

## Emergence, longevity and fecundity of *Trissolcus basalis* and *Telenomus podisi* after cold storage in the pupal stage

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Abstract – Pupae of *Trissolcus basalis* (Wollaston) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) were stored at 12°C and 15°C for 120-210 days, after different periods of parasitism at 18°C in order to evaluate adult emergence, longevity and ovipositional capacity. There was no emergence of adults at 12°C. The rate of emergence of parasitoids transferred to 15°C at the beginning of the pupal stage was 1.5% and 26.3%, for *T. basalis* and *T. podisi* respectively, whereas those parasitoids transferred one day before the expected date of emergence at 18°C showed 86.4% of emergence for *T. basalis* and 59.9% for *T. podisi*. Mean adult longevity was also significantly lower when pupae were transferred to 15°C at the beginning of the pupal stage. Females emerged after storage and maintained for 120 to 210 days at 15°C parasitized host eggs after transference to 25°C; however, fecundity of *T. podisi* was reduced in about 80% after cold storage.

Index terms: Insecta, hibernation, egg parasitoids, biological control.

## Emergência, longevidade e fecundidade de *Trissolcus basalis* e *Telenomus podisi* após estocagem no estágio pupal

Resumo – Pupas de *Trissolcus basalis* (Wollaston) e *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) armazenadas a 12°C e 15°C por 120 a 210 dias, após diferentes períodos de parasitismo a 18°C, foram avaliadas quanto à emergência, longevidade e capacidade de parasitismo dos adultos. Não houve emergência de adultos a 12°C. Os índices de emergência de parasitóides transferidos a 15°C no início do estágio pupal foram 1,5% e 26,3%, em *T. basalis* e *T. podisi*, respectivamente, enquanto os transferidos um dia antes da data prevista para a emergência dos adultos a 18°C apresentaram índices de emergência de 86,4% em *T. basalis* e 59,9% em *T. podisi*. De forma semelhante, a longevidade média dos adultos foi significativamente menor quando a transferência para 15°C foi realizada no início do estágio pupal. Fêmeas emergidas após estocagem das pupas e mantidas por 120 a 210 dias a 15°C parasitaram ovos hospedeiros após transferência para 25°C; no entanto, a fecundidade de *T. podisi* foi reduzida em cerca de 80% quando mantida em baixa temperatura.

Termos para indexação: Insecta, hibernação, parasitóides de ovos, controle biológico.

### Introduction

Several egg parasitoids of subtropical and temperate regions overwinter in a hibernation state (Boivin, 1994), which can determinate their population dynamics, geographic distribution and potential as biological control agents (Mansingh, 1971; Boivin, 1994). The developmental stage susceptible to hibernation differs between species; however, most egg parasitoids hibernate as larvae or pupae within the host egg (Boivin, 1994).

Low temperatures and short photoperiods can induce hibernation on egg parasitoids and this procedure is useful

to optimize mass-production of these natural enemies and to improve synchronization between the pest and the parasitoid (Boivin, 1994).

Most research on cold storage of egg parasitoids has been conducted on *Trichogramma* species (Stinner et al., 1974; Curl & Burbutis, 1977; Jalali & Singh, 1992; Laing & Corrigan, 1995), *Ooencyrtus ennemus* Yoshimoto (Encyrtidae) (Anderson & Kaya, 1974, 1975) and *Encarsia formosa* Gahan (Aphelinidae) (Lacey et al., 1999).

The storage of *Telenomus remus* Nixon in the pupal stage at 10°C for seven days did not influence parasitoid survival (Gautam, 1986). Storage of *Trissolcus basalis*

(Wollaston) and *Telenomus podisi* Ashmead eggs and larvae was unsuitable for parasitoid emergence. However, adults of *T. basalis* and *T. podisi* that emerged at 15°C when pupae were stored one day before the predicted date for emergence survived for about five months at the low temperature (Foerster & Nakama, 2002).

The objective of this work was to determine the effect of pupae storage at 12°C and 15°C after different periods of parasitism on adult emergence, longevity and fecundity of *Trissolcus basalis* and *Telenomus podisi*.

