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Effects of *Beauveria bassiana* on the growth and reproductive rates of *Nezara viridula*

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Abstract A laboratory colony of the southern green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae) was used to evaluate the effect of two strains of *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae) on its growth and reproductive rates. Four concentrations (10^6, 10^7, 10^8, and 10^9 conidia g^{-1}) of native (NI8) and commercial (GHA) entomopathogenic fungi alongside a water control were used. Cumulative oviposition and survival of nine groups (ten couple per group per concentration) were used to calculate the demographic parameters of this insect. Two computations were done based on total offspring (fertile and infertile eggs) and eggs with developed embryo (fertile eggs). Net reproductive rates (R_o) on insect sprayed with NI8 calculated based on total offspring showed a dose-dependent effect (72.55, 85.50, 58.15, and 37.60 females per newborn female) compared with water control (87.65). In populations sprayed with GHA, only the highest concentration (10^9 conidia g^{-1}) was lower than control (27.15). The calculated R_o values based on fertile eggs were much lower as it was expected with 60.75, 61.45, 45.45, and 32.05 females per newborn female from lowest to highest concentrations of NI8 and 21.50 for GHA highest concentration compared with water control (78.45). These results demonstrated that both native and commercial strains affected embryo development, decreasing growth and reproductive rates of *N. viridula* populations. Further field testing is needed to evaluate the potential for in-field control.

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Introduction

The southern green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae) is a destructive and highly polyphagous global pest that causes severe damage to several agricultural crops, including peanut, *Arachis hypogaea* L., soybean, *Glycine max* L (Fabales: Fabaceae), tomato, *Solanum lycopersicum* L. tobacco, *Nicotiana tabacum* L., potatoes, *Solanum*

tuberosum L. (Solanales: Solanaceae), wheat, *Triticum aestivum* L., rice, *Oryza sativa* L., corn, *Zea mays* L. (Poales: Poaceae), cotton, *Gossypium arboretum* L. (Malvales: Malvaceae), and all cruciferous vegetables (Musolin 2012). Both nymphs and adults cause damage piercing and sucking part of the host plant tissues and developing fruits, which reduce photosynthesis and impedes plant growth (Esquivel et al. 2018). The potential for transmission of fungal and bacterial diseases directly to the plant's vasculature system during feeding is heightened and often detrimental to yield, grain, and seed quality (Yukawa and Kiritani 1965; Esquivel and Medrano 2020). Combined, these factors continue to negatively impact the production of crop-derived food, feed, and fuel around worldwide.

Nezara viridula is widely established and distributed throughout tropic and subtropic areas (Esquivel et al. 2018) and still spreading to new locations (Esquivel and Medrano 2020). Worldwide control measures are based on chemical tactics contributing to insecticidal resistance and outbreak populations (Knight and Gurr 2007; Permadi et al. 2018). Infestations of *N. viridula* seasonally occur in the most valuable commodities including cotton and soybean across the Southern USA now require multiple insecticide applications during a growing season (Portilla et al. 2022a). Significant increases in insecticidal applications are costly for producers and may also have unintentional ecological impacts including mammalian toxicity and non-target effects on communities of beneficial insects (Esquivel et al. 2018). New approaches for managing the pest stink bug complex including *N. viridula* that are ecologically friendly are needed. The use of the entomopathogenic fungi such us *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae) has been used historically with successful integration in modern IPM in several integrated pest management programs where the climate conditions were favorable for fungal developments, with high susceptibility of the insect target, and a better conidium acquisition of the conidia from contact rather than direct spray. This is the case, for example, for the integrated management of the coffee berry borer, *Hypothenemus hampei* F. (Coleoptera: Curculionidae) in Colombia (Bustillo and Posada-Florez 1996) and Hawaii (Aristizabal et al. 2016). In these cases, the programs were developed after expending considerable efforts

in screening candidate fungi, emphasizing virulence and spore production (Bustillo and Posada-Florez 1996). However, these variables will not be enough to develop a program for *N. viridula*, that is well known for its highly resistance to pathogenic fungi, high mobility, and frequently molting (Sosa-Gomez et al. 1997; Nada 2015; Gad and Nada 2020; Portilla et al. 2022a, b; Soliman et al. 2022).

Although there are many limitations to using *B. bassiana* to control *N. viridula*, a recent study demonstrated that *N. viridula* immature stages could easily acquire spores from treated surfaces and through direct spray (Portilla et al. 2022a). Portilla et al. (2022b) also demonstrated that high concentrations of native (NI8) (10^8 and 10^9 conidia g^{-1} and a commercial (GHA) (10^9 conidia g^{-1}) strains of *B. bassiana* affected its fecundity. Although no significant differences in male survival were found among concentrations, they reported that females were much more susceptible than males. Moreover, several investigations have found effects of *B. bassiana* on insect populations dynamics including significant reduction in lifetime fecundity, egg hatchability and viability, and reduce fecundity of insect hosts (Noma and Strickler 2000; Fernandez-Ruvalcaba et al. 2010; Ugine 2012). Therefore, the objective of this study was to quantify the embryo development from the cumulative oviposition of *N. viridula* treated with different concentration of native (NI8) and commercial (GHA) strains of *B. bassiana*. Demographic parameters, in addition to growth and reproductive rates, were calculated based on total offspring and fertile eggs. This is the first study that estimate the reproductive rates of infected *N. viridula* populations with *B. bassiana* and compares the fecundity and fertility values to quantify its natural growth and reproduction after *B. bassiana* infection.

