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Lethal and sub-lethal effects of *Beauveria bassiana* (Cordycipitaceae) strain NI8 on *Chrysoperla rufilabris* (Neuroptera: Chrysopidae)

Maribel Portilla[1,], Gordon Snodgrass[1], and Randall Luttrell[1]*

Abstract

A Mississippi Delta native strain (NI8 ARSEF8889) of *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae), isolated from *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), was tested on green lacewings, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) at 4 spray concentrations (7.02×10^4, 10^5, 10^6, and 10^7 spores per mL) to evaluate effects on reproductive rates and adult life expectancy of this insect predator. The application method simulated atomized spray, and concentrations tested were similar to those used to measure impacts of the fungus on *L. lineolaris*. Significant effects of *B. bassiana* on *C. rufilabris* adults were found, and the severity of impact depended on the concentrations tested. *Beauveria bassiana* impacted all demographic measurements of *C. rufilabris* reproduction and survival. Intrinsic and finite rates of increase and gross and net reproductive rates of adults treated with the highest concentrations tested were significantly decreased, whereas doubling time increased for adults treated with the lowest test concentrations. Based on these observations, *C. rufilabris* will be affected by sprays of *B. bassiana* targeted at *L. lineolaris* if adults are present at the time and location of treatment. The measured lethal concentration, LC_{50}, of 2.11 viable spores per mm^2 compares to an LC_{50} of 2.75 spores per mm^2 determined previously for *L. lineolaris*. Higher concentrations of spores per mm^2 were required for sporulation (SR_{50}) of the entomopathogenic fungus on *C. rufilabris* (13.60 viable spores per mm^2) than concentrations required for mortality (LC_{50}).

Key Words: lacewing; entomopathogenic fungus; demographic parameter; life expectancy; solid diet

Resumen

Se hizo un bioensayo utilizando una cepa nativa del Delta del Mississippi (NI8 ARSEF8889) de *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae), aislada de *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), en una crisopa, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) a 4 concentraciones de pulverización (7.02×10^4, 10^5, 10^6, and 10^7) esporas por mL) para evaluar su efecto sobre la tasa reproductiva y duración de la vida de los adultos de este predador de insectos. El método de aplicación simuló pulverización atomizada y las concentraciones probadas fueron similares a las utilizadas para medir el impacto del hongo en *L. lineolaris*. Se encontraron efectos significativos de *B. bassiana* en adultos de *C. rufilabris* y la gravedad del impacto dependió de la concentración probada. *Beauveria bassiana* impactó todas las medidas demográficas de la reproducción y sobrevivencia de *C. rufilabris*. La tasa de aumento intrínseco y finito y las tasas de reproducción bruta y neta de adultos tratados con las concentraciones de prueba más altas disminuyeron significativamente, mientras que el tiempo de duplicación aumentó para adultos tratados con las concentraciones de pruebas más bajas. Basándose en estas observaciones, *C. rufilabris* si será afectado por aerosoles de *B. bassiana* dirigidos a *L. lineolaris* si los adultos están presentes en el momento y lugar del tratamiento. La concentración letal medida, CL_{50}, de 2.11 esporas viables por mm^2 se compara con una CL_{50} de 2,75 esporas por mm^2determinada previamente para *L. lineolaris*. Se requirieron mayores concentraciones the esporas por mm^2 para la respuesta de esporulación (RS_{50}) del hongo entomopatógeno sobre *C. rufilabris* (13,60 esporas viables por mm^2) de las concentraciones que se necesitaron para la respuesta de mortalidad (LC_{50}).

Palabras Clave: crisopa; hongos entomopatogenos; parámetros demográficos; esperanza de vida; dieta solida

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The entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae) has great potential as a biological control agent against many insect pests of agricultural importance especially those with piercing-sucking mouth parts that do not consume biological control agents applied to the surface of host plants (Thungrabeab & Togma 2007). This hyphomycete fungus with contact activity has been employed worldwide with success, and interest in its use has increased as evidenced by the number of commercial products available and under development (Butt et al. 2001; Jaronski 2014). Today, there are more than 40 products based on the entomopathogenic fungus *B. bassiana*, but only 11 are commercially available worldwide. In the United States, there are 4 *B. bassiana* mycoinsecticides currently registered by the U.S. Environmental Protection Agency (Jaronski 2014). Commercial mycoinsecticides can regulate insect populations through inundative and inoculative application (Mahdavi et al. 2013). *Beauveria bassiana* has a number of positive attributes including potential mortality of up to 80% of the targeted pest population, great diversity and high genetic variability among different strains, potential infection of different stages of the targeted pest host, cutaneous penetration through the integument, and capacity for horizontal and vertical dispersal depending on the host pest environment involved (Destefano et al. 2004; Jaronsky 2014).

