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ASPECTOS DA BIOLOGIA DE *TACHINAEPHAGUS ZEALANDICUS* ASHMEAD (HYMENOPTERA: ENCYRTIDAE), PARASITÓIDE DE LARVAS DE DÍPTEROS SINANTRÓPICOS

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SUMÁRIO

RESUMO

Tachinaephagus zealandicus Ashmead é um endoparasitóide gregário que ataca larvas de terceiro instar de dípteros muscóideos no Hemisfério Sul. O objetivo deste estudo foi avaliar a influência de seis temperaturas constantes (16, 18, 20, 22, 25 e 27°C) no tempo de desenvolvimento, a influência da ordem de emergência na longevidade, o efeito da temperatura e tratamento alimentar na longevidade, a influência do tratamento alimentar, densidade do hospedeiro e temperatura na taxa de ataque de *T. zealandicus* e também avaliar a influência de quatro temperaturas constantes (15, 20, 25, 30° C) no tempo de desenvolvimento e longevidade de colônias infectadas e não infectadas por um microsporídeo. O sucesso na emergência foi maior em 22°C tanto para machos quanto para fêmeas; um número significativamente menor (24.1-30.4%) de parasitóides emergiu em 16°C e 25°C comparados com 22°C. O tempo de desenvolvimento foi de 24.0 a 56.9 dias para os dois sexos. Não foi observada emergência em 27°C. O parasitóides da mesma coorte que emergiram primeiro apresentaram maior longevidade que aqueles que emergiram mais tarde. A longevidade das fêmeas que receberam mel e água decresceu com o aumento da temperatura e aquelas mantidas em 16°C viveram três vezes mais que aquelas mantidas em 27°C. As fêmeas que receberam mel e água apresentaram longevidades semelhantes entre 16-20°C, e fêmeas que receberam somente água viveram de 4.8-7.6 dias em todas as temperaturas. As fêmeas viveram significativamente mais que os machos em todas as temperaturas, exceto em 16°C, mas as diferenças devido ao sexo foram pequenas se comparadas com os efeitos da temperatura e tratamento alimentar. Os tratamentos alimentares (mel, mel + água, água, e jejum) apresentaram efeitos estatisticamente significativos nas taxas de ataque em *C. putoria* e *M. domestica*, contudo nenhum efeito significativo foi constato nos tratamentos quando analisada a progênie produzida. Tanto para *M. domestica* quanto *C. putoria* foi observado que a porcentagem de hospedeiros mortos decresceu com o aumento da densidade do hospedeiro. A temperatura apresentou efeito significativo na taxa de ataque do parasitóide sobre *C. putoria*. Durante 24 horas de exposição, as maiores taxas de ataque foram observadas em 22°C. Para o número de hospedeiros mortos, não foram encontradas significativas diferenças entre 20, 22, 25 e 27°C. Um microsporídeo não determinado foi encontrado infectando *T. zealandicus*. Esporos estavam presentes em todas as regiões do corpo do inseto e em todos os estágios de desenvolvimento. Adultos infectados apresentaram cerca de 3.75×10^5 esporos. Para separar a colônia em indivíduos infectados e não infectados, as fêmeas infectadas foram tratadas com Rifampicin misturado ao mel como alimento e após 8 dias cerca de 37% dos indivíduos da

progênie examinada ainda estavam infectados. Uma cultura não infectada foi estabelecida e as duas colônias foram testadas para o modo de transmissão. Foi observado que a eficiência na transmissão materna foi de 96.3%. Parasitóides infectados desenvolveram significativamente mais rápido que os não infectados em 15, 20 e 25°C. Pupários que não apresentaram emergência foram dissecados e os resultados mostraram que os parasitóides infectados apresentaram maior dificuldade para emergir de seus pupários, especialmente em 20 e 25°C. A longevidade das fêmeas decresceu com o aumento das temperaturas. Os efeitos da infecção na longevidade foram fortes em todas as temperaturas, entre os parasitóides que receberam mel e água; a longevidade foi pequena em todos os grupos que receberam somente água. Os resultados de ANOVA mostraram que não somente a temperatura e o tratamento alimentar, mas também a infecção afetou significativamente a longevidade de fêmeas e de machos. Fêmeas e machos da colônia infectada apresentaram tempos de vida similares (3.7-3.9 dias) quando os parasitóides receberam mel, água e larvas de mosca doméstica continuadamente após emergência. Parasitóides infectados produziram progênie significativamente menor que os não infectados, e uma grande proporção de parasitóides infectados falharam para emergir do pupário do hospedeiro. Grande parte do ataque ao hospedeiro e parasitismo ocorreu durante o primeiro dia de emergência, indicando que esta espécie é proovigênica. Foram comparados o ataque ao hospedeiro e o parasitismo sobre duas espécies de mosca, mosca doméstica e *Sarcophaga bullata* indicando que o efeito da infecção foi modulado pela espécie de hospedeiro presente. Números similares foram observados para ambos hospedeiros para número de pupas mortas por infectados e não infectados (70.2-74.1 hospedeiros atacados por grupo de 5 fêmeas de *T. zealandicus*). Contudo, parasitóides infectados produziram substancialmente menos progênie de mosca doméstica (311.1 para não infectados comparados com 138.3 para infectados), porém a infecção não apresentou efeito significativo na progênie produzida para o hospedeiro maior (*S. bullata)* (588.2 e 460.1 progênie produzida por não infectados e infectados, respectivamente). Diferenças na atuação para as duas espécies de hospedeiro pode ser devida a diferenças qualitativas entre ambas ou mesmo a pronunciada diferença entre tamanhos das duas espécies (190 mg/larva para *S. bullata* versus 20 mg/larva para *M. domestica*). *T. zealandicus* não infectados estocados em 15°C apresentaram maior taxa de ataque ao hospedeiro (58-62 hospedeiros mortos por grupo de 5 fêmeas do parasitóide em 25°C) e progênie produzida (173.8-261.2 indivíduos) após 6-12 dias estocados nesta temperatura. Relativamente poucos hospedeiros foram parasitados no primeiro dia em 15°C.

ABSTRACT

Tachinaephagus zealandicus Ashmead is a gregarious endoparasitoid that attacks third instars of muscoid flies in the Southern Hemisphere. The purpose of the present study was to evaluate the influence of six constant temperatures (16, 18, 20, 22, 25 and 27°C) on development time, the influence of emergence order on longevity, and the effects of temperature and food treatment on longevity, the influence of nutritional treatment, host density and temperature on attack rates on *T. zealandicus* and also evaluated the influence of four constant temperatures (15, 20, 25, 30° C) on development time and longevity of microsporidium-infected and uninfected colonies. Emergence success was greatest at 22°C for both males and females; significantly fewer (24.1-30.4%) parasitoids emerged at 16°C and 25°C compared with 22°C. Development time ranged from 24.0 to 56.9 days for both sexes. No emergence was observed at 27°C. Early-emerging parasitoids had greater longevity than parasitoids that emerged later from the same cohorts. The longevity of females given honey and water decreased with increasing temperature, and those reared at 16°C lived about three times longer than those kept at 27°C. Females given honey and water had similar longevities at 16-20°C, and females that were given only water lived for only 4.8-7.6 days at all temperatures. Females lived significantly longer overall than males at all temperatures except 16°C, but differences due to sex were small compared to the effects of temperature and nutrition. Nutritional treatment (honey, honey+water, water, and starvation) had statistically significant effects on rates of attack on *C. putoria* and *M. domestica*, however no significant treatment effects were observed in rates of progeny production. With both *M. domestica* and *C. putoria* it was observed that the percentage of killed hosts decreased as host density increased. Temperature had significant effects on host attacks on *C. putoria*. During 24 hours of exposure, highest rates of host attacks were observed at 22°C. For the number of killed hosts, differences were not statistically significant among 20, 22, 25 and 27°C. An undetermined microsporidium was found infecting *Tachinaephagus zealandicus*, a gregarious parasitoid that attacks third instar larvae of muscoid flies. Spores were present in all body regions and in all stages of development. Infected adults contain an average of 3.75×10^5 spores. In order to separate the colony into infected and uninfected individuals, we treated infected females on rifampicin mixed with honey as food and after 8 days ca. 37% of individuals of progeny examined were still infected. An uninfected culture was established and the two colonies were tested for infection transmission. It was observed that the efficiency of maternal transmission was 96.3%. Infected parasitoids developed significantly faster than uninfected ones at 15, 20 and 25°C. Uneclosed puparia were dissected and the results showed that infected parasitoids had difficulty emerging from host puparia, especially at 20 and 25°C. The longevity of females decreased as temperatures increased. Effects of infection on longevity were strongest at all temperatures among parasitoids that were given honey and water; longevity was short in all parasitoid groups that were only given water. ANOVA results showed that not only temperature and feeding treatment but also the infection affected significantly the longevity of females and males. Females and females from a colony infected with

the undetermined microsporidium had similar lifespans (3.7-3.9 days) when parasitoids were provided with honey, water and house fly larvae continously after emergence. Infected parasitoids produced significantly fewer progeny than uninfected parasitoids, and a greater proportion of infected parasitoid progeny failed to emerge from host puparia. Most host attacks and parasitism occurred on the first day of emergence, and little parasitism was observed by day 3 after emergence for both uninfected and infected females, indicating that this is a proovigenic species that emerges with a predetermined number of oocytes that are deposited when hosts are available for parasitism. Comparison of host attacks and parasitism on two species of flies (house flies and *Sarcophaga bullata* (Diptera: Sarcophagidae) indicated that the effects of infection are modulated by the species of host present. Similar numbers of both species fly hosts were killed by uninfected and infected parasitoids (70.2-74.1 hosts attacked per group of 5 female *T. zealandicus*). However, infected parasitoids produced substantially fewer progeny from house fly hosts (311.1 for uninfected parasitoids compared with 138.3 progeny from infected parasitoids), wheras infection had no significant effect on progeny production from the much larger *S. bullata* hosts (588.2 and 460.1 progeny produced by uninfected and infected parasitoids, respectively). Differences in performance on the two host species may be due to innate differences in host quality or to the pronouced size differences of the two species (190 mg/larva for *S. bullata* versus 20 mg/larva for *M. domestica*). Uninfected *T. zealandicus* that were stored at 15°C had highest rates of host attacks (58-62 hots killed per group of five female parasitoids at 25°C) and progeny production 173.8-261.2 progeny) after 6-12 days of storage at this temperature; relatively few hosts were attacked or parasitized after 0 or 1 day at 15°C.

1-INTRODUÇÃO

O acúmulo de fezes em locais de criação animal como as granjas de aves poedeiras, gera a proliferação de moscas sinantrópicas, o que muitas vezes compromete as condições de higiene local, e conseqüentemente, a qualidade dos ovos. Dentre estas moscas, *Musca domestica* L., 1758 (Muscidae) é uma das mais importantes que compõe esta variada fauna (Povolný, 1971). No Brasil, foi observada também a proliferação das espécies *Chrysomya putoria* (Wiedemann, 1830) (Calliphoridae) e *Muscina stabulans* (Fallén, 1817) (Muscidae) na granja Capuavinha, município de Monte Mor, Estado de São Paulo (Monteiro & Pires do Prado, 2000) e na granja Piva, município de Santa Cruz da Conceição, Estado de São Paulo (dados não publicados).

Dados de levantamentos realizados em ambientes de granjas de aves poedeiras e esterqueiras de bovinos, por Silveira *et al.* (1989), e somente em granjas por Costa (1989) e Monteiro & Pires do Prado (2000) demonstraram que o endoparasitóide larval-pupal de dípteros sinantrópicos, *Tachinaephagus zealandicus* Ashmead, 1904 (Hymenoptera: Encyrtidae) foi encontrado associado a diferentes hospedeiros como: *Cochliomyia hominivorax* (Coquerell, 1858) (Calliphoridae), *Synthesiomyia nudiseta* (Wulp, 1883), *M. domestica, Stomoxys calcitrans* (L., 1758), *M. stabulans* (Muscidae) e *C. putoria.*

1.1 - Locais de ocorrência de *Tachinaephagus zealandicus*

1.1.1 - Oceania e Ásia

Uma das primeiras ocorrências registradas para *T. zealandiucus* foi atacando *Chrysomya megacephala* (Fabricius, 1794) (Calliphoridae), *Chrysomya rufifacies*

(Macquart, 1842) (Calliphoridae), *Lucilia* sp. e *Sarcophaga* sp. (Johnston & Tiegs, 1921). Mais tarde, Ferriere (1933) relatou pela primeira vez a ocorrência de *T. zealandicus* associado às pupas de *Haematobia (Lyperosia) exigua* Meijen coletadas em fezes de gado bovino, em Java (Sudeste da Ásia), sendo esta a primeira ocorrência reportada fora da Austrália e Nova Zelândia. O parasitóide foi encontrado também atacando *Sarcophaga* sp. na Nova Caledônia (Melanésia) (Risbec, 1956), atacando *M. domestica e Muscina* sp., (Legner & Olton, 1968) na Austrália e Nova Zelândia; atacando *Fannia canniculáris* (L.) (Muscidae) e *S. calcitrans*, na Nova Zelândia (Prinsloo, 1979).

1.1.2 – Continente Africano

Prinsloo (1979) registrou a ocorrência de *T. zealandicus* atacando pupas de *Chrysomya chloropyga* (Wiedemann, 1818) (Calliphoridae), *Sarcophaga haemorroidalis* (Fallén, 1817) (Sarcophagidae) e *Lucilia sericata* (Meigen, 1826) (Calliphoridae) na África do Sul.

1.1.3 – Continente Americano

Foram registradas várias ocorrências de *T. zealandicus* atacando pupas de dípteros sinantrópicos no Continente Americano. No sul do Uruguai, por Legner *et al.* (1967) e De Santis (1979) atacando pupas de *M. domestica* que se desenvolviam em fezes de gado bovino. De Santis & Sureda (1988) registraram também a ocorrência do parasitóide no Chile e na Argentina, atacando pupas de *M. domestica, M. stabulans* e *S. calcitrans* e De Santis & Fidalgo (1994) registraram a ocorrência do parasitóide em pupas de *Chrysomya*

sp. na Argentina (Castelar, Buenos Aires) e na Ilha de Páscoa (Chile). Walsh & Gordo (1997) coletaram *T. zealandicus* em pupas de moscas, numa estação de pesquisa em agricultura em Buenos Aires, Argentina.

No Brasil foram registradas as seguintes ocorrências: atacando pupas de *Chrysomya* sp. coletadas por T.L. Aagesen, em Bastos, Estado de São Paulo (De Santis & Fidalgo, 1994); Bruno *et al.* (1988) verificaram o parasitóide atacando pupas de *S. calcitrans*, em ambiente de granja de aves poedeiras; De Santis & Sureda (1988) verificaram o parasitóide atacando pupas de *M. domestica* e *S. calcitrans* em São Paulo; Pinheiro *et al.* (1989) relataram a ocorrência de *T. zealandicus* em Lavras (Minas Gerais) atacando pupas de *M. domestica;* Silveira *et al.*, (1989) registraram o parasitóide atacando pupas de *C. hominivorax* coletadas em esterqueira de gado bovino, no Estado de São Paulo, e atacando *S. nudiseta* em granjas do Estado de Minas Gerais. No mesmo ano, Costa (1989) verificou a ocorrência de *T. zealandicus* associado às pupas de *M. domestica, S. calcitrans* e *M. stabulans* coletadas em granjas de aves poedeiras, localizadas no interior do Estado de São Paulo e Bruno (1991) verificou o parasitóide atacando *M. domestica* e *S. calcitrans* também em granjas do Estado de São Paulo. De Santis & Fidalgo (1994) registraram a ocorrência do parasitóide atacando pupas de *M. domestica* coletadas por V.H.P. Bueno em Lavras, Estado de Minas Gerais e Carvalho *et al.* (1995) registraram a ocorrência de *T. zealandicus* pupas de *Pattonella intermutans* (Walker) (Sarcophagidae) coletadas em carcaça animal, em Campinas, no interior do Estado de São Paulo. Recentemente, Monteiro & Pires do Prado (2000) descreveram a ocorrência de *T. zealandicus* atacando pupas de *M. domestica, C. putoria* and *M. stabulans* coletadas em uma granja de Monte Mor, interior de São Paulo.

As evidências indicam que possivelmente esta espécie seja endêmica no Hemisfério Sul (Olton, 1971). Amostragens foram realizadas no sudeste dos Estados Unidos, com a

finalidade de se determinar os inimigos naturais associados aos dípteros sinantrópicos nesta região. Os resultados revelaram haver pequena ou nenhuma atividade deste parasitóide no local em estudo (Legner, 1966 e Legner & Olton, 1971). Em 1967, *T. zealandicus* foi importado de regiões da Austrália e Nova Zelândia para o sul da Califórnia (Legner & Olton, 1968), e o fato de serem regiões climaticamente semelhantes proporcionou possibilidade de estabelecimento desta espécie, em locais que anteriormente ocupavam somente nichos muito restritos (Olton, 1971). Uma colônia passou a ser mantida, no sul da Califórnia, e estudos mais detalhados da biologia desta espécie foram realizados por Olton (1971).

Outras ocorrências também foram verificadas para *T. zealandicus* como: atacando *Chrysomya* sp. no Havaí (La Plante, 1970); atacando *M. domestica* e *S. calcitrans* coletadas em Saint Kitts (Mar das Antilhas) e Trinidad (De Santis, 1981) e atacando pupas de *Protocalliphora* sp. (Calliphoridae), na Califórnia, USA (Gold & Dablsten, 1989).

1.2 – Biologia de *Tachinaephagus zealandicus*

Johnston *et al.* (1921) fizeram as primeiras observações sobre o ciclo de vida de *T. zealandicus* e o presente estudo surgiu da necessidade de conhecermos mais sobre a biologia deste parasitóide, pois o único trabalho realizado que reúne este tipo de dado pertence à tese de doutoramento de Gary Stanley Olton defendida em 1971, pela Universidade da Califórnia, Riverside (USA). Os resultados apresentados pelo autor se referem a experimentos realizados com a colônia estabelecida por ele e o Dr. E. F. Legner, em 1967. Os resultados apresentados nesta tese serão discutidos e comparados com os obtidos e apresentados em cada um dos cinco capítulos deste estudo.

1.3 - Histórico da colônia e um breve relato de cada capítulo

Amostragens realizadas na Granja Piva, localizada no município de Santa Cruz da Conceição, Estado de São Paulo, Brasil (21° 59'S; 47° 21'W e 597,0 m), em 1997 e 1998, apontaram que a ocorrência de *T. zealandicus* se deu principalmente entre os meses de Agosto e Dezembro. No final de 1998 conseguimos definitavamente estabelecer uma colônia de laboratório, cuja identificação dos espécimes fora confirmada pelos taxonomistas Dr. Mike Schauff (USNM, USA) e Dr. John Heraty (UC Riverside, USA). A partir desta, iniciamos alguns ensaios que nos dariam mais detalhes sobre a biologia desta espécie e registramos algumas características da biologia de *T. zealandicus* como: o aspecto morfológico dos ovários (ANEXO 1), do ovócito (ANEXO 2) e do ovo extraído do hospedeiro (ANEXO 3). Registramos algumas fases do desenvolvimento do parasitóide dentro do hospedeiro (ANEXOS 4, 5, 6, 7, 8 e 9); a constatação de que o hospedeiro continuou o desenvolvimento e chegou a concluir a formação de asas, pernas e olhos (ANEXO 10); a emergência de uma fêmea de *T. zealandicus* do pupário de *C. putoria* (ANEXO 11); e um caso esporádico de multiparasitismo com *Muscidifurax zaraptor* Kogan e Legner, observado em laboratório (ANEXO 12).

O primeiro capítulo deste trabalho trata da influência da temperatura no tempo de desenvolvimento e longevidade de *T. zealandicus* em seis temperaturas constantes e o segundo capítulo trata da influência do tratamento nutricional, da densidade e da temperatura na taxa de ataque ao hospedeiro por *T. zealandicus.*

Após estes estudos da biologia de *T. zealandicus* e contando com o apoio do Dr. Christopher J. Geden (United States Department of Agriculture, Agricultural Research Service, Center of Medical, Agricultural and Veterinary Entomology, Gainesville, Florida,

USA) constatamos que parte da colônia estava infectada por um microsporídeo. Iniciamos assim, tentativas de tratamento da parte infectada da colônia e uma delas foi a aplicação do tratamento pelo calor. Tentamos a exposição às temperaturas de 42 e 45°C durante períodos que são terapêuticos para *Nosema* sp. em *Muscidifurax raptor* Girault e Sanders, contudo este tipo de tratamento foi letal para *T. zealandicus.* No tratamento com drogas foram utilizados Albendazol e Rifampicin (3% adicionados ao mel oferecido às fêmeas), porém somente obtivemos sucesso no tratamento com este último.