Materials and methods

Insects

A laboratory-reared *N. viridula* colony has been maintain at the United State Department of Agriculture, Agriculture Research Service (USDA-ARS), Southern Insect Management Research Unit (SIMRU) in Stoneville, MS, USA since 2018. This colony is regularly maintained following procedures

outlined by Portilla and Reddy (2021), which was designed for medium-scale production of even-aged individuals. This study used a F_n generation of female and male adults 3–5 d old fed with an artificial diet (Portilla and Reddy 2021).

Bioassay procedure

The application of *B. bassiana*, and bioassay procedures were conducted following processes described in Portilla et al. (2022b). Briefly, technical spore powder (10^6, 10^7, 10^8, and 10^9 conidia g^{-1}) of the native strain NI8 (collected, isolated, and genetically identified in 2005 (McGuire et al. 2006) and produced since then at USDA-ARS Southern Insect Management Research Unit) and the commercial strain GHA (Botani-Gard 22WP) were diluted to obtain concentrations of 5×10^4, 5×10^5, 7×10^6, 7×10^7 conidia ml^{-1}. Aliquots of 6 ml of each spore suspension and water control were applied to groups of ten adult females and ten adult males. The suspensions of both strains were sprayed from the lowest to the highest concentrations using a spray tower modified for Burgerjon tower (Portilla et al. 2022a, b). Conidia mm^{-2} were quantified by counting spores dropped on disposable microscope cover slips of 2.2 cm^2 (S1752) placed under the sprayer during treatment applications. After each application, the nozzles were rinsed once with 10 ml of 10% hypochlorite solution and twice with 10 ml of reverse-osmosis water. Spray nozzles were changed for each strain to avoid cross-contamination. Treated insects (females and males) were released into an insect rearing cage (30×30 cm) (1466 PB) (ten cages: ten couples per cage) and placed in environmental chambers (model I36VLC8) set at $27 \pm 2C$, $55 \pm 10\%$RH, and a L:D 12:12 photoperiod (environmental conditions ideal for *N. viridula* rearing and reproduction). Insects were fed with artificial diet and kept for oviposition until all adults were dead. Diet was changed twice a week or when needed. The total eggs production of the nine groups (ten couples per group) sprayed with the four concentrations of native (NI8) and commercial (GHA) strains of *B. bassiana* including water control and their daily survival were used for growth and reproductive rates calculations.

Adult survival and egg collection

Eggs masses laid and pasted by the treated females to the wall of the oviposition cages (30×30 cm) were carefully removed every other day and total number of egg mases per cage, number of eggs per egg mass, number of eggs with developed embryo per egg mass, number of eggs with undeveloped embryo per egg mass, and number of eggs with no embryo per egg mass were recorded until all adults died. Adult mortality also was recorded every second day and female and male longevity was calculated. The Image Pro Plus 7.0.1 software was used for morphological differences for both sexes (males have claspers on the terminal abdominal segment), egg counting, and embryo recognition. From the total egg masses collected from each treated group, percentage of egg with undeveloped embryo and egg mass sizes were calculated. The morphological differences for both sexes were accessed to ensure that each treatment contain ten females and ten males.

Embryo recognition, classification, and eggs mass sizes of *N. viridula*

Egg masses collected from each treated group of adults were stored in plastic solo-cups T-125 with a modified lid (three small openings added to the lids for ventilation, 3–4 mm in diameter) and maintained in environmental incubators (I36VLC8) at 27 ± 2 °C, $75 \pm 10\%$ RH, and a L:D 16:8 photoperiod for 4–6 days until fully embryo developed. After 4–6 days of egg storage, the embryo recognition tests were performed according to the description in Portilla and Reddy (2021) as follow: (1) eggs with no embryo—translucent appearance due to the absence of the embryo, (2) undeveloped embryo—opaque-yellow color egg, and (3) developed embryo—orange to reddish coloration that resides primarily in the eye-spots formation and other body parts of the developing embryo. The Image Pro Plus 7.0.1 software was used for egg recognition. Two classified group of oviposition were created for each treated population: (1) total offspring and (2) eggs with developed embryo. Therefore, to evaluate the actual effect of both strains and concentrations of the entomopathogenic fungi, each oviposition group was used separately to

calculate each population's demographic parameters, growth, and reproductive rates. This was performed assuming that, using the total offspring for life fertility table calculation, the values could be greater than the group with fertile eggs only.

Demographic parameters of treated *N. viridula* populations

Over 14,000 eggs were collected and individually classified. A total of 18 fertility life tables were calculated using codified Excel spreadsheets (Portilla et al. 2014). Calculation of egg-specific survival rate (l_x) and age-specific fecundity (m_x) was used to estimate the gross fecundity rate (M_x), net reproductive rate (R_o), doubling time (DT), mean generation time (T), finite rate of increase (λ), and intrinsic rate of increase (r_m) which was obtained from the Lotka formula (Carey 1993; Krebs 2001). The adult life expectancy (e_x) and reproductive values (V_x) were calculated as an additional column in the life and fertility table according to Carey (1993). The method of trial value r was used, incorporating a sex ratio of 50:50 (F:M) and developmental time (egg-adult) of 37 days according to Portilla and Reddy (2021). The experiment was repeated one time and each couple per treatment (ten couples per treatment) were considered replications.

