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Contents

Preface

The use of biological control (BC), especially to control agricultural pests, has been increasing by 10–15% annually worldwide. Insect rearing is of prime importance for the success of BC programs, especially for macro-organisms. Many commercial firms currently mass-rear and sell these control agents. The programs using control agents, especially in Brazil, were initially developed by government agencies and today are conducted by commercial firms. Some of these companies have developed the capacity to supply large-scale control programs, and some are start-ups or smaller companies that meet local requirements. Whatever the eventual purpose, all BC programs must start with small-scale insect rearing, so-called "research rearing", in order to learn the capabilities of both the pest and the natural enemy.

For releases of macro-organisms in greenhouses or in open fields, smaller or larger rearing systems are used, depending on the number of insects needed. Small- and medium-scale production of insects is used for classic biological control, with inoculative releases, and largest-scale production is for augmentative biological control, with "inundative releases". Natural enemies are usually produced on the host that they parasitize in the wild (natural host), or when possible, on a factitious host in order to simplify the rearing procedure or to reduce the production cost.

The development of artificial diets for pest rearing has greatly simplified the process of rearing natural enemies. These artificial diets have significantly advanced mass-rearing techniques, especially for pests of the orders Lepidoptera, Coleoptera and Diptera, on which the natural enemies are reared. Research on diets, beginning in the 1960s, has contributed to the advances in BC and integrated pest management (IPM). Despite these advances, the basic rearing procedures must always be developed at universities or research institutes, and only later scaled up and modified for large-scale rearing (mass rearing), where the problems of sanitation, cost, quality control, and storage will inevitably increase.

For any BC program, it is necessary to rear two species of insects: a natural host (which may be the pest) or a factitious host; and the natural enemy for release. In a few cases, a factitious host can be used to replace the natural

host. A well-known example is rearing egg-parasitoid wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), which parasitize several species of lepidopterans, in stored moth eggs. This possibility has been known since 1927, when the American researcher S.E. Flanders published a note stating that species of *Trichogramma* could be reared in eggs of the Angoumois grain moth *Sitotroga cerealella*.

Subsequent investigators found that it was possible to rear *Trichogramma* spp. in eggs of other moth species, which provide some advantages, such as *Anagasta*[1] *kuehniella* and *Corcyra cephalonica*. The Chinese use eggs from silkworm species such as *Bombyx mori*, *Antheraea pernyi* or *Samia cynthia* to rear this egg parasitoid. There are other examples of factitious hosts, such as *Galleria mellonella*, a factitious host for the tachinid flies that parasitize *Diatraea saccharalis*. Obviously, when the natural enemy is produced in a factitious host, it must have similar qualities to natural enemies reared in the natural host. Brazil has been increasing the use of BC, especially in sugarcane, to control the sugarcane borer *Diatraea saccharalis* with releases of the introduced parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae), reared on the natural host, which in turn is reared on an artificial diet. *Diatraea saccharalis* is also controlled with the egg parasitoid *Trichogramma galloi* (Hymenoptera: Trichogrammatidae), reared in the factitious host *Anagasta kuehniella* (Lepidoptera: Pyralidae). Currently, *C. flavipes* is being used on more than 3 million hectares, and *T. galloi* on about 2 million hectares of sugarcane. Predators, being non-specific, are reared on different prey (hosts),

This technical guide makes available to professors, researchers, undergraduate and graduate students, technicians, extension workers, and farmers the techniques for small-scale rearing of the egg parasitoids *Trichogramma* spp. in the factitious host *Anagasta kuehniella*. These parasitoids are among the most widely used natural enemies in the world, and in Brazil are used to control lepidopteran pests in a wide variety of crops, including sugarcane, corn, soybean, cotton, vegetables (tomatoes, potatoes), fruit (avocado, citrus, grapes), and tobacco.

[1]The genus *Anagasta* is used by American taxonomists, while *Ephestia* is used by Europeans. Thus, depending on the local publication, *Anagasta kuehniella* or *Ephestia kuehniella*

1 Introduction

More than 235 species of *Trichogramma* have been described worldwide, 29 of which are reported in Brazil (Querino and Zucchi, 2019) (**Table 1**). These tiny egg parasitoids (0.25 mm) are associated mainly with lepidopteran pests and have been released on 18 million hectares, primarily in socialist countries (the former Soviet Union and China) (Filippov, 1992; Hassan, 1994). Today, the wasps continue to be used and marketed in many countries to control pests of various crops, along with the 440 other species of natural enemies that are currently available commercially (van Lenteren *et al.*, 2018). Around 28 species of *Trichogramma* had already been released to control pests of 28 crops (Hassan, 1988).

Table 1. *Trichogramma* species described in Brazil (from Querino and Zucchi, 2019)

Trichogramma acacioi Brun, Moraes & Soares, 1984
Trichogramma acuminatum Querino & Zucchi, 2003
Trichogramma alloeovirilia Querino & Zucchi, 2003
Trichogramma atopovirilia Oatman & Platner, 1983
Trichogramma atropos Pinto, 1992
Trichogramma bertii Zucchi & Querino, 2003
Trichogramma bruni Nagaraja, 1983
Trichogramma clotho Pinto, 1992
Trichogramma demoraesi Nagaraja, 1983
Trichogramma dissimilis Zucchi, 1988
Trichogramma distinctum Zucchi, 1988
Trichogramma esalqueanum Querino & Zucchi, 2003
Trichogramma exiguum Pinto & Platner, 1978
Trichogramma galloi Zucchi, 1988
Trichogramma iracildae Querino & Zucchi, 2003
Trichogramma jalmirezi Zucchi, 1988
Trichogramma lasallei Pinto, 1999
Trichogramma manicobai Brun, Gomez de Moraes & Soares, 1984
Trichogramma marandobai Brun, Gomez de Moraes & Soares, 1986
Trichogramma maxacalii Voegelé & Pointel, 1980

Continued

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Table 1. Continued.

Trichogramma parrai Querino & Zucchi, 2003
Trichogramma piracicabense Querino & Zucchi, 2017
Trichogramma pratissolii Querino & Zucchi, 2003
Trichogramma pretiosum Riley, 1879
Trichogramma pusillum Querino & Zucchi, 2003
Trichogramma rojasi Nagaraja & Nagarkatti, 1973
Trichogramma tupiense Querino & Zucchi, 2003
Trichogramma valmiri Querino & Zucchi, 2017
Trichogramma zucchii Querino, 2003

One of the great advantages of these parasitoids is the possibility of rearing them on factitious hosts, which costs significantly less than rearing them on the natural host (Parra, 1997).

Flanders (1927) demonstrated the feasibility of using eggs of the Angoumois grain moth, *Sitotroga cerealella*, to rear species of *Trichogramma*. Following this discovery, mass rearing of species of *Trichogramma* on *S. cerealella* eggs came to be a routine procedure.

