Low doses of selenium nanoparticles enhance the performance of the generalist parasitoid *Anisopteromalus calandrae*

RUNNING TITLE: SELENIUM NANOPARTICLES AND A PARASITOID

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ABSTRACT

BACKGROUND: The environmental and economic costs of conventional insecticides have stirred an interest in alternative management tactics, including the use of nanotechnologies. Selenium nanoparticles (SeNPs) have many applications in agriculture but may not be compatible with biological control; however, low concentrations of SeNPs may benefit natural enemies via hormesis. This study investigates the dose-dependent effects of SeNPs (0–1000 mg L^{-1}) on *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae), a generalist parasitoid of stored product pests.

RESULTS: The LC_{50} of SeNPs was 1540 mg L^{-1} for female parasitoids and 1164 mg L^{-1} for males (which was not significantly different between sexes). SeNPs had a significant hormetic effect; average lifespan increased by 10% at a dose of 4.03 mg L^{-1} for females and by 35% at 13.83 mg L^{-1} for males. In a bioassay including hosts, low-dose SeNPs (25 mg L^{-1}) enhanced female performance; lifespan increased by 23% and the number of offspring increased by 88%. However, the number of emerging hosts (azuki bean beetle *Callosobruchus chinensis* (L.) (Coleoptera: Chrysomelidae: Bruchinae)) did not significantly decrease; in the absence of parasitism, SeNPs improved emergence by 17%.

CONCLUSION: Because higher concentrations of SeNPs reduced parasitoid lifespan, whereas low doses enhanced not only parasitoid performance but also host emergence, practitioners will need to pay special attention when optimizing SeNP formulations to maximize their contribution to pest control or in the mass rearing of insect parasitoids.

Keywords: integrated pest management, biological control, nanotechnology, biological control, stored product pests, dose-response, hormesis

1. INTRODUCTION

The complementarity of chemical, cultural, biological, and other control strategies is a key aspect of integrated pest management (IPM)[1,2]. Because there tend to be compatibility issues between conventional insecticides and natural enemies[3–5], and due to the environmental and economic costs of these pesticides[6,7], there are ongoing efforts to counter an overreliance on chemical control and incorporate safer biorational alternatives to improve management outcomes and meet sustainable development goals (SDGs)[8–10]. Emerging research is also investigating the possible roles of nanotechnology in pest management, including the use of entomotoxic nanoparticles (NPs) or nano-encapsulated pesticides[11,12]; despite this effort, the ecological risks of NPs remain relatively unknown[11,13–15]. Due to the nonspecific mode of action of elemental (or elemental oxide) NPs[16], these products might pose a risk towards nontarget organisms, including natural enemies.

Even though the sublethal effects of insecticides on natural enemies are typically negative[3,17], there are also instances in which they can be positive[18]. The term "hormesis" (or "hormoligosis" in insects[19]) refers to the positive or stimulatory effect of low doses of a chemical stressor, which can be characterized by a biphasic dose-response curve[20]. Since pesticide residues are often present throughout the agroecological landscape, it is important to understand how these (and other stressors) influence the biological control services provided by natural enemies. A hormetic response may improve pest suppression or be applicable to the mass rearing and release of commercial biological control agents[18,21]. Although there are many variables that affect the persistence of anthropogenic NPs in the environment (and their relationship to naturally occurring NPs)[15,21,22], relatively small or residual amounts of NPs might offer a direct benefit to biological control agents by hormesis.

Anisopteromalus calandrae (Howard) (Hymenoptera: Pteromalidae) is a cosmopolitan parasitoid that attacks a broad range of hosts, including the azuki bean beetle *Callosobruchus chinensis* (L.) and the cowpea seed beetle *C. maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae)[23]. As solitary idiobiont ectoparasitoids, females typically lay one egg after paralyzing a host[24] (usually a late-instar larva or pupa), and the developing parasitoid larva feeds from outside of its host; additional parasitoid-induced mortality can arise via host feeding[25], a process through which adult female *A. calandrae* attack and consume the hemolymph of hosts (preferably younger instars[25]) to acquire nutrients for egg maturation[25–27] (and, when feeding on *C. chinensis*, to enhance longevity as well; JRM, personal observation). Because of the importance of *A. calandrae* in the control of bruchine beetles (and other coleopteran pests)[28–32], it is important to test their compatibility with the entomotoxic NPs that have garnered recent attention—selenium nanoparticles (SeNPs), in particular[33,34]—as a possible new control strategy for these insects.

