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ORIGINAL RESEARCH ARTICLE

Effect of diet, maintenance frequency, and environmental conditions on the rearing of *Orius insidiosus* (Hemiptera: Anthocoridae) in Neotropical highlands

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Abstract

To standardize and optimize the mass rearing of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), an important natural enemy of the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), a method using a production batch system for the rearing of this predator was evaluated. A total of 28 production batches (one batch was defined as a set of cohorts) were evaluated, in which some variables were alternated, such as the rearing conditions (laboratory or greenhouse), type of diet provided (*F. occidentalis* individuals alone or with *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), and frequency of maintenance (24 or 48 h). Each batch was monitored by recording parameters necessary for rearing, such as the survival of initial individuals, oviposition, time taken for new adult individuals to develop, and the total production of new adults. It was determined that when using a mixed diet, the production of new adult individuals was 8.4 times greater than when only a single food source was provided. Additionally, the constant conditions of the laboratory resulted in 4.9 times greater production than in the greenhouse and reduced the time required to obtain new individuals by 11.8 days. Further, a 48-h feeding frequency produced 4.2 times more individuals than a 24-h frequency. Control of these parameters will allow the standardization of a batch production process for ensuring the continuation of *O. insidiosus* production for use in biological control programs.

Keywords *Frankliniella occidentalis* · Mass rearing · Factitious prey · Oviposition · Survival · Mixed diet

Introduction

The predatory bug *Orius insidiosus* (Say 1832) (Hemiptera: Anthocoridae) has been recognized as a promising natural enemy of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Gholami and Sadeghi 2016), an important pest of crops in Neotropical Highlands in Colombia (Reitz 2015). *Orius* bugs are produced in different countries for marketing and implementation in integrated pest management programs, especially for ornamental crops and vegetables (Kim et al. 2004; Silveira et al. 2004; Thomas et al. 2012; Bernardo et al. 2017).

The use of *O. insidiosus* as a natural enemy of thrips is due to its capacity to predate *F. occidentalis* adults—A couple consumes approximately 21.7 ± 2.4 thrips per day under laboratory conditions (Tommasini et al. 2004), although it can also subsist on pollen, nectar, or other prey, such as whiteflies, aphids, and mites (Rutledge and O'Neil 2005; Butler and O'Neil 2007; Bosco et al. 2008; Weintraub et al. 2011; Lefebvre et al. 2013; Avellaneda et al. 2016).

In Europe, Anthocoridae of the genus *Orius,* especially *O. laevigatus*, are presently used to control different species of thrips in commercial crops. This has been achieved due to a series of studies on the recognition and identification of *Orius* species native to this region and their potential as biological controls under both laboratory and commercial conditions (greenhouses), which has led to the standardization of methods of mass rearing and commercialization of these species (van Lenteren and Tommasini 2003; van Lenteren 2012).

Since the last century, the rearing of biological control agents has been considered as a means of strengthening augmentative biological control programs (de Bach 1964; van Lenteren and Tommasini 1999). The mass production of most beneficial insects requires highly defined, complex, automated, and specialized processes for each species of interest;

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however, several mass rearing programs have been developed through artisanal processes (van Lenteren and Tommasini 2003; Thomas et al. 2012). Therefore, there are presently numerous standardized rearing systems and methods for different biological control agents (Leppla and De Clercq 2019).

In Colombia, the production of *O. insidiosus* tends to be low because there is low market demand for this species due to ignorance regarding its use as a biological control in pest management as an alternative to chemical pesticides. Additionally, difficulties faced in production methods do not allow producers of this species to ensure constant production throughout the year. This has raised the possibility of importing it from European countries; however, current regulations do not allow this. Further, organisms produced in Europe are adapted to the climatic conditions of the Mediterranean, and when introduced to other regions like Neotropical Highlands, their efficiency as predators of thrips is lower than in their country of origin (van Lenteren 2012). Thus, there is a need to produce predators from spontaneous or native populations by means of a batch method that ensures a constant supply of individuals.

Considering this, the objective of the present work was to evaluate a method of rearing *O. insidiosus* in a system of continuous production in batches, determining the effect of environmental conditions, type of diet, and frequency of maintenance on the total production of individuals. With this information, one could optimize the mass rearing methods of this important predator to support integrated *F. occidentalis* management programs for the flower crops in Neotropical Highlands.

Material and methods

Study site

The experiments in the present study were carried out under two sets of environmental conditions, laboratory and greenhouse, at an average altitude of 2563 m.a.s.l in the Sabana de Bogotá. In the laboratory, a rearing chamber with a regulated temperature of 24 °C ± 1 °C, a relative humidity of 64% ± 3%, and a photoperiod of 12:12 was used. The greenhouse experiments were performed in metallic tables covered with shading mesh at an average temperature of 17.9 °C ± 5.1 °C and a relative humidity of 69.2% ± 20.3%.

