Title

Development, predation, and prey preference of *Chrysoperla externa* on *Liorhyssus hyalinus* and *Nysius simulans,* two emerging pests of quinoa

Author affiliations

- Author names: Luis Cruces[1,2], Eduardo de la Peña[2,3], Carmen Livia[1], Patrick De Clercq[2]
- Institutional affiliation of the authors:

  [1]Department of Entomology, Faculty of Agronomy, Universidad Nacional Agraria La Molina, Lima, Peru.

  [2]Department of Plants & Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium.

  [3]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora (IHSM-UMA-CSIC), Spanish National Research Council 5CSIC, Estación Experimental "La Mayora", Malaga, Spain

- Corresponding author: Luis Miguel Cruces, luiscruces@lamolina.edu.pe, tel. 051-999448427
- Orcid ID: 0000-0002-8055-020X (L. Cruces); 0000-0002-6057-6664 (E. de la Peña); 0000-0003-4456-322X (C. Livia); 0000-0003-0664-1602 (P. De Clercq)

Abstract

In recent years, *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Rhopalidae) and *Nysius simulans* Stål (Hemiptera: Lygaeidae) have emerged as important pests of quinoa in Peru, when the crop started to be cultivated at relatively low elevations. The potential of the native lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) was evaluated as a biological control agent of these two pest species. Prey consumption on all immature stages of *L. hyalinus* and *N. simulans* was assessed, as well as development on first instars of these heteropterans and eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Pyralidae) as a factitious prey. In addition, prey preference was examined in the absence and presence of a preferred prey, *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae). Larvae of the predator were not able

to feed on *L. hyalinus* eggs, but they effectively did on *N. simulans* eggs as well as on all nymphal instars of both species. Nymphs of *L. hyalinus* were less suitable prey for larval development of *C. externa* than eggs of *S. cerealella*, whereas *N. simulans* was overall an unsuitable prey. There was a clear prey preference of *C. externa* for aphids over the two heteropteran species, as well as a preference for *N. simulans* over *L. hyalinus*. The predation rates in this study indicate the potential of *C. externa* as a predator of these heteropterans pests that can play a role in both conservation and augmentation biological control programs.

Keywords

Chrysoperla externa, Liorhyssus hyalinus, Nysius simulans, quinoa, biological control

Author contributions

Conceptualization: Luis Cruces, Eduardo de la Peña, Patrick De Clercq; Methodology: Luis Cruces, Eduardo de la Peña, Carmen Livia, Patrick De Clercq; Formal analysis and investigation: Luis Cruces, Eduardo de la Peña, Carmen Livia, Patrick De Clercq; Writing - original draft preparation: Luis Cruces; Writing - review and editing: Luis Cruces, Eduardo de la Peña, Patrick De Clercq; Supervision: Eduardo de la Peña, Patrick De Clercq.

Statements and Declarations

The authors have no competing interests to declare that are relevant to the content of this article.

Acknowledgments

We thank the following professors from the Universidad Nacional Agraria La Molina in Peru, for offering facilities and permits: Luz Gómez, from the Cereals and Native Grain program, Clorinda Vergara, chief of the Museum of Entomology “Klaus Raven Büller”, and Alexander Rodriguez, chief of the Department of Entomology. Finally, we acknowledge VLIR-UOS/UNALM, for funding this study and professor Eddie Schrevens of KU Leuven for his unwavering support.

Introduction

Over the last decade, quinoa (*Chenopodium quinoa* Willd.) has been increasingly cultivated outside of its Andean origin, not only in South America but also around the world (Alandia et al. 2020). Since the crop was introduced at lower elevations, several insect pests have emerged causing losses to the quinoa crop (Latorre 2017; Cruces et al. 2020). Two of these pests in South America are *Liorhyssus hyalinus* (Fabricius) (Hemiptera: Rhopalidae) and *Nysius simulans* Stål (Hemiptera: Lygaeidae), which were not part of the known pest complex of quinoa in the traditional cultivation areas (Saravia et al. 2014). These true bugs have been reported to cause serious problems in quinoa grown at the coastal level to about 1,400 m a.s.l., in Peru (Gómez and Aguilar 2016; Cruces et al. 2016; Latorre 2017). In Argentina and Chile, both species have also been documented to be part of the quinoa pest complex (Dughetti 2015a, b; Chorbadjian et al. 2021).

Both species, *L. hyalinus* and *N. simulans*, may infest quinoa fields at high densities, mainly from the grain filling to maturation stage, when they climb to the panicle to suck the photosynthates of seeds, causing direct damage by reducing weight of the grains (Dughetti 2015a, b). At this crop stage, the application of insecticides involves the risk of harvesting grains with residues, which may eventually be rejected in the market due to the potential health hazard (Bedoya-Perales et al. 2018). Under this context, a biological control method can be a more appropriate option that needs to be explored (Baker et al. 2020).

Among the predatory species that are part of the natural enemy guild in quinoa agroecosystems are the green lacewings (Neuroptera: Chrysopidae) (Valoy et al. 2015; Cruces et al. 2020). Lacewing larvae are voracious generalist predators, feeding on a wide range of insects. Prey of lacewings may include species of Sternorrhyncha (aphids, whiteflies, psyllids, mealybugs, scale insects), Auchenorrhyncha (leafhoppers), Thysanoptera, Lepidoptera (eggs and small larvae) and Acari (Canard 2007). However, heteropterans as lacewing prey are barely studied, with only a few cases provided in literature (e.g. the plant bug *Creontiades pallidus* (Rambur) and the *Stephanitis pyrioides* (Scott)) (Jafari et al. 2006; Rinehart and Boyd, 2006).

Green lacewings have been widely studied and successfully used in biological control programs (Senior and McEwen 2007; Souza et al. 2019; Venzon et al. 2021). One of the most important species with a neotropical distribution is *Chrysoperla externa* (Hagen) (Albuquerque et al. 2007; Gamboa et al. 2016).

This lacewing species is very common in the coastal areas of Peru and has been collected in quinoa fields (Sánchez & Vergara, 2005; Cruces et al. 2016). Moreover, it has been deemed as an excellent potential biological control agent because of its ability to adapt to different cropping ecosystems where they prey on a range of economically important pests (Albuquerque et al. 1994; Garzón et al. 2015; Gamboa et al. 2016).