SeNPs have several potential applications in agriculture: promoting plant growth, bolstering plant defenses, and acting as an antimicrobial, nematocidal, or insecticidal agent, depending on the

concentration or formulation[35–37]. The entomotoxic effects of SeNPs arise through the slow release of elemental selenium, which, depending on the route of entry, may amass in the Malpighian tubules and midgut, negatively impacting the growth, development, and survival of the target insect[38–40]. While SeNPs have shown some promise as an alternative control method for bruchine beetles, including *C. chinensis*[33,34], there is evidence they may also harm the parasitoid *A. calandrae* when applied concurrently[34]. However, lower doses of SeNPs may actually enhance parasitoid performance via hormesis. The purpose of the present study is to investigate the dose-dependent effects of SeNPs (0–1000 mg L^{-1}) on the lifespan of the generalist parasitoid *A. calandrae* and to test the hormetic effects of low doses of SeNPs (25 mg L^{-1}) on parasitoid performance (lifespan, fecundity, offspring emergence time, age-dependent parasitism rate, and host suppression), all of which is relevant in the control of the azuki bean beetle and has important implications for other IPM programs that incorporate *A. calandrae* or related generalist parasitoids.

2. METHODS

Colonies of the azuki bean beetle, *C. chinensis* (strain jC, originating from Japan), and its parasitoid *A. calandrae* (also from Japan) have been maintained under laboratory conditions (30°C, 60% R.H., 16:8 L:D) for more than 20 years on dried azuki beans, *Vigna angularis* var. *angularis* (Willdenow) Ohwi & Ohashi (Fabaceae) (Daiwa Grain Co., Obihiro, Japan). The chemical synthesis of SeNPs[41,42] (amorphous and approximately 5–10 and 60–100 nm in diameter) was previously described by Miksanek & Tuda[33]; in brief, a 20-mL solution of SeNPs (1000 mg L^{-1}) was prepared by reducing sodium selenite (43.8 mg) with ascorbic acid (2 mL, 0.633 M) in ultrapure water (17.9 mL; Milli-Q, 18.2 MΩ·cm) and stabilizing with polysorbate 20 (100 µL). Additional SeNP concentrations of 1, 10, 25, 50, 100, 200, and 500 mg L^{-1} were made by diluting the original solution with ultrapure water; a 0 mg L^{-1} control solution (containing only ultrapure water) was also prepared.

2.1 Dose-dependent toxicity

2.1.1 Bioassay

Newly emerged (< 24 h) adult parasitoids (males and females) were placed individually in mini-sized Petri dishes (35 mm in diameter, 10 mm in height) before adding a 20-µL droplet of SeNPs (0, 1, 10, 50, 100, 200, 500, or 1000 mg L^{-1} (0–20 µg SeNPs)). Each Petri dish was gently agitated to evenly coat all surfaces as well as the parasitoid with SeNPs. After this initial treatment, the parasitoids were placed in a climate-controlled chamber (30°C, 60% R.H., 16:8 L:D) and monitored daily until death. All eight SeNP concentrations were replicated 25 times for each sex, for a total of 400 parasitoids.

114 **2.1.1 Analysis**

115 The concentration-dependent effects of SeNPs on parasitoid lifespan (age-at-death, x) were described
116 using the Cedergreen–Ritz–Streibig modification of a log-logistic model, which allows for an effect of
117 hormesis[43,44]. This model takes the form:

118

$$f(x) = \frac{d + f\exp(-1/x^{\alpha})}{1 + \exp(b(\log(x) - \log(e)))} \quad (1)$$

119

120 in which d is the upper horizontal asymptote and f is the magnitude of the effect of hormesis (with α
121 affecting the shape of the peak); the parameters b and e have no direct biological interpretation in the
122 Cedergreen–Ritz–Streibig modification of the log-logistic model (in the related three-parameter log-
123 logistic model, b is a scaling factor and e is an inflection point that corresponds with the EC_{50} or
124 LC_{50})[43,44]. If the parameter f is equal to 0, then there is no hormetic effect and the model simplifies to a
125 three-parameter log-logistic curve[43,44].

126 Model fitting and analysis was conducted in R version 4.2.2. The term α was set to 0.5 when
127 fitting the four-parameter Cedergreen–Ritz–Streibig model[43,44]. Parasitoid sex was included as a grouping
128 factor when estimating the parameters b and d (the model was simplified because preliminary analyses
129 showed no significant effect of sex on estimates of e and f). The package *drc* was used in the analysis,
130 which also allowed for estimating the maximum effect of hormesis from the mean response predicted by
131 the model (using the "MAX" function[44], as described by Cedergreen et al.[43]), lethal concentrations (e.g.
132 LC_{50}), and calculating t-statistics for comparisons between sexes[43,44].

133

134 **2.2 Low-dose SeNPs and parasitoid performance**

135 **2.2.1 Bioassay**

136 The effects of a low dose of SeNPs on the lifespan and reproductive success of *A. calandrae*, as well as
137 offspring emergence timeline, were tested by exposing parasitoids to infested azuki beans treated with
138 either SeNPs or water. The experimental design consisted of a full factorial for parasitism (*A. calandrae*
139 present or absent) and SeNP application (0 or 25 mg L^{-1} SeNPs). For this bioassay, a concentration of 25
140 mg L^{-1} SeNPs was chosen because a preliminary analysis of dose-dependent survivorship suggested that
141 this approximate concentration may best enhance parasitoid performance.