Biological materials

Individuals of *F. occidentalis* used in this study were obtained from a population previously established in the laboratory rearing chamber from adults collected from flora, especially purple clover *Trifolium pratense* L. (Fabales: Fabaceae), around greenhouses of ornamental and horticultural plants in the Bogotá savannah.

These individuals were introduced to 4000 mL plastic containers with rectangular openings in both the lid and lateral sides covered with stainless steel woven mesh (Icomallas® 304 mesh, 325 × 325, 0.03 mm wire) that allowed ventilation of but not the exit or entry of individuals. In each container, 200 *F. occidentalis* adults were placed on absorbent sheets of paper with 15 to 20 pompom *Dahlia* hybrid (Asterales: Asteraceae) (Cav. 1791) flowers. After eight days, fresh flowers were introduced to feed the thrips and new adults were obtained two weeks after the start of rearing. These individuals were used as prey for *O. insidiosus* in the subsequent trials.

Bean pods (*Phaseolus vulgaris* L.) of the "ICA Cerinza" variety were obtained from a clean crop maintained in the greenhouse. The pods used had completed filling but were not in the period of seed maturation (two weeks old).

The initial adult *O. insidiosus* individuals used for each experiment were obtained from a small group kept in the laboratory. This group was obtained from a population of *O. insidiosus* obtained commercially (Scientia S.A.S) mixed with individuals collected from the field. The individuals were kept in plastic boxes as described above. Two-week-old bean pods and *Sitotroga cerealella* eggs (Lepidoptera: Gelechiidae) (Olivier 1789) were provided ad libitum.

Orius insidiosus rearing methodology

In both laboratory and greenhouse experiments, *O. insidiosus* was reared following the same methodology (Bueno et al. 2006). Rearing took place in 500 mL cylindrical glass bottles with plastic screw lids. Each lid had a hole 4 cm in diameter covered with the same metal mesh as that described above. Two circles of two-ply filter paper were inserted in the bottom of each bottle to maintain humidity and serve as a refuge for insects. Four bean pods as described above were used as an oviposition substrate.

Next, 20 *O. insidiosus* adults (0.5♂:0.5♀) aged 48 h were introduced into each of the bottles. The feed source varied according to the evaluation. The adults were removed from the bottle after 24 or 48 h according to the experiment and introduced into a new bottle with the same characteristics and fresh bean pods.

This procedure was repeated with the same frequency until the death of the initial adult individuals. A new bottle was obtained every 24/48 h from the eggs laid by the females of the original group. Each set of bottles produced during the time that the adults survived was considered one production batch; in other words, each batch comprised all the cohorts of new individuals produced daily from the initial parental adults until those initial adults died. The frequency of maintenance is the frequency at which bean pods are changed, food is provided, and individuals are moved between bottles.

Each day, pods were examined under a stereomicroscope (Leica DM 500) to count eggs laid. Later, they were introduced into the bottles and kept under the conditions to be evaluated (laboratory or greenhouse) until the emergence of nymphs. Nymphs were supplied with the same sources of nutrition as adults, and the number of individuals that completed development to the adult stage was recorded.

Experiments

To evaluate the effect of different environmental conditions, type of diet, and frequency of maintenance on the total production of adults, tests were performed under both laboratory and greenhouse conditions.

Laboratory trials

The effect of two food sources on the development of individuals and the total production of adults at the end of the cycle was evaluated under laboratory conditions. The food sources evaluated were as follows: 1) five *F. occidentalis* per live *O. insidiosus* individual in each bottle and 2) a combination of 30 adult *F. occidentalis* individuals and *S. cerealella* eggs ad libitum. Six production batches were fed the former diet, while seven batches were fed the latter diet. For both treatments, a maintenance frequency of 48 h was used. For this trial, the only variable that changed between treatments was the type of prey used.

Greenhouse trials

For the trials under greenhouse conditions, the effect of the frequency of maintenance activity on development parameters was evaluated. The frequency of maintenance refers to the time in hours during which the tasks of maintenance are performed, both in the oviposition phase of the initial *O. insidiosus* adults and in the maintenance of new eggs until the adult stage. The following two rearing maintenance frequencies were evaluated: daily (every 24 h) and every other day (every 48 h). The food source was the same for both treatments: immature and adult *F. occidentalis* individuals in a ratio of five thrips for each living *O. insidiosus* adult in each bottle. Seven batches were monitored in each treatment group.

Comparison of environmental conditions

To compare the effect of environmental conditions on production, data from previous trials using laboratory and greenhouse batches were examined. These previous batches had the same frequency of maintenance, every 48 h, and were fed with *F. occidentalis* individuals. As such, the variables evaluated were neither frequency of maintenance nor type of diet, but different environmental conditions.

