Maria João Lima Araújo Lemos de Medeiros Melo

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Dissertação para obtenção do Grau de Mestre em Biotecnologia em Controlo Biológico

Orientador: Doutora Luísa Oliveira

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A ela dedico este trabalho.

Resumo

Os factores ambientais, tais como a temperatura, afectam a fisiologia e comportamento dos insectos. Nos parasitóides de ovos de lepidópteros, este efeito pode ser verificado na alteração de parâmetros biológicos como a longevidade, parasitismo e emergência da descendência. Para além disto, características dos ovos, como o seu conteúdo nutricional ou tamanho, podem afectar a descendência.

O presente trabalho teve como objectivo o estudo da dinâmica, em campo, das duas espécies de *Trichogramma* estabelecidas na ilha de São Miguel: *Trichogramma cordubensis* Vagas & Cabello e *Trichogramma achaeae* Nagaraja & Nagarkatti. Também foi determinado em laboratório o efeito da temperatura em vários dos seus parâmetros biológicos: esterilidade e mortalidade das fêmeas, tempo de desenvolvimento, parasitismo, emergência e a relação entre o número de fêmeas e machos na descendência.

T. achaeae foi observado em todos os locais de recolha, ao contrário do *T. cordubensis* que apenas foi encontrado em dois deles. A primeira espécie, embora estabelecida há menos tempo, encontrava-se em maior quantidade no campo. Verificou-se que em condições naturais os parasitóides partilham espécies hospedeiras. Em relação à influência da temperatura, tal como esperado, ocorreram alterações nos parâmetros biológicos estudados em ambas as espécies. *T. cordubensis* demonstrou ser a espécie mais eficiente no parasitismo de ovos de *Ephestia Kuehniella* Zeller (Lep. Pyralidae).

Abstract

The environmental factors such as the temperature, affect the physiology and behavior of the insects. In lepidopteran eggs parasitoid wasps this effect can be seen in biological parameters such as longevity, parasitic performance and emergence. Also, the host eggs characteristics such as the nutritional content or size can have an effect on the parasitoids offspring.

The present work had as the objectives the study of the field dynamics of the two Trichogramma species established on the island of São Miguel: *Trichogramma cordubensis* Vargas & Cabello and *Trichogramma achaeae* Nagaraja & Nagarkatti. In addition, the effect in the following biological parameters, for both species, was determined in the laboratory: sterility and mortality of females, time of development, parasitism, emergence and sex-ratio.

T. achaeae was observed in all collection points while *T. cordubensis* was only registered for two. Even though the first parasitoid species established more recently, in the field, than *T. cordubensis*, nevertheless it is present in a larger amount. It was verified that under field conditions the parasitoids shared host species. As expected, temperature had an impact on the studied parameters between the two *Trichogramma* species. *T. cordubensis* proved to be the more efficient in the parasitism of *Ephestia Kuehniella* Zeller (Lep. Pyralidae), eggs.

Introduction

The egg parasitoids *Trichogramma* (Hymenoptera: Trichogrammatidae) are used worldwide against Lepidoptera as natural enemies in pest management strategies (Smith, 1996; Roriz *et al*., 2006; Pizzol *et al*., 2010). One advantage of is the reduction of insecticide application resulting in a lower impact on the non-target species (Pizzol *et al*., 2010).

Two species of *Trichogramma* are known to have established on São Miguel Island, Azores (Portugal): *Trichogramma cordubensis* Vargas & Cabello and *Trichogramma achaeae* Nagaraja & Nagarkatti (Pintureau *et al*., 1990; Polaszek, 2010). *T. cordubensis* was first collected and identified during an inventory made in 1989 (Pintureau *et al.*, 1990). This is an important thelytokous species of egg parasitoids in the island (Pinto & Tavares, 1992; Garcia *et al*., 2002; Roriz *et al*., 2006). It has been studied with the prospect of implementing pest management programs against local agricultural pests (Roriz, 2004). Studies of dynamics, longevity, fertility, parasitic capacity, host suitability and preference, diapause and several others were performed for this *T. cordubensis* strain (Pinto & Tavares 1990; Pinto & Tavares, 1992; Garcia, 1995; Garcia *et al*., 1995; Garcia *et al.*, 2002). Under field conditions *T. cordubensis* parasitized eggs of several Lepidoptera (Noctuidae) species: *Autographa gamma* (L.), *Chrysodeixis chalcites* (Esper), *Noctua pronuba* (L.), *Peridroma saucia* (Hübner), *Phlogophora meticulosa* (L.), *Xestia c-nigrum* (L.) (Roriz *et al.,* 2006).

T. achaeae was collected on São Miguel in 2006 (unpublished data), and no biological studies were performed locally with it, until now. This is an arrhenotokous species native to India (Nagaraja & Nagarkatti, 1969). There are few developed studies for this species, such as the response of low temperatures for storage purpose, field survey, behavior in presence of kairomones, and crossing tests (Lewis *et al*., 1975; Sekhon & Varma, 1983; Nagaraja, 1987;

Jalali & Singh, 1992). Polaszek (2010) refers that *T. achaeae* parasitizes eggs from the following Noctuidae species: *Achaea janata* (L.), *Anticarsia gemmatalis* (Hübner), *C. chalcites* (Esper), *Earias sp.*, *Earias. insulana* (Boisduval), E*arias vittella* (Fabricius), *Helicoverpa armigera* (Hübner) , *Helicoverpa zea* (Boddie), *Mamestra brassicae* (L.), *Spodoptera sp.*, *Tiracola plagiata* (Walker) and *Trichoplusia ni* (Hübner).

