EFFECTS OF DURATION OF COLD STORAGE OF HOST EGGS ON PERCENT PARASITISM AND ADULT EMERGENCE OF EACH OF TEN TRICHOGRAMMATIDAE (HYMENOPTERA) SPECIES

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ABSTRACT

Improving parasitoid mass rearing techniques is important to reduce costs of biological control programs and supply natural enemies at times of high demand. The objective of this study was to evaluate the suitability of *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs stored at 5 °C for different time periods as a host for *Trichogrammatoidea annulata* (Hymenoptera: Trichogrammatidae) and for 9 *Trichogramma* species (*T. acacioi*, *T. atopovirilia*, *T. benneti*, *T. brasiliensis*, *T. bruni*, *T. demoraesi*, *T. galloi*, *T. pretiosum*, and *T. soaresi*). The experiment had a factorial design with 10 parasitoid species, 9 host storage periods (5, 10, 15, 20, 25, 30, 35 and 40 days, and a no storage control) and 20 replications, each consisting of one card (0.4 × 2.0 cm) with 40.70 ± 0.03 eggs of *A. kuehniella*. *Trichogrammatoidea annulata*, *T. acacioi*, *T. brasiliensis*, *T. bruni*, *T. pretiosum*, and *T. soaresi* parasitized eggs of *A. kuehniella* stored up to 24 days; *T. atopovirilia* parasitized eggs stored up to 16 days. *Trichogramma demoraesi* and *T. benneti* parasitized eggs stored for 15 days and *T. galloi* parasitized eggs stored for up to 13 days. The percentage of parasitized eggs decreased when the storage period increased. Among the tested parasitoids *T. acacioi* parasitized eggs stored for longer periods and showed the highest percentage both of parasitism and adult emergence.

Key Words: Biological control, insects rearing, Lepidoptera, *Trichogramma*

RESUMEN

Mejorar las técnicas de cría masiva es importante para reducir los costos de programas de control biológico y proveer enemigos naturales en periodos de alta demanda. El objetivo de este estudio fue evaluar la idoneidad de huevos de *Anagasta kuehniella* (Lepidoptera: Pyralidae), almacenados a 5 °C por varios periodos de tiempo, como hospederos de *Trichogrammatoidea annulata* (Hymenoptera: Trichogrammatidae) y nueve especies de *Trichogramma* (*T. acacioi*, *T. atopovirilia*, *T. benneti*, *T. brasiliensis*, *T. bruni*, *T. demoraesi*, *T. galloi*, *T. pretiosum*, and *T. soaresi*). El experimento tuvo un diseño factorial con 10 especies de parasitoides, 9 periodos de almacenamiento (5, 10, 15, 20, 25, 30, 35 y 40 días; y no almacenamiento como control) y 20 réplicas, cada una consintiendo en un pedazo de cartón (0.4 × 2.0 cm) con 40.70 ± 0.03 huevos de *A. kuehniella*. *Trichogrammatoidea annulata*, *T. acacioi*, *T. brasiliensis*, *T. bruni*, *T. pretiosum*, y *T. soaresi* parasitaron huevos de *A. kuehniella* almacenados hasta 24 días; *T. atopovirilia* parasitó huevos almacenados hasta 16 días. *Trichogramma demoraesi* y *T. benneti* parasitaron huevos almacenados 16 días y *T. galloi* parasitó huevos almacenados hasta 13 días. El porcentaje de huevos parasitados disminuyo con incrementos al periodo de almacenamiento. Dentro de los parasitoides evaluados *T. acacioi* parasitó huevos almacenados por periodos más largos y presento los niveles de parasitismo y de emergencia de adultos más altos.