142 Adult parasitoids were collected from a laboratory colony within 24 h of emergence. Next, each
143 male–female pair was treated with a 20-µL droplet of SeNPs (0.5 µg SeNPs) or ultrapure water (as
144 previously described) and provided 8 azuki beans (1.1 g) treated concurrently and in the same manner (by
145 gently agitating each dish evenly coat all surfaces as well as the beans with SeNPs) with a 30-µL droplet[33]

146 (0.75 μg SeNPs). Each bean was infested by eight mixed-stage *C. chinensis* larvae (indirectly counted as
147 the number of eggs on the surface of the bean); by the time of parasitoid exposure, most larvae were 15–
148 16 days old, which is the favored host stage for parasitism; the smaller number of younger larvae are the
149 preferred size for host-feeding. Beans and parasitoids were placed in a small Petri dish (55 mm in
150 diameter, 15 mm in height). The male parasitoid was discarded after 24 h to prevent excessive sexual
151 harassment. Parasitoid mortality was monitored at 24-h intervals. After four days, females were provided
152 with a fresh set of infested beans (of the same treatment conditions), and the original sets of beans were
153 checked daily to record the number of emerging adults (hosts and parasitoids). The second set of beans
154 were not monitored for daily emergence, but the total number of emerged parasitoids was recorded three
155 weeks after the death of the parental parasitoid in order to calculate lifetime reproduction. Each
156 combination of treatments was replicated 30 times, for a total of 120 replicates; daily emergence was only
157 recorded from a subset of replicates (24 replicates for each of the treatments in the absence of parasitism
158 and 25 from each of those with a parasitoid present).
159
160 **2.2.2 Analysis**
161 The effects of SeNPs on the lifespan and fecundity of adult female parasitoids were tested with a Cox
162 proportional hazards model and linear regression model, respectively. A binomial GLM was used to test
163 the effects of SeNP treatment on the sex ratio of the resulting parasitoid offspring. The effects of SeNPs
164 on the development time of offspring (days to adult emergence) were assessed with a Cox proportional
165 hazards model, with sex and the interaction between sex and SeNP treatment as additional factors; the
166 identity of the maternal parasitoid was included as a random effect by clustering.
167 To further investigate the reproduction of female parasitoids, a linear regression was used to
168 assess the relationship between the log-transformed early rate of reproduction (the age-corrected number
169 of offspring per day, calculated as the number of offspring produced from days 0–3 (inclusive) divided by
170 the lifespan of the parasitoid) and SeNP treatment on the reproductive rate later in life (from day 4–death,
171 calculated in the same fashion); only parasitoids with a lifespan greater than 4 days and a nonzero early
172 reproductive rate were included in this analysis. Next, to investigate the influence of SeNPs on the
173 lifetime reproductive success (total number of offspring) of female parasitoids as a function of age, an
174 asymptotic Michaelis–Menten-type model was fit to the data. This model predicts the total number of
175 offspring of a female parasitoid living to age *t* as:
176

$$f(t) = \frac{m(t - t_{\mathrm{pre}})}{\beta \exp(-\gamma[\mathrm{Se}]) + t - t_{\mathrm{pre}}} \tag{2}$$

177

178 Analogous to the standard Michaelis-Menten model, the parameter m denotes the upper asymptote of the
179 saturating curve and β is associated with the midpoint of the saturating curve; biologically, m represents
180 the maximum number of parasitoid offspring (limited by host depletion or as a more general effect of
181 parasitoid senescence). The modifications on the Michaelis-Menten model include an x-intercept (t_{pre}) and
182 the effect of SeNPs ($\exp(-\gamma[\mathrm{Se}])$). The term t_{pre} applies a horizontal transformation to the curve;
183 biologically, t_{pre} represents the length of the pre-oviposition period of the adult female (a female might
184 use this time for mating, sperm storage, host feeding, or egg maturation). The function $\exp(-\gamma[\mathrm{Se}])$
185 applies an effect γ of SeNPs ([Se] = 0 or 25 mg L^{-1}) to β; thus, at a concentration of 0 mg L^{-1} (or if there
186 is no effect γ), then β is unaffected. For simplicity, the Michaelis–Menten-type model only considers the
187 effect of SeNPs on the parameter β; however, this formulation was also supported by an informal analysis
188 of the shortest- and longest-lived parasitoids (0–3 and 9–12 days, respectively), in which were there no
189 differences in the reproduction of control and SeNP-treated parasitoids in either age class, suggesting that
190 m and t_{pre} are likely independent of SeNP treatment.
191 Finally, the effects of SeNPs and parasitism on the number and sex ratio of emerging hosts were
192 tested with a linear mixed-effects model (LMM) and binomial generalized mixed-effects model (GLMM),
193 respectively. In both models, the identity of the source colony was included as a random effect to account
194 for potential variation in the stage structure or exact number of hosts; because source colony did not
195 improve the statistical models of parasitoid life history (described above), this random effect was only
196 included in these analyses of the host population.
197 All modeling and statistical analyses were conducted in R version 4.2.2. The Michaelis–Menten-
198 type model was fit using nonlinear least squares; confidence intervals for model predictions were
199 calculated by Monte Carlo simulation (n = 100000). The packages *car*, *lme4*, *merTools*, *survival*, *coxed*,
200 and *propagate* were also used in the analyses.
201
202 **3. RESULTS**
203 **3.1 Dose-dependent toxicity**
204 The lifespan of adult parasitoids was measured in response to a range of SeNP concentrations (0–1000 mg
205 L^{-1}). In fitting the Cedergreen–Ritz–Streibig-modified log-logistic model to the concentration-dependent
206 effects of SeNPs on the lifespan of male (n = 200) and female (n = 200) parasitoids (with parasitoid sex as
207 a grouping factor when estimating b and d), the estimates for all parameters were significant (Figure 1,
208 Table 1). The upper asymptote d—which represents the maximum lifespan in the absence of SeNPs—was
209 4.55 ± 0.15 d (estimate ± SE) for females and 3.01 ± 0.16 d for males, and the difference between sexes
210 was significant (t = 6.27, p < 0.001) (Figure 1; Table 1); the estimates of parameter b (a scaling factor)
211 were marginally different between sexes as well (t = −1.91, p = 0.057) (Table 1).

