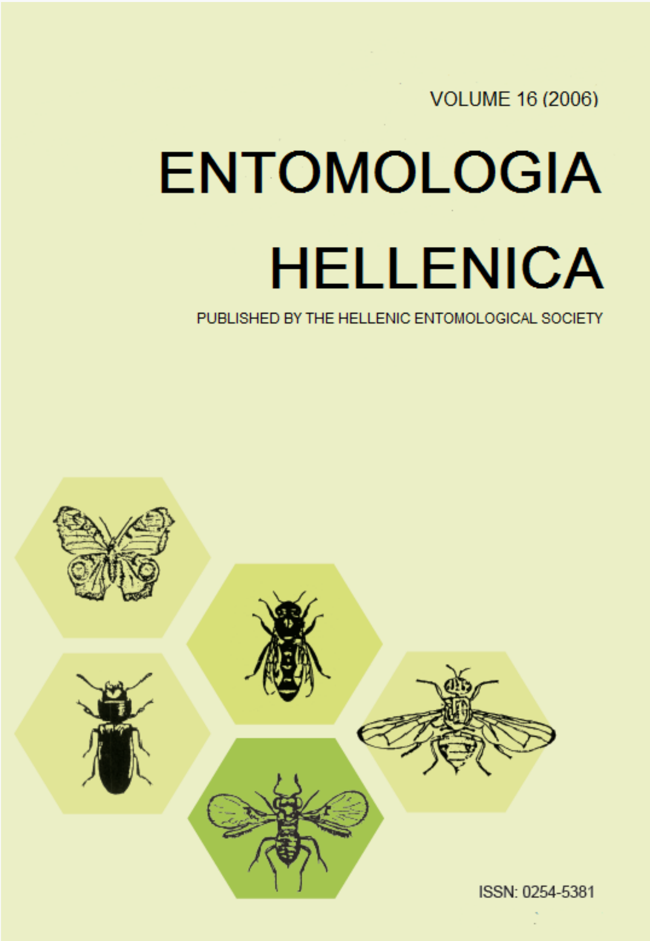
ENTOMOLOGIA HELLENICA

Τόμ. 16 (2006)



Επίδραση της ηλικίας των θηλυκών ενηλίκων του Trichogramma cacoeciae και των αυγών του ξενιστή του στην παρασιτική του ικανότητα

F. Vourlioti, P. G. Milonas

doi: 10.12681/eh.11620

ENTOMOLOGIA HELLENICA **16** (2005-2006): 3-10

Influence of female age of *Trichogramma cacoeciae* and host eggs age on its parasitic effectiveness

F. VOURLIOTI AND P. G. MILONAS*

Biological Control Lab. Department of Entomology, Benaki Phytopathological Institute, 8 S. Delta str. 145 61 Kifissia, Greece
e-mail: p.milonas@bpi.gr

ABSTRACT

Laboratory experiments were conducted to investigate the influence of female age of *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) and egg age of *Lobesia botrana* (Denis & Schiffermueller) (Lepidoptera: Tortricidae) on parasitoid effectiveness. It was found that *T. cacoecciae* females parasitize more frequently 4 days old *L. botrana* eggs than younger ones. Furthermore, developmental time of their offspring retarded and percentage of viable eggs decreased when parasitoid larvae fed with older eggs. *T. cacoecciae* females that were 4 days old were more effective in parasitising *L. botrana* eggs. Rearing the parasitoids on *Sitotroga cerealella* eggs for one generation resulted in a decreased number of parasitised eggs of *L. botrana* than when reared on *Ephestia kuehniella* eggs. The implications of these results in selecting a candidate species for biological control are discussed.

