Neotrop Entomol (2019) 48:476–483
https://doi.org/10.1007/s13744-018-0664-x

BIOLOGICAL CONTROL

A Scouting Method for Estimating Insect Populations in an *Encarsia formosa* (Hymenoptera: Aphelinidae) Mass Rearing System

MA Díaz, D Rodríguez, F Cantor

Basic and Applied Sciences Faculty, Nueva Granada Military Univ, Bogota, Colombia

Keywords

Whiteflies, sampling methods, production costs, production efficiency, Hemiptera, Aleyrodidae

Correspondence
MA Díaz, Basic and Applied Sciences Faculty, Nueva Granada Military Univ, Bogota, Colombia; marco.diaz@unimilitar.edu.co

Edited by Christian S Torres – UFRPE

Received 31 March 2018 and accepted 11 December 2018
Published online: 3 January 2019

Abstract

Encarsia formosa (Gahan) (Hymenoptera: Aphelinidae) has been used to control for the biological control of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in greenhouse tomato crops. One aspect that influences the success of this method is the continuous availability of large numbers of *E. formosa* that enable the grower to release them at the proper time and in the quantity required. Rearing facilities of this parasitoid require reliable and low time-consuming methods for scouting populations of insects to forecast production. In this work, we develop a time-effective method for estimating insect populations in a mass rearing system with *T. vaporariorum*, using common bean plants as hosts. The population density of *T. vaporariorum* in highly infested leaves was determined to be 27.5 nymphs/cm^2 using a linear regression model. Using an effort curve and binomial distribution, we determined that 14 and 54 leaves are the minimum number of sampling units required to estimate the *T. vaporariorum* nymphs and *E. formosa* pupae populations, respectively. A wasp ratio was determined by dividing the number of *E. formosa* produced by the total number used in the process. This index was higher when less than 7000 *E. formosa* were released per production batch in 1 week. When high populations of *E. formosa* are released in a batch, the production efficiency decreases, producing fewer new adults per adult used in the batch.

Introduction

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is an important natural enemy used for the control of whiteflies *Trialeurodes vaporariorum* Westwood and *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), which are major pests of Solanaceae and Fabaceae crops. *Encarsia formosa* is considered a cosmopolitan species because it is widely used in several countries in biological control programs under greenhouse conditions (van Lenteren 2012).

In Colombia, the supply of this natural enemy for biological control programs is limited. Van Lenteren & Bueno (2003) reviewed the state of commercial augmentative biological control in the country and found that most of the efforts have focused on the production of *Trichogramma* species for sugarcane pests. Although research on *E. formosa* has been ongoing in Colombia since the 1980s (Español & Corredor 1988, Tello *et al* 2007, Aragón *et al* 2008, de Vis & van Lenteren 2008), a successful commercial mass rearing process that can offer *E. formosa* to growers has not yet been established. For mass production, one of the problems is the labor cost for scouting populations, because it is a time-demanding activity that increases the production cost (Sørensen *et al* 2012).

The number of insects used and produced during the mass rearing process is usually unknown because counting each insect takes a considerable amount of time. This problem could be solved by estimating the total number of insects in a production batch using sampling methods (Pedigo & Buntin 1994), but determining which method to use

depends on the insect population parameters. Few studies have estimated insect population sizes in the *E. formosa* mass rearing process, and those studies have only reported the quantity of *E. formosa* at the end of the process (Scopes 1969, Scopes & Biggerstaff 1971). No studies have determined the density of whitefly nymphs or the population of *E. formosa* pupae during the process. In fact, over the past 5 years, only one study aimed to develop indirect methods to measure populations in an insect mass rearing; however, this study avoided using direct counts (Zheng *et al* 2015).

Therefore, this study aims to adapt a method for quantifying populations of insects in the mass rearing of *E. formosa*. Specifically, as part of this method, we estimated (i) the *T. vaporariorum* population density that occurs in a production batch, using the leaf area of the host plant as a base, (ii) the minimum representative sample unit to accurately estimate the density of *E. formosa* pupae, and (iii) the relationship between the number of *E. formosa* adults released and the total number of individuals produced in a production batch.

Material and Methods

The study was conducted in a plastic film greenhouse (L, 8 × W, 6.4 × H, 3.8 m) with roof ventilation located on the Nueva Granada Military University Campus in Cajicá, Cundinamarca, Colombia (4°56′49.1″N 74°00′50.8″W), at 2.558 m above sea level. The mean annual temperature inside the greenhouse was 17.07 ± 0.11°C, the maximum temperature was 30.17 ± 0.29°C, and the minimum temperature was 10.09 ± 0.085°C. The mean relative humidity was 72.94 ± 0.38%, and the photoperiod was 12:12 during the experiment, which used natural light. The temperature and relative humidity were not controlled because the facility aims to produce insects that are acclimated to commercial greenhouses.