Parameters of production and statistical analysis

During monitoring, the fecundity of females, survival of adults, production of individuals (eggs and adults), time of development from egg to adult, and survival of eggs until the adult stage were recorded. Although these variables are biological, they were measured from a productive point of view; in other words, variables such as the total number of adults or the total number of eggs indicate how optimal the process was in terms of potential (eggs) and final production (total adults). Likewise, other variables such as the time of development from egg to adult were taken as indicators to monitor and maintain the population, rather than to assess biological development.

Additionally, the production index, a monitoring and efficiency of rearing parameter, was calculated. This parameter indicates how many new adult individuals are produced from the initial individuals. It establishes in productive terms the effectiveness of the process, since it is expected that for each *O. insidiosus* adult used to start a production batch, more adults will be produced, thus multiplying the production units. A value less than one indicates that fewer adults are produced than are used to start the batch, while a value greater than one indicates that more individuals are produced than initially used. Values higher than one were expected in the present trials (Díaz et al. 2019).

Additionally, survival during rearing was determined by determining the relationship between potential production (total eggs obtained) and total (adults produced per batch upon completion). With this data, it is possible to determine how efficient the process was in terms of ensuring the survival of all individuals during development. Low survival rates indicate that the management characteristics, food source, or environmental conditions are causing higher mortality than the natural mortality rate for that species.

The non-parametric Kruskal–Wallis test was used together with special multiple comparison tests for non-parametric statistics to compare the four production schemes analyzed. Box plots and bar graphs were constructed to show the means and standard errors of the total numerical variables for each test, and scatter plots were produced for variables that were recorded across time. Student's *t*-tests were used to determine whether there were significant differences between the two diet treatments in the laboratory, between the two frequencies of maintenance in the greenhouse, and between the two different environmental conditions (laboratory and greenhouse). Statistical analyses were performed using the R-Cran program nparcomp package (R Core Team 2018).

Results

Effect of type of diet on laboratory production

For this trial, two types of diets were compared, one comprising *F. occidentalis* adults and larvae (FO) and another comprising a mixture of FO and *S. cerealella* eggs (FOSC). The diet that resulted in the highest production of *O. insidiosus* eggs and adults was FOSC, which produced 378.7 ± 85 eggs and 264.7 ± 41 adults. These values are approximately three and nine times higher, respectively, than those obtained with the FO diet (Fig. 1). The survival of the eggs to the adult stage was 70% with the FOSC diet and 22% with the FO diet; a marked difference that was evidenced in the production index, which was eight times higher with the FOSC diet. The production index with the FO diet was 1.6, which indicates that it is not possible to obtain twice as many individuals at the end of the production cycle as were used to start the batch using this diet. However, with the FOSC diet, the production index reached 13.2, indicating that for each adult initially used to start the process, 13 new adults were obtained for commercialization. Statistically significant differences were found between the two types of diets in egg production ($p = 0.033$, $t = -2.60$, $df = 7.49$), production of adults ($p = 0.0012$, $t = -5.55$, $df = 6.3$) and production index ($p = 0.0012$, $t = -5.5$, $df = 6.3$). However, no statistically significant differences were found in the time taken to develop from egg to adult ($p = 0.053$, $t = 2.2$, $df = 9.5$), although the time was slightly higher with the FO diet.

Effect of frequency manipulation on greenhouse production

In this test, two maintenance frequencies were evaluated (24 and 48 h), and significant differences were identified between frequencies in all variables analyzed: egg production ($p = 0.028$, $t = -2.85$, $df = 6.17$), production of adults ($p = 0.03$, $t = -2.77$, $df = 6.3$), development from egg to adult ($p = 0.001$, $t = 4.33$, $df = 11.8$), and production index ($p = 0.03$, $t = --2.77$, $df = 6.3$).

The optimum frequency was 48 h, with egg and adult production at this frequency four times greater than with a maintenance frequency of 24 h. This also influenced the production index, which was 1.9 ± 0.3 and 7.8 ± 2 for frequencies of 24 and 48 h, respectively (Fig. 2). The egg-to-adult development time was slightly longer for the 24 h frequency, with a difference of three days, which according to statistical analysis is significant ($p = 0.001011$, $t = 4.33$, $df = 11.8$).