O terceiro capítulo descreve a infecção de *T. zealandicus* pelo microsporídeo. Mais tarde, como dispunhamos de uma colônia infectada e outra não infectada, realizamos alguns experimentos comparando o desempenho de indivíduos provenientes das diferentes colônias.

O quarto capítulo descreve a influência da temperatura no tempo de desenvolvimento e longevidade de *T. zealandicus* infectados e não infectados em quatro temperaturas constantes. Finalmente, o quinto capítulo descreve o ataque ao hospedeiro e a progênie resultante de fêmeas de *T. zealandicus* infectadas e não infectadas.

CAPÍTULO I

Influence of temperature on development time and longevity of *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae), and effects of nutrition and emergence order on longevity

ABSTRACT

Tachinaephagus zealandicus Ashmead is a gregarious endoparasitoid that attacks third instars of muscoid flies in the Southern Hemisphere. The purpose of the present study was to evaluate the influence of six constant temperatures (16, 18, 20, 22, 25 and 27°C) on development time, the influence of emergence order on longevity, and the effects of temperature and food treatment on longevity. Emergence success was greatest at 22°C for both males and females; significantly fewer (24.1-30.4%) parasitoids emerged at 16°C and 25°C compared with 22°C. Development time ranged from 24.0 to 56.9 days for both sexes. No emergence was observed at 27°C. Early-emerging parasitoids had greater longevity than parasitoids that emerged later from the same cohorts. The longevity of females given honey and water decreased with increasing temperature, and those reared at 16°C lived about three times longer than those kept at 27°C. Females given honey and water had similar longevities at 16-20°C, and females that were given only water lived for only 4.8-7.6 days at all temperatures. Females lived significantly longer overall than males at all temperatures except 16°C, but differences due to sex were small compared to the effects of temperature and nutrition. Further investigations will be necessary to determine the climatic zones in which *T. zealandicus* is most likely to be an effective biological control agent of muscoid flies.

INTRODUCTION

Tachinaephagus zealandicus Ashmead is a gregarious endoparasitoid of muscoid fly third instars that appears to be endemic to the Southern Hemisphere (Olton 1971). Legner et al. (1967) observed *T. zealandicus* parasitizing *Musca domestica* L. breeding in bovine manure in southern Uruguay. The importation of *T. zealandicus* from areas of Australia and New Zealand (Legner and Olton 1968) into climatically similar sections of southern California provided an opportunity for establishment of this species in a relatively empty niche (Olton 1971). Thus, colonies were established in southern California in 1967 (Legner and Olton 1968) and aspects of the biology of this species were studied by Olton (1971).

Silveira *et al.* (1989) provided the first report of *T. zealandicus* attacking *Cochliomyia hominivorax* (Coquerell) (Calliphoridae) in the State of São Paulo, Brazil, and attacking *Synthesiomyia nudiseta* (Wulp) (Muscidae) in the State of Minas Gerais, Brazil. Costa (1989) found *T. zealandicus* associated with pupae of *M. domestica, Stomoxys calcitrans* (L.) and *Muscina stabulans* (Fallén) (Muscidae) collected in a poultry house located in the interior of State of São Paulo. Monteiro and Pires do Prado (2000) found *T. zealandicus* emerging from pupae of *M. domestica, Chrysomya putoria* (Wiedemann) and *Muscina stabulans* collected in poultry facilities in the interior of São Paulo.

From monthly samples collected in a poultry facility in the interior of São Paulo, during 1997 and 1998, we collected *T. zealandicus* from pupae of *M. domestica* and *C. putoria.* Parasitism at this site was highest from August to December in both years (unpublished data). A colony of *T. zealandicus* was established from these samples, and

laboratory studies of the biology of *T. zealandicus* were conducted. The objectives of the present study were to evaluate the influence of different constant temperatures on development time and longevity of this species and to evaluate the effects of nutrition and emergence order on longevity.

MATERIALS AND METHODS

Insect colonies

Tachinaephagus zealandicus were from a colony originally established from samples collected from a poultry farm in Santa Cruz da Conceição (21° 59'S; 47° 21'W and 597.0 m), São Paulo, Brazil, and had been maintained on *C. putoria* for 12 generations at the time of testing.

Parasitoids were maintained in 2-l clear plastic boxes covered by snap-on plastic lids with screened openings. Cages were stocked with approximately 2,000 parasitoids per cage and held at 25±1°C, 60±10% RH, with a photoperiod of 12L:12D. Honey and water were provided to the insects and *C. putoria* larvae were exposed to 2-day-old females at a host:parasitoid ratio of 20:1 for 4 consecutive days. Parasitized pupae were removed from the cages daily and held in a rearing chamber at 22±1°C.

C. putoria were also from a colony originally established from the poultry farm in Santa Cruz da Conceição. Larvae were reared using the diet described by Leal *et al.* (1982), and adults were held in cages under the environmental conditions described above for *T. zealandicus.* Flies were given water and sugar *ad libitum* and periodically given liver for egg maturation and oviposition.

Influence of constant temperatures on development time

T. zealandicus females were exposed to 1300 *C. putoria* larvae at a host:parasitoid ratio of 10:1. Twenty four hours later, parasitized pupae were placed into individual gelatin capsules (size no. 00) and placed in groups of 100 in 100-ml clear plastic cups with snap-on plastic lids with screened openings. The pupae were then transferred to rearing chambers set at 16, 18, 20, 22, 25 and 27°C (2 replications of 100 pupae per temperature) and 70% RH and a 12D:12L photoperiod. In addition, 100 unparasitized pupae were placed in each chamber as controls. Parasitism under these test conditions was 98%. Pupae were monitored twice daily for parasitoid emergence and the development time at each temperature was determined. The relative emergence success of parasitoids reared at different temperatures was determined by comparing emergence at 22°C with emergence at other temperatures using G-tests of independence to obtain estimates of χ^2 (Sokal and Rohlf 1994).

Development data were fit to the thermodynamic model of Sharpe and DeMichele (1977) to describe the effects of temperature on development rates for *T. zealandicus*. The four-parameter form of the model, with high-temperature inhibition, was selected:

$$r(K) = \{Rho25*K/298.15*\exp[HA/298.15*(1/298.15-1/K)]\}/\{1+\exp[HH/1.987*(1/TH-1/K)]\}$$

where $r(K)$ = development rate at temperature K (in degrees Kelvin), and Rho25, HA, TH, and HH are constants associated with a hypothetical single rate-controlling enzyme that is limited by high temperatures (Schoolfield et al. 1981). Briefly, Rho25 is the development rate at 25°C (298.15K), HA is the enthalpy of activation of the hypothetical enzyme, TH is

the temperature (in degrees Kelvin) at which the enzyme is half-inactivated by heat, and HH is the change in enthalpy associated with high-temperature inhibition. Estimates of Rho25, HA, TH, and HH were obtained using the algorithm of Wagner *et al.*(1984), which uses a sequence of SAS Procedures to estimate the parameters (SAS Institute, 1992). Minor changes were made in the algorithm to accommodate changes in SAS command language since it was published. Regression analysis (observed versus predicted rate data) was performed to evaluate goodness-of-fit of the models to the constant temperature data.

Influence of emergence order on longevity at different temperatures

Parasitoids that emerged from pupae in the above experiment were isolated daily and placed individually in 100-ml plastic cups with screen lids, given honey and water and kept in the same chambers (at the same temperatures) in which they had completed development. Parasitoid mortality was monitored daily until all parasitoids had died. In this way, we were able to determine whether parasitoid longevity was affected by the order in which parasitoids emerged within a cohort at each temperature. Longevity differences within each temperature as a function of the day of emergence were evaluated by ANOVA using the GLM Procedure of SAS (SAS Institute, 1992). Sample sizes varied depending on the numbers of parasitoids that emerged on any given day, for an overall average of 32.1 parasitoids per day of emergence and temperature (range, 5-84).

Influence of temperature and food treatment on longevity

Five hundred parasitized pupae of *C. putoria* were isolated in gelatin capsules for emergence of *T. zealandicus* at 25°C. Individual female and male parasitoids were collected within one hour of emergence and placed in 100-ml clear plastic cups covered by snap-on plastic lids with screened openings. Forty parasitoids of each sex were placed in each of six rearing chambers set at 16, 18, 20, 22, 25 and 27°C, 60±10% RH and a photoperiod of 12L:12D. Half of the parasitoids were given water and honey as food, and the other half were given only water (20 male and female parasitoids for each food and temperature combination). Parasitoid mortality was recorded daily. Differences in parasitoid mortality at six temperatures were evaluated by ANOVA, and means separated using Tukey's method under the GLM Procedure of SAS ($P<0.05$) (SAS Institute, 1992).

RESULTS

Influence of different temperatures on development time

Emergence success was greatest at 22°C for both males and females, although there were no significant differences in emergence success at 18, 20 and 22°C. Significantly fewer (24.1-30.4%) parasitoids emerged at the temperature extremes of 16 and 25°C compared with 22°C (100%) (Table 1). No emergence was observed at 27°C.

Development times for females and males varied from 24.0 to 56.9 days over the range of temperatures from which parasitoids emerged (Table 1). Males and females had nearly identical development times at all temperatures. Median values for development

time are also shown in Table 1. The time to 50% emergence was 23.5 days at 25°C for both females and males.

Parameter estimates for Sharpe and De Michele thermodynamic model are presented in Table 2, with model predictions and observed data presented in Table 1. The models fit the data well in both cases ($R^2 > 0.972$).

TABLE 1

Emergence success and development times of *T. zealandicus* reared at constant temperatures.

Temp (°C)	No. of emerged parasitoids	Relative emergence success[1]	χ^2 (df=1)	Development time (days)		
				Mean (SE)	Median	
					Observed	Predicted[3]
				Females		
16	120	30.4	46.22**[2]	56.9 (0.17)	56.3	55.7
18	317	80.2	1.88ns	42.3 (0.08)	41.7	42.6
20	340	86.1	0.88ns	33.6 (0.05)	33.3	32.9
22	395	100.0	-	27.1 (0.05)	26.1	26.2
25	140	35.4	36.29**	24.0 (0.01)	23.5	26.2
27	0	0.0	261.4**	-	-	-
				Males		
16	156	24.1	71.45**	56.9 (0.15)	56.2	55.7
18	501	77.4	2.77ns	42.2 (0.07)	41.6	42.4
20	515	79.6	2.21ns	33.6 (0.04)	33.0	32.6
22	647	100.0	-	27.0 (0.04)	25.9	25.9
25	182	28.1	28.13**	24.0 (0.01)	23.5	23.5
27	0	0.0	337.6**	-	-	-

[1] Parasitoid emergence as a percentage of emergence at 22°C.

[2] ns, P>0.05, ** P>0.01 (G-test of independence for parasitoid emergence at 22°C compared with other termperatures)

[3] Predicted values from the Sharpe and DeMichele model using parameters presented in Table 2

TABLE 2

Parameter estimates (SE) for the models of temperature-dependent rates of development of male and female *T. zealandicus*, and regression coefficients for goodness of fit of predicted versus observed development rates.

Sex	Rho25	HA	TH	HH	R^2
females	0.0590	22040.8	299.5	122889	0.972
	(0.00760)	(2779.6)	(0.4834)	(92337.9)	
males	0.0603	22437.2	299.5	118934	0.999
	(0.00723)	(2565.2)	(0.3292)	(73310.6)	

Influence of emergence order on longevity at different temperatures

Adults of *T. zealandicus* emerged on different days, although larvae were parasitized at the same time. The longevity of adults from the same cohort, but which emerged on different days, is presented in Table 3. There was a tendency for greater longevity among parasitoids that emerged earlier rather than later at 16-22°C, although this difference was statistically significant only at 16, 18, and 22°C. At 16-20°C the latest-emerging parasitoids only lived 45-60% as long as earliest-emerged individuals.

TABLE 3

Mean (SE) longevity in days of adults (males and females combined) of *T. zealandicus* as a function of the order of emergence at five constant temperatures.

Temp,°C	Mean (SE) longevity of parasitoids that emerged in the following order (in days)						ANOVA F[1]	Overall longevity
	1st	2nd	3rd	4th	5th	6th		
16	16.9 (0.7)	12.4 (0.6)	12.4 (0.5)	10.5 (0.6)	7.9 (0.8)	8.2 (0.9)	15.14 **	10.7 (0.2)
18	20.1 (0.6)	15.1 (0.9)	15.1 (0.9)	17.9 (0.5)	17.1 (0.6)	11.2 (1.5)	7.67 **	16.4 (0.3)
20	17.8 (1.3)	15.7 (0.6)	14.1 (0.9)	15.2 (1.5)	11.0 (4.6)	-[2]	1.63 ns	15.2 (0.4)
22	15.7 (0.9)	16.2 (0.9)	18.5 (0.9)	10.7 (1.1)	10.8 (3.6)	15.9 (1.1)	5.18 **	15.5 (0.2)
25	6.4 (0.8)	6.4 (1.1)	7.0 (0.5)	7.2 (1.0)	-[2]	-[2]	0.16 ns	6.9 (0.3)

[1] Results of one-way ANOVA's run separately for each temperature (**, P≤0.01; *, P≤0.05; ns, P>0.05).

[2] No parasitoids emerged

Influence of temperature and food treatment on longevity

The longevity of females given honey and water decreased with increasing temperature (Table 4). Females reared at 16°C lived about 3 times longer (18.0 days) than those kept at 27°C (6.2 days). Females given honey and water had similar longevities at 16-20°C (14.8-18.0 days). In contrast, females that were given water but not honey only lived for 4.8-7.6 days at all temperatures, and there were no significant differences in longevity as a function of temperature except at the two temperature extremes (7.6 and 4.8 days at 16 and 27°C, respectively).

Similar results were obtained with males. Males that were given honey and water lived about three times longer at 16°C (14.5 days) than those held at 27°C (4.4 days)

(Table 4). Males that were given water but not honey lived for 1.8 (27°C) to 7.1 days (16°C), but differences in longevity were small (and not statistically significant) in the temperature range of 16-25°C.

Females lived significantly longer overall than males at all temperatures except 16°C, but differences due to sex were small compared to the effects of temperature and nutrition. The effect of sex on longevity was modulated by nutrition in some cases, as indicated by significant sex x food treatment interactions at 18 and 22°C. Differences in longevity between males and females were small among parasitoids that were given only water and were only statistically significant at 27°C. In contrast, females that were given honey and water lived significantly longer than males in the same food treatment groups at all temperatures except 16 and 20°C.

TABLE 4

Mean (SE) longevity of females and males of *T. zealandicus* that received food treatments at six constant temperatures

Temp., °C	Mean (SE) longevity in days[1]						
	females		males		ANOVA F[2]		
	honey+water	water	honey+water	water	sex	food treat.	Sex x food treat.
16	18.0 (1.27) Aa	7.6 (1.15) Ba	14.5 (1.03) Aa	7.1 (0.94) Ba	3.19 ns	65.19 **	1.90 ns
18	17.6 (1.20) Aa	6.4 (0.34) BCab	8.9 (1.31) Bbc	5.1 (0.47) Cab	28.61 **	65.24 **	15.67 **
20	14.8 (1.02) Aab	7.1 (0.16) Ba	12.9 (1.01) Aab	4.7 (0.34) Bb	8.41 **	114.93 **	0.07 ns
22	13.6 (0.79) Ab	6.8 (0.33) Cab	9.9 (1.18) Bbc	6.6 (0.45) Cab	6.50 *	43.62 **	5.24 *
25	8.6 (0.80) Ac	6.7 (0.14) Bab	6.3 (0.51) Bcd	5.9 (0.25) Bab	9.29 **	5.94 **	2.02 ns
27	6.2 (0.58) Ac	4.8 (0.94) Bb	4.4 (0.37) Bd	1.8 (0.13) Cc	46.64 **	30.94 **	2.62 ns

[1] Means within columns followed by the same lowercase letter and means within rows followed by the same uppercase letter are not significantly different at P=0.05 using Tukey's method.

[2] Results of two-way ANOVA's run separately for each temperature. Df= 1,76 for all model effects (**, P≤0.01; *, P≤0.05; ns, P>0.05).

DISCUSSION

One of the purposes of this study was to evaluate the response of *T. zealandicus* to selected constant temperatures. Understanding the response of parasitoids to temperature is critical to elucidation of the numerical relationships between a parasitoid and its host (Ables et al. 1976). *T. zealandicus* is commonly collected in poultry facilities located in the State of São Paulo (Brazil), where it is associated with *M. domestica* and other muscoid flies such as *C. putoria* (Silveira et al. 1989; Costa 1989; Monteiro and Pires do Prado 2000). In field observations during 1998 we observed that *T. zealandicus* was most prevalent from August to December (unpublished data). Data provided by CATI (Coordenadoria de Assistência Técnica Integral, from State of São Paulo) indicated that average temperatures for the nearby city of Santa Cruz da Conceição varied from 17.0°C to 24.6°C (minimum and maximum, respectively) in August; 18.4°C to 25.8°C in September; 19.0°C to 25.4°C in October; 19.9°C to 28.0°C in November and 21.7°C to 28.9°C in December. In the final sample that was collected (Dec. 23. 1998) the rate of parasitism by *T. zealandicus* was ca. 90% (unpublished data).

Field records made by Bishop et al. (1996) showed that *T. zealandicus* isolations were made mostly (22/23 samples) from January through April in New Zealand. Twenty-one of the samples of parasitized larvae were from North Island localities, with *Lucilia* spp. and *Calliphora stygia* (Fabricius) identified as hosts in all but four cases. *T. zealandicus* was also reared from *Chrysomya rufifacies* (Macquart) collected from a sheep carcass in the Wairarapa.

Legner et al. (1975) found no evidence of *T. zealandicus* establishment on California poultry farms after making a large release of *T. zealandicus* when the *M. domestica* population was at its peak during January. The reasons of this are not understood, as our data indicate that cooler weather should have favored establishment of this species. The apparently greater activity of *T. zealandicus* during cooler weather in Brazil may be due to innate preferences of this parasitoid for lower temperatures or to increases in the developmental time of its hosts, thus making them available for a longer period of time (Olton and Legner 1974).

No parasitoids emerged in this study when they were held at a constant temperature of 27°C. Olton (1971) also observed no development when parasitoids were held at 29 and 32°C. This does not necessarily mean that short exposures to these higher temperatures are lethal when they occur in a fluctuating temperature regime. Liu et al. (1995) reported that conclusions drawn from studies using constant temperatures should be viewed with caution and that the effect of variable temperatures on development rates merits more extensive analysis. Differences in development times between constant and varying temperatures can usually (but not always) be accounted for by the effects of simple rate summation used on the curvilinear relationship between temperature and rate of development. For example, in a related study Geden (1997) observed that constant exposure to 35°C was lethal for *Muscidifurax raptor* Girault and Sanders and three *Spalangia* spp, but that fluctuating temperature regimes that included >6 hr/day of exposure to this temperature resulted in very low mortality.

High temperatures can also have indirect effects on the host:parasitoid relationship. Olton (1971) observed that a variable number of parasitoid eggs and early instars were

completely encapsulated when their development was retarded by high temperature. Upon eclosion, the first instar larvae of *T. zealandicus* retains the chorion for 8-12 hours. In many cases the chorion is melanized when it is displaced into the hemolymph of the host. This melanization of cast chorions accounts for the black bodies present in hosts containing parasitoid early instars (M.A.F.A., unpublished observations). Apparently, incomplete melanization in this species is a normal oxidative phenomenon and a host defense reaction. The process of cellular encapsulation results when the host's hemocytes surround and adhere to the surface of the invading object, forming a multicellular capsule-like envelope. Factors that can affect the frequency of parasitoid encapsulation include host and parasitoid species, the host's physiological age and physiological condition, the host origin (or strain), superparasitism, and the rearing and/or ambient temperature (Blumberg, 1997).

