Controlled Mass Rearing of Cochineal Insect (Hemiptera: Dactylopiidae) Using Two Laboratory-Scale Production Systems in Peru

Francisco Javier Roque-Rodriguez

Department of Pharmacy, Bio-Chemistry and Biotechnology, Faculty of Pharmaceutical, Biochemical and Biotechnological Sciences, Universidad Católica De Santa María, Urb. San José s/n Umacollo, Arequipa 0401, Peru, and Corresponding author, e-mail: froque@ucsm.edu.pe

The research was conducted at the Laboratory of Bioprocess Research (LBR), Department of Pharmacy, Bio-Chemistry and Biotechnology, Universidad Católica de Santa María (UCSM), Arequipa, Perú. The insects collected in field were maintained under laboratory-controlled conditions (27 ± 0.5°C, 40 ± 5% RH, and a photoperiod of 16:8 (L:D) h).

Subject Editor: Muhammad Chaudhury

Received 22 August 2021; Editorial decision 5 November 2021

Abstract

The carmine cochineal (Dactylopsius coccus Costa) has high economic value as it is a natural source of carminic acid, an organic chromophore used in a wide range of sectors including pharmaceutics, food, and cosmetics. High demand is fuelling the search for innovative production techniques in order to move away from dependence on the prickly pear, which carries a number of limitations. The aim of this study was to establish cochineal colonies and breed and mass-produce the insects using two laboratory-scale production systems. The first system (STC-01) comprised a prismatic acrylic box with three compartments; synthetic matrices were placed vertically inside the box to provide support and a source of nutrients for the cochineal, and the system was lit artificially during fixed daylight periods. The second system (STC-02) comprised an automated micro-tunnel allowing the insects to move towards the sunlight, containing synthetic matrices arranged horizontally. There was a significant difference in yield between the two systems in a cochineal total life cycle of 120 d (80–90 d harvest period in both cases), with STC-01 being superior and producing a maximum yield of 4.86 ± 0.68 g fresh weight per day per square metre compared with 3.20 ± 0.14 g fresh weight per day per square metre production yield in STC-02. We conclude that cochineal production under controlled artificial conditions is feasible and sustainable, removing the need for natural and biological support and overcoming the environmental limitations posed by traditional production methods.

Key words: artificial rearing device, Peruvian cochineal, collagen synthetic matrix, natural host-free, increased fresh weight

In all the world, and specifically in Peru and other peripheral countries, a large variety of edible insects are harvested in wild conditions since several centuries ago but a few insect species have could be domesticated in controlled environments. Nowadays, due to the huge demand for products of biological origin, there has been an increase in the use of insects to produce commercially-valuable products including proteins and fats (Gasco et al. 2020), oils (Kierończyk et al. 2018), margarines (Smetana et al. 2020), and insect gut enzymes have also been used for the bioengineering of proteases (Kannan et al. 2019). Therefore, it is important to automate the rearing, production, harvesting, and processing of insects to make this sector more attractive, profitable, and competitive (Kroncke et al. 2020). Carmine cochineal Dactylopsius coccus Costa (DCC) is a sap-sucking insect belonging to the order Hemiptera that feeds on the cactus Opuntia ficus-indica (L.) Miller (OFI) commonly known as the prickly pear or barbary fig. This insect is commercially and economically very valuable because it is a natural biological source of carminic acid, a red dye from the anthraquinone group with a vast range of industrial uses and applications in sectors including pharmaceutics, food, and cosmetics (Méndez-Gallegos et al. 2010, Campana et al. 2015, Serrato et al. 2017). On the other side, this cactus specie also has huge socio-economic value, producing nutritional fruits and young edible cladodes that are used as vegetables (Cota-Sánchez 2015). In addition, mucilage from the cladodes has a number of uses, mainly in the environmental sector, for instance, as a natural flocculant, emulsifier, and stabilising agent (Bouaouine et al. 2019, de Andrade Vieira et al. 2021). However, maintenance and production of this cactus specie are affected by biotic (invasive) factors including the carmine cochineal, which is parasitic to the plant and sucks its sap. This, lead to chlorosis and other damage to the...
cladodes, including necrosis and eventually the death of the plant, causing serious agro-industrial problems (Palacios-Mendoza et al. 2004, Flores-Hernández et al. 2006, Rodríguez-Leyva et al. 2010).

Cocinheal insect has a natural binomial symbiotic relationship comprising a host and parasite (plant and insect) with specific affinity and a particular preference for Opuntia ficus-indica (Falcão et al. 2013), therefore, leads to a natural symbiosis wherein O. ficus-indica cladodes provides support, shelter protection, and nutrition for the breeding and growth of this scale insect, target of this study, as well as serving as a site for copulation and oviposition, which ultimately leads to necrosis of the plant (Méndez-Gallegos et al. 2010, Serrato et al. 2017). Studies of the mass-rearing released from the natural hosts of various commercially-valuable insects (parasites) have considered ways to reduce and completely eliminate damage to the host plant species. Current review in this topic reports some successful systems, both traditional and modern, for artificially rearing different insects in various parts of the world, using synthetic nutrients and substrates. These allow mass production of parasites like Hypothenemus hampei (Portilla et al. 2006, 2018), Sitotroga cerealella (Palma et al. 2011), and Anastrepha fraterculus (Flores et al. 2012), including some biocontrol agents like Ladybird Coleomegilla maculata (Riddick and Zhixin 2015), at various stages without the need for a plant host; however, no reports have been found related to unnatural or artificial systems for breeding cochineal insects that do not use their common and natural host (Opuntia sp).