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Host-predator-entomopathogen interactions in agricultural systems can be synergistically or antagonistically harmful to beneficial arthropods, other non-target insects, and ecological communities (Fuentes-Contreras & Niemeyer 2000; Roy & Cottrell 2008; Meyling et al. 2011). Therefore, the successful use of *B. bassiana* for targeted pest control depends not only on high efficacy against insect pests, but also potential selectivity and low virulence against non-target insects. There are several studies that have demonstrated that *B. bassiana* has been employed with success against a variety of insects in a number of different agro-ecosystems with no significant ecological implications (Lipa 1985; Kimtova & Bajan 1982; Hajek et al. 1987; Weiser 1987; Groden & Lockwood 1991). More recently, Rossini et al. (2014) observed high compatibility of *B. bassiana* and the parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) when applied against *Reticulitermes* spp. (Isoptera: Rhinotermitidae), *Metamasius hemipterus* (Coleoptera: Curculionidae), and *Sphenophorus levis* Vaurie (Coleoptera: Curculionidae). Similarly, several studies have shown under laboratory conditions that application of commercial concentrations of *B. bassiana* is compatible with beneficial insects. Thungrabeab and Tongma (2007) indicated that *B. bassinna* was found to be non-pathogenic to several natural enemies including *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and *Dicyphus tamaninii* Wagner (Hemiptera: Miridae), and the beneficial soil-dwelling insect *Heteromurus nitidus* (Templeton) (Collembola: Entomobryidae). Al mazra'awi (2007) exposed honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), hives to high inoculum densities of *B. bassiana,* which resulted in very low mortality that was not different from the untreated control regardless of the isolate tested. Two of the strains tested were isolated from *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) collected in Arkansas and New York. Todovora et al. (1996) fed *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae) with *B. bassiana* infected Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) and *B. bassiana* contaminated pollen and found no mortality of *C. maculata*. Leyva et al. (2011) found that larvae and pupae of *Chrysoperla exotera* (Navás) (Neuroptera: Chrysopidae) submerged in high concentration suspensions of *B. bassiana* were not affected at any developmental stages of this predator.

Mycopesticides are often based on an indigenous rather than exotic fungal pathogens (Butt et al. 2001; Inglis et al. 2001). The native strain NI8 of *B. bassiana* (ARSEF8889) was originally isolated from insects in the Mississippi Delta and its frequency of natural infection on *L. lineolaris* is higher in areas undisturbed by agriculture practices, which are also areas often preferred by arthropod predators (Leland & Snodgrass 2005; Portilla et al. 2016). Investigations are underway to measure the impact of the native strain NI8 on *L. lineolaris* populations in wild hosts and crops in the Mississippi Delta. Portilla (2014) found under laboratory conditions that the Mississippi Delta native strain NI8 *B. bassiana* isolated from *L. lineolaris* can kill predators such as minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), jumping spiders (Aranea: Salticidae), and crab spiders (Aranea: Thomosidae), but that LC_{50} values were greater than 3- to 90-fold those needed to kill the tarnished plant bug, *L. lineolaris*. Knowledge of the impact of *B. bassiana* on target and non-target insects is critical for potential registration and expanded use of this fungus as a microbial control agent, especially as the targeted host may be located in ditches and field borders early in the growing season (Abel et al. 2007).

The green lacewing, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) is a polyphagous predator that has potential as a biological control agent against several species of pests including *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Breene et al. 1992), *Aphis gossypii* Glover (Hemiptera: Aphididae) (Nordlund & Morrison, 1990), *L. decemlineata* (Nordlund & Morrison, 1992), *Heliothis virescens* F. (Lepidoptera: Noctuidae) (Nordlund & Morrison 1990), and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) (Lingren et al. 1968). Adult lacewings are often found in high numbers in corn and cotton fields (Sheldon & Macleod 1971, 1974). However, to the best of our knowledge, no research has examined the effect of *B. bassiana* on adults of this predator. The purpose of this study was to measure the impact of atomized sprays of the entomophatogenic fungus *B. bassiana* strain NI8 on *C. rufilabris* by quantification of the lethal effects and sub-lethal impacts on reproductive rates by estimating LC_{50} (lethal concentration), SR_{50} (sporulation response), and ratio-response impacts. *Chrysoperla rufilabris* is one of the most common lacewings species preying on nymphs of *L. lineolaris* and many other insects in the Mississippi Delta. Sprays of *B. bassiana* targeted at *L. lineolaris* would likely expose this and other predators to the entomopathogenic fungus.