Our results about development times were similar to those observed by Olton (1971). Although *T. zealandicus* is active in the field during months when ambient temperatures are near levels that appear to be lethal, it is possible that parasitoid and host behaviors limit their exposure to high-temperature extremes. Because *T. zealandicus* is a parasitoid of dipteran third instars, the temperature conditions that the parasitoids experience as immatures are determined by the habitat and microclimate selections of the hosts after they have been parasitized. Little is known about the depth at which host larvae pupate in the field, but they are commonly found several cm below the surface of the soil in open-sided poultry houses. Temperature conditions in the soil are less volatile than ambient air temperatures. For example, we observed in the field that if the air temperature was about 28°C, the temperature of the soil (ca. 5 cm of depth) was 23-24°C (unpublished

data). The high humidity (ca. 70%) and relatively low temperatures experienced by parasitized pupae under these conditions would favor parasitoid survival even when ambient air temperatures routinely exceed the upper developmental limit of 25°C. Presumably, parasitoid survival would be even higher at greater depths. Olton (1971) found that the lower threshold for development of *T. zealandicus* is near 15°C. The lower temperature that we used was 16°C. It is possible that host encapsulation is promoted by low temperatures as well as at the higher temperatures. It would be interesting to investigate development rates and host encapsulation using other species of flies as hosts such as *M. domestica* and *Sarcophaga bullata.*

Muscoid flies in poultry houses are often attacked by other parasitoids, mostly in the pteromalid genera *Muscidifurax* and *Spalangia*. The rates of development of *M. raptor* and *S. endius* observed by Ables et al. (1976) showed that *M. raptor* undergoes limited development at 12.8°C and after 7 months, when transferred to 26.7°C produced adult parasitoids within 10 days. The upper threshold for this species appears to be between 32.2 and 35°C. (Ables *et al.*, 1976, Geden, 1997, Mann *et al.*, 1990). For *S. endius,* these authors reported that the parasitoids cannot survive prolonged exposure to low temperatures. Development rates at 22°C for *M. raptor* and *S. endius* were 28 and 39 days, respectively, compared to our results of 27 days at this temperature for *T. zealandicus* (Table 1). These three parasitoid genera occur sympatrically in our region (Ferreira de Almeida and Pires do Prado, 1999; Monteiro and Pires do Prado, 2000).

Our results about observed development times are similar to those of two other gregarious encyrtid parasitoids of squash bug eggs: *Ooencyrtus anasae* (Ashmead) and a

closely related species *Ooencyrtus* sp. near *anasae* (Tracy and Nechols, 1987). These species complete development in ca. 18 days at 26.6°C to ca. 32 days at 20.8°C.

In our study, longevity of *T. zealandicus* was examined from two standpoints. First, we evaluated the longevity of individuals as a function of emergence order after having been reared at different temperatures. Emergence at all temperatures was distributed over several days, an observation that agrees with those of Olton (1971). Our results indicate that the parasitoids that emerge early have greater fitness, at least regarding longevity, than those that emerge later (Table 3). This response was strongest at the lower temperatures of 16-20°C. Legner (1969) tested several solitary endo- and ectoparasitic species of muscoid Diptera and found that the size and density of hosts influenced the distribution of emergence times. He alluded to various hypotheses proposed to explain this variation and found that differential larval and pupal development and delayed adult emergence were the causes for differences in emergence patterns among closely related species.

The other aspect of longevity that we examined was the influence of food treatment on longevity at different temperatures. Parasitoids that were given honey and water lived 2-3 times longer than those that were only given water. Madar and Miller (1983) found that adult longevity of *Apanteles yakutatensis* (Hymenoptera: Braconidae), a primary and gregarious larval endoparasitoid of *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) was significantly increased when the parasitoids were provided with a sugar-water food source. Nothing is known about the feeding behavior and nutritional ecology of *T. zealandicus* in the field, so it is not known whether this species forages for nectar sources in nature. They do not host-feed and do not require a protein meal to develop their ovaries. If host larvae are present, the mortality of females increases rapidly after they

have oviposited, and longevity is actually reduced substantially in the presence of hosts (M.A.F.A., unpublished data). This is why we did not include host larvae as a "food" treatment in the tests. Our results may thus be seen as representing the longevity of parasitoids that are searching for but have not yet located host larvae to attack. Fly larvae under field conditions vary widely in abundance and are patchily distributed, so the parasitoids must be able to survive until they find them. Field studies are needed to evaluate possible and actual carbohydrate sources for *T. zealandicus* under natural conditions.

Further investigations also will be necessary to compare host attack rates and progeny production of *T. zealandicus* at different temperatures. Data on temperature-dependent host attacks are available for other parasitoids that occupy the same niche as *T. zealandicus*, and there are substantial differences among these species in their temperature optima for host attacks. For example, *S. gemina* is most effective at 25°C, *S. cameroni* is similar in the 20-30°C range but less effective of 15 and 35°C, and *M. raptor* is equally effective at killing fly pupae at all temperatures tested except at the high extreme of 35°C (Geden 1996). Similar data are needed for *T. zealandicus* in order to determine the climatic zones in which it is most likely to be an effective biological control agent of muscoid flies.

CAPÍTULO II

Influence of nutritional treatment, host density, temperature on attack rates of *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae)

ABSTRACT

Tachinaephagus zealandicus Ashmead is a gregarious parasitoid that attacks third instar larvae of muscoid flies and a colony was originally established from samples collected from a poultry farm in Santa Cruz da Conceição, São Paulo, Brazil. The purpose of this study was to evaluate the influence of nutritional treatment, host density and temperature on attack rates on *T. zealandicus*. Nutritional treatment (honey, honey+water, water, and starvation) had statistically significant effects on rates of attack on *C. putoria* and *M. domestica*, however no significant treatment effects were observed in rates of progeny production. With both *M. domestica* and *C. putoria* it was observed that the percentage of killed hosts decreased as host density increased. Temperature had significant effects on host attacks on *C. putoria*. During 24 hours of exposure, highest rates of host attacks were observed at 22°C. For the number of killed hosts, differences were not statistically significant among 20, 22, 25 and 27°C. These results provide substantial information about the biology of *T. zealandicus*, since this species is a strong candidate as a biological control agent of muscoid flies in Brazil.

INTRODUCTION

Tachinaephagus zealandicus Ashmead is a gregarious parasitoid that attacks third instar larvae of muscoid flies. Silveira *et al.*, 1989 described *T. zealandicus* attacking *Cochliomyia hominivorax* (Coquerel) (Calliphoridae) in the State of São Paulo, Brazil, and attacking *Synthesiomyia nudiseta* (Wulp) (Muscidae) in the State of Minas Gerais, Brazil. Costa (1989) found *T. zealandicus* associated with pupae of *Musca domestica* L., *Stomoxys calcitrans* (L.) and *Muscina stabulans* (Fallén) (Muscidae) collected in a poultry house located in the interior of State of São Paulo. Monteiro and Prado (2000) found *T. zealandicus* emerging from pupae of *Musca domestica, Chrysomya putoria* (Wiedemann) and *Muscina stabulans* collected in poultry facilities in the interior of São Paulo.

Because of the potential importance of this species as a fly biological control agent in the Southern Hemisphere, a colony of *T. zealandicus* was established in our laboratory in 1998. Studies about biology of *T. zealandicus* were conducted by Olton and Legner (1974), and Ferreira de Almeida (Chapter I) examined the development time and longevity of this species at six different constant temperatures. Although these studies provided substantial information about the biology of *T. zealandicus,* little is known about host attack rates and progeny production by this species under different environmental conditions. The purpose of the present study was to evaluate the influence of nutritional treatment, host density, temperature, on attack rates on *T. zealandicus.*

MATERIALS AND METHODS

Insect colonies

Tachinaephagus zealandicus were from a colony originally established from samples collected from a poultry farm in Santa Cruz da Conceição, São Paulo, Brazil, and had been maintained on *Chysomya putoria* for 14 generations at the time of testing.

Parasitoids were maintained in 2-l clear plastic boxes covered by snap-on plastic lids with screened openings. Cages were stocked with ca. 2,000 parasitoids per cage and held at 25±1°C, 60±10% RH, under a photoperiod of 12L:12D. Honey and water were provided to the insects and *C. putoria* larvae were exposed to 2-d-old females at a host:parasitoid ratio of 20:1 for 4 consecutive days. Parasitized pupae were removed from the cages daily and held in a rearing chamber at 22±1°C.

Chrysomya putoria were also from a colony originally established from the poultry farm in Santa Cruz da Conceição. Larvae were reared using the diet described by Leal *et al.*(1982), and adults were held in cages under the environmental conditions described above for *T. zealandicus.* Flies were given water and sugar *ad libitum.* and periodically given liver for egg maturation and oviposition.

Musca domestica were from a colony originally established from poultry farms in central Florida in the early 1980's. Larvae were reared on a diet of 50% wheat bran, 30% ground alfalfa, and 20% corn meal plus water, and adults were fed a mixture of powdered milk, sugar, and powdered egg yolk.

Influence of nutritional treatment on fecundity

One hundred sixty females were placed individually in 100-ml clear plastic cups covered by snap-on plastic lids with screened openings. The parasitoids were less than 6 hours old at the time of the experiment and were assumed to have mated (unpublished observations). Females were assigned to four different nutritional treatments: treatment I (honey plus water); treatment II (only honey); treatment III (only water); and treatment IV (starvation). In addition, the parasitoids were provided with 16 fly larvae for oviposition. Half of the parasitoids were provided with larvae of *M. domestica* and the other half were provided with larvae of *C. putoria* (n=20 females for host species and nutrition combination). Larvae were removed and replaced with new larvae each day for three days. Larvae that had been exposed to parasitoids were held for fly and parasitoid emergence under the conditions described above.

Differences in the number of host attacks and progeny for each nutritional treatment were evaluated by ANOVA, and means were separated using Tukey's method ($P \leq 0.050$) under the General Linear Models Procedure of the Statistical Analysis System (SAS Institute, 1992).

Influence of host density on fecundity

One hundred individual mated females within 24 hr of emergence were placed individually in 100-ml clear plastic cups covered by snap-on plastic lids with screened openings. The parasitoids had been provided with water and honey before being removed for the test. Females were provided with five different densities of either *M. domestica* or

C. putoria larvae: 2, 4, 8, 16, 32 larvae per female (n=10 females per host species and density). After 24 hr the females were removed and the exposed fly immatures held for fly and parasitoid emergence as before. No food was provided to the females during the exposure to host larvae.

Differences in the number of host attacks and progeny production were evaluated by two-way ANOVA using host species, density and host species x density as model effects using the GLM Procedure of SAS (SAS Institute, 1992).

Influence of temperature on fecundity

One hundred individual mated females within 24 hr of emergence were placed individually in 100-ml clear plastic cups covered by snap-on plastic lids with screened openings and placed in rearing chambers maintained at either 20, 22, 25, 27 or 30°C (20 females per temperature). The parasitoids were then provided with 30 *C. putoria* larvae and honey during a 24-hr exposure. After exposure, the females were removed and the fly immatures were held at 25°C for fly and parasitoid emergence.

Differences in the number of host attacks and progeny for females at each temperature were evaluated by ANOVA, and means separated using Tukey's method under GLM Procedure of SAS as before (SAS Institute, 1992).

RESULTS

Influence of nutritional treatment on fecundity

The effect of nutritional treatment was analyzed with respect to host attacks and progeny production. The results showed that food treatments had significant effects on both variables (Table 1). When *M. domestica* larvae were used as hosts, *T. zealandicus* killed significantly more hosts when honey was included in their diets than when they were either starved or given only water. The parasitoids killed about three times as many hosts (24.9 hosts killed) when they were given honey and water than when they were given neither (8.4). When *C. putoria* larvae were used as hosts the greatest numbers of fly immatures were killed when the parasitoids were given honey and water (18.9 hosts killed), but this was only significantly different from the treatment where no honey or water were provided (11.4). Progeny production among individuals was more variable than host attacks, and this variation prevented the detection of significant treatment effects (Table 2), however, very few progeny were produced by parasitoids that were starved and exposed to larvae of the house fly. Two-way ANOVA's indicated that the host species (house fly or *C. putoria*) had no significant effect on overall attack rates or on the production of female progeny by *T. zealandicus* (Table 3). Significantly more male progeny were produced when *C. putoria* were provided as hosts than when house fly larvae were used. There was a significant feeding treatment x host species interaction for host attacks (Table 3), reflecting the greater sensitivity of the parasitoids to honey deprivation when house fly larvae were provided as hosts.

TABLE 1

Mean (SE) number of hosts killed and progeny produced (males plus females) per female *T. zealandicus* during three days of exposure to four feeding treatments.

Feeding Treatment	Flies species			
	M. domestica		*C. putoria*	
	hosts killed[1]	Progeny produced	hosts killed	Progeny produced
Honey & water	24.9 (2.7) a	4.1 (1.5) a	18.9 (1.9) a	11.6 (3.9) a
Honey only	24.6 (2.5) a	7.1 (3.8) a	16.4 (1.6) ab	7.4 (2.5) a
Water only	13.1 (2.2) b	8.2 (4.0) a	14.6 (1.4) ab	9.2 (4.0) a
No honey or water	8.4 (1.3) b	1.1 (0.6)a	11.4 (1.2) b	8.8 (2.6) a
ANOVA F[2]	14.00**	1.24 ns	4.29**	0.28 ns

[1] Means within columns followed by the same letter are not significantly different at P=0.05 using Tukey's method.
[2] Results of one-way ANOVA's run separately for each fly species and feeding treatment (**, P≤0.01; *, P≤0.05; ns, P>0.05).

TABLE 2

Mean (SE) number of female and male progeny produced per female *T. zealandicus* during three days of exposure to four feeding treatments.

Feeding treatment	Fly species			
	M. domestica		*C. putoria*	
	females	males	females	males
Honey & water	2.6 (1.1) a	1.5 (0.6) a	7.5 (2.0) a	4.1 (2.3) a
Honey only	6.4 (3.5) a	0.6 (0.3) a	5.5 (2.0) a	1.9 (0.7) a
Water only	6.3 (3.2) a	1.8 (0.8) a	6.9 (2.9) a	2.3 (1.1) a
No honey or water	0.9 (0.5) a	0.2 (0.2) a	6.0 (1.7) a	2.8 (0.9) a
ANOVA F[1]	1.28 ns	2.09 ns	0.17 ns	0.46 ns

[1] Means within columns followed by the same letter are not significantly different at P=0.05 using Tukey's method.
[2] Results of one-way ANOVA's run separately for each nutritional treatment (**, P≤0.01; *, P≤0.05; ns, P>0.05).

TABLE 3

Two-way ANOVA results for effects of feeding treatment and host species on host attacks and progeny production by *T. zealandicus*.

Source of variation	ANOVA F[1]		
	Hosts killed	Progeny produced	
		females	males
Feeding treatment (FT)	17.58 **	0.69 ns	0.83 ns
Host species (HS)	3.20 ns	2.13 ns	5.39 *
FT x HS	4.14 **	0.87 ns	0.48 ns

[1] **, P≤0.01; *, P≤0.05; ns, P>0.05.

Influence of host density on fecundity

The average numbers of hosts killed as a function of host density are presented in Table 4. With both *M. domestica* and *C. putoria,* the percentage of killed hosts decreased as host density increased, reaching a maximum attack rate of 21-22 hosts killed per female at the highest density of 32 larvae per female (Table 4). There were no significant differences in host attacks due to host species or to species x density interactions (Table 6). Similar results were observed with progeny production, with overall production reaching a maximum of 12.9 progeny per female with both host species at the highest density of 32 larvae per female (Table 5). Significantly more males emerged from *C. putoria* than from *M. domestica*, but there were no differences in female emergence from the two fly species (Table 6).

TABLE 4

Mean (SE) number of hosts killed by female *T. zealandicus* in 24 hour at different host densities using two species of flies.

Density (larvae per female)	Fly species	
	M. domestica	*C. putoria*
2	1.9 (0.1)	1.9 (0.1)
4	3.8 (0.2)	3.4 (0.2)
8	7.0 (0.4)	6.3 (0.5)
16	11.9 (0.9)	12.8 (1.0)
32	21.7 (1.8)	21.0 (2.1)
ANOVA F[1]	66.15 **	56.47 **

[1] One-way ANOVA (**, P≤0.01; * P≤0.05; ns, P>0.05).

TABLE 5

Mean (SE) number of progeny produced per female *T. zealandicus* in 24 hours at different host densities using two species of flies.

Density	Fly species					
	M. domestica			*C .putoria*		
	females	males	total	females	males	total
2	1.1(0.4)	0.8(0.4)	2.0(0.7)	2.0(0.7)	0.6(0.2)	2.6(0.9)
4	6.1(1.2)	1.3(0.4)	7.4(1.5)	3.0(1.0)	1.2(0.5)	4.2(1.4)
8	7.9(1.9)	3.5(0.8)	11.5(2.5)	4.1(0.9)	1.1(0.3)	5.2(1.2)
16	8.5(1.8)	3.3(1.2)	11.7(2.8)	6.3(1.3)	1.2(0.4)	7.5(1.4)
32	9.6(2.5)	3.3(0.8)	12.9(3.2)	10.0(2.2)	2.8(0.9)	12.9(3.0)
ANOVA F[1]	3.69 **	2.48 *	3.72 **	5.49 **	2.61 *	5.23 **

[1] One-way ANOVA's (**, P≤0.01; * P≤0.05; ns, P>0.05).

TABLE 6

ANOVA results for effects of host species and density on host attacks and progeny production by *T. zealandicus*.

Source of variation	ANOVA F[1]		
	hosts killed	progeny produced	
		females	males
Host species (HS)	0.01 ns	2.36 ns	6.18 *
Host density (HD)	121.6 **	7.85 **	3.75 **
HS x HD	0.36 ns	0.90 ns	1.29 ns

[1] **, P≤0.01; *, P≤0.05; ns, P>0.05.

Influence of temperature on fecundity

Temperature had significant effects on attack rates and progeny production by *T. zealandicus* (Table 7). The parasitoids killed the greatest numbers of *C. putoria* immatures at 22°C (12.6 host attacks per female) and the fewest at 29°C (6.3). Differences in attack rates over the range of 20-27°C were small and not statistically significant. Similarly, progeny production ranged from 6.0 to 10.9 progeny per female in the range of 20-27°C, whereas few progeny were produced (1.8) at the high temperature of 29°C.

TABLE 7

Mean (SE) number of hosts (*C. putoria*) killed and progeny produced by *T. zealandicus* during a 24-hour exposure to host larvae at five different temperatures.

Temp. (°C) during exposure	Hosts killed[1]	Progeny produced		
		females	males	total
20	11.4 (1.57) ab	7.8 (1.84) a	3.0 (0.68) a	10.9 (2.20) a
22	12.6 (1.67) a	5.8 (1.66) ab	3.0 (0.74) a	8.7 (2.21) a
25	10.9 (1.61) ab	4.5 (1.56) ab	1.4 (0.56) ab	6.0 (2.05) ab
27	10.6 (1.25) ab	3.2 (1.34) ab	1.6 (0.86) ab	4.8 (1.82) ab
29	6.3 (1.19) b	1.2 (0.58) b	0.6 (0.28) b	1.8 (0.79) b
ANOVA F[1]	3.33 *	3.44 *	3.97 **	4.57 **

[1] Means within columns followed by the same letter are not significantly different at P=0.05 using Tukey's method.
[2] One-way ANOVA's (**, P≤0.01; *, P≤0.05; ns, P>0.05)

DISCUSSION

The purpose of this study was to evaluate the response of *T. zealandicus* to nutritional treatment, host density and temperature with respect to attack rates and progeny production. *T. zealandicus* is a proovigenic parasitoid that emerges with a full compliment of eggs and dies soon after commencing oviposition and each ovary of a newly emerged adult female contains approximately 12-16 ovarioles, with a variable number of mature oocytes present in the ovarioles (Olton 1971). Female abdomens dissected after a 48-hr oviposition period are sunken and nearly devoid of eggs. From laboratory observations we noted that females did not appear to feed on their hosts but were readily attracted by honey when this food was offered to them.