In the present study, we assessed *C. externa* as a predator of *L. hyalinus* and *N. simulans* by determining its developmental and predation rates when feeding on the immature stages of both pest species. Furthermore, in the field the predator may be diverted by the presence of other potential prey; thus, prey preference was studied using *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), a polyphagous aphid that regularly infests quinoa fields (Cruces et al. 2020).

Materials and Methods

Insect cultures

Colonies of the predator *C. externa* and the hemipterans *L. hyalinus* and *N. simulans* were established and maintained in the laboratories of the Museum of Entomology "Klaus Raven Büller" at the National Agrarian University La Molina, at ambient laboratory conditions at around 26-28 °C. The aphid *M. euphorbiae* was reared at around 20-22 °C.

Chrysoperla externa

Eggs of *C. externa* were obtained from the Peruvian National Service for Plant and Animal Health (SENASA) in Lima, Peru. The eggs were placed in plastic containers of 35 x 24 x 11 cm (LxHxW) lined with paper towels, and supplied with folded cardboard to reduce larval cannibalism. The top was covered with a piece of tulle and with a perforated lid to provide ventilation. The eclosed larvae were fed every other day with frozen eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), which was also obtained from SENASA.

When larvae showed pre-pupation behavior, pieces of corrugated carboard were placed inside the rearing containers in order to provide a hiding site for pupation. Pupae were removed after 5-6 days and then transferred to an acrylic box of 40 x 30 x 30 cm (LxWxH) lined with paper towels and provided with a

piece of kraft paper as oviposition substrate on top, covered with a perforated lid to provide ventilation. The emerging adults were fed with a mixture of honey, bee pollen, yeast, and water (6:0.25:10:15); water was provided through a moistened cotton pad. The kraft paper containing the eggs was replaced daily or every 12 h (for the experiments).

Eggs of < 12 h old were used to initiate the predation experiments. They were placed in Petri dishes (9 cm diameter, 1.5 cm high, lined with white cardboard) and kept in a climatic cabinet at 26 ± 0.5 °C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h to complete the egg incubation period. Emerging larvae were used directly or reared on to the 2nd or 3rd instar, depending on the experiment, using *S. cerealella* eggs as food. First and/or second generation of the lacewings obtained in the laboratory were used in the experiments.

Liorhyssus hyalinus and Nysius simulans

Colonies of *L. hyalinus* and *N. simulans* were established in December 2018 with nymphs and adults collected in the quinoa fields of the Cereal and Native Grains Program at the National Agrarian University La Molina, in Lima, Peru. The insects were housed separately in acrylic boxes of 20 x 20 x 20 cm (LxWxH) lined with paper towels. The identification of *L. hyalinus* was confirmed based on DNA extraction and PCR procedures performed at the Department of Plants and Crops of Ghent University in Belgium (Cruces et al. 2021); while *N. simulans* was identified by Pablo Dellapé from the Museo de La Plata in Argentina.

Adults and nymphs of both species were fed with fresh grains at milk stage of amylaceous corn (*Zea mays*), which also served as a water source. For the adults of *N. simulans*, cotton rolls were provided as an oviposition substrate. For *L. hyalinus* no oviposition substrate is needed because eggs are laid on the corn grains and on the walls of the acrylic cages. Maintenance of the colony was done every 2-3 days, replacing food and removing the dead individuals. The containers with adults were inspected for collecting eggs to start a new generation, or to be used in the predation experiments.

Eggs collected for the experiments were less than 24 h old and were placed in Petri dishes (9 cm diameter, 1.5 cm high, lined with white cardboard) to complete the incubation period and different nymphal stadia (N1-N5), depending on the experiment.

Macrosiphum euphorbiae

A colony of *M. euphorbiae* was established in October 2021 with nymphs and adults collected in the quinoa fields of the Cereal and Native Grains Program at the National Agrarian University La Molina, in Lima, Peru. The collected specimens were identified by taxonomic keys provided in the literature (Blackman and Eastop 2000; Blackman and Eastop 2006).

The aphid was reared modifying the method of Sidney et al. (2010) as follows: pieces of infested quinoa plants were placed in Petri dishes (9 cm diam. x 1.5 cm H), with a piece of midrib of butterhead lettuce (*Lactuca sativa* var. *capitata* L.). The aphids moved to lettuce when the quinoa tissues started to dry out. Pieces of lettuce were replaced every other day at which time dead or parasitized aphids were removed.

The new healthy aphid colonies were transferred to a circular plastic container (11 cm diam. x 5 cm H) with paper towelling on the bottom and covered with tulle and a perforated lid at the top, containing three pieces of lettuce midribs lined in the perimeter of the container. Every three days (when lettuce midrib showed signals of dehydration), adult females were moved to new piece of lettuce with the aid of a paint brush to start a new rearing container. For the experiments 2-day-old aphids (i.e., late first to early second instar nymphs) were used, which belonged to the second or third generation of rearing.

Experimental set up

All experiments were done in the laboratories of the Museum of Entomology "Klaus Raven Büller", in a climatic cabinet (VISION SCIENTIFIC VS-3DM, South Korea) set at 26 ± 0.5 °C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h.

Predation rates

Predation rates of *C. externa* of different larval instars were assessed using the immature stages (eggs and all nymphal instars) of *L. hyalinus* and *N. simulans* as prey (Table 1).

Preliminary assays with 5-10 replicates of every predator/prey combination determined the number of prey needed for offering *ad libitum* to each lacewing instar (Table 1). In these trials, newly emerged (<24 h) lacewing larvae were caged in individual plastic Petri dishes (5 cm diam. x 1.3 cm H) lined with white cardboard and kept for 24 h of starvation; thereafter, they were offered the prey *ad libitum* during 24 h, after which the prey consumption was quantified.

The number of prey items needed for each predator-prey combination in the final experiment (Table 1) were transferred with a fine brush to 5-cm Petri dishes containing a single fresh grain of corn (as a source

of water and food for the prey). The Petri dishes were transferred to the climatic cabinet and after another 12 h, any nymphs that died as a result of manipulation were replaced by healthy ones.

Newly emerged first instar larvae of *C. externa* were individually housed in 5-cm Petri dishes and initially fed with *S. cerealella* eggs. After the first six to 12 h within each tested instar, larvae were starved for 24 h without access to water. After starvation, each larva was individually transferred to a Petri dish containing the number of prey of the corresponding predator-prey combination. At least 15 replicates were tested for each combination of predator and prey. After 24 h, the number of dead and live prey were counted.