There are evidences that parasitism efficacy of female *Trichogramma* is affected directly or indirectly by biotic and abiotic factors of the habitat, making these parasitoids more of a habitat-specific species than host-specific. The multi-trophic interactions of the wasps with their environment can be determinant for the efficacy of the biological control in the field (Smith, 1996; Romeis *et al.*, 2005). Because of their small size *Trichogramma* are influenced by plant characteristics being more common on specific plants or habitats. Host plant characters such as structure, color, chemistry and volatiles, space between plants and even the quality of food provided by the plant are factors that can influence wasp searching and the parasitism behavior (Romeis *et al.*, 2005). Host eggs characteristics such as its volume and nutritional contents can also influence the parasitism rate, number of wasps per host egg as well as offspring fecundity (Smith, 1996; Martel & Boivin, 2004; Roriz *et al.*, 2006; Andrade *et al.*, 2011).

Temperature is one of the most important abiotic factors because it may directly or indirectly affect physiology and behavior of insects. Biological traits like survival, parasitic performance and emergence of the parasitoid wasps can be strongly affected by this factor (Smith, 1996; Pizzol *et al.*, 2010; Andrade *et al.*, 2011). Not only it produces differences in the biological traits between *Trichogramma* species but also within strains of the same species (Andrade *et al.*, 2011).

In order to better know the present dynamics of *T. cordubensis* and *T. achaeae*, a field survey was performed in three different locations on the Island, having in account the Lepidoptera host plant and respective egg species. The second objective was to expose reared females *Trichogramma* of both species at different temperatures to compare the response of biological parameters such as, development time, parasitism rate and offspring sex-ratio.

Materials and Methods

Field trial

The field work was carried out weekly between May and December of 2010. Lepidoptera eggs were collected in three different locations of S. Miguel Island, at variable altitudes: Relva (0612387 N, 4179768 O, at 140m altitude), Santana (0626483 N, 4185365 O, at 85m altitude) and Chã da Macela (0628437 N, 4180056 O, at 253m altitude).

The host eggs survey were carried out in the following plants: *Mentha suaveolens* (Ehrh), *Conyza bonariensis* (L.), *Phytolacca americana* (L.), *Solanum nigrum* (L.), *Rumex* sp., *Ballota nigra* (L.), *Veronica persica* (Poiret), *Lavatera cretica* (L.), *Galinsoga parviflora* (Cav.), since they provided a suitable number of Lepidoptera eggs. The eggs were obtained with a small portion of the plant where they were found, usually on the leaves. The plants were identified. The time spent weekly on each collection points was about 45 minutes.

These eggs were registered according to the location and the plant species where they were found. The identification of the egg species was made on the laboratory with the aid of a binocular lens (80x). The collected eggs were grouped by their morphology and placed individually in glass tubes (7 x 1cm), closed with cotton wool. They were maintained at room conditions, 21±1ºC of temperature, 70±5% relative humidity, with natural photoperiod, until the completion of their development and identification of larvae or hatched parasitoids**.** The hatched larvae were fed with artificial diet (Poitout & Bues 1974). After emergence, the adults were used for identification of the Lepidoptera species.

Trichogramma stock cultures

Emerged parasitoids were maintained in eggs of *Ephestia kuehniella* Zeller (Lep. Pyralidae). These were used has hosts and reared in the biofactory of the University of Azores according to Tavares & Vieira (1992). *E. kuehniella* eggs were previously irradiated by ultra-violet for 20 minutes, were glued with gum Arabic (diluted in water at 30%) into a yellow cardboard (0.8cm x 7cm) and then inserted into a glass tube (10 x 1cm). The eggs were exposed to several *Trichogramma*, the tubes closed with cotton wool and placed on a support until adults emerged. Parasitoids cultures were maintained at 21±1ºC of temperature, 70±5% relative humidity, and natural photoperiod

Temperature effects on biological trait

The females used for this test were taken from a tube containing a population with 24 hours of life (with several hundred individuals) to allow previous matting.

They were placed individually on test tubes (10 x 1cm) with a of yellow card (7 x 0.8cm) with UV irradiated *E. kuehniella* eggs and a drop of honey 50 % diluted as food, stooped up with cotton wool. The strips had about 400 eggs glued with gum Arabic solution on an area of 1 x 0.7cm.

Groups of four replicates, each with 15 individuals, a total of 60 isolated females from each species, were exposed to one of six different temperatures. The study temperatures were: 10±2, 12±2, 15±1, 20±1, 25±1 and 30±1ºC. Parasitism was allowed for seven days with 70±5% of humidity and photoperiod of L16: D8. After that period the female was removed and the number of dead ones was recorded. Once the progenitor was removed, the eggs were kept until the end of development at previous conditions. The evaluated parameters for both species were: sterile females, progenitor mortality, development time, number of parasitized eggs, emergence rate, and sex-ratio.