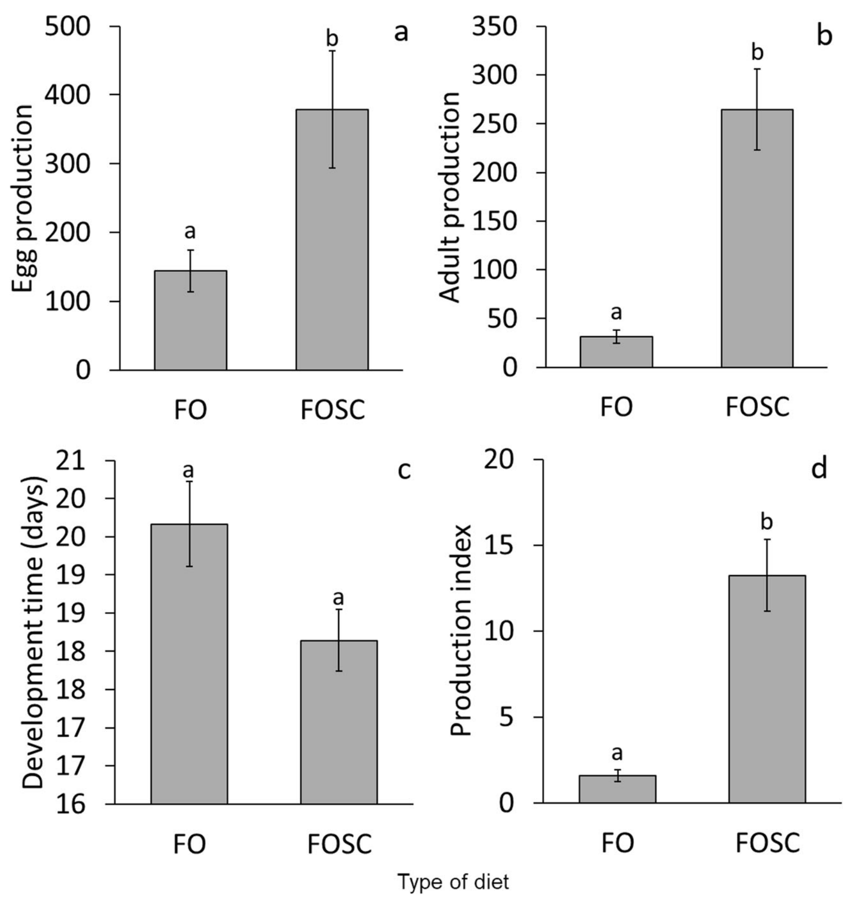

Fig. 1 Effect of two types of diets evaluated on the production parameters analyzed. (a) Egg production, (b) adult production, (c) development time, (d) production index. FO: diet of *Frankliniella occidentalis* (immature and adult), FOSC: mixed diet of FO and *Sitotroga cerealella* eggs. Gray bars correspond to the mean, means with the same letters are not significantly different at the 0.05 level. Error bars correspond to standard error

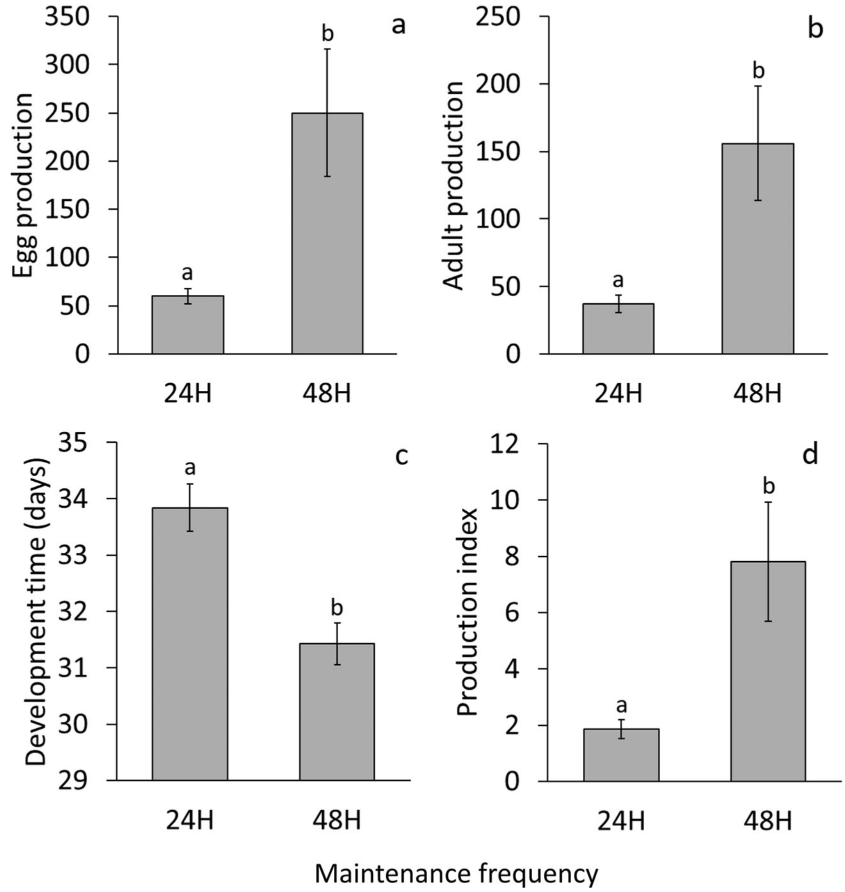

Fig. 2 Effect of two maintenance frequencies, 24 and 48 h, on the production parameters analyzed. (a) Egg production, (b) adult production, (c) development time, (d) production index. Gray bars correspond to the mean, means with the same letters are not significantly different at the 0.05 level. Error bars correspond to standard error

Effect of type of environment on production

No significant differences between environments were found for egg production ($p = 0.182$, $t = 1.456$, $df = 8.36$), although it was higher in the greenhouse, with an average of 250 ± 66 eggs produced compared to 143.8 ± 30 eggs in the laboratory (Fig. 3a).