To check for any natural mortality of the prey in the absence of the predator, 6-10 replicates were considered as controls, using the same prey density of the corresponding experiment (Table 1), but without predator.

Table 1. Number of prey offered for each combination of predator (*Chrysoperla externa* larvae) and prey (eggs or nymphs of *Liorhyssus hyalinus* or *Nysius simulans*).

Prey			Predator: *C. externa*		
Species	Life-stage		1st instar	2nd instar	3rd instar
L. hyalinus	Egg		30	30	30
		N1	5	15	50
		N2	5	10	30
	Nymph	N3	5	5	20
		N4	5	5	10
		N5	5	5	5
N. simulans	Egg		20	50	160
		N1	10	40	120
		N2	10	20	60
	Nymph	N3	5	10	35
		N4	5	5	20
		N5	5	5	10

Effect of prey on development

Larval development of *C. externa* fed on nymphs (first to early second instar) of *L. hyalinus* and *N. simulans* was studied, as compared to *S. cerealella* eggs as a factitious prey.

From 77 to 100 first instars (< 2 h old, which corresponds with a brief feeding period on *S. cerealella* eggs) of *C. externa* were individually caged in 5-cm plastic Petri dishes and offered prey ad libitum. In the treatments with *L. hyalinus* and *N. simulans* as prey, a single fresh grain of corn was offered as a source of water and food for the pest. Developmental time and survival were daily monitored to determine the larval and pupal period. Newly emerged lacewing adults (< 12 h old) were sexed and weighed using a Mettler Toledo AL204 balance (Mettler-Toledo Group, China).

Prey preference

Third instar larvae of *C. externa* were tested in prey preference experiments. Newly emerged first instar lacewing larvae were individually housed in 5-cm Petri dishes and fed with *S. cerealella* eggs until 24 h before the experiment. Newly moulted 3rd instars of *C. externa* (6-12 h old) were starved for 24 h and then each larva was individually transferred to a larger Petri dish (9 cm diam. x 1.5 cm H) lined with white cardboard and provided the following prey combinations: (1) *N. simulans* and *L. hyalinus*; (2) *M. euphorbiae* and *N. simulans*; (3) *M. euphorbiae* and *L. hyalinus*.

Each combination had 20 to 30 replicates and 5 controls without predators. Based on preliminary assays, the number of nymphs of each species offered in each combination was 40 and the experimental period was set to 12 h allowing a sufficient level of predation with minimal natural mortality of the prey. Third instar *C. externa* were offered nymphs of similar size of each studied prey species: for *L. hyalinus,* these were 1-day-old first instars; for *N. simulans*, 3-day-old first instar; and for *M. euphorbiae*, <2-day-old nymphs (i.e. late first to early second instar). For the aphid/heteropteran prey combinations, two fresh grains of corn were placed in one side of the Petri dish, to feed *L. hyalinus* or *N. simulans*, and a piece of lettuce midrib (of about 6 cm long and 2 cm wide) with the colony of the aphids at the opposite side; for the *L. hyalinus*/*N. simulans* combination, two grains in one side and two in the opposite were placed.

After 12 h, the numbers of live and dead nymphs were counted with the aid of a binocular stereoscope (Carl Zeiss, Stemi 508 LAB, Zeiss, Jena, Germany).

Statistical analysis

All statistical analyses were performed using R software, version 3.4.2 (R Core Team, 2017) and all tests were analyzed at a significance level of $\alpha = 0.05$.

Predation rates of *C. externa* larvae on immatures stages of *L. hyalinus* and *N. simulans* were analyzed using a generalized linear model, with a Poisson distribution and groups were identified by the Tukey test.

Data of developmental time that was normally distributed and homoscedastic, as indicated by Shapiro Wilk and Bartlett test, respectively, was analyzed using analysis of variance (ANOVA). Means were separated using a Tukey test. When data was not normally distributed, the non-parametric Kruskal Wallis test was used to compare multiple treatments (prey), followed by a Fisher's least significant difference test as a post hoc test, or Mann-Whitney U test to compare two treatments (prey). The percent of lacewing larvae and pupae survival was compared by means of a logistic regression and groups were identified by the Tukey test. Sex ratios were evaluated versus an equal male:female distribution (1:1 ratio) by way of a non-parametric Chi-square test.

Prey preference was analyzed by means of Manly's preference index calculated with the formula (Manly 1974; Huang and Enkegaard 2010):

$$\beta = \frac{\text{Log}\,(\frac{e_1}{A_1})}{\text{Log}\,\left(\frac{e_1}{A_1}\right) + \text{Log}(\frac{e_2}{A_2})}$$

Where β is the preference to prey species 1, e_1 and e_2 are the numbers of prey species 1 and species 2 alive after the experiment, A1 and A2 are the numbers of prey species 1 and prey species 2 offered to the predator. An index value close to 1 indicates a preference for prey species 1 by the predator, while an index value close to 0 indicates a preference for species 2. Significant differences between the preference indices and the value 0.5 (meaning no preference) were analyzed by a one sample t-test. Prey species 1 were chosen as follows: *M. euphorbiae* in the prey combination with *N. simulans* or *L. hyalinus* and *N. simulans* in the prey combination with *L. hyalinus*.

Results

Predation rates

Natural mortality of *L. hyalinus* and *N. simulans* nymphs observed in the control groups over a 24-h period was zero. No predation on *L. hyalinus* eggs was observed for any of the larval instars of *C. externa*. On the other hand, predation upon *N. simulans* eggs significantly increased as a function of lacewing instar (χ^2 = 2297.4, p < 0.001) (Table 2). Predation upon all nymphal instars of *L. hyalinus* and *N. simulans* by lacewing larvae significantly increased from the first to the third instar (p < 0.001) (Table 2).

First instars of *C. externa* killed more first instars of *L. hyalinus* than older instars (χ^2= 83.68; p < 0.001). The number of third to fifth instar nymphs killed was similar (p > 0.05). Likewise, a larger number of younger instars of *N. simulans* was killed as compared to the older ones (χ^2= 322.85; p < 0.001). Despite that, the number of third and fourth instar nymphs killed by the lacewings, as well as the number of fourth and fifth instars, was similar (Table 2).