Introduction

An economically important pest of vineyards in Europe is the European grape vine moth *Lobesia botrana* (Denis & Schiffermueller) (Lepidoptera: Tortricidae). It completes 3 generations per year and exceptionally a partial or complete 4^{th} one in the southern regions. Larvae of the first generation damage the inflorescence and those of the following generations damage the green, ripening and ripe grape berries (Tzanakakis et al. 1988). The exploitation of *Trichogramma* species against *L. botrana* is an issue that had taken a considerable effort by many researchers in the past (Babi 1990; Hassan 1997; Zimmermann et al. 1997; 2; Barnay et al. 2001; Hommay et al. 2002). Generally, in European vineyards *Trichogramma* parasitoids are commonly found parasitizing eggs of *L. botrana* and *Eupoecilia ambiguella*. Barnay et al. (2001) in a thorough survey of *Trichogramma* wasps in vineyards in France reported 4 species, namely *T. cacoeciae* (Marchal), *T. daumalae* (Dugast & Voegele), *T. evanescens* (Westwood) and *T. principium* (Sugonjaev and Sorokina), which were found on host eggs. Although eggs of *L. botrana* can be parasitized by several *Trichogramma* species (Dugast and Voegele 1984; Leisse 1987; Fursov 1994; Barnay et al. 2001; Ibrahim et al. 2004), research effort focused mainly on *T. cacoeciae* and *T. evanescens* for deploying a biological control program using egg parasitoids against *L. botrana* (Tavares et al. 1988; Zimmermann et al. 1997; Hassan and Wuhrer 1997; Hommay et al. 2002). Only the species *T. brassicae, T. cacoeciae, T.*

dendrolimi and *T. evanescens* are currently listed for use in countries of the EPPO-region (EPPO 2002).

Although some positive results were obtained in previous years using *T. cacoeciae* against *L. botrana* they are not yet used commercially, because of fluctuating efficiency (Hassan and Wuhrer 1997; Hommay et al. 2002).

Numerous factors are used in determining the parasite's effectiveness as an agent for biological control of a certain pest. These factors should be thoroughly studied before a selection of a candidate *Trichogramma* species or strain for field releases takes place. Host age is among these factors and may be critical in selecting a species/strain to be used in a biological control program (Schmidt 1970; Pak et al. 1986). Furthermore, parasitoid age and rearing host are also of interest in evaluating the effectiveness of a parasitoid host complex, as both factors are known to contribute to the final outcome of a biological control effort (Barnay et al. 1999; Monje et al. 1999; Smith 1996).

Since *L. botrana* is a multivoltine species, inundative releases of *Trichogramma* wasps, at the early stage of each generation, when egg laying starts, is necessary for *Trichogramma* to be a successful biological control agent. However, before an inundative release becomes successful thorough research is needed to understand the biology of the candidate *Trichogramma* species or strain better (Pak et al. 1986; Smith 1996; Calvin et al. 1997; Hassan 1997).

In the current study we examined the capacity of a commercially available species, *T. cacoeciae,* to parasitize eggs of *L. botrana,* in relation to a number of factors in the laboratory. We determined the possible influence of host and parasitoid age and of rearing host on parasitization by the parasitoid and on host suitability, development and mortality of the parasitoid offspring.

Materials and Methods

Insects' rearing. Insects used in this study were obtained from a laboratory colony of *T. cacoeciae* that was established using commercially available cards from a strain in Germany. This colony was used experimentally for the control of the olive moth *Prays oleae* (Bern) (Lepidoptera, Yponomeutidae) in olive groves in central Greece. Parasitoids were reared at 25°C, 60% R.H. and 16:8 (L:D) photoperiod using *Ephestia kuehniella* eggs as a host. These were obtained from a laboratory colony maintained at the insectary of Benaki Phytopathological Institute on semolina wheat flour at 20°C. Twenty four - hours old *Trichogramma* females were placed in glass rearing vials (10 X 2.5 cm). In each vial a strip of paper containing 500 host-eggs, pasted onto it by non-toxic water-soluble glue, was placed. Honey streaks were offered as a food source. Fresh eggs of *L. botrana* were obtained from a laboratory colony maintained in the laboratory for many years on artificial diet. Newly emerged adults of *L. botrana* were kept for 24h in a plastic cup with a piece of cotton submerged in a sugar solution placed at the bottom of the cup. Eggs were individually deposited on the walls of the cup.

Influence of host age. In a non-choice experiment, 30 eggs of *L. botrana* that were 1, 2, 3 and 4 days old were offered to a newly emerged female wasp in a glass tube (10 x 1 cm) covered by a cotton cloth. The parasitoids were removed after 24 h and the tubes bearing the eggs were kept under controlled conditions (25°C, 60% R.H. and 16:8 (L:D) photoperiod). Fifteen parasitoids were tested for each host age. The parameters recorded were the number of parasitized eggs, developmental time of the parasitoids offspring and percentage of emerging parasitoids (emergence rate).