Half a century later, Lewis and collaborators (1976) showed that some cases of failure in the use of *Trichogramma* species were due to the nutritional inadequacy of the host that was used, and recommended that *S. cerealella* be replaced by the flour moth *Anagasta kuehniella* to produce long-lived and more fecund parasitoids (**Table 2**). As a result, several laboratories in Europe started to use this host for rearing. The Chinese, who are among the largest users of *Trichogramma*, use eggs of the rice moth *Corcyra cephalonica* or ova of silkworm species such as *Antheraea pernyi* and *Samia cynthia ricini* (Parra, 1997).

In Brazil, studies with these egg parasitoids began in the 1940s, in order to control *Neoleucinodes elegantalis* in tomato (Gomes, 1963). These studies were followed by the work of Moraes *et al.* (1983) to control forest pests. For the Brazilian species of *Trichogramma* that are currently mass reared for field release, *T. pretiosum*, *T. galloi* and *T. atopovirilia* (**Table 3**), *A. kuehniella* has proven to be the most suitable factitious host because it is easiest to rear, even though *C. cephalonica* is also efficient for rearing *T. galloi* (**Table 3**). Although *S. cerealella* is the poorest performer of the three host species, it is the easiest to rear in the laboratory and is still widely used in many countries, such as Colombia and Germany.

In studies initiated at Piracicaba, São Paulo, Brazil in the 1980s to control several agricultural pests, *A. kuehniella* has been used to rear *Trichogramma* species (Parra, 1997; Parra and Coelho Jr, 2019). The appropriate generic placement of this moth is currently under discussion; in general, European taxonomists assign it to *Ephestia*, and American workers to *Anagasta*.

This technical guide describes the details of a rearing system for *A. kuehniella*, for research purposes. This system produces 100 g of eggs per

Table 2. Fecundity and longevity of *Trichogramma pretiosum* reared on *Sitotroga cerealella* and *Anagasta kuehniella* (Lewis *et al*., 1976)

Host	Parasitized eggs/female	Longevity (days)
Anagasta kuehniella	147.9 ± 6.1	19.9 ± 1.0
Sitotroga cerealella	9.9 ± 1.3	4.5 ± 0.4

Table 3. Most suitable factitious (alternative) hosts for rearing *Trichogramma* species used in Brazil

Species	Factitious host
Trichogramma pretiosum	*Anagasta kuehniella* (Parra *et al*., 1991)
Trichogramma galloi	*Anagasta kuehniella* or *Corcyra cephalonica* (Gomes and Parra, 1998)
Trichogramma atopovirilia	*Anagasta kuehniella* or *Corcyra cephalonica* (Dias *et al*., 2010)

day (1 g corresponds to approximately 36,000 eggs). For commercial or other mass-rearing purposes the production scale must be adjusted. Today, some commercial firms are capable of producing 40 kg of *A. kuehniella* eggs per day, for rearing not only *Trichogramma* but also other predators such as *Orius*, coccinellids, chrysopids, etc. (CropLife Brasil, personal communication[1]).

Note

[1]CropLife Brasil-integration of different associations, including ABCBio Brazilian Association of Biological Control Companies

2 Taxonomy, Biology, and Behavior of *Trichogramma* Species

Of the 29 species of *Trichogramma* described in Brazil (Table 1), *T. galloi*, *T. pretiosum* and *T. atopovirilia* are currently used by farmers. These three species can be reliably identified only by specialists, following the illustrated key presented by Querino and Zucchi (2011). Molecular techniques can also be used to identify these wasps (Ciociola Jr *et al.*, 2001; Stouthamer, 2006) and hyperspectral imaging shows promise for this purpose (Nansen *et al.*, 2014).

These tiny wasps should be reared in separate rooms to avoid mixing species. Although *Trichogramma* species are non-specific parasitoids, they may have different and well-defined intrinsic microclimatic requirements. For this reason, *Trichogramma* strains must be kept isolated by collection site and host, because sometimes a strain collected in a cold region may not perform efficiently in a warm region and vice versa (Bleicher and Parra, 1990a; Coelho Jr *et al.*, 2018). In this case, strains are defined as collections made in the same place on a given host. They should be identified by a code that indicates the species, collection site, and season of the year (Geremias, 2008) (Fig. 1).

Fig. 1. Coding system used to label strains. The first letter (1) indicates the species (in this example, *Trichogramma galloi*); the next numbers (2) provide the postal address code of the collection site (first five numbers of the postal code, here the code for Jacarezinho, Paraná); and the last letter (3) represents the season when the strain was collected (in this case, S = summer) (Geremias, 2008).

(1) Species
(2) Postal address code
(3) Harvest season

Females of *Trichogramma* lay 70 to 120 eggs on average (Parra and Zucchi, 2004) and the number of eggs is proportional to the size of the host. Thus,

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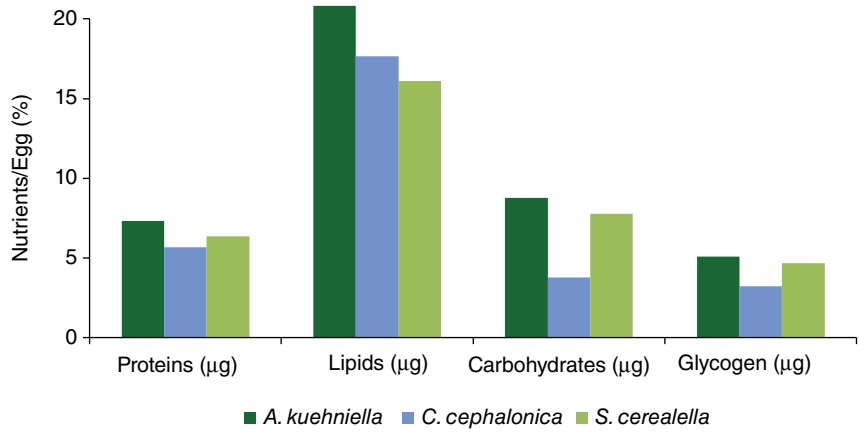

Fig. 2. Percentage of nutrients in relation to weight of eggs of three alternative hosts (Coelho Jr and Parra, unpublished data).

an egg of *S. cerealella* provides enough nutrients for one parasitoid individual to develop; *A. kuehniella* enough for one or two; and *Erinnyis ello* (L.) for more than 50 parasitoids. Eggs of the different species differ not only in size but also in their nutritional content (**Fig. 2**). In eggs parasitized by *Trichogramma*, multiparasitism can occur, i.e. two different species of parasitoids can emerge from the same egg.

The sequence of behaviors by *Trichogramma* wasps in parasitizing an egg is well characterized (Parra and Cônsoli, 2009) (**Fig. 3**). The wasp (the natural enemy) is attracted by a kairomone (a chemical substance that can be perceived by the wasp) emitted from the scales of lepidopterans (the host). Certain substances, such as tricosane, are deposited on egg surfaces at the time of laying and attract the female wasps to parasitize the eggs.