Sublethal and lethal dose of *N. viridula* populations exposed to *B. bassiana*

From the survival column (l_x) of the 18 fertility tables, mortality data from 15-, 20-, and 30-days post exposure (DPE) were used to estimate sublethal and lethal mortality of the total unsexed population. Estimations were done using spores mm^{-2}.

Statistical analysis

Total number of eggs per collection, number of fertile eggs per collection, eggs with undeveloped embryos per collection, size of egg masses, and female and male longevity were analyzed using ANOVA in SAS Institute (2013) followed by Tukey's test HSD. Slopes, sublethal (LC$_{15}$, LC$_{30}$), lethal concentrations (LC$_{50}$), and resistance ratios (RR$_{50}$) were calculated using the PROC PROBIT in SAS and using log$_{10}$ of the concentrations. Mortality for each treated group was corrected for control effects using Abbott's formula (Abbott 1925) and confidence intervals for RR$_{50}$ were calculated using the formula from Robertson and Priesler (1992).

Results

Longevity of *N. viridula* exposed to different concentrations of *B. bassiana*

There were no statistically significant differences in male longevity among concentrations sprayed with NI8 (Table 1). However, high significant differences in longevity were found for females sprayed with the same strain (F=15.19; df=4, 9; $p \leq 0.0001$). There were statistically significant differences in males and females sprayed with GHA strain with F=19.63 and 15.67, respectively (df=4, 9; $p \leq 0.0001$). There was no dose-dependent between concentration and longevity, but in females treated with the highest

Table 1 Female and male longevity of *N. viridula* sprayed with two strains: native NI8 and commercial GHA of *B. bassiana* at different concentrations and fed with an artificial diet and fresh corn

Adult-Strain	*Beauveria bassiana* concentrations—conidia g^{-1} Longevity ($\pm$SE) (Days)				
	Water control	10^6	10^7	10^8	10^9
Male—NI8	45.80 ± 6.02 a	34.60 ± 7.20 b	51.60 ± 8.36 a	49.10 ± 5.41 a	35.10 ± 6.32 b
Female—NI8	41.90 ± 4.96 a	47.20 ± 4.71 a	40.40 ± 5.50 a	32.60 ± 5.54 b	30.20 ± 4.44 b
Male—GHA	45.80 ± 6.02 a	45.10 ± 8.09 a	47.20 ± 7.09 a	44.00 ± 6.05 a	43.70 ± 9.97 a
Female—GHA	41.90 ± 4.96 abc	47.40 ± 3.81 ab	51.90 ± 5.51 a	35.40 ± 7.35 bc	38.64 ± 6.84 bc

Means $\pm$ SE within row followed by the same letters are not significantly different among treatments (concentrations) (Tukey test; $p < 0.05$)

Table 2 Egg mass size and eggs fertility of *N. viridula* sprayed with two native and commercial strains of *B. bassiana* at different concentrations. Adults were fed with an artificial diet and fresh corn

Parameter	Concentrations (spores ml^{-1})					ANOVA
	Water control	10^6	10^7	10^8	10^9	
NI8 Strain						
Egg mass size	60.83 ± 4.47 a	52.03 ± 3.14 ab	49.72 ± 4.01 ab	53.71 ± 5.89 ab	43.07 ± 5.38 b	$F_{4, 133} = 2.14$; $p = 0.0797$
Total eggs per col[1]	109.56 ± 19.05 a (16)[2]	103.64 ± 18.79 a (14)	114.66 ± 21.44 a (15)	116.30 ± 23.56 a (10)	107.42 ± 34.63 a (7)	$F_{4, 15} = 0.89$; $p = 0.4782$
Fertile eggs per col	98.06 ± 16.42 a	85.21 ± 16.41 a	78.60 ± 16.52 a	81.00 ± 20.14 a	75.57 ± 28.81 a	$F_{4, 15} = 1.31$; $p = 0.2823$
Unfertile eggs per col	11.50 ± 3.12 b	18.42 ± 5.55 ab	36.10 ± 8.68 a	35.30 ± 8.76 a	31.85 ± 6.26 a	$F_{4, 15} = 2.60$; $p = 0.0497$
GHA Strain						
Egg mass size	60.83 ± 4.47 ab	62.17 ± 3.65 a	56.74 ± 4.32 ab	46.16 ± 4.94 b	46.07 ± 8.35 b	$F_{4, 156} = 2.51$; $p = 0.0442$
Total eggs per col	109.56 ± 19.05 b (16)	178.00 ± 29.92 a (16)	113.56 ± 21.31 b (18)	106.31 ± 20.40 b (16)	90.50 ± 20.85 b (6)	$F_{4, 17} = 2.94$; $p = 0.0293$
Fertile eggs per col	98.06 ± 16.42 ab	141.25 ± 26.04 a	85.33 ± 20.49 ab	72.93 ± 15.00 b	61.66 ± 19.13 b	$F_{4, 17} = 3.70$; $p = 0.0103$
Unfertile eggs per col	11.50 ± 3.12 b	36.75 ± 7.07 a	28.22 ± 3.33 ab	33.37 ± 10.63 ab	28.83 ± 2.86 ab	$F_{4, 17} = 3.84$; $p = 0.0084$

Means $\pm$ SE within rows followed by the letter are not significantly difference (Tukey test; $p = 0.05$)

[1] Egg collections obtained every other day until all females die (ten couples)

[2] Number of collections

concentrations (10^8 and 10^9 conidia g^{-1}) of both NI8 and GHA lived shorter than the control, and only GHA highest concentration (10^9 conidia g^{-1}) affected male longevity (Table 1).