212 The LC_{50} was 1540 ± 391 mg L^{-1} for females (estimate ± SE) and 1164 ± 224 mg L^{-1} for males,
213 but there was no significant difference in the estimates of LC_{50} between sexes (t = 1.19, p = 0.237). For
214 females, the average age at death of SeNP-treated individuals was 5.02 d at a concentration of 4.03 mg
215 L^{-1}; for males, the average age at death of SeNP-treated individuals was 4.06 d at a concentration of 13.83
216 mg L^{-1} (Figure 1; Table 1).
217

218 **3.2 Low-dose SeNPs and parasitoid performance**

219 Parasitoid performance was assessed in response to a low dose (25 mg L^{-1}) of SeNPs to investigate the
220 effects of hormesis on a generalist biological control agent. The performance parameters that were
221 measured included parasitoid lifespan and fecundity, offspring sex ratio and development time, parasitoid
222 reproductive rate and lifetime reproductive success, and host emergence and sex ratio.
223 Adult female parasitoids (n = 60) treated with 25 mg L^{-1} SeNPs lived significantly longer than
224 control parasitoids; adult lifespan was 7.53 ± 0.52 and 6.10 ± 0.48 d, respectively (mean ± SE) (Figure 2a;
225 Table 2). SeNP-treated females also produced a greater number of offspring; the total number of offspring
226 was 13.0 ± 1.1 vs. 6.9 ± 1.1 (Figure 2b; Table 2). The sex ratio (n = 56) and development time (n = 285)
227 of the offspring of SeNP-treated parasitoids did not differ from those of the control (Table 2). Regardless
228 of SeNP treatment, female offspring (n = 233) emerged later than males (n = 52); the time to emergence
229 was 13.66 ± 0.09 and 12.73 ± 0.18 d, respectively (Table 2).
230 For ovipositing female parasitoids with an age of death > 4 d (n = 47), the early reproductive rate
231 was inversely correlated with the rate of reproduction later in life (offspring per day, days 4–death) ($F_{3,43}$
232 = 14.94, multiple R^2 = 0.510, p < 0.001) (Figure 3a). As the early rate of reproduction increased, there
233 was a decrease in the rate of reproduction later in life; treatment with 25 mg L^{-1} SeNPs increased the rate
234 of reproduction overall (Figure 3a; Table 3a). To investigate the lifetime reproductive success of all
235 female parasitoids (n = 60), a Michaelis–Menten-type model was implemented, in which all parameter
236 estimates were significant (Figure 3b; Table 3b). The maximum number of offspring m was 44.7 ± 17.8
237 (estimate ± SE) and the preoviposition period was 1.90 ± 0.30 d (Table 3b). There was also a significant
238 effect of SeNP treatment γ, with 25 mg L^{-1} SeNPs increasing the lifetime reproductive success of female
239 parasitoids (Figures 3a and 3b; Tables 3a and 3b).
240 Finally, the effects of SeNPs and parasitism on the host population were evaluated as the number
241 and sex ratio of the emerging hosts in each Petri dish (n = 120). The number of emerging hosts was lower
242 in the presence of a parasitoid than in the absence of the parasitoid but, when treated with SeNPs, was
243 higher in the absence of parasitism (Figure 4a, Table 4). When treated with 0 mg L^{-1} SeNPs, there were
244 an estimated 21.5 (95% prediction interval: 15.0–28.8) emerging hosts per Petri dish in the absence of
245 parasitism and 19.1 (12.3–26.1) emerging hosts per dish in the presence of a female parasitoid; when

246 treated with 25 mg L^{-1} SeNPs, there were 25.1 (18.6–31.4) emerging hosts per dish in the absence of
247 parasitism and 17.1 (10.9–23.4) emerging hosts with a parasitoid present (Figure 4a, Table 4). Finally, the
248 sex ratio of the emerging hosts shifted from a male bias (0.54 [0.51–0.57]) at 0 mg L^{-1} SeNPs to a slight
249 female bias (0.47 [0.45–0.51]) when treated with 25 mg L^{-1} SeNPs (Figure 4b, Table 4).
250