Host feeding in parasitic Hymenoptera is related to the need to secure nutrients, especially protein, for the continued production of oocytes. Proovigenic species such as *T. zealandicus* typically do not host-feed, whereas most adult parasitoids that continue to develop oocytes after emergence (synovigenic species) do engage in this behavior (Jervis and Kidd, 1986). Frequent observations (by M.A.F.A.) of oviposition revealed that females of *T. zealandicus* may either alight on or walk to a prospective host larva. The host is usually mounted in the posterior dorsal region and inspected with antennae and the tip of the ovipositor. If the host is acceptable, the ovipositor is inserted either inter- or intra-segmentally at a point just beneath the cuticle. The eggs are deposited in 20-45 seconds or longer, depending on the activity of the larva (Olton, 1971). *T. zealandicus* females are very aggressive and tenacious, and host inspection/oviposition is prabably energetically demanding (Olton, 1971). This may explain why the availability of a sugar source such as honey promotes greater numbers of host attacks but does not necessarily result in increased progeny production (Table 1). Our nutrition treatment experiment indicated that there is no significant difference in progeny production when females are given honey, although honey is known to promote longevity in this species (Chapter I). According to Lewis *et al.* (1998), parasitoids periodically have to interrupt host foraging in order to seek food sources that supply energy for maintenance and locomotion to allow them to sustain high fertility and to survive until hosts are located. Some adult parasitoids feed on host hemolymph or host-associated honeydew, (Jervis and Kidd, 1986; Kidd and Jervis, 1989) whereas others are feed independently of the host on resources such as floral and extrafloral nectaries, honeydew, or pollen (Jervis *et al.*, 1993).

Most parasitoids require food as adults such as nectar, pollen, homopteran honeydew or host hemolymph. In the laboratory, substitutes for these can include diluted honey, cut raisins, and sucrose solutions. When food is not available, energy resources bound up in eggs, fat body or muscle tissue can be converted into usable energy, but the parasitoids may starve before such conversions release necessary quantities of nutrients for immediate metabolic needs (Heimpel and Collier, 1996). Studies have shown that the longevity of both sexes and the fecundity of females typically are significantly reduced in the absence of food (Jervis *et al*, 1992). Females of *Spalangia endius* Walker, for example, had higher longevity when they were provided with a combination of honey and host (*M. domestica)* pupae than when they were given only hosts or honey (Arellano and Rueda, 1988).

Parasitoids that do not host-feed can be divided into two groups on the basis of their food searching strategies. The first group finds food resources in the same part of the environment as the hosts, such as aphid parasitoids feeding on the honeydew produced by their hosts (Jervis and Kidd, 1996). The second group includes those parasitoids that find food resources and hosts in different parts of the environment and feed, for example, on nectar, pollen and extrafloral nectar (Jervis *et al*, 1993). No information is available on natural food sources of *T. zealandicus* in the field. Further investigation will be necessary to evaluate the suitability of potential food resources that are available to the parasitoids in poultry facilities such as fermenting spilled poultry feed and cracked hen eggs. Regardless of the natural food preferences of *T. zealandicus*, these results and those of (Chapter I) indicate that provision of honey is beneficial for promoting longevity of this species under colony conditions during times when host larvae are not immediately available.

Results of influence of host density on attack rates and progeny production showed that females killed ca. 95% of exposed hosts at the low densities of 2-4 host larvae/female, and killed 65.6-87.5% of hosts at the higher densities of 8 through 32 hosts/female (Table 4). ANOVA results presented in Table 6 showed that host density but not host species was important in determining rates of host attacks and production of female progeny, indicating that both *M. domestica* and *C. putoria* are suitable hosts for this species. Data suggest that *T. zealandicus* is able to search and survive at low host densities, as revealed by high parasitization of hosts in isolated fly breeding situations (Johnston and Tiegs, 1922). The influence of host density on reproductive period and longevity indicate that *T. zealandicus* is a short-lived species capable of depositing a fixed number of eggs during a short reproductive period, regardless of host density. When host densities are low, the parasitoids allocate more eggs among the available hosts, resulting in shortened development times because of accelerated host resource depletion (Olton, 1971). There also appears to be a critical minimum number of eggs that must be deposited to result in successful parasitism. Solitary parasitism in this species is uncommon, and the number of parasitoids produced per host varies with host size. We have also observed that when large hosts such as *Sarcophaga bullata* are lightly parasitized (<5 parasitoid immatures/host larva), the larvae of *T. zealandicus* grow to very large sizes but fail to complete development to the pupal stage (unpublished data).

Evaluation of temperature effects on attack rates and progeny production demonstrated that *T. zealandicus* was inhibited by temperatures near ca. 30°C and that temperature effects were small in the range of 20-27°C. The modest differences in response rates to temperature were somewhat surprising. This may have been due in part to

possible temperature effects on development of the host larvae during the 24-hr exposure period. Differences in attack rates on live dipteran hosts and prey can be partially masked if the host/prey species develops beyond the window of susceptibility to attack during the experiment, as has been observed with both predators and parasitoids of house fly immatures (Geden and Axtell, 1988, Geden, 1996). Thus, for example, at higher temperatures the host larvae may develop to a stage where they are less prone to parasitism before the parasitoids have attacked as many larvae as they are capable of attacking. Further experimentation with more frequent provisioning of hosts would be required to test this hypothesis with *T. zealandicus*.

The lower threshold of activity for this species has not been established, however, we have observed that the parasitoids are sluggish and show negligible interest in host larvae at 15°C (personal observations). Geden (1996) observed that other parasitoids that occur sympatrically with *T. zealandicus* (*Spalangia gemina, S. cameroni* and *Muscidifurax* spp.) are more effective near ca. 25°C. *M. raptor* killed more house fly pupae at 30°C than either *S. gemina* or *S. cameroni*. The two *Spalangia* species had similar attack rates overall, although attack rates of *S. gemina* were more depressed by both low and high temperature than were those of *S. cameroni*. The *Spalangia* species studied by Geden (1996) were also collected on a dairy farm ca. 15 km far from the source population of *T. zealandicus* in this study, with the highest prevalence occurring between September and December (Ferreira de Almeida and Pires do Prado, 1999). Field observations during 1998 showed that *T. zealandicus* was most prevalent from August to December and that the maximum temperatures during the latter month was ca. 29°C (Chapter I). Parasitoids in the field may

be able to mitigate the apparently inhibitory effects of high ambient air temperatures by foraging in microhabitats with more moderate local environmental conditions.

CAPÍTULO III

Microsporidiosis of *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae)

ABSTRACT

An undetermined microsporidium was found infecting *Tachinaephagus zealandicus*, a gregarious parasitoid that attacks third instar larvae of muscoid flies. Spores were present in all body regions and in all stages of development. Infected adults contain an average of 3.75×10^5 spores. In order to separate the colony into infected and uninfected individuals, we treated infected females on rifampicin mixed with honey as food and after 8 days ca. 37% of individuals of progeny examined were still infected. An uninfected culture was established and the two colonies were tested for infection transmission. It was observed that the efficiency of maternal transmission was 96.3%. We are postulanting that the transmission of the microsporidian of *T. zealandicus* occurs by contamination of the honey that is offered *ad libitum* to adults when infected and uninfected individuals are reared together in the same cage and when the same host is superparasitized by infected and uninfected females. The method of transmission within superparasitized hosts is not known.

larvae, pupae and adults stages (n=10) were made with a hemocytometer using the method of Cantwell (1970).

Drug treatment

Adult parasitoids (n=ca. 300 females) within five hours of emergence were assigned to different drug treatments: treatment I (honey with 3% rifampicin), treatment II (honey with 3% albendazole), and treatment III (honey-only). Preliminary testing established that this was the upper limit of rifampicin that the parasitoids could tolerate without causing unacceptably high mortality. They were maintained at 20°C, 60±10% RH, and in addition, the parasitoids were provided with host larvae on days 2, 6 and 8 after emergence. Progeny (n=30/ treatment) were assessed for infection after being presented with host larvae to parasitize and clean family lines were used to establish an uninfected colony.

Efficiency of maternal transmission within superparasitized hosts

Twenty individual larvae of *Sarcophaga bullata* Parker were placed in 30-ml clear plastic cups covered by snap-on plastic lids with screened openings and exposed to female parasitoids in the following four combinations: 1) 2 infected females [8h]; 2) 2 uninfected females [8h]; 3) 2 uninfected females [4h] then 2 infected females [4h]; 4) 2 infected and 2 uninfected females [8h]. Progeny were counted and assessed for infection status. Differences in the number of progeny infected with the microsporidium

for each combination were evaluated by ANOVA using GLM Procedure of SAS ($P<0.05$) (SAS Institute, 1992).

RESULTS

Description of microsporidian infection

Spores (Figs. 1, 2, 3) were present in all body regions, but greatest spore concentrations occurred in the abdomen in male and female parasitoids in all stages of development (Figs. 1, 2, 3). Staining with Giemsa revealed the presence of parasites in the oöcytes (Figs. 8, 9) and in newly deposited parasitoid eggs (Fig. 10). All of the spores observed in *T. zealandicus* were ovocylindrical in shape (Fig. 3). Infected *T. zealandicus* pupae contained an average of 1.4×10^5 spores and 3.75×10^5 spores per parasitoid at adult (Table 1). Examining some whole-animal mounts stained by Giemsa, it was possible to identify some phases of presporulation development and sporogony. This study was made comparing our figures with those of Becnel and Geden (1994). Some phases were recognized as:

During presporulation development: uninucleate schizonts stained intensely and were generally of two sizes; they were spherical in shape with a large centrally located nucleus (Figs. 4d; 6b,d); stages binucleate (not diplokariotic) (Figs. 4c; 5a,b,c; 6c; 7a,b,e); cytokinesis of meront (Figs. 5e; 7f).

During sporogony: transitional forms were larger than meronts, slightly elliptical to fusiform in shape with a lightly stained cytoplasm (Figs. 4a; 5f; 6a); early

sporoblasts that had a small chromophilic granulate at one pole (Fig. 7c); later sporoblasts had a small chromophilic granulate at one pole (Fig. 7d).

Spores: Spores were generally ovocylindrical (Figs. 4b; 5d; 7g).

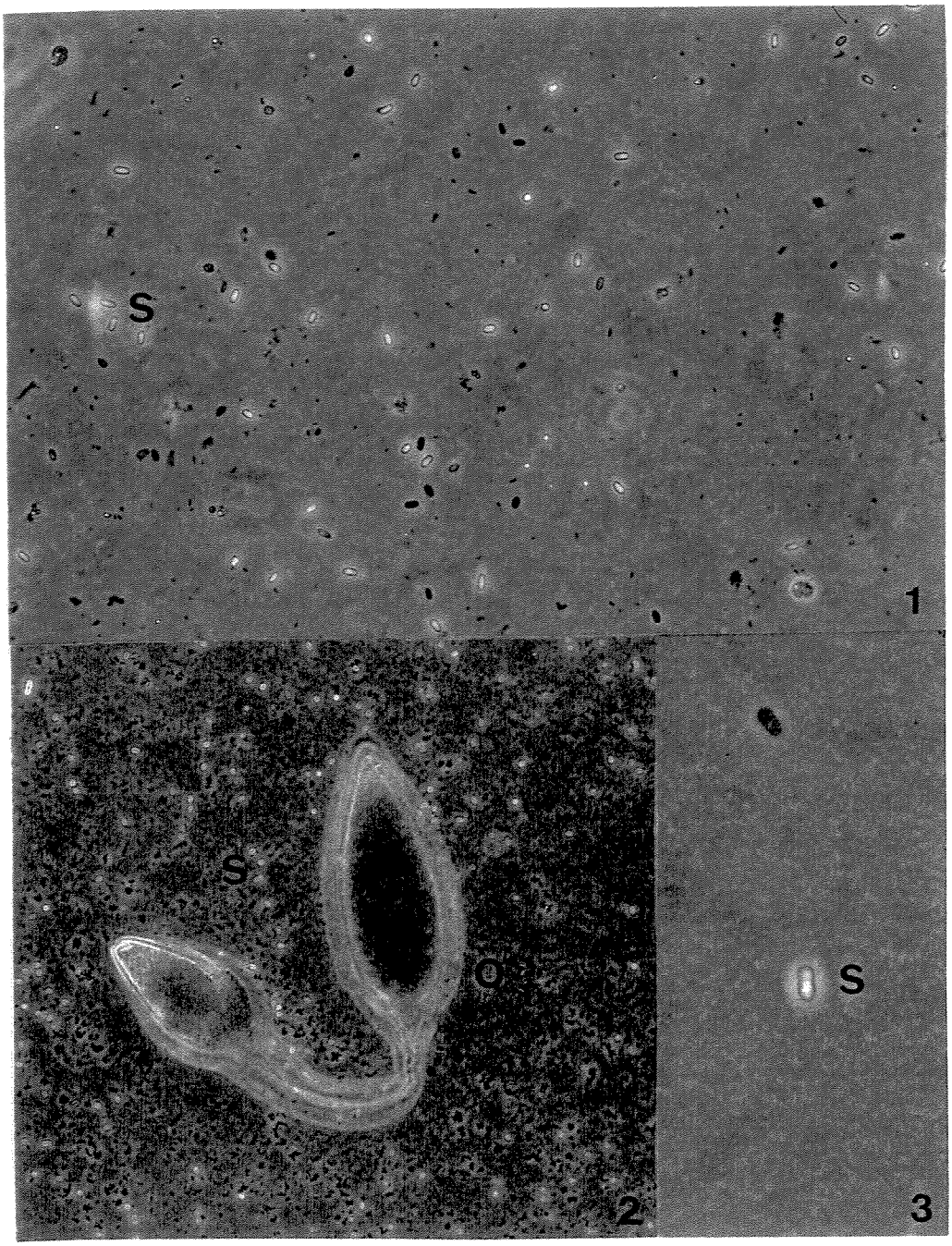
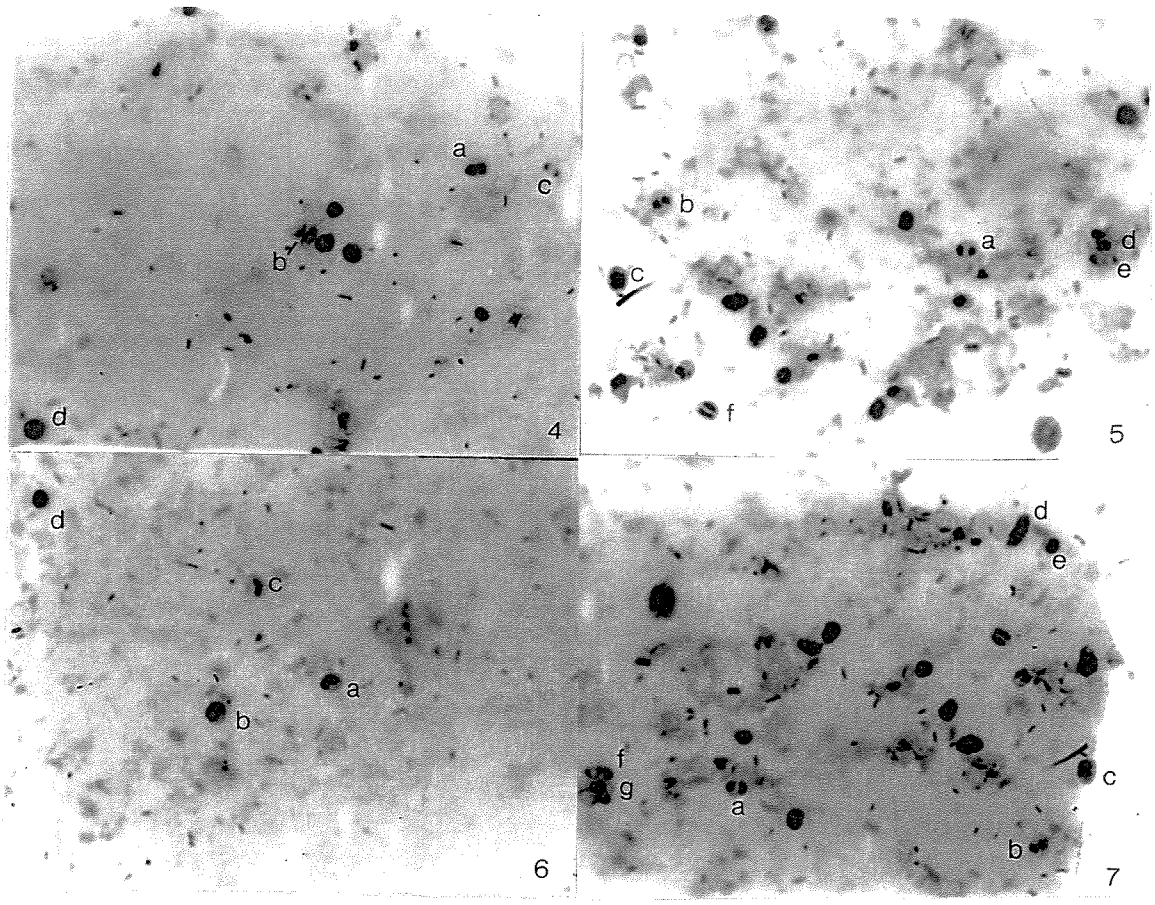
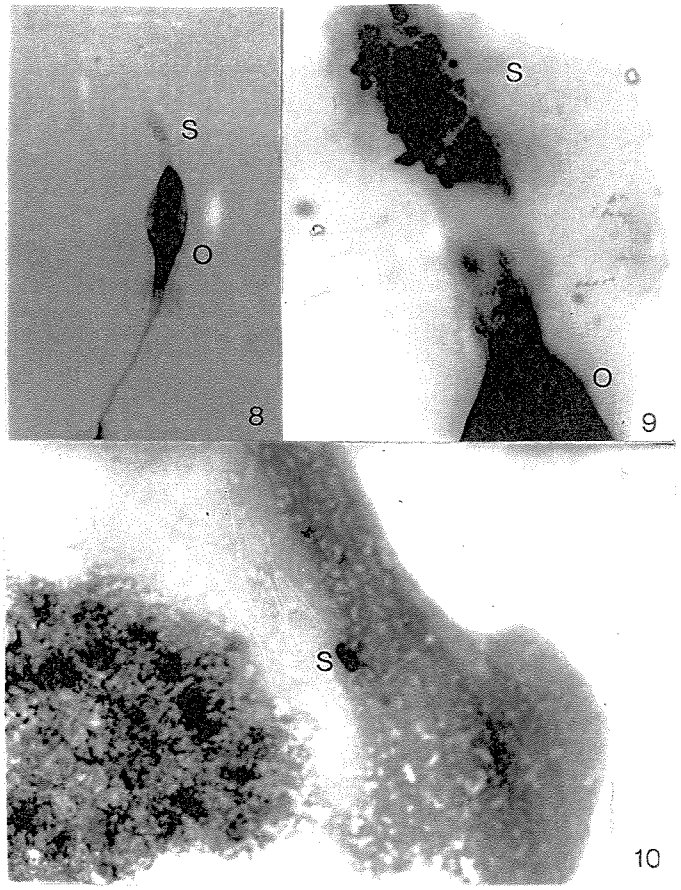


Fig. 1: Wet mount of an infected female of *T. zealandicus* showing the spores (S) (phase-contrast compound microscope) (1740X). **Fig. 2:** Wet mount of an infected female of *T. zealandicus* showing the spores (S) compared with an oöcyte (O) of the parasitoid (phase-contrast compound microscope) (870X). **Fig. 3:** Wet mount of an infected female of *T. zealandicus* showing the spores (S) (phase-contrast compound microscope) (4350X).



Photomicrographs of stages of the microsporidium in adults of *T. zealandicus.* Giemsa-stained and x 2,500. **Fig. 4: (a)** Diplokaryotic sporont; **(b)** Two diplokaryotic spores; **(c)** Binucleate stage; **(d)** Large uninucleate schizont. **Fig. 5: (a, b, c)** Binucleate stages; **(d)** Diplokariotic spore; **(e)** Cytokinesis of meront; **(f)** Fusiform sporont. **Fig. 6: (a)** Diplokaryotic sporont; **(b)** Large uninucleate schizont; **(c)** Binucleate stage; **(d)** Small uninucleate schizont. **Fig. 7: (a, b, e)** Binucleate stages; **(c)** Early diplokaryotic sporoblast; **(d)** Immature diplokaryotic spore; **(f)** Cytokinesis of meront; **(g)** Two diplokaryotic spores.