Influence of parasitoid age. In a non-choice experiment, 30 fresh eggs of *L. botrana* were placed in glass tubes, each with a single female wasp that had emerged 1, 2, 4 and 8 days earlier. Parasitoids were unexperienced and they had access only to drops of honey solution. The wasps were removed after 24 h and the eggs incubated at 25°C, 60% R.H. and 16:8 (L:D) photoperiod. Fifteen parasitoids were tested for each age group. Parameters recorded were the number of parasitized eggs, developmental time of the parasitoids offspring and emergence rate.

Influence of rearing hosts. Parasitoids after being reared for 5 generations on *E. kuehniella*, were reared for one generation on *Sitotroga cerealella* eggs. Fifteen females emerged from *S. cerealella* eggs and 15 females from *E. kuehniella* eggs were placed individually for 24 h with 30 fresh eggs of *L. botrana*. Further treatment followed the method for testing the influence of host and parasitoid age. The number of parasitised eggs was recorded after 4 days. Parasitized eggs were checked daily until adult emergence and finally, developmental time of the parasitoids' offspring and emergence rate were recorded.

Statistical analysis. One-way ANOVA was performed to detect any difference on the number of parasitized eggs, parasitoid developmental time and emergence rate influenced by host or parasitoid age. In all cases proportional data were normalized with arcsine transformation and development and the number of parasitized eggs were normalized with Log10 (X+1) to minimize heterogeneity of variances (22). Tukey HSD or t-test was used to compare any difference among treatments.

Results and Discussion

Influence of host age. The mean number of parasitized eggs ranged from 8.5 to 18 for 4 and 3 days old host eggs respectively. The number of parasitized eggs was significantly influenced by the host egg age (Table 1) (F=7.88; df=3, 49; P=0.0002). The number of parasitized eggs on 4 days old eggs was significantly lower than on all other host egg ages (Table 1). Host egg age also influenced the development of parasitoid offspring. Development of immature parasitoids was slightly retarded on 4 days old eggs (Table 1). Parasitoids reared on 4 days old eggs, emerged after 14.6 days whereas on 3 days-old eggs they emerged after 12.4 days (F=6.5; df=3, 38; P=0.001). Host age also had a strong influence on emergence rate (Table 1). On 4-days old eggs parasitoids emerged from 75% of parasitized eggs, which was significantly different from 94% and 95% on 2nd, and 3rd days old eggs respectively (F=4.6; df=3, 39; P=0.008).

TABLE 1. Influence of host egg age on parasitization of *T. cacoeciae* on *L. botrana* eggs (mean ± se).

Age of host eggs (days)	Mean no. parasitized eggs	Developmental time (days)	Emergence rate (%)
1	15.6 ± 2.1 a *	13.3 ± 0.5a	91.7 ± 4.1ab
2	16.3 ± 2.3a	13.3 ± 0.3ab	93.9 ± 3.4a
3	18.0 ± 1.8a	12.4 ± 0.3ab	95.4 ± 4.4a
4	8.5 ± 0.9b	14.6 ± 0.3b	75.7 ± 6.1b

*Means followed by the same letter within a column are not statistical different (Tukey HSD; P=0.05)

Although size is often related to host age selection by larval parasitoids (Vinson 1976), in the case of egg parasitoids any difference for host age acceptance and suitability is based on chemical or physiological internal or external differences (Schmidt 1970; Pak et al. 1986). The influence of host age on parasitization by *Trichogramma*, although a trivial one, has been widely demonstrated. Generally, younger host eggs are parasitized more frequently than older ones (Hintz and Andow 1990; Reznik et al.1990; Liu et al. 1998; Monje et al. 1999). Host age influences parasitism and mortality of several *Trichogramma* species (Pak et al. 1986). Whenever a preference in host age for parasitism exists in a parasitoid-host complex, release strategies should take into account host age to obtain the maximum efficiency from a release. In our case, host age not only affected host acceptance by adult parasitoids, but also the development and emergence rate were negatively affected. This implies that the timing of a release of *Trichogramma* against *L. botrana* could play a role in obtaining the desired outcome of a biological control effort. Calvin et al. (1997) reported a longer developmental period for *T. pretiosum* (Riley) on older eggs of *Diatraea grandiosella* (Dyar). They attributed this slower development on the fewer larvae per host egg on old eggs (black-headed). *Trichogramma* parasitoids do not pupate unless all the yolk of an egg is consumed and a lower number of larvae per host egg means that the *Trichogramma* larvae have more food to consume and presumably a longer developmental period. However in our case, the number of adults emerged from parasitized eggs was slightly lower in the case of the 4-day-old eggs than it was in the case of eggs, which were from 1 to 3 days old eggs (data not shown). Probably the slower development most likely resulted of nutritional deterioration of the egg yolk for the immature parasitoid. Survival of immature parasitoids was decreased on older eggs. Although parasitoids were able to parasitize 4 days old host eggs in some cases neither host larva nor parasitoid emerged. A similar observation was reported by Takada et al. (2000) for *T. dendrolimi* Matsumura on *Mamestra brassicae* L. They attributed the arrest in development of the host to a kind of venom injected by the parasitoid during oviposition.