Palabras Clave: control biológico, cría de insectos, Lepidóptera, *Trichogramma*

Biological control is important in Integrated Pest Management (IPM) in different crops in Brazil (Pires et al. 2011; Soares et al. 2011, 2012; Bueno et al. 2012). Parasitoids of the family Trichogrammatidae can suppress populations of agricultural and forest pests, and reduce the excessive use of insecticides (Soares et al. 2007; Oliveira et al. 2011; Goulart et al. 2012). Development of large scale production and release technologies for these natural enemies is necessary (van Lenteren 2012). Improved mass rearing techniques can provide parasitoids at times of high demand with reduced costs. Trichogrammatidae demand may be low when there are surplus of host (rearing) eggs (Lohmann & Santos 2007). By contrast, shortage in host eggs during periods of high demand can hinder biological control programs and require the use of insecticides for pest control. Storage at low temperatures can increase the lifetime of parasitoids and hosts. This technique allows synchronizing releases of natural enemies with pest outbreaks (Pitcher et al. 2002; Colinet & Boivin 2011).

Eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) may be kept in a refrigerator for up to 30 days without losing their viability as hosts for Trichogrammatidae (Pratissoli et al. 2003). Parasitism rates of stored *Trichogramma ostriniae* (Pang & Chen) (Hymenoptera: Trichogrammatidae) were similar to controls after 2 to 4 weeks' storage at 9-12 °C but declined with storage periods longer than 4 weeks (Pitcher et al. 2002). However, parasitoids reared on refrigerated eggs may have reduced performance because long exposure to low temperatures reduces host quality (Flanders 1938; Kostal et al. 2004, 2006). Eggs of *Riptortus pedestris* (F.) (Hemiptera: Alydidae) maintained at 2 °C were less parasitized (34%) by *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) than eggs kept under natural conditions (62%) (Alim & Lim 2011). Storage at 4 ± 1 °C reduced fecundity and longevity of *Trichogramma brassicae* Bezd. (= *T. maidis* n. sp.), *Trichogramma cacoeciae* Marchal and *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) (Özder 2008). Studies to determine ideal temperatures and storage times of host eggs for parasitoid rearing are needed.

The objectives of this study were to determine the emergence and parasitism potentials of 10 Trichogrammatidae species reared in eggs of *A. kueniella* stored at 5 °C for different time periods.

Material and Methods

Experimental Design

This study was conducted at the George Washington Gomez de Moraes Insectarium of the Institute of Agricultural Sciences, Federal University of Minas Gerais (ICA/UFMG) in Montes Claros, Minas Gerais State, Brazil. A 10 (parasitoid spp.) × 9 (host storage periods) factorial design with 20 replications was used. The experimental unit was a navy blue card (0.4 × 2.0 cm) with 40.70 ± 0.03 eggs of *A. kuehniella* (total of 1,800 cards).

Treatments were 10 Trichogrammatidae species, including 9 *Trichogramma* spp. (*T. acacioi* Brun, Moraes and Soares, *T. atopovirilia* Oatman and Platner, *T. bennetti* Nagaraja and Nagarkatti, *T. brasiliensis* (Ashrnead), *T. bruni* Nagaraja, *T. demoraesi* Nagaraja, *T. galloi* Zucchi, *T. pretiosum* Riley, and *T. soaresi* Nagaraja) and *Trichogrammatoidea annulata* (De Santis). All parasitoids were obtained from the ICA/UFMG Insectarium. *Anagasta kuehniella* eggs subject to 9 storage periods (0, 5, 10, 15, 20, 25, 30, 35 and 40 days in a refrigerator at 5 °C and the control-no storage) were used as hosts.

Eggs of *Anagasta kuehniella*

Anagasta kuehniella was reared on wheat bran, corn meal (1:1) and 3% brewer's yeast (Tavares et al. 2009; Soares et al. 2007, 2012). Adults were placed in cages for mating and oviposition. Eggs were collected and washed with distilled water to eliminate dirt and scales.

Experimental Setup

Eggs of *A. kuehniella* were fixed to pieces of card board (0.4 × 2.0 cm) with 10% arabic gum, exposed to ultraviolet (UV) for 60 min (Soares et al. 2007, 2012), placed in glass bottles (7.5 cm diam × 13 cm H) sealed with PVC plastic and an elastic film, and stored in a refrigerator at 5 °C and 80% RH (Pratissoli et al. 2003). This procedure was repeated every 5 days during 40 consecutive days in order to obtain the storage periods. Then, each card was placed inside a microtube (model MCT= - 200 - C 2.0 mL, clear scientific axygen Lot: 070507-262) with a single 1-day old parasitoid female for 24 h at 12:12 h L:D and 24.39 ± 0.01 °C (Soares et al. 2007, 2012). After this period, the cards were transferred to glass test tubes until emergence of adult Trichogrammatidae. The tested parasitoids reproduce by thelytokous parthenogenesis in absence of males (Soares et al. 2012).

