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Influence of the Entomopathogenic Fungus *Beauveria bassiana* on *Prynocaria congener* (Billberg) (Coleoptera: Coccinellidae) Under Laboratory Conditions

Zhen Huang,[1] Shaukat Ali[1], Shunxiang Ren[1]*, Jianghui Wu and Yang Zhang[2]
[1]*Engineering Research Center of Biological Control, Ministry of Education, College of Natural Resource and Environment, South China Agricultural University, P.P. China, 510642.*
[2]*Plant Protection Institute, Guangdong Agricultural Science Academy, P.P. China, 510641.*

Abstract.- Effects of entomopathogenic fungus *Beauveria b*assiana (Hypocereales:Cordycipitaceae) on biological characteristics and life table parameters of *Prynocaria congener* (Coleoptera: Coccinellidae), a predator of *Aleurodicus disperses* and *Bemisia tabaci* (Hemiptera: Aleyrodidae), were studied using three different conidial concentrations under laboratory conditions. Results indicated that significant differences were not observed among different fungal concentrations on the survival percentages of all immature stages of *P. congener*. The developmental periods for all immature stages (from eggs, first, second, third, fourth instar larvae and pupae up to adult hatching) among the fungal treatments were not statistically significant when compared to control. Moreover, fecundity, longevity, egg viability and life table parameters of *P. congener* females did not differ significantly among the different tested concentrations. It can be concluded that control strategies comprising *P. congener* and *B. bassiana* could be used in an integrate manner for controlling *A. disperses* and *B. tabaci*.

Keywords: *Beauveria bassiana*; *Prynocaria congener*; biological characteristics; life table.

INTRODUCTION

Studies regarding biological control of spiraling whitefly, *Aleurodicus disperses* Russel, a serious pest in all tropical and subtropical regions of the world (Avidov and Harpaz, 1969; Mani and Krishnamoorthy, 2002), have indicated that the coccinellid predator are consistently performing as the best predator in the field as well as under laboratory conditions (Goolsby *et al.*, 1996; Legaspi *et al.*, 1996; Ren *et al.*, 2004; Yao, 2003). *Prynocaria congener* (Billberg) is an important coccinellid predator that feeds on different life stages of whitefly, such as *Aleurodicus disperses* and *Bemisia tabaci* (Mani, 2010; Wu *et al*., 2010), and has shown considerable potential for biological control of *B. tabaci* (Wu *et al*., 2010).

Beauveria bassiana (Bals) Vuill., *B. brongniortii* Sacc. and *B. velata* Sans and Evans cause white muscardine disease in a wide range of insects. *B. bassiana* isolated from over 700 species of insects from nine orders most frequently from infected Lepidopteran and Coleopteran hosts (Li, 1988). *B. bassiana* is now exploited in greenhouse and outdoor crops as a tool for the control of many agricultural arthropod pests including whiteflies, aphids, thrips, psyllids, mealybugs and weevils (Shah and Goettel, 1999).

Our earlier investigations on whitefly demonstrate that integration of biocontrol agents can substantially contribute to sustainable management of whitefly damage both in greenhouse and environments (Fazal, 2004). The biological control agents may act synergistically, additively or antagonistically. Synergetic interactions between pathogens and insect natural enemies can enhance control efficacy, whereas antagonistic interactions would reduce total control efficacy (Roy *et al.*, 1998). Lethal and sub-lethal effects of entomopathogens on the biology of insects in general and on predators in particular are too complex to be observed (Brooks, 1993). In cases of entomopathogens, the lethal and sub-lethal effects of the pathogen on beneficial insects (predators and parasitoids) with regard to fecundity, longevity and survivorship among others, are worth evaluating.

Life table studies provide a powerful technique for quantitative evaluations of natural

* Corresponding author: hzscau@scau.edu.cn.
Shaukat Ali, the joint fist author.
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enemies in terms of detailed description of age-specific mortality of individuals in the population (Luck *et al.*, 1988). When information on the insect's fecundity and age-specific mortality is available, the effect of the natural enemy can be easily expressed in terms of its effects on the pest population growth rate (van Driesche and Bellows, 1996). Nevertheless, there is very little information on the compatible utilization of *B. bassiana* and *P. congener* for integrated management of *A. disperses*, by using the life table method. Therefore, the objectives of this study were to evaluate the impact of *B. bassiana* on the survival and reproduction of *P. congener* in relation to natural mortality factors, including natural enemies and their efficient ratio on *A. disperses* population dynamics.

MATERIALS AND METHODS

Insects

Prynocaria congener and *Bemisia tabaci* were obtained from stock colonies kept in a greenhouse at the Engineering Research Center of Biological Control, South China Agricultural University (SCAU) on poinsettia plants, *Euphorbia pulcherrima* Willd, which were grown in plastic pots (15 cm diameter). Sufficient slow release fertilizer (N: P: K = 13: 7: 15) unit was added when needed to maintain normal plant growth. Intact plants were maintained in another greenhouse. Insects used in these studies were moved into a room maintained at 26±2°C, RH 80-90%, L:D= 14:10 h, after being maintained on host plants for several generations.