The symbiont *Wolbachia* (Stouthamer *et al.*, 1993), a bacterium that causes feminization, parthenogenesis or cytoplasmic incompatibility, is commonly present in *Trichogramma*. *Wolbachia* can be eliminated with antibiotics or by keeping the insects at a temperature above 28°C (Stouthamer *et al.*, 1990).

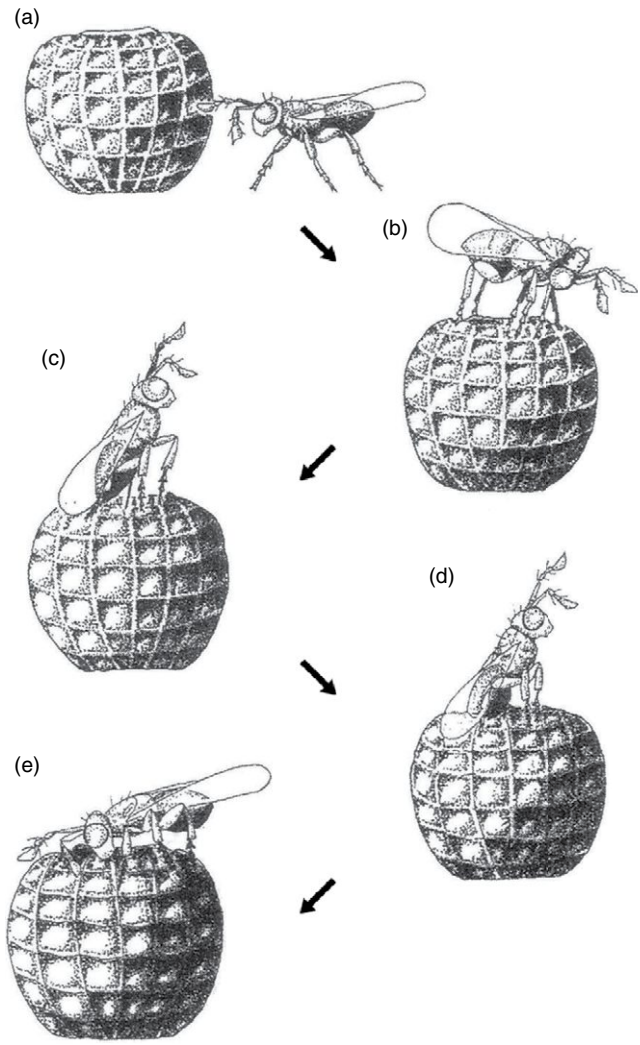

Fig. 3. Sequence of events as a *Trichogramma* female wasp parasitizes a host egg (Parra and Cônsoli, 2009, adapted from Pak, 1988): (a) contact; (b) evaluation; (c) drilling; (d) oviposition; (e) host feeding.

3 Production Sequence of *Anagasta kuehniella* and *Trichogramma* Species

Rearing of *A. kuehniella* must be started with eggs from a laboratory that maintains strict quality control of the insects produced (Parra, 1997).

Five rooms are needed for the different stages of rearing:

1. Room for rearing the larval phase
2. Room for moths to emerge
3. Room for collection of eggs
4. Room for preparation of materials, diet, and cleaning and disinfection of materials
5. Room for *Trichogramma* rearing.

Rearing of *A. kuehniella*

Early studies developed small-scale production systems (Strong *et al.*, 1968; Bournier and Peyrelongue, 1973) and then extended to mass rearing of *A. kuehniella* (Daumal *et al.*, 1975, 1985). In Brazil, the first *Trichogramma* rearing sytem using *A. kuehniella* as a factitious host was developed by Moraes *et al.* (1983), aiming to control forest pests. Later, for agricultural pests, a small-scale rearing technique was developed by Parra *et al.* (1985), followed by the work of Stein and Parra (1987a) and Parra *et al.* (1989a, b). Subsequent studies by Parra *et al.* (1994) and Magrini *et al.* (1993, 1995) aimed at developing a more economical diet to produce good-quality insects. Innovations in the rearing model for *A. kuehniella* were proposed by Parra *et al.* (2010), Parra *et al.* (2013), Coelho Jr and Parra (2013a, b) and Coelho Jr *et al.* (2016a).

Rearing Techniques

The most frequently used diets are composed of whole-wheat flour (97%) plus brewer's yeast (3%) (Parra *et al.*, 1989b) or wheat flour (40%) plus corn (maize) flour (non-GMO) (60%) (Parra *et al.*, 1994). The diets can be used

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alternately to avoid problems with insects due to occasional contamination of the components by agrochemicals.

Based on the weight of adults reared and the number of eggs laid, a diet consisting of common hybrid corn (yellow corn) can be comparable to the conventional diet (wheat flour and yeast) and also to a diet of white corn, which contains larger amounts of certain amino acids (lysine and tryptophan) (Magrini *et al*., 1995) but is more difficult to obtain in the Brazilian market.

After the techniques for rearing moths in the various stages of development are established, in order to schedule production on small or, mainly, large scales, the effect of temperature on the duration of the insect life cycle must be known (**Table 4**). Knowledge of the temperature requirements makes it possible to forecast or even control insect production in the laboratory. The temperature requirements of insects are evaluated by the thermal constant (K), expressed in degree-days (DD), which starts from the hypothesis that the duration of development at a certain temperature is a constant, computed from the lower developmental threshold, called the temperature threshold (Tt). This thermal constant is expressed by the formula: $K = D \times (T - Tt)$, where D is the duration of development and T is the environmental temperature (Bean, 1961; Silveira Neto *et al*., 1976).

Knowledge of the temperature threshold (Tt) of the egg phase is important for storing eggs that have not been used (at times when no release is planned). This value is close to 10°C for *A. kuehniella*, according to several studies such as those of Davison (1944), Bell (1975), and Jacob and Cox (1977), and coincides with the results obtained by Stein and Parra (1987a) and Coelho Jr (2011).

Optimal container capacity for the different rearing stages

In this kind of rearing, the aim is to maximize egg production. In the case of *A. kuehniella*, using plastic trays (boxes) (35 × 29 × 6 cm) for development

Table 4. Duration of the embryonic and egg-adult periods of *Anagasta kuehniella* at different temperatures and the respective thermal requirements (Stein and Parra, 1987a)

	Length (days)	
Temperature (°C)	Egg	Egg-adult
18	10.2	108.4
20	9.5	89.5
22	6.7	68.3
25	5.1	51.0
30	4.0	41.0
32	3.8	–
Tt (°C)	10.5	11.0
K (DD)	79.2	760.8

Tt = temperature threshold. K = thermal constant, expressed in degree-days (DD).

of the larval and pupal stages, the highest production is obtained with 0.3 g of eggs (≈ 10,800 eggs) per rearing tray (Coelho Jr *et al.*, 2016 a.). As the number of eggs per tray increases, the weight of the resulting adults decreases, and these lighter adults will produce fewer eggs (Parra *et al.*, 1989a; Coelho Jr and Parra, 2013a).