*Production scheme for mass rearing **E. formosa** on common bean plants using **T. vaporariorum** as a host*

The mass rearing production scheme of *E. formosa* was based on one reported in the literature (Scopes 1969, van Lenteren & Tommasini 2003) and was modified to achieve a weekly batch scheme production. Each batch consists of 30 pots with 90 plants of common bean *Phaseolus vulgaris* L. (Fabales: Fabaceae) as described below.

Three common bean plants (cultivar ICA-Cerinza) were grown in 1-kg pots using soil and rice husk as substrates. The apical growth of the plants was limited by cutting off the first shoot of trifoliate leaves, and only the primary leaves remained. Each week, new leaves were removed to ensure that only the primary leaves were presented. At the sixth week of development, plants were put inside an insect cage (L, 120 × W, 80 × H, 80 cm). Next, whitefly adults were collected from a separated mass rearing greenhouse using an ecoVAC® Insect vacuum (ecoTech, Bonn, Germany) and released inside the cage were the plants were held during 24 h. Approximately 2000 adults of *T. vaporariorum* per plant were released to ensure that the total area under the leaf was covered. After the 24 h, adults were removed using the insect vacuum and taken back to the separate rearing greenhouse in order to stop egg-laying.

Subsequently, the plants with *T. vaporariorum* eggs were maintained in the cage until nymphs reached the third instar. A × 40 magnifying lens was used to count nymphs and determine the instar using morphological characteristics. Once the third instar stage was reached, adults of *E. formosa* were released as follows.

Previously to the release, *E. formosa* pupae from old batches were collected and kept in a dark cage with glass jars embedded in its walls. Recently emerged (24 h) *E. formosa* adults were collected from the glass jars using a mouth-operated insect aspirator. Then, the *E. formosa* adults were released inside cages with infested plants by opening the container of the aspirator upside down and gently hitting in the base. The number of wasps released was established using the reported relationship of one wasp for each 17 nymphs (Aragón *et al* 2008). Six weeks after the release of *E. formosa*, the leaves with *E. formosa* black pupae were harvested and placed in a dark cage to obtain *E. formosa* adults.

*Determination of **T. vaporariorum** population density on the plant*

In this study, population density was determined using the number of third instar nymphs of *T. vaporariorum* on the leaf (nymphs/cm^2). This parameter was used to estimate rapidly and reliably the total number of nymphs on a leaf without counting the total number of nymphs. To determine the dispersion of this parameter, 30 leaves with *T. vaporariorum* third instar nymphs were collected from different production batches of the mass rearing. Each leaf was analyzed in the laboratory; the total number of nymphs on the leaf was counted using a stereomicroscope, and the leaf area (cm^2) was measured using ImageJ® software (ver. 1.46a) (Abramoff *et al* 2004).

The recorded data were used to adjust a multiple linear regression model using the statistical package R (R Core Team 2018). The dependent variable was the total number of nymphs per leaf, and the explanatory variables were the leaf area and the density of third instar nymphs.

To accurately estimate the mean of the explanatory variables, the minimum sample size for each variable was estimated. The sample size was calculated using the standard deviation and the mean of each explanatory variable, as

shown in Eq. 1. This equation is based on a binomial negative distribution that is appropriate for species with an aggregated population, as in the case of whiteflies.

$$n = \frac{1}{\frac{\overline{X} + 1/\hat{k}}{D^2}} \quad (1)$$

where n is the minimum sample size for each explanatory variable (i.e., leaf area or density), X is the sample mean, D is the significance level (0.05), and k is the binomial negative distribution parameter that is calculated using Eq. 2.

$$\hat{k} = \frac{\overline{X}}{s^2 - \overline{X}} \quad (2)$$

where s^2 is the sample variance. K values less than 1 represent a population with an aggregated distribution. The data were plotted in an effort curve using the sample size on the x-axis and the mean and standard deviation (bars) for each sample size on the y-axis. The minimum sample unit was established as the value of the x-axis where the mean and standard deviation values stabilized (Kranz 1988).

*Determination of the minimum sample size of **E. formosa** pupae for three different sample units*

Three different sample units and their corresponding sample sizes were evaluated for the quantification of *E. formosa* population pupae. The sample units evaluated were the pot, plant, and leaf. These three units can be used to report the total number of *E. formosa* pupae in a production batch. A total of 19 pots with three plants each and having 90 leaves were randomly collected from different production batches that were obtained under the conditions described.