Regarding the other variables, significant differences were noted in favor of the greenhouse in adult production ($p = 0.026$, $t = 2.9$, $df = 6.3$), with an average of 156 ± 42 adults produced in the greenhouse versus 31.7 ± 6.7 in the laboratory. Development time was significantly longer for individuals reared in the greenhouse ($p = 3e^{-8}$, $t = 17.6$, $df = 8.9$), with 31 ± 0.4 days taken to complete the egg-to-adult cycle in the greenhouse, while in the laboratory it took 20 ± 0.6 days.

The survival percentage during egg-to-adult development was greater under greenhouse conditions at 62.4%, while in the laboratory it was 22%. This was evidenced in the production index, which was 4.9 times higher in the greenhouse than in the laboratory ($p = 0.026$, $t = 2.9$, $df = 6.3$).

Effect of production scheme on oviposition and survival of initial adults

A preoviposition period of 24 to 48 h was observed in all evaluated batches. In the laboratory batch fed the FO diet with a maintenance frequency of 48 h (Fig. 4a, LABFO48H), oviposition peaked between four and five days from the beginning of rearing. In other batches, several oscillatory peaks were recorded. This was more evident in the greenhouse batch fed the FO diet with a maintenance frequency of 48 h (Fig. 4a, INVFO48H). The lowest oviposition rate was in the greenhouse batch fed the FO diet with a maintenance frequency of 24 h (Fig. 4a, INVFO24H). The longevity of the initial individuals from which rearing was started was lower for the laboratory batch fed the FO diet with a maintenance frequency of 48 h; the mortality of the initial individuals of this batch was high, and survival reached a maximum of 11 d. This influenced the production of eggs. The highest survival was observed in the scheme INVFO48H, followed by the laboratory batch fed the FO diet with a maintenance frequency of 48 h, which had a maximum longevity of 33 and 56 days, respectively. It should be noted that there does not seem to be a relationship between the peaks of egg production and the survival of the adults, since the latter remained approximately constant while oviposition varied over time.

Determination of optimum production scheme

Lastly, a comparison was made between the four production schemes evaluated to establish which performed best

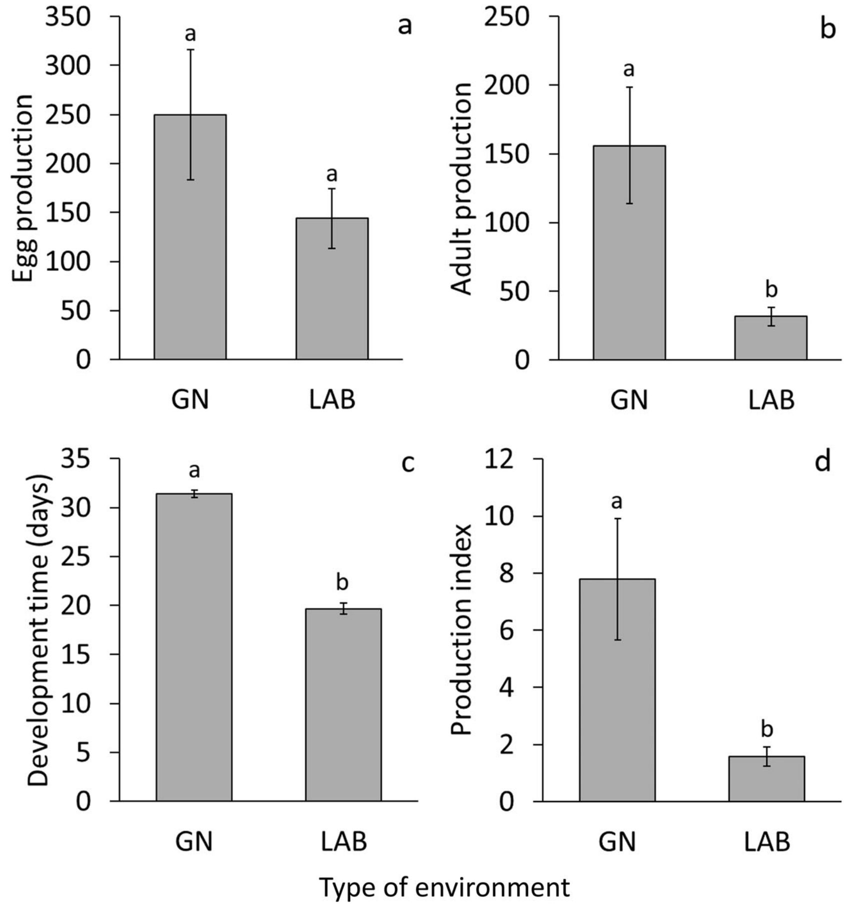

Fig. 3 Effect of two types of environments, GN: greenhouse; LAB: laboratory, on production parameters analyzed. (a) Egg production, (b) adult production, (c) development time, (d) production index. Gray bars correspond to the mean, means with the same letters are not significantly different at the 0.05 level. Error bars correspond to standard error

