JEN **129**(1) doi: 10.1111/j.1439-0418.2005.00929.32–38

Effects of toxins from two strains of *Verticillium lecanii* (Hyphomycetes) on bioattributes of a predatory ladybeetle, *Delphastus catalinae* (Col., Coccinellidae)

L. Wang[1,2], J. Huang[1], M. You[1], X. Guan[1] and B. Liu[3]
[1]Key Laboratory of Pesticide Biochemistry, Fujian Agriculture & Forestry University, Ministry of Education, Fuzhou, China; [2]Institute for Plant Pathology and Plant Protection, Georg-August-University, Goettingen, Germany; [3]Biotechnology Center, Fujian Academy of Agricultural Sciences, Fuzhou, China

Ms. received: August 23, 2004; accepted: November 11, 2004

Abstract: The aim of this study was to test the hypothesis that the whitefly (*Bemisia tabaci*; Hom., Aleyrodidae) predator ladybird beetles, *Delphastus catalinae* (Col., Coccinellidae), are not adversely affected in the field by the crude insecticidal toxins extracted from two strains of the fungus *Verticillium lecanii*, V3450 and Vp28. We developed a method to evaluate sublethal toxicity and its effects on consumption and functional response of *D. catalinae*. The crude toxins have low toxicity against beetle larva with LC_{50} values of 1942 (1393–2710) and 2471 (1291–4731) p.p.m., respectively (approximately 10- and 12-fold of field rate of application 200 p.p.m.). The adult beetles had less sensitivity to crude toxins with LC_{50} values of 4260 (3376–5375) and 4426 (1734–11298) p.p.m., respectively (approximately 20- and 22-fold of field rate 200 p.p.m.). The consumption and foraging capacity were significantly impaired especially in the second-instar larval beetles which took longer time (more than twice of the control beetles) to consume whitefly eggs after exposure to toxins, although *D. catalinae* suffered no significant effect on fecundity and longevity, when exposed to a toxin dilution of field rate. The data suggest that spraying of *V. lecanii* or its toxins should be avoided in the field having immature stages of *D. catalinae*.

Key words: *Bemisia tabaci*, *Delphastus catalinae*, *Verticillium lecanii*, crude toxins, fecundity, functional response, LC_{50}, longevity, predation capacity

1 Introduction

Verticillium lecanii (Zimm.) Viegas is a major microbial biocontrol agent of whiteflies and aphids. It is a widespread entomopathogen (Hall, 1981). The pathogenicity involves adhesion of spores on the insect cuticle, their germination, penetration and internal colonization culminating in host death. In addition, the toxic substances they secrete may play an important role in host mortality (Gindin et al., 1994). High humidity is an absolute requirement for germination, establishment of infection, and sporulation, and consequent epizootics (Lacey et al., 1996). Therefore, high humidity facilitating the epizootics of plant disease is a common phenomenon. The application of *V. lecanii* as a microbial agent has its limitations (Li et al., 1995). It also produces secondary metabolites with insecticidal properties during colonization of the host tissue (Kanaoka et al., 1978; Claydon and Grove, 1982). Recently, the toxic substances extracted from *V. lecanii* have been found to be potential against whiteflies and aphids in the laboratory and field levels (Gindin et al., 1994; Li et al., 1995; Wang et al., 2000). However, the application of *V. lecanii* or its metabolic toxin extracts might adversely affect non-target invertebrates (Kanaoka et al., 1978). Therefore, it is important to test their impact on non-target invertebrates.

Delphastus catalinae (Horn) (Col., Coccinellidae) is considered one of the major predators of whiteflies and a potential biocontrol agent for *Bemisia argentifolii* (Hoelmer et al., 1993). Both adult male and female beetles exhibit high prey consumption rates (Hoelmer et al., 1993; Heinz and Parrella, 1994) and females are highly fecund (Heinz et al., 1994). *Encarsia formosa* (Gahan) (Hym., Aphelinidae) is one of the widely studied biocontrol agents of whiteflies (Van Lenteren et al., 1980). However, in certain situations, *Encarsia* sp. only partially controlled against *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Hamdan, 1997). Therefore, considerable multi-agency efforts have been expended on simultaneous utilization of fungi or its metabolic extracts with predators or parasitoids to control whitefly populations (USDA, 1995). *Paecilomyces fumosaroseus* (Wize) and *Beauveria bassiana* (Balsamo) exhibited compatibility with *D. catalinae* to control whiteflies (Wraight et al., 1998). Nevertheless, there is very little information on the compatible utilization of *V. lecanii* and *D. catalinae*. For integrated management of *B. tabaci*, the information of

compatible utilization of *D. catalinae* with *V. lecanii* or its metabolic toxins is mandatory. Therefore, we hypothesize that the whitefly predator beetles *D. catalinae* is not adversely affected by the insecticidal crude toxins extracted from *V. lecanii* at field rate of application. We developed a method to evaluate the sublethal toxicity of the crude metabolic toxins extracted from two strains of *V. lecanii* and their effects on consumption and functional response of *D. catalinae*.