Large populations of larvae (caterpillars) raise the temperature inside the trays by 7–9°C at the end of development, due to the high metabolic activity of the insects (Cerutti *et al.*, 1992; Coelho Jr and Parra, 2013b). This temperature increase not only prolongs the larval period, but also produces smaller adults that have short life spans and do not lay eggs. Parra *et al.* (1989a) found that adults which emerged by the second week laid more eggs (432) than those which emerged in the seventh week (315). CO_2 concentrations above 1200 ppm in the rearing rooms may reduce egg production (Coelho Jr and Parra, 2013a).

A similar principle of space requirements applies to the adult collection cage. As mentioned by Peters and Barbosa (1977), there is always an optimal space for maximum egg production. In cylindrical containers 24 cm in diameter and 27 cm high, there is an optimal number of adults to obtain the maximum egg production per female (Parra, J.R.P., personal information).

Rearing system used in the Entomology and Acarology Department at USP/ESALQ

The rearing system for *A. kuehniella* uses white plastic trays measuring 35 × 29 × 6 cm and each containing 1 kg of artificial diet (**Fig. 4A**), one of the two diets mentioned above (whole-wheat flour plus brewer's yeast or wheat flour plus corn flour). In each tray, 0.3 g of *A. kuehniella* eggs (about 10,800 eggs) is "inoculated" (Coelho Jr and Parra, 2013b; Coelho Jr *et al.*, 2016a), with or without strips of corrugated cardboard for pupation (**Fig. 4B**). The cardboard can be eliminated, because if the diet is well compacted the webs secreted by the caterpillars will provide an appropriate consistency for pupation (**Fig. 4C**).

The trays are covered with a lid with an opening in the middle that is covered with two layers of voile fabric separated by polystyrene cubes. This opening allows air to circulate and reduces attacks on the *A. kuehniella* caterpillars by the braconid wasp *Habrobracon hebetor* (**Fig. 4D**). The space between the lid and the tray is sealed with adhesive tape 4.5 cm wide to prevent the moth caterpillars from escaping and *H. hebetor* from entering (**Fig. 4D**). The trays are then transferred to shelves in an air-conditioned room, with a total of 27 trays per shelf (**Fig. 4E**).

In the late larval stages, the trays should be kept at a lower temperature, because, depending on the larval metabolism, the temperature may increase by 7–9°C as mentioned above. Therefore, if the starting temperature is 25°C, at the end of the larval stage the temperature in the tray will reach 32–34°C, which may affect the number of eggs laid by the resulting adults (Coelho Jr and Parra, 2013b; Coelho Jr *et al.*, 2016a.).

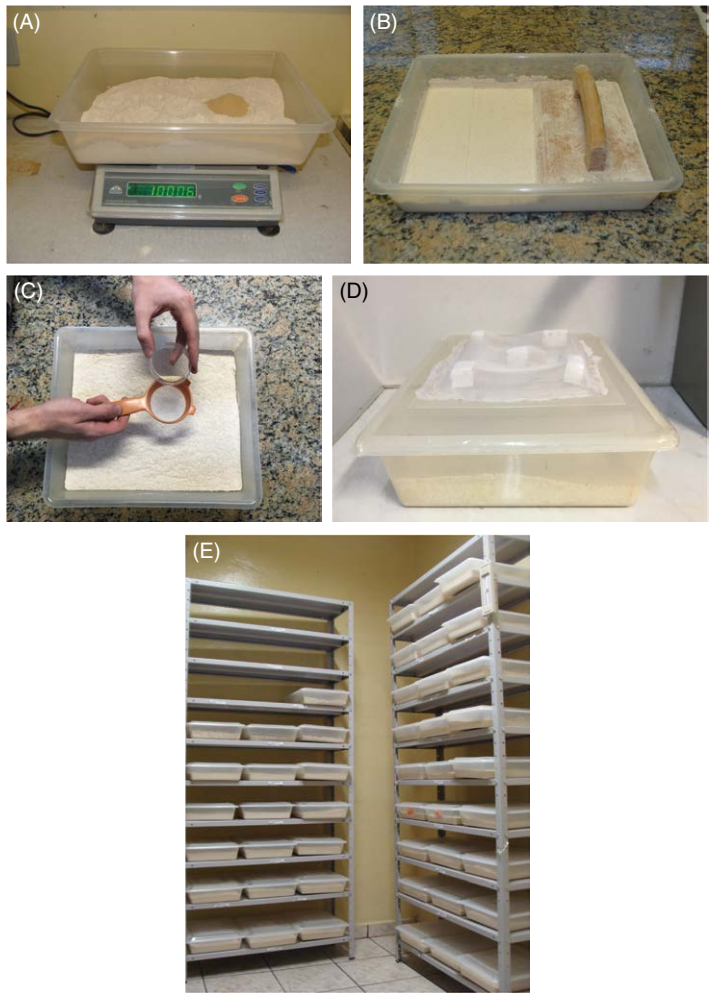

Fig. 4. Preparing a tray for rearing *Anagasta kuehniella* from the egg to the pupal stage. **(A)** Adding 1 kg of diet (97% whole-wheat flour + 3% brewer's yeast). **(B)** Compressing the diet to facilitate pupation. **(C)** Spreading 0.3 g of eggs evenly over the diet. **(D)** Tray tightly closed with a transparent plastic cover with a voile-covered opening for oxygenation, to prevent parasitism by *Habrobracon hebetor*. **(E)** Trays kept in a climate-controlled room for larva-to-pupa development.

The first adults emerge about 45 days later (**Fig. 5A**). They are collected daily, directly from the trays by means of a system consisting of a polyvinyl chloride (PVC) tube coupled to a suction pump (vacuum cleaner), which is used to transfer the insects to the oviposition cage (**Fig. 5B**, **C**, **D**, **E**, **F**, **G**,). This cage consists of a PVC tube 15 cm long containing a folded nylon net for the adults to land on; the tube is closed at both ends with a net secured by a metal clamp (**Fig. 5H**). The cage is placed on a Petri dish to facilitate egg collection