The total number of pupae for each sample type was counted, and the data were recorded in a spreadsheet and randomized in a table of two columns; a random function was used to avoid sampling bias. The first column of the table denominated the number of the sample and was organized from one to the maximum value. The second column represented the total number of pupae in each sample. Then, third and fourth columns were calculated based on the accumulative mean and standard deviation, and the sample numbers ranged from sample two to the maximum value. Next, the data calculated for each sample type were plotted using an effort curve as previously described. Finally, the total production of a batch was estimated by multiplying the mean number of pupae from the sample leaves randomly collected from the batch by the total number of leaves that were harvested (Eq. 3).

$$N_T = P_m \times N_h \quad (3)$$

where N_T is the total number of pupae in a batch, P_m is the number of pupae in a leaf sample, and N_h is the total number of leaves in a batch at the end of production.

*Production efficiency of **E. formosa***

For the purpose of this work, production efficiency was defined as the relationship between the number of new individuals produced and the number of adults released during the mass rearing process (produced/released). This ratio is used to measure the efficiency of mass rearing and indicates how many new individuals are produced for those released. This ratio should not be confused with the net reproduction rate, although they are similar. A value of one for this ratio means that the individuals of *E. formosa* that are released have exactly enough progeny to replace them and continue the cycle, but no new individuals are produced for sale. A ratio less than one suggests that there are losses of individuals during a certain step of the process. For mass rearing production, values higher than one are needed. This ratio implicitly includes *E. formosa* fecundity, parasitism rate, and immature mortality. These are important parameters for mass-reared natural enemies (van Lenteren & Tommasini 2003).

To estimate the efficiency ratio, the number of released *E. formosa* adults was recorded for 17 production batches (one batch = one production cycle) that were reared in the same production scheme as before. The batches were monitored until harvest, at which time the number of pupae produced per leaf was registered. The data were fitted to a second-degree polynomial regression model with the efficiency ratio as the dependent variable and the number of released *E. formosa* as the explanatory variable (Hothorn & Everitt 2009). The analysis was performed using the lm procedure in R.

Results

T. vaporariorum population density estimation

Estimation of the total number of third instar nymphs per leaf

The mean density of *T. vaporariorum* third instar nymphs was 27.5 ± 1.94 nymphs/cm^2 ranging from 10.8 to 49.6 nymphs/cm^2. It is possible to produce as many as 3241 ± 232 nymphs when the mean leaf area is 118 ± 2.47 cm^2. The total number of nymphs on the leaf was estimated by multiplying the density (nymphs/cm^2) by the total leaf area. The total number of nymphs of *T. vaporariorum* on the whole leaf (N) is significantly positively related to the density (Nc) and the leaf area available for feeding (A) (Eq. 4) (Fig 1).

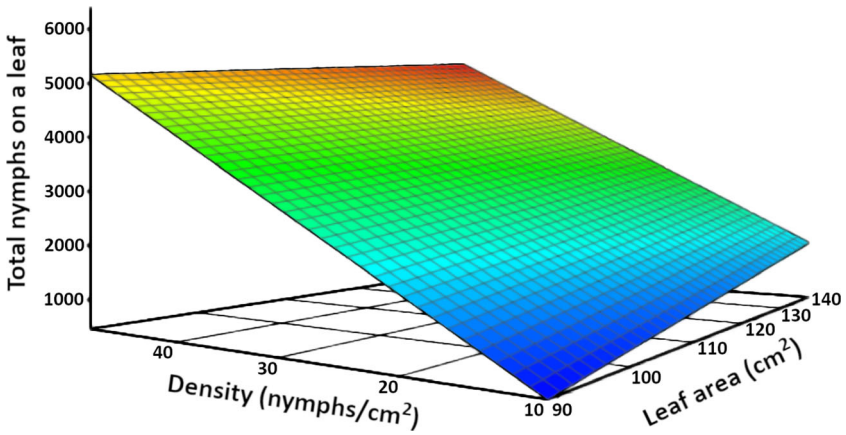

Fig 1 Surface plot of the total number of third instar nymphs of *Trialeurodes vaporariorum* in a leaf as a function of the density and leaf area as described in Eq. 4: $N = -3019.16 + 25.63A + 117.4Nc$. $R^2 = 98.93\%$, p value < $2.2e^{-16}$, $n = 30$.

$$N = -3019.16 + 25.63A + 117.4Nc \quad (4)$$

Estimation of the sample size for leaf area and density of nymphs

The minimum number of samples that must be collected in a production batch to estimate the mean leaf area and the population density was calculated using Eqs. 1 and 2. The estimated values were 13.5 samples for the leaf area and 14.5 samples for the density (nymphs/cm^2), which is supported by the graphic analysis (vertical lines in Fig 2).