Owing to the trend for natural and environmentally friendly products, the past two decades have seen an increase in demand for natural dyes, which is expected to continue (Lokeshwari et al. 2010) so the improved natural breeding of the cochineal insect has prompted efforts to design and run large-scale high-yield and rearing systems without leaving the natural host (OFI) in different configurations since several years ago (Aldama-Aguilera and Llanderal-Cázares 2003, Campos-Figueroa and Llanderal-Cázares 2003, Tovar et al. 2005, Flores-Hernández et al. 2006, Diodato et al. 2009, Méndez-Gallegos et al. 2010, Paul-Fils et al. 2020). Such systems enable optimal control of the environmental parameters (temperature, relative humidity, daylight, etc.) that influence the establishment and growth of Dactylopius coccus Costa, while minimising antagonists such as extreme temperatures, dehydration, and adverse weather conditions such as rain, wind, and excessive solar radiation. Mexico and Peru have reported the use of alternative DCC mass-rearing methods using greenhouses for the infestation and establishment of cochineal colonies on cut stems hung vertically. These methods, which were intended to resolve the recurring biotic and abiotic problems that inhibit normal development of the insects, have met with relative success (Campos-Figueroa and Llanderal-Cázares 2003). Alternatively, micro-tunnels (Aldama-Aguilera et al. 2005) and the called ‘Nopalotecas’ (Hernández et al. 2017, Zacarias-Alvarado et al. 2020) are outstanding and have been used to protect infested Opuntia ficus cladodes, and have produced excellent results with respect to the carminic acid content of the final harvest ensuring a large fresh weight of the reared cochineal (Rodríguez et al. 2005, Alonso et al. 2013, Flores-Alatorre et al. 2014, Zacarias-Alvarado et al. 2020); however, the dependence of these systems on OFI cladodes result in serious problems with the preservation of this cacti specie. This study, therefore, reports the use of artificial supports for the establishment and growth of DCC rather than natural cladodes in two engineered controlled systems. These devices used synthetic matrices made of hydrolysed collagen that replaced OFI's natural cladodes taking advantage of its permeability, gelling, texture, thickness, and water-binding properties (Gómez-Guillén et al. 2011). The objective of this investigation was to establish, rear, and mass-produce carmine cochineal colonies in two laboratory-scale production systems to efficiently substitute natural rearing in prickly pear or barbary fig in open field defining a good quality and concentration of carminic acid with that produced in natural conditions by ensuring a highest value of D. coccus fresh weight using artificial and functional synthetic medium (Hendrikson and Merkle 2014).

Materials and Methods

Source of Clean and Infested Cladodes

Cladodes were collected from Opuntia ficus-indica plants of between 1 and 2 years old from the region of Tiabaya (16° 29′ 32.80″ S, 71° 50′ 54.07″ W, altitude 1,513 m) in Arequipa (Peru) as a natural source of both host and parasite (Fig. 1A); healthy samples (host only) and infested samples (host + parasite) were collected from different plants in a typical production zone in this geographic locality (Fig. 1B). Cladodes were harvested by cutting the base of the plant, leaving a clean and healthy stump, then transported to the laboratory and stored whole in metal cages (60 cm wide × 60 cm long × 30 cm high) before being covered with transparent polypropylene sheets and kept at 27 ± 0.5 C and 40 ± 5% RH (Fig. 2).

Taxonomic Identification of DCC

Wild insects were harvested from the infested cladodes and preserved in 70% ethanol, before being mounted on slides using the method for coccidian species proposed by Solis (1993) and the keys described by De Lotto (1974) related to the status and identity of
the native cochineal insects at the considered geographical location in South America. Taxonomic identification of these cochineal insects was performed by the Entomology Department of the Faculty of Biological Sciences at Universidad Nacional de San Agustín de Arequipa based on the study of the main morphological features distributed along the observed Tiabaya region in Peru, who studied the physical anatomy of three adult females using a stereoscope and optical microscope.

**Biological Characteristics**

The growth cycle, biological cycle, preoviposition period, oviposition ability, postoviposition, and egg viability of insects were all examined to obtain a description of insect behaviour, based mainly on movement, copulation, and feeding in 12 clean and cut stems of *O. ficus-indica* in laboratory (27 ± 0.5°C, 40 ± 5% RH, and photoperiod of 16:8 (L:D) h). In each cladode, fastened by an ironmade hook in vertical inverted position, 10 adult and egg-filled females of *D. coccus* collected from original infested samples were put into a small folded paper envelope (Zhang 2017).