Photomicrographs of stages of the microsporidium in adults of ***T. zealandicus.*** Giemsa-stained. **Fig. 8:** Spores (S) inside the oöcyte (O) (1000 x). **Fig. 9:** Fig 8 in detail (2,500x). **Fig. 10:** one spore (S) isolated from a newly deposited egg of *T.* ***zealandicus*** (2,500x).

Voucher specimens of infected parasitoids have been deposited for identification/description with J. Becnel, Insects Affecting Man and Animals Laboratory (USDA), Gainesville, Florida, USA.

Drug treatment

The results of the drug treatment applied to infected females of *T. zealandicus,* indicated that the infection of their progeny was partially controlled (63% of progeny uninfected) after 8 days of receiving rifampicin mixed with honey as food, *ad libitum* (Fig. 11). All of the progeny from the albendazole-treated and honey-only-treated parasitoids were infected, as were those of the rifampicin-treated parasitoids in the first two exposures to host larvae (days 2 and 6 post-treatment).

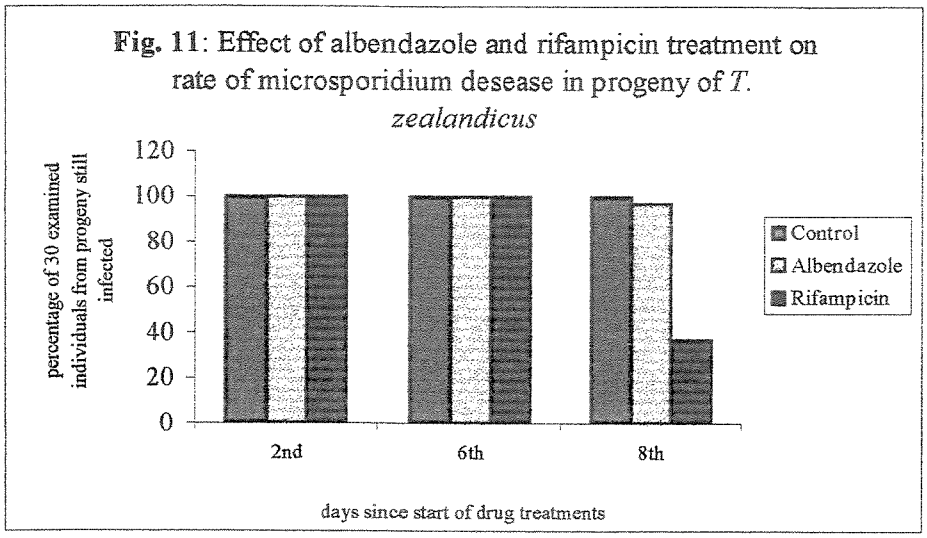
Efficiency of maternal transmission within superparasitized hosts

When individual host larvae were exposed to two infected parasitoids for 8 hours the rate of maternal transmission was 96.3% (Table 2). None of the progeny from the uninfected parasitoids were infefcted. When hosts were exposed to equal numbers of infected and uninfected parasitoids, the rate of infection among progeny was about 83%, indicating that horizontal transmission occurred within superparasitized hosts. The number of progeny produced per larva ranged from 40.9 to 55.0 across the treatments and did not appear to vary with the infection status of the parents.

TABLE 1

Density of microsporidian spores observed in infected stages of adults of *T. zealandicus*.

Stage of development	Mean no. of spores per parasitoid
eggs inside the host	~ 6
larvae	9.5×10^4
pupae	1.40×10^5
adults	3.75×10^5



Drugs mixed with honey and given to adult parasitoid *ad libitum* starting on the day of emergence

TABLE 2

Progeny production and infection rates of *T. zealandicus* progeny reared from individual *Sarcophaga bullata* larvae exposed to microsporidium-infected and/or uninfected female parasitoids.

Infection status of parents (exposure time)	Mean (SE) parasitoid progeny/host	Mean (SE) % progeny infected with the microsporidium
2 infected (8 hours)	42.5 (4.67)	96.3 (2.72)
2 uninfected (8 hours)	40.9 (4.41)	0.0 (0.0)
2 uninfected (4 hours) **then** 2 infected (4 hours)	52.3 (4.00)	83.2 (5.57)
2 uninfected **and** 2 infected (8 hours)	55.0 (2.52)	82.5 (6.32)
ANOVA F[1]	3.27 *	82.02 **

[1] Results of one-way ANOVA's (**, $P \leq 0.01$; * $P \leq 0.05$; ns, $P > 0.05$).
N= 20 larvae per treatment

DISCUSSION

Since 1998, when a colony of *T. zealandicus* was established in our laboratory we have studied this parasitoid and some aspects of its biology as described by Ferreira de Almeida (Chapters I and II). During a visit to the U.S. Department of Agriculture's Center for Medical, Agricultural and Veterinary Entomology, located in Gainesville, Florida, our attention was called by Dr. C.J. Geden to the possibility of infection of our *T. zealandicus* colony by a microsporidium. Our investigations provided positive results and we observed that part of the colony was infected by a microsporidium that appears to belong to the genus *Nosema*. All microsporidia are obligatory intracellular organisms, multiplying in the host cell in the form of small paucinucleate meronts or plasmodia. Excessive mortality, reduced longevity, or reduced fecundity are sometimes the indications of a microsporidium in laboratory-reared insects (Undeen and Vávra, 1997). According to morphological observations conducted at the light level with wet mounts and Giemsa-stained smears we feel that all characteristics presented here agree with those used by other authors to describe the genus *Nosema*. We also observed the presence of binary fission as described by Becnel and Geden (1994) in *N. muscidifuracis*. The spores are generally small, several micrometres in size. Most are ovoid to tubular in shape. They are refringent under phase-contrast microscopy and often a vacuolar space can be seen at the posterior pole of the spore (Undeen and Vávra, 1997). Details of the pathogen life cycle were provided by Becnel and Andreadis (1999).

Spores were observed in newly deposited eggs of *T. zealandicus* indicating transovarial transmission, and we also observed spores inside the oöcytes (Figs. 8, 9).

Spores of *N. muscidifuracis* within *M. raptor* eggs were revealed only after crushing them; no spores were observed adhering to the outside of the egg and also no spores were observed in the remains of hosts that were attacked by infected parasitoids (Zchori-Fein *et al.*, 1992). Details of these observations must be carefully reviewed. The infection in *M. raptor* also intensifies as the host parasitoid develops from one stage to another (Table 1). For example, *Muscidifurax raptor* females contained an average of 1.8×10^4 spores per parasitoid at emergence, and infection intensity increased to 2.7×10^4 spores after 5-7 days (Zchori-Fein *et al.*, 1992). For *T. zealandicus* we found that adults contain ca. 3.75×10^5 spores. Some investigations were conducted to reveal the transmission of *N. muscidiruracis* to house flies and the results showed that neither fly adults nor larvae become infected and that there was no increase in mortality at any dose compared with controls (Geden *et al.*, 1995).

In order to obtain an uninfected colony, we also tried heat shock treatment by exposing newly parasitized hosts to elevated temperatures for varying amounts of time (personal observations). Low temperatures for this kind of treatment such as 40°C had no therapeutic value, whereas higher temperatures (up to 50°C) killed the host. As *T. zealandicus* is a koinobiont parasitoid this fact caused the death of the parasitoid (unpublished data). A 45- to 60-min exposure to a 47°C water bath greatly suppressed *Nosema* disease in *M. raptor,* but resulted in substantially lower parasitoid survival (Geden at al. 1995). *Urolepis rufipes* (Ashmead) did not survive to any of the treatments that had therapeutic value for *M. raptor* (Geden *et al.*, 1995).

Our results showed that the treatment using albendazole was not effective compared to the treatment with rifampicin (Table 2). After obtaining an absolute

uninfected colony we conducted the first experiment in order to investigate the efficiency of transmission of this pathogen. We observed that the efficiency of the maternal transmission by infected females was very high (96.3 %) (Table 3) and is close to the 100% transmission efficiency typically observed with transmission *of N. muscidifuracis* (Geden *et al.*, 1995). Further experiments would be required to evaluate the role of males in possible paternal and venereal transmission, but neither of the routes of infection is likely to occur (Zchori-Fein *et al.*, 1992, Geden *et al.*, 1995).

High rates of infection of progeny (ca. 83%) were observed when larvae were exposed to equal numbers of infected and uninfected females (Table 3). Horizontal transmission of *N. muscidifuracis* occurs in *M. raptor* through at least two mechanisms. First, healthy parasitoid immatures can become infected when they feed on infected immatures within superparasitized host puparia; and second, healthy parasitoid adults can become infected by feeding on infected parasitoid immatures within host puparia (Geden *et al.*, 1995). Transmission by the first mechanism is intensified by the cannibalistic nature of *M. raptor* larvae that ensures that parasitism is nearly always solitary. With *T. zealandicus* these two mechanisms probably do not occur because this species is a gregarious parasitoid and it does not appear to host feed (Olton 1971 and Ferreira de Almeida [Chapter II]). There are other possible mechanisms for horizontal infection in *T. zealaniducs*. Although larval cannibalism is unlikely, it is possible that infected eggs or larvae could die within the host and release spores that could be ingested by uninfected larvae in the same host. A second possible mechanism could be the deposition of spores into the host hemolymph at the time of oviposition that are consumed later by uninfected larvae. Another possible source of horizontal

transmission in colonies (but not in our experiment) is through contamination of honey when infected and uninfected parasitoids share the same food source. Because *T. zealandicus* feeds acidly on honey in colony containers this could be a significant source of disease amplification in the generations following establishment of new colonies from the field. Further investigations will be necessary to evaluate the prevalence of the infection in field-collected parasitoids, since laboratory studies indicate that the disease is amplified in culture.

CAPÍTULO IV

Influence of microsporidian infection on development time, emergence success, and longevity of *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae)

ABSTRACT

An undetermined microsporidium was found infecting *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae), a gregarious parasitoid that attacks third instar larvae of muscoid flies. This study evaluated the influence of four constant temperatures (15, 20, 25, 30° C) on development time and longevity of microsporidium-infected and uninfected colonies. No development was observed at 30°C and infected parasitoids developed significantly faster than uninfected ones at 15, 20 and 25°C. Uneclosed puparia were dissected and the results showed that infected parasitoids had difficulty emerging from host puparia, especially at 20 and 25°C. The longevity of females decreased as temperatures increased. Effects of infection on longevity were strongest at all temperatures among parasitoids that were given honey and water; longevity was short in all parasitoid groups that were only given water. ANOVA results showed that not only temperature and feeding treatment but also the infection affected significantly the longevity of females and males.

INTRODUCTION

An undetermined microsporidium recently has been found infecting *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae), a gregarious parasitoid that attacks third instar larvae of muscoid flies. The transmission of this pathogen is probably transovarial (Chapter III) and this seems to be an effective means of maintaining these infections (Zchori-Fein *et al.* 1992; Ni *et al.*, 1995). Insects are the most important hosts of microsporidia and approximately half the number of species are parasites of insects (Larsson, 1988). Excessive mortality, reduced longevity, or reduced fecundity are sometimes the indications of a microsporidium in laboratory-reared insects (Undeen and Vávra, 1997) and an example is the deterioration observed in colonies of *Muscidifurax raptor* infected by *Nosema muscidifuracis* (Geden *et al.*, 1992).

The purpose of this study was to evaluate the influence of infection on development time and emergence success of *T. zealandiscus* at four constant temperatures, and to assess the effect of infection on longevity parasitoids at different temperatures and under different nutitional regimes.

MATERIALS AND METHODS

Insect Colonies

Infected *T. zealandicus* were from a colony originally established from samples collected on a poultry farm in Santa Cruz da Conceição, São Paulo, Brazil, and had been mantained on *Chysomya putoria* for 16 generations at the time of testing. Uninfected *T. zealandicus* were from the same colony that had been treated with rifampicin and subjected to the Pasteur method to produce an uninfected culture (Chapter III).

The parasitoids were maintained at 25±1°C, 60±10% RH, under a photoperiod of 12L:12D. Honey and water was provided to the insects and mature *C. putoria* larvae were exposed to 2-d-old females. Parasitized pupae were removed from the cages daily and held in a rearing chamber at 22±1°C.

Chrysomya putoria were also from a colony originally established from the poultry farm in Santa Cruz da Conceição. Larvae were reared using the diet described by Leal *et al.*(1982). Adults were given water and sugar *ad libitum* and periodically given liver for egg maturation and oviposition.

Influence of four constant temperatures on development time and emergence success of infected and uninfected colonies

Groups of microsporidium-infected and uninfected *T. zealandicus* females were provided with 1200 *C. putoria* larvae at a host:parasitoid ratio of ca. 5:1. Twenty four hours later, parasitized pupae were placed in individual gelatin capsules and placed in

groups of 100 in 100-ml clear plastic cups with snap-on plastic lids with screened openings. The pupae were then transferred to rearing chambers set at 15, 20, 25 and 30°C (3 groups of infected and 3 groups of uninfected per temperature) and 60±10% RH, 12D:12L photoperiod. In addition, 100 unparasitized pupae were placed in each chamber as controls. Parasitism under these test conditions was 98%. Pupae were monitored twice daily for parasitoid emergence and the development time at each temperature was determined. Uneclosed host puparia were dissected. Differences in parasitoid development time at the four temperatures was evaluated by ANOVA using the GLM Procedure of SAS ($P<0.05$) (SAS Institute, 1992) and either the adult emergence or emergence success of infected versus uninfected individuals were evaluated by simultaneous G tests of independence comparing the proportion of emerged and dead adults and the proportion males and females emerged to obtain estimates of χ^2 (Sokal and Rohlf, 1994).

Influence of temperature and food treatment on longevity of infected and uninfected colonies

Five hundred infected and five hundred uninfected parasitized pupae of *C. putoria* were isolated in gelatin capsules for emergence of *T. zealandicus* at 25°C. Individual female and male parasitoids were collected within one hour of emergence and placed in 100-ml clear plastic cups covered by snap-on plastic lids with screened openings. Forty infected parasitoids of each sex and infection status were placed in each of four rearing chambers set at 15, 20, 25 and 30°C, 60±10% RH, and photoperiod 12L:12D. Half of the

parasitoids were given water and honey as food, and the other half were given only water (20 male and 20 female parasitoids for each combination of temperature, food treatment and infection status). Parasitoid mortality was recorded daily. Differences in parasitoid longevity were evaluated by ANOVA, and means separated using Tukey's method under the GLM Procedure of SAS ($P<0.05$) (SAS Institute, 1992).

RESULTS

Influence of four constant temperatures on development time and emergence success of infected and uninfected colonies

Development time of microsporidium-infected and uninfected *T. zealandicus* at four different constant temperatures is presented in Table 1. No development was observed at 30°C. Infected parasitoids developed significantly faster than uninfected parasitoids at 15, 20 and 25°C., however the differences in development times between infected and uninfected parasitoids was very small at 20 and 24°C. Substantially more parasitoids emerged from the uninfected than the infected groups at all three temperatures from which parasitoids emerged (Table 2). At 15°C there was no significant difference in the sex ratios of the infected versus uninfected adults, but overall emergence of uninfected parasitoids was 16 times greater than emergence of infected parasitoids. Sex ratios were significantly more male-biased in the infected groups at 20 and 25°C, and total emergence of uninfected was 11- and 3-fold greater among uninfected parasitoids at these two temperatures, respectively (Table 2).

Results of dissections of uneclosed puparia are presented in Table 3. Mortality of immature parasitoids was high at 15°C for both infected and uninfected groups, although significantly higher mortality was observed among uninfected parasitoids. Emergence success of infected parasitoids was much lower than emergence of uninfected parasitoids at 20 and 25°C. At 20°C, only 10.5% of the infected parasitoids emerged successfully compared with 85.6% of the uninfected parasitoids. At 25°C, 40.3% of the infected parasitoids were successful in emerging from their hosts, whereas 94.63% of the uninfected parasitoids emerged successfully.

TABLE 1

Development time of microsporidium-infected and uninfected *T. zealandicus* reared in *C. putoria* at four temperatures

Temp.(°C)	Mean (SE) development time (days)				ANOVA F [1]		
	Infected		Uninfected		Sex	Infection	Sex x Inf.
	Female	Male	Female	Male			
15	58.60	58.50	63.51	63.22	0.41 ns	32.78 **	0.01 ns
	(0.24)	(0.19)	(0.32)	(0.27)			
20	33.12	33.02	33.24	33.20	2.07 ns	6.45 **	0.11 ns
	(0.15)	(0.07)	(0.03)	(0.02)			
25	22.73	22.78	22.97	22.96	1.57 ns	27.18 **	0.49 ns
	(0.08)	(0.03)	(0.03)	(0.02)			
30	-	-	-	-			

[1] df= 1,222 (15°C); 1,2136 (20°C) and 1,2821 (25°C)

Overall model F=11.07 (15°C), 2.88 (20°C) and 9.75 (25°C)

TABLE 2

Adult emergence of microsporidium-infected and uninfected *T. zealandicus* from *C. putoria* at four temperatures

Temp. (°C)	No. adult parasitoids emerged				Chi-square [1]
	Infected		Uninfected		
	Females	Males	Females	Males	
15	5	8	78	135	0.01 ns
20	24	152	725	1239	44.28 **
25	101	592	775	1357	128.11 **
30	-	-	-	-	

[1] G-test of independence for proportion of males and females emerged, infected versus uninfected parasitoids

TABLE 3

Emergence success microsporidium-infected and uninfected *T. zealandicus* from *C. putoria* at four temperatures

Temp. (°C)	No. adult parasitoids observed				Chi-square [1]
	Infected		Uninfected		
	Emerged	Dead[2]	Emerged	Dead	
15	13	316	213	527	105.04 **
20	176	1502	1964	330	2,466.09 **
25	693	1026	2132	128	1,490.35 **
30	-	-	-	-	

[1] G-test of independence for proportion of males and females emerged, infected versus uninfected parasitoids

[2] Parasitoids that failed to emerge based on dissections of uneclosed host puparia.

Influence of temperature and food treatment on longevity of infected and uninfected colonies

The longevity of females decreased as temperatures increased and significant differences due to infection status were only observed in the groups that were given with honey and water (Tables 4 and 6). Differences in longevity of females due to infection were most pronounced at the temperature extremes of 15 and 30°C (infected females lived 32 and 52% as long as uninfected females at these temperatures, respectively). Infected and uninfected females that were only given water had similar longevities and lived for a much shorter time than the honey-fed females in all cases. Similar results were observed with males (Tables 5 and 6). ANOVA results that all of the model main effects

were significant (temperature, feeding treatment, and infection status) and their respective interaction terms were highly significant for both males and females (Table 6).

TABLE 4

Longevity of microsporidium-infected and uninfected *T. zealandicus* females held under different temperatures and feeding conditions

Temp. (°C)	Mean (SE) longevity in days of parasitoids given food treatment			
	Water only		Water and honey	
	Uninfected	Infected	Uninfected	Infected
15	5.4 (0.39)c	3.5 (0.34)c	30.1 (1.17)a	16.0 (2.05)b
20	2.9 (0.19)b	2.2 (0.15)b	12.8 (0.76)a	10.0 (1.32)a
25	2.9 (0.18)c	2.2 (0.15)c	9.6 (0.60)a	6.0 (0.87)b
30	1.8 (0.12)bc	1.1 (0.07)c	8.7 (0.47)a	2.8 (0.39)b

Means within rows followed by the same letter are not significantly different at P=0.05 (Tukey's method)

TABLE 5

Longevity of microsporidium-infected and uninfected *T. zealandicus* males held under different temperatures and feeding conditions

Temp. (°C)	Mean (SE) longevity in days of parasitoids given food treatment			
	Water only		Water and honey	
	Uninfected	Infected	Uninfected	Infected
15	5.4 (0.34)c	4.1 (0.33)c	23.4 (2.73)a	12.7 (2.34)b
20	2.6 (0.13)b	1.8 (0.14)b	13.6 (1.18)a	4.0 (0.71)b
25	1.9 (0.14)c	1.4 (0.11)c	8.3 (0.57)a	4.4 (0.78)b
30	2.1 (0.14)b	1.2 (0.10)b	4.5 (0.18)a	3.8 (0.38)a

Means within rows followed by the same letter are not significantly different at P=0.05 (Tukey's method)

TABLE 6

Anova results for longevity of microspodium-infected an uninfected *T. zealandicus* adults held under different temperatures and feeding condictions.