2 Materials and Methods

2.1 Insect maintenance

A laboratory colony of the coccinellid *D. catalinae* was established in a controlled rearing room at 25 ± 3°C, 75–78% relative humidity (RH) and 14 : 10 h (L : D) photoperiod, feeding on *B. tabaci*, which were maintained on sweetpotato seedlings (variety 'You 98588'). The source of the colony was a culture kept in the Faculty of Plant Protection, Fujian Agriculture and Forestry University (the stock from NIC, Toronto, Canada). Second, third and fourth instars and adult beetles (24-h-old) were used for the experiments.

The whitefly, *B. tabaci* used in the experiments was maintained on sweetpotato plants (variety 'You 98588') in greenhouse (27 ± 3°C) following Wang et al. (2004). Only eggs (< 24-h-old) were used as prey.

2.2 Toxins

$Toxin_{V3450}$ and $toxin_{Vp28}$ were extracted from strains V3450 and Vp28 of *V. lecanii*, respectively, obtained from the cultures maintained in the Forestry Academy of Guangdong Province, China. Conidia used for fermentation were harvested in sterile 0.03% Tween® 80 (Fluka, Neu-Ulm, Germany) from 7-day-old cultures grown on slants of Sabouraud dextrose agar (Fluka, Neu-Ulm) with 1% yeast extract at pH 6.8, 25 ± 1°C, 90 ± 5% RH and 14 : 10 h (L : D) photoperiod. Concentrations of conidial suspensions were determined using a haemocytometer. Suspensions of approximately 1×10^6 conidia/ml were made by dilution of initial suspensions in sterile 0.03% Tween® 80. These were used to inoculate 100-ml batches of Czapeck-Dox (Difco, Albany, NY, USA) liquid medium with 1% bactopepton (Scharlau, Barcelona, Spain) in 250 Erlenmeyer flasks. The flasks were inoculated with 1 ml of conidial suspension containing 1×10^6 conidia/ml and incubated at 25 ± 1°C on a rotary shaker (100 rpm) for seven days. Ten replicates was produced for each strain. The cultures were harvested after 7 days of growth by vacuum filtering the mycelium. The culture filtrate was filtered through a Buchner funnel lined with Whatman No. 1 filter paper to ensure complete removal of conidial and hyphal debris. The culture filtrates were extracted four times with ethyl acetate (Fluka, Steinhein, Germany), and evaporated by a rotary evaporator (Glasapparatefabrik, Flawil, Switzerland) in vacuum at 40°C until the appearance of a yellow-brown viscous precipitates. The precipitates were dissolved in 1.5 ml acetone (Fluka, Steinhein) and designated as crude toxin preparation. The viscous crude toxins were dissolved in acetone, transferred into Eppendoff cups and centrifuged at 28,700 **g** for 10 min. The supernatant was transferred to a vial and the acetone was evaporated overnight at 40°C in a hot block.

The crude extracts [100 mg dry weight (DW) in 1 ml absolute acetone] were diluted to tested concentrations with sterile distilled water containing 0.03% Tween® 80 and used for bioassay. Distilled water containing 0.03% Tween® 80 and 1 ml absolute acetone was used as controls.

2.3 Toxicity test

For each treatment, ten coccinellids were treated on the ventral side singly with 1 μl of the appropriate solution using a Pipetman P20 micropipette (Gilson Medical Electronic, Villiers-le-Bel, France). Treated beetles were then placed in a single 90-mm Petri dish with water-saturated Whatman no. 1 filter paper and eggs of *B. tabaci* as food. This procedure was repeated five times, for at least 50 beetles tested per concentration. Each crude toxin was tested at concentrations of 1000, 2000, 4000, 5000 and 10 000 p.p.m. (5×, 10×, 20×, 25×, 50× of field application rate, respectively). Mortality was recorded every 24 h over a 5-day period.

2.4 Effect of crude toxins on consumption capacity of *D. catalinae*

For each treatment, ten *D. catalinae* were treated in the same dilutions of the crude toxins ($toxin_{V3450}$ and $toxin_{Vp28}$) at concentrations of 200 and 400 p.p.m. (one- and twofold of field application rate, respectively), and transferred singly to a single 140-mm Petri dish, and a sweetpotato leaf with 160–180 eggs of *B. tabaci* per Petri dish per day. The leaflet petioles were wrapped in water-saturated cotton. The number of whitefly eggs consumed and mortality of *D. catalinae* were recorded daily. This procedure was repeated five times, for at least 50 beetles tested per concentration.