Second and third instars of *C. externa* significantly killed more younger nymphs than older nymphs for both *L. hyalinus* (χ^2= 633.17; p < 0.001; χ^2= 1430; p < 0.001) and *N. simulans* (χ^2= 1112.7; p < 0.001; χ^2= 3799.8; p < 0.001) (Table 2).

Table 2 Predation rates (means ± SE), expressed as the number of prey killed in 24 h, by different larval instars of *Chrysoperla externa* on egg and nymphal stages of *Liorhyssus hyalinus* and *Nysius simulans*.

Prey			*C. externa* (number of replicates)			χ^2
Species	Life-stage		1st instar	2nd instar	3rd instar	
L. hyalinus	Eggs		0 ± 0 (20)	0 ± 0 (30)	0 ± 0 (30)	N.A.
	Nymphs	N1	2.8 ± 0.78 cA (23)	13.8 ± 3.34 bA (30)	39.6 ± 4.39 aA (25)	967.7
		N2	1.5 ± 0.68cB (30)	6.7 ± 1.53bB (23)	21.4 ± 3.14aB (31)	675.4
		N3	0.7 ± 0.55cC (30)	3.5 ± 0.52bC (15)	12.1 ± 1.16aC (15)	286.5
		N4	0.5 ± 0.52cC (15)	1.3 ± 0.47bD (29)	5.7 ± 1.08aD (31)	143.5
		N5	0.1 ± 0.26bC (15)	0.6 ± 0.49bE (30)	3.7 ± 0.80aE (33)	127.3
N. simulans	Eggs		9.5 ± 1.96cA (15)	36.6 ± 6.57bA (18)	138.3 ± 13.17aA (15	2297.4

Nymphs	N1	7.4 ± 0.86cAB (18)	25.8 ± 4.52bB (19)	97.7 ± 9.90aB (15)	1715.9
	N2	3.4 ± 0.91cC (15)	14.6 ± 2.00bC (18)	50.3 ± 6.94aC (15)	816.2
	N3	1.6 ± 0.51cD (15)	6.8 ± 0.92bD (18)	28.8 ± 3.15aD (17)	547.3
	N4	0.7 ± 0.46cDE (15)	3.5 ± 0.74bE (15)	17.7 ± 2.19aE (15)	339.9
	N5	0.1 ± 0.26cE (15)	1.5 ± 0.52bF (15)	6.8 ± 1.93aF (19)	161.5

Different lowercase letters within a row, or uppercase letters within a column and a prey species indicate significant differences at $\alpha = 0.05$.

N.A. Not applicable, *L. hyalinus* eggs were not observed to be fed upon by the lacewings in any of the experiments.

Effect of prey on development

Prey species significantly affected immature survival of *C. externa* (χ^2 = 140.02; p < 0.001). Survival up to the adult stage of the lacewing was highest when feeding on the factitious prey (*S. cerealella* eggs) and lowest when feeding on *N. simulans* nymphs (Table 3).

Survival of *C. externa* when presented with *L. hyalinus* was more affected in the pupal stage (χ^2 = 43.97; p < 0.001), and only 53.6% of the initial number of individuals tested survived to the adult stage. However, survival of the lacewing when offered *N. simulans* was significantly and gradually affected from the first instar to the pupal stage (χ^2 = 102.93; p < 0.001) and only 10.3% of the initial number of individuals survived to the adult stage. Survival of the lacewing with *S. cerealella* eggs was high and similar in all larval instars and in the pupal stage (χ^2 = 0.49; p = 0.919), and 93% of the individuals reached the adult stage (Fig 1, Table 3).

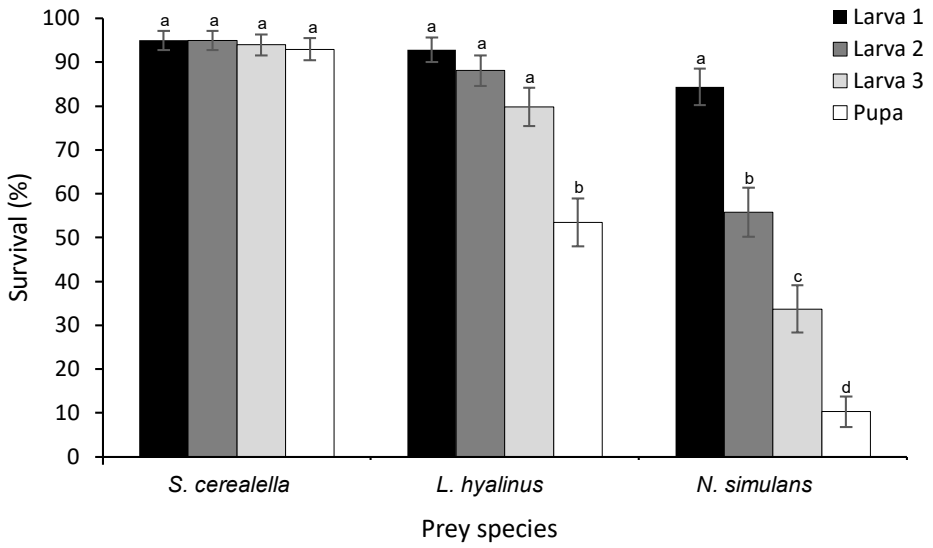

Fig 1 Survival during larval and pupal development of *Chrysoperla externa* fed with *Sitotroga cerealella* eggs, or nymphs of *Liorhyssus hyalinus* or *Nysius simulans.* Different letters within a treatment indicates significant differences at α = 0.05.

No differences in the larval period were found among *C. externa* males fed on *L. hyalinus* nymphs and *S. cerealella* eggs, but offering *N. simulans* as prey yielded a significantly shorter male larval period (χ^2 =

17.55, df=2, p < 0.001). Larval period of lacewing females was similar when fed with *L. hyalinus* nymphs and *S. cerealella* eggs (W=540, df=1, p=0.751). Since only two lacewing females reached adulthood when offered *N. simulans*, this data was excluded from the analysis (Table 3).

Male pupal period of the lacewing was longer with *L. hyalinus* as prey and shortest with *N. simulans* (χ^2 = 15.89, df=2, p < 0.001). Female pupal period was longer on with *S. cerealella* eggs than on *L. hyalinus* nymphs (W=882.5, df=1, p < 0.001) (Table 3).