Source of variation	df	ANOVA F[1]	
		Females	Males
Influence of Temperature (TM)	1	56.62 **	131.35 **
Influence of Feeding treatment (FT)	1	179.22 **	565.12 **
Influence of Infection status (IS)	3	49.57 **	93.98 **
TM x FT	3	21.51 **	69.81 **
TM x IS	3	6.06 **	13.45 **
FT x IS	1	28.15 **	50.45 **
TM x FT x IS	3	5.01 **	8.71 **

[1] Denominator df=304. Overall model F=34.97 (males) and 91.97 (females)
(**, P≤0.01; * P≤0.05; ns, P>0.05).

DISCUSSION

We were studying aspects of the biology of *T. zealandicus* such as the influence of temperature on development rate and longevity (Chapter I) when we detected that part of our colony, established in the Department of Parasitology (IB, UNICAMP) might be infected by a microsporidium (Chapter III). Geden *et al.* (1992) had already described that profound losses in fitness and searching ability could be observed in colonies of *Muscidifurax raptor* (Pteromalidae) infected by a microsporidium. Releases of infected parasitoids also may have the perverse effect of introducing massive levels of disease into field populations where it is rare. Cultures of these natural enemies should therefore be subject to rigorous quality control to ensure that only healthy parasitoids are released in integrated management programs for flies (Geden *et al.*,

1995). The use of predatory arthropods in biological control necessitates that the predators are pathogen-free to avoid the risk of introduction of pathogens which could destabilize the natural fauna (Haque *et al.* 1993). Since we expected to promote the release of *T. zealandicus* on poultry farms located in the interior of São Paulo in order to control fly populations, we decided to investigate the development time, adult emergence success, and longevity of microsporidium-infected and uninfected colonies. Although, according to Dry *et al.*, 1999, among-species comparisons of microsporidian infection prevalence revealed differences between wasps from release and nonrelease farms, prevalence was often higher in pteromalids from farms with no history of parasitoid releases.

A 100% uninfected colony was established three generations before the tests presented here when we were succesful in eliminating the disease by a combination of rifanpicin/honey trreatment followed by the culling of infected family lines (Chapter III). The disease in *T. zealandicus* appears to be similar to *N. muscidifuracis* in *M. raptor* in that it is rapidly amplified under normal rearing conditions (Zchori-Fein *et al.* 1992).

Our results showed that no development was observed at 30°C for either infected or uninfected parasitoids. This result was expected, since no development had been observed by Olton (1971) at 29°C. Infected parasitoids developed significantly faster than uninfected parasitoids at 15, 20 and 25°C, however the differences were extremely small (only a few hours) except at 15°C. These results were surprising, because infected *Muscidifurax raptor* required 10 to 15% more time to complete development from egg to adult (24-26 days) than uninfected parasitoids (22 days) (Zchori-Fein et al. 1992).

Another point investigated here was the average number of adults that emerged at each temperature. Uninfected parasitoids produced vastly more adult progeny than infected parasitoids at all temperatures. These results induced us to examine uneclosed pupae in order to determine whether infected parasitoids simply produced fewer progeny or whether they also had higher rates of mortality during immature stages. Pupal dissections revealed that the adult emergence success of infected parasitoids was due in large measure to pre-eclosion mortality. Immune responses to microsporidia sometimes show up as dark spots of melanized parasites or infected tissues. Infected tissues are identifiable during dissections of larger hosts by swelling and altered colour due to masses of spores or spore-filled cysts (Undeen and Vávra, 1997). We did not observe any such gross signs of infection during the dissections. Indeed, most of the infected dead parasitoids that were observed were healthy-appearing teneral adults that had simply arrested development.

Newman and Andrewartha (1930) also observed that a large number of parasitized blowfly pupae contained mature *T. zealandicus* which failed to emerge during the dry mid-summer conditions of Perth, Australia. They speculated that prevailing hot-arid conditions either decreased parasitoid vitality or hardness of the puparium was more pronounced. This inability to emerge was particularly evident when the "hairy maggot", *Chrysomya rufifacies* Macquart, was the host. However, during the spring and autumn months, the parasitoids emerged easily. Sajap and Lewis (1988) analysed the effect of *Nosema pyrausta* infecting *Trichogramma nubilale* and observed a significant decrease in adult emergence of about 36% due to *N. pyrausta.*

We also investigated the longevity of infected and uninfected males and females. The infection influenced only individuals treated with water and honey. For example, uninfected females treated with honey and water and maintained at 15°C lived two times longer than infected females, and those maintained at 30°C lived three times longer than infected ones. Longevity in the absence of food, which is perhaps more typical of field conditions for this proovigenic species (Olton, 1971; [Chapter II]) was not significantly different among infected and uninfected parasitoids. Sajap and Lewis (1988) showed that the longevity of infected adults of *Trichogramma nubilale* was not significantly reduced by *Nosema* disease.

According to Geden *et al.* (1992), infected *Muscidifurax raptor* live half as long and produce about 10% as many progeny as uninfected parasitoids in the laboratory. It was also speculated that such compromised parasitoids could also be more vulnerable to pesticides and other stresses encountered under field conditions, although this was not known. After two generations in the laboratory, the culture was 100% infected, indicating the speed with which such infections can be amplified under normal rearing conditions after establishment of a new colony from field material. The effects of infection on the fitness of *Muscidifurax raptor* were sufficiently severe that selection against infected individuals would be expected to reduce the occurrence of disease in laboratory cultures if this pathogen relied solely on vertical transmission (Zchori-Fein *et al.*, 1992). According to Ni *et al.* (1997) *Nosema empoascae* causes little mortality to its cicadellid host and is not efficiently transmitted horizontally. Normally when microsporidia occur in hosts confined in a common cage, as is the case with *Empoasca fabae* (Homoptera: Cicadellidae) colonies, the prevalence approaches 100% and the

insect colony cannot be maintained indefinitely. It is possible that relatively inconspicuous microsporidia such as *Nosema empoascae* commonly occur in insects. They are likely to be identified only when insects are examined routinely for microsporidian infections and when transmission mechanisms and host pathogenicity are investigated.

In summary, the pathogen found infecting *T. zealandicus* has little impact on the development time of its host except at low temperatures. Infected colonies produce many fewer progeny, and this is the result of lower parasitism rates as well as difficulties that infected parasitoids have in completing development and escaping fron the host puparium. Infection reduces adult longevity when parasitoids are given high-quality food sources but not under nutritional conditions more typical of those encountered in the field. From the standpoint of applied biological control programs, the pathogen clearly has deleterious effects on parasitoid production and quality and efforts should be made to prevent its introduction into colonies of this beneficial insect. Further research is needed to assess how the pathogen is transmitted and the impact of the disease on parasitoid fecundity.

CAPÍTULO V

Host attack rates and progeny production of microsporidium-infected and uninfected *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae)

ABSTRACT

Uninfected *T. zealandicus* Ashmead (Hymenoptera: Encyrtidae) females and females from a colony infected with an undetermined microsporidium had similar lifespans (3.7-3.9 days) when parasitoids were provided with honey, water and house fly (*Musca domestica* L. [Diptera: Muscidae]) larvae continously after emergence. Infected parasitoids produced significantly fewer progeny than uninfected parasitoids, and a greater proportion of infected parasitoid progeny failed to emerge from host puparia. Most host attacks and parasitism occurred on the first day of emergence, and little parasitism was observed by day 3 after emergence for both uninfected and infected females, indicating that this is a proovigenic species that emerges with a predetermined number of oocytes that are deposited when hosts are available for parasitism. Comparison of host attacks and parasitism on two species of flies (house flies and *Sarcophaga bullata* (Diptera: Sarcophagidae) indicated that the effects of infection are modulated by the species of host present. Similar numbers of both species fly hosts were killed by uninfected and infected parasitoids (70.2-74.1 hosts attacked per group of 5 female *T. zealandicus*). However, infected parasitoids produced substantially fewer progeny from house fly hosts (311.1 for uninfected parasitoids compared with 138.3 progeny from infected parasitoids), wheras infection had no significant effect on progeny production from the much larger *S. bullata* hosts (588.2 and 460.1 progeny produced by uninfected and infected parasitoids, respectively). Differences in performance on the two host species may be due to innate differences in host quality or to the pronouced size differences of the two species (190 mg/larva for *S. bullata* versus 20 mg/larva for *M. domestica*). Uninfected *T. zealandicus* that were stored at 15°C had highest rates of host attacks (58-62 hots killed per group of five female parasitoids) and progeny production 173.8-261.2 progeny) after 6-12 days of storage at this temperature; relatively few hosts were attacked or parasitized after 0 or 1 day at 15°C.

INTRODUCTION

Tachinaephagus zealandicus Ashmead (Hymenoptera: Encyrtidae) is a gregarious parasitoid that attacks third instar larvae of muscoid flies and appears to be endemic to the Southern Hemisphere (Olton, 1971). Because of the potential importance of this species as a biological control agent of house flies (*Musca domestica* L.) and other pest species, a colony of *T. zealandicus* was established in our laboratory in 1998. Basic life history characteristics of the parasitoid were studied in the first few months after establishment of the colony (Chapters I and II). A microsporidium was discovered infecting the parasitoids in some of the colony cages in late 1999 and rapidly spread through the colony (Chapter III). This pathogen resembles *Nosema muscidifuracis* in its morphology and life cycle (Chapter III, Becnel and Geden 1995), and the two pathogens are nearly indistinguishable based on analysis of DNA sequences from the small ribosomal subunit (B. Moser, personal communication). *N. muscidifuracis* is a cosmopolitan pathogen that infects pteromalid parasitoids of muscoid fly pupae, especially *Muscidifurax* spp. (Becnel and Geden 1995, Geden et al. 1995). *Nosema* diseases have also been found in the parasitoid genera *Spalangia*, *Urolepis*, and *Encarsia* (Zchori-Fein et al. 1992, Dry et al. 1999, Boohene 2001)

Nosema disease in *M. raptor* is characterized by a rapid decline in colony fitness characteristics such as host attack rates, fecundity and longevity (Geden et al. 1992, 1995). Infected *M. raptor* also have longer immature development times and produce higher proportions of males than uninfected parasitoids (Boohene et al. 2001). In a previous study, Ferreira de Almeida (Chapter IV) observed that *Nosema* disease had little effect on development time of *T. zealandicus*, however, infected colonies had

lower proportions of females and low rates of emergence success (Ferreira de Almeida (Chapter 4). Dissections of uneclosed hosts revealed that many infected parasitoids developed to the adult stage but were apparently unable to chew through the host puparium and escape. Olton (1971) also observed high rates of parasitoid emergence failure in *T. zealandicus* and attributed this to low relative humidities, but *Nosema* disease may have been involved as well. Ferreira de Almeida (Chapter IV) also observed that infection reduced the longevity of *T. zealandicus*, but only among parasitoids that were given high-quality food; no differences were observed between infected and uninfected parasitoids that were only given water.

The objectives of this study were to continue evaluating the effect of microsporidiosis on *T. zealandicus*, specifically: 1) to compare rates of host attacks and progeny production by healthy and infected parasitoids on two species of hosts, 2) to determine the effect of infection on parasitoid longevity, attack rates, and progeny production during the first few days after emergence; and 3) to determine whether uninfected parasitoid immatures can become infected when they share superparasitized hosts with infected immatures.

MATERIALS AND METHODS

Colonies

Infected *T. zealandicus* were from a colony originally established from samples collected on a poultry farm in Santa Cruz da Conceição, São Paulo, Brazil, and had been mantained on *Chysomya putoria* or *Sarcophaga bullata* for 16 generations at the time of testing. Uninfected *T. zealandicus* were from the same colony that had been treated with rifampicin and subjected to the Pasteur method to produce an uninfected culture (Ferreira de Almeida [Chapter III]). The parasitoids were maintained at 25±1°C, 60±10% RH, under a photoperiod of 12L:12D. Honey and water were provided to the insects and mature *C. putoria* or *S. bullata* larvae were exposed to 2-d-old females at a host:parasitoid ratio of 20:1 for 4 consecutive days. Parasitized pupae were removed from the cages daily and held in a rearing chamber at 22±1°C.

Chrysomya putoria were also from a colony originally established from the poultry farm in Santa Cruz da Conceição. Larvae were reared using the diet described by LEAL *et al.*(1982), and adults were held in cages under the environmental conditions described above for *T. zealandicus*. Flies were given water and sugar *ad lib.* and periodically given liver for egg maturation and oviposition. *S. bullata* were reared in the same manner as *C. putoria* except that the larvae were reared on calf liver. House fly larvae were reared at 28°C on a mixture of wheat bran, ground alfalfa, corn meal and water; adult house flies were given water and dry food (powdered milk, sugar, and dried egg yolk) *ad libitum*.

Host attack rates and fecundity of infected and uninfected parasitoids

Infected and uninfected females were collected within 24 hours of emergence, counted into groups of 5 females each, and placed in 100-ml clear plastic cups covered by snap-on plastic lids with screened openings It was assumed that the females had mated, as mating typically occurs within the host before emergence (personal observations). Five groups were provided with 75 mature *M. domestica* larvae/ group (mean weight, 20 mg/larva) and the other five groups were provided with 75 larvae of *S. bullata* (mean weight, 190 mg/larva). The *M. domestica* larvae were removed from by hand from rearing pans as mature third instars within 24 hours of pupation (4 days after placement of eggs under our rearing conditions). *S. bullata* larvae were collected as they migrated from the liver in search of pupation sites. Fly immatures (mostly pupae) were removed from the containers after 24 hours and held for fly and parasitoid emergence. Uneclosed host puparia (those that produced neither a fly nor any parasitoids after 35 days) were dissected for the presence of parasitoid immatures. The experiment was replicated twice using different emergence cohorts of parasitoids and flies (total n=10 sets of 5 females per host species and infection status)..

Differences in the number of killed hosts, number of host pupae with dead parasitoid larvae, number of pupae with adult parasitoids, number of parasitized pupae (larvae+adults), number of adult progeny (males+females) and the number of parasitoid progeny/parasitized host were evaluated separately for each fly species as a function of infection status, replication, and infection status x replication using the GLM Procedure of the Statistical Analysis System (SAS Institute, 1992).

Longevity, host attacks and progeny production of infected and uninfected females

Twenty individual mated infected females and 20 uninfected females within 10 hours of emergence were placed in 100-ml clear plastic cups covered by snap-on plastic lids with cloth-screened openings and were provided with water, honey, and 16 *M. domestica* larvae. Water was provided by placing a soaked cotton ball on a portion of the screen lid of each cup; honey was rubbed into another portion of the cloth lid. Host larvae were replaced daily and the water and honey replenished as necessary until each parasitoid died. Longevity, host attacks, and progeny production were evaluated as a function of infection status using the GLM Procedure of SAS (SAS Institute, 1992).

Results of the previous experiment indicated that some individual parasitoids were capable of attacking more than the 16 fly larvae that were provided and that nearly all host attacks were restricted to the first 2-3 days after emergence. A second test was therefore conducted using a daily host provisioning rate of 30 larvae/female, with the observation period restricted to the first three days after emergence (n=20 females per infection status). Uneclosed puparia were dissected after 35 days, and any dead adult parasitoids present within the puparium were counted. Host attacks, parasitism, and progeny production (including emerged as well as unemerged adults) were evaluated as a function of infection status using the GLM Procedure of SAS (SAS Institute, 1992).

Effect of storage at 15°C on rates of host attacks and progeny production

Uninfected females were collected from colony cages within several hours of emergence and transferred to a rearing chamber maintained at 15°C. Parasitoids in this cool chamber were provided with water and honey (but not hosts) for 24 days, during which time samples of parasitoids were removed and assessed for host attack rates and fecundity. Five groups of five females each were removed from the chamber on days 1, 2, 6, 12 and 24 and presented with 75 larvae of *C. putoria*/group for 24 hours at 25±1°C, 60±10% RH. Exposed hosts were removed from the parasitoids after 24 hours and held for fly and parasitoid emergence. Differences in the number of killed pupae and progeny production as a function of storage time (days since emergence) at 15°C were evaluated by ANOVA, and means separated using Tukey's method under the GLM Procedure of SAS ($P<0.05$) (SAS Institute, 1992).

RESULTS

Host attack rates and fecundity of infected and uninfected parasitoids

Infected and uninfected parasitoids attacked similar numbers of house fly larvae during a 24-hour exposure (72-73 hosts killed per group of 5 female parasitoids), and there were no differences in the numbers of hosts found with dead parasitoid larvae (Table 1). Uninfected parasitoids parasitized significantly more house fly hosts (59.7) and produced more than twice as many adult progeny (311.1) as infected parasitoids (45.1 hosts parasitized, 138.3 adult progeny produced). Parasitoid progeny production

from each parasitized host was significantly higher for uninfected (5.7 adult *T. zealandicus*/pupa) than for infected (3.8) parasitoids.

In tests with *Sarcophaga bullata* the effect of infection was less pronounced (Table 2). Infected and uninfected parasitoids killed similar numbers of larvae of this fly, and although uninfected parasitoids produced somewhat more progeny than infected parasitoids (588.2 and 460.1, respectively), the difference was not statistically significant. The only parameters that were affected by infection status were total numbers of parasitized pupae (69.5 and 57.4 for uninfected and infected parasitoids. respectively) and adult progeny produced per parasitized pupae (11.7 and 10.2 for uninfected and infected parasitoids, respectively).

Longevity, host attacks and progeny production of infected and uninfected females

Infected and uninfected parasitoids had similar lifespans (3.7-3.9 days) when they were provided with honey, water and hosts (house fly larvae) continuously after emergence (Table 3). Infected parasitoids attacked significantly fewer house fly larvae overall (16.5 hosts killed) than uninfected parasitoids (28.2), and produced about half as many progeny (9.6 and 21.4 progeny produced per infected and uninfected female, respectively).

Results of the second test, in which daily attack rates were examined for the first three days after emergence, are presented in Table 4. In contrast to the previous experiment, there were no significant differences in host attack rates, with infected and uninfected parasitoids attacking 19-26 house fly larvae over the three-day observation period. Uninfected females successfully parasitized 6.8 house fly larvae (hosts that

produced live adult parasitoid progeny) compared with only 1.3 larvae successfully parasitized by infected parasitoids. Uninfected parasitoids produced about five times as many live progeny (29.7 adult progeny per female) as infected parasitoids (5.9). Dissections of uneclosed puparia indicated the presence of substantial numbers of adult parasitoids that were unable to chew their way out the puparium, but the proportion of unsuccessful adult emergence was higher among infected parasitoids (60% of the total of 15 adult progeny) than among uninfected parasitoids (44% of the total of 52.7 adult progeny). Host attacks and parasitism by both uninfected and infected parasitism were strongest on day 1 after emergence, and relatively little activity was observed by day 3, indicating that *T. zealandicus* is a proovigenic species.

TABLE 1

Host attacks and progeny production of microsporidium-infected and uninfected *T. zealandicus* using *Musca domestica* larvae as hosts.

Mean (SE) rates of attack and progeny production	Infection status		ANOVA F
	Uninfected	Infected	
No. killed hosts (uneclosed pupae)	72.6 (0.2)	71.5 (1.4)	0.47 ns
No. pupae with dead parasitoid larvae	5.9 (1.4)	9.4 (2.1)	0.90 ns
No. pupae with parasitoid adults	53.8 (2.1)	35.7 (4.4)	15.31 **
No. parasitized pupae (larvae + adults)	59.7 (2.2)	45.1 (4.2)	11.59 **
No. adult progeny (males + females)	311.1 (24.1)	138.3 (18.0)	35.57 **
No. parasitoid progeny/parasitized host	5.7 (0.4)	3.8 (0.2)	22.68 **

n= 10 groups of 5 female parasitoids given 75 host larvae
ANOVA F for effect of infection status (**, $P \leq 0.01$; *, $P \leq 0.05$; ns, $P > 0.05$).

TABLE 2

Host attacks and progeny production of microsporidium-infected and uninfected *T. zealandicus* using *S. bullata* larvae as hosts.