2.5 Effect of crude toxins on functional response of *D. catalinae*

The female adult (10–15-day-old)/larval (second, third and fourth instar) beetles were treated with 200 p.p.m. concentration of crude $toxin_{V3450}$. The treated beetles were then transferred singly to test tube (diameter 3.5 cm, height 10 cm). To standardize hunger, the predators were fed with prey (eggs of *B. tabaci*) for 24 h then starved for 24 h. The prey densities exposed for predation were: 80, 100, 120, 160, 200, 240 and 280 eggs per sweetpotato leaflet (surface area approximately 9.6 cm^2) with one predator for each density in a vial. The leaflet petioles were wrapped in water-saturated cotton. We tested one individual predator and one density of prey per arena (9.6 cm^2) and the number of prey consumed was recorded after 24 h. The prey consumption at different densities by crude toxins treated or control predator was recorded on 10 replications and prey was not replaced during the experiment.

2.6 Effect of crude toxins on fecundity and longevity of *D. catalinae* adult

For each treatment, 20–25 second-instar larval beetles were treated with 200 and 400 p.p.m. of $toxin_{V3450}$ following the aforementioned method. For each concentration, 100, 150, 200 and 250 eggs of *B. tabaci* per sweetpotato leaflet were offered daily to treated and control beetles. After adults' emergence from the treated and control groups, male and female were paired and introduced into a vial with sweetpotato leaf with same density of prey eggs. Five pairs of *D. catalinae* were observed for each treatment. The fecundities of the adult females of *D. catalinae* were recorded. For longevities test of *D. catalinae*, enough prey (eggs) were

offered daily until the adults died. Five replications were conducted for each treatment with a total of at least 50 beetles tested.

2.7 Data analysis

Mortality rates for all stages of *D. catalinae* were corrected using Abbott's formula (Abbott, 1925), and the corrected mortality rates for each treatment were used for probit analysis (Finney, 1971) performed by with a data processing system (DPS) software (Tang and Feng, 1997). Failure of 95% confidence intervals (CI) to overlap was used as the criteria for identifying significant differences among LC_{50} values for different development stages of *D. catalinae* to two crude toxins. The functional response of *D. catalinae* on *B. tabaci* was described by the Holling's disc equation (Holling, 1959)

$$N_a = \frac{aTN}{(1 + aThN)}$$

where N_a is the number of prey consumed, N is the prey density and T is the total time of exposure. Th and a are two biological significant constants in the model: Th represents the handling time (including piercing, feeding and digestion times) and a stands for the attack rate. The parameters of the equations were estimated by nonlinear regression. Three-way ANOVA were used for analysis of crude toxin, treatment concentration and predator stage effect on daily consumption. Two-way ANOVA was performed for analysis of treatment concentration and prey density effect on fecundity. Other statistical differences between control and treatment were compared by one-way ANOVA. Nonlinear regression and ANOVA were performed using SYSTAT® 10 (SPSS Inc, 2000).

3 Results

3.1 Toxicity of crude toxins

The application of crude acetone extracts from two strains of *V. lecanii* resulted in lower toxicity to larvae and adults of *D. catalinae* (table 1). It was relatively higher toxicity against larvae than against adults. The crude extract $toxin_{V3450}$ was found to have an LC_{50} value of 1924 p.p.m. Crude extract $toxin_{Vp28}$ had relatively lower toxic effects against larvae (LC_{50} value of 2471 p.p.m.). Adults of *D. catalinae* had no sensitivity to crude toxins from two strains (LC_{50} values were 4260 and 4426 p.p.m., respectively).

3.2 Effect on consumption capacity of the *D. catalinae*

Adult beetles mortalities were $<0.5\%$ and $<1\%$ at the concentration of 200 and 400 p.p.m. of crude toxins, respectively; the larval mortalities were $<0.5\%$ and approximately 0.5% at the concentration of 200 and 400 p.p.m., respectively. Therefore, the concentrations of 200 and 400 p.p.m. can be considered as sublethal concentrations.

Three-way ANOVA indicated that consumption capacities were not only significantly affected by the crude toxin and the treatment concentration, but also by the predator stage and the interaction toxin × predator stage (table 2). The crude toxins from *V. lecanii* impaired the daily feeding capacity of *D. catalinae* larvae. Crude toxins from two strains caused a significant decrease in feeding capacity at treated concentrations (fig. 1a,b, $P < 0.01$), with the exception of non-significant difference of consumption on first day of treatment with crude $toxin_{Vp28}$ ($F = 1.21$; d.f. = 2, 12; $P = 0.351$).

For adults of *D. catalinae*, the effects of treatment with crude $toxin_{V3450}$ and $toxin_{Vp28}$ on consumption of whitefly eggs were different (fig. 1c,d). Significant decrease ($P < 0.001$) of feeding capacity was detected from day 1 to 4 at treated concentrations of crude $toxin_{V3450}$, with the exception of no significant difference at day 5 ($F = 1.1061$; d.f. = 2, 12; $P = 0.362$). However, no significant differences were found in the feeding capacities between treated and control groups at day 4 and 5 ($F = 0.61$; d.f. = 2, 12; $P = 0.559$ and $F = 0.772$; d.f. = 2, 12; $P = 0.484$, respectively). No significant differences were observed at day 2 and 3 in the feeding capacities between control groups and 200 p.p.m. crude $toxin_{Vp28}$ treated groups ($F = 3.554$; d.f. = 1, 8; $P = 0.096$ and $F = 0.890$; d.f. = 1, 8; $P = 0.373$, respectively). Its significant effect on the feeding capacities was only revealed at day 1 after treatment with crude $toxin_{Vp28}$ ($F = 38.736$; d.f. = 2, 12; $P < 0.001$).