P. congener (Billberg) can feed on different life stages of *A. disperses* and *B. tabaci*. *A. disperses* is a serious quarantine pest in China, it distributes only in Hainan province. So *B. tabaci* was used in this study as food instead of *A. disperses*. A large number of *B. tabaci* adults were placed into the plastic bags with small holes made by needle punctures. The plastic bags were fixed on the leaves of poinsettia plants and adults of *B. tabaci* were allowed to oviposit for 6 h then they were taken out. Leaves bearing eggs of *B. tabaci* were monitored until hatching. Poinsettia plants bearing nymphs of whiteflies were kept in an air-conditioned room as above described. When nymphs of *B. tabaci* entered into the second instar, 100 nymphs per leaf were reserved for treatment.

Fungal material

Strain PF01-N4 of *B. bassiana*, maintained in tubes containing Sabouraud Dextrose Agar (SDA) and deposited at the Engineering Research Center of Biological Control, SCAU was used in all the assays. *B. bassiana* was cultured on potato dextrose agar (PDA) and incubated at 26±2°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 *B. bassiana*. Lower concentrations of 1×10^7 to 1×10^4 were prepared by serial dilutions, and adjusted by counting as described above. The concentration of 0 conidia/mL only contained 0.02% Tween 80 solution prepared in distilled water.

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 of germination was estimated at > 95% for all experiments.

LC_{50} of B. bassiana *on* A. disperses *immatures*

Six different concentrations of *B. bassiana* (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 *A. disperses* were treated by dipping infested leaves (not excised leaves) into each of the six concentrations of *B. bassiana* (freshly prepared suspensions) for 15 s and removing to air dry before transferring to the cages (60×60×60 cm) along with the plant. Water was added to the bottom of the cage daily to maintain high relative humidity (> 95%). Each fungal

concentration was repeated three times with a new batch of insects and fresh conidial suspension and four leaves with 100 whitefly nymphs per leaf were treated each time. All treatments were done at one time, using randomized groups of insects from a single batch.

Treated insects were placed in a temperature controlled room as previously described and monitored daily until adult hatching. Mortalities of *A. disperses* were recorded after 6 and 12 days of treatment. To determine the infective mortalities, the cadavers were taken out and separately incubated at 26±2°C, >95% RH to encourage sporulation. If sporulation of *B. bassiana* was observed on a cadaver, the cadaver was considered as having been infected by *B. bassiana*.

Influence of B. bassiana *on the survival and developmental time of* P. congener *immatures*

The different life stages of *P. congener* (eggs, first, second, third, and fourth instars, and pupae) with poinsettia leaves having eggs and immatures of *B. tabaci* as food, were directly dipped into one of the prepared suspensions (0, 1×10^4, 1×10^8 conidia/ mL) for 15 s and then placed on moist 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 L:D = 14:10. Following the initial 24 h, the *P. congener* 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.

The egg hatchability and developmental time of each stage until adult hatching was recorded. Mortality of beetles was also recorded at 24-h intervals until adult hatching. 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 and incubated in a temperature controlled room. Beetles from which mycelia and conidia of *B. bassiana* 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.

Fecundity, adult longevity and egg viability of newly emerged females

Newly emerged adults fed on *B. tabaci* as prey and treated with one of the tested conidial concentrations (0, 1×10^4, 1×10^8 conidia/mL) were separated in a small cage and allowed to mate for about 5 h, 4 days later, with 6 days for the preoviposition periods (Wu *et al*., 2010). The mating adult pairs of beetles from each concentration were dipped into either of three different conidial concentrations (0, 1×10^4, 1×10^8 conidia/mL) of *B. bassiana* for 15 s. Adults were treated with the same conidia concentration as in the treatment from which they emerged. After being maintained in nearly saturated humidity for 24 h as previously described, these adults were then transferred to plastic Petri dishes with poinsettia plant leaves. Petri dishes were kept in the laboratory under the previously mentioned conditions. 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 for 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, 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 randomly. The selected eggs were placed in a temperature controlled room as previously described. The number of eggs enclosed and first instar larvae emerged was counted and comparisons were made between treatment groups and their respective untreated controls. The viability of beetle eggs descended from *B. bassiana* treated insects was calculated as the percentage of hatching , i.e., the number of hatching first instar larvae 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, 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):

$r_0=\sum l_x m_x,$
$T=1/r_0\sum l_x m_x,$
$r_m= \ln r_0/T,$
$\lambda=\exp(r_m),$

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 B. bassiana *on* A. disperses *immatures*

The mean mortalities for second instars of *A. disperses* treated with the six conidial concentrations were 2.3, 3.4, 4.6, 9.3, 17.1 and 28.2% after 6 days and 3.6, 14.5, 23.6, 35.8, 53.6 and 69.4% after 12 days, for the 0, 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 conidia/ml, respectively. Based on the above initial mortality data, the concentration mortality response regression analysis for *B. bassiana* was calculated by assaying seven concentrations against newly molted *A. disperses* nymphs or larvae (Table I). The LC_{50} values of *B. bassiana* against *A. disperse* at different time intervals are presented in Table I. There was a decrease in LC_{50} values with the passage of time after the fungal treatment (Table I).