Egg mass sizes, total number of eggs, fertile eggs, and eggs with undeveloped and no embryo of *N. viridula* populations exposed to different concentrations of *B. bassiana*

There were statistically significant differences in the number of egg per egg mass among concentrations for NI8 (F = 2.14; df = 4, 133; $p \leq 0.0797$) and GHA (F = 2.51; df = 4, 159; $p \leq 0.0442$). Table 2 shows that the lowest concentration (10^6 conidia g^{-1}) of GHA (62.17 ± 3.65 [SE] egg per mass) did not differ from the control (60.84 ± 4.47 eggs per mass) where both were larger than the other treatments. The smallest egg masses were obtained from couples sprayed with NI8 with the highest concentration (10^9 conidia g^{-1}) (43.07 ± 5.39 egg per mass), followed by insects sprayed with the GHA 10^8 and 10^9 conidia g^{-1} with 46.08 ± 29.67 and 46.17 ± 29.67 eggs per mass,

respectively. Similarly, Table 2 shows that although there were not statistically significant differences between couples sprayed with NI8 on the total number of eggs and number of fertile eggs per collection, there was a dose-dependent effect. Statistically significant differences among concentrations were observed for the total eggs and fertile eggs on couples treated with GHA, and eggs with undeveloped embryo on couple spray with NI8 and GHA strains (Table 2). The lowest number of eggs with undeveloped embryo was obtained in water control (11.50 ± 3.12) followed by 18.42 ± 5.55, 36.07 ± 8.68, 35.30 ± 8.76, and 31.86 ± 6.26 for NI8 and 36.75 ± 7.07, 28.22 ± 3.33, 33.37 ± 10.63, and 28.83 ± 2.86 for GHA from lowest to highest concentrations, respectively.

Total offspring, demographic parameters, and growth and reproductive rates of *N. viridula* treated with different concentration of *B. bassiana*

Nezara viridula sprayed with the native strain NI8

All demographic parameters were affected by NI8 applications at all concentrations using both

Table 3 Growth rate statistics for *N. viridula* sprayed with *B. bassiana* NI8 strain at different concentrations and fed with an artificial diet and fresh corn. Calculations were done using total offspring (CTO) and eggs with developed embryo (CDE)

Parameters	*Beauveria bassiana* concentrations—conidia g^{-1} (conidia ml^{-1}) (conidia mm^{-2})									
	Water control		10^6 (5×10^4) (22)		10^7 (5×10^5) (77)		10^8 (7×10^6) (159)		10^9 (7×10^7) (356)	
	CTO	CDE	CTO	CDE	CTO	CDE	CTO	CDE	CTO	CDE
Total offspring (ten couples)	1753	1569	1551	1215	1710	1229	1163	909	752	541
M_x (offspring per female)[1]	246.06	218.59	189.97	163.04	226.13	169.46	161.21	121.76	114.25	97.00
m_x (females per female)[2]	123.03	109.30	94.99	81.52	113.07	84.73	80.61	60.88	57.13	48.50
R_o (Daughter per new-born female)[3]	87.65	78.45	72.55	60.75	85.50	61.45	58.15	45.45	37.60	32.05
T (d)[4]	60.06	59.97	62.35	62.77	58.19	60.13	54.65	54.14	57.53	57.43
DT (d)[5]	8.70	8.94	9.25	9.71	8.56	9.64	9.00	9.63	10.74	11.26
r_m (GCER[6] per individuals per day)[7]	0.079	0.077	0.074	0.071	0.081	0.072	0.077	0.072	0.064	0.062
λ (Individuals per female per day)[8]	1.083	1.081	1.077	1.074	1.084	1.075	1.080	1.075	1.067	1.063

[1]Gross fecundity

[2]Net fecundity

[3]Net reproductive rate

[4]Mean generation time

[5]Doubling time

[6]Growth constant exponential rate

[7]Intrinsic rate of increase

[8]Finite rate of increase

calculations of total offspring and eggs with developed embryo (Table 3). The M_x, m_x, R_o, r_m, and λ values had a negative correlation with concentration. As expected, the demographic parameter values with the total offspring calculation were greater than that of egg with developed embryo calculation suggesting that the population treated with *B. bassiana* NI8 reduced *N. viridula* net reproductive rate (R_o) from 17% (72.55 female per new-born female) with the lowest concentration (10^6 conidia g^{-1}) to 57% (37.60 females per new-born female) with the highest concertation (10^9 conidia g^{-1}) compared to control (water) (87.65 females per new-born female). These values were even lower when the eggs with undeveloped or no embryo were removed from the calculation. Therefore, the true R_0 value was reduced from 33% (60.75 females per new-born female) with the lowest concentration to 60% (32.05 females per new-born female) with the highest concentration compared

with control (78.45 female per new-born female). No correlation was observed on T and DT when calculations were done with either total offspring or eggs with developed egg (Table 3), but adults sprayed with the highest concentration duplicate its population (11.26 d) almost three days slower than the control (8.94 d). The total population's life expectancy (e_x) of the total population (Fig. 1a) showed a high variability between control and the infected populations. At the same time, the calculation of the e_x of the females (Fig. 1b) showed the susceptibility of females except for one of the lower concentrations (10^7 conidia g^{-1}) that was higher than the control. The calculation of the gross fecundity (M_x) and reproductive value (V_x) are presented in Figs. 1cdef. Results demonstrated how the population of undeveloped and no embryo from the total offspring impacted *N. viridula* growth and reproductive rates as shown in Fig. 1c vs. Fig. 1d (M_x) and Fig. 1e vs. Fig. 1f (V_x).