**Physical and Chemical Composition of OFI Stems (Cladodes)**

An uninfested stem was washed, cut, and peeled to produce two fractions: skin and flesh. The first fraction was subjected to scanning electron microscopy (SEM) to determine its structural morphology and surface microstructure using a scanning electron microscope VEGA II LSH; the second fraction was homogenised in order to determine its physical and chemical properties including pH, total titratable acidity (TTA) expressed as a percentage of malic acid (MA), conductivity (G, mS/cm), total dissolved solids (TDS, ppm), oxidation-reduction potential (ORP, mV), and heavy metals, as described by Terán et al. (2013) and Hernández-Carranza et al. (2019). Finally, the second fraction was separated in a solid and liquid fraction for use as precursors of the mucilage (liquid phase) and pulp solids (solid phase).

**In Vitro Mass-rearing of DCC on an Artificial Medium**

Live cochineal were reared in vitro using the artificial medium described by Hendrickson and Merkle (2014), free of predators, and under optimal growth laboratory conditions at 27 ± 0.5°C, to obtain clean and egg-filled females free of dust, sericins, remains of exuviae, male cocoons, or another environmental contaminant (Aldama-Aguilera and Llanderal-Cázares 2003). The synthetic base medium was prepared by mixing 1.125 ml water, 37 g agar-agar, and 175 ml corn syrup. The mixture was sterilised at 121°C and 15 psi for 20 min and then cooled to 40°C, before 80 mg methyl paraben was added as an antifungal agent, ensuring it was fully dissolved. Finally, 15 g mucilage, 15 g pulp solids, and 15 g pulvérised skin obtained from the uninfested cladodes were added to the base medium as additives and attractants to stimulate its appeal to the insects and achieve normal and optimal growth. The medium was then poured into six 10 cm Petri dishes and left to cool completely and harden at laboratory temperature. Each plate was inoculated under sterile conditions with 5 egg-filled females (approximately 0.40–0.50 g fresh weight) removed from the infested cladodes harvested in the field; the cultures were then incubated under controlled conditions at 27 ± 0.5°C for 70–90 d, 40 ± 5% RH with 16 h light per day, until harvested near infested cladodes anchored in laboratory in order to prove the artificial diet.

**Preparing Three-dimensional (3-D) Permeable Hydrolysed Collagen Support Matrices**

To elaborate 3-D permeable hydrolysed collagen support as hosts for biomimetic controlled production of cochineal, were manufactured synthetic membranes (1–2 cm thick) using a modified synthetic medium proposed by Hendrickson and Merkle (2014), replacing the agar with food-grade hydrolysed collagen and adding a hardening solution (50% water, 25% formaldehyde, 15% ethanol v/v), then combining 1.125 ml agar-free Hendrickson medium with 35 g hydrolysed collagen powder type I (2,000–4,000 Da) and 2.5 ml hardening solution. This modified medium was poured into transparent acrylic moulds measuring 10 cm wide × 25 cm long × 2.5 cm thick, then left to gelify for 2 h at 27 ± 0.5°C under completely sterile conditions. The 3-D support matrices were then removed from the moulds and used to construct the cochineal mass-rearing systems. In addition, for the purposes of comparative statistical analysis, blank permeable 3-D matrices were prepared without the natural OFI additives (mucilage, pulp solids, and skin obtained from healthy cladodes).

**Experimental Configuration of the Cochineal Mass-rearing Systems**

Two different controlled engineered systems were used to house the artificial host (3-D hydrolysed collagen matrices) and parasites (insects) to encourage infestation and mass breeding.
System 1 (STC-01)
This system comprised a prismatic box made of transparent acrylic and measuring 40 cm long × 30 cm wide × 25 cm high with a metal support structure. The box was divided into three 10 cm chambers (I, II, and III) separated by a metal mesh, such that the chambers were linked but the insects could not invade or cross the barriers to contaminate a different chamber.

The structure also included four 0.64 cm stainless steel crossbars for holding the hydrolysed collagen matrices in the V position (vertically) in each of the three chambers; one of the chambers (III) contained the blank matrices. The unit was fitted with two 45-W high-pressure sodium lights (F20 T12-PL, GRO, and SHO PL., General Electric) to provide 16 h light per day controlled by a simple on-off system; a humidity, temperature, and carbon dioxide concentration (CO2) datalogger (Extech Mod. RHT-50), and a container of a supersaturated potassium carbonate solution to provide constant relative humidity of 45%, prevent the collagen matrices from drying out, and generate the modified atmosphere needed for establishment of the insect colonies.

System 2 (STC-02)
This system comprised a micro-tunnel 15 cm wide × 20 cm long with a 316-L stainless steel base, a finely textured hemispherical cupula of tulle net, an automatically-controlled electric servomotor, an Arduino UNO microcontroller, PWM communication, LDR photoresistors, and BPW34 photodiodes to determine the path of maximum luminosity during the day and for use as sunlight-tracking sensors, in order to reduce energy consumption and the use of sodium lights to provide the required 16 h of light per day.

Unlike the first system, this structure included tracking of the sun as a source of natural light to optimise energy consumption. In addition, the 3-D hydrolysed collagen membranes were arranged as a single layer in the H position (horizontally), with the same instruments for monitoring RH (%), temperature (°C), and CO2 (ppm).