Male and female adult weights were heaviest with *S. cerealella* as prey, and there were no differences in lacewing male weights when fed on *L. hyalinus* and *N. simulans* (males: F = 19.54, df=2, p<0.001; females: F = 47.07, df = 1, p < 0.001). Sex ratios of *C. externa* did not significantly deviate from a 1:1 ratio, with either *S. cerealella* (χ^2 = 2.42, p = 0.119) or *L. hyalinus* (χ^2 = 3.76, p = 0.053) as prey (Table 3).

Table 3 Developmental characteristics (means ± SE) of *Chrysoperla externa* fed with *Sitotroga cerealella* eggs, or with nymphs of *Liorhyssus hyalinus* or *Nysius simulans*.

Prey species	Survival (%)	Larval period (days)		Pupal period (days)		Adult weight (mg)		Sex Ratio
		Males	Females	Males	Females	Males	Females	♀/(♀+♂)
S. cerealella	93.0 ± 2.6a (100)	11.2 ± 0.38a	11.2 ± 0.36a	10.8 ± 0.35b	10.9 ± 0.33a	6.9 ± 0.69a	7.4 ± 0.14a	0.42
L. hyalinus	53.6 ± 5.4b (84)	11.4 ± 0.54a	11.5 ± 0.66a	10.9 ± 0.43a	10.6 ± 0.37b	5.8 ± 0.70b	7.1 ± 0.81b	0.64
N. simulans	10.3 ± 3.5c (77)	9.7 ± 0.42b	11.3 ± 0.35*	10.1 ± 0.49c	10.3 ± 1.06*	5.7 ± 0.57b	8.4 ± 0.72*	0.25*

Different letters within a column indicate significant differences at α = 0.05: Tukey test (survival), Kruskal Wallis test (larval and pupal male period), Mann-Whitney U test (larval and pupal female period); Tukey test (adult weight).
The initial number of first instars tested is given in parentheses.
* Data excluded from the analysis given the low number of females surviving

Prey preference

Third instar larvae of *C. externa* preferred *N. simulans* to *L. hyalinus* (β = 0.76 ± 0.09), and *M. euphorbiae* to *L. hyalinus* (β = 0.86 ± 0.06) or *N. simulans* (β = 0.71 ± 0.09). All preference index values were significantly different from 0.5 (Table 4). Natural mortality in 12 h was 3.3 % for *M. euphorbiae*, whereas there was no control mortality for *L. hyalinus* and *N. simulans*. As natural mortalities of the prey in 12 h were lower than 5%, observed values were not corrected (Bonte et al. 2015).

Table 4 Number (means ± SE) of *Liorhyssus hyalinus*, *Nysius simulans* and *Macrosiphum euphorbiae* nymphs killed in 12 h by third instars of *Chrysoperla externa* and prey preference index (β, mean ± SE) calculated for different prey combinations

No. of prey killed		No. of replicates	β	t	p-value
N. simulans	*L. hyalinus*				
24.8 ± 6.94	9.6 ± 4.02	30	0.76 ± 0.09	16.35	< 0.001
M. euphorbiae	*L. hyalinus*				
29.0 ± 4.88	7.8 ± 4.04	21	0.86 ± 0.06	27.72	< 0.001
M. euphorbiae	*N. simulans*				
28.1 ± 4.45	16.5 ± 6.95	20	0.71 ± 0.09	9.28	< 0.001

Preference index value (β) tested for difference from 0.5 with a one-sample t-test at a significant level α = 0.05.

Discussion

To date, no studies have addressed the potential of *C. externa* to suppress pests in quinoa. The present laboratory study comprises the first effort to explore the role of *C. externa* for conservation or augmentative biological control of *L. hyalinus* and *N. simulans*, serving to manage population densities of these two emerging pests of quinoa.

Chrysoperla externa was not able to feed on the eggs of *L. hyalinus* in any of the larval ages, probably due to the inability to penetrate the chorion with its mandibles; when examined under the stereoscope, larvae of the lacewing did try to pierce the *L. hyalinus* eggs but without success of feeding or inflicting damage, with pest nymphs successfully hatching. This was not the case for *N. simulans* eggs, which were effectively consumed by all larval instars of the predator.

All instars of the lacewing were able to kill individuals of the different nymphal ages of *L. hyalinus* and *N. simulans*. Larvae of the predator became more voracious with increasing instar as seen in other studies (Bastidas et al. 2010; Tavares et al. 2011; Fonseca et al. 2015; Cuello et al. 2019; Luna-Espino et al. 2020). For instance, the third instar larvae killed more than three times the number of prey killed by the second instar. This is in line with Canard (2007) who stated that third instar larvae of lacewings account

for the major part of the total larval prey consumption; for instance, third instars of *C. carnea* killed between 72 to 80% of the total number of prey killed during the larval stage, when they were offered *Tetranychus urticae* Koch (eggs), *Mamestra brassicae* (L.) (eggs or first instars), *Myzus persicae* (Sulzer) (second instars), *Ephestia kuehniella* Zeller (eggs) or *Pectinophora gossypiella* (Saunders) (eggs or first instars) as prey.

Second and third instar larvae killed a significantly larger number of young instars than older instars, both of *L. hyalinus* and *N. simulans*. However, first instars of *C. externa*, were able to successfully catch only first and second instars of *L. hyalinus*, or first to third instar of *N. simulans*, whereas older nymphs of both pests easily escaped from the smaller lacewing larvae. These older nymphs were often attacked by being pierced at the tarsus. Similarly, smaller individuals of different prey types were reportedly killed in higher numbers by *C. externa* than their larger conspecifics (Pacheco-Rueda et al. 2015). Further, *C. externa* killed a larger number of *N. simulans* nymphs than *L. hyalinus* nymphs, which may be primarily due to the overall larger size of *L. hyalinus* as compared with *N. simulans,* although other factors may have also affected the prey consumption observed (e.g. mobility).