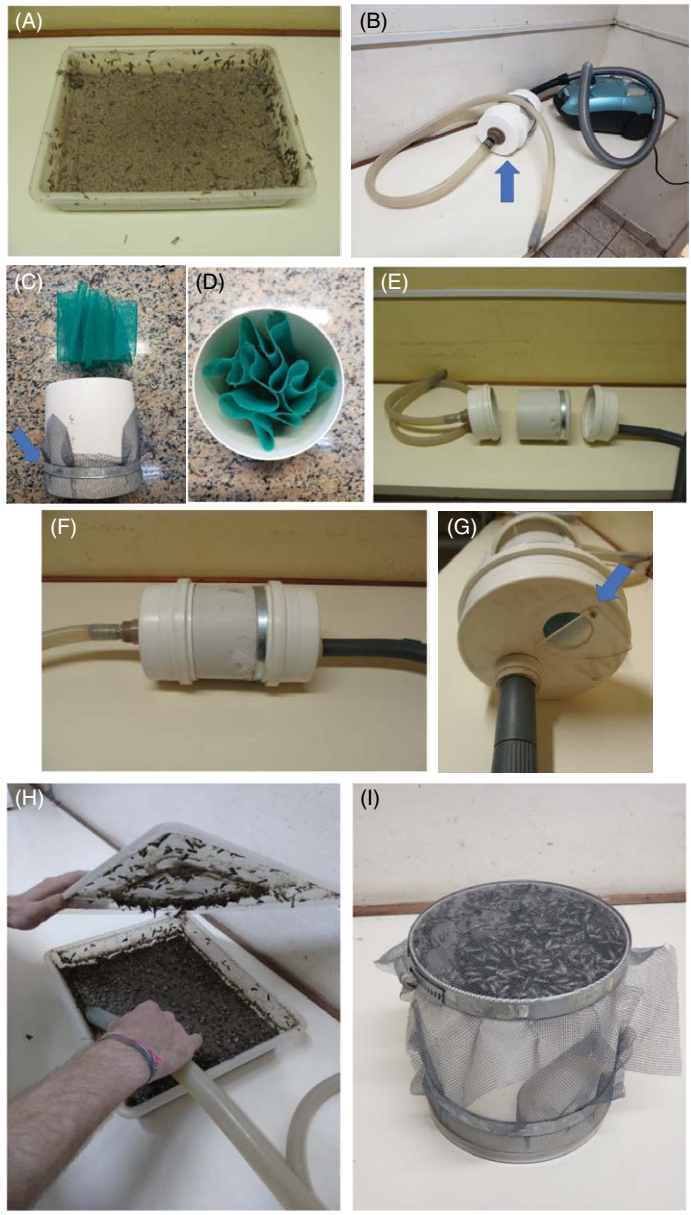

Fig. 5. System for collecting adults of *Anagasta kuehniella*: **(A)** Tray with emerging adults. **(B)** Complete collection system (arrow indicates oviposition cage). **(C, D)** Preparation of the cage with green netting for oviposition (arrow indicates the clamp used to attach the screen, to prevent the aspirator from sucking out the adults). **(E)** Fitted PVC covers for coupling to the cage and the aspirator. **(F)** Collection system ready for use. **(G)** Valve (arrow) to adjust the aspirator pressure. **(H)** Collecting insects directly from the tray. **(I)** Closing the cage containing the adults with plastic netting attached with another clamp.

Fig. 6. Collecting and cleaning *Anagasta kuehniella* eggs. **(A)** Cage set above Petri dish (arrow) to facilitate egg collection. **(B)** Group of cages in climate-controlled room (controlled temperature, RH and photoperiod). **(C)** General appearance of the vacuum hood where eggs are gently brushed to remove the lightest scales. **(D)** Transferring eggs from the Petri dish to the plastic tray. **(E)** Sieving to separate larger impurities. **(F)** Separating scales with hydrophilic cotton, using static electricity. **(G)** Cleaned eggs sticking to the plastic. **(H)** Eggs with impurities. **(I)** Cleaned eggs.

(**Fig. 6A**). The cage must be kept in a room with controlled temperature, air relative humidity, and photoperiod (**Fig. 6B**).

Each tray (1 kg of diet inoculated with 10,800 eggs) produces about 6800 adults (63% viability). Assuming a sex ratio of 0.5, we have 3400 females, each of which lays around 250 eggs, and therefore each tray should produce 850,000 eggs or 23 g of eggs (1 g = 36,000 eggs) (Coelho Jr, 2011; Coelho Jr and Parra, 2013a, b; Coelho Jr. *et al.*, 2016a). However, in mass-rearing conditions a production rate of 23 g of eggs per kg of diet is difficult to reach, and 10 g of eggs per kg of diet is a reasonable amount (Cerutti *et al.*, 1992). Eggs are collected daily. The process of separating and cleaning the eggs is laborious and requires special care to prevent the moths' wing scales from scattering and being inhaled by the laboratory personnel. The eggs are first cleared of the lightest scales by gentle brushing under a vacuum hood that passes the air through a plastic-fiber filter before expelling it into the environment (**Fig. 6C**). The eggs are then cleaned by successive sieving to eliminate the remaining powdery material, using hydrophilic cotton pads (static electricity) for a final cleaning (**Fig. 6D**, **E**, **F**, **G**, **H**, **I**).

To produce 100 g of eggs per day, 60 trays must be "inoculated" every 4 days. Inoculating trays and collecting insects requires about 6 hours per day, and collecting and cleaning eggs requires an additional 2 hours per day, by two laboratory technicians. For safe disposal, the material is stored in a freezer and then tightly sealed in plastic bags to prevent dissemination of pathogens, especially *H. hebetor*, an ectoparasitoid of *A. kuehniella* that is attracted by frass from the moth's fifth-instar larvae (Parra *et al.*, 1996).

Coelho Jr and Parra (2013b) found that the best temperature for maintaining *A. kuehniella* from the egg stage to the death of adults is 25°C, which corresponds to obtaining 6.6 generations of moths per year and a fertility of 400 eggs per female. Temperatures of 30–32°C cause deformation in the coupling structures of *A. kuehniella* males, and reduce the viability and weight of the eggs.

If the CO_2 concentration in the rearing room is higher than 1200 ppm, egg production will be reduced (Coelho Jr and Parra, 2013a).

Inactivation of *A. kuehniella* embryos

Embryos are inactivated (sterilized) with a germicidal (ultraviolet) lamp (15 W) in small closed chambers. The period of exposure to this radiation and the distance between the source and the eggs must be appropriate to avoid altering the characteristics of the egg for parasitism. This is essential because, even if there is a high percentage of parasitized eggs, some non-parasitized eggs will remain, and the larvae (caterpillars) hatched from these eggs will indiscriminately destroy both the parasitized and non-parasitized eggs. In order to become inactivated and suitable for parasitism, *A. kuehniella* eggs should be irradiated for 40–50 minutes at a distance of 15 cm from the light source (Stein and Parra, 1987b) (**Fig. 7**).

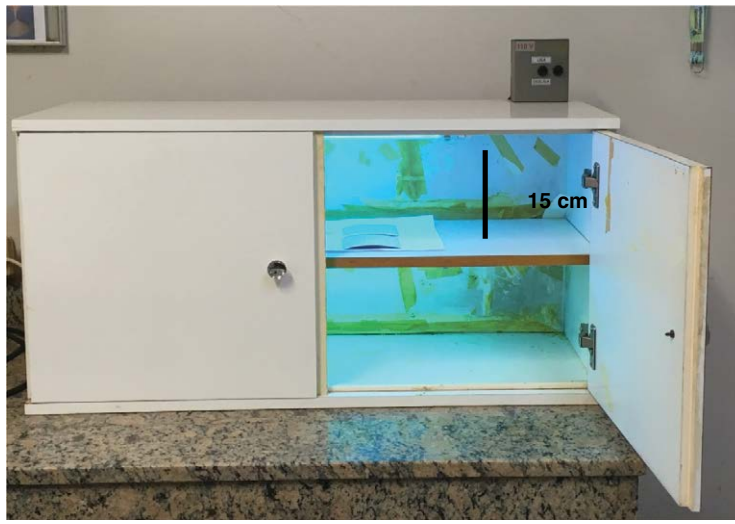

Fig. 7. Formica box used to sterilize *Anagasta kuehniella* eggs with a 15 W germicidal UV lamp for 40–50 minutes, before the eggs are offered for parasitism by *Trichogramma* species (Stein and Parra, 1987b).