Start Up, Inoculation, and Induced Infestation in STC-01 and STC-02
The experiment used a single STC-01 unit and a set of five STC-02 units so that the predetermined operational and functional parameters could be studied in quadruplicate. In STC-01, four hydrolysed collagen membranes were placed in the V position in chambers I and II, and four blank membranes were placed in chamber III.

For STC-02, four 3-D hydrolysed collagen membranes were placed in the H position in each unit, with one unit used for the blank membrane.

Infestation was induced using nests of sterile filter paper containing 10 clean egg-filled females (Diodato et al. 2009) in an optimal state, bred in laboratory from the in vitro culture.

Four nests were placed in each of the chambers of the STC-01 unit, ensuring an equal ratio of the number of inoculated insects to membrane to total number of usable surfaces (NIE:M:NUS) of 40:4:8 or 10:1:2, i.e., 10 insects per membrane with both sides used, until the end of their total life cycle. The infesting nest was placed in the same location on each membrane and in each chamber to avoid introducing an additional variable.

For STC-02, one 3-D hydrolysed collagen membrane was placed on the stainless steel base in the H position, creating a single surface for infestation. One infesting nest of 20 insects was placed in each of the STC-02 units to ensure the same NIE:M:NUS ratio as that used in the STC-01 unit, given that the STC-02 units had just one available surface per hydrolysed collagen membrane.

Once the systems had been set up and inoculated using the process described by Diodato et al. (2009), the colonies were left to grow and infest for a total period of 120 d.

Carmine Cochineal Insects Production Using the STC-01 and STC-02 Systems Under Controlled Conditions
Once infestation had been established in all STC-01 and STC-02 units under controlled conditions protected from biotic and abiotic factors, the colonies were monitored to avoid cross-contamination until the gravid female phase was reached (Tekelenburg 1995). The cochineal were then collected, the total number of insects and fresh weight per 3-D hydrolysed collagen membrane in each system were recorded, and the average yield was calculated using the following equation (Feng et al. 2010):

\[
R_{NHC} = \frac{NHC}{t \times S \times n}
\]

where:
- \(R_{NHC}\) is the production yield per system, i.e., the number of insects per day per square metre;
- \(NHC\) is the number of adult females harvested;
- \(t\) is the time to reach the gravid phase in days;
- \(S\) is the membrane surface area (m²);
- \(n\) is the number of surfaces exposed to infestation.

Likewise, the yield was calculated in terms of fresh weight (\(R_{WHC}\), replacing \(NHC\) by \(WHC\) the fresh weight of adult females harvested, g fresh weight per day per square metre).

Statistical Analysis
The results obtained for yield (\(R_{NHC}\) and \(R_{WHC}\)) in terms of gravid and non-gravid insects harvested on the two different systems (STC-01 and STC-02) were subjected to Barlett and Kolmogorov tests to determine the normality and homogeneity of variance needed for analysis of variance. One-way analysis of variance (ANOVA) was conducted to assess the effect of the STC-01 chambers I and II and four STC-02 units on yield (\(NHC\) and \(WHC\)) results. Post hoc multiple comparison, Games Howell’s method, was conducted to evaluate differences among means for those data due to unequal variances. All set of experimental data were analyzed with Microsoft SPSS software version 26 (IBM SPSS Statistics 2019). In this study a P-value < 0.05 was considered statistically significant.

Results
Taxonomic Identification and Biological Characteristics of DCC
The Entomology Laboratory of the Faculty of Biological Sciences at Universidad Nacional de San Agustín de Arequipa produced a taxonomic identification of the collected biologic units of Dactylmapus sp. (report no. 179-2016-LE-UNSA), reported the individuals belong to Family Dactylopiidae, Genus Dactylmapus and Species Dactylmapus coccus Costa 1835.

In all cut stems of O. ficus-indica used in laboratory were observed that eggs emerged irregularly, occurring over a period of 3–11 d of initial infestation (7.15 ± 2.32 d; F = 1.01; df = 9,11; P ≤ 0.2896), observed incubation period before they hatched was less than 30 min in all cases and the hatching process was similar to that described by Guerra and Kosztarab (1992) for other species of this genus and characteristic of D. coccus specie. There were no
Table 1. Main developmental times (days) of *Dactylopius coccus Costa 1835* reared in cut infected cladodes (27 ± 0.5°C, 45 ± 5% RH, and a photoperiod of 16:8 (L:D) h)

| Stages          | Mean ± SD | df | F value | P value |
|-----------------|-----------|----|---------|---------|
| Eggs            | 7.15 ± 2.32 | 9,11 | 1.01     | 0.2896  |
| First-instar nymph | 15.00 ± 2.83 | 9,11 | 1.05     | 0.2488  |
| Second-instar nymph | 19.25 ± 2.18 | 9,11 | 0.99     | 0.3497  |
| Adult           | 69.66 ± 18.8 | 9,11 | 1.09     | 0.2133  |
| Growth life cycle | 74.75 ± 5.51 | 9,11 | 0.91     | 0.3754  |
| Pre-oviposition period | 42.75 ± 2.18 | 9,11 | 0.98     | 0.3521  |
| Oviposition period | 91.33 ± 6.80 | 9,11 | 0.88     | 0.4725  |
| Post-oviposition period | 102.75 ± 11.47 | 9,11 | 1.32     | 0.2875  |