Table 2. *Three-way ANOVA on factors affecting consumption of eggs of* Bemisia tabaci *by different stages (larvae/adults) of* Delphastus catalinae

Source	d.f.	MS	*F*-value	P-value
Toxin	1	1070.700	40.031	0.000
Predator stage	1	2776.849	103.82	0.000
Toxin concentration	2	3222.844	120.495	0.000
Toxin × predator stage	1	128.071	4.788	0.034
Toxin × toxin concentration	2	41.885	1.566	0.219
Predator stage × toxin concentration	2	57.002	2.131	0.130
Toxin × predator stage × toxin concentration	2	50.419	1.885	0.163
Error	48	26.747		

Table 1. *The toxicity of crude toxins from* Verticillium lecanii *on larvae and adults of* Delphastus catalinae

						Statistic analysis of regression			
Toxin	Stage	Insect	n (p.p.m.)	LC_{50} (95% CI)	Slope ± SE	d.f.	*F*-value	P	R^2-value
$Toxin_{V3450}$	Adult	50	4260	(3376–5375)	2.231 ± 0.047	1, 23	2443.13	<0.001	0.991
$Toxin_{V3450}$	Larvae	50	1942	(1393–2710)	2.979 ± 0.030	1, 23	9890.82	<0.001	0.998
$Toxin_{Vp28}$	Adult	50	4426	(1734–11298)	2.205 ± 0.064	1, 23	1176.62	<0.001	0.981
$Toxin_{Vp28}$	Larvae	50	2471	(1291–4731)	3.179 ± 0.092	1, 23	1264.37	<0.001	0.982