Influence of B. bassiana *on the survival of* P. congener

The percent survivals of each stage (eggs, 1st, 2nd, 3rd, 4th instar nymphs, and pupae) treated with different concentrations up to hatching were not significantly different from the control (Table II). The survival rates decreased slightly with the increasing concentrations from 1×10^4 to 1×10^8 conidia/ml when compared with the control. *B. bassiana* was found to have no adverse effect on survival of *P. congener* (Table II).

Influence of B. bassiana *on the developmental time of different life stages of* P. congener

The developmental period for all immature stages (eggs, 1st, 2nd, 3rd, 4th instar nymphs and pupae up to hatching) at different conidial concentrations was not significantly different when compared with the control (Table III). There was less than 0.5 day longer for the developmental period for the colony treated with conidial concentrations of 1×10^8 conidia/ml when compared with the control and that treated with conidial concentrations of 1×10^8 conidia/ml. The pre-imaginal developmental time was shortest for first, second and third instars, and longest for eggs, fourth instar larvae and pupae at different concentrations (0, 1×10^4, 1×10^8 conidia/ml) as shown in Table III.

Fecundity, egg viability and adult longevity of newly emerged P. congener *females*

The fecundity of females showed significant differences among the treatments including the control, 1×10^4 and 1×10^8 conidia/mL (F =71.26, df = 2, P = 0.0490). The maximum number of eggs

Table I.- Regression analysis of probit mortality and log-concentration data of bioassay with *B. bassiana* against 2nd instar of *A. disperses.*

Insect	Days	Slope (M±SE)	χ^2	LC_{50} value (95% FL)	LC_{95} value
A. disperses	6	0.43 ± 0.02	6.68	2.9×10^{10} (3.0×10^{9} – 2.8×10^{11})	2.1×10^{14}
	12	0.42 ± 0.10	2.63	8.2×10^{7} (3.7×10^{7} – 1.8×10^{8})	6.3×10^{11}

Table II.- Percentage survival (Mean±SE) of *P. congener* immature stages treated with different concentrations of *B. bassiana.*

Treatments (conidia/ml)	Egg	1st instar	2nd instar	3rd instar	4th instar	Pupa
Control	81.9±4.52 A	76.3±3.85 A	75.9±4.07 A	83.4±4.13 A	80.4±3.92 A	84.0±3.83 A
1×10^4	80.6±5.23 A	76.1±4.61 A	75.3±3.13 A	83.0±3.81 A	79.6±3.05 A	83.6±2.92 A
1×10^8	79.8±4.27 A	75.8±3.68 A	74.6±3.19 A	82.7±3.09 A	79.1±3.02 A	82.5±3.83 A
df, F,	2, 1.40,	2, 1.13,	2, 2.07,	2, 2.93,	2, 2.03,	2, 1.10,
P	0.5704	0.6268	0.8057	0.5068	0.4972	0.8823

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

Table III.- Developmental periods in days (Mean±SE) of *P. congener* immature stages treated with different concentrations of *B. bassiana.*

Treatments (conidia/ml)	Egg-adult	L1-adult	L2-adult	L3-adult	L4-adult	Pupa-adult
Control	13.3 ±1.02 A	10.9±1.15 A	9.1±1.31 A	7.8±1.06 A	6.6±1.32 A	3.9±1.04 A
1×10^4	13.6±1.38 A	11.1±1.15 A	9.3±1.03 A	7.9±1.12 A	6.8 ±1.10 A	4.0±0.92 A
1×10^8	13.8±1.01 A	11.3 ±1.13 A	9.5±1.00 A	8.1 ±1.02 A	6.9±1.07 A	4.1 ±0.89 A
F, df,	0.54, 2,	0.41, 2,	0.45, 2,	0.62, 2,	0.46, 2,	0.48, 2,
P	0.8192	0.8974	0.9316	0.9051	0.8912	0.8796

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (Tukey's Studentized RangeTest, P= 0.05). L1, first instar; L2, second instar; L3, third instar; L4, fourth instar.