in terms of the productive variables evaluated. The Kruskal–Wallis test showed that there were significant differences between the four production schemes for the four variables evaluated: egg production ($p = 0.0007832$, $x^2 = 15.2$, df = 3), adult production ($p = 0.0004665$, $x^2 = 17.8$, df = 3), survival percentage ($p = 0.00154$, $x^2 = 15.3$, df = 3), egg-to-adult development time ($p = 0.00004326$, $x^2 = 22.9$, df = 3), and production index ($p = 0.0004665$, $x^2 = 17.9$, df = 3). The production scheme that performed best was a diet of both *F. occidentalis* individuals and *S. cerealella* eggs, laboratory conditions, and a maintenance frequency of 48 h (LABFOSC48H). This scheme produced the highest number of eggs and adults (Fig. 5a), the highest survival percentage of the eggs obtained (Fig. 5b), a shorter egg-to-adult development time (Fig. 5c), and a greater production index (Fig. 5d).

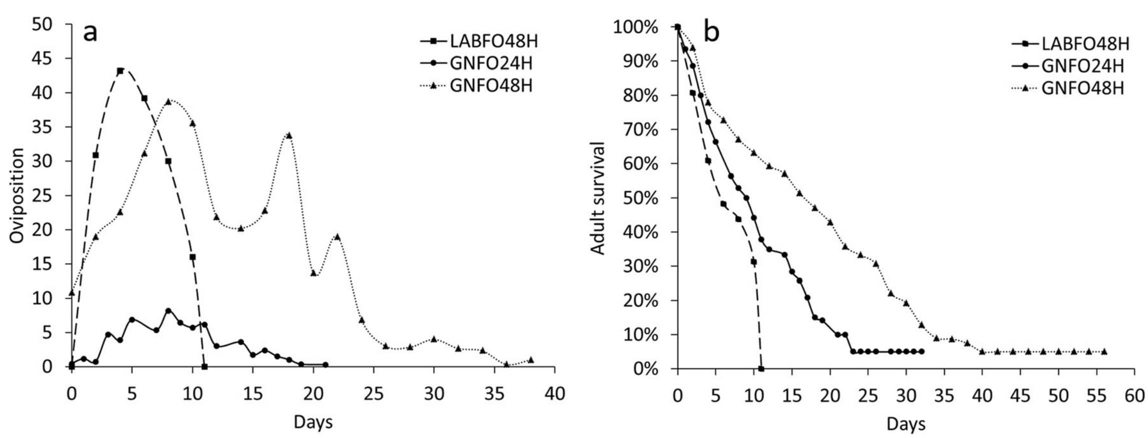

Fig. 4 Oviposition curves (a) and adult survival (b) in an average batch. The curves in each graph correspond to three of the production schemes evaluated. LAB: laboratory; GN: greenhouse, FO: *Frankliniella occidentalis* as food; 24 and 48 h: frequency of maintenance