Statistical Analysis

The percentage of parasitized eggs and number of emerged parasitoids per egg and per card board were analyzed using the program SAEG 9.1 (UFV). ANOVAs ($P < 0.05$) and Sigmoidal Weibull regression (5 parameters) ($P < 0.05$) were conducted to determine the maximum storage period of *A. kuehniella* eggs for parasitism by *Trichogramma* or *Trichogrammatoidea* species. Scott-Knott (P < 0.05) tests were used to determine the species with better biological performance (i.e., parasitized eggs, number of adults emerged). In probability theory and statistics, the Weibull distribution is a continuous probability distribution. We used 5 parameters, where: a = max (y)-min (y), b = xwtr (x,y-min (y), c = 1 ''Auto", x0 = x50 (x,y-min (y), and y0 = min(y) ''Auto"; equation: f= if (x< = x0-b*ln(2)^(1/c), y0, y0 + a*(1-exp(-(abs(x-x0 + b*ln(2)^ (1/c)) / b) ^c))).

Results

Parasitism by all Trichogrammatidae species decreased as the storage periods increased (Fig. 1). *Trichogrammatoidea annulata*, *T. acacioi*, *T. brasiliensis*, *T. bruni*, *T. pretiosum*, and *T. soaresi* parasitized eggs stored up to 24 days; *T. atopovilia* parasitized eggs stored up to 16 days, *T. demoraesi* and *T. bennetti* parasitized eggs stored up to 15 days, and *T. galloi* eggs stored up to 13 days (Fig. 1).

The storage period for maximum parasitoid emergence was 23 days for *T. acacioi*, *T. annulata*, *T. brasiliensis*, *T. bruni*, and *T. pretiosum*, 15 days for *T. atopovilia*, *T. benneti*, *T. demoraesi*, and *T. soaresi*, and 13 days for *T. galloi* (Fig. 3). The highest number of *T. acacioi*, *T. annulata*, *T. atopovilia*, *T. bennetti*, *T. demoraesi*, *T. galloi*, *T. pretiosum*, and *T. soaresi* adults emerged from *A. kuehniella* eggs stored up to 23 days; The highest number of *T. brasiliensis* and *T. bruni* adults emerged from *A. kuehniella* eggs stored up to 17 days (Fig. 2).

Trichogramma galloi showed the highest parasitism and adult emergence rates per card with eggs stored for 5 days, followed by *T. acacioi*. The lowest parasitism and adult emergence rates were observed in *T. soaresi* and *T. benneti* on eggs stored for < 1 day, *T. pretiosum* on eggs stored for 5 days, and *T. brasiliensis* and *T. bruni* on eggs stored for 10 days (Table 1). The number of emerged parasitoids per host egg was similar between treatments with an average of 1.0006 ± 0.0002.

Discussion

The success of biological control programs with egg parasitoids depends on the ability to produce these natural enemies in high numbers when pest problems occur. Storing host eggs for different time periods can help synchronize parasitoid production with demand.

The higher parasitism by *T. acacioi*, *T. annulata*, *T. brasiliensis*, *T. bruni*, *T. pretiosum*, and *T. soaresi* on eggs of *A. kuehniella* stored for longer periods may be related to factors such as these species having: 1) stronger ovipositors than the other parasitoids tested, 2) the ability to recognize chemical signals more efficiently, and/or 3) higher tolerance to changes in physical stimuli such as color, size and shape of chilled eggs (Stoepler et al. 2011). Except *T. soaresi*, the above species also showed high adult emergence from host stored for longer periods. The "exposure dose to cold" is a parameter use to describe a combination of exposure time and temperature. A decrease in temperature and/or an increase in exposure time can result in cumulative and irreversible chilling injuries (Colinet & Hance 2010). Several authors agree that survival of parasitoids decreases when host storage periods increase (Flanders 1938; Ayvaz et al. 2008; Chen et al. 2008; Colinet & Hance 2010; Abd El-Gawad et al. 2010).