*Observed period in which eggs appeared in infested cladodes (incubation period < 30 min in all cases).*
observed previous oviposition. Final harvest of DCC insects obtained and withdrawn from STC-01 (19.69 ± 2.77 g fresh weight) was significantly higher to STC-02 results (8.45 ± 0.37 g fresh weight) in, approximately, 100% in number and fresh weight desired of cochineal insects was achieved with the same operational conditions. NHC and WHC result in chambers I, II, and III in STC-01 and in each of one STC-02 units followed a normal distribution but with significantly different variances when the Barlett and Kolmogorov test was applied. Box-and-whisker diagrams for the NHC and WHC results from chambers I and II (STC-01) and from STC-02 units show statistical differences between systems (Fig. 9). Means and variances for chambers I and II were similar \((n = 4; P > 0.05)\) but were so different with STC-02 \((n = 4; P < 0.001)\).

NHC was significantly influenced by the system structure used in this study \((F_{2,9} = 51.84, P < 0.01)\) and the same for WHC \((F_{2,9} = 52.626, P < 0.001)\); the highest NHC \((F_{1,6} = 0.21, P > 0.05)\) and WHC \((F_{1,6} = 0.005, P > 0.05)\) values were obtained from STC-01 in chambers I and II compared with the results from STC-02 (Tables 4 and 5).

Experimental values were used to calculate an average final yield for STC-01 unit of \(R_{NHC} = 22.81 ± 2.04\) adult females per day per
exposed square metre and $R_{WHC} = 4.85 \pm 0.50$ g fresh weight per day per exposed square metre; the blank membrane produced only $5.74 \pm 0.58$ adult females per day per exposed square metre and $1.14 \pm 0.19$ g fresh weight per day per exposed square metre (Table 4); that is, a difference of more than 75% in each case showing significant difference ($n = 4; P < 0.05$) determined, conclusively, by the absence of attractant additives in their elaboration.

For STC-02, the average final yield was $14.96 \pm 1.36$ adult females per day per exposed square metre and $3.20 \pm 0.54$ g fresh weight per day per exposed square metre (Table 5); the blank membrane produced $6.82$ adult females per day per exposed square metre, and $0.81$ g fresh weight per day per exposed square metre, that is, a difference in yield of more than 45% NHC and 25% WHC.

**Discussion**

In this study was found that a typical Peruvian cochineal insect characterized as *Dactylopius coccus* Costa 1835 obtained in open field from infested cactus *Opuntia ficus-indica* (L.) Miller could be conveniently reared and massified in two different controlled systems (STC-01 and STC-02) without using the natural host in laboratory conditions (Tables 4 and 5); however, it is unknown whether the concentration, quality, and yield of carminic acid are different from that produced under natural conditions. Therefore, to promote normal and efficient induced growth in artificial hydrolyzed collagen supports, three different fractions of the natural cladodes of *O. ficus* (skin, pulp solids, and mucilage) were used in the synthetic growth medium with components of specific function such as corn syrup as a carbon source, because they can be added to facilitate the growth of insects, as well as to promote crop health, insect health and potentially shorten the life cycle of insects to increase carminic acid/carmine yield (Hendrickson and Merkle 2014).

Obtained values of biological characteristics of the DCC population are comparable to that found by Guerra and Kosztarab (1992) for first and second instars nymphs and Zhang (2017) for nymphs and adult stages developed normally (Table 1) under laboratory conditions ($27 \pm 0.5^\circ C$, $40 \pm 5\%$ RH, and $16$ h light per day) defining a specific *Dactylopius* genus like carminic acid producer since this *D. coccus* specie is reported as a source of this red dye when hosted and reared in cactus of *O. ficus-indica* specie (Rodríguez et al. 2005, Hernández et al. 2017). Zacarias-Alvarado et al. (2020) reported that the concentration of carminic acid and the fresh weight of the reared cochineal increase proportionally, when one of the two variables is increased to a certain limit; suggesting that *D. coccus* has specific thresholds for optimal development; in addition, Rodríguez et al. (2005) determined that cochineal with the highest fresh weight had the lowest carminic acid concentration, even though they can have more eggs, which means an inverse relationship between carminic acid concentration and fecundity. In this study, the harvest was carried out before $91.33 \pm 1.96$ d when was observed the oviposition period (Table 1); $81$ d for STC-01 (Table 4) and $88$ d for STC-02 (Table 5) ensuring high fresh weights values and adult females in a period prior to oviposition in both systems.