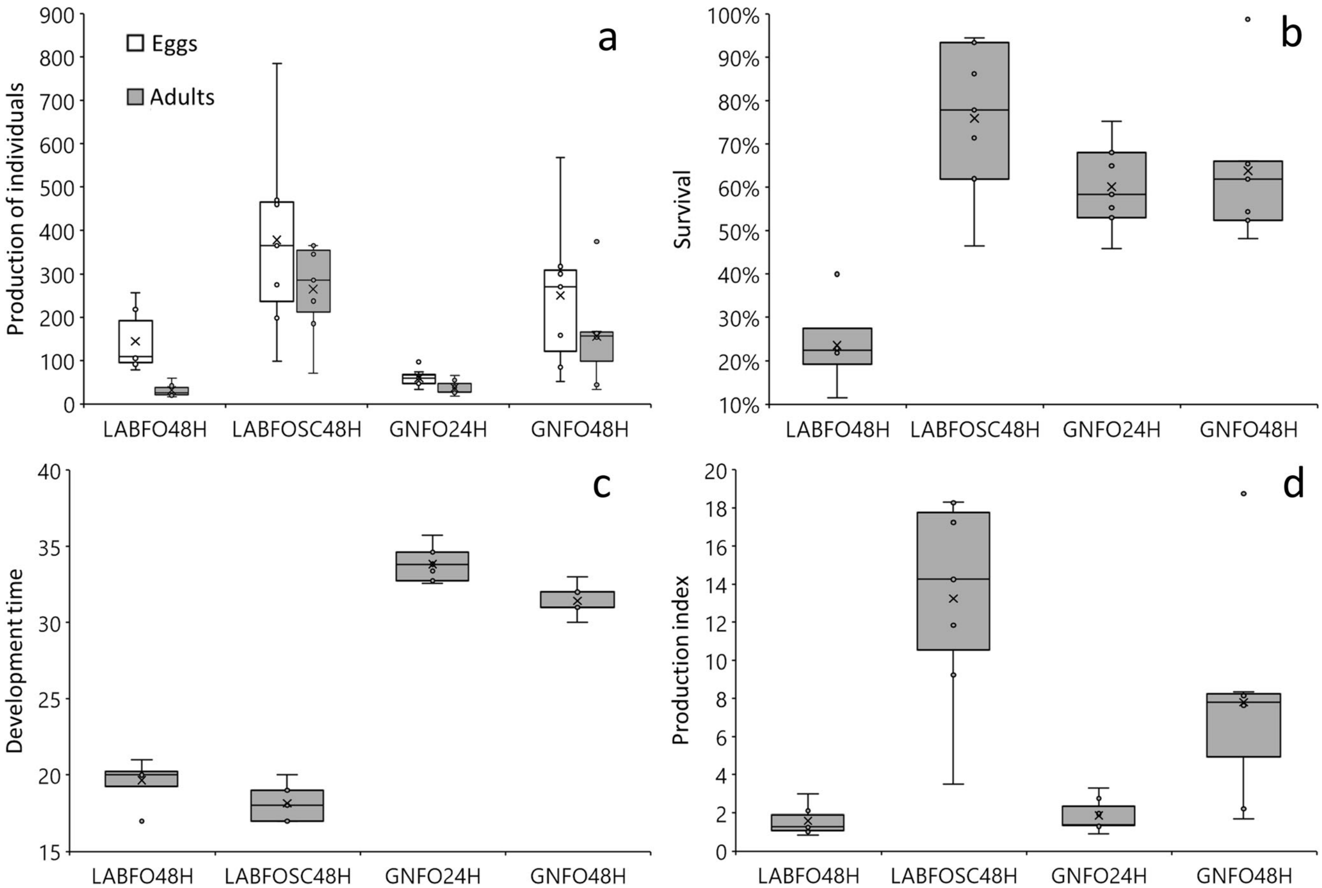

Fig. 5 Box plots showing the four variables analyzed in the four production schemes. (a) Production of eggs and adults, (b) survival percentage from egg to adult, (c) egg-to-adult development time, (d) production index. FO: diet of adult and immature individuals of *Frankliniella occidentalis*, FOSC: diet of *Frankliniella occidentalis* and eggs of *Sitotroga cerealella*. LAB: laboratory; GN: greenhouse; 24 and 48 h: maintenance frequencies in hours. Whiskers correspond to the minimum and maximum data. The average is marked by an "X" and the median by a straight line through the box

Discussion

The rearing of a beneficial insect or biological control agent (parasitoid, predator, or entomopathogen) has different purposes, among which are the following: the study of the biological and ecological parameters of said species, achieving wider distribution of a species previously introduced, or the production of a supply of organisms for release within a program of augmentative biological control (Singh et al. 2012; van Lenteren et al. 2018). For achieving these aims, it is necessary to develop rearing programs or systems that allow the continuous maintenance of populations of relevant species and do not depend on their natural occurrence, which would make biological control programs very challenging and time-consuming (Guedes et al. 2000).

During the present investigation, an optimal scheme of *O. insidiosus* production was determined by studying production under different conditions and with different diets and frequencies of maintenance using biological variables that are more practical and easier to measure from an industrial point of view, intentionally avoiding other more sophisticated ecological variables like those traditionally used in life table studies (Tommasini et al. 2004). These variables, although rich in terms of the scientific information they contain, are not suitable under commercial conditions (Pilkington and Hoddle 2007). Hence, it is believed that non-scientific readers involved in the commercial production and massification of natural enemies could understand the scope of the present research thanks to its practical language, but without any loss of scientific quality, thus reducing the gap between science and industry.