Influence of parasitoid age. The age of female wasps significantly affected the mean number of parasitized *L. botrana* eggs ($F=9.1$; $df=3, 59$; $P<.0001$). The highest parasitism observed with 4-days-old parasitoid (11.4) and the lowest with 8-days-old parasitoids (2.3) (Table 2). Parasitoid age influenced the development of the parasitoids offspring (Table 2). The developmental time of parasitoids produced by 4-days-old adult females was shorter than those from all other age groups though the difference was only significant with that from 2-days-old adult females ($F=10.9$; $df=3, 46$; $P<0.001$). No substantial difference was observed for emergence rate (Table 2) ($F=0.6$; $df=3, 47$; $P=0.6$). The age of female wasps had a great influence on the number of progeny emerged, as it is observed for other egg parasitoids (Hintz and Andow 1990; Miura and Kobayashi 1998). Young parasitoids at the age of 24 to 48 h after emergence were less capable of parasitising than 4-days-old females. They may need an initial food source before they are ready to parasitize. Host acceptance of *L. botrana* eggs was not influenced, as the wasps were reared for many generations on factitious hosts.

TABLE 2. Influence of *T. cacoeciae* females age on parasitization of *L. botrana* eggs (mean ± se).

Age of host eggs (days)	Mean no. parasitized eggs	Developmental time (days)	Emergence rate
1	6.8± 1.3ab*	13.4 ± 0.1a	87.1 ± 4.6a
2	5.5 ± 1.5b	15.3 ± 0.6b	88.6 ± 6.4a
4	11.4 ± 1.4a	12.6 ± 0.2a	82.6 ± 4.9a
8	2.3 ± 0.6b	13.7 ± 0.3a	84.6 ± 5.3a

*Means followed by the same letter within a column are not statistical different (Tukey HSD; P=0.05)

Influence of rearing host. The rearing host had an apparent influence on mean number of *L. botrana* parasitized eggs (Table 3). Females reared on *S. cerealella* eggs for just one generation, parasitized significantly fewer eggs of *L. botrana* than those reared on *E. kuehniella* (11.7 and 16.5 respectively) (F=7.1; df=1, 29; P=0.012). Developmental time and emergence rate was not influenced by the rearing host (Table 3). It is known that rearing hosts have a significant effect on parasitization capacity of *Trichogramma* parasitoids (Monje et al. 1999; Kuhlmann and Mills 1999). However, any difference in the performance of *Trichogramma* reared from *S. cerealella* and *E. kuehniella* that are detected in the laboratory are not necessarily relevant to the field conditions (Smith 1996).

The success of inundative biological control programs using *Trichogramma* can be improved through a better understanding of their biology and response to environmental conditions of the commercially produced parasitoid species.

TABLE 3. Influence of rearing host on parasitization of *L. botrana* eggs by *T. cacoeciae* (mean ± se).

Rearing Host	Mean no. parasitized eggs	Developmental time (days)	Emergence rate
S. cerealella	11.7 ± 0.7a*	7.8 ± 0.1a	72.7 ± 5.5a
E. kuehniella	16.5 ± 1.5b	7.9 ± 0.2a	84.9 ± 2.6a

*Means followed by the same letter within a column are not statistical different (T-test; P=0.05)

References

Babi, A. 1990. Bioécologie de *Trichogramma cacociae* Marchal et *T. daumalae* Dugast et Voegelé (Hym. Trichogrammatidae). Utilisation en lutte biologique contre *Lobesia botrana* Den. & Schiff. (Lep.Tortricidae). PhD Thesis, Université Aix-Marseille, 143pp.