Ideal combination of thermal conditions for immature and adult forms and laying rhythm of the moth at different temperatures

According to Coelho Jr and Parra (2013b), the most favorable temperature range for development of *A. kuehniella* is between 20°C and 25°C. At 30°C and above, the viability of the egg stage decreases considerably, and a temperature of 34°C or higher will kill the larvae. The laying capacity of adults resulting from larvae that were reared in the range of 18–32°C will be affected, even if the adults are transferred to a constant temperature (25°C).

Keeping adult moths within a temperature range of 18–32°C, after their immature stages were reared at a constant temperature of 25°C, does not affect their laying capacity (Coelho Jr and Parra, 2013b).

Parra *et al.* (1993) and Coelho Jr and Parra (2013b) found that when insects are kept at a constant temperature from the beginning of development until the adults die, the best temperature for egg production is 22°C. Of course, this temperature extends the egg-adult period, which is a disadvantage for mass rearing.

The laying rhythm is important because it is advisable to concentrate the egg-laying period, to increase the efficiency of mass-rearing operations. In optimal conditions (around 25°C), females live from 5 to 7 days. A female produces 75% of its eggs in the first 3 days (**Table 5**).

Exploitation time of adult and egg collection trays

In any insect mass-rearing routine, the workforce represents 60–80% of the total production cost, depending on the location. Therefore, each step

Table 5. Average daily percentage of total egg production by females of *Anagasta kuehniella* at a temperature of 25°C (Parra *et al.*, 1989a)

Day	%
1	30.0
2	27.0
3	18.1
4	11.3
5	6.5
6	5.0
7	2.1

of the production process must be optimized. The length of time that each rearing container is in use must be adjusted so that no problems occur and consequently increase the production cost.

The cage that holds adults for egg collection is used for 5–7 days. Adults are collected for a maximum of 3 weeks, because more than 80% of the moths emerge during this period and from then on problems in rearing may arise (see **Rearing problems**). This illustrates the need for all stages of rearing to be recorded, so that the entire moth production process can be strictly controlled.

Rearing problems

Rearing of this species of moth involves certain characteristic problems. Other problems are common to insect rearing in general (Parra *et al.*, 1989b).

General problems

- **Ants:** Ant invasions cause problems in rearing, mainly due to the use of pure honey to feed *Trichogramma* adults, and in some laboratories, to the use of gum arabic to glue *A. kuehniella* eggs.
- **Mites:** Large mite populations can be a serious problem for rearing colonies, especially if the relative humidity of the room used to rear *A. kuehniella* is high. Reducing the relative humidity to 30–40%, together with continuous cleaning of the laboratory, can help to eliminate the mites.

Specific problems

- ***Habrobracon hebetor*:** This braconid wasp is an efficient natural enemy of the last instar of *A. kuehniella* caterpillars and is attracted by the frass that they produce (Parra *et al.*, 1996). If no action is taken, large populations of the insect can completely destroy a rearing colony. The reproductive potential of *H. hebetor* is higher at high temperatures (**Fig. 8** and **Table 6**). The only way to eliminate *H. hebetor* is to physically exclude it from rearing rooms (hermetic sealing of doors, use of netting, etc.).

- **Humidity:** High relative humidity favors the development of fungi and impairs the larval development of *A. kuehniella*. It is recommended that adults in the emergence room be collected for only a limited period of time, because if the collection period is more than 3 weeks, then mites, the *H. hebetor* population, and the humidity inside the emergence trays will increase.

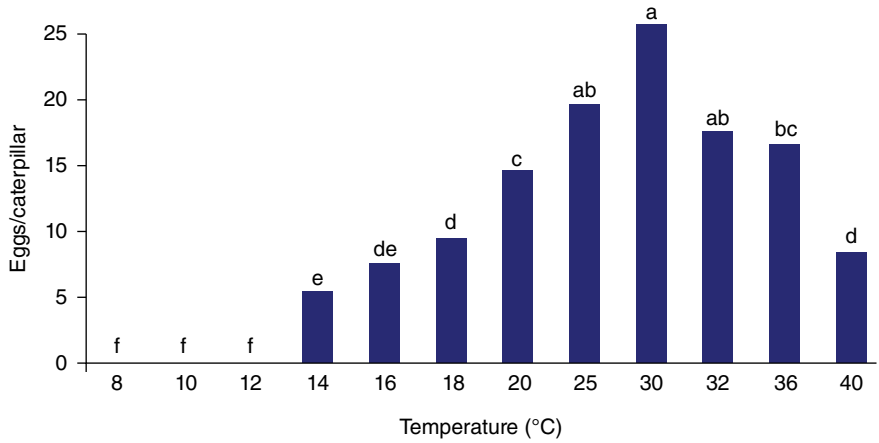

Fig. 8. Number of *Habrobracon hebetor* eggs laid on *Anagasta kuehniella* caterpillars over 60 hours at different temperatures. RH: 60 ± 10%; photophase: 14 hours (Serra, 1992).

Table 6. Percentage of *Anagasta kuehniella* caterpillars parasitized by *Habrobracon hebetor* at different temperatures over 60 hours. RH: 60 ± 10%; photophase: 14 hours (Serra, 1992).

Temperature (°C)	Parasitism %[a]
8	3.3 a
10	86.7 b
12	90.0 bc
14	93.3 bc
16	90.0 bc
18	89.3 bc
20	96.7 bc
25	96.6 bc
30	100.0 c
32	96.4 bc
36	100.0 c
40	100.0 c

[a]Averages followed by the same letter do not differ statistically from each other, by Student's *t* test at the 1% probability level.

General care

Daily cleaning of the rearing rooms to eliminate the remains of flour, insects, scales, etc. helps to reduce mites and *H. hebetor* (Parra *et al.*, 1996). Disinfection of counters, shelves and floors reduces the possibility of contamination by microorganisms. Corrugated cardboard (when used), after it is removed from the emergence trays, should be burned or frozen. All materials used in rearing (trays and cages for adults) can be stored in a freezer in order to eliminate the mites.

When the mite population reaches alarming levels, the use of acaricides can be considered. These products should be applied during washing and sterilization of the rearing trays.

Rearing of *Trichogramma* Parasitoids

A fifth room for rearing the parasitoid is essential in mass rearing programs. This room can also be used for material preparation and processing for rearing operations of different factitous hosts. Some aspects that should be taken into consideration are as follows.