Materials and Methods

COLONIES OF *CHRYSOPERLA RUFILABRIS*

Chrysoperla rufilabris adults used in this study were obtained from a commercial supplier (Biocontrol Net Work, Brentwood, Tennessee). About 400 adults (2–3 d old) were received overnight. To ensure copulation, insects were maintained collectively in the original container obtained from the commercial supplier (3 L cylindrical cardboard carton covered with organdy cotton cloth). A sponge with sugar-water solution (10%) was placed in an 11 cm diameter Petri dish inside the cage. Insects were held in a growth chamber at 25 °C, 55% relative humidity (RH), and a photoperiod of 12:12 h L:D until first oviposition was observed.

CULTURE OF *BEAUVERIA BASSIANA* STRAIN NI8

The NI8 strain of *B. bassiana* was obtained from stored sources of spore powder maintained at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Southern Insect Research Unit (SIMRU). NI8 is produced at SIMRU regularly for the *L. lineolaris* research program (Portilla et al. 2016). The inoculum concentration (1.20×10^{11} spores per g) was suspended in 50 mL of 0.04% Tween-80 (Sigma-Aldrich P8074, St. Louis, Missouri) and diluted to obtain final concentrations of 7×10^7 spores per mL. The inoculum viability was measured according to the methodology of Portilla et al. (2014a, 2016). Lower test concentrations (7×10^4, 10^5, and 10^6) for this study were extrapolated based on dilution of the highest concentration (7×10^7). Resulting data were analyzed by analysis of variance (SAS 2013). Aliquots (6 mL) of the highest concentration suspension (7×10^7) provided 395 viable spores per mm^2 on the targeted sprayed area when applied using a Potter Precision Laboratory Spray Tower (Burkard Scientific, Uxbridge, UK) following the procedures of Portilla et al. (2014a).

BIOASSAY PROCEDURE

Serial dilutions of 4 test concentrations of NI8 strain (1.2×10^4, 10^5, 10^6, and 10^7 spores per mL) were prepared to treat *C. rufilabris* females and evaluate the effect of the NI8 strain on their reproductive rates. To avoid cross infection, only 4- to 5-d-old adult females received from the commercial supplier were used. Selected females were sprayed with NI8 using the direct inoculation method (atomized spray delivery) described by Portilla et al. (2014a, 2016). Treated insects were held in a growth chamber at 25 °C, 55% RH, and a photoperiod of 12:12 h L:D. Each assay treatment (individual concentration) was replicated 4 times with 10 adult females per replicate (200 females total). Control insects were sprayed similarly (12.5 kPa per $inch^2$) with 6 mL of water (water control). Treatments of NI8 concentrations (1.20×10^4, 10^5, 10^6, and 10^7 spores per mL) were similarly delivered in a 6 mL spray volume of *B. bassiana* solution. After application, *C. rufilabris* females were placed individually into a 29.7 mL cups with a solid diet developed for *L. lineolaris* bioassays (Portilla et al. 2014a). No additional food source was provided. Females were examined daily for mortality and oviposition. The numbers of eggs oviposited every day by each female were counted. Females with eggs were removed and placed in a new diet cup until the last female died. Dead insects were retained in individual diet cups for 10 d and were observed daily for sporulation.