251 **4. DISCUSSION**

252 Because of the environmental and economic costs of conventional chemical insecticides, there is ongoing
253 interest in exploring alternative management tactics, including the use of nanotechnologies. Selenium
254 nanoparticles (SeNPs) have many applications in agriculture, but their nonspecific entomotoxic mode of
255 action may limit their compatibility with biological control agents; however, low concentrations may
256 benefit natural enemies via hormesis. The present study found that, for the generalist parasitoid *A.*
257 *calandrae*, the LC_{50} of SeNPs was 1.5 g L^{-1} for female parasitoids and 1.2 g L^{-1} for males, and an
258 analysis of the dose-response curves identified a significant hormetic effect; for females, the average age
259 at death increased by 10% at a dose of 4.03 mg L^{-1} and, for males, increased by 35% at 13.83 mg L^{-1}. In a
260 follow-up bioassay that provided female parasitoids with hosts, low-dose SeNPs (25 mg L^{-1}) were found
261 to enhance parasitoid performance; female lifespan increased by 23% and the number of offspring
262 increased by 88%. Regardless, the number of emerging azuki bean beetles (the host *C. calandrae*) did not
263 significantly decrease after treatment with low-dose SeNPs but rather, in the absence of parasitism,
264 increased by 17%.

265 There exists a delicate balance in the essential role of selenium as a micronutrient and its dose-
266 dependent toxicity in both vertebrate and invertebrate animals[35–40,45–49]. In insects, diets with supplemental
267 selenium can benefit growth, survival, and reproduction, but higher concentrations can have the opposite
268 effect[36,38–40,46]. While insects play a role in the biogeochemical cycling of selenium[47,49], little is known of
269 the impact of selenium on predator–prey or host–parasitoid ecology; the biotransfer or biomagnification[50]
270 of selenium can negatively affect the development and fitness of predators[51], and higher concentrations of
271 selenium can potentially disrupt host–parasitoid population dynamics, with *Cotesia marginiventris*
272 (Cresson) (Hymenoptera: Braconidae) and the beet armyworm, *Spodoptera exigua* (Hubner)
273 (Lepidoptera: Noctuidae), serving as an example[49,52]. Even though SeNPs have a lower toxicity and
274 higher bioavailability than inorganic or organic forms of selenium[37], applications of SeNPs ranging in
275 concentration from 100–500 mg L^{-1} can still have significant entomotoxic effects[37,53], including on the
276 azuki bean beetle[33,34] (although its endosymbiont *Wolbachia* can mediate resistance[33]).

277 In the present study of the *C. chinensis–A. calandrae* system, the parasitoid *A. calandrae* also
278 began showing adverse effects within the 100–500 mg L^{-1} range[33,34]. Thus, the concurrent use of SeNPs
279 and biological control would not be compatible in this case. Similarly, since the pest species also appears

280 to exhibit a hormetic response to low-dose SeNPs (25 mg L^{-1}), this limits the complementary of SeNPs
281 and biological control, at least in the short term; additional studies that extend beyond the single-
282 parasitoid, single-generation paradigm (and that also address the timing of SeNP applications and residual
283 effects), would be required to identify any long-term consequences that might be relevant for pest
284 suppression. But first, there is the fundamental question of whether the performance of *A. calandrae* is
285 enhanced as a result of a response to SeNPs as a chemical stressor (i.e. hormoligosis/hormesis), via the
286 potential benefits of selenium as a micronutrient (parasitoids could be observed drinking from dispersed
287 droplets of SeNPs [or water] before the application completely dried, although this would not offer the
288 same benefits as supplemental honey or sugar[26,54]), or through some combination of the two.
289 The positive effects of low-dose SeNPs (25 mg L^{-1}) on lifespan (and fecundity) were more
290 prominent when hosts were available for host feeding and oviposition, which suggests that SeNPs may at
291 least alter the nutritional physiology of female parasitoids (and, if linked to host feeding, this may
292 introduce an additional source of density dependence in the system[55,56]). However, because selenoproteins
293 have largely been lost in the related parasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae)
294 and the honey bee *Apis mellifera* L. (Hymenoptera: Apidae)[57,58], the positive effects of low-dose SeNPs
295 are more likely to be a hormetic response to small amounts of oxidative stress or via a reduced stress
296 threshold rather than as a positive response to an essential micronutrient[69–61]. (Note that most Coleoptera
297 also lack selenoproteins[57,58,61] as well, so a hymenopteran parasitoid and its coleopteran host might be
298 expected to exhibit similar physiological responses to SeNPs, which could complicate control efforts.)
299 Regardless, the ultimate result is an enhancement of parasitoid performance—at least for the treated
300 parasitoids, since any effects on subsequent generations are currently unknown. So, while not a
301 complementary control strategy on its own, and offering no net gain as a tactic for enhancing parasitism
302 over a single generation in the field, the use of low-dose SeNPs (in conjunction with supplemental
303 nutrients, although this would require further study) may instead have more promising applications in the
304 mass rearing of commercial control agents for inoculative or inundative release in augmentation
305 biological control[18].
306
307 **5. CONCLUSION**
308 Overall, this study provides insight into the role of SeNPs in the control of the azuki bean beetle and has
309 important implications for other programs that incorporate the generalist *A. calandrae* or closely related
310 parasitoids; there are also potential applications in the mass rearing of biological control agents. The
311 results of the present study indicate that, because higher concentrations of SeNPs reduced parasitoid
312 lifespan—whereas a low-dose treatment benefitted not only the parasitoid but the host as well—the
313 effects of SeNPs and biological control may neither be compatible nor complementary tactics. However,