In most rearing programs for biological control agents, natural prey diets are supplied, and in the case of pathogens and parasitoids, the natural host is used as a substrate for development, which is in turn maintained on the plant on which it naturally occurs (De Clercq 2005). Hence, it is normally necessary to maintain three trophic levels (the beneficial species reared, its host or prey species, and the host plant). In several cases, this is not possible since it results in an increase in the cost of production due to the labor required for the maintenance of the hots plant. Thus, various mass rearing programs for biological control agents begin with a diet or alternate host (van Lenteren and Tommasini 1999). Lepidoptera eggs (Honda et al. 1998; Yano et al. 2002; Brito et al. 2009; Avellaneda Nieto et al. 2015) are often considered the best diet as they result in high fecundity and high survival of immature predators, which corroborates the results obtained in the present research, in which adding eggs of *S. cerealella* to the diet of *O. insidiosus* increased fertility and the survival of immature individuals. Other diets reported in alternative hosts include cysts of *Artemia franciscana* (Kellogg) (Anostraca: Artemiidae) (Arijs and De Clercq 2001), which gave results close to those obtained with a diet of Lepidoptera eggs and mobile states of the mite *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) (Husseini et al. 1993). Additionally, the use of artificial diets has been reported, although they tend to produce significantly less optimal results than Lepidoptera eggs or *A. franciscana* cysts (Bonte and De Clercq 2008). On the contrary, the effect of aphids mixed with thrips as a food source for *O. insidiosus* was shown to be positive; reportedly, when the mixture contains more thrips than aphids, greater adult longevity and a development time of 17 days are observed at a temperature of 22 °C (Rutledge and O'Neil 2005; Butler and O'Neil 2007). Another beneficial addition the *O. insidiosus* diet is pollen. Several authors have shown that the addition of pollen can increase the fertility and survival of individuals (Baniameri et al. 2005; Wong and Frank 2013), although it is not a food source that can be used alone (Bernardo et al. 2017).

According to the literature, immature Anthocoridae individuals have a survival percentage of 50% when they are fed with only thrips (Avellaneda et al 2014) and more than 60% when they are fed with eggs of *S. cerealella* (Avellaneda et al. 2016). In the present study, it was found that with a mixture of these two diets, survival rates close to 90% can be obtained, with the advantage that the reared individuals will not become accustomed to a single type of prey, which can have negative effects upon release as learning has been reported during the immature states of *O. insidiosus* (Hénaut et al. 1999).

For rearing biological control agents, micro-climatic conditions approximating the optimum conditions of their natural environment or the conditions under which they will have to function when released must initially be established, either under greenhouse conditions or in open fields (van Lenteren and Tommasini 1999). Maintaining optimal abiotic conditions in rearing systems is essential so that the organisms will be able to normally perform all necessary biological processes, such as mating, oviposition, and feeding when they are released into the field (Parra 2002). The literature on the development and life cycle of *Orius* species is extensive and includes studies under controlled conditions at temperatures between 23 °C and 32 °C (Husseini et al. 1993, Schmidt et al. 1995, Honda et al. 1998, Arijs and De Clercq 2001, Murai et al. 2001, Yano et al. 2002, Bueno et al. 2006, Ito 2007, Bonte and De Clercq 2008). However, few studies have focused on the rearing of this predator under greenhouse conditions. Studies on the effect of temperature in the laboratory show a tendency for the number of days required for an egg to develop into an adult to decrease as temperature increases (Baniameri et al. 2005).

In the present research, the following two sets of environmental conditions used by *O. insidiosus* producers were evaluated: laboratory and greenhouse. The former has the advantage of not requiring large spaces and allowing homogenization of the insect development cycle; however, it can be more expensive due to the equipment used to control the environment. Additionally, low fecundity and high mortality were observed in the laboratory compared to the greenhouse. Laboratory conditions are more practical and affordable for producers of beneficial insects. Further, in the laboratory it is possible to generate the environmental conditions under which release will take place once the natural enemy is produced. However, it has been found that greenhouse production takes more time and, therefore, more working hours for maintenance (Sørensen et al. 2012), as evidenced in the present study, with the production of adults taking approximately 10 more days in the greenhouse. The main factor influencing this is temperature; there is an inverse relationship between the temperature and length of development, with an average base temperature of 12.3 °C for *O. insidiosus* (Baniameri et al. 2005; Mendes et al. 2005; Yanik and Unlu 2011). The accumulation of temperature measured in degree days (°D) in the present study was 381.3 °D for the development of eggs to adult in the greenhouse and 246 °D in the laboratory.

Another parameter that can influence the development of *O. insidiosus* according to the type of environment is lighting (Branco and De Lavras 2002). Although we attempted to keep this parameter similar under both greenhouse and laboratory conditions in the present investigation, it was more constant in the laboratory because in the greenhouse the luminosity changed during the day depending on the weather, especially in the mornings.

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Authors' contributions MD, JA, and DR conceived the research idea. MD designed and performed the experiments and took the lead in writing the manuscript. DR verified the methods and performed the analysis. JA contributed to the interpretation of the results. All authors discussed the results and commented on the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Ethical Commite Universidad Militar Nueva Granada, Research project CIAS-1919). This article does not contain any studies with human participants performed by any of the authors.

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