Nezara viridula sprayed with the commercial strain GHA

Compared to NI8, all demographic parameters were affected by GHA applications only with the highest concentration (10^9 conidia g^{-1}) using both the total offspring calculation and the eggs with the developed embryo calculation (Table 4). The m_x, R_o, r_m, and λ values were affected with concentrations of 10^8 and 10^9 conidia g^{-1}. Unexpectedly, the demographic parameters values of the couples sprayed with the lowest concentrations (10^6, 10^7 conidia g^{-1}) were higher than the control. However, and similarly to NI8, the values calculated using the total offspring were greater than those of the egg with developed embryo calculation. The population treated with GHA 10^9 conidia g^{-1} reduced *N. viridula* net reproductive rate (R_o) to 69% (27.15 females per new-born female) that increased to 73% (21.5 females per new-born female) when removed from the calculation eggs with undeveloped and no embryo (Table 4). Both GHA calculations were higher than NI8 (57% and 60%, respectively) at the highest concentration. Like NI8, no correlation was observed on *T* and *DT* when calculations were done with either total offspring or eggs with developed eggs (Table 4). No difference in *T* and *DT* value was observed between GHA and NI8 in couples sprayed with the highest concentration: both groups duplicate their population three days slower (12.02 d) than the control (8.94 d). The life expectancy (e_x) of the total population (Fig. 1a) and the e_x of the females (Fig. 1b) showed similar trends among concentrations, suggesting that the commercial GHA will not kill *N. viridula* adults even with the highest concentration. However, and similarly to NI8, this commercial entomopathogenic fungi will affect reproduction and prevent embryo development which will reflect in its growth and reproductive rates mainly with the highest concentration as shown in Fig. 2c vs. Fig. 2d (M_x) and Fig. 2e vs. Fig. 2f (V_x).

Sublethal and lethal mortality of *N. viridula* to *B. bassiana* strains NI8 and GHA.

Although, no significant regression was obtained at any time of evaluation as LC_{15}, and LC_{50} was determined by PROC PROBIT (Table 5), results show that the unsexed population of *N. viridula* was more susceptible to the native NI8 than the commercial GHA. Sublethal and lethal mortality reduced over the post-exposure period for both strains, dropping

the LC_{50} from 10,443 conidia mm^{-2} (15 DPE) to 391 spores mm^{-2} (30 DPE) and from 10^6 conidia mm^{-2} (15 DPE) to 424 conidia mm^{-2} (30 DPE) for NI8 and GHA, respectively.