Mean (SE) rates of attack and progeny production	Infection status		ANOVA F
	Uninfected	Infected	
No. killed hosts (uneclosed pupae)	74.1 (1.0)	70.2 (1.6)	3.77 ns
No. pupae with dead parasitoid larvae	19.2 (3.3)	13.9 (3.8)	1.19 ns
No. pupae with parasitoid adults	50.2 (0.5)	43.6 (5.8)	1.10 ns
No. parasitized pupae (larvae + adults)	69.5 (1.0)	57.4 (4.1)	7.28 *
No. adult progeny (males + females)	588.2 (44.6)	460.1 (72.4)	2.13 ns
No. parasitoid progeny/parasitized host	11.7 (0.5)	10.2 (0.4)	5.04 *

n= 10 groups of 5 female parasitoids given 75 host larvae

ANOVA F for effect of infection status (**, $P \leq 0.01$; *, $P \leq 0.05$; ns, $P > 0.05$).

TABLE 3

Longevity and lifelong rates of host attacks and progeny production by microsporidium-infected and uninfected *T. zealandicus*. Tests were done with individual mated parasitoids (n=40) given 16 house fly larvae/day, honey and water throughout life.

Mean (SE) longevity, attack rate or fecundity	Infection status		ANOVA F
	Uninfected	Infected	
Longevity (days)	3.9 (0.46)	3.7 (0.42)	0.10 ns
No. hosts killed	28.2 (3.06)	16.5 (1.32)	12.65 **
No. male progeny	13.4 (3.40)	4.4 (1.59)	5.93 *
No. female progeny	8.5 (2.44)	5.6 (1.83)	0.87 ns
No.progeny (total)	21.4 (4.87)	9.6 (2.95)	4.34 *

Results of one-way ANOVA's run separately for each result (**, $P \leq 0.01$; *, $P \leq 0.05$; ns, $P > 0.05$).

Effect of storage at 15°C on rates of host attacks and progeny production

When T. zealandicus were stored at 15oC immediately after emergence and assessed periodically, maximum rates of host attacks (58-62.4 C. putoria killed per group of 5 female parasitoids) and progeny production (174-261 progeny per group) were observed among parasitoids assessed after days 6 and 12 of storage (Table 5). Cold storage for as long as 24 days had only modest effects on attack rates and total progeny production compared to 6 and 12 days, and no statistically significant differences were observed in the numbers of female progeny produced after 6, 12 or 24 days of storage. Comparatively few hosts were attacked or parasitized when parasitoids were tested immediately after emergence or after 1 day of storage at 15°C.

TABLE 4

Host attacks and parasitism of house fly immatures by *Nosema*-infected and uninfected *T. zealandicus* during the first 3 days after emergence. Tests were done with individual mated parasitoids (n=40) given 30 house fly larvae/day, honey and water.

Test day	Infection status	Mean (SE) no. hosts			Mean (SE) no. parasitoid progeny	
		Killed[1]	w/ emerged adult parasitoid	Total parasitized[2]	Emerged	Total[3]
Day 1	Uninfect	14.5 (2.5)	5.3 (1.2)	8.6 (1.6)	22.1 (5.2)	33.8 (6.6)
	Infected	12.6 (1.8)	1.2 (0.3)	3.4 (0.9)	5.4 (1.8)	12.8 (3.7)
	ANOVA	0.35 ns	10.93 **	7.93 **	9.28 **	7.72 **
Day 2	Uninfect	8.3 (1.7)	1.2 (0.4)	3.5 (1.0)	5.1 (2.1)	14.3 (4.0)
	Infected	5.5 (1.1)	0.2 (0.1)	0.5 (0.3)	0.6 (0.4)	2.2 (1.1)
	ANOVA	1.85 ns	5.63 *	7.29 *	4.87 *	8.82 **
Day 3	Uninfect	4.1 (1.6)	0.5 (0.3)	1.3 (0.9)	3.4 (2.8)	6.2 (4.6)
	Infected	0.8 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	ANOVA	5.09 *	2.91 ns	2.72 ns	1.95 ns	2.09 ns
Days 1-3	Uninfect	25.6 (3.6)	6.8 (1.4)	13.0 (2.1)	29.7 (6.0)	52.7 (9.2)
	Infected	19.0 (2.1)	1.3 (0.3)	4.0 (0.9)	5.9 (1.8)	15.0 (3.7)
	ANOVA	2.51 ns	15.72 **	15.23 **	14.34 **	14.49 **

[1] House fly pupae that did not produce adult flies
[2] Includes pupae with adult parasitoids that did not emerge based on dissections uneclosed pupae
[3] Includes adult parasitoids that did not emerge based on dissections of uneclosed pupae
(**, $P \leq 0.01$; * $P \leq 0.05$; ns, $P > 0.05$).

TABLE 5

Mean (SE) for the total of pupae killed and emerged parasitoids, for each day of exposure after emergence of non-infected females of *T. zealandicus* stored at 15°C and with honey using *C. putoria* larvae as host.

Day after emergence	Killed pupae	Progeny		
		females	males	total
0	6.0 (0.3) d	5.0 (0.5) d	4.0 (1.1) d	9.0 (1.3) d
1	8.8 (0.4) d	4.8 (0.7) d	1.60 (0.2) d	6.40 (0.7) d
6	62.4 (1.3) a	85.8 (28.6) a	175.4 (39.7) a	261.2 (53.9) a
12	58.0 (1.7) a	32.4 (7.5) ab	141.4 (32.8) ab	173.8 (32.3) ab
24	40.2 (5.9) b	33.0 (14.0) ab	42.20 (28.2) bc	75.2 (39.4) bc
ANOVA F[1]	90.28 **	5.10 **	9.41 **	11.12 **

[1] (**, $P \leq 0.01$; * $P \leq 0.05$; ns, $P > 0.05$).
Means within columns followed by the same letter are not significantly different at P=0.05 using Tukey's method.
N= 5 sets of 5 females for each day after emergence

DISCUSSION

A colony of *T. zealandicus* was established in the Departament of Parasitology (Institute of Biology-UNICAMP) in 1998, and after ten generations, we observed that part of this colony was infected by a microsporidium (Ferreira de Almeida [Chapter III]). Other studies were conducted in order to investigate the comparisons between development time and longevity of infected and uninfected colonies, as described in Ferreira de Almeida (Chapter IV) and the present study increases the available information about some aspects of the biology of microsporidium-infected *T. zealandicus*. One of these aspects studied was the host attack and fecundity of infected

and uninfected colonies using two different hosts, *M. domestica* and *S. bullata*. It was not observed significative differences in the number of hosts killed (Tables 1 and 2) indicating that host attack was not affected by the infection, for the two species of host. For the bigger host *S. bullata* it was not observed differences in the number of adult progeny production. The same was not observed when the host used was *M. domestica*. In this case, it was observed that the progeny produced for the uninfected group was bigger than that produced for infected one. The longevity of infected and uninfected colonies had been investigated before by Ferreira de Almeida (Chapter IV) and these authors observed that there were no significative differences between longevity of infected and uninfected colonies mantained only with water. These results confirm that *T. zealandicus* is proovigenic (Olton, 1971; Ferreira de Almeida (Chapter II) and that the infection does not influence the longevity. Infected *Muscidifurax raptor* live half as long and produce about 10% as many progeny as uninfected parasitoids in the laboratory (Geden *et al.*, 1992).

The results presented in Table 3 showed that the number of hosts killed was significantly different between infected and uninfected parasitoids, however the other results presented in Table 1, also using *M. domestica* as host were different. These differences may be correlated with the number of females used in each experiment. It is evident that superparasitism is very common among *T. zealandicus* females and probably this habit promotes that individual differences in the females fitness become lower. The influence of host density on reproductive period and longevity indicate that *T. zealandicus* is a short lived species capable of depositing a fixed number of eggs during a short reproductive period, regardless of host density. The probability of an increased

number of eggs per host was a function of host density. A frequency distribution of the number of parasitoids emerged per host over a 4 day spread from a "mass attack" culture. A range of 3-18 parasitoids emerged per host. Apparently, a high multiplicity of oviposition occurred with the high parasitoid host ratio. In the retrospect, results of the reproductive potential tests indicated females deposited an average 4-6 eggs per host during her life span. There was enough food present in a single standardized host to sustain development of at least 3-4 average egg depositions. However, resulting individuals were usually small and stunted at the upper limit (Olton,1971). Studies conducted with *Muscidifurax raptor* showed thata infected parasitoids lived about half as long (8.8 versus 16.5 days) and produced about half as many progeny (102.6 versus 228.6) as uninfected parasitoids. Certain observations were made by Ni *et al.* (1997) that support the hypothesis that the microsporidium causes little mortality and is not efficiently transmitted horizontally. Normally when microsporidia occur in hosts confined in a common cage, as is the case with our *Empoasca fabae* (Homoptera: Cicadellidae) colonies, the prevalence approaches 100% and the insect colony cannot be maintained indefinitely. It is possible that benign microsporidia such as *Nosema empoascae* commonly occur in insects. They are likely to be indentified only when insects are examined routinely for microsporidian infections and when transmission mechanisms and host pathogenicity are investigated.

CONCLUSÕES GERAIS

O tempo de desenvolvimento de machos e fêmeas de *T. zealandicus* variou de 24,0 a 56,9 dias (temperaturas de 25 a 16°C) e nenhum indivíduo emergiu em 27°C. Aqueles mantidos durante o desenvolvimento em 22°C apresentaram maior sucesso na emergência. Adultos da mesma "cohort" emergiram em diferentes dias e aqueles que emergiram nos primeiros dias apresentaram maior longevidade. A longevidade das fêmeas que receberam mel e água diminuiu com o aumento da temperatura e aquelas mantidas em 16°C viveram três vezes mais que as mantidas em 27°C. Fêmeas mantidas somente com água viveram de 4,8 - 7,6 dias em todas as temperaturas (de 16 a 27°C). As fêmeas viveram significativamente mais que os machos em todas as temperaturas, exceto em 16°C.

Fêmeas de *T. zealandicus* mataram larvas de *Chrysomya putoria* (Calliphoridae) e *Musca domestica* (Muscidae), de forma significativamente diferente para cada tratamento nutritional, contudo as diferenças detectadas entre os tratamentos para fêmeas e machos da progênie que emergiu destes hospedeiros não foram estatiscamente diferentes. Tanto para *M. domestica* quanto para *C. putoria* foi observado que a porcentagem de hospedeiros mortos decresceu com o aumento da densidade do hospedeiro. Fêmeas de *T. zealandicus* mataram pupas de *C. putoria* significantivamente diferente para cada temperatura. Durante 24 horas de exposição, a melhor temperatura para as fêmeas atacarem as larvas foi 22°C. Para o número de hospedeiros mortos não houve diferença entre 20, 22, 25 e 27°C.

Um microsporídeo indeterminado foi constatado infectando *T. zealandicus*. Os esporos foram encontrados em todas as regiões do corpo e em todos os estágios de desenvolvimento do parasitóide. Os adultos infectados apresentaram em média 3.75×10^5 esporos. Fêmeas infectadas e tratadas com Rifampicin misturado ao mel como alimento apresentaram após 8 dias, 63% dos indivíduos da progênie não infectados. Nenhum resultado positivo foi constatado para as fêmeas tratadas com Albendazol. A eficiência na transmissão transovariana do microsporídeo foi de 96.3%.

A comparação entre indivíduos infectados e não infectados confirmou não haver desenvolvimento em 30°C. Os resultados da ANOVA mostraram que somente a infecção foi fator significativamente importante para as diferenças obtidas entre tempo de desenvolvimento em cada temperatura. Pupários não abertos e dissecados mostraram que os parasitóides infectados apresentaram dificuldade para emergir especialmente em 20 and 25°C. Foram encontradas significativas diferenças entre as longevidades de indivíduos infectados e não infectados tratados com mel e água. Para aqueles tratados somente com água não houve significativa diferença.

Os resultados obtidos para ataque ao hospedeiro demonstraram que exceto para o número de larvas de *M. domestica* mortas e número de larvas mortas do parasitóide não houve significativa diferença entre infectados e não-infectados. Para *Sarcophaga bullata* (Sarcophagidae) foi observado que não somente o número de hospedeiros mortos e pupários contendo larvas do parasitóide mortas foi significativamente diferente para infectados e não-infectados, mas também foi para o número de indivíduos adultos. Nem para longevidade, nem para número de fêmeas da progênie foi observada significativa diferença entre grupos infectados e não infectados. Para os outros aspectos avaliados como: número de hospedeiros mortos, número de machos da progênie e total de indivíduos da progênie, não houve significativa diferença entre as médias calculadas para infectados e não-infectados. Nenhuma diferença foi observada entre número de hospedeiros mortos por infectados e não-infectados durante os primeiros dias de vida do parasitóide. Do 6° ao 24° dias de vida, fêmeas não infectadas, mantidas em 15°C e alimentadas com mel apresentaram bom desempenho reprodutivo com relação ao número de hospedeiros mortos e o total de parasitóides da progênie, indicando que estas constituem adequadas condições para armazenamento dos insetos em cultura.

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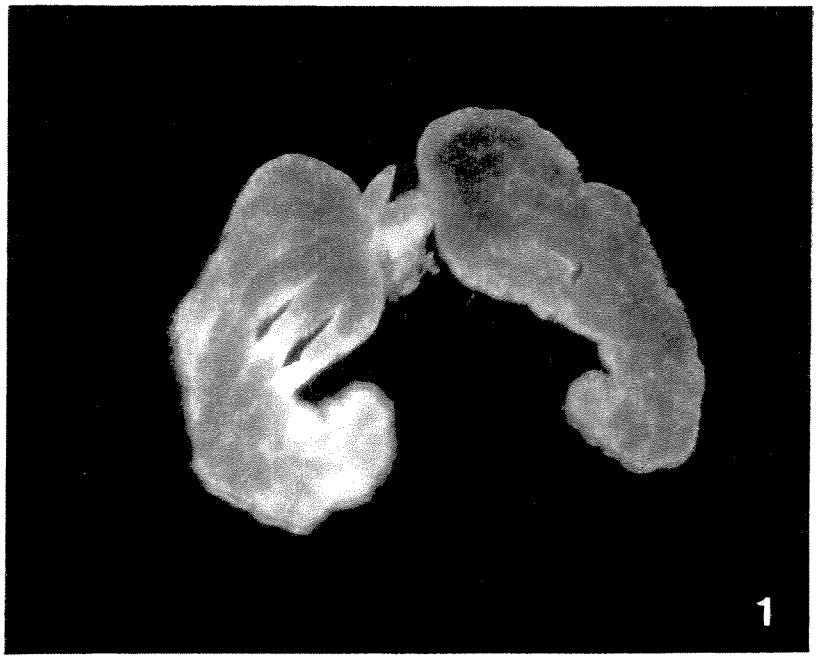
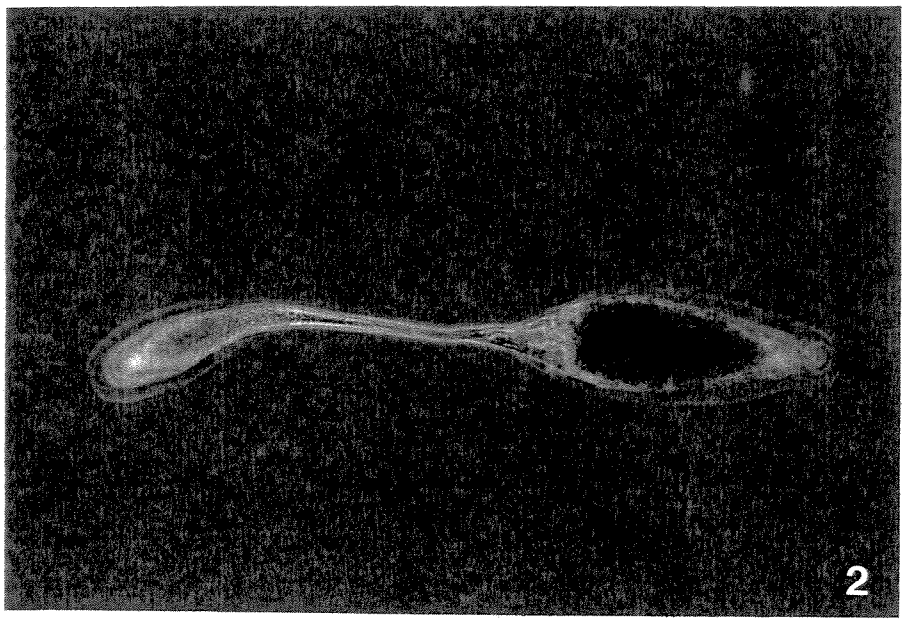
ANEXO 1



Ovários de *T. zealandicus*, material fresco (230X).

ANEXO 2



Ovócito extraído do ovário de *T. zealandicus*,

material fresco (940X).

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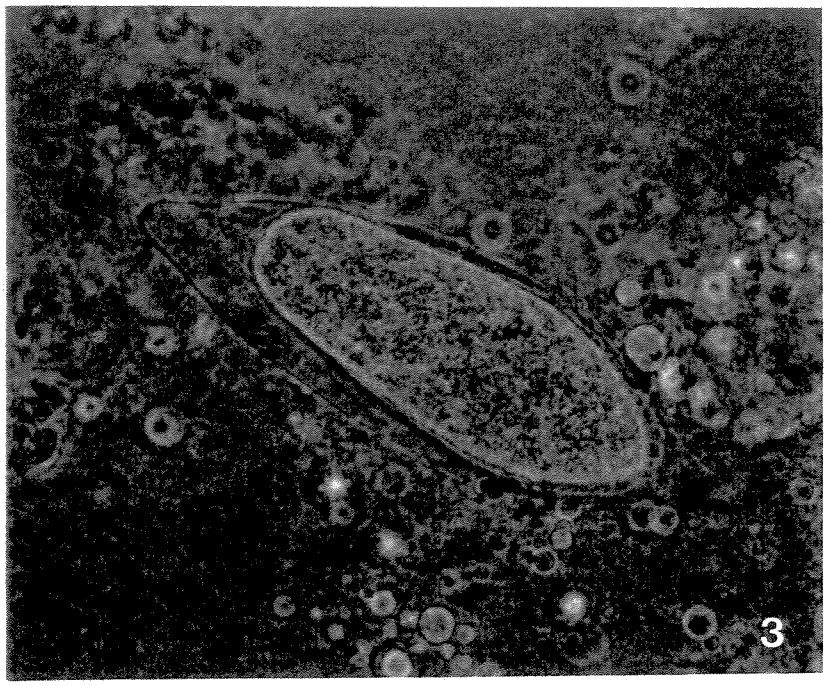
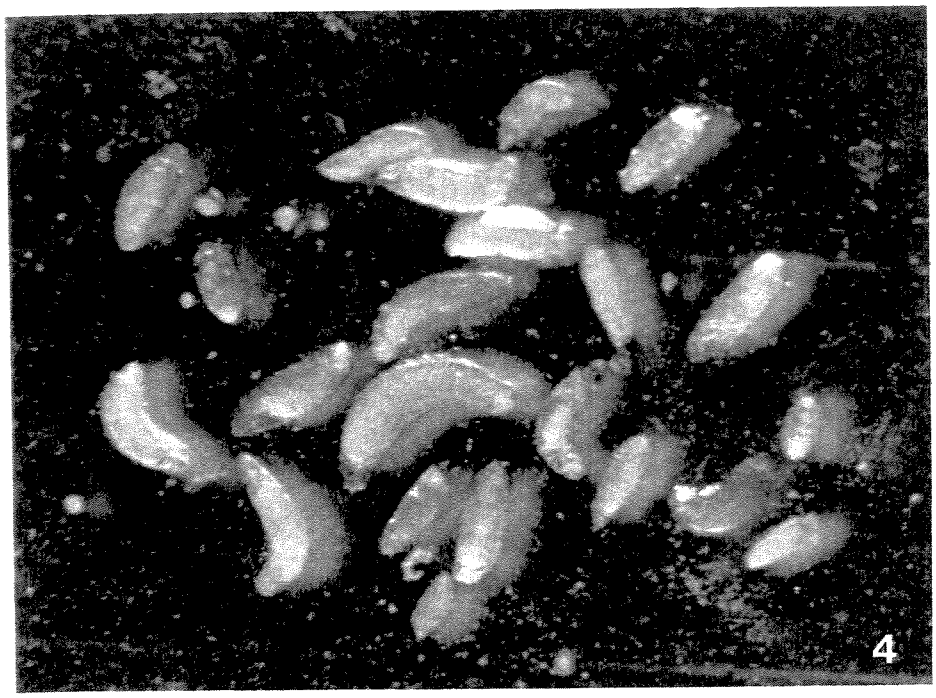


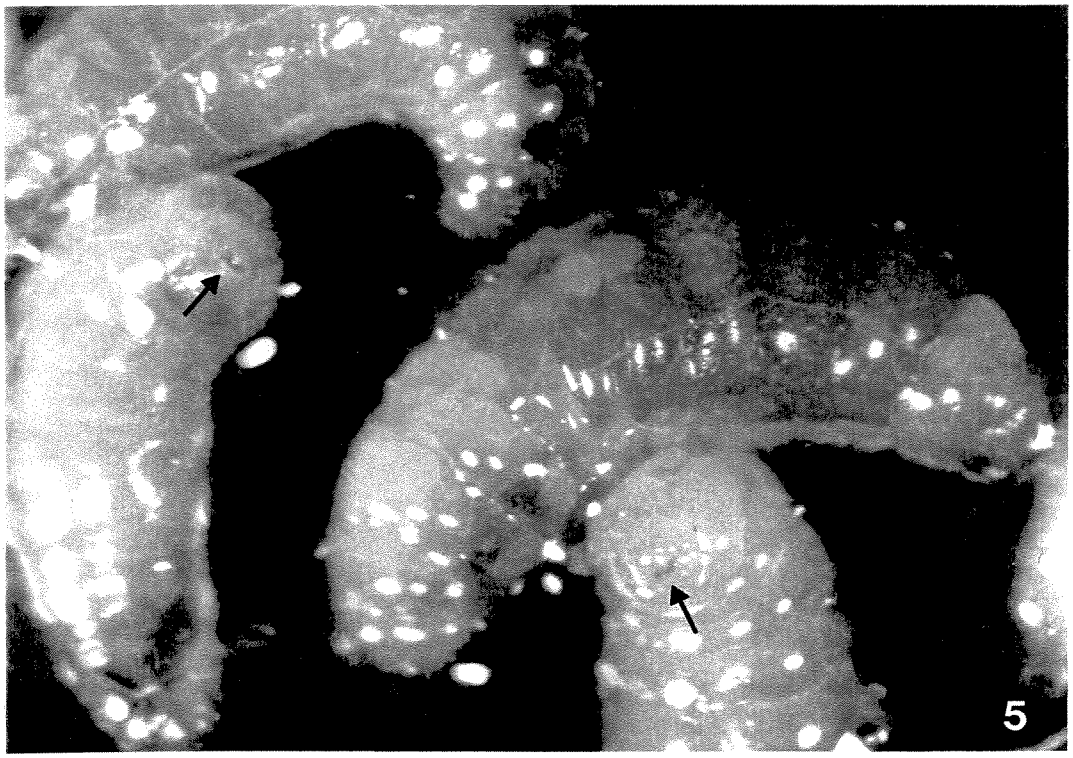
Ovo de *T. zealandicus* extraído da pupa de *Chrysomya putoria*, 30 horas após exposição e mantidas em 25°C, material fresco (1800X).