Total dissolved solids ($355 \pm 5.66$ ppm) and pH ($3.65 \pm 0.02$) values of *O. ficus* cladode mucilage (Table 2) and aluminium, calcium, and magnesium content in the microstructure of skin (Fig. 4) and mucilage (Table 2) obtained from our study are in accordance to

Table 2. Physical and chemical properties of the mucilage fraction of clean cladodes of *O. ficus-indica* harvested in Tiabaya region (Peru)

| Parameter    | Unit    | Mean ± SD ($n = 2$) |
|--------------|---------|---------------------|
| pH           | –       | 3.65 ± 0.02b        |
| TTA          | g MA/100 ml | 0.84 ± 0.03cd      |
| G            | mS/cm   | 8.16 ± 0.21a        |
| TDS          | ppm     | 355 ± 5.66bc        |
| ORP          | mV      | +509 ± 1.45bc       |
| Aluminum     | ppm     | 0.31 ± 0.03d        |
| Iron         | ppm     | 0.09 ± 0.01d        |

*Means ± SD followed by same letter in last column are not significantly different ($P < 0.05$, Tukey’s test).
that reported by Rykaczewski et al. (2016), Barazarte et al. (2017) and Hernández-Carranza et al. (2019) provided a nutritionally well-balanced diet in the modified synthetic medium of Hendrickson and Merkle (2014) used to build their aluminum complex carmine, precursor of the anthraquinone carminic acid that would have metabolized our Peruvian cochineal insect during their total life cycle evidenced by a typical and intense red colour in the adult females bodies collected.

It is important to clarify that although two different systems were compared, both laboratory-scale production systems are free of natural host and use a stationary in V-membrane position (STC-01) and mobile in H-membrane position (STC-02) operation mode for rearing D. coccus. Our data showed that the production NHC and WHC estimated from the collected period was statistically higher \( (n = 4; F = 3.52, P < 0.05) \) in STC-01 (19.63 ± 2.03 g fresh weight, 0.025 m\(^2\), 81 d, Fig. 9B) with respect to STC-02 (8.45 ± 0.37 g fresh weight, 0.03 m\(^2\), 88 d, Fig. 9B) and, between STC-01 chambers I and II there was no significant difference so this system behaved much more homogenously. Aldama-Aguilera and Llanderal-Cázares (2003) noted that to produce one kilogram of fresh cochenille, a mean of 100 cladodes were needed in 120 d; however, results obtained in our study indicate, comparatively, a use of only 23 cladodes in 81
d (STC-01) and 32 cladodes in 88 d (STC-02) achieving four harvests per year providing all the biotic and abiotic key factors (operation mode, carbon source, and cladode fractions added in synthetic medium, membrane position, environmental constant parameters) necessary for the insects to complete their life cycle in continuous generations, successfully replacing the prickly pear cactus (OFI) in field as reported by other authors including other insect species (Sprague and Brent 2016, Morales-Ramos et al. 2015, Ramírez-Cruz et al. 2008, Chaudhury and Alvarez 1999, Barriga-Ruiz 1994). The largest cochineal population was obtained with STC-01, which used constant daylight times (Table 4), it probably resulted in smaller temperature fluctuations (Fig. 8A) and no movement requirement for its operation compared to the other system. This meant a better establishment and development of migratory and fixed nymphs in the first system since synthetic permeable matrices were used in a vertical position and their infestation was allowed on both sides simulating natural growth (Aldama-Aguilera et al. 2005). Therefore, our method could be adaptable for scale-up and start-up in continuous production mode owing were found that the cochineal presented a similar characteristic feeding behavior, similar digestive and nutritional demands, and similar reproductive and gas exchange requirements; Veldkamp et al. (2012) reported that is technically feasible to produce insects on a large scale in artificial containers under controlled conditions. Used synthetic matrices (V-position) in STC-01 produced larger populations of cochineal owing to their uniform structure and composition, avoiding the issues associated with use of a natural support (OFI) and plant age; cladode colour and age can have a negative impact on cochineal growth because of variations in nutritional content (Rodríguez et al. 2005).

Obtained experimental results allowed to warn that there was a statistically significant difference (N = 4; F = 2.45; P < 0.05) in fresh weight and number of insects values obtained in the blank membranes used in both systems that did not contain skin, pulp solids, and mucilages in their composition compared to those membranes evaluated during the total life cycle of cochineal, which would mean that these fractions are a key factor affecting the production yield of a mass rearing insect colony, 1.14 ± 0.19 g gravid insects/day/m² in STC-01 (Table 4) and 0.81 ± 0.08 g gravid insects/day/m² in STC-02 (Table 5) resulting in 76.54% and 74.68% lower than the maximum RWHC obtained, respectively.

There are no published mass rearing systems for D. coccus without natural host (O. ficus) nor using artificial diets; therefore, the results obtained in this study were compared to the fresh weight production of cochineal insect (Dactylopius coccus C.) (Hemiptera: Dactylopiidae) using natural and induced host. Fresh weight and number of reared insects harvested from our study were, in all cases, higher than those reported and found in open field with native and transplanted plants (Santibañez 1992, Barriga-Ruiz 1994, Tovar et al. 2005, Feng et al. 2010), in greenhouses using cut cladodes (Aldama-Aguilera and Llanderal-Cázar 2003, Campos-Figueroa and Llanderal-Cázar 2003, Alonso et al. 2013), in microtunnel greenhouses (Aldama-Aguilera et al. 2005), and in ‘Nopaloteca’ systems (Hernández et al. 2017, Zacarias-Alvarado et al. 2020).