Discussion

Life and fertility tables constructions are typically used to determine the contribution to the future population that individual females will make (Carey 1993; Krebs 2001) varying depending on the population's exposure factors. In the case of *N. viridula*, the growth and intrinsic reproductive rates under field conditions are unknown. However, a few studies previously calculated its demographic parameters under laboratory conditions using host plants and an artificial diet (Fortes et al. 2006; Gonzales and Ferrero 2008; Portilla et al. 2015; Rojas and Morales-Ramos 2023). The r_m values (0.077–0.079) obtained from control (water) in our study using the rearing method from Portilla and Reddy (2021) differed from Portilla et al. (2015) (0.074) using an artificial diet but were similar to those found by Fortes et al. (2006) (0.076) who used green beans for *N. viridula* reproduction. R_o values, however, were much lower (87.65, 78.45) in our control than that in Portilla et al. (2015) (130.8) and Fortes et al. (2006) (132.7), but they were similar to the R_o value (117.5, 142.40) found in our infected population with the lower concentration of the commercial GHA. The rearing method using in this study was appropriate for *N. viridula* rearing and suitable to demonstrate the impact of *B. bassiana* on its reproduction. The interpretation of the fertility tables is the speed of a population increase, measured by λ and determined by r_m and estimated for future populations (Krebs 2001). In our case the fertility tables calculated using total offspring should represent this insect's true and ecological relevant growth and reproductive rates. However, it must be considered that a significant proportion of the total oviposition included eggs that do not survive to contribute to the future population (eggs with undeveloped or no embryo) even for control. The rearing method used in this study (Portilla and Reddy 2021) reported a rate of 13.49% of not fertile eggs, which was close to the rate that was observed in our control (10.10%) (Table 2). For example, NI8 reduced *N. viridula* R_o from 17% (72.55 female per new-born female) with
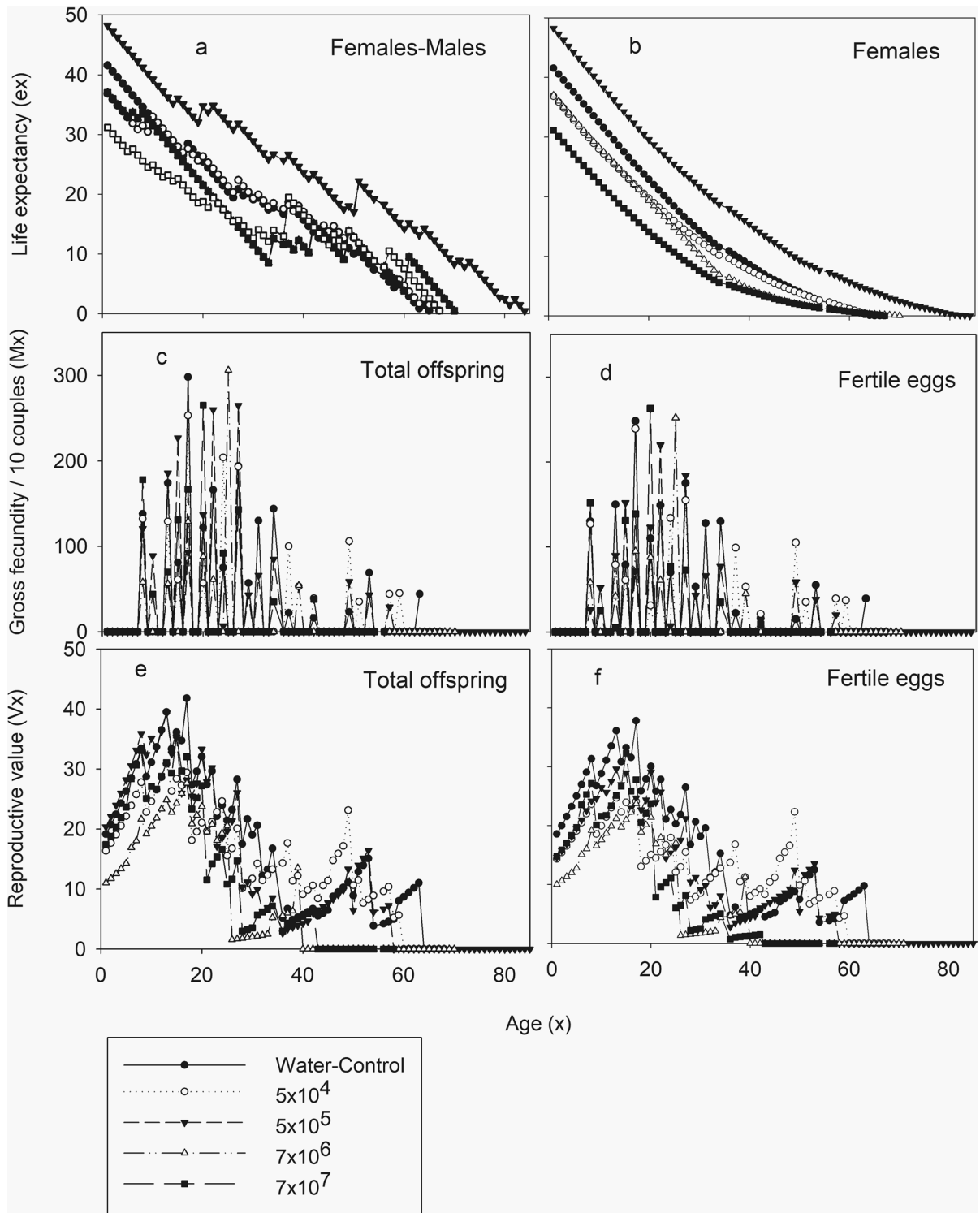
◀**Fig. 1** Growth and reproductive values of *N. viridula* populations sprayed with different concentrations of NI8 native strain of *B. bassiana*. **a**. Life expectancy calculated using the entire population (females-males) vs. **b**. Life expectancy calculated using females. **c, e**. Gross fecundity and the reproductive value calculated using the total offspring vs. **d, f**. calculated using fertile eggs with developed embryo only. Adults were fed with an artificial diet and fresh corn. Concentrations presented in spores ml^{-1}

the lowest concentration (10^6 conidia g^{-1}) to 57% (37.60 females per new-born female) with the highest concertation (10^9 conidia g^{-1}) compared to control (water) (87.65 females per new-born female). These values were lower when the eggs with undeveloped or no embryo were removed from the calculation (10.10, 17.88, 31.67, 30.43, and 29.76% for control, 10^6, 10^7, 10^8, and 10^9 conidia g^{-1}, respectively), reducing its R_o from 33% (60.75 females per new-born female) with the lowest concentration to 60% (32.05 females per new-born female) with the highest concentration compared with control (78.45 females per new-born female). In the case of GHA,

the R_o for the lowest concentrations were unexpectedly higher than the control, but as shown in Table 4, Fig. 1c-f and Fig. 2c-f, the values of the actual growth and reproductive rates were much lower after removing 20.64, 24.97, 31.48, and 32.03% of not fertile eggs from the total oviposition of populations exposed from the lowest to the highest concentration of GHA, respectively, compared with the water control (10.10%). This is the first study that reports that *B. bassiana* affects embryo development on *N. viridula,* and no comparative study was available. However, there are reports that this entomopathogenic fungus affected the feeding and ovipositional behavior of *Lygus hesperus* knight (Hemiptera: Miridae) (Noma and Strickler 2000), decreased rate of reproduction of *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae) (Ugine 2012), and reduced oviposition and hatchability on *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) (Fernandez-Ruvalcaba et al. 2010).