At 26 °C, *L. hyalinus* and *N. simulans* have an oviposition period of about two months with an average oviposition rate of 4 to 5 eggs per day (unpublished data). The average predation rates of third instar *C. externa* observed in the present study (39.6 and 97.6 first instars nymphs of *L. hyalinus* and *N. simulans*, respectively), exceed by far the fertility rates of individual females of these pests under laboratory conditions. This indicates a promising perspective for use of *C. externa* in augmentative biological control programs during peak numbers of the heteropterans in quinoa. However, even for a highly acceptable prey in the laboratory there may be a different outcome as to the predation rate under field conditions, where a complex of ecological interactions is expected to affect the performance of an insect predator (Canard 2007).

Although lacewing larvae avidly fed on first and second instars of both *L. hyalinus* and *N. simulans*, these species were not as suitable as prey to support the larval development as were the eggs of *S. cerealella*. This may in part be attributed to the nutritional value of the lepidopteran eggs (Albuquerque et al. 1994; López-Arroyo et al. 1999; Pappas et al. 2007; Huang and Enkegaard 2010) and more in particular the efficiency of *S. cerealella* eggs as a factitious food for *C. externa* has been widely documented (McEwen and New 2007; Haramboure et al. 2015; Bezerra et al. 2017). The worst prey for the larval development of the lacewing appeared to be *N. simulans* nymphs, with only 10.3% of immature survival of the

lacewing and high mortality in both the larval and pupal stage. Unsuitability of prey for growth and survival was also observed for *Chrysoperla rufilabris* (Burmeister), whose larvae voraciously consumed individuals of *Tetranychus gloveri* Banks offered in the laboratory, but they could not support the full development of the lacewing (Canard 2007). Likewise, *Chrysoperla carnea* (Stephens) and C. *rufilabris* reared on *Drosophila melanogaster* Meigen larvae suffered high mortality in the larval and pupal stages (Hydorn and Whitcomb 1979; Osman and Selman 1996).

The unsuitability of the studied heteropteran prey for growth and survival of *C. externa* observed in our laboratory study does not necessarily reduce its potential to suppress populations of these true bugs, because the predator may complement its nutritional requirements for optimal development on other prey (McEwen and New 2007). However, in an augmentation program, inundative releases of *C. externa* larvae might be more suitable than inoculative releases (Senior and McEwen 1998)

Third instars of *C. externa* showed a clear preference for the aphid *M. euphorbiae* over both *L. hyalinus* and *N. simulans*, when they were offered in two-prey combinations. Preference for aphid prey was also reported in other species of green lacewings (Ables et al. 1978; Ding and Chen 1986; Nordlund and Morrison 1990; Huang and Enkegaard 2010). Although aphids have been demonstrated to be suitable for larval development of lacewings, the reason for these reported preferences is not clear and may be attributed to physical attractiveness determined by such factors as size, colour, mobility or chemical cues of the prey, more than a perception of their nutritional value (El-Arnaouty et al. 1996; Cardoso and Lazzari 2003; Canard 2007; Huang and Enkegaard 2010; Garzón et al. 2015; Gamboa et al. 2016). This is supported by the fact that green lacewing larvae preferred *N. simulans* over *L. hyalinus* nymphs, although the former were found to be the worst prey for growth and survival of *C. externa*. In addition, Canard (2007) stated that the discovery of the prey by lacewings is random but can be slightly stimulated, within a very short distance, by the honeydew of sap-sucking insects such as aphids, mealybugs or other species of Sternorrhyncha.

Macrosiphum euphorbiae is an aphid species that infests quinoa fields in the lowlands of Peru throughout the crop phenology, with peak numbers during the vegetative stage and decreasing populations towards the end of the cropping season. The aphid population is regulated by species from the aphidophagous guild that usually appear in large numbers, such as Aphidiinae wasps, lady beetles, and hoverfly larvae besides green lacewings (Cruces et al. 2020). On the other hand, *L. hyalinus* and *N. simulans* start the infestation during the grain filling stage, but peak numbers are found at the maturation stage, not

coinciding with the highest infestation of *M. euphorbiae* (Gómez and Aguilar 2016; Latorre 2017; Cruces et al. 2020, 2021). Moreover, being a generalist predator, the preference of *C. externa* for aphids over the heteropterans does not necessarily indicate a limited potential to suppress significant densities of *L. hyalinus* or *N. simulans* in quinoa fields. For instance, in a field-cage study on cotton, *C. carnea* larvae were able to kill substantial numbers of the lepidopterans *Chloridea virescens* (Fabricius) and *Helicoverpa zea* (Boddie) in the presence of high numbers of other prey, including the preferred prey *Aphis gossypii* Glover, in spite of the negative influence of the latter on the efficiency of the lacewing in controlling the target pests (Ridgway and Jones 1968; Ables et al. 1978). Likewise, in another field-cage study on cotton, the presence of *A. gossypii* as alternative prey did not significantly affect the predation by the lacewing *Mallada signatus* (Schneider) on larvae of *Helicoverpa armigera* (Hübner) (Bahar et al. 2013).

Given the results of the present predation experiments and the fact that *C. externa* naturally occurs in quinoa fields (Valoy et al. 2015; Cruces et al. 2016), this predator might be a target for conservation biological control programs. Moreover, as *C. externa* is easily mass reared and commercially available in Peru (e.g., by SENASA), the lacewing might also be considered for augmentation biological control programs, particularly during the late crop phenology when pest density is expected to be high. Field studies are needed, however, to better understand the potential of *C. externa* as a biological control agent in quinoa and to determine the most suitable strategies, taking into account the complexity of the quinoa agroecosystem.

Conflict of Interest

The authors declare that they have no conflict of interest

References

Ables JR, Jones SL, McCommas Jr. DW (1978) Response of selected predator species to different densities of *Aphis gossypii* and *Heliothis virescens* eggs. Environ Entomol 7(3): 402-404. https://doi.org/10.1093/ee/7.3.402.

Alandia G, Rodriguez JP, Jacobsen SE, Bazile D, Condori B (2020) Global expansion of quinoa and challenges for the Andean region. Global Food Security 26: 100429. https://doi.org/10.1016/j.gfs.2020.100429.

Albuquerque GS, Tauber CA, Tauber MJ, (1994) *Chrysoperla externa* (Neuroptera: Chrysopidae): life history and potential for biological control in Central and South America. Biol control 4(1): 8-13. https://doi.org/10.1006/bcon.1994.1002.

Albuquerque GS, Tauber CA, Tauber MJ (2007) *Chrysoperla externa* and *Ceraeochrysa* spp.: potential for biological control in the New World tropics and subtropics. In: McEwen P, New T (eds) Lacewings in the Crop Environment, Cambridge University Press, New York, pp 408-423.

