

Host specificity of *Telenomus nitidulus* (Thomson) (Hymenoptera: Scelionidae), egg parasite of the satin moth, *Leucoma salicis* L.¹⁾

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Introduction

Members of the genus *Telenomus* parasitize insect eggs pertaining primarily to the Lepidoptera and Heteroptera but they have also been found on other insect orders such as the Diptera, Homoptera and Neuroptera. Their natural range in host specificity is extremely varied (Bin and Johnson, 1982): e.g. *Telenomus podisi* Ashmead has been found in 12 genera of the Pentatomidae and Sculleridae (Heteroptera), whereas *Telenomus alsophilae* Viereck is a common egg parasite of the fall canker worm *Alsophila pometaria* (Harris) and the looper *Phigalia titea* Cramer (Talerico, 1968) both belonging to the Geometridae. Fedde (1977), in an experiment to determine the parasitization capabilities of *T. alsophilae* on other hosts, offered eggs of 22 different species in the Geometridae, Noctuidae, Arctiidae, Sphingidae and Saturniidae. Of these 12 species of geometrids (including a species of *Oxydia*) and 2 species of noctuids were successfully parasitized. *T. alsophilae* was introduced in Colombia (South America) and successfully controlled an outbreak of *Oxydia trychiata* (Guenée) in plantations of *Pinus patula* and *Cupressus lusitanica* (Bustillo and Drooz, 1977; Drooz et al. 1977).

On the other hand, *T. clisiocampae* Riley appears to be oligophagous; it has only been reared from eggs of a number of *Malacosoma* spp. (Lasiocampidae) (Bin and Johnson, 1982), whereas *T. californicus* Ashmead is thought to be host-specific for the Douglas-fir tussock moth (*Orgyia pseudotsugata*) (McDunnough) (Lepidoptera: Lymantriidae) (Torgersen and Ryan, 1981). Torgersen and Ryan came to this conclusion because in field plots female *T. californicus* attacked offered egg masses of *O. pseudotsugata* as late as November and as early as April 1, suggesting that the egg parasites had overwintered as adults. The tussock moth egg masses were obtained from rearings on artificial diet and were wired to Douglas fir foliage in the

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field at times when natural oviposition by *O. pseudotsugata* could not possibly have occurred.

T. californicus has been recorded as parasitizing the eggs of the satin moth (Burgess, 1921; Burgess and Crossman, 1972; Muesebeck et al., 1951; Thompson, 1958). However, Johnson, cited by Torgersen and Ryan (1981) suggested that these records are probably erroneous because incomplete taxonomical knowledge and the lack of keys hampered correct identification.

With respect to the host specificity of *Telenomus nitidulus*, Brown (1931) states: "Eggs from about 25 different species of Lepidoptera were tried but only slight and not at all natural oviposition was secured on eggs of two species, namely *Porthetria* (*Lymantria*) *dispar* and *Olene* sp. It is evident that this species attacks the eggs of other Lepidoptera, for it is multiple-brooded and cannot hibernate in satin moth eggs. It may, however, hibernate as an adult, but this has not as yet been determined".

Apart from *Leucoma salicis* and the two above-mentioned Lepidoptera the following alternative hosts have been mentioned in the literature: *Agrotis segetum* (Noctuidae) (Kozlov and Kononova, 1983) and *Mamestra brassicae* (Noctuidae) (Bírová, 1979).

This research on the host specificity of *T. nitidulus*, resulted from an outbreak of the satin moth in Flevo-land, The Netherlands, where 258 ha of poplar plantations were defoliated in 1983². The plantations consisted mainly of clones belonging to the Aigeiros section, such as *Populus* 'Robusta', P. 'Zeeland', and P. 'Flevo'. However, clones of *P. canescens* in the area were also attacked.

The main purpose of the research project is to investigate whether a biological control method can be developed in which "open" pheromone traps loaded with a pathogen can be used to disseminate the disease among the satin moth population. Part of the research programme, however, is dedicated to making an inventory and investigating promising biological control agents, such as the egg parasite *Telenomus nitidulus*.

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Material and methods

Parasitized egg masses of the satin moth were collected in July 1983 in the Hollandse Hout, Oostelijk Flevoland. Emerged specimens of *Telenomus* were sent for identification to Dr. Lars Huggert, Zoological Institute, Lund, Sweden whereas emerged specimens of *Trichogramma* were sent to Dr. B. Pintureau, Station de Zoologie et de Lutte Biologique, Antibes, France. The remaining *Telenomus* population that emerged was used to study the biology and oviposition behaviour of this egg parasite and to investigate its hibernating capacity. As Brown (1931) noted, the adult stage of this egg parasite must hibernate if it is host specific, unless it can hibernate in its host eggs in the egg or larval stage (which he ruled out). The following experiments were carried out to locate the hibernating site and to determine the hibernating capacity of *T. nitidulus* in the field as well as under more controlled conditions.