Data analysis

All field data were analyzed with a One-way ANOVA (analyses of variance): number sp. and percentage of parasitized eggs by *Trichogramma* spp. One–way ANOVA was also used to compare developmental time, number of parasitized eggs emergence rate and sex-ratio data within species at the different temperatures. When ANOVA showed significant differences ($P<0.05$) among data sets, paired comparisons of each mean were made by LSD test. A t-test was used to compare the parameters between the two species. Sterile females were excluded for analyze of the number of parasitized eggs. Data were transformed by $\sqrt{(x+0.5)}$ and arcsin $\sqrt{x}$ for percentage.

Female mortality and the number of sterile female data were analyzed through a non parametric test, Kruskal-Walls test, to establish a comparison of values among temperatures.

A Two-Sample Kolmogorov-Smirnov test was used to compare data between species. Data were analyzed using SPSS statistic package, software version 15.0 for Windows (SPSS 2006).

The threshold temperature for development was estimated by the x-intercept method, i.e., through least squares regression of the mean development rate over temperature, and extrapolating the regression line back to the x-axis. The day-degrees (D^o) required for development of all life stages were estimated using the method reported by Arnold (1959). The meteorological data, from two agro-climatic stations at 35 and 309m, were provided by the Institute of Meteorology/Azores. Calendar monthly averages of temperature recorded daily at 12:00 GMT for 30 years (1961-1990) were used.

Results

Field trial

During the seven months field survey, the number of collected eggs on Relva (n=782), Chã da Macela (n=1387) and Santana (n=1396) (Figure 1) were similar and not significantly different among localities (*F*=2.485; df=2, 90; *P*=0.089). The same pattern was observed for the percentage of parasitized eggs (*F*=711; df=2, 79; *P*=0.711), percentage of eggs parasitized by *Telenomus* sp. (*F*=0.158; df=2.79; *P*=0.854), and percentage of eggs parasitized by *Trichogramma* spp. (*F*=0.052; df=2, 79; *P*=0.949)

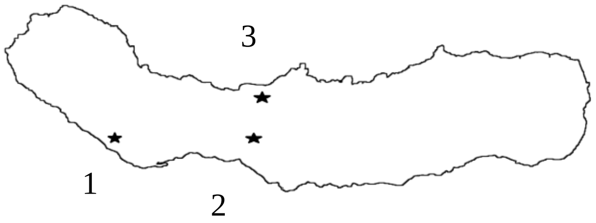

Figure 1-Representation of the three collection sites at S. Miguel Island. Relva (1); Chã da Macela (2); Santana (3).

The Lepidoptera egg species collected were identified as: *Thysanoplusia orichalcea* (Fabricius), *C. chalcites, A. gamma, P. meticulosa, Udea ferrugalis* (Hübner), and one other host species in which the identification was not conclusive. More than 98% of the total collected eggs were identified as *T. orichalcea*, *C. chalcites*, *A. gamma* and *P. meticulosa* eggs. The eggs of the first two species combined represented more than 80% of the total collected (Figure 2).

The highest percentage of eggs collected was in *M. suaveolens* with 86.2, 93.97 and 88.25% at Relva, Chã da Macela and Santana, respectively (Figure 3).

From the collected eggs, two different *Trichogramma* species were obtained and reared. One of the two populations collected was thelytokous and showed a light color body. On the contrary, the other one had a dark color body and an arrhenotokous reproduction. The preliminary identification was made according the color and the offspring sex of virgin females indicated that the populations could correspond to *T. cordubensis* and *T. achaeae* respectively, since they are the only *Trichogramma* species known on the island and correspond to the characteristics mentioned before. The identification was later confirmed by molecular methods (unpublished data).

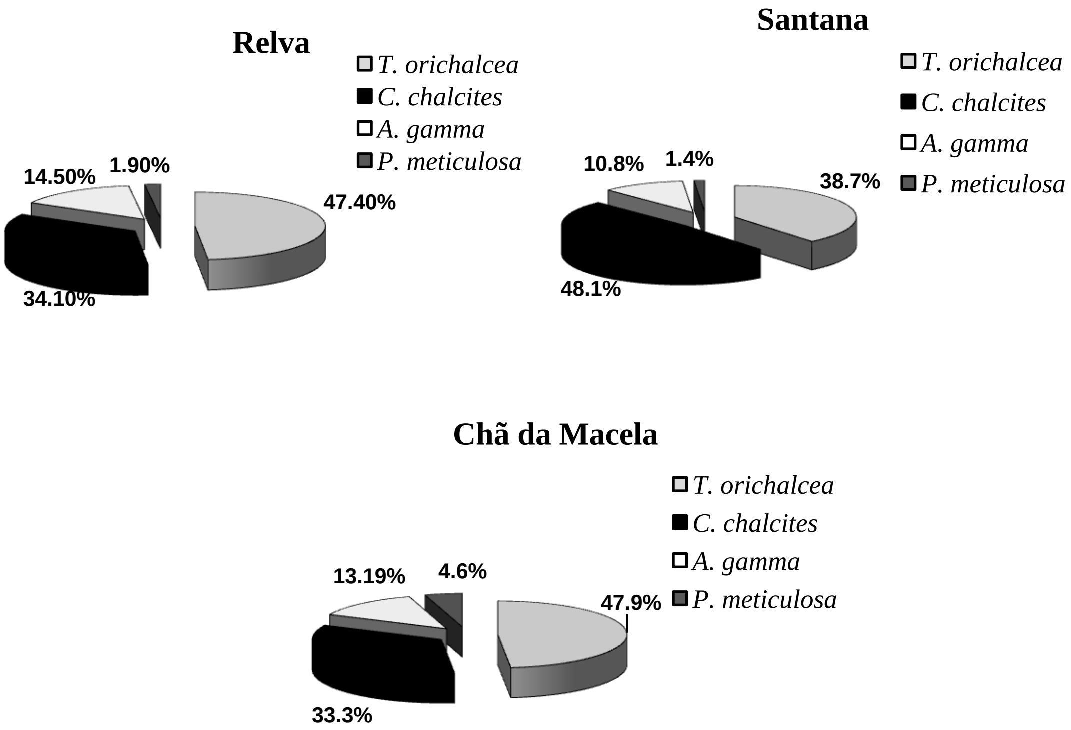

Figure 2- Percentage of the different host eggs species collected at the three locations.