REPRODUCTIVE RATES OF *C. RUFILABRIS*

Fertility life tables were calculated according to Portilla et al. (2014b). Life fertility tables were determined by selecting age class (x) and the number of females surviving to age x (N_x). Using these parameters the following model was determined: $l_x = N_x/N_o$ (where l_x = the proportion of females surviving to age x, and where N_o = the number of initial females) (Carey 1993). The daily calculation of age-specific survival rate (l_x) and age specific fecundity (m_x) was used to estimate net reproductive rate ($R_o = \Sigma^{\beta}_{x=\alpha} l_x m_x$), doubling time (DT = 1n(2)/r_m), mean generation time ($R_o = \Sigma^{\beta}_{x=\alpha} l_x m_x / \Sigma^{\beta}_{x=\alpha} x l_x m_x$), intrinsic rate of increase ($r_m = \Sigma^{\beta}_{x=\alpha} e^{-r(x+0.5)} l_x m_x = 1$) and finite rate of increase ($\lambda = e^{-r_m}$) (Carey 1993; Krebs 2001). Calculations were done by assuming a 1:1 sex ratio, an immature survival of 0.7 (due to their cannibalistic nature) and a developmental time (egg to adults) of 25 d based on quality assessment data obtained from *C. rufilabris* producers in California (Silvers et al. 2002) and published work by Nordlund & Morrison (1990, 1992), Legaspi et al. (1994), and Giles et al. (2000). By using the fertility tables, reproductive values were calculated, which is defined as the contribution in population numbers that 1 newly hatched individual will make over the remaining life of the female where *y* and *x* are age and *w* is the age of the last successful reproduction (Krebs 2001).

STATISTICAL ANALYSES

One-way ANOVA followed by the Tukey Honest Significant Difference test was used to compare fertility table parameters on *C. rufilabris* sprayed with different *B. bassiana* concentrations. Non-parametric estimates of the survival function of *C. rufilabris* females were compared between treatments by using PROC LIFETEST procedure in SAS (SAS 2013). Statistical differences in the survival of *C. rufilabris* females were declared based on the log-rank statistic and by using the PROC GLM procedure to detect differences between concentrations at 3, 5, and 10 d after application. Mortality and sporulation data for each group of *C. rufilabris* females and each concentration were analyzed by PROBIT (SAS 2013) using common logarithm (log to the base 10) of the concentration value.