Selection of *Trichogramma* strains

Since the behavior of *Trichogramma* species depends on macro- and microclimate conditions, it is essential to collect the strains of a species in a location similar to the area where the parasitoid is intended to be released, preferably on the target insect (Parra *et al.*, 2015). If this is not possible, a selection of strains of the species should be evaluated for parasitism capacity, life cycle duration, viability, sex ratio, parasitoid size, longevity, percentage of deformed wings, and flight capacity. The best strain(s) to use can be identified via a cluster analysis (Pratissoli and Parra, 2001; Geremias, 2008). As mentioned previously, *A. kuehniella* and *C. cephalonica* are the most suitable factitious hosts for Brazilian species, because they have higher nutritional quality (**Fig. 2**), support higher levels of parasitism (**Table 7**), multiply more with each generation (R_0) (**Table 8**), and produce larger parasitoids (**Table 9**).

Studies of the biology of parasitoids at different temperatures and their thermal requirements

Since populations of the same species may vary (Bleicher and Parra, 1990b) (**Table 10**), it is useful to determine the temperature requirements of different strains. This information is obtained by studying the insects' biology

Table 7. Parasitism of *Trichogramma pretiosum* within 24 hours and *Trichogramma galloi* within 48 hours on different factitious hosts. Temperature: 25 ± 1°C; RH: 60 ± 10%; photophase: 14 hours (Gomes, 1997)

Species	*Anagasta kuehniella*	*Corcyra cephalonica*	*Sitotroga cerealella*
Trichogramma pretiosum	23.9 ± 1.92 a	23.36 ± 1.75 a	18.59 ± 1.69 b
Trichogramma galloi	47.0 ± 8.20 a	–	35.00 ± 6.08 b

Table 8. Net reproductive rate (R_0) of *Trichogramma bruni*, *Trichogramma atopovirilia*, and *Trichogrammatoidea annulata* on three factitious hosts (Dias *et al*., 2010)

	R_0 on different factitious hosts		
Species	*Anagasta kuehniella*	*Corcyra cephalonica*	*Sitotroga cerealella*
Trichogramma bruni	47.07 ± 3.95 a	27.58 ± 3.88 b	14.72 ± 1.90 c
Trichogramma atopovirilia	77.74 ± 6.90 a	96.74 ± 8.78 a	28.68 ± 3.11 b
Trichogrammatoidea annulata	64.40 ± 6.91 a	77.00 ± 5.71 a	27.29 ± 1.95 b

Table 9. Size of the tibia (μm) of *Trichogramma pretiosum* reared on *Sitotroga cerealella* and *Anagasta kuehniella*, when one or two individuals per egg emerged (Parra *et al*., unpublished data)

Host	Size of the tibia (μm) and no. of individuals/egg[a]	
	1 individual	2 individuals
Sitotroga cerealella	146.6 ± 0.84 aa	125.3 ± 3.18 aB
Anagasta kuehniella	154.6 ± 1.59 ba	125.9 ± 4.41 aB

[a]Equal uppercase letters in a column do not differ from each other by Tukey's test at the 1% probability level; equal lowercase letters in a row do not differ by the same test

at different temperatures (**Table 11**) to improve forecasts of the production parameters, and in some cases, the best conditions for short periods of storage.

Some researchers have been concerned that maintenance of a parasitoid at constant temperatures could affect its development and its aggressiveness when released in field conditions, where temperatures fluctuate. Coelho Jr *et al*. (2016b) demonstrated that an isoline that performed best in the laboratory also performed best in the field. Cônsoli and Parra (1995a, b) found that *T. galloi* showed no major differences in biology when reared at

constant or fluctuating temperatures, indicating that it can be produced in the laboratory at constant temperatures and released into the field without loss of quality.

Site separation for the production of different species

Some species are more aggressive and "win" in interspecific competition, eliminating the other species. This is the case for *T. pretiosum*, which is much more aggressive than *T. galloi*, and if reared in the same room with *T. galloi* becomes the predominant species (Parra, J.R.P., personal information).

Appropriate ratio of parasitoids to host eggs

To obtain the maximum parasitism, the ratio of parasitoids to host eggs varies according to the species. In addition to the ratio (proportion), the period of time that the eggs are exposed for parasitism should be taken into account. For *T. galloi* reared on *A. kuehniella*, the ideal proportion is three or four parasitoids per ten eggs of the factitious host, exposed for 24 hours.

For *T. pretiosum* the ratio is 1:10, for the same period. The egg density (the kairomone concentration increases with the number of eggs) can affect parasitism, as observed by Lopes and Parra (1991) (**Table 12**) and Pak and Oatman (1982) under laboratory conditions, by Sá (1991) in a greenhouse, and by Neil and Specht (1990) under field conditions.

The relationship (ratio) between parasitoid and host eggs cannot be too high, to avoid superparasitism, or too low so that a low rate of parasitism is obtained.

Age of host egg most susceptible to parasitism and capable of producing aggressive and highly competitive parasitoids

In general, younger (newly laid) eggs are preferred for parasitism. Exceptions exist, as recorded by Lopes and Parra (1991) with *Trichogramma distinctum*,

Table 10. Mean generation time (T), net reproductive rate (R_0), intrinsic rate of increase (r_m), and finite rate of increase (λ) for two populations of *Trichogramma pretiosum*. Temperature: 25 ± 1°C; RH: 70 ± 10%; photophase: 14 hours (Bleicher and Parra, 1990b)

Population	T (days)	R_0	rm	λ
Trichogramma pretiosum (Iguatu population)	15.47	102.13	0.2990	1.3485
Trichogramma pretiosum (Goiânia population)	14.15	44.38	0.2680	1.3074

Table 11. Life cycle (days) of *Trichogramma* species at different temperatures and their respective thermal requirements (Parra, 1997; Molina *et al*., 2005)

Temperature (°C)	*Trichogramma galloi*	*Trichogramma pretiosum*	*Trichogramma atopovirilia*	*Trichogramma distinctum*
18	31.3	27.3	23.6	34.2
20	19.5	19.4	16.1	15.2
22	15.6	15.4	15.4	15.3
25	12.1	9.7	8.7	11.4
28	–	7.1	7.1	–
30	8.0	8.6	6.3	9.1
32	7.1	6.7	6.2	9.2
Tt (°C)	13.7	12.8	13.5	9.6
K (DD)	131.1	133.1	107.8	191.0

Tt = Temperature threshold
K = Thermal constant, expressed in degree-days

Table 12. Total parasitism of a *Trichogramma galloi* strain at two densities of *Diatraea saccharalis* eggs. Temperature: 25 ± 10°C; RH: 60 ± 10%; photophase: 14 hours (Lopes and Parra, 1991).

Density (no. eggs/day)	Total number of parasitized eggs
25	26.99 ± 1.27[a]
75	37.12 ± 2.21[b]

[a]Means followed by the same letter do not differ by Tukey's test at the 1% probability level.
[b]Two or three *T. galloi* developed in one *D. saccharalis* egg

which preferred to parasitize eggs of *Diatraea saccharalis* on the fourth day of embryonic development.