As observed in similar works in Azores (Garcia *et al*., 1995; Garcia, 1995) a third species of Lepidoptera egg parasitoid was collected, *Telenomus* sp.. *Telenomus* sp. was found in the three locations and was the most abundant parasitizing 19.3% of the collected eggs, whereas *Trichogramma* species parasitized 3.0% of the eggs.

Regarding *Trichogramma* species, we observed that *T. cordubensis* was present in a lower amount, representing 0.1% of the total eggs collected at Chã da Macela and 0.3% of the eggs collected at Santana. However*, T. achaeae* were found in all collection points, representing 3.6% of the eggs collected at Relva, 2.5 % at Chã da Macela and 2.4% of the obtained at Santana.

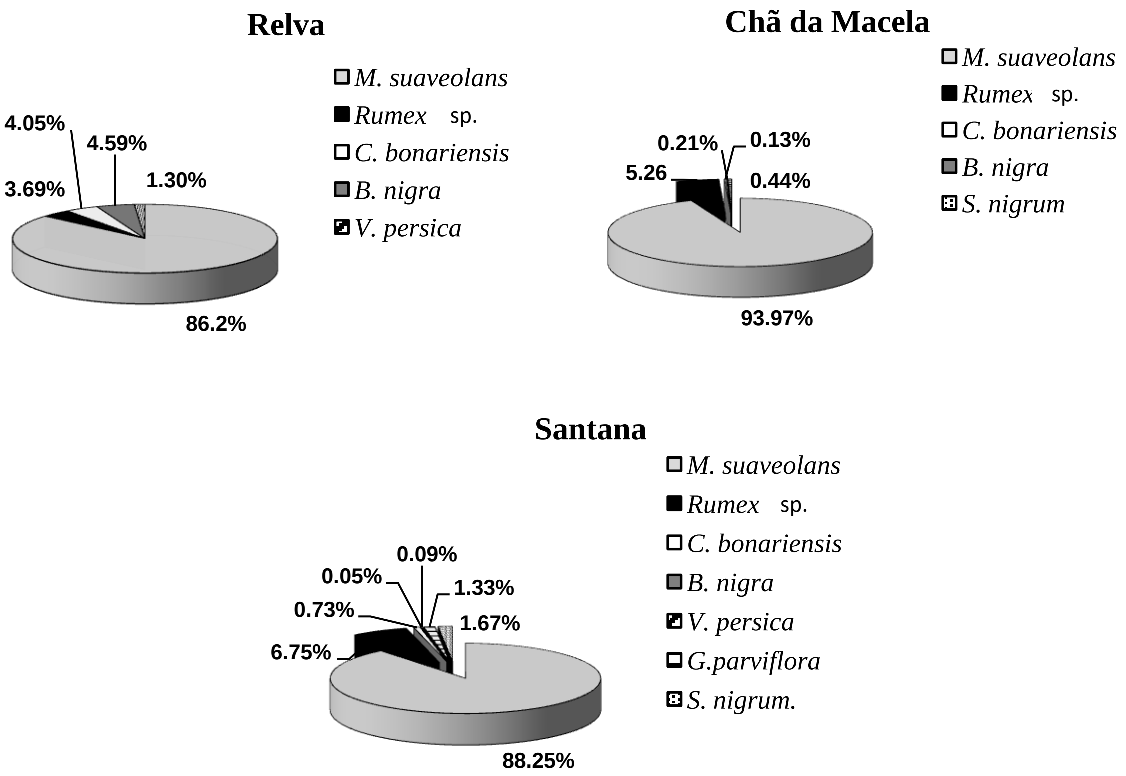

Figure 3- Percentage of the different plant species collected at the three locations.

From a total of 101 host eggs parasitized by *Trichogramma* species, 94.1 % were parasitized by *T. achaeae* and only 5.9% by *T. cordubensis*.

Regarding host plant preferences, *M. suaveolens* was the plant with more parasitized eggs by *Trichogramma*, (89.1 %) followed by *Rumex* sp. (6.9%) and *G. parviflora* (4.0%) (Table 1).

Table 1- Total *Trichogramma* emerged from each host egg species and percentage of parasitism on host plant species, per collection local.

Local	Host egg species	Total *Trichogramma* emerged (n)	Egg parasitism per plant species (%)		
			M. suaveolen	*Rumex* sp.	*G. parviflora*
Relva					
	T. orichalcea	32	100.00		
	C. chalcites	26	100.00		
	A. gamma	3	100.00		
	P. meticulosa	11	100.00		
Chã Macela					
	T. orichalcea	30	100.00		
	C. chalcites	36	100.00		
	A. gamma	17	83.33	16.67	
	P. meticulosa	4		100.00	
Santana					
	T. orichalcea	24	40.00	60.00	
	C. chalcites	45	80.95		19.05
	A. gamma	14	83.33	16.67	

Only four of the six species of Lepidoptera eggs collected will be exploited as they are responsible for more than 99% of the parasitism: *T. orichalcea*, *C. chalcites*, *A. gamma* and *P. meticulosa*.