Estimation of the minimum sample size and sample unit for ***E. formosa***

The approximate mean number of pupae in each sample unit was 7045 ± 93 for "Pot," 2350 ± 30 for "Plant," and 1500 ± 14 for "Leaf." Graphical analysis showed that the minimum sample sizes were 13 pots (Fig 3a), 42 plants (Fig 3b), and 54 leaves (Fig 3c). Each type of sample unit could be used to determine the number of pupae in a production batch; however, the leaf is a practical sample unit because counting the number of pupae on 54 leaves is an activity that can be performed in parallel with the harvest during the last stage of the production process, where it is necessary to remove and count the total number of leaves per batch. This count requires approximately 1 h per week and can be performed without equipment because pupae are black in color and can be differentiated easily. The counting cost is 1.3 USD per sampling event.

Evaluation of production efficiency of ***E. formosa*** adults

The relationship between the number of produced *E. formosa* and the number of released *E. formosa* was, on average, 3.82. Variation was high among different production process cycles, with values between 1 and 8.9 and a coefficient of variation of 0.58. An inverse relationship was established with higher production efficiency when fewer adults were released (Fig 4).

Discussion

In this study, a method to scout populations in a tritrophic mass rearing facility was adapted using the leaf area of the plant and the population density of insects as main parameters. Standardizing this kind of method is important because it could help producers accurately determine the required production and detect possible anomalies in the process; additionally, it is possible to estimate certain costs using the data that are collected during scouting.

The density parameter found in this research could be used as an indicator for the production and as a standard for other mass rearing producers, but it is important to clarify that this parameter was measured using common bean plants for the rearing process; thus, parameter values could vary for other host plant species. The value found in this study ranged from 10 to 50 nymphs/cm^2 and could be explained by the high number of adults used to infest the leaves and the high supply of nitrogen through fertilization that has a positive effect on oviposition (Jauset *et al* 1998, 2000, Ortega-Arenas *et al* 2006, Park *et al* 2009).

No previous studies have measured the population density of *T. vaporariorum* nymphs under mass rearing conditions using common bean as the host plant. Some authors have measured the density in commercial crops using other host plant species (i.e., tomato, gerbera, strawberry, and tobacco). Due to this lack of information for mass rearing, we use the data from these commercial crops to analyze the variability of the density parameters as shown below.

Ortega-Arenas *et al* (2006) reported that *T. vaporariorum* population density could range from 2.7 to 13.9 nymphs/cm^2

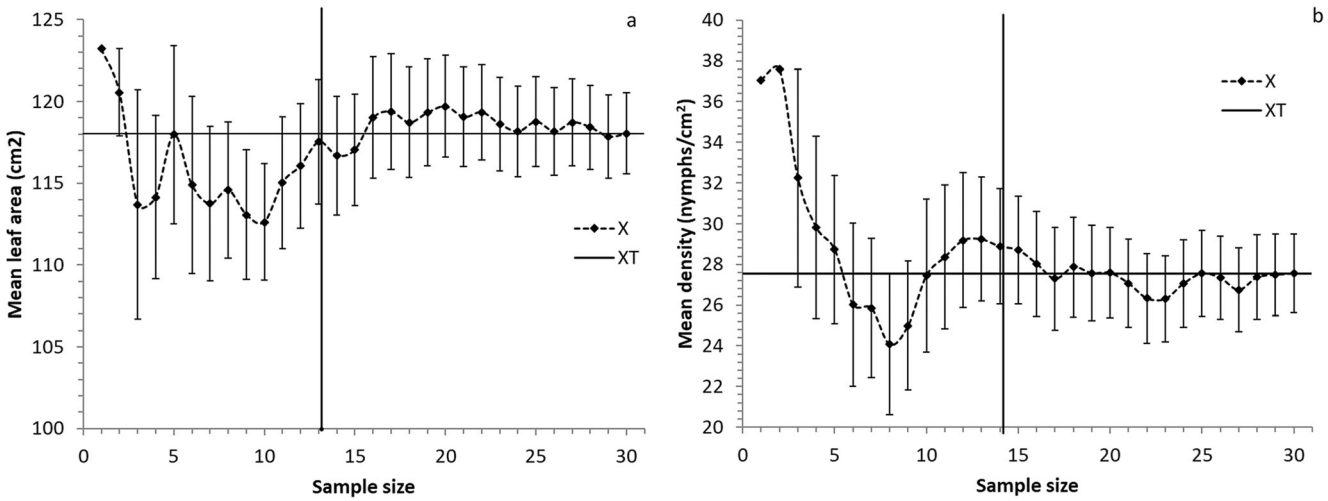

Fig 2 Graphical analysis of the minimum sampling size for the parameters leaf area (**a**) and density (**b**). X, mean of the parameter in each sample size; XT, mean of the parameter at the maximum sample size. Vertical line, minimum sample size. Error bars, standard deviation.

in *Gerbera jamesonii* (Bolus ex Hook.f.) (Asterales: Asteraceae) plants, depending on the plant cultivar and its nitrogen concentration. The whitefly population was larger when the nitrogen fertilization level is high. Bi *et al* (2002)