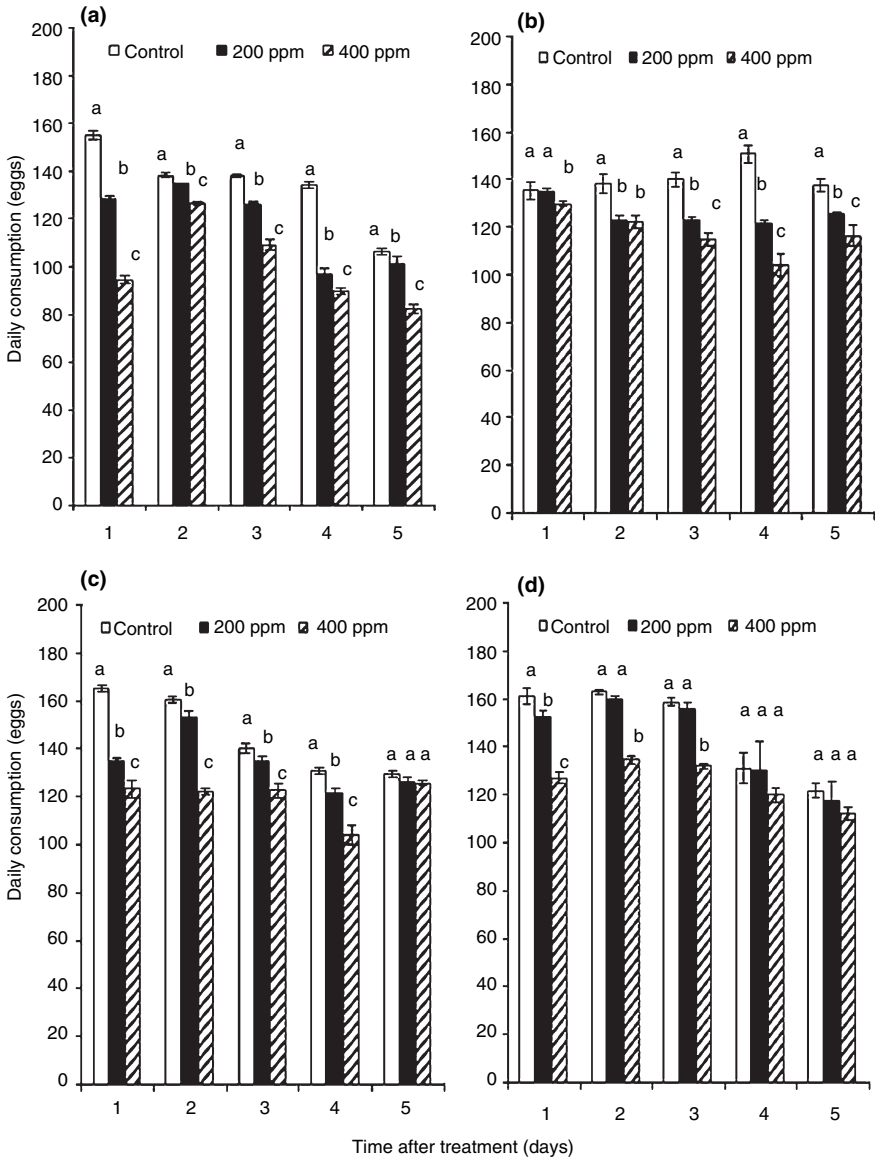

Fig. 1. *Consumption of* Bemisia tabaci *by* Delphastus catalinae *after treatment with dilutions of crude toxins from* Vertcillium lecanii. *(a) Larvae treated with crude toxin$_{V3450}$; (b) larvae treated with crude toxin$_{Vp28}$; (c) adults treated with crude toxin$_{V3450}$; (d) adults treated with crude toxin$_{Vp28}$. Bars (mean ± SE) within a day topped by the same letters are not significantly different [P > 0.05; Tukey's Studentized range test, HSD (SPSS Inc, 2000)]*

3.3 Effects on functional response of *D. catalinae*

The functional response of *D. catalinae* on *B. tabaci* was presented in fig. 2. We fitted the type II functional response equation to our data and estimated the parameters *a* and *Th* for the different stages of *D. catalinae* treated with the toxins. In control trials, the third-, fourth-instar larvae and adults of *D. catalinae* showed similar patterns of functional response (fig. 2) with similar attack rate ($a = 0.0485$–0.0494 arena/h) and similar handling time ($Th = 0.049$–0.067 h). The second-instar larvae of *D. catalinae* performed lower searching with attack rate (*a*) of 0.0489 arena/h and handling time (*Th*) of 0.255 h.

The searching capacities of predatory stages of *D. catalinae* decreased after treatment with 200 p.p.m. crude toxin$_{V3450}$ with lower attack rate ($a = 0.0403$–0.0445 arena/h) and longer handling time ($Th = 0.064$–0.558 h). Among the stages of *D. catalinae*, the effect on the searching capacities and functional response of second-instar larvae was the most with handling time prolonged more than twice (0.558 : 0.255 h), as compared with the control.

3.4 Effect on fecundity and longevity of female *D. catalinae* adult

The data on the effect of *V. lecanii* of toxin (toxin$_{V3450}$) on the ovipsition of *D catalinae* under various densities of whitefly eggs were presented as fig. 3. Two-way ANOVA revealed a significant effect of prey densities and treatment concentrations on the fecundities ($F = 639.75$; d.f. = 3, 48; $P < 0.001$ and $F = 43.75$; d.f. = 3, 48; $P < 0.001$, respectively). However, when being offered with same prey density, no significant differences were recorded between control and crude toxin (200 p.p.m.) treated groups ($F = 0.430$; d.f. = 1, 32; $P = 0.516$), while the fecundities were significantly different between control and crude toxin (400 p.p.m.) treated groups ($F = 72.88$; d.f. = 1, 32; $P < 0.001$).

Higher concentration (400 p.p.m.) of crude toxin had significant effect on the longevity of *D. catalinae* ($F = 6.14$; d.f. = 1, 8; $P = 0.038$), but lower concentration (200 p.p.m.) did not affect the longevities of treated and untreated predators ($F = 0.31$; d.f. = 1, 8; $P = 0.593$) (fig. 4).