T. cordubensis only parasitized *T. orichalcea* and *C. chalcites* eggs, and in a very lower percentage (0.1 and 0.2% of the eggs), whereas, *T. achaeae* parasitized 2.5, 3.6, 2.3 and 5.1% of the eggs of *T. orichalcea*, *C. chalcites*, *A. gamma* and *P. meticulosa,* respectively.

The mean number of adults per host egg was lower for *T. cordubensis*, when comparing with *T. achaeae* (Table 2).

Table 2- Number of parasitized host eggs, medium number of emerged adults per egg, and sex-ratio for both species: *T. achaeae* and *T. cordubensis,* for each host species and collection local.

		T. achaeae			*T. cordubensis*		
Local	Host egg species	Parasitized eggs (n)	Medium number of aduls per egg	Sex-ratio (♀ %)	Parasitized eggs (n)	Medium number of aduls per egg	Sex-ratio (♀ %)
Relva							
	T. orichalcea	12	2.67	68.56	0		
	C. chalcites	10	2.60	72.78	0		
	A.gamma	2	1.50	25.00	0		
	P. meticulosa	4	2.75	70.83	0		
Chã Macela							
	T. orichalcea	13	2.31	69.44	0		
	C. chalcites	14	2.43	75.37	2	1	100
	A. gamma	6	2.83	69.44	0		
	P. meticulosa	1	4.00	75.00	0		
Santana							
	T. orichalcea	8	2.50	56.67	2	2	100
	C. chalcites	19	2.16	71.43	2	2	100
	A. gamma	6	2.33	67.92	0		

Temperature effects on biological traits

Sterile female percentage varied between species when those where compared to 12ºC and 30ºC temperatures (p<0.005) (Figure 4). Yet *T. cordubensis* did not manifested significant variation for the percentage of sterile females at the tested temperatures (χ^2= 2.443, df=5, *P*=0,785), unlike *T. achaeae* (χ^2=99.737, df=5, *P*=0.00) (Table 3 and 4).

The mortality of female progenitors at the 7th day of parasitism, was not significantly different between the species when exposed to the various temperatures (P>0.05) (Figure 5). On the other hand there were differences within the species and those occurred at the same temperatures either for *T. achaeae* and *T. cordubensis* (χ^2= 204.575, df=5, *P*<0.001 and χ^2=140.739, df=5, *P*<0.001, respectively), (Table 3 and 4).

Both species completed their development at all six temperatures. As temperature increased, the time of development decreased (Table 3 and 4). The developmental time was statistically different between the two species when those were exposed to 10ºC, 15ºC and 20ºC, with. *T. achaeae* having a longer time of development (*t*=9.837, df=59, *P*=0.00; *t*=2.116, df=87.518, *P*=0.37 and *t*= -3.067, df=88.988, *P*=0.003, respectively) (Figure 6).

The number of parasitized eggs had significant differences within the species along the several temperatures tested (Table 3 and 4). It also differed significantly when compared both species at 10ºC, 12ºC, 20ºC and 25ºC (*t*=-12.626, df=99, *P*=0.00; *t*=-5.693, df=83, *P*=0.00; *t*=-3.231, df=111, *P*=0.002; *t*=-3.231, df=111 , *P*=0.02 and *t*=-3.621, df=97, *P*=0.000), being larger in *T. cordubensis* at all those temperatures (Figure 7).

Comparing the emergence rate between species it can be concluded that it differed at all tested temperatures (*t*=-27.512, df=69.048, *P*=0.000; *t*=-9.010, df=83, *P*=0.000; *t*=2.600, df=102, *P*= 0.011; *t*=-3.659, df=65, *P*=0.001), except at 20ºC and 25ºC (*P*>0.05). *T. cordubensis* was the species with higher emergence rate at five of the total six tested temperatures (Figure 8).

Due to its mode of reproduction *T. cordubensis* presented 100% of female offspring at all temperatures. *T. achaeae* had an elevated sex ratio (above 50%) at the five highest temperatures (Figure 10) decreasing only at 10 ºC.

Table 3- Sterile female (%), mortality of female progenitor on the 7th day (%), preimaginal development time (days), number of parasitized eggs (mean ± SE), adult emergence rate (%), and offspring sex-ratio (%) of *T. achaeae* when exposed to six different temperatures.

Temperature (ºC)	Sterile♀ (%)	♀Mortality (%)	Development time (days)	Parasitized eggs (n)	Emergence rate (%)	Sex-ratio (♀%)
30	76.67d	93.33c	7.64±0.23a	30.21±3.01bd	78.70±3.85c	64.16±7.49b
25	28.33bc	15.00a	11.71±0.24b	35.37±1.93cd	94.50±0.89d	72.21±3.17b
20	1.67a	65.00b	15.21±0.23c	38.88±1.69c	95.46±0.78d	68.72±3.07b
15	13.33ab	3.33a	30.56±0.29d	25.31±1.03b	95.99±0.01d	74.10±3.03b
12	43.33ce	10.00a	72.75±0.39e	9.03±0.58a	4.98±1.47b	75.00±4.90b
10	20.00abe	1.67a	95.50±0.42f	7.46±0.56a	0.95±0.40a	20.00±6.20a

Values in each column that are followed by different letter are significantly different ($P<0.05$).