314 future studies on the trade-offs between lifespan and reproduction as well as on population-level
315 responses (extending beyond single-parasitoid, single-generation experiments) may offer additional
316 insight into the possible roles of SeNPs in the field of pest control.

6. ACKNOWLEDGEMENTS

6.1 Author contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by JRM and CA. JRM and MT participated in data analysis. The first draft of the manuscript was written by JRM, and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

6.2 Data availability

The datasets and R script associated with the current study are available from the primary corresponding author on request.

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7. CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest and affirm that the present study was carried out in adherence to all applicable ethical standards.

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477 **Table 1.** Parameter estimates for the Cedergreen–Ritz–Streibig-modified log-logistic model for the
478 concentration-dependent effect of selenium nanoparticles (0–1000 mg L^{-1}) on the lifespan of the
479 parasitoid *Anisopteromalus calandrae* in the absence of hosts.

Parameter	Grouping (sex)	Estimate	SE	*t*-value	*p*-value
b	Female	0.47	0.09	5.29	**< 0.001**
	Male	0.84	0.16	5.24	**< 0.001**
d (upper asymptote)	Female	4.55	0.15	29.60	**< 0.001**
	Male	3.01	0.16	19.21	**< 0.001**
e	–	494.80	118.95	4.16	**< 0.001**
f (effect of hormesis)	–	1.63	0.44	3.73	**< 0.001**

480

481 **Table 2.** Statistical tests of the effects of selenium nanoparticles (25 mg L^{-1}) on the life history
482 parameters of adult female parasitoids (*Anisopteromalus calandrae*) and offspring.

Response (Model)	Predictor	df	χ^2	*p*-value
Age at death (Cox prop. hazards)	SeNP treatment	1	4.27	**0.039**
Reproduction (linear regression)	SeNP treatment	1	16.24	**< 0.001**
Sex ratio (binomial GLM)	SeNP treatment	1	0.89	0.346
Development time (Cox prop. hazards)	Sex	1	18.38	**< 0.001**
	SeNP treatment	1	0.03	0.856
	Sex × SeNP treatment	1	0.02	0.884

483

484 **Table 3a.** Correlation between early and late reproduction (number of offspring per day) by female
485 *Anisopteromalus calandrae* and the effects of selenium nanoparticles (25 mg L^{-1}).

Predictor	df	χ^2	*p*-value
Early reproductive rate [log(age-adjusted number of offspring per day)]	1	42.97	**< 0.001**
SeNP treatment	1	6.32	**0.016**
Early reproductive rate × SeNP treatment	1	0.00	0.946

486

487 **Table 3b.** Parameter estimates for the Michaelis–Menten-type model of the lifetime reproductive success
488 of selenium nanoparticle-treated (25 mg L^{-1}) female parasitoids (*Anisopteromalus calandrae*).

Parameter	Estimate	SE	*t*-value	*p*-value
Maximum number of parasitized hosts, m	44.72	17.85	2.51	**< 0.001**
β	21.94	11.91	1.84	**< 0.001**
Preoviposition period, t_{pre}	1.90	0.30	6.35	**< 0.001**
Effect of SeNP treatment, γ	0.02	0.01	3.62	**< 0.001**

489

490 **Table 4.** Statistical tests of the effects of the parasitoid *Anisopteromalus calandrae* and selenium
491 nanoparticles (25 mg L^{-1}) on the host/pest (azuki bean beetle) population.

Response (Model)	Predictor	df	χ^2	*p*-value
Host emergence (LMM)	Parasitoid presence	1	26.91	**< 0.001**
	SeNP treatment	1	0.61	0.436
	Parasitoid presence × SeNP treatment	1	8.14	**0.004**
Host sex ratio (binomial GLMM)	Parasitoid presence	1	0.06	0.808
	SeNP treatment	1	9.22	**0.002**
	Parasitoid presence × SeNP treatment	1	0.13	0.715