Bahar MH, Stanley J, Gregg P, Socorro AD (2013) Predation of cotton bollworm by green lacewings in the presence of cotton aphid as alternative prey on transgenic Bt and conventional cotton. Entomol Exp Appl 146(2): 224-231. https://doi.org/10.1111/eea.12017.

Bastidas JS, Devia EH, Amaya ÓS (2010) Cría y evaluación de la capacidad de depredación de *Chrysoperla externa* sobre *Neohydatothrips signifer*, trips plaga del cultivo de maracuyá. Ciencia & Tecnología Agropecuaria 11(1): 31-40.

Bedoya-Perales NS, Pumi G, Mujica A, Talamini E, Domingos Padula A (2018) Quinoa expansion in Peru and its implications for land use management. Sustainability 10(2): 532. https://doi.org/10.3390/su10020532.

Baker BP, Green TA, Loker AJ (2020) Biological control and integrated pest management in organic and conventional systems. Biol Control 140: 104095. https://doi.org/10.1016/j.biocontrol.2019.104095.

Bezerra CES, Amaral BB, Souza B (2017) Rearing *Chrysoperla externa* larvae on artificial diets. Neotrop Entom 46(1): 93-99. https://doi.org/10.1007/s13744-016-0427-5.

Blackman R, Eastop V (2000) Aphids on the world's crops: An identification and information guide, 2nd edn. Vol 1,2. John Wiley and Sons, Chichester.

Blackman R, Eastop V (2006) Aphids on the world's herbaceous plants and shrubs. John Wiley and Sons, Chichester.

Bonte J, De Hauwere L, Conlong D, De Clercq P (2015) Predation capacity, development and reproduction of the southern African flower bugs *Orius thripoborus* and *Orius naivashae* (Hemiptera: Anthocoridae) on various prey. Biol Control 86: 52-59. https://doi.org/10.1016/j.biocontrol.2015.04.007.

Canard M (2007) Natural food and feeding habits of lacewings. In: McEwen P, New T (eds) Lacewings in the Crop Environment, Cambridge University Press, New York, pp 116-129.

Cardoso JT, Lazzari S (2003) Development and consumption capacity of *Chrysoperla externa* (Hagen) (Neuroptera, Chrysopidae) fed with *Cinara* spp. (Hemiptera, Aphididae) under three temperatures. Rev Bras Zool 20: 573-576.

Chorbadjian RA, Ahumada MI, Urra F, Elgueta M, Gilligan TM (2021) Biogeographical patterns of herbivore arthropods associated with *Chenopodium quinoa* grown along the latitudinal gradient of Chile. Plants 10(12): 2811. https://doi.org/10.3390/plants10122811.

Cruces LM, Callohuari Y, Carrera C (2016) Quinua: Manejo integrado de plagas. Estrategias en el cultivo de la quinua para fortalecer el sistema agroalimentario en la zona andina. Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), Santiago de Chile.

Cruces L, de la Peña E, De Clercq P (2020) Seasonal phenology of the major insect pests of quinoa (*Chenopodium quinoa* Willd.) and their natural enemies in a traditional zone and two new production zones of Peru. Agriculture 10(12): 644. https://doi.org/10.3390/agriculture10120644.

Cruces L, de la Peña E, De Clercq P (2021) Field evaluation of cypermethrin, imidacloprid, teflubenzuron and emamectin benzoate against pests of quinoa (*Chenopodium quinoa* Willd.) and their side effects on non-target species. Plants 10(9): 1788. https://doi.org/10.3390/plants10091788.

Cuello EM, Andorno AV, Hernandez CM, Lopez SN (2019) Prey consumption and development of the indigenous lacewing *Chrysoperla externa* feeding on two exotic *Eucalyptus* pests. Biocontrol Science and Technology 29(12): 1159-1171.

Ding YQ, Chen YP (1986) Predation pattern of *Chrysoperla (Chrysopa) sinica* on cotton aphid and cotton bollworm. Chin J Biol Control 2: 97–102.

Dughetti A (2015a) Plagas de la quinua y sus enemigos naturales en el valle inferior del río Colorado, Buenos Aires, Argentina. Estación Experimental Hilario Ascasubi, INTA Edditions. Buenos Aires. https://inta.gob.ar/sites/default/files/script-tmp-inta-manual-plagas-de-la-quinua-y-sus-enemigos-natura.pdf. Accessed 20 January 2022

Dughetti A (2015b) La chinche diminuta *Nysius simulans*: plaga emergente en quinua y otros cultivos en el valle bonaerense del Río Colorado. Departamento de Agronomía de la Universidad Nacional del Sur. http://www.servicios.uns.edu.ar/institucion/files/1_AP_0_68.pdf. Accessed 20 January 2022

El-Arnaouty SA, Ferran A, Beyssat-Arnaouty V (1996) Food consumption by *Chrysoperla carnea* (Stephens) and *Chrysoperla sinica* (Tjeder) of natural and substitute prey: determination of feeding efficiency (Insecta: Neuroptera: Chrysopidae). In: Canard M, Aspöck H, Mansell MW (eds) International Symposium on Neuropterology (5th: 1994: Cairo, Egypt). Pure and applied research in neuropterology. Toulouse, France.

Fonseca AR, Carvalho CF, Cruz I, Souza B, Ecole CC (2015) Development and predatory capacity of *Chrysoperla externa* (Neuroptera: Chrysopidae) larvae at different temperatures. Revista Colombiana de Entomología 41(1): 5-11.

Gamboa S, Souza B, Morales R (2016) Predatory activity of *Chrysoperla externa* (Neuroptera: Chrysopidae) on *Macrosiphum euphorbiae* (Hemiptera: Aphididae) in *Rosa* sp. crop. Rev Colomb Entomol 42(1): 54-58.

Garzón A, Freire BC, Carvalho GA, Oliveira RL, Medina P, Budia F (2015) Development and reproduction of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) fed on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) vectoring Potato leafroll virus (PLRV). Neotrop Entomol 44(6): 604-609. https://doi.org/10.1007/s13744-015-0329-y.

Gómez L, Aguilar E (2016) Guía de cultivo de la quinua. Organización de las Naciones Unidas para la Alimentación y la Agricultura, Universidad Nacional Agraria La Molina, Lima.