Table IV.- Fecundity, egg viability and longevity of *P. congener* female treated with different concentrations of *B. bassiana.*

Treatments (Conidia/ml)	Fecundity (eggs/female)	Egg viability	Female longevity (days)
Control	389.5 ± 36.48 A	7.0 ± 1.31 A	64.5 ± 6.45 A
1×10^4	378.8 ± 43.57 A	6.8 ± 0.64 A	63.3 ± 6.49 A
1×10^8	366.6 ± 40.81 B	6.0 ± 1.32 A	62.4 ± 6.83 A
F, df, P	71.26, 2, 0.0490	2.37, 2, 0.8032	3.19, 2, 0. 3198

Egg viabilities were subjected to square root arcsine transformation prior to computation. Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (Tukey's Studentized Range Test, P= 0.05)

were laid by beetles in Control colony, whereas the lowest fecundity was observed for 1×10^8 conidia/ml treatment (Table IV). The duration of the egg viability of *P. congener* showed no significant differences among the different treatments and the control, with viabilities ranging from 6.0±1.32 in the 1×10^8 conidia/ml treatment to 7.0±1.31 in the control (Table IV). Longevity of adult females treated with different concentrations did not vary significantly (F = 3.19, df = 2, P = 0.3198) among the treatments and the control, with longevity ranging from 62.4 days in 1×10^8 conidia/ml treatment to 64.5 days in the control (Table IV).

Life table characteristics

The net reproduction rate (R_0) was highest in control with a mean value of 119.7 progeny/ female while the lowest net reproductive rate (117.7

Table V.- Life table parameters (Mean ± SE) of *P. congener* treated with different concentrations of *B. bassiana*.

Treatments (Conidia/ml)	Number of adult pairs	R_0 (Progeny per female)	r_m (Progeny per female)	T (days)	λ
Control	36	119.7	0.0974	48.1	1.1004
1×10^4	36	118.6	0.0964	47.8	1.0982
1×10^8	36	117.7	0.0958	47.2	1.0856

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (Tukey's Studentized Range Test, P= 0.05)

progeny/female) was observed for 1×10^8 conidia/ml treatment (Table V). 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 *A. disperses*. To use both *B. bassiana* and *P. congener* it is important to select the optimum concentration of *B. bassiana* which is effective against *A. disperses* and at the same time is compatible with *P. congener*. The highest concentration of fungus used in this experiment was 1×10^9 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 *A. disperses* (Unpublished data).

Little information is available on sub-lethal and chronic effects of entomopathogenic fungi on the developmental time of beetles when a fungus is directly applied. The total developmental period from egg to adult of *S. japonicum* feeding on nymphs of *B. tabaci* on eggplant (17.42 days) recorded by Yao (2003) is almost same as that recorded in this study in the control. The developmental period of each immature stage of *S. japonicum* was within 13–14 days and remained unaffected by the fungi (Fazal, 2004). Similar to Poprawaski *et al.* (1998), eggs, larval and pupal developmental times were not significantly different for all application dosages with as compared with controls. Thus, it can be concluded that *B. bassiana* had no sub-lethal effects on developmental biology of *P. congener* surviving direct contamination by the entomopathogenic fungi.

Fertility of females varied substantially over the different concentrations (Table 3). This decrease in the rate of fertilization might be derived from a decline in the female physiological state related to: (i) fungal colonization of tissues, such as fat body (source of vitellogeins) and ovaries (Blay and Yuval, 1999); (ii) fungal toxin production to overcome insect cellular and humoral immune reactions (Inglis *et al.*, 2001; Quesada-Moraga and Vey, 2004); and (iii) depletion of resources needed for vital egg production such as proteins (Carey *et al.*, 1998). 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 hatching of adults. Feeding of the larvae on infected aphids had similar effects, and also decreased fecundity. Wang *et al.* (2005) studied the effect of two strains on *Delphastus catalinae*, a predator of whitefly. They observed that *D. catalinae* suffered no significant effects on fecundity and longevity, when exposed to *V. lecanii*. 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. These results are also in-line with the findings of Nielson *et al.* (2005), who studied the effect of *M. anisopilae* on survival and reproduction of *Spalangia cameroni*. They showed that by testing the sensitivity of r_m, where the infection rate of fungal treated females (1×10^8 conidia/mL) was increased from 50% to 100%. A pronounced decline in survival and reproduction from day 7 onwards had no impact on the value of

r_m, which was changed from 0.1350 to 0.1321 days^{-1}. However, if the mortality of 100% infected females had occurred 3 days earlier, the value of r_m would have been reduced to 0.1137 per days.

It is apparent from our research results that there are no adverse effects of *B. bassiana* application on *Prynocaria congener*. It can be concluded that *B. bassiana* control strategies tested are compatible with natural predators and their integration has promising prospects for the control of *A. disperses* and *B. tabaci*. The results mentioned above indicate that the interaction among biocontrol agents is positive to a greater extent with minimum risk hazards to natural enemies.

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