The highest parasitism and adult emergence rates were observed in *T. galloi* parasitizing eggs stored for 5 days. Longer storage times may result in decreased nutritional quality for this parasitoid's embryos by deterioration of the yolk (Pratissoli et al. 2003). Parasitoid females can reject eggs with altered chemical and physical stimuli (Soares et al. 2009; Goubault et al. 2011; Penaflor et al. 2011; Stoepler et al. 2011) such as eggs that contain cryoprotectants (glycerol, alanine) to resist cooling (Rivers et al. 2000). Storage can also modify the egg shape and affect the host recognition by the parasitoids (Conti et al. 1996). Parasitism of *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) on *Coagulata homalodisca* (Say) (Hemiptera: Cicadellidae) eggs was reduced when cold storage period increased (Chen & Leopold 2007). Emergence of *G. ashmeadi* from *C. homalodisca* eggs stored for 70 days was reduced by 48%, fecundity by 53%, female production by 19%, the development time was extended, and female longevity shortened, compared to parasitoids reared on non-stored hosts (Chen & Leopold 2007). Thus, storage of host eggs for long periods could lead to unfavorable conditions for parasitoids and only those most adapted can reproduce.

Emergence of Trichogrammatidae parasitoids per parasitized egg in our experiments (1.0006 ± 0.0002) was low compared with reports by Andrade et al. (2011) [1.5 *T. pretiosum* individuals per egg of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae)], but similar to reports of *Trichogramma maxacalii* Vogelé & Pointel reared also on *H. virescens* (Oliveira et al. 2000; Soares et al. 2007). Larger eggs are the most important factor involved in allowing the emergence of more individuals per host (Andrade et al. 2011).

Trichogrammatidae species differ in their adaptability to use chilled hosts, and this fact should be considered when using this technology to mass rear parasitoids for biological control programs.