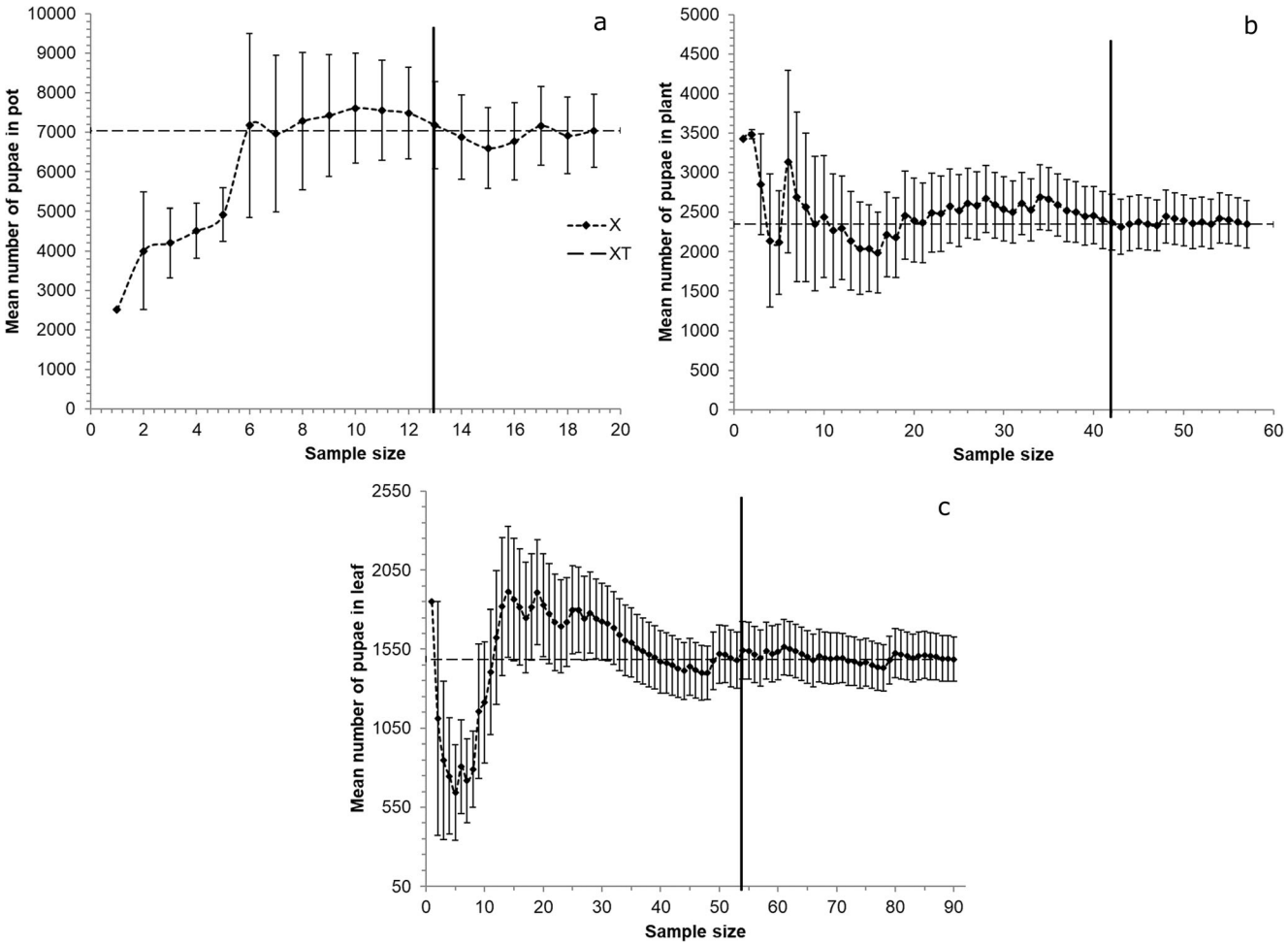

Fig 3 Graphical analysis of the minimum sampling size for each sample unit for the mean number of pupae parameter. **a** Pot. **b** Plant. **c** Leaf. X, mean of the parameter in each sample size; XT, mean of the parameter at the maximum sample size. Vertical line, minimum sample size. Error bars, standard deviation.