Barnay, O., J. Pizzol, C. Gertz, J.C. Kienlen, G. Hommay and L. Lapchin. 1999. Host density dependence of discovery and exploitation rates of egg patches of *Lobesia botrana* (Lepidoptera: Tortricidae) and *Ephestia kuehniella* (Lepidoptera: Pyralidae) by the parasitoid *Trichogramma cacoeciae* (Hymenoptera Trichogrammatidae). J. Econ. Entomol. 92: 1311-1320.

Barnay, O., G. Hommay, C. Gertz, J.C. Kienlen, G. Schubert, J.P. Marro, J. Pizzol and P. Chavigny. 2001. Survey of natural populations of *Trichogramma* (Hym., Trichogrammatidae) in the vineyards of Alsace (France). J. Appl. Entomol. 125: 469-477.

Calvin, D.D., J.E. Losey, M.C. Knapp and F.L. Poston. 1997. Oviposition and development of *Trichogramma pretiosum* (Hymenoptera Trichogrammatidae) in three age classes of southwestern corn borer eggs. Environ. Entomol. 26: 385-390.

Dugast, J.F. and J. Voegele. 1984. Les trichogrammes parasites des vers de la grappe; découverte d'une nouvelle espéce: *Trichogramma daumalae* (Hym., Trichogrammatidae). Actes Inst. Agro. Vét. 4,11-21.

EPPO, 2002. PM 6/3(2). Safe use of biological control. List of biological control agents widely used in the EPPO region. Bulletin OEPP/EPPO Bulletin 32: 447-461.

Fursov, V. 1994. The taxonomic control of *Trichogramma* production in Ukraine. In: *Trichogramma* and Other Egg Parasitoids. Les colloques l'inra no. 73, Cairo (Egypt), October 4-7 1994 .Ed by WAJNBERG, E. PARIS: INRA.

Hassan, S.A. 1997. Zum Stand der Forschung und Kommerziellen Nutzung von Eiparasiten der Gattung *Trichogramma* in Deutschland. Gensunde Pflanzen 49: 75.

Hassan, S.A. and B.G. Wuhrer. 1997. Present status of research and commercial utilisation of egg parasitoids of the genus *Trichogramma* in Germany. Gensunde Pflanzen 49: 68-75.

Hintz, J.L. and D.A. Andow. 1990. Host age and host selection by *Trichogramma nubilale*. Entomophaga 35: 141-145.

Hommay, G., C. Gertz, J.C. Kienlen, J. Pizzol and P. Chavigny. 2002. Comparison between the control efficacy of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) and two *Trichogramma cacoeciae* Marchal strains against grapevine moth (Lobesia *botrana* Den. & Schiff.), depending on their release density. Biocon. Sc. Techn. 12: 569-581.

Ibrahim, R., H. Holst and T. Basedow. 2004. Natural occurrence and distribution of *Trichogramma* spp. In vineyards of Rheingau (Hessia, Germany). Mitt. Dtsch. Ges. Allg. Ent. 14: 213-216.

Kuhlmann, U. and N.J. Mills. 1999. Comparative analysis of the reproductive attributes of three commercially-produced *Trichogramma* species (Hymenoptera: Trichogrammatidae). Biocon. Sc. Techn. 9: 335-346.

Liu, S.S., G.M. Zhang and F. Zhang. 1998. Factors influencing parasitism of *Trichogramma dendrolini* on eggs of the Asian corn borer, *Ostrinia furnacalis*. Biocontrol 43: 273-287.

Miura, K. and M. Kobayashi. 1998. Effects of host-egg age on the parasitism by *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), an egg parasitoid of the diamondback moth. Appl. Entomol. Zool. 33: 219-222.

Monje, J.C., C.P.W. Zebitz and B. Ohnesorge. 1999. Host and host age preference of *Trichogramma galloi* and *T. pretiosum* (Hymenoptera: Trichogrammatidae) reared on different hosts. J. Econ. Entomol. 92:97-103.

Pak, G.A., H.C.E.M. Buis, I.C.C. Heck and M.L.G. Hermans. 1986. Behavioral variations among strains of *Trichogramma* spp.: Host age selection. Entomol. Exp. Appl. 40:247-258.

Reznik S.Ya., T.Ya. Umarova and N.D Voinovich. 1997. The influence of previous host age on current host acceptance in *Trichogramma*. Entomol. Exp Appl. 82: 153-157.