Haramboure M, Mirande L, Schneider MI (2015) Improvement of the mass rearing of larvae of the neotropical lacewing *Chrysoperla externa* through the incorporation of a new semiliquid artificial diet. BioControl 61(1): 69-78. https://doi.org/10.1007/s10526-015-9699-7.

Huang N, Enkegaard A (2010) Predation capacity and prey preference of *Chrysoperla carnea* on *Pieris brassicae*. BioControl 55(3): 379-385. https://doi.org/10.1007/s10526-009-9254-5.

Hydorn SB, Whitcomb WH (1979) Effects of larval diet on *Chrysopa rufilabris*. The Florida Entomologist 64(4): 293-298. https://doi.org/10.2307/3493982.

Jafari AA, Fathipour Y, Hosseini SM, Talebi AA, Moharamipour S (2006) Preference of *Nabis capsiformis* and *Chrysoperla carnea* to different nymph instars of *Creontiades pallidus*. Journal of Agricultural Sciences 12(1): 57-65.

Latorre F (2017) Is quinoa cultivation on the coastal desert of Peru sustainable? A case study from Majes, Arequipa. Dissertation, Aarhus University, Denmark.

López-Arroyo JI, Tauber CA, Tauber MJ (1999) Effects of prey on survival, development, and reproduction of trash-carrying chrysopids (Neuroptera: Ceraeochrysa). Environ Entomol 28(6): 1183-1188. https://doi.org/10.1093/ee/28.6.1183.

Luna-Espino HM, Jiménez-Pérez A, Castrejón-Gómez VR (2020) Assessment of *Chrysoperla comanche* (Banks) and *Chrysoperla externa* (Hagen) as biological control agents of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) on tomato (*Solanum lycopersicum*) under glasshouse conditions. Insects 11(2): 87. https://doi.org/10.3390/insects11020087.

Manly BFJ (1974) A model for certain types of selection experiments. Biometrics 30(2): 281–294. https://doi.org/10.2307/2529649.

McEwen P, New T (2007) Lacewings in the Crop Environment, Cambridge University Press, New York.

Nordlund DA, Morrison RK (1990) Handling time, prey preference, and functional response for *Chrysoperla rufilabris* in the laboratory. Entomol Exp Appl 57(3): 237-242. https://doi.org/10.1111/j.1570-7458.1990.tb01435.x.

Osman MZ, Selman BJ (1996) Effect of larval diet on the performance of the predator *Chrysoperla carnea* Stephens (Neuropt., Chrysopidae). J Appl Entomol 120(1-5): 115-117. https://doi.org/10.1111/j.1439-0418.1996.tb01575.x.

Pacheco-Rueda I, Lomeli-Flores JR, López-Arroyo JI, González-Hernández H, Romero-Napoles J, Santillán-Galicia MT, Súarez-Espinoza J (2015) Preferencia de tamaño de presa en seis especies de

Chrysopidae (Neuroptera) sobre *Diaphorina citri* (Hemiptera: Liviidae). Rev Colomb Entomol 41(2): 187-193.

Pappas ML, Broufas GD, Koveos DS (2007) Effects of various prey species on development, survival and reproduction of the predatory lacewing *Dichochrysa prasina* (Neuroptera: Chrysopidae). Biol Control 43(2): 163-170. https://doi.org/10.1016/j.biocontrol.2007.07.006.

R Core Team (2017) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; R Core Team: Vienna, Austria. Available online: https://www.r-project.org/ Accessed 10 January 2022

Ridgway RL, Jones SL (1968) Field-cage releases of *Chrysopa carnea* for suppression of populations of the bollworm and the tobacco budworm on cotton. J Econ Entomol 61(4): 892-898. https://doi.org/10.1093/jee/61.4.892.

Rinehart TA, Boyd DW (2006) Rapid, high-throughput detection of azalea lace bug (Hemiptera: Tingidae) predation by *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), using fluorescent-polymerase chain reaction primers. J Econ Entomol 99(6): 2136-2141. https://doi.org/10.1093/jee/99.6.2136

Sánchez G, Vergara C (2005) Control Biológico Aplicado. Universidad Nacional Agraria La Molina. Lima, Peru.

Saravia R, Plata G, Gandarillas A (2014) Plagas y enfermedades del cultivo de quinua. Fundación PROINPA, Cochabamba, Bolivia.

Senior LJ, McEwen PK (1998) Laboratory study of *Chrysoperla carnea* (Stephens) (Neuropt., Chrysopidae) predation on *Trialeurodes vaporariorum* (Westwood) (Hom., Aleyrodidae). J Appl Entom 122(1-5): 99-101. https://doi.org/10.1111/j.1439-0418.1998.tb01469.x.

Senior LJ, McEwen, PK (2007) The use of lacewings in biological control. In: McEwen PK, New T (eds) Lacewings in the Crop Environment. Cambridge University Press, New York, pp 296-302.

Sidney LA, Bueno VHP, Lins Jr. JC, Sampaio MV, Silva DB (2010) Larval competition between *Aphidius ervi* and *Praon volucre* (Hymenoptera: Braconidae: Aphidiinae) in *Macrosiphum*

euphorbiae (Hemiptera: Aphididae). Environ Entomol 39(5): 1500-1505. https://doi.org/10.1603/EN09366.

Souza B, Vázquez LL, Marucci RC (2019) Natural Enemies of Insect Pests in Neotropical Agroecosystems: Biological Control and Functional Biodiversity. Springer Nature, Switzerland.

Tavares WS, Cruz I, Silva RB, Serrão JE, Zanuncio JC (2011). Prey consumption and development of *Chrysoperla externa* (Neuroptera: Chrysopidae) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs and larvae and *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs. Maydica, 56(3): 283-289.

Valoy M, Reguilón C, Podazza G (2015) The potential of using natural enemies and chemical compounds in quinoa for biological control of insect pests. In: Murphy KS, Matanguihan J (eds) Quinoa: Improvement and sustainable production. John Wiley & Sons, New Jersey, pp 63-86.

Venzon M, Martins EF, Batista MC, Botti JMC, Andrade FP, Barroso AM (2021) Green lacewings and their role in pest management. Controle alternativo de pragas e doenças: opção ou necessidade? Belo Horizonte: Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Minas Gerais, Brazil.