Table 4- Sterile female (%), mortality of female progenitor on the 7th day (%), preimaginal development time (days), number of parasitized eggs (mean ± SE), adult emergence rate (%), and offspring sex-ratio (%) of *T. cordubensis* when exposed to six different temperatures.

Temperature (ºC)	Sterile♀ (%)	♀Mortality (%)	Development time (days)	Pararasitized eggs (n)	Emergence rate (%)	Sex-ratio (♀%)
30	11.70a	88.33c	8.02±0.02a	35.57±1.13d	90.79±1.74c	100.00±0.00a
25	8.33a	16.67a	11.27±0.08b	44.34±1.56e	93.91±1.03cd	100.00±0.00a
20	11.67a	51.67b	15.98±0.18c	46.04±1.48e	96.08±0.59de	100.00±0.00a
15	10.00a	10.00a	29.54±0.43d	27.35±0.99c	93.18±1.00ce	100.00±0.00a
12	18.30a	8.30a	73.16±0.74e	17.00±0.90a	36.29±3.07a	100.00±0.00a
10	11.70a	10.00a	77.92±0.48f	20.38±0.71b	71.06±2.83b	100.00±0.00a

Values in each column that are followed by different letter are significantly different ($P<0.05$).

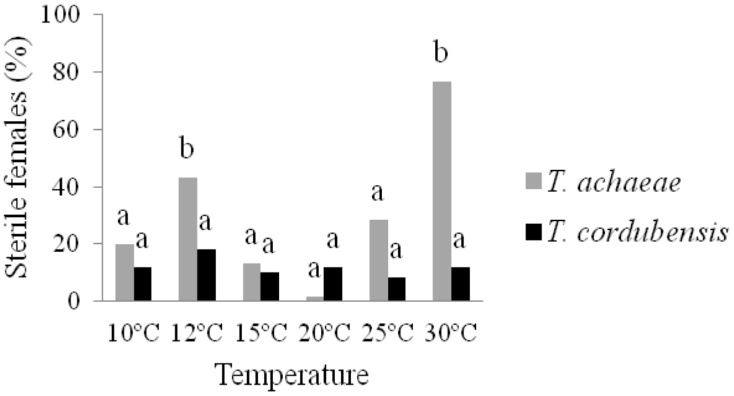

Figure 4- Sterile females (%) of *T. achaeae* and *T. cordubensis* when exposed to each of the six tested temperatures. For each temperature, bars regarding the two species with different letters are significantly different at *P*= <0.05 (t-test).

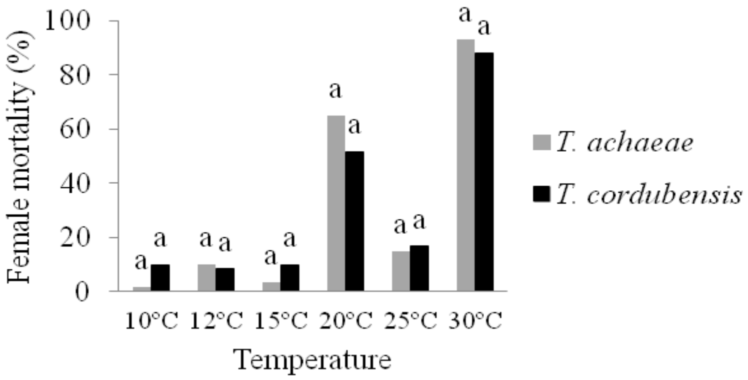

Figure 5- Number of death female progenitors on the 7th day (%) of *T. achaeae* and *T. cordubensis* when exposed to each of the six tested temperatures. For each temperature, bars regarding the two species with different letters are significantly different at *P*= <0.05 (t-test).

For the range of temperatures used, the lower temperature threshold (t_b) and the day-degrees (D^o) required for the total development time of *T. achaeae* and *T. cordubensis* were effectively determined by the linear model, as shown by the high coefficient of determination obtained for all the developmental stages (both R^2>0.98).

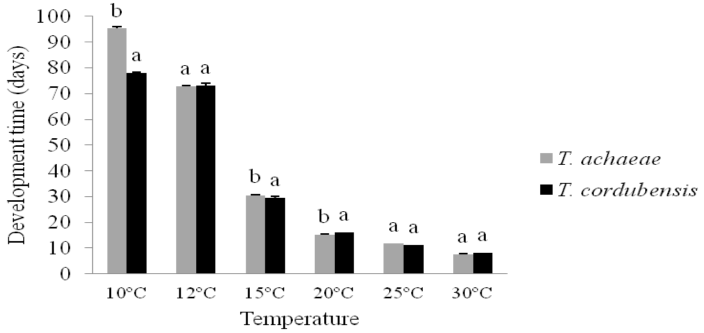

Figure 6- Development time (days), of *T. achaeae* and *T. cordubensis* when exposed to each of the six tested temperatures. For each temperature, bars regarding the two species with different letters are significantly different at *P*= <0.05 (t-test).