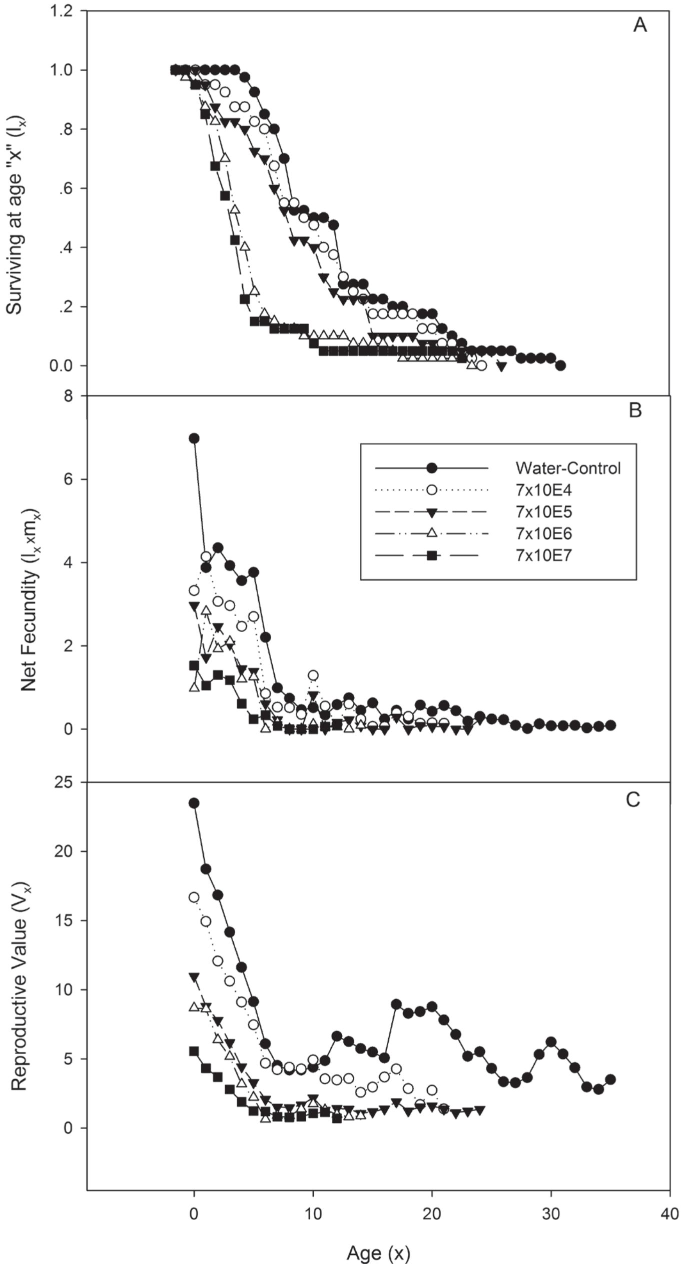

Fig. 1. Fertility table data and reproductive values of *Chrysoperla rufilabris* females exposed to *Beauveria bassiana* strain NI8 at different concentrations (spores per mm^2) under laboratory conditions. Insects were fed with a *Lygus* species solid diet after being sprayed with fungus. A. Survival probability at age x (l_x) (p = 0.05, LIFETEST of Equality over Strata); B. Net fecundity ($l_x m_x$); and C. Reproductive value (V_x).

Results

TIME-MORTALITY RESPONSE OF *C. RUFILABRIS* TO *B. BASSIANA* STRAIN NI8

The survivorship of *C. rufilabris* females treated at different concentrations of *B. bassiana* strain NI8 is shown in Fig. 1A. Survival was measured through daily post-treatment observations until all females died. Survival rates of treated females varied among the 4 *B. bassiana* concentrations, where those at higher concentrations died faster than those at lower concentrations. The earliest mortality recorded was observed at the highest concentration (7×10^7) followed by 7×10^6 at 2 and 3 d after treatment, respectively. Mortality at lower concentrations (7×10^4 and 7×10^5) was recorded 4 and 5 d after treatment, yet the first mortality in the water control was not recorded until 9 d after application. Mortality analyzed by the test of equality with the strata statement in −log (survival probability) PROC LIFETEST indicated significant differences among concentrations (Log-Rank X^2 = 23.99, df = 4, $p < 0.0001$) (Fig. 1A).

MORTALITY-RESPONSE AND SPORULATION RESPONSE OF *CHRYSOPERLA RUFILABRIS* TO *B. BASSIANA* STRAIN NI8

The *B. bassiana* strain NI8 was pathogenic to *C. rufilabris* females. However, the levels of mortality and resulting sporulation in cadavers were highly variable between concentrations (Fig. 2). Mortality 3 d af-

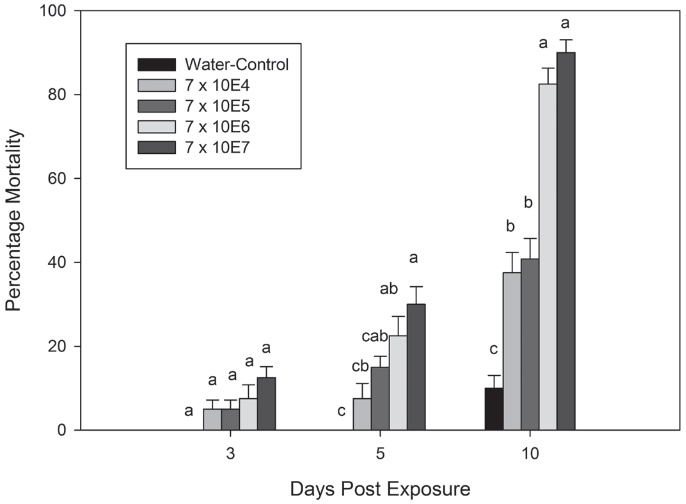

Fig. 2. Cumulative mortality of *Chrysoperla rufilabris* females at 3, 5, and 10 d exposed to *Beauveria bassiana* strain NI8 at different concentrations (spores per mm^2) under laboratory conditions. Insects were fed with a *Lygus* species solid diet after being sprayed with fungus. Columns within the group labeled with a different letter were significant different at $P = 0.05$ (Tukey Honest Significant Difference test).

ter spray at the lowest concentration (7×10^4) was 2.5-fold lower than that observed at the highest concentration (7×10^7), but no significant differences were found among those insects exposed to water alone ($F = 1.47$, df = 4, 199; $p = 0.2135$). Mortality at 5 d ($F = 4.73$, df = 4, 199; $p = 0.0012$), and 10 d ($F = 1.47$, df = 4, 199; $p < 0.0001$) after spray was significantly different among treatments. The percentage of individuals resulting in sporulating cadavers at 10 d was significantly affected by spore concentration ($F = 43.34$, df = 4, 199; $p < 0.0001$). Sporulation increased with concentration tested (Table 1). Time of sporulation after death was significantly different among treatments ($F = 57.87$, df = 4, 115; $p < 0.0001$). Sporulation took longer at lower concentrations. Analyses of concentration–mortality and sporulation responses are shown in Table 2. *Chrysoperla rufilabris* females were highly affected at low concentrations of *B. bassiana* (LC_{50} = 2.11 viable spores per mm^2); higher concentration were needed for sporulation (SR_{50} = 3.60 viable spores per mm^2).