Studies on *N. viridula* and other Pentatomid's mating behavior (Evans 1982; Panizzi and Mourao 1999; Portilla et al. 2015; Portilla and Reddy 2021)

Table 4 Growth rate statistics for *N. viridula* sprayed with *B. bassiana* GHA strain at different concentrations and fed with an artificial diet and fresh corn. Calculations were done using total offspring (CTO) and eggs with developed embryo (CDE)

Parameters	*Beauveria bassiana* concentrations—conidia g^{-1} (conidia ml^{-1}) (conidia mm^{-2})									
	Water control		10^6 (5×10^4) (22)		10^7 (5×10^5) (82)		10^8 (7×10^6) (165)		10^9 (7×10^7) (352)	
	CTO	CDE	CTO	CDE	CTO	CDE	CTO	CDE	CTO	CDE
Total offspring (ten couples)	1753	1569	2848	2341	2240	1903	1671	1211	543	430
M_x (offspring per female)[1]	246.06	218.59	360.01	299.09	307.52	256.22	342.91	251.53	62.29	49.42
m_x (females per female)[2]	123.03	109.30	180.01	149.55	153.76	128.11	121.45	125.76	31.15	24.71
R_o (Daughter per new-born female)[3]	87.65	78.45	142.40	117.05	112.00	95.15	83.55	60.55	27.15	21.50
T (d)[4]	60.06	59.97	60.41	60.28	64.73	63.62	62.53	63.25	53.60	53.79
DT (d)[5]	8.70	8.94	7.81	8.14	8.46	8.67	8.92	9.83	11.06	12.02
r_m (GCER[6] per individuals per day)[7]	0.079	0.077	0.089	0.085	0.082	0.079	0.077	0.070	0.063	0.058
λ (Individuals per female per day)[8]	1.083	1.081	1.093	1.089	1.085	1.083	1.081	1.073	1.065	1.059

