrol of diamond back moth. *J. Inverteb. Pathol*., **61**: 75-80.

PORAWSKI, T.J., LEGASPI, J.C. AND PARKER, P., 1998. Influence of Entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae) an important predator of whiteflies (Homoptera: Aleyorodidae). *Biol. Cont.,* **27**: 785–795.

QUESADA-MORAGA, E. AND VEY, A., 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. *Mycol. Res.*, **108**: 441–452.

REN, S. X., HUANG, Z. AND YAO, S. L., 2004. Advances in studies on predators of *Bemisia tabac*i Gennadius. *Nat. Enem. Insects*, **26**: 34-42. (in Chinese)

REN, S.X. AND PANG, X.F., 1992. The A*xinoscymnus cardilobus* (Coleoptera: Coccinellidae) from China. *Coccinellidae*, **4**: 20-23.

SAS INSTITUTE INC., 2000. *SAS/STAT Version 8.1: Statistics*. SAS Institute, Cary, NC.

SEWIFY, G.H. AND EL-ARNAOUTY, S.A., 1998. The effect of the entomopathogenic fungus *Verticillium lecanii* (Zimm.) Viegas on mayure larvae of *Chrysoperla carnea* Stephens (Neuroptera, Chrysopidae) in the laboratory. *Acta Zool. Fenn.*, **209**: 233–237.

TODOROVA, S.I., CÔTÉ, J.C., MARTEL, P. AND CODERRE, D., 1994. Heterogeneity of two *Beauveria bassiana* strains revealed by biochemical tests, protein profiles and bio-assays on *Leptinotarsa decemlineata* (Col: Chrysomelidae) and *Coleomegilla maculate* Lengi (Col.: Coccinellidae) larvae. *Entomophaga*, **39**: 159-169.

WANG, L., HUANG, J., YOU, M. AND LIU, B., 2005. Effects of toxins from two strains of *Verticillium lecanii* (Fungi: Hyphomycetes) on bioattributes of a predatory lady beetle, *Delphastus catalinae* (Coleoptera: Coccinellidae). *J. appl. Ent.*, **129**: 32–38.

YAO, S., 2003. *Ecological studies on* Serangium japonicum *(Coleoptera: Coccinellidae) and its potential biological control of sweet potato whitefly* Bemisia tabaci *(Homoptera: Aleyrodidae)*. Ph.D. dissertation. South China Agricultural University, Guangzhou, China

(Received 17 December 2008, revised 19 February 2009)