EFFECTS OF *B. BASSIANA* NI8 STRAIN ON THE FERTILITY TABLE PARAMETERS OF *C. RUFILABRIS*

All demographic measurements for *C. rufilabris* females obtained from the water controls were significantly higher than those for insects treated with different concentrations of *B. bassiana* except for doubling time (DT) ($F = 2.13$, df = 4, 19; $p = 0.1402$) which, did not differ among treatments. However, high variation among treatment values of DT were observed (Table 3). Water control females doubled their populations in 6.28 ± 0.39 (SE) d and females sprayed with the highest concentration of *B. bassiana* doubled their population in 20.11 ± 17.21 (SE) d. Total egg production varied among the 4 test concentrations ($F = 18.13$, df = 4, 19; $p < 0.0001$). Egg production from females exposed to water control (822.25 ± 141.70 [SE] eggs per 10 females) was 1.68-fold (488.5 ± 156.74 [SE]) and 7.18-fold (114.5 ± 70.35 [SE]) greater than those obtained from females exposed to the lowest and highest concentrations of *B. bassiana*, respectively. The highest intrinsic rate of increase (r_m) was found in females sprayed with water alone (0.111 ± 0.003 [SE]) and r_m values varied significantly among treatments ($F = 10.41$, df = 4, 19; $p = 0.0007$). Daily rate of increase of 1.12 females per female per d, a doubling time of 6.28 ± 0.39 (SE), a gross fecundity (R_o) of 127.15 ± 44.39 (SE) for female and male eggs per female, and a mean generation time (T) of 31.89 ± 1.81 (SE) d were observed for females exposed to water alone. The mean generation time of females from the water control was significantly higher than all other treatments ($F = 3.41$, df = 4, 19; $p = 0.0289$) with a prolonged mean age of reproduction of about 1 and 4 d longer than that of females sprayed with lowest and highest concentrations of *B. bassiana,* respectively. Females sprayed with the lowest concentration had a gross fecundity (R_o) of 66.47 ± 31.19 (SE) eggs per female; those sprayed with the highest concentration had a gross fecundity of 17.51 ± 9.39 (SE) eggs per female. Significantly shorter longevity also was found ($F = 21.90$, df = 4,199; $p < 0.0001$) in treated insects. Females sprayed with the highest concentration lived 6 d shorter than those females sprayed with the lowest concentration and 13 d shorter than females sprayed with water alone (Table 3). Figure 1A, B, and C showed that trends of survival (l_x), fecundity function ($l_x m_x$), and reproductive values (V_x) were inversely related to spore concentrations. Higher concentrations resulted in lower survival and reproduction.

Discussion

The significant differences in −log survival probability among concentrations indicated that *C. rufilabris* females obtained lethal concentrations of conidia directly from the *B. bassiana* spray (Fig. 1A). Low mortality and survival noted for insects in the water controls suggests that the *Lygus* species diet (Portilla et al. 2014a) may be an acceptable diet for rearing *C. rufilabris* females. Preliminary assays (data not shown) indicated that this predator survived better on the *Lygus* diet than when females were fed individually with a nutrient-rich slurry consisting of brewer's yeast, sugar, and water (1:1:1) (Cohen & Smith 1998). Cohen (1993, 1995) explained the extra-oral digestive nature of feeding by Neuropteran predators; predators such as *C. rufilabris* thrive on solid lipid- and protein-rich diets. Portilla et al. (2016) similarly demonstrated that the *Lygus* diet facilitated a comparison of pathogenesis and sporogenesis phases of 3 *B. bassiana* strains tested against *Megacopta cribraria* F. (Heteroptera: Plataspidae).

Mortality and sporulation are the main evaluation factors used to determine levels of *B. bassiana* pathogenicity (Portilla et al. 2016). Results presented in this investigation indicated that under laboratory conditions *C. rufilabris* adult females are highly susceptible to

Table 1. Mean (± SD) percentage sporulation in *Chrysoperla rufilabris* sprayed with 4 concentrations of *Beauveria bassiana* strain NI8 and fed with a solid *Lygus* species diet.