In this context, based in the obtained results, is referring to that using proposed facilities, STC-01 specifically, up to four cochineal harvests could be produced per year, compared with two or three obtained in open field or greenhouses that used natural host. Santibañez (1992), Barriga-Ruiz (1994), and Tovar et al. (2005) reared D. coccus species using natural O. ficus-indica in open and...
Table 4. Final population number (NHC) and fresh weight (WHC) of Dactylopius coccus Costa at harvest in STC-01 system\(^a\) (30.08 ± 2.33°C, 45.21 ± 2.87% RH, and a photoperiod of 16:8 (L:D) h)

| Position | Chamber |
|----------|---------|
|          | I       | II       | III\(^6\) |
|          | NHC, adult females | WHC, g fresh weight | NHC, adult females | WHC, g fresh weight | NHC, adult females | WHC, g fresh weight |
| Membrane 1 | 86      | 18.40    | 95       | 20.05    | 22       | 4.38  |
| Membrane 2 | 78      | 16.69    | 84       | 17.72    | 20       | 3.98  |
| Membrane 3 | 96      | 20.54    | 94       | 19.83    | 22       | 4.38  |
| Membrane 4 | 108     | 23.11    | 98       | 20.69    | 29       | 5.77  |
| Mean ± SD (n = 4) | 92.00 ± 12.96ab | 19.69 ± 2.77cd | 92.75 ± 6.08ab | 19.57 ± 1.28cd | 23.25 ± 3.95e | 4.63 ± 0.79e |
| R\(^b\) NHC | 22.72 ± 2.77ab | –        | 22.90 ± 1.30ab | –        | 5.74 ± 0.84c | –     |
| R\(^b\) WHC | –       | 4.86 ± 0.68ab | –        | 4.83 ± 0.32ab | –        | 1.14 ± 0.19c |

\(a\)Membrane area = 0.025 m\(^2\); Harvest period (oviplane phase) = 81 d; Number of used infestation surfaces = 2.

\(b\)Production yield reported as number of adult females per day per square metre.

\(c\)Production yield reported as g fresh weight per day per square metre.

\(d\)Blank values; Mean ± SD followed by the same letter are not significantly different (\(P < 0.05\), Tukey’s test).

Table 5. Final population number (NHC) and fresh weight (WHC) of Dactylopius coccus Costa at harvest in STC-02 system\(^a\) (28.55 ± 3.04°C, 47.35 ± 1.09% RH, and a photoperiod of 16:8 (L:D) h)

| Replicate | NHC, adult females | WHC, g fresh weight |
|-----------|--------------------|---------------------|
| Blank     | 18                 | 2.13                |
| R\(^b\) NHC | 6.82             | –                   |
| R\(^b\) WHC | –                 | 0.81                |
| 1         | 42                 | 8.99                |
| 2         | 39                 | 8.35                |
| 3         | 38                 | 8.13                |
| 4         | 39                 | 8.35                |
| Mean ± SD (n = 4) | 39.50 ± 1.73 | 8.45 ± 0.37        |
| R\(^b\) NHC | 14.96 ± 1.36     | –                   |
| R\(^b\) WHC | –                 | 3.20 ± 0.54         |

\(a\)Membrane area = 0.03 m\(^2\); Harvest period = 88 d; Number of used infestation surfaces = 1.

\(b\)Production yield reported as number of adult females per day per square metre.

\(c\)Production yield reported as g fresh weight per day per square metre.

In summary, the observed properties of permeability and porosity of the hydrolysed collagen membranes used to replace the natural process were promising to achieve the establishment and development of insects in both systems, but the V position turned out to be more significant to define this variable in the systems under analysis. STC-01 used in this study provided full protection from biological and physical contamination and ensure optimal levels of biotic and abiotic factors but the uncontrolled factors such as stray sounds or vibrations (STC-02) influence negatively the rearing process as reported by Choi and Park (2019).

Currently, controlled environment monitored in this study was found superior to natural conditions because the biological growth of cochineal may be limited by meteorological factors such as heavy rain, sub-zero temperatures, direct sunlight, and strong winds, especially during the early stages of development of cochineal (Flores-Hernández et al. 2006, Diodato et al. 2004). In addition, a controlled environment protects the insects from exposure to parasitic and predatory insects that feed on them, including Hymenoptera, Diptera, Coleoptera, Lepidoptera, and Neuroptera (Cohen 2018, Zimmermann et al. 1979).

The economic value of OFI and DCC can be multiplied through the use of controlled systems, with box and stationary system (STC-01) proving superior to mobile and dynamic system with membranes in H-position (STC-02). South America is largely arid, and the farming of OFI for infestation with DCC is a widespread practice requiring vast areas of farmland that could be used for other economic purposes. Growing DCC under controlled conditions means a host is no longer required, thus freeing up extensive areas of farmland. Finally, no or very little research has yet focused on the establishment of cochineal insects in this type of controlled or semi-controlled systems free of OFI; this is one of the innovative aspects of the current study. However, additional investigation is needed that examines the potential of the proposed methods and innovative controlled production systems to evaluate quality, yield, and concentration of carminic acid in order to verify no detrimental changes of DCC reproduction with this important response.