ANEXO 4



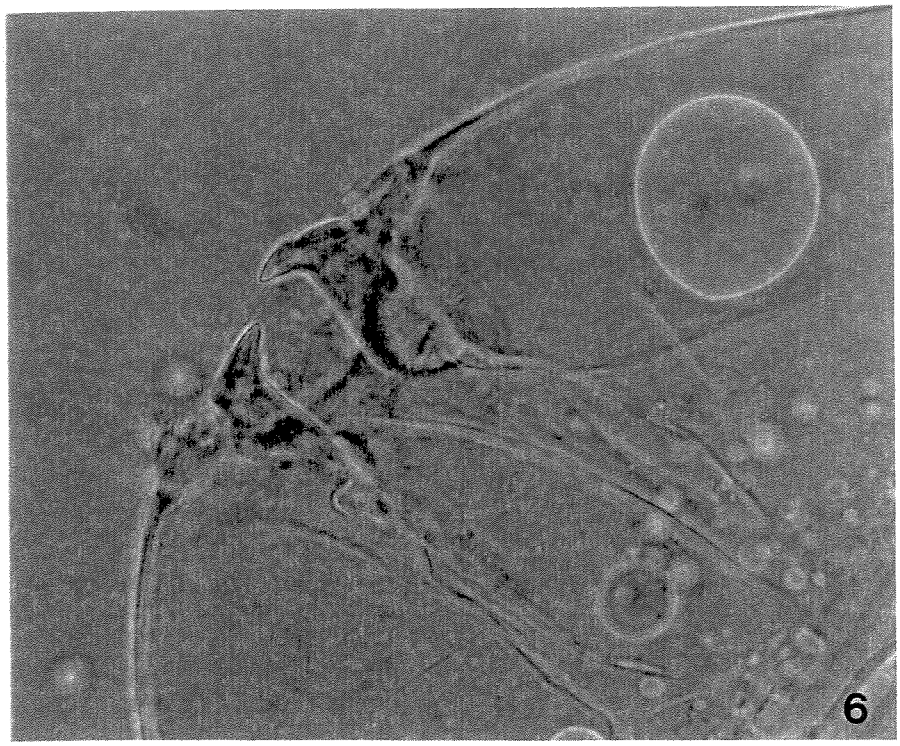
Larvas de *T. zealandicus* extraídas do pupário dissecado de *C. putoria*, 4 dias após exposição e mantidas em 25°C, material fresco (92X).

ANEXO 5



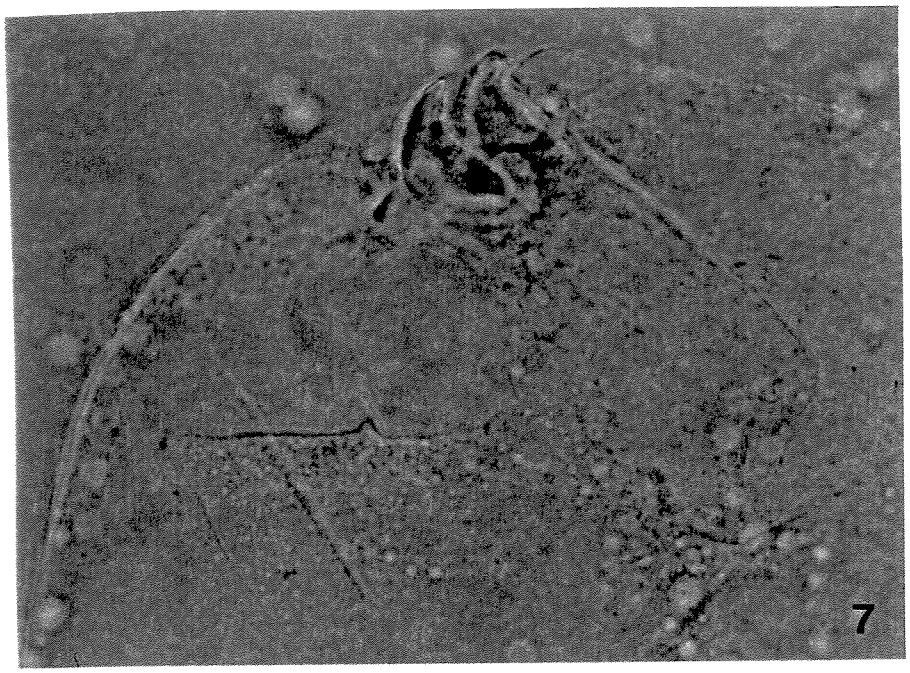
Larvas de *T. zealandicus* extraídas do pupário dissecado de *C. putoria*, 7 dias após exposição e mantidas em 25°C, material fresco. A coloração vermelha da larva se deve à ingestão de pigmentos dos olhos do hospedeiro. Par de mandíbulas quitinosas (setas) (144X).

ANEXO 6



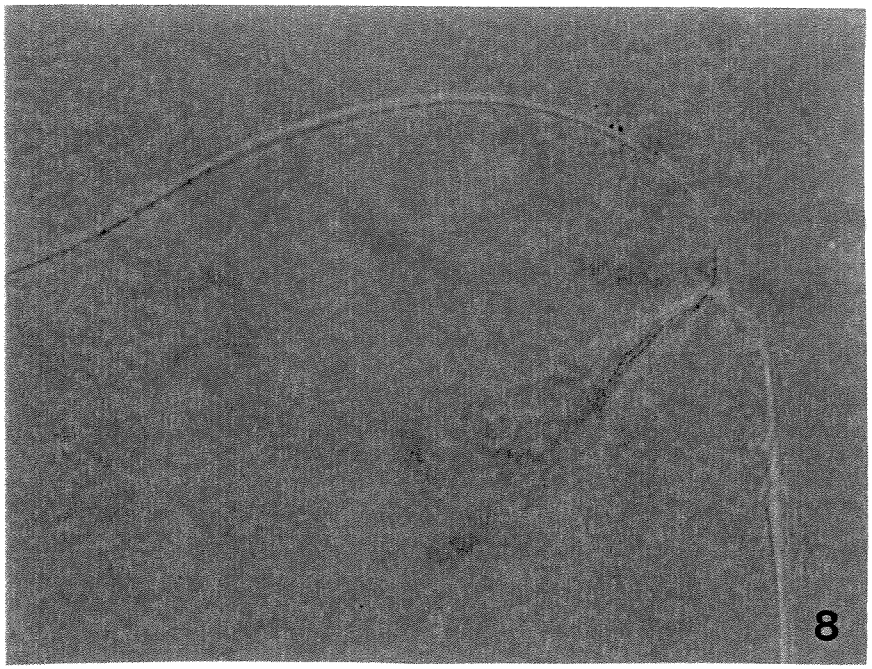
Vista ventral das mandíbulas da larva de *T. zealandicus* extraídas do pupário dissecado de *C. putoria*, 5 dias após exposição e mantidas em 25°C, material fixado em solução de Hoyer (2000X).

ANEXO 7



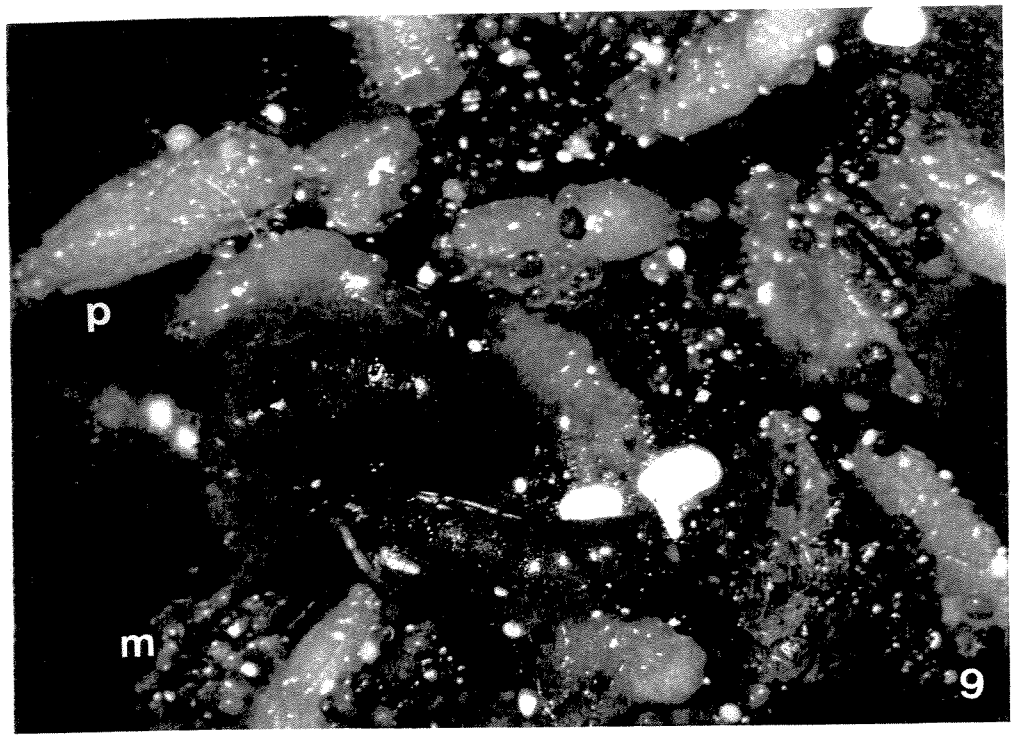
Vista lateral das mandíbulas da larva de *T. zealandicus* extraídas do pupário dissecado de *C. putoria*, 5 dias após exposição e mantidas em 25°C, material fixado em solução de Hoyer (2000X).

ANEXO 8



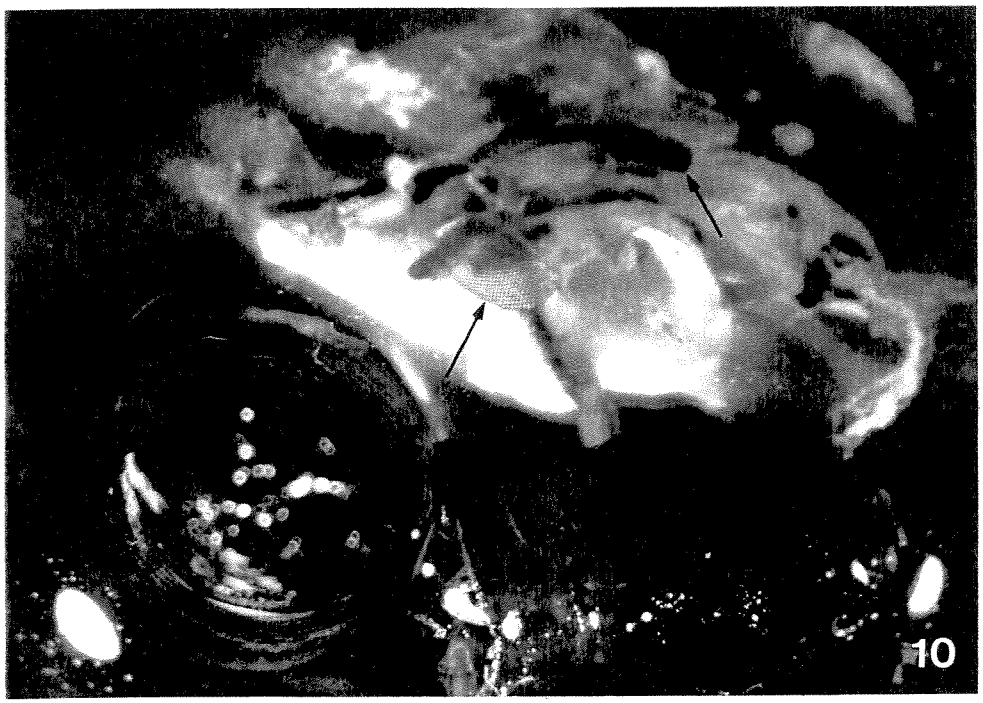
Extremidade distal da larva de *T. zealandicus* extraída do pupário dissecado de *C. putoria*, 11 dias após exposição e mantidas em 25°C, material preparado em Hoyer (2000X).

ANEXO 9



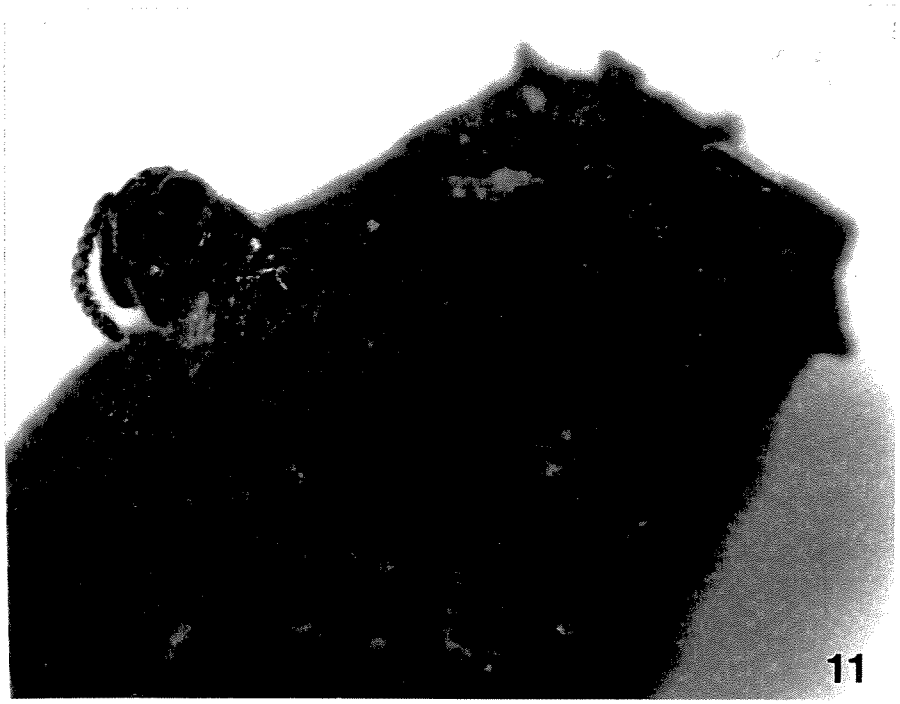
Larvas de *T. zealandicus* extraídas do pupário dissecado de *C. putoria*, 14 dias após exposição e mantidas em 25°C, material fresco. A coloração vermelha da larva se deve à ingestão de pigmentos dos olhos do hospedeiro. Notar a presença de pupas do parasitóide (p) e mecônio (m) com forma característica eliminado pela larva de último instar (72X).

ANEXO 10



Restos da pupa de *C. putoria*, 7 dias após exposição ao parasitóide e mantidas em 25°C, material fresco. Notar os restos de olhos e pernas do hospedeiro (setas) (72X).

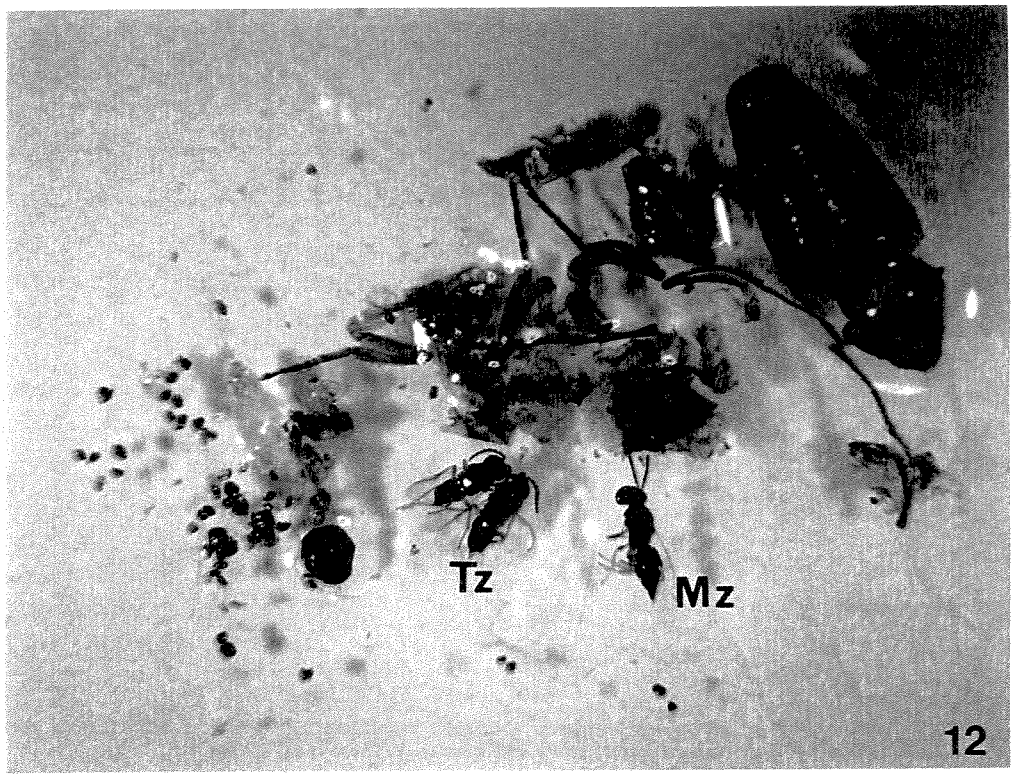
ANEXO 11



Fêmea *de T. zealandicus* emergindo do pupário de *C. putoria*

(140X).

ANEXO 12



Pupário de *C. putoria* dissecado após a observação de um caso de multiparasitismo de *T. zealandicus* (Tz) e *Muscidifurax zaraptor* (Mz) (26X).