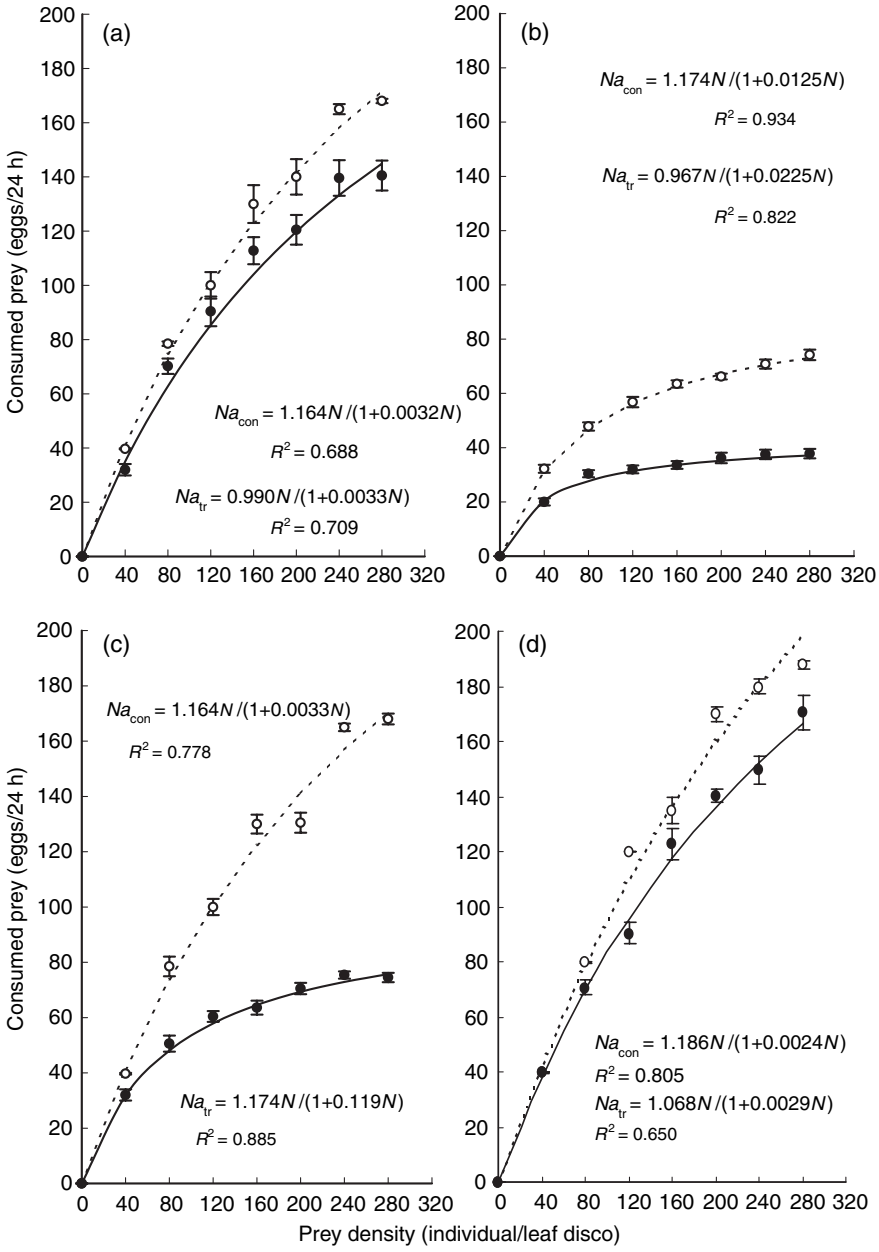

Fig. 2. *Functional response fit (curve) and mean (± SE) number of prey (eggs of* Bemisia tabaci*) consumed by of* Delphastus catalinae *treated with 200 p.p.m. dilutions of crude toxin$_{V3450}$ (solid line, closed circles) and control (dotted line, open circles) on arena of a tomato leaf disc (9.6 cm^2). (a) Adult stages of predator; (b) second-instar larvae of predator; (c) third-instar larvae of predator; and (d) fourth-instar larvae of predator. Error bar: SE*

4 Discussion

The present work clearly demonstrated that, the toxicities of the crude toxins of two strains of *V. lecanii* to the different stages of *D. catalinae* were significantly different, being more toxic against larvae than against adults. Crude toxin$_{V3450}$ extracted from the strain V3450 caused relatively higher toxicity to beetles than crude toxin$_{Vp28}$ extracted from the strain Vp28. Nevertheless, the LC_{50} values of two toxins are much lower than that of the field application rate for the control of whiteflies (Wang et al., 2000). The application of crude toxins of *V. lecanii* highly influenced the foraging and feeding behavior of the predator *D. catalinae* of *B. tabaci* under laboratory condition.

Our study revealed that the crude toxins from two strains at the field application rate significantly decreased the feeding capacity of larval beetles. The effects on the feeding capacity of adult beetles were different, the adverse effect of toxin$_{V3450}$ being more significant than toxin$_{Vp28}$. As for a concentration of 400 p.p.m. (twice of field application rate), significant decreases in the feeding capacities of adults were observed between the control and treated groups with either toxin$_{V3450}$ or toxin$_{Vp28}$. Toxins significantly reduced the fecundity and longevity of *D. catalina* at a concentration of 400 p.p.m. Reduction of consumption capacity, reproduction and longevity of predators by other natural pesticides such as neem and avermectins have been previously reported (Tedeschi et al., 2001; Jiang et al., 2002). Few cases of the effects of fungal on behavioral response of predators have been published. Sewify and El Arnaouty (1998) reported that *V. lecanii* impaired the feeding and searching capacity of green lacewing *Chrysoperla carnea* Stephens. Our study demonstrated that toxins from *V. lecanii* are able to impair the foraging capacities and functional response of *D. catalinae* by prolonging the handling time. For control treatment, younger larvae took significantly longer to consume prey than