[1] Gross fecundity

[2] Net fecundity

[3] Net reproductive rate

[4] Mean generation time

[5] Doubling time

[6] Growth constant exponential rate

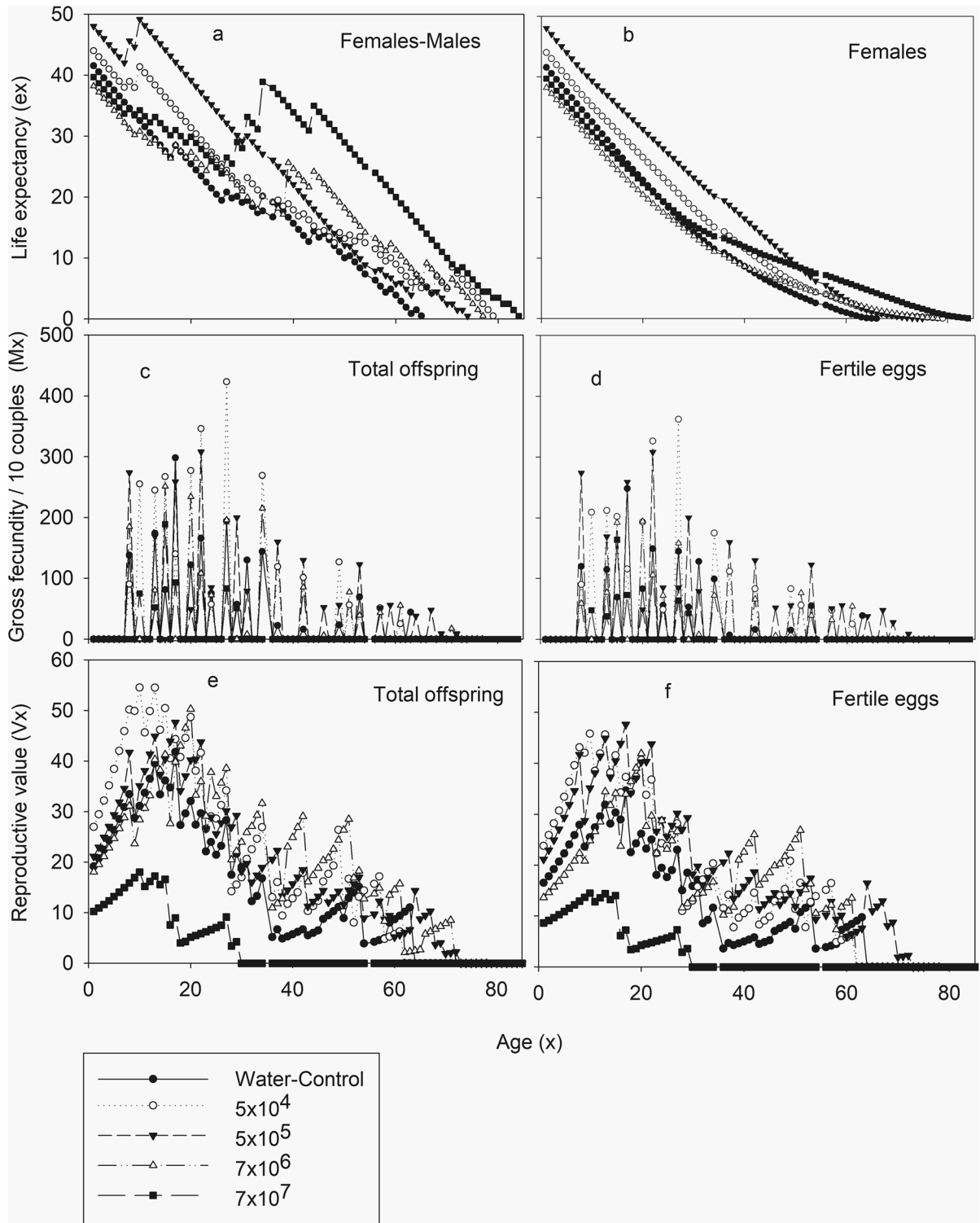[7] Intrinsic rate of increase

[8] Finite rate of increase

◄**Fig. 2** Growth and reproductive values of *N. viridula* populations sprayed with different concentrations of GHA commercial strain of *B. bassiana*. **a**. Life expectancy calculated using the entire population (females-males) vs. **b**. Life expectancy calculated using females. **c, e**. Gross fecundity and the reproductive value calculated using the total offspring vs. **d, f**. calculated using fertile eggs with developed embryo only. Adults were fed with an artificial diet and fresh corn. Concentration presented in spores ml^{-1}

reported that fecundity depends on the quantity and quality of mating events. Portilla et al. (2015) found oviposition in *N. viridula* 2–3 days after every mating. Therefore, including males in e_x calculation is fundamental for this insect to determine the true expected total lifetime fecundity at a given age of both sexes. This study corroborated many previous studies (Sosa-Gomez et al. 1997; Nada 2015; Gad and Nada 2020; Portilla et al. 2022a, b; Soliman et al. 2022) showing that *N. viridula* adults exhibit resistance to *B. bassiana*. The high variability of the life expectancy (e_x) of *N. viridula* (total population) exposed to NI8 and GHA among concentrations including control (Fig. 1a and 2a) suggested that both strains did not significantly affect the population for this insect, which was verified in Table 5 where the concentration of 29.6-fold (10,443 spores mm^{-2}) higher than the highest concentration of NI8 (356 spores mm^{-2}) and 28.5×10^5-fold (100,000 conidia mm^{-2}) higher than the highest concentration of GHA (352 conidia mm^{-2}) required to kill 50% of unsexed population of *N. viridula* 15 days after exposure. Similar values were found

by Portilla et al. (2022b) (1.9×10^3 conidia mm^{-2} by contact and 3.3×10^6 conidia mm^{-2} by direct spray) under laboratory conditions and Panizzi and Mourao (1999) (19.6×10^7 spore ml^{-1}) under field conditions. However, it is essential to clarify, that females were to be found 10^6-fold (236 conidia mm^{-2}) and 10^7-fold (326 conidia mm^{-2}) more susceptible than males sprayed with NI8 and GHA, respectively (20 DAE). This behavior can be easily observed when the e_x values were independently calculated for females (Fig. 1b and 2b). The female susceptibility based on e_x trend showed a superior performance of NI8 compared to GHA.

In general, our results indicated that *N. viridula* female and male populations are highly resistant to *B. bassiana* and the concentration of both NI8 and GHA suggested in this study and in Portilla et al. (2022a) are considered impractical for its control. However, it is essential to consider that the real impact of this fungus is caused primarily by preventing embryo development. The life fertility tables constructed in this study indicated variation depending on the concentration of the *B. bassiana* strain even at sublethal doses (22 conidia mm^{-2}). Conversely, the environmental conditions ($27 \pm 2C$, $55 \pm 10\%$RH, and a L:D 12:12 photoperiod) used in these experiments were optimal for *N. viridula* rearing and reproduction, not for *B. bassiana* development, meaning that these effects could be found in *N. viridula* populations under field conditions. Therefore, confirmations in-field studies are needed.

Table 5 Sublethal and lethal dose of *N. viridula* were treated with native and commercial *B. bassiana* strains at different concentrations and fed with an artificial diet and fresh corn

Strain-Days	n	Slope $\pm$ SE	LC$_{15}$ (95% CI)*	LC$_{50}$ (95% CI)*	Test for slope		Goodness of fit		RR$_{50}$ (95% CI)*
					χ^2 (df)	$P > \chi^2$	χ^2 (df)	$P > \chi^2$	
NI8-15	50	0.23 ± 0.45	109 (-)	10,443 (-)	0.26(1)	0.6103	0.21(2)	0.8030	1
GHA-15	50	0.12 ± 0.18	21 (-)	10^6 (-)	0.43(1)	0.5142	0.02(2)	0.9787	10.23 (9.9^{-9}–10^9)
NI8-20	50	0.37 ± 0.27	57 (-)	970 (-)	1.80(1)	0.1803	0.12(2)	0.8911	1
GHA-20	50	0.30 ± 0.32	141 (-)	4287 (-)	0.89(1)	0.3428	0.002(2)	0.9979	4.15 (0.0098–1768)
NI8-30	50	1.16 ± 1.09	160 (-)	391 (-)	1.13(1)	0.2869	0.30(2)	0.7401	1
GHA-30	50	1.05 ± 0.82	158 (-)	424 (-)	1.66(1)	0.1971	0.07(2)	0.9303	1.12 (0.42–2.97)

(-) Values for CI not calculated duo to probit model did not produce a good fit among concentrations

*Lethal concentration (LC$_{50}$) values were calculated in conidia mm^{-2}. Resistance ratios (RR$_{50}$) and 95% CI were calculated using Robertson and Priesler (1992). Differences among RR$_{50}$ values are significant if 95% CI do not include 1.0

Declarations

Conflict of interest The authors declare no conflict of interest.

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