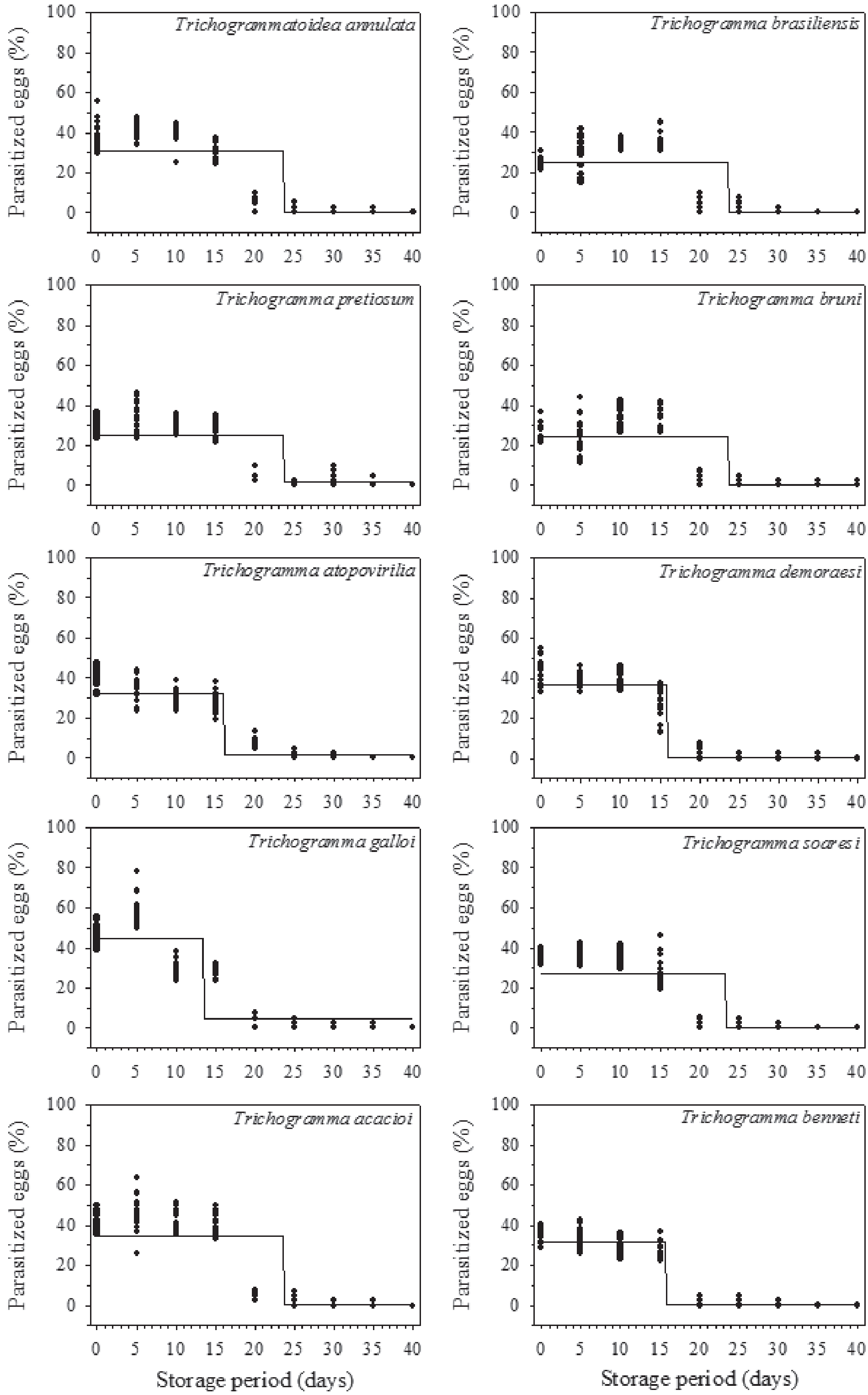

Fig. 1. Percentages of *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs parasitized by each of 10 Trichogrammatidae species as function of the number of days that these eggs had been stored at 5 °C before they were parasitized. Each point on the graph represents 20 replications.