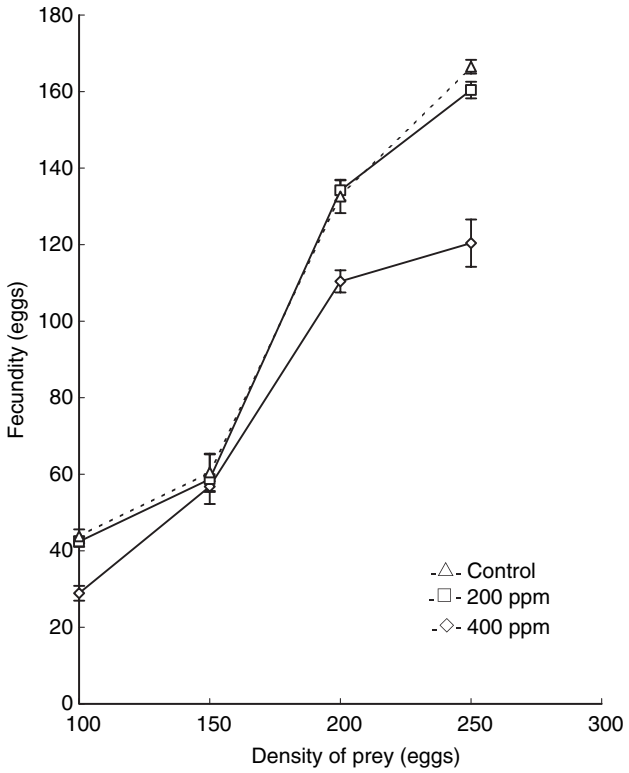

Fig. 3. *Oviposition of* Delphastus catalinae *under various densities of prey (eggs of* Bemisia tabaci*) after treatment with different concentrations of dilutions crude toxin$_{V3450}$. Error bar: SE*

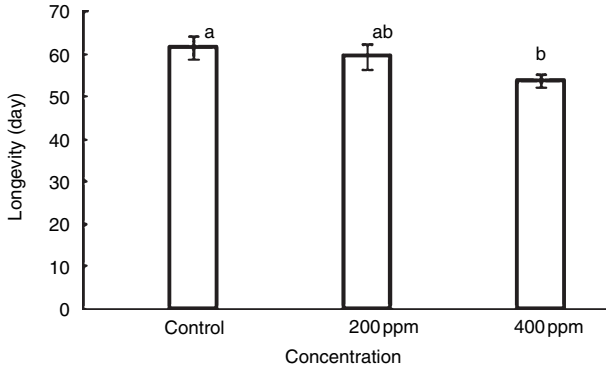

Fig. 4. *Longevity of female adults of* Delphastus catalinae *after treatment with different concentration dilutions of crude toxin$_{V3450}$. Bars (mean ± SE) topped by the same letters are not significantly different [P > 0.05; Tukey's Studentized range test, HSD (SPSS Inc, 2000)]*

older larvae or adults. This is similar to findings in the previous studies (Hoelmer et al., 1993; Guershon and Gerling, 1999; Liu and Stansly, 1999). In our study, we observed that the fourth instars took a shorter time than adults did in handling whitefly eggs. This might have been due to control having a solution containing 0.3% Tween® 80 and solvent (1 ml absolute acetone). After treatment with dilutions of crude toxins, the deleterious effect on the foraging capacity of the beetles is different with different stages. The second-instar larvae exhibited the most prolonged handling time. This result revealed that the younger larvae are more sensitive to the crude toxins.

Fungal secondary metabolite toxins play a great role in infection and pathogenesis (Gillespie and Claydon, 1989; Gindin et al., 1994). These categories of low-molecular-weight secondary metabolites produced by insect pathogens were considered to be of a potential value as biocontrol agents (Gillespie and Claydon, 1989; Gindin et al., 1994). Furthermore, we recommend that spraying of entomopathogenic fungi or their toxins should be avoided when the immature stage of *D. catalinae* is present in field. Nevertheless, more field experiments are needed to test the possible incompatibility of *V. lecanii* and *D. catalinae* as a dual biocontrol agent against *B. tabaci* under natural conditions.

Acknowledgements

The authors thank Professor Stefan Vidal and Dr Dereje Dugassa-Gobena (Institute for Plant Pathology and Plant Protection, Georg-August-University, Goettingen, Germany) for revising the manuscript. Thanks are also extended to Mr Guyan Lin and Zhisheng Liang (Fujian Agriculture & Forestry University) for help with the experiments, and two anonymous referees for numerous insightful comments. This research was funded by Grant no.30270904 from National Natural Science Foundation of China (NSFC) and no. 21835033 from China Scholarship Fund.

References

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

Claydon, N.; Grove, J. F., 1982: Insecticidal secondary metabolic products from the entomologenous fungus *Verticillium lecanii*. J. Invertebr. Pathol. **40,** 413–418.

Finney, D. J., 1971: Probit Analysis. Cambridge, UK: Cambridge University Press.

Gillespie, A. T.; Claydon, N., 1989: The use of entomogenous fungi for pest control and the role of toxin in pathogensis. Pestic. Sci. **27,** 203–215.

Gindin, G.; Barash, I.; Harari, N.; Raccah, B., 1994: Effect of endotoxic compounds isolated from *Verticillium lecanii* on the sweetpotato whitefly, *Bemisia tabaci*. Phytoparasitica **22,** 189–196.

Guershon, M.; Gerling, D., 1999: Predatory behavior of *Delphastus pusillus* in relation to the phenotypicplasticity of *Bemisia tabaci* nymphs. Entomol. Exp. Appl. **92,** 239–248.

Hall, R. A., 1981: The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In: Microbial Control of Pests and Plant Diseases 1970–1980. Ed. by Burges, H. D. New York: Academic Press, 483–498.