Experiment 1. Egg masses of the satin moth obtained from a laboratory rearing on poplar leaves, were attached to poplar trees in the outbreak area on 10 May 1983. In The Netherlands, satin moth larvae never pupate before June; thus parasitization in May would indicate that *Telenomus* adults were already present in the adult stage before eggs of the host were available. On 18 May the egg masses were inspected.

Experiment 2. At the end of August 1983, 100 heavily parasitized egg masses in the outbreak area were marked with coloured pins to facilitate recovery at a later stage. During autumn and winter, old egg masses are difficult to locate because they become covered with green algae. This experiment was set up because it had been noted earlier in laboratory studies that after having been denied food or new host eggs, *Telenomus* would re-enter old egg masses that had little or no spumilin cover and hide in empty egg cases from which *L. salicis* larvae had already emerged. A very typical resting posture (antennae folded underneath bowed head and thorax) was adopted in these cases. On 10 January 1984, forty of these egg masses were retrieved and placed in a climatic chamber (L:D = 16:8 h; temp. = 24 °C;) to allow any eggs or larvae to develop into adults, or to encourage hiding adults to emerge.

Experiment 3. Female adults of *T. nitidulus* emerging from field-collected egg masses on 24 June and during the month of July 1983, were placed in polystyrene vials of 6 cm length and 2 cm diameter, provided with droplets of honey and closed off with air-permeable polyether stoppers. The females were kept in reserve for studying the oviposition behaviour of the parasite. For this reason no males were included in this experi-

ment. The vials were kept in the same climatic chamber as described earlier. On 11 and 12 October 1983, surviving females were given new vials and honey; these vials were then packed in plastic boxes and placed on trays in a large perforated plastic bag on top of the soil, under the canopy of a small stand of Douglas fir. A few fir branches were used to prevent direct sunlight from penetrating the boxes.

On 3 November 1983 when the vials were checked for survival of the parasites it appeared that many had drowned in the honey. Therefore it was decided to divide the vials into three groups, each of which was given an additional treatment:

Treatment I: fresh droplets of honey were provided.

Treatment II: a small wad of filter paper was inserted into the vials to provide parasites with a dry hiding place.

Treatment III: a small wetted plug of cotton was inserted to provide the parasites with water instead of honey.

On 13 and 27 April and on 2 May 1984, the vials were checked for surviving *T. nitidulus* females. One vial containing the oldest batch of 8 females was checked on 29 June 1984, 370 days after the parasites had emerged.

The dead specimens were classified into three categories on the basis of possible cause of death: drowned in honey; fungal attack, and other causes. The latter category comprised adults that had died because of lack of vigour.

Specimens of *T. nitidulus* from which fungi were growing were sent to Dr. R. A. Samson, Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands, for identification.

Records of minimum temperature and relative humidity during the period were obtained from the weather station of the Agricultural University at Wageningen, in order to gain a general impression of the frost conditions during the winter 1983/84.

An analysis of variance was carried out to determine any significant differences in mortality between the three treatments.

Experiment 4. On 3 April 1984, samples of soil, litter, bark and hibernacula of overwintering satin moth larvae were collected from the outbreak area, to locate possible hibernation sites of adult *T. nitidulus*. These samples were again taken from the poplar stands in the outbreak area. Soil and litter samples were taken directly at the foot of the trees; bark samples were chiselled out and cut off from protruding bark, mostly from branch scars at heights between 0.60-1.60 m. Hibernacula from *L. salicis* larvae were collected from crevices in the bark at the same heights. Between 15 and 25 trees were sampled in this manner. The sam-

ples were then placed in the climatic chamber for a period of three weeks to monitor the emergence of any parasite adults.

Experiment 5. On 21 October 1983, in order to test whether *T. nitidulus* could hibernate in the eggs of *L. salicis*, 6 satin moth egg masses, each containing between 30-40 eggs, were offered upside down (with the spumilin layer down, to obtain maximum parasitization) to *T. nitidulus* females in the climatic chamber for a period of three days. On 24, 25 and 26 October 1983 respectively, two vials containing one egg mass each were placed under the canopy of the Douglas-fir trees in the same manner as earlier described. The maximum ages of any developing *T. nitidulus* in each pair of vials on the day the vials were placed outdoors would therefore have been three, four, or five days old, respectively, and the corresponding minimum ages would have been zero, one, or two days old.