492

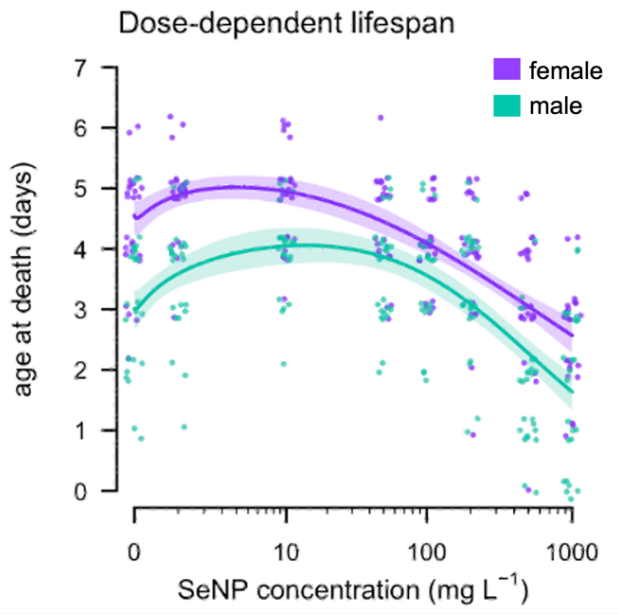

Figure 1. Dose-dependent lifespan of the parasitoid *Anisopteromalus calandrae* (adult lifespan, i.e. age at death) treated individually with different concentrations of selenium nanoparticles (SeNPs) in the absence of hosts; the solid line plots the Cedergreen–Ritz–Streibig-modified log-logistic model (with 95% CI); points plot observed values (jittered for clarity).

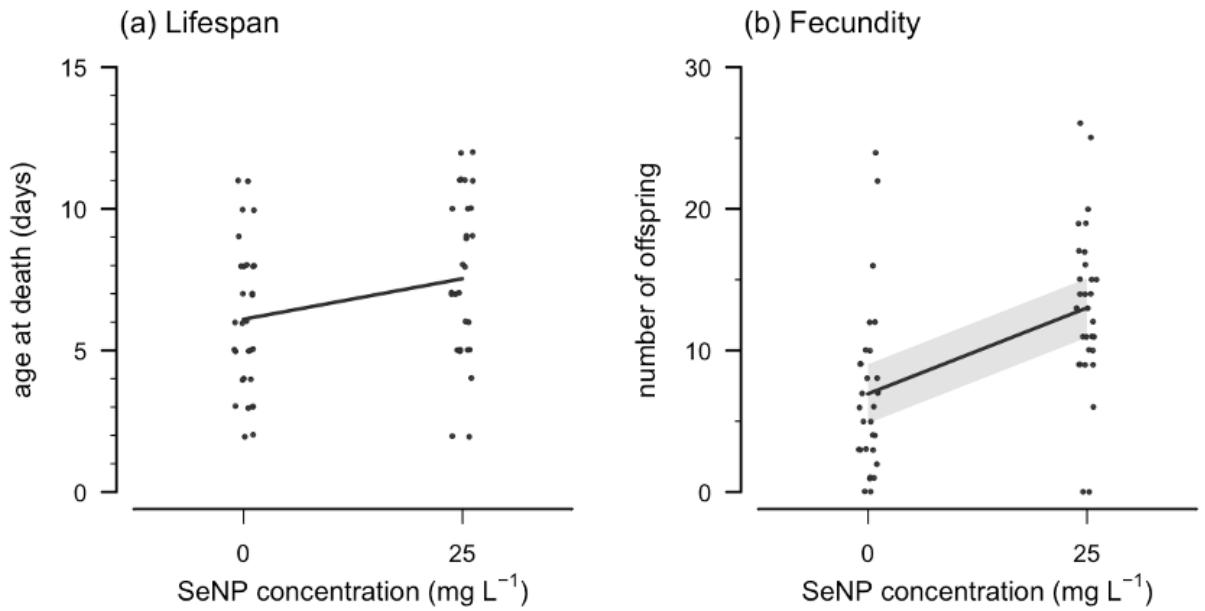

Figure 2. Lifespan and fecundity of adult female *Anisopteromalus calandrae* as a function of selenium nanoparticle (SeNP) treatment (0 or 25 mg L^{-1}). **(a)** Lifespan of adult female parasitoids; the solid line indicates the average age at death. **(b)** Fecundity (total number of offspring); the solid line plots the linear regression model (with 95% CI). In both panels, points plot observed values (jittered for clarity).