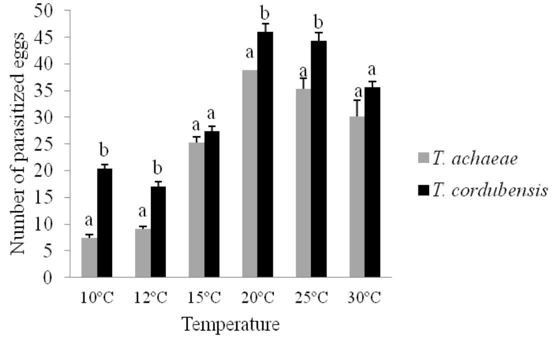

Figure 7- Number parasitized eggs by *T. achaeae* and *T. cordubensis* when exposed to each of the six tested temperatures. For each temperature, bars regarding the two species with different letters are significantly different at *P*= <0.05 (t-test).

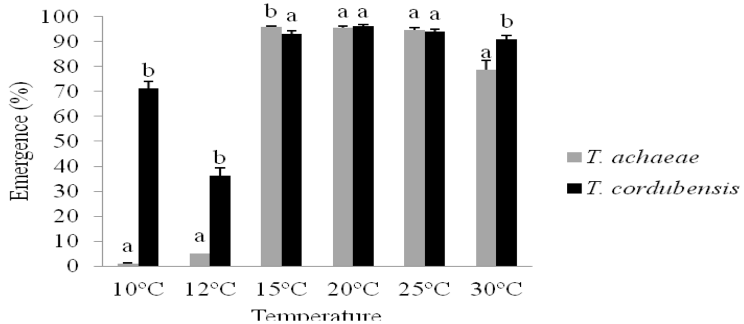

Figure 8- Emergence rate (%) of *T. achaeae* and *T. cordubensis* when exposed to each of the six tested temperatures. For each temperature, bars regarding the two species with different letters are significantly different at *P*= <0.05 (t-test).

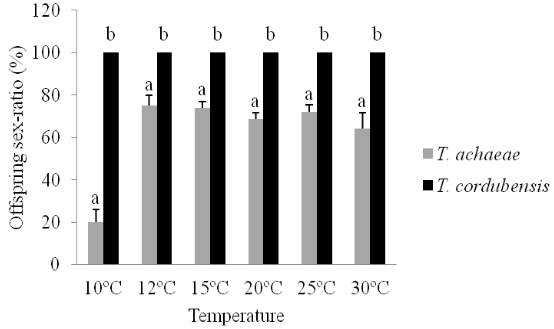

Figure 9- Female offspring (%) of *T. achaeae* and *T. cordubensis* when exposed to each of the six tested temperatures. For each temperature, bars regarding the two species with different letters are significantly different at *P*= <0.05 (t-test).

The lower developmental thresholds (t_b) for egg to adults of *T. achaeae* and *T. cordubensis* were 9.13 ºC and 8.86 ºC, respectively. The number of day-degrees required for the development from egg to adult emergence was 163.68 Dº for *T. achaeae* and 171.36 Dº for *T. Cordubensis.*

For the calculation of the day-degrees required for the development of *T. achaeae* and *T. cordubensis* in the field. January 1st was used as the initial date because it is commonly used for agronomic purposes (López *et al.*, 2001). This and the meteorological data for two localities at 35m and 309m of altitude, indicate that the estimated number of generations of *T. achaeae* per year were: 18.3 generations in Ponta Delgada (=Relva), and 12.9 in Chã da Macela (Figure10). The predicted number of generations per year of *T. cordubensis* at Ponta Delgada and Chã da Macela are: 17.5 and 12.3 generations respectively. (Figure11).

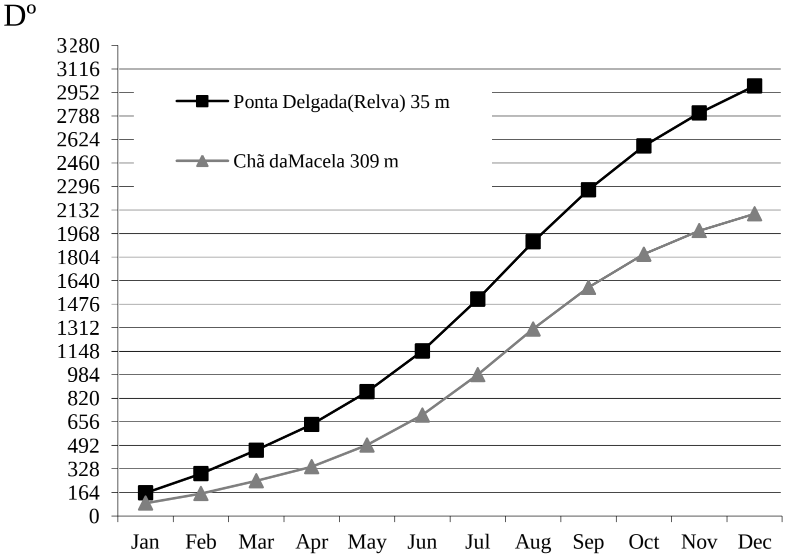

Figure 10- The accumulation of day-degrees during the course of a year for *T. achaeae*, assuming a t_b= 9.13 ºC, at the localities at two altitudes (35 and 309m) on S. Miguel Island.