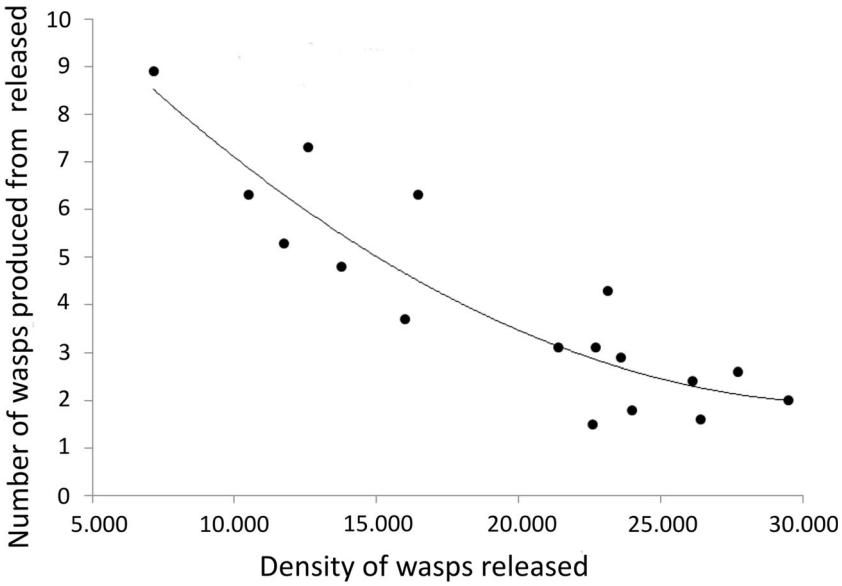

Fig 4 Number of *Encarsia formosa* produced in function of the density of wasps released (production efficiency ratio) in different production batches. $y = 1e^{-08}\ x^2 - 0.0007x + 12.867$ $R^2 = 0.822$.

found that the maximum population density in strawberry plants under cover was 12 nymphs/cm^2. In greenhouse tomato crops, values ranging from 4 to 30 nymphs per leaf have been reported for a minimum leaf area of 100 cm^2 (Jauset *et al* 2000, Basso *et al* 2001, Park *et al* 2009). In string bean and common bean, Bueno *et al* (2005) reported that when adults covered 60% of the infested leaf area, the population density of nymphs was 25.2 nymphs/cm^2.

Those studies present population density values that vary in the range determined in the present research, and those other values suggest there is a host plant effect on the density of nymphs. No information about the density parameter in tobacco plants was found, although it has been reported as the best plant for mass rearing whiteflies (Scopes & Biggerstaff 1971). Oviposition preference for whiteflies have been reported to correlated positively with leaf morphology characteristics as leaf trichome density, trichome length, and leaf lamina thickness (Hasanuzzaman *et al* 2016), so differences in oviposition between host plants also could be related to leaf morphology.

The monitoring cost in this study was approximately 1.60 USD for the 54 leaves (0.03 USD/leaf), and approximately 1-h labor was required to perform the sampling. This activity must be performed weekly. No previous studies have estimated this cost under mass rearing conditions; however, Bernal *et al* (2008) estimated that the monitoring cost for *T. vaporariorum* nymphs in commercial greenhouse tomato crop was 1.00 USD at the density of 12 nymphs per leaf with a precision level of 5%, assuming a sample size of 198 leaves (0.0050 USD/leaf). The monitoring cost per leaf found in this study was higher than that reported under commercial conditions, which was possibly due to the higher density of nymphs on the common bean leaf under mass rearing. Although this cost was higher, it is necessary to complete this task to estimate the population level in the mass rearing.

The efficiency ratio stablished in this work (Fig 4) shows that as the number of *E. formosa* released in a production cycle increased, the number of *E. formosa* adults that were produced diminished, suggesting that the optimal number should be approximately 7000 in a weekly production batch. Similar results were reported by Berndt & Meyhöfer (2007), which analyzed the control efficiency of *E. formosa* released at high densities (1.000) over a *T. vaporariorum* population in *Gerbera*. According to the authors, the use of this high *E. formosa* density caused a decrease in the rate of parasitism, which was possibly related to the parasitoid selection process for oviposition and/or predation (i.e., *host-feeding*) of the prey. Netting & Hunter (2000) reported that *E. formosa* females are able to recognize nymphs of *T. vaporariorum* that have been previously parasitized, and instead of discarding the host, they kill the egg from their congener to oviposit their own eggs. The authors also reported that this behavior has no significant effect on the handling time; thus, the effect of interference found in this study is not due to the loss of search time between parasitized and non-parasitized hosts but rather to the ovicidal behavior among congeners, which will increase if the *E. formosa* population is very high.

Previous authors have used the term "efficiency" to refer to the parameters of time or cost in natural enemy rearing conditions. Nasreen *et al* (2011) established that the production efficiency for a *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) rearing system was the proportion between the reproductive potential (total fecundity) and the development time. Using this concept, the authors estimated the production of females per time unit, which they

reported was affected positively by prey egg quantity. De Bortoli *et al* (2011) used efficiency, but they used cost terms rather than time. They established that the efficiency of the process was affected by the net reproduction rate (*R*o), and the efficiency was higher when the *R*o values were high. In the present research, neither time nor cost was used to establish efficiency; rather, a gross marginal index was used because it helps better understand how productive the rearing in numbers was, i.e., how many insects *i* produced for each one *i* used.