Acknowledgments

I acknowledge the financial support and the ideal infrastructure to ensure the quality of the results obtained in this study by all the staff of the office of Vice-Rectorate for Research of Universidad Católica de Santa María (Arequipa, Peru). This work was funded by a grant from the Vice-Rectorate for Research of Universidad Católica de Santa María (Arequipa 21906-R-2015).
Portilla, M., and M. Grodowitz. 2018. A novel method to evaluate the reproductive potential of Phymastichus coffee (Hymenoptera: Eulophidae) in Hypothenemus hampei (Coleoptera: Curculionidae: Scolytinae) under laboratory conditions. J. Insect Sci. 18: 15; 1–7.

Portilla, M., and D. Streett. 2006. Nuevas técnicas de producción masiva automatizada de Hypothenemus hampei sobre la dieta artificial Cenibroca modificada. Rev. Col. Entomol. 57: 37–50.

Ramírez-Cruz A., C. Llanderal-Cázares, and R. Racotta. 2008. Ovariole structure of the cochineal scale insect, Dactylopius coccus. 5pp. J. Insect Sci. 8: 20.

Riddick, E., and W. Zhixin. 2015. Effects of rearing density on survival, growth, and development of the ladybird coleomegilla maculata in culture. Insects. 6: 858–868.

Rodríguez, L., E. Faúndez, J. Seymour, C. A. Escobar, L. Espinoza, M. Petrousta, A. Ayres, and H. M. Niemeyer. 2005. Factores bióticos y concentración de ácido carmínico en la cochinilla (Dactylopius coccus Costa) (Homoptera: Dactylopiidae). Agric. Técnica. 65: 323–329.

Rodríguez-Leyva, E., J. R. Lomeli-Flores, and J. M. Vanegas-Rico. 2010. Enemigos naturales de la grana cochinilla del nopal Dactylopius coccus Costa (Hemiptera: Dactylopiid), pp. 101–112. In L. Portillo, A. L. Vigueras (eds.), Conocimiento y Aprovechamiento del Nopal. Universidad de Guadalajara, Guadalajara, Jalisco, México.

Rykaczewski, K., J. S. Jordan, R. Linder, E. T. Woods, X. Sun, N. Kemme, and L. C. Majure. 2016. Microscale mechanism of age dependent wetting properties of Prickly Pear Cacti (Opuntia). Langmuir. 32: 9335−9341.

Santibañez, M. T. 1992. Formas de explotación de grana-cochinilla en Valles Centrales en Oaxaca, pp. 69–72. In Memories of the III International and V National Congress on knowledge and uses of nopal. Universidad Autónoma de Chapingo and CONACYT, Chapingo, México.

Serrato, J., G. Arroyo, and R. Rojas. 2017. Producción y control de calidad del insecto Grana Cochinilla. Jóvenes en la Ciencia. 2: 1444–1449.

Smetana, S., L. Leonhardt, S. M. Kauppi, A. Pajic, and V. Heinz. 2020. Insect margarine: processing, sustainability and design. J. Clean. Prod. 264: 121670.

Solís, A. J. F. 1993. Escamas (Homóptera: Coccoidea); descripción, morfología y técnica de montaje. Departamento de Parasitología Agrícola. Universidad Autónoma Chapingo. Estado de México, 34–36 p.

Spruceon, D. W., and C. S. Brent. 2016. Development, survival, and hatching periodicity of Lygus hesperus (Hemiptera:Miridae) eggs under constant and variable temperatures. J. Entomol. Sci. 51: 292–304.

Tekelenburg, A. 1995. La producción de cochinilla (Dactylopius coccus Costa) en ambientes semicontrolados. pp. 48–55. In E. Pimiento, C. Neri, A. Muñoz, F. M Huerta (eds.), Memorias del 6to Congreso Nacional y 4to. Internacional sobre el Conocimiento y Aprovechamiento del Nopal. Universidad de Guadalajara, Guadalajara, Jalisco, México.

Tovar, A., M. Pando-Moreno, and C. Garza. 2005. Evaluation of three varieties of Opuntia ficus-indica (L.) Miller as hosts of the cochineal insect Dactylopius coccus Costa (Homoptera: Dactylopiidae) in a semiarid area of Northeastern Mexico. Econ. Bot. 59: 3–7.

Veldkamp, T., G. Van Duinkerken, A. Van Huis, C. M. Lakemond, E. Ottevanger, G. Bosch, and M. A. Van Boekel. 2012. Insects as a sustainable feed ingredient in pig and poultry diets : a feasibility study = Insecten als duurzame diervoedergrondstof in varkens- en pluimveevoeders : een haalbaarheidstudie. (Report / Wageningen UR Livestock Research; No. 638). Wageningen UR Livestock Research.

Zacarías-Alvarado, J. R., C. L. Tovar-Robles, S. de J. Méndez-Gallegos, R. Magallanes-Quintanar, and G. Aquino-Pérez. 2020. Yield and quality of carminic acid of Dactylopius coccus Costa in different height levels of the Nopaloteca. Agrociencia. 54: 705–716.

Zhang, Z. 2017. The life tables of Dactylopius Coccus Costa (Homoptera: Dactylopiidae) at different temperatures and humidities. Agric. Forest. Fish. 6: 45–48.