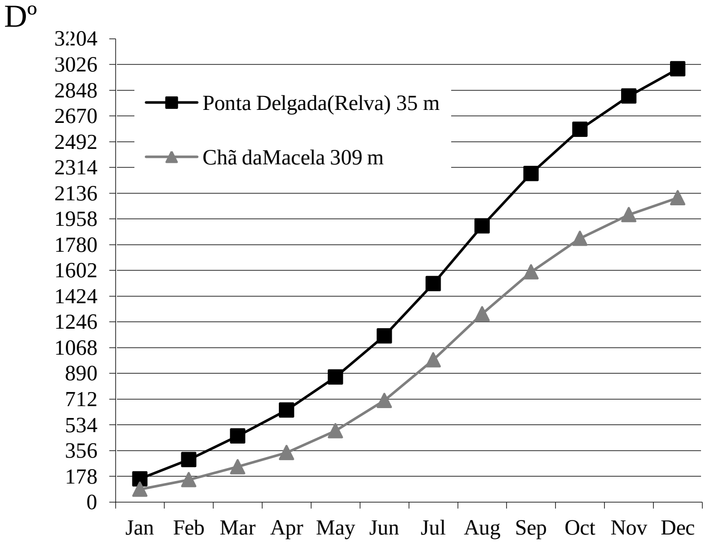

Figure11- The accumulation of day-degrees during the course of a year for *T.cordubensis*, assuming a t_b= 98.86 ºC, at the localities at two altitudes (35 and 309m) on S. Miguel Island.

Discussion

It is known that the host plant can be an important factor for differential parasitism under natural conditions due to structural complexity and chemistry cues (Romeis *et al.*, 2005). Although there was a small amount of collected Lepidoptera eggs parasitized by *Trichogramma* (especially *T. cordubensis*), the higher incidence on *M. suaveolens* and *Rumex* sp. may demonstrate a preference to this plant by these parasitoids. According to Garcia *et al.*, (1995) the highest number of collected parasitized host egg by *T. cordubensis* were collected from *Scrophularia auriculata* (L.) and *Eupatorium adenophorum* (Sprengel) plants followed by *Rumex* sp., in similar survey conducted in S. Miguel. In the present work, the first two plants species did not occur in the surveyed habitat.

Among the several host species collected during this work, *T. orichalcea* and *C. chalcites* were the most parasitized by both *Trichogramma* species, suggesting that these hosts were more attractive to the parasitoids. According to Polaszek (2010), it is the first time that *T. orichalcea, A. gamma* and *P. meticulosa* were cited as *T. achaeae* hosts.

The field survey showed that under natural conditions *T. achaeae* and *T. cordubensis* can parasitize eggs from the same Lepidoptera species.

The highest number of emerged adults per egg was obtained from eggs of *P. meticulosa*. According to Roriz *et al.* (2006) larger host eggs permits the development of a higher number of *Trichogramma* wasps. This suggests that the eggs of *P. meticulosa* were the largest from all collected (Roriz *et al.*, 2006).

Temperature is one of the most important abiotic factors that influence insect physiology and behavior, more than any other environmental conditions (Pizzol *et al.*, 2010; Andrade *et al.*, 2011). This study demonstrated that temperature directly affected biological parameters of *T. achaeae* and *T. cordubensis*, as observed on similar works with other *Trichogramma* species (Andrade *et al*., 2011).

When selecting a species for pest management programs it is important to know how that species biological trait varies with temperature, helping to predict *Trichogramma* biological responses to specific conditions. Those biological variations related to temperature suggest the importance of a careful choice about where the parasitoid is going to be used when selecting a species for pest management (Andrade *et al.*, 2011).

The developmental time was one of the most affected biological parameters by temperature. The increase of the temperature originated a decrease in the duration of development in both species, as observed in previous studies (Jalali & Singh, 1992; Garcia *et al*., 2002).

Parasitism capacity and the emergence rate are probably the most important parameters to take into account for a successful pest management program. In the present study *T. cordubensis* had a higher parasitism capacity than *T. achaeae*, suggesting that *T. cordubensis* is the most efficient in parasitizing *E. kuehniella* eggs at the tested temperatures.

The temperature that parasitoid development takes place influences the emergence rate of *Trichogramma* spp. (Andrade *et al.*, 2011). The emergence rate of *T. achaeae* was much lower than the one for *T. cordubensis* when exposed to the lowests temperatures. Jalali & Singh (1992) showed that emergence rate of *T. achaeae* at 10 ºC for 14 days was about 97.7%, decreasing with the increase of the exposure time. After 49 days the emergence rate was considerably lower.

The percentage of females offspring differed significantly between the two species at all temperatures. This result is due to the difference in the mode of reproduction of the two *trichogramma* species. Due to the symbiont bacteria *Wolbachia*, *T. cordubensis* offspring is composed only by females. This can be changed by treatment with antibiotics or high temperatures (>30ºC) (Stouthamer *et al.*, 1990). On the present study, the exposure temperature did not change the reproduction mode leading to an offspring composed by 100% of females at all temperatures. *T. achaeae* arrhenotokous reprodution leads to an offspring composed by males and females. The number of females was higher than 50% at temperatures superior to 12ºC but much lower at 10ºC, which demonstrates that the temperature affected the sex-ratio.

The estimated lower developmental thresholds (t_b) and the number of day-degrees (D^o) required for the development from egg to adult emergence of the *Trichogramma* species indicate that the predicted number of generations for both species will be approximate.

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