### Material and Methods

Stink bugs *Nezara viridula* (L.) and *Euschistus heros* (Fabr.) (Heteroptera: Pentatomidae) were used as hosts for *Trissolcus basalis* and *Telenomus podisi*, respectively. Specimens used in this work were obtained from the Laboratory of Integrated Control of Insects at the Universidade Federal do Paraná, Brazil.

Egg masses with 40 eggs of *N. viridula* and *E. heros* were exposed respectively to five females of *T. basalis* and *T. podisi*, at 18±0,5°C (12L: 12D), during 48 hours. After this period, the parasitoids were discarded and the parasitized eggs remained on moistened filter paper. At different dates after parasitism, these eggs were transferred from 18°C to either 15±0,5°C or 12±0,5°C (10L:14D) to evaluate adult emergence. Storage at 12°C was made 15, 20 and 25 days after parasitism; at 15°C, both parasitoids were stored 15, 20, 25, 26, 28 and 30 days after parasitism and for *T. podisi*, the parasitoids were also stored 33 days after parasitism, because this species has lower developmental rate than *T. basalis*. Emergence rate, developmental time and progeny sex ratio were recorded in each treatment.

Adults were fed with honey and maintained in glass tubes (17x 2 cm) partially covered with black paper; previous results showed that this procedure increases their longevity (Doetzer, 2003). Mortality of *T. basalis* and *T. podisi* was daily recorded.

The effect of pupal storage at 15°C on the fecundity of parasitoids emerged was evaluated. Females of *T. basalis* and *T. podisi* emerged after storage one day before the predicted date for emergence were maintained at 15°C between 120 and 210 days and then transferred to 25°C at 30 days intervals. Ten females of each species were individually exposed to 50 *E. heros* eggs for 24 hours one day after transference to 25°C. Parasitoids were discarded after this period and the eggs

were maintained on moistened filter paper. The number of parasitized eggs per female and progeny sex ratio was recorded. Results were analyzed in relation to a control treatment, conducted with females developed continuously at 25°C and exposed to host eggs one day after emergence.

Data were submitted to analysis of variance and means compared by Tukey's test ( $p < 0.05$ ). Comparisons between species were done by t test ( $p < 0.05$ ). The relationships between storage age and emergence rate, developmental rate, progeny sex ratio and adult longevity were evaluated by linear regression analysis.

### Results and Discussion

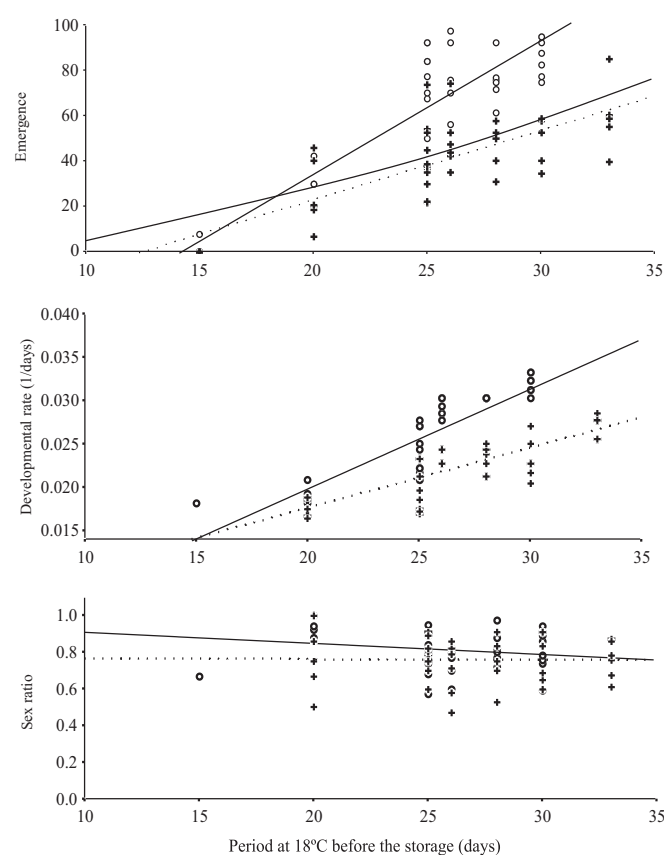
Adults failed to emerge when *T. basalis* and *T. podisi* pupae were transferred from 18°C to 12°C at any of the pupal ages evaluated, showing that storage at this temperature is lethal to the parasitoids in the pupal stage.