	Beauveria bassiana concentrations (spores per mm^2) (means ± SD)				
Variable	Water control	7×10^4	7×10^5	7×10^6	7×10^7
Sporulation (%)	0 ± 0d	17.50 ± 3.84dc	22.50 ± 4.22c	67.96 ± 4.74b	90.00 ± 3.03a
Sporulation after dead (d)	0 ± 0a	3.71 ± 1.38b	3.44 ± 1.13c	2.25 ± 1.28b	2.17 ± 0.87c

Means ± standard deviation (SD) followed by the same letter in each row are not significantly different ($P < 0.05$ Tukey Honest Significant Difference test).

Table 2. Mortality-response (LC$_{50}$) and sporulation-response (SR$_{50}$) of adult female of *Chrysoperla rufilabris* treated with *Beauveria bassiana* strain NI8 applied at 4 concentrations (± 95% CI [confidence interval]).

	Concentration response (spores per mm^2)				
Variable	Slope ± SE	LC$_{50}$/SR$_{50}$ (95% CI)	Slope[a]	GoF[b]	Ratio response
Mortality	0.271 ± 0.047	2.110 (0.252–10.832)	$X^2 = 33.560$, $P < 0.0001$	$X^2 = 1.1013$, $P = 0.3551$	1.000
Sporulation	0.396 ± 0.056	13.595 (3.397–49.025)	$X^2 = 48.960$, $P < 0.0001$	$X^2 = 0.9695$, $P = 0.4718$	6.443

[a] Test for slope, significance indicates concentration affects mortality or sporulation (SE = standard error)
[b] Test for goodness of fit (GoF), significance indicates error from Probit trend is greater than expected for simple binomial response

B. bassiana infection by direct exposure. Infectivity and sporulation of entomopathogenic fungi has been shown to increase under high humidity in field, laboratory, and green house conditions (Barson 1976). However, the number of conidia acquired by the host is probably the key factor that increases propagation of conidia by sporogenesis. Mortality and sporulation levels gradually increased when concentrations of conidia increased, even when the humidity condition that occurred in the closed diet cup (> 80% RH) was consistent for all treatments (Table 1; Fig. 2). The LC$_{50}$ reported in this study (Table 2) showed that *C. rufilabris* mortality could be affected at very low concentrations of *B. bassiana* strain NI8 (2.11 viable spores per mm^2), which is comparable to that found for *L. lineolaris* using the same strain (2.75 viable spores per mm^2) (Portilla 2014). Both *C. rufilabris* and *L. lineolaris* need higher concentrations of conidia for sporulation (SR$_{50}$), but those needed for sporulation in *C. rufilabris* (13.60 spores per mm^2) were 5.4-fold greater than those needed for *L. lineolaris* (5.81 spores per mm^2) (Portilla 2014). Other chrysopids (Neuroptera: Chrysopidae) including *Chrysoperla externa* Hagen (Pessoa et al. 2005), *C. carnea* (Thungrabeab & Tongma, 2007), and *Chrysoperla exterior* (Navás) (Leyva et al. 2011) also have shown to have concentration-dependent responses to entomopathogenic fungi. Pessoa et al. (2005) observed that *C. externa* third instar larvae were affected by suspensions of 1.0 × 10^7 conidia mL of *B. bassiana*; but, there was no fungal effects on egg viability or developmental time of first and second instar larvae. Leyva et al. (2011) obtained similar results when *C. exterior* was exposed to different concentrations of *B. bassiana*. No significant effects were measured with 1 × 10^6 and 1 × 10^7 on any immature stages, but adults showed 10% mortality 4 d after application with concentrations of *B. bassiana.*

Measurements of fundamental reproductive components are essential for understanding the population dynamics of *C. rufilabris* when exposed to *B. bassiana*. Based on the present results, applications of *B. bassiana* to *C. rufilabris* adult females will decrease survival and reproduction. Exposure to higher concentrations will exhibit greater effects (Fig. 1A, B, C; Table 3). According to Donegan (1989) temperature, starvation, and nutrition stresses significantly affect susceptibility of *C. carnea* to *B. bassiana*, but nutrition is the most important. With the present study, it should be noted that the use of *Lygus* diet in this research could impact some aspects of *C. rufilabris* female biology and behavior such as longevity and estimates of production. However, results in this study were comparable to those on the quality assessment of *C. rufilabris* producers in California (Silvers et al. 2002), where a female fed with artificial diet deposited more than 200 eggs in her 4 to 6 wk lifespan under laboratory conditions, which is similar to the gross fecundity of 127.15 eggs per female obtained in an approximate 4 wk period (25.17 ± 8.95 SE d) for the water control. It should also be noted that the egg production in the present study was obtained from females that were exposed to males only from emergence to the mating period (2 d after received from commercial supplier). This could explain the shorter longevity obtained in infected females.