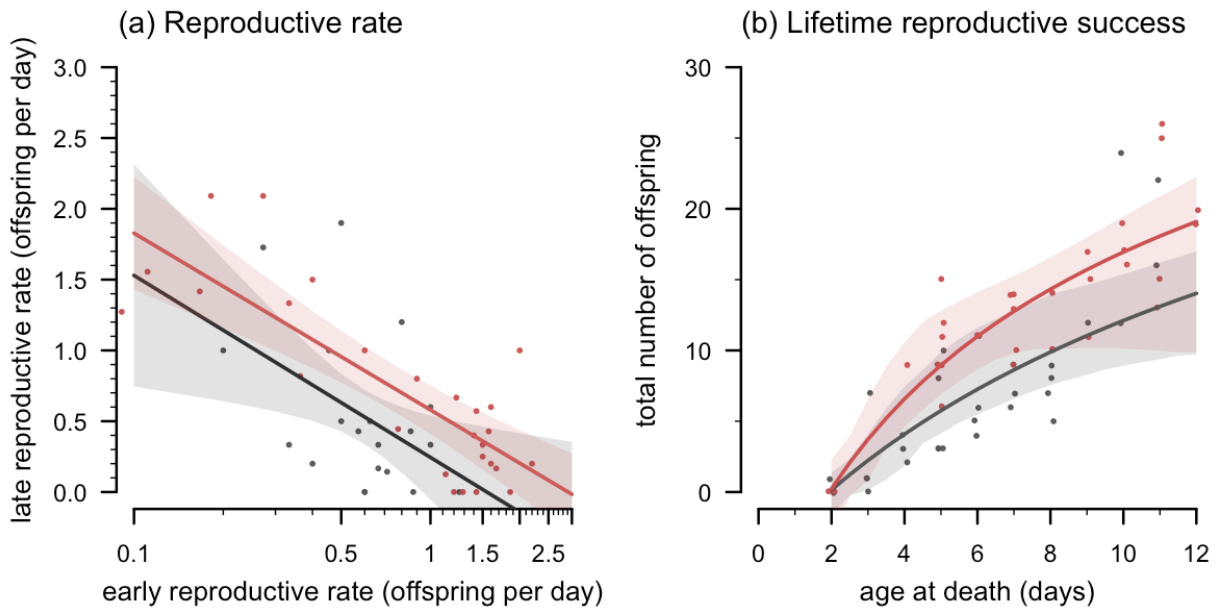

Figure 3. Reproduction of the generalist parasitoid *Anisopteromalus calandrae* as a function of age and selenium nanoparticle (SeNP) treatment (0 or 25 mg L^{-1}). **(a)** Correlation between the early and late reproductive rates of female parasitoids (number of offspring per day [age-adjusted], days 0–3 vs. day 4–death); the solid line plots the linear regression model (with 95% CI). **(b)** Lifetime reproduction, or the total number of offspring [per female]; solid lines plot the Michaelis-Menten-type models (with 95% CI). In both panels, points plot observed values (jittered for clarity in panel b).

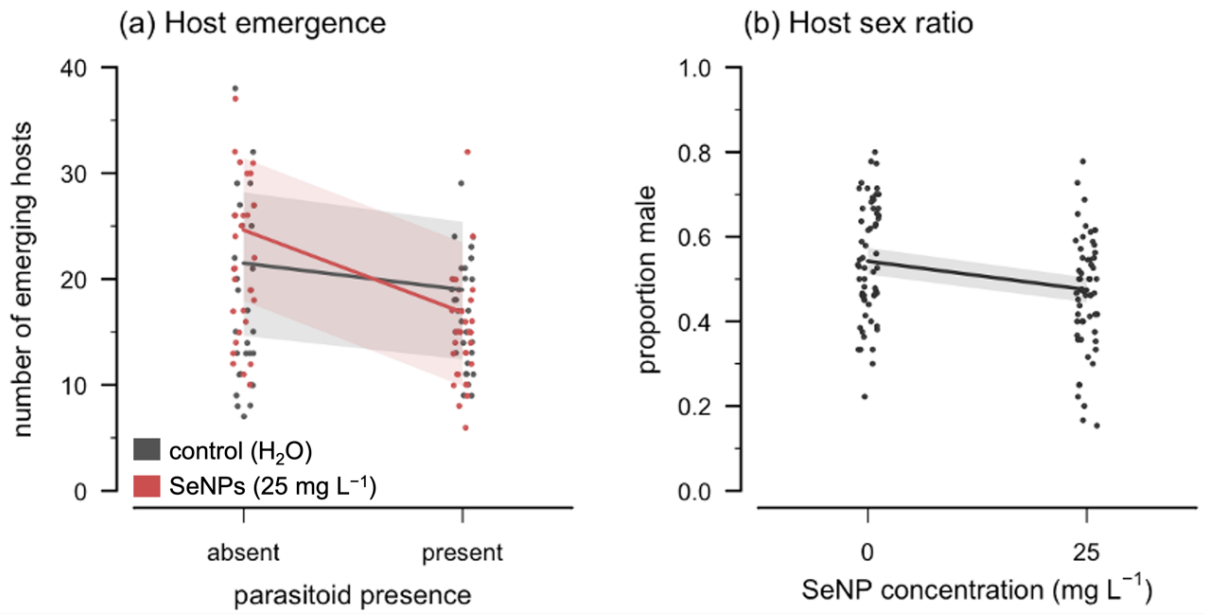

Figure 4. Influence of parasitism and selenium nanoparticle (SeNP) treatment (0 or 25 mg L^{-1}) on the host/pest population. **(a)** Host suppression, or the number of emerging adult azuki bean beetles; solid lines plot the population-level predictions from the LMM (with 95% prediction interval). **(b)** Sex ratio of emerging beetles; the solid line plots the population-level predictions from the binomial GLMM (with 95% prediction interval). In both panels, points plot observed values (jittered for clarity).