Parasitoids stored at 15°C in the pupal stage continued to develop and emerged at this temperature. Emergence of *T. basalis* occurred in all treatments at 15°C, whereas no adults of *T. podisi* emerged when their pupae were stored 15 days after parasitism (Table 1). Pupal age at storage significantly influenced adult emergence (*T. basalis*:  $y = -84.06 + 5.89x$ ,  $R^2 = 0.80$ ,  $p < 0.05$ ; *T. podisi*:  $y = -38.23 + 3.05x$ ,  $R^2 = 0.59$ ,  $p < 0.05$ ) and developmental rate of the parasitoids (*T. basalis*:  $y = 89.04 - 1.92x$ ,  $R^2 = 0.77$ ,  $p < 0.05$ ; *T. podisi*:  $y = 85.29 - 1.47x$ ,  $R^2 = 0.60$ ,  $p < 0.05$ ) (Figure 1).

Cold storage of pupae did not affect the sex ratio of the parasitoids, as also found for *T. remus* (Gautam, 1986). Progeny sex ratio of *T. basalis* ranged from 0.67 to 0.95 and for *T. podisi*, ranged from 0.71 to 0.80 (Table 1). Relationships between the age of the stored pupae and progeny sex ratio were not detected (*T. basalis*:  $y = 96.23 - 0.59x$ ,  $R^2 = 0.04$ ,  $p > 0.05$ ; *T. podisi*:  $y = 75.98 + 0.01x$ ,  $R^2 = 0.01$ ,  $p > 0.05$ ) (Figure 1).

Adult parasitoids that emerged after pupal storage at 15°C hibernated with a drastic reduction in mobility and feeding (Mansingh, 1971). Pupal age at storage affected adult longevity for both species ( $p < 0.05$ ) (*T. basalis*, males:  $y = 5.38 + 6.34x$ ,  $R^2 = 0.11$  and females,  $y = -263.56 + 18.23x$ ;  $R^2 = 0.42$ ; *T. podisi*, males:  $y = -78.09 + 6.34x$ ,  $R^2 = 0.32$  and females,  $y = -93.61 + 10.29x$ ,  $R^2 = 0.25$ ) (Figure 2). Highest longevity was verified when parasitoids were stored at 15°C one day before the predicted date of emergence (nine and eight

months, for *T. basalis* and *T. podisi*, respectively) and decreased when the pupae were stored at the beginning of the stage. Females survived longer than males and *T. basalis* longevity was higher than *T. podisi* (Table 2).



**Figure 1.** Relationship between the period at 18°C before the storage and the percentage of emerged adults, developmental rate and sex ratio of *Trissolcus basalis* (—○—) and *Telenomus podisi* (—+—) stored at 15°C at different dates after parasitism.

Parasitoids stored at earlier stages of pupal development spent more energy to complete their development and, therefore, their longevity was shorter. Similarly, the longevity of females of *T. oenone* that emerged at 15°C was lower than of those that emerged at 17.5°C (James & Warren, 1991). Adults of *T. basalis* emerging at 15°C were small, inactive and survived less than one day (Orr et al., 1985).