In a tritrophic system, population parameters are strongly related between species; for example, it has been reported that the number of *E. formosa* adults to be released depends on the number of *T. vaporariorum* third instar nymphs present (Tello *et al* 2007), and a 1 to 17 ratio is preferred. This optimized relationship maximizes the parasitization activity and prevents interference between *E. formosa* adults (Netting & Hunter 2000). This reported ratio was used in this research and is supposedly to allow a 77.13% rate of parasitism (Aragón *et al* 2008). This same proportion was reported by Roermund & Lenteren (Van Roermund *et al* 1997), who used a simulation model to establish that the maximum parasitic capacity of *E. formosa* on *T. vaporariorum* was 17 nymphs. Considering these previous reports, it is possible to use the data in the "T. vaporariorum Population Density Estimation" section to estimate that the number of *E. formosa* to be released per leaf must be 190 *E. formosa* (3241 nymphs/17 *E. formosa*).

Finally, the consolidated sampling method can be resumed in six steps as follows. (1) Take a random sample of 14 leaves from a production batch with third instar nymphs; (2) calculate the density of third instar nymphs on a 1-cm^2 section of the leaves and measure the leaf area; (3) use Eq. 4 to calculate the mean number of nymphs on a leaf and multiply this value by the approximate number of leaves in the batch (each plant in the batch has two leaves); (4) calculate the number of *E. formosa* adults to release, factoring the total number of nymphs in the batch into 17; (5) when it is time to harvest the *E. formosa* pupae, take 54 leaves of the plant and calculate the mean number of *E. formosa* pupae on the leaves; and (6) calculate the total production using Eq. 4.

To estimate the total population of *T. vaporariorum* on a leaf, it is necessary to determine the density of nymphs (nymphs/cm^2) and the area of the leaf from the samples in a production batch. The results of this study suggest that the optimal number of *E. formosa* released in each batch depends on the *T. vaporariorum* density, which should be approximately 27.5 individuals/cm^2 for a leaf with an area of 118 cm^2. At this density, the number of *E. formosa* adults released on a leaf should be approximately 190 individuals. When high populations of *E. formosa* are released in a batch, the production efficiency decreases, producing fewer new adults per adult used in the batch.

Acknowledgements The authors thank Mary Rozo for the great contribution of experience to the proper development of the research process.

Author Contributions MD, DR, and FC 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. FC contributed to the interpretation of the results. All authors discussed the results and commented on the manuscript.

Funding information This work was supported by the Research Vicerectory of Nueva Granada Military University through the research project CIAS1175 entitled "OPTIMIZACION DE LA PRODUCCIÓN DE *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) ENEMIGO NATURAL DE LA MOSCA BLANCA DE LOS INVERNADEROS *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) MEDIANTE UN MODELO DE SIMULACION."

References

Abramoff MD, Magalhães PJ, Ram SJ (2004) Image processing with ImageJ. Biophoton Int 11:36–42

Aragón S, Rodríguez D, Cantor F (2008) Release criteria of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) for the control of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) on tomato. Agron Colomb 26:277–284

Basso C, Franco J, Grille G, Pascal C (2001) Distribución espacial de *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) en plantas de tomate. Bol San Veg Plagas 27:475–487

Bernal L, Pesca L, Rodríguez D, Cantor F (2008) Direct sampling plan for *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in commercial tomato crops. Agron Colomb 26:266–276

Berndt O, Meyhöfer R (2007) Whitefly control in cut gerbera: is it possible to control *Trialeurodes vaporariorum* with *Encarsia formosa*? BioControl 53:751–762. https://doi.org/10.1007/s10526-007-9123-z

Bi J, Toscano N, Ballmer G (2002) Greenhouse and field evaluation of six novel insecticides against the greenhouse whitefly *Trialeurodes vaporariorum* on strawberries. Crop Prot 21:49–55. https://doi.org/10.1016/S0261-2194(01)00063-1

Bueno JM, Cardona C, Chacón P (2005) Fenología, distribución espacial y desarrollo de métodos de muestreo para *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) en habichuela y fríjol (*Phaseolus vulgaris* L.). Rev Colomb Entomol 31:161–170

De Bortoli SA, Otuka AK, Vacari AM et al (2011) Comparative biology and production costs of *Podisus nigrispinus* (Hemiptera: Pentatomidae) when fed different types of prey. Biol Control 58:127–132. https://doi.org/10.1016/j.biocontrol.2011.04.011

de Vis R, van Lenteren JC (2008) Biological control of *Trialeurodes vaporariorum* by *Encarsia formosa* on tomato in unheated greenhouses in the high altitude tropics. Bull Insectology 61:43–57