Hamdan, A. J. S., 1997: Biological and ecological studies on the predatory bug *Macrolophus caloginosus* (Heteroptera: Miridae) as a biocontrol agent against the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hompotera: Aleyrodidae). Ph.D. thesis, Wye College, University of London.

Heinz, K. M.; Parrella, M. P., 1994: Poinsettia (*Euphorbia pucherrima* Willd. ex Koltz.) cultivars mediated differences in performance of five natural enemies of *Bemisia argentifolii* Bellows & Perring, n. sp. (Homoptera: Aleyrodidae). Biol. Contr. **4,** 305–318.

Heinz, K. M.; Brazzle, J. R.; Pickett, C. H.; Natwick, E. T.; Nelson, J. M.; Parrella, M. P., 1994: *Delphastus pusillus* as a potential biological control agent for sweetpotato (silverleaf) whitefly. Calif. Agric. **48,** 35–40.

Hoelmer, K. A.; Osborne, L. S.; Yokomi, R. K., 1993: Reproduction and feeding behavior of *Delphastus pusillus* (Coleoptera: Coccinellidae), a predator of *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. **86,** 322–329.

Holling, C., 1959: Some characteristics of simple type of predation and parasitism. Can. Entomol. **91**:385–398.

Jiang, Y.; Zhang, Z. N.; Niu, C. Y.; Deng, J. H.; Lei, C. L., 2002: Effects on avermecting on the suppression of the peach aphid, *Myzus persicae*, by *Orius similes*. Acta Entomologica Sinica **45,** 215–220. (in Chinese).

Kanaoka, M.; Isogal, A.; Murakoshi, S., 1978: Bassianolide, a new insecticidal cyclodpsipeptide from *Beauveria bassiana* and *Verticillium lecanii*. Agri. Biol .Chem. **42,** 629–635.

Lacey, L. A.; Fransen, J. J.; Carruthers, R., 1996: Global distribution of naturally occurring fungi of *Bemisia*, their biologics and use as biological control agents. In: Bemisia: Taxonomy, Biology, Damage, Control and Management. Ed. by Gerling, D.; Mayer, R. T. Andover, UK: Intercept, 401–434.

Li, G. X.; Gao, X. W.; Liu, Q. C., 1995: Preliminary study on the culture of *Verticillium lecanii* and the insecticidal effect of its metabolites on insects. J. Beijing Agric. Univ. **21,** 409–415.

Liu, T. X.; Stansly, P. A., 1999: Searching and feeding behavior of *Nephaspis oculatus* and *Delphastus catalinae* (Coleoptera: Coccinellidae), predators of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Environ. Entomol. **28,** 901–906.

Sewify, G. H.; 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. Fennica. **209,** 233–237.

SPSS Inc 2000: SYSTAT® 10 Statistics II. Chicago, IL: SPSS Science Marketing Department SPSS Inc.

Tang, Q. Y.; Feng, M. G., 1997: Practical Statistics and DPS data Processing System. Beijing: Chinese Agricultural Press. (in Chinese).

Tedeschi, R.; Alma, A.; Tavella, A., 2001: Side-effects of three neem (*Azadirachta indica* A. Juss) products on the predator *Macrolophus caliginosus* Wagner (Het., Miridae). J Appl. Ent. **125,** 371–402.

USDA 1995: Silverleaf Whitefly: 1995 Supplement to the 5-year National Research and Action Plan, USDA-ARS 1995–2. Beltsville, MD: USDA.

Van Lenteren, J. C.; Nell, H. W.; Sevenster-Van Der Lelie, L. A., 1980: The parasite-host relationship between *Encarsia formosa* (Hym., Aphelinidae) and *Trialeurodes vaporariorum* (Hom., Aleyrodidae). IV. Oviposition behaviour of the parasite, with aspects of host selection, host discrimination and host feeding. Z. Ang. Enotmol. **89,** 442–454.

Wang, K. Q.; Li, X. M.; Liu, C. L., 2000: Preliminary study on the control of greenhouse whitefly, *Trialeurodes vaporariorum* with toxin from *Verticillium lecanii* (Zimm.). Plant Protection **26,** 44–45. (in Chinese).

Wang, L.; Huang, J.; You, M.; Liu, B., 2004: Time-dose-mortality modeling and virulence indices for six strains of *Verticillium lecanii* against sweetpotato whitefly, *Bemisia tabaci* (Gennadius). J. Appl. Ent. **128,** 494–500.

Wraight, S. P.; Carruthers, R. I.; Bradley, C. A.; Jaronski, S. T.; Lacey, L. A.; Wood, P.; Galaini-Wraight, S., 1998: Pathogenicity of the entomopathogenic fungi *Paceilomyces* spp. and *Beauveria bassiana* against the silver whitefly, *Bemisia argentifolii*. J. Invertebr. Pathol. **71,** 217–226.

Author's address: Liande Wang (corresponding author), Institute for Plant Pathology and Plant Protection, Georg-August-University, Goettingen, Grisbachstr.6, 37077, Goettingen, Germany. E-mail: wangliande@yahoo.com