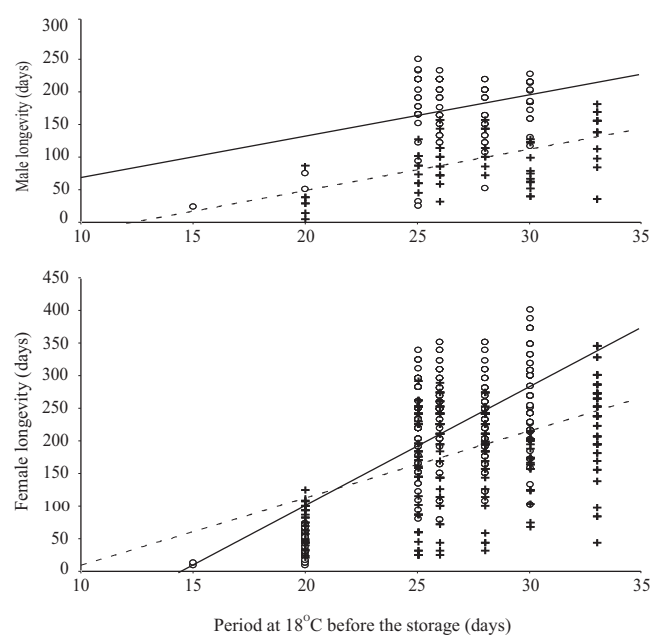
Foerster & Nakama (2002) found that the mean longevity of *T. basalis* and *T. podisi* transferred from 25°C to 15°C one day before the predicted date for emergence was of about five months; this value is lower than the results obtained in this work. Longevity of *T. basalis* and *T. podisi* at 15°C was also higher than that of other Scelionidae species such as *Ceratobaeus masneri* Austin (Austin, 1984) and *Trissolcus oenone* (Dodd) (James & Warren, 1991).

Females of *T. basalis* and *T. podisi* emerging after pupal storage one day before the predicted date for emergence and maintained at 15°C for 120 to 210 days, parasitized host eggs (Figure 3). Fecundity of *T. basalis* decreased as storage period increased from 120 to 180 days; however, after storage for 210 days, fecundity values increased as those recorded for females reared at 25°C (Figure 3). This increased fecundity after storage for seven months could not be explained, which shows the need for additional research in order to fully understand the reproductive dynamics of this species. Females of *T. podisi* were more affected by adult maintenance at 15°C, than *T. basalis*. The fecundity of *T. podisi* after storage at 15°C was lower than that of females reared at 25°C and the mean number of parasitized eggs decreased as the storage period increased, ranging from 0.0 to 6.4 parasitized eggs (Figure 3). Progeny sex ratio ranged from 0.87 to 0.92 for *T. basalis*, and from 0.34 to 0.69 for *T. podisi* and was

**Table 1.** Emergence (%), developmental time (days) and progeny sex ratio (number of females/total number of emerged adults) of *Trissolcus basalis* and *Telenomus podisi* stored at 15°C in different dates after parasitism at 18°C<sup>(1)</sup>.

Period at 18°C before the storage (days)	Emergence		Developmental time		Sex ratio	
	<i>T. basalis</i>	<i>T. podisi</i>	<i>T. basalis</i>	<i>T. podisi</i>	<i>T. basalis</i>	<i>T. podisi</i>
15	1.5±1.49c	0.0±0.00	55.0±0.00a	-	0.67±0.00b	-
20	28.5±4.15bA	26.3±7.23bA	52.2±1.16aA	57.0±1.58aB	0.95±0.02aA	0.80±0.08aA
25	70.7±6.02aA	41.0±5.06aB	42.3±1.48bA	49.8±2.15bB	0.80±0.04abA	0.80±0.03aA
26	78.5±7.49aA	49.3±5.56aB	34.2±0.58cA	42.5±0.67cB	0.74±0.04bA	0.71±0.06aA
28	75.5±4.11aA	46.8±3.96aB	35.7±1.67cA	43.5±1.23cB	0.83±0.04abA	0.78±0.06aA
30	86.4±3.12aA	46.3±5.09aB	31.3±0.42cA	43.3±1.75cB	0.79±0.04abA	0.77±0.06aA
33	-	59.9±5.97a	-	36.8±0.70c	-	0.76±0.04a

<sup>(1)</sup>Means ± standard error followed by the same letter, low in the columns (Tukey test) and capital in the lines (t test), do not differ statistically (p≤0.05) (n = 10).