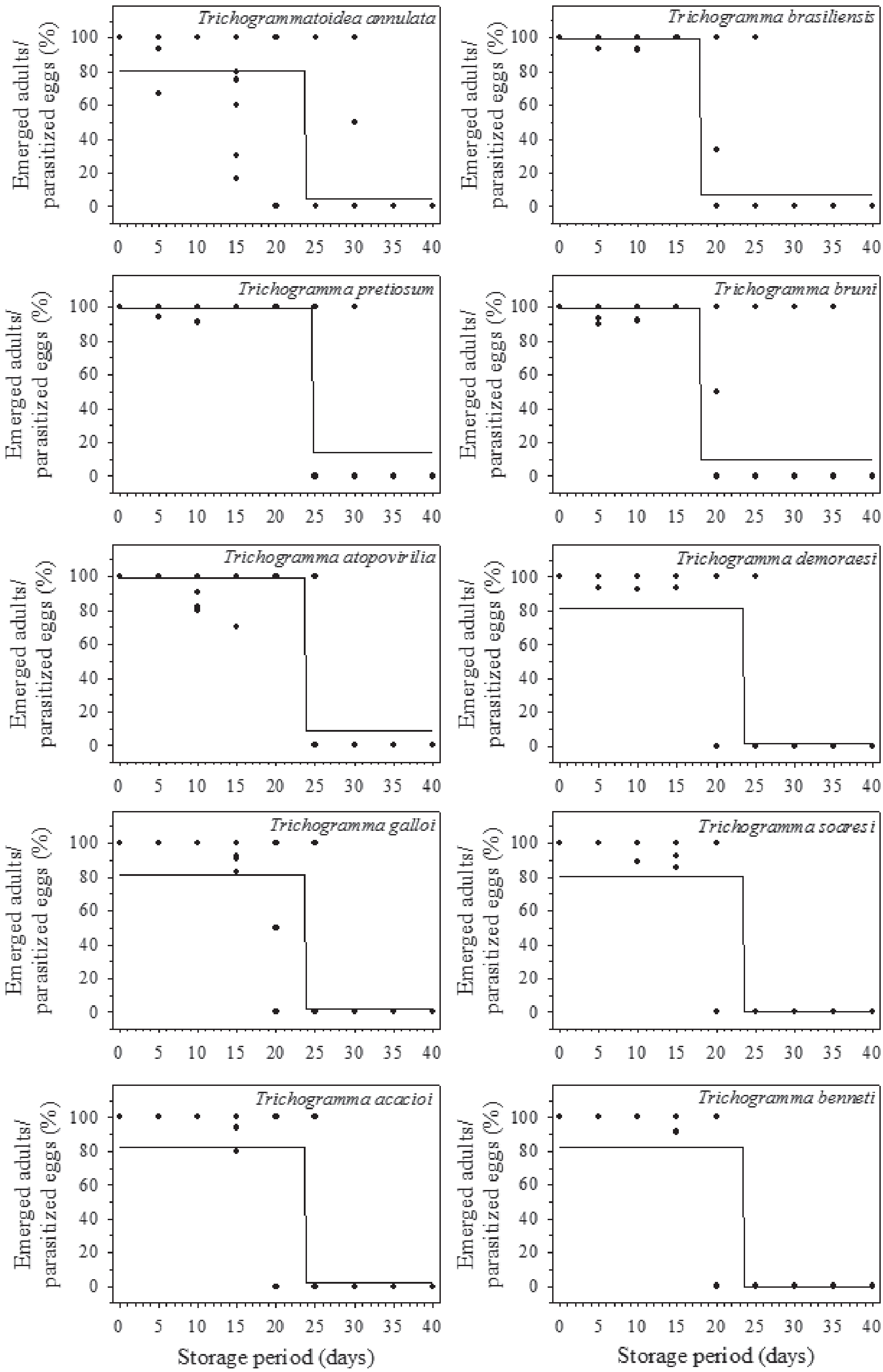

Fig. 2. Percentages of adults of each of 10 Trichogrammatidae species that emerged from parasitized *Anagasta kuehniella* eggs as function of the number of days these eggs had been stored at 5 °C before they were parasitized. Each point on the graph represents 20 replications.