Schmidt, G.T. 1970. The effect of host development on parasitism and mortality of two pests attacked by Trichogramma evanescens (Hymenoptera: Trichogrammatidae). Ann. Entomol. Soc. Am. 63: 1319-1322.

Sengonca, C. and N. Leisse. 1987. Vorkommen und bedeutung von *Trichogramma semblidis* (Auriv.) (Hym., Trichodermmatidae) als Eiparasit beider Traubenwicklerarten im Ahrtal. J. Appl. Entomol.103,527-531.

Smith, S.M. 1996. Biological control with *Trichogramma*: advances, successes, and potential of their use. Ann. Rev. Entomol. 41, 375--406.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry, 3rd ed. Freeman, New York, USA.

Takada, Y., S. Kawamura and T. Tanaka. 2000. Biological characteristics: growth and development of the egg parasitoid *Trichogramma dendrolini* (Hymenoptera: Trichogrammatidae) on the cabbage armyworm *Mamestra brassicae* (Lepidoptera: Noctuidae). Appl. Entomol. Zool. 35:369-379.

Tavares, J., L.Oliveira, R. Teixeira, L. Anunciada, I. Moreira, F. Santos, D. Madeira, L. Henriques and H. Matias. 1988. Biological control of *Lobesia botrana* Den & Schiff (Lep.: Tortricidae) using *Trichogramma cacoeciae* Marchal (Hym.: Trichogrammatidae). Bol. Soc. Portug. Entomol. 4-1(103): 11.

Tzanakakis, M.E., M. Savopoulou-Soultani, C.S. Oustapassidis, S.C. Verras and H. Hatziemmanuel. 1988. Induction of dormancy in *Lobesia botrana* by long day and high temperature conditions. Entomol. Hell. 6: 7-10.

Vinson, S.B. 1976. Host selection by insect parasitoids. Ann. Rev. Entomol. 21:109-133.

Zimmermann, O., H. Holst and B. Wűhrer. 1997. Biologische Bekämpfung der Traubenwickler *Eupoecilia ambiguella* Hb. und *Lobesia botrana* Schiff. Mit *Trichogramma cacoeciae* Marchal. Mitteilungen der Deutscen Gesellschaft für Allgemeine und Angewandte Entomologie 11: 363-366.

KEY WORDS: Biological control, egg parasitoid, Trichogrammatidae, host suitability

Επίδραση της ηλικίας των θηλυκών ενηλίκων του *Trichogramma cacoeciae* και των αυγών του ξενιστή του στην παρασιτική του ικανότητα

Φ. ΒΟΥΡΛΙΩΤΗ ΚΑΙ Π. ΜΥΛΩΝΑΣ

Τμήμα Εντομολογίας και Γεωργικής Ζωολογίας, Μπενάκειο Φυτοπαθολογικό Ινσατιτούτο, Στ. Δέλτα 8, 145 61 Κηφισιά.
e-mail: p.milonas@bpi.gr

ΠΕΡΙΛΗΨΗ

Σε εργαστηριακά πειράματα εξετάστηκε η επίδραση της ηλικίας των αυγών του εντόμου *L. botrana* ως προς την αποδοχή τους για παρασιτισμό από το ωοπαρασιτοειδές *T. cacoecciae*. Θηλυκά άτομα του *T. cacoecciae* παρασιτούν σε μικρότερο βαθμό τα αυγά ηλικίας 4 ημερών από ότι αυγά ηλικίας 1 ή 2 ημερών. Επίσης, η διάρκεια ανάπτυξης ως την ενηλικίωση του *T. cacoecciae* σε 4 ημερών αυγά του *L. botrana* ήταν σημαντικά μεγαλύτερη από ότι σε αυγά μικρότερης ηλικίας. Επιπλέον, εξετάστηκε και η επίδραση της ηλικίας των παρασιτοειδών ως προς την ικανότητα παρασιτισμού αυγών του *L. botrana*. Η ηλικία του παρασιτοειδούς επηρέασε σημαντικά τον αριθμό των παρασιτισμένων αυγών του *L. botrana*. Περισσότερα αυγά παρασιτίστηκαν όταν χρησιμοποιήθηκαν θηλυκά άτομα του *T. cacoecciae* ηλικίας 4 ημερών.