Español JA, Corredor D (1988) Contribución al estudio de la biología y capacidad de control de *Encarsia formosa* Gahan en la Sabana de Bogotá. Agron Colomb 5:97–102

Hasanuzzaman ATM, Islam MN, Zhang Y, Zhang CY, Liu TX (2016) Leaf morphological characters can be a factor for intra-varietal preference of whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) among eggplant

varieties. PLoS One 11:1–15. https://doi.org/10.1371/journal.pone.0153880

Hothorn T, Everitt BS (2009) A handbook of statistical analyses using R, second edition, 2nd edn. CRC Press, London

Jauset AM, Sarasua MJ, Avilla J, Albajes R (1998) The impact of nitrogen fertilization of tomato on feeding site selection and oviposition by *Trialeurodes vaporariorum*. Entomol Exp Appl 86:175–182. https://doi.org/10.1046/j.1570-7458.1998.00278.x

Jauset AM, Sarasúa MJ, Avilla J, Albajes R (2000) Effect of nitrogen fertilization level applied to tomato on the greenhouse whitefly. Crop Prot 19:255–261. https://doi.org/10.1016/S0261-2194(00)00016-8

Kranz J (1988) In: Kranz J, Rotem J (eds) Experimental techniques in plant disease epidemiology. Springer Berlin Heidelberg, Berlin, pp 35–50

van Lenteren JC, Tommasini MG (2003) Quality control and production of biological control agents: theory and testing procedures, 1st edn. CABI, Wallingford

Nasreen A, Gillespie DR, Mustafa G (2011) Graphical marginal analysis of the economics of natural enemy production: an example using a pilot mass rearing system for green lacewing. Biol Control 57:44–49. https://doi.org/10.1016/j.biocontrol.2011.01.003

Netting J, Hunter M (2000) Ovicide in the whitefly parasitoid, *Encarsia formosa*. Anim Behav 60:217–226. https://doi.org/10.1006/anbe.2000.1463

Ortega-Arenas LD, Miranda-Aragón DA, Sandoval-Villa M (2006) Densidad de huevos y ninfas de mosca blanca *Trialeurodes vaporariorum* (WEST.) en *Gerbera jamesonii* H. Bolus con diferentes regímenes de fertilización nitrogenada. Agrociencia 40:363–371

Park MK, Kim JG, Song YH, Lee JH, Shin KI, Cho K (2009) Effect of nitrogen levels of two cherry tomato cultivars on development, preference and honeydew production of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). J Asia Pac Entomol 12:227–232. https://doi.org/10.1016/j.aspen.2009.04.004

Pedigo LP, Buntin G (1994) Handbook of sampling methods for arthropods in agriculture, 1st edn. CRC Press, Boca Raton

R Core Team (2018) R: a language and environment for statistical computing. R A Lang. Environ. Stat. Comput. R Found. Stat. Comput., Vienna, Austria. ISBN 3–900051–07-0. http://www.R-project.org/

Scopes NEA (1969) The economics of mass rearing *Encarsia formosa*, a parasite of the whitefly *Trialeurodes vaporariorum*, for use in commercial horticulture. Plant Pathol 18:130–132. https://doi.org/10.1111/j.1365-3059.1969.tb00481.x

Scopes NE a, Biggerstaff SM (1971) The production, handling and distribution of the whitefly *Trialeurodes vaporariorum* and its parasite *Encarsia formosa* for use in biological control programmes in glasshouses. Plant Pathol 20:111–116. https://doi.org/10.1111/j.1365-3059.1971.tb00525.x

Sørensen JG, Addison MF, Terblanche JS (2012) Mass-rearing of insects for pest management: challenges, synergies and advances from evolutionary physiology. Crop Prot 38:87–94. https://doi.org/10.1016/j.cropro.2012.03.023

Tello P, Cantor F, Rodríguez D, Cure JR (2007) Release densities and frequencies of *Encarsia formosa* (Hymenoptera: Aphelinidae) on *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) in tomato. Agron Colomb 25:314–319

van Lenteren JC (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57:1–20. https://doi.org/10.1007/s10526-011-9395-1

Van Lenteren JC, Bueno VHP (2003) Augmentative biological control of arthropods in Latin America. BioControl 48:123–139. https://doi.org/10.1023/A:1022645210394

Van Roermund HJW, Van Lenteren JC, Rabbinge R (1997) Analysis of foraging behaviour of the whitefly parasitoid *Encarsia formosa* on a plant: a simulation study. Biocontrol Sci Tech 7:131–152. https://doi.org/10.1080/09583159731117

Zheng M-L, Zhang D-J, Damiens DD, Yamada H, Gilles J (2015) Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) - I - egg quantification. Parasit Vectors 8:42. https://doi.org/10.1186/s13071-014-0631-2