Host adaptation

For laboratory rearing, the parasitoid often requires a few generations to adapt to the factitious host. *Trichogramma galloi* requires four or five generations to adapt to *A. kuehniella*, while *T. pretiosum* adapts in the first generation (Rossi, unpublished data). According to Bertin *et al*. (2017), rearing *T. galloi* for successive generations in a factitious host leads to a decrease in the performance of this BC agent. The authors suggested that this decrease is related to adaptation to laboratory conditions, which reduces the genetic variability of a population.

Storage and transport

Storage techniques for parasitized eggs (**Fig. 9**), using low temperatures or diapause induction (in temperate-zone countries), can help to use biological material obtained in the laboratory more efficiently, without interrupting the rearing process during the year. In general, parasitized eggs can be stored for up to 20 days at 10°C, which is close to the temperature threshold (Tt). Because the mechanisms of developmental disruption of neotropical species are not known, one way of extending the storage period is to store the parasitized eggs at 18°C immediately after the parasitism and, on the 20th day, cool them to 10°C. This will allow the eggs to be stored for up to 40 days, thus facilitating scheduling for field release (Schmidt, 1991). Concerning the atmospheric gases, ventilation of rearing cages is recommended in order to maintain CO_2 below 4.3% and O_2 above 18.5%, allowing maximal parasitoid quality (Coelho Jr *et al.*, 2017).

After the eggs of the factitious host are produced, they are exposed for parasitism and then taken to the field to be released. To offer them for parasitism, the eggs can be glued on pieces of cardboard or capsules with gum arabic (diluted with water). Instead of using gum arabic, the eggs can be offered loosely arranged on trays. This method facilitates field release, especially using drones.

A flowchart of *Trichogramma* production in *Anagasta kuehniella* eggs is shown in **Fig. 10**.

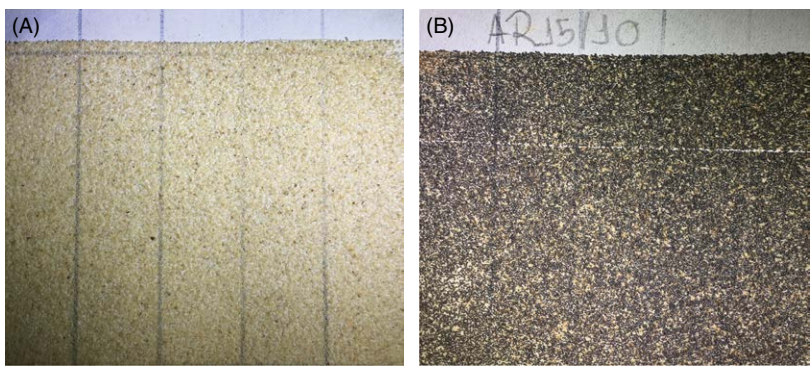

Fig. 9. *Anagasta kuehniella* eggs glued with gum arabic on blue cards. **(A)** Unparasitized eggs. **(B)** Parasitized eggs (dark).

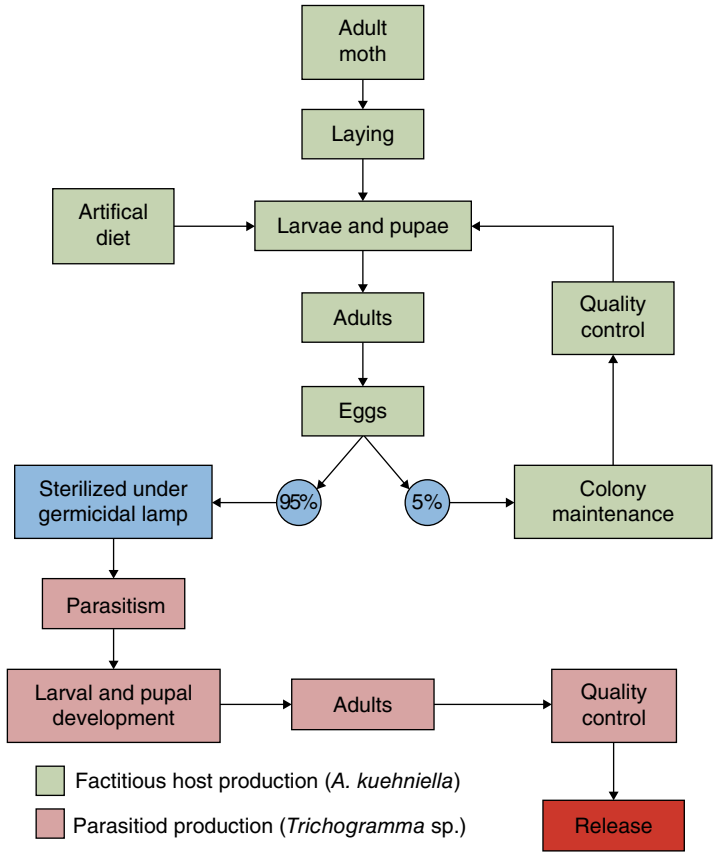

Fig. 10. Scheme of production of *Trichogramma* species in the factitious host *Anagasta kuehniella*.

Concluding Remarks

4

The quality of both the factitious host and the parasitoid should be evaluated based on biological or biotechnological criteria (Prezotti and Parra, 2002; van Lenteren, 2003; Prezotti *et al.*, 2004; Coelho Jr *et al.*, 2016b; Bertin *et al.*, 2017).

Rearing the parasitoid is only one step in a biological control program, which includes:

1. Collection, identification and maintenance of *Trichogramma* strains
2. Selection of a host for parasitoid mass rearing
3. Study of biological and behavioral aspects of *Trichogramma*
4. Dynamics of the target pest eggs
5. Parasitoid release: number of parasitoids released and release points, time and method of release
6. Quality control
7. Agrochemical selectivity
8. Efficiency evaluation: parasitoid/pest simulation model (Parra, 2002).

After 35 years of studies (Parra and Zucchi, 2004; Parra, 2010; Parra and Coelho Jr, 2019), biological control using *Trichogramma* species is already a routine technique in Brazil, where *T. galloi* is being released on almost 2,000,000 ha of sugarcane to control *Diatraea saccharalis* (Parra and Coelho Jr, 2019). In addition, *T. pretiosum* is being released in several areas to control *Chrysodeixis includens* in soybean, *Tuta absoluta* in tomato, and more recently, *Helicoverpa armigera* in different crops, on 250,000 ha in total.

Studies in Brazil have advanced significantly, with increasing potential for use of *Trichogramma* species on sugarcane, cotton, soybean, corn, forests, fruit trees, and vegetables, among others, in large and small areas and in organic agriculture and greenhouses. Mastery of the techniques for rearing the factitious host, detailed here, is essential for these applications to become practical.

The rearing protocol described here is for a daily production of 100 g of eggs. For mass rearing the scale must be expanded, which may require a period of time to make the necessary industrial scale-up adjustments.

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Index

Note: bold page numbers indicate tables; italic page numbers indicate figures.