In general, the reproductive estimates shown here assumed a hypothetical cohort subjected throughout its lifetime from egg to adult females mortality that could be measured for an actual population of *C. rufilabris* exposed to the entomopathogenic fungus *B. bassiana*. The speed at which a population increased (r_m) is the most important parameter (Carey 1993; Krebs 2001) and *C. rufilabris* individuals in the water control obtained the highest intrinsic rate value (0.111). The r_m calculation of *C. rufilabris* agrees closely with Jokar & Zarabi (2012)

Table 3. Life table statistic for *Chrysoperla rufilabris* sprayed with *Beauveria bassiana* strain NI8 at different concentrations and fed with a *Lygus* species solid diet.

	Beauveria bassiana concentrations (spores per mm^2) (means ± SE [standard error])				
Statistic	Water control	7 × 10^4	7 × 10^5	7 × 10^6	7 × 10^7
Longevity (after treated)	25.17 ± 8.84a	18.65 ± 6.67b	18.85 ± 6.85b	13.02 ± 5.78c	12.30 ± 5.95c
Total egg[a]	822.25 ± 141.70a	488.50 ± 156.74b	297.75 ± 44.46bc	199.00 ± 96.35bc	114.50 ± 70.35c
Gross fecundity[b]	127.15 ± 44.39a	66.47 ± 31.19ab	39.59 ± 17.54b	28.43 ± 15.38ab	17.51 ± 9.39b
Net fecundity[c]	63.57 ± 22.19a	33.23 ± 19.60ab	19.79 ± 8.02b	14.21 ± 8.69ab	8.76 ± 5.19b
Net reproductive rate[d]	28.82 ± 4.49a	17.09 ± 6.41b	10.42 ± 1.78cb	6.96 ± 3.32cb	4.08 ± 2.32c
Mean generation time[e]	31.89 ± 1.81a	30.02 ± 2.15ab	29.74 ± 2.09ab	28.41 ± 0.55ab	27.22 ± 0.48b
Doubling time[f]	6.28 ± 0.39a	7.87 ± 1.76a	8.83 ± 0.12a	12.26 ± 3.65a	20.57 ± 17.21a
Intrinsic rate of increase[g]	0.111 ± 0.003a	0.091 ± 0.018ab	0.078 ± 0.001abc	0.062 ± 0.020bc	0.048 ± 0.020c
Finite rate of increase[h]	1.120 ± 0.007a	1.090 ± 0.010ab	1.080 ± 0.001bac	1.060 ± 0.022bc	1.050 ± 0.025c

Means **±** SE (standard error) followed by the same letter in each row are not significantly different (*P* < 0.05 Tukey Honest Significant Difference test)
[a] 10 females per replicate
[b] Total offspring per female
[c] Females per female at age x
[d] Daughters per newly hatched female
[e] Mean age of reproduction (d)
[f] Time required for (λ) to doubling number
[g] Rate of natural increase (daughters per female per d)
[h] Individuals per female per d

when *C. carnea* was reared under laboratory conditions (r_m values of 0.074, 0.162, and 0.185) and fed with different media. Amarasekare & Shearer (2013) reported similar r_m values calculated for *C. carnea* and *Chrysoperla johnsoni* Henry (Neuroptera: Chrysopidae) of 0.161 and 0.132, respectively.

This laboratory experiment provides information needed to understand the effect of *B. bassiana* on *C. rufilabris*. Strain NI8 affects this predator by direct mortality effects and indirect reproductive impacts. The r_m values reported in this study may vary under field conditions, where chrysopids directly interact with pests and the environment. Further studies are under way that will examine the pathogenicity of *B. bassiana* strain NI8 to predator and other non-targets arthropods under field conditions. Decisions to deploy NI8 as a biological control for *L. lineolaris* in different host environments should be based on an overall assessment of ecological and economic benefits and costs.

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