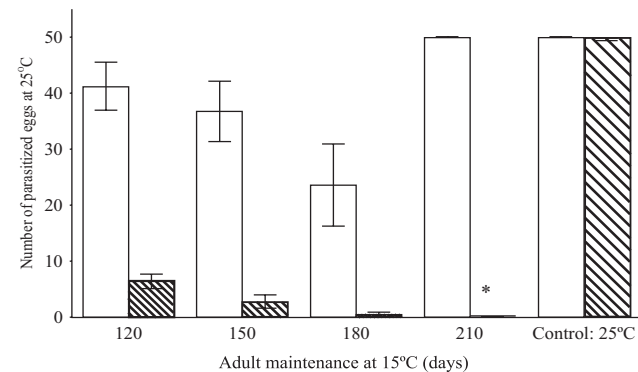


**Figure 2.** Relationship between the period at 18°C before the storage and the adult longevity of *Trissolcus basalis* (—○—) and *Telenomus podisi* (---+---) emerged and maintained at 15°C after storage at different dates after parasitism.

**Table 2.** Longevity (days) of *Trissolcus basalis* and *Telenomus podisi* emerged at 15°C after pupal storage in different dates after parasitism at 18°C<sup>(1)</sup>.

Period before storage (days)	<i>T. basalis</i>			
	Males	Range	Females	Range
15	24.0 (1)	-	12.0±2.00 (2) <sup>3</sup>	10-14
20	75.3±14.14 (3) <sup>3</sup>	51-100	42.3±3.21c (35)	10-74
25	175.8±11.03a (26)	25-250	231.6±6.61b (76)	81-340
26	186.0±6.65a (28)	122-233	249.2±8.34ab (59)	80-352
28	166.7±8.39a (24)	52-219	224.7±6.11b (83)	108-352
30	188.7±6.34a (21)	117-228	266.1±6.77a (81)	103-401
33	-	-	-	-
Mean	175.4B		220.3A	
	<i>T. podisi</i>			
	Males	Range	Females	Range
15	-	-	-	-
20	35.1±9.99c (17)	5-88	71.5±5.65d (28)	22-126
25	82.9±8.61abc (19)	46-128	170.0±9.56c (65)	26-293
26	97.8±8.33abc (17)	32-158	204.0±8.53b (60)	25-290
28	118.1±9.00ab (20)	73-158	203.4±6.59b (68)	32-275
30	79.7±8.41bc (19)	41-124	169.4±9.01bc (24)	69-216
33	136.9±9.32a (16)	37-182	240.6±8.19a (63)	44-346
Mean	98.4C		189.2B	

<sup>(1)</sup>Means ± standard error followed by the same letter, low in the columns or capital in the lines, do not differ statistically according to Tukey test ( $p \leq 0.05$ ); number of replicates inside brackets. <sup>(2)</sup>Statistic analysis was not done due to the low number of emerged adults.



**Figure 3.** Number of parasitized eggs (means±standard error) at 25°C by *Trissolcus basalis* (□) and *Telenomus podisi* (▨) emerged after pupae storage one day before the predicted date for emergence and maintained as adults at 15°C for 120 to 210 days (50 eggs offered to one female during 24 hours; \*eggs were not parasitized).

not influenced by adult maintenance at 15°C. Several studies have shown the impact of cold storage on the fecundity of egg parasitoids; Flanders (1938) states that this impact is due to the fact that the reproductive organs are the ones most likely to be affected by the low temperature. Foerster & Nakama (2002) found that *T. basalis* and *T. podisi* kept for 20 days at 15°C had their fecundity reduced by more than 80% and 95%, respectively, and as reported for *T. remus*, parasitoids maintained at 5°C for three days had a significant reduction in fecundity (Gautam, 1986).

### Conclusions

1. Pupae of *T. basalis* and *T. podisi* do not survive at 12°C, independently of their developmental stage at the time of storage.
2. Adult longevity at 15°C is higher when the pupae are transferred to 15°C at the end of the pupal stage.
3. Females of *Trissolcus basalis* parasitize eggs of *Euschistus heros* at 25°C after remaining in hibernation at 15°C for 120–210 days; females of *Telenomus podisi* do not parasitize eggs after 180 days at 15°C.

### Acknowledgements

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