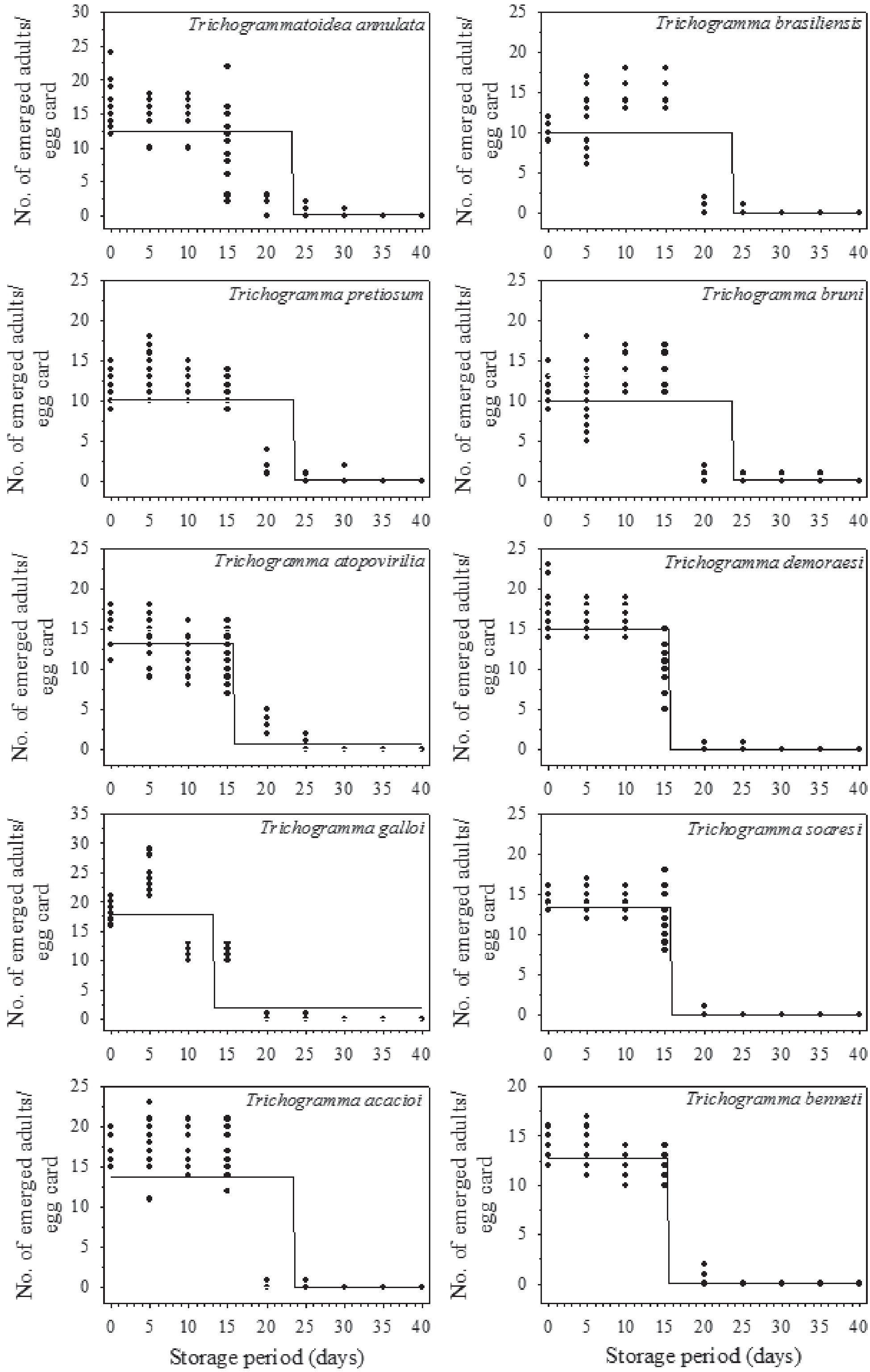

Fig. 3. Number of adults of each of 10 Trichogrammatidae species that emerged per card of 40.70 ± 0.03 *Anagasta kuehniella* eggs as function of the number of days that these eggs had been stored at 5 °C before they were parasitized. Each point on the graph represents 20 replications.

TABLE 1. HIGHEST PERCENTAGES OF PARASITIZED EGGS AND NUMBER OF EMERGED ADULTS OF *TRICHOGRAMMATOIDEA ANNULATA* AND NINE SPECIES OF *TRICHOGRAMMA* (HYMENOPTERA: TRICHOGRAMMATIDAE) PER CARD OF *ANAGASTA KUEHNIELLA* (LEPIDOPTERA: PYRALIDAE) EGGS, AS A FUNCTION OF STORAGE PERIOD AT MONTES CLAROS, MINAS GERAIS STATE, BRAZIL.

Species	Days of storage	Parasitized eggs (%)	Emerged adults/egg card
T. annulata	5	41.25 ± 0.83 c	16.45 ± 0.43 c
T. brasiliensis	10	34.08 ± 0.37 d	13.95 ± 0.26 d
T. pretiosum	5	33.88 ± 1.69 d	13.65 ± 0.62 d
T. bruni	10	34.16 ± 1.20 d	14.00 ± 0.52 d
T. atopovirilia	< one day	39.40 ± 1.00 c	16.15 ± 0.40 c
T. demoraesi	< one day	41.99 ± 1.30 c	17.20 ± 0.52 c
T. galloi	5	57.49 ± 1.48 a	23.40 ± 0.50 a
T. soaresi	< one day	35.29 ± 0.57 d	14.55 ± 0.21 d
T. acacioi	5	45.35 ± 1.67 b	18.45 ± 0.69 b
T. bennetti	< one day	35.31 ± 0.73 d	14.65 ± 0.24 d
		ANOVA	
F	—	40.568	42.030
P	—	0.00001	0.00001
CV	—	13.020	12.619

Means (mean ± standard error) followed by the same letter per column do not differ by Scott-Knott test ($P < 0.01$). $df = 171$. 100% wasps emerged/parasitized egg.

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