All vials were retrieved on 2 May 1984 and placed in a climatic chamber to monitor any further development of the parasite larvae. On that date all eggs were already black, indicating that parasitization and development had taken place. The vials were kept in the climatic chamber for a period of 14 days, after which the eggs were opened to check if any living larvae or pupae of *T. nitidulus* were present.

In the same experiment unparasitized egg masses of mated females of *L. salicis* from the rearing, oviposited on 15 October 1983 were kept for three days in a refrigerator at 6 °C and thereafter placed under the previously described outdoor conditions. In addition, one batch of unparasitized egg masses of mated female *L. salicis* was placed in a climatic chamber for 3 days, and another batch for 6 days, to allow partial development of the larvae before they were included in the outdoor experiment. These batches were also retrieved on 2 May 1984, placed in the climatic chamber for a period of 14 days and subsequently checked for the hatching or development of *L. salicis* larvae.

Experiment 6. During 1983 and 1984 fresh eggs of the following lepidopterans were offered for a period of 10 days to female *T. nitidulus* for parasitization: *Mamestra*

brassicae (Noctuidae), *Lymantria dispar* (Lymantriidae) and *Laotoë populi* (Sphingidae). Subsequent oviposition behaviour of the parasites in vials was followed under laboratory conditions.

Results and discussion

The results of the six experiments carried out to locate the hibernation site, capacity to hibernate and host specificity are given in the sequence indicated under materials and methods. The parasites that emerged from the egg masses of the satin moth were identified as *Telenomus nitidulus* (Thomson) and *Trichogramma embryophagum* Hartig. The latter egg parasite was far less frequent than *T. nitidulus*.

Experiment 1. On 18 May 1984, several *T. nitidulus* females were found on the egg masses that had been attached to the boles of the poplar trees in the Hollandse Hout, indicating that the parasite probably hibernates in the adult stage.

Experiment 2. No *Telenomus* adults emerged from the heavily parasitized egg masses that had been marked in August 1983 and were retrieved in January 1984 from the poplar stands, suggesting that in The Netherlands the parasite does not hibernate as egg, larva or adult in egg masses of the satin moth parasitized at the end of June or in July. The hiding of parasite adults in empty egg shells left by first instar *L. salicis* larvae must be considered as abnormal behaviour caused by the fact this was only hiding place offered in the vials. The propensity of *T. nitidulus* to hide itself and to adopt a typical "resting" posture was also observed under outdoor conditions in the vials containing a small wad of paper. Several of the egg parasites had also entered cells of the polyether stopper to hide and shelter themselves.

Experiment 3. Survival of female *T. nitidulus* in vials under a canopy of Douglas fir trees. Although clear differences exist in the number of surviving parasites in the three treatments, it is obvious that this egg parasite hibernates in the adult stage (Table 1). During the peri-

Table 1 Survival of hibernating female *T. nitidulus* in vials under the canopy of a Douglas fir stand (Experiment 3). Survival ascertained on 27 April 1984.

Treatment in vials on 3 November 1983	no. of vials	original no. of <i>T. nitidulus</i>	surviving no. of <i>T. nitidulus</i>	average age (days)	mortality causes		
					drowned in honey	fungi	other
wet cotton plug	14	115	11 (9.5%)	273.8	48 (41.7%)	32 (27.8%)	24 (20.9%)
small paper wad	23	162	85 (52.4%)	281.4	41 (25.3%)	0 (0%)	36 (22.2%)
fresh honey	13	86	65 (75.6%)	279.5*)	17 (19.8%)	0 (0%)	4 (4.7%)

*) In one vial of this treatment, 7 females (87.5% survival) were 370 days old on the day the manuscript was completed (29 June 1984).

od of observation, light, moderate and heavy frosts occurred on 74, 13 and 1 days, respectively. The lowest temperature recorded was $-11,1^{\circ}\text{C}$. The relative humidity varied between 41 and 98 per cent, averaging 80 per cent.

Of the parasites that had received additional drop-lets of honey on 3 November 1983, there was 75.6 per cent survival on the days of inspection (13 and 27 April 1984). In the vial that contained the oldest batch of 8 female *T. nitidulus* (emergence of the egg parasites on 24 June 1983), 7 females (87.5% survival) were 370 days old on 29 June 1984 and very vital. Second best results were obtained from the treatment in which a small wad of paper was provided to give adult females shelter and a hiding place. In these vials more than 52 per cent of the females had survived on 27 April 1984. The addition of a wet cotton plug in the vials was very detrimental to the survival of the egg parasite. Only in this treatment did fungi grow on the parasites (approximately 28 per cent dead specimens). However, the fungus (*Penicillium? brevicompactum* Dierckx) is thought to be saprophytic. Differences in survival between the three treatments were highly significant ($P < 0.001$). A better method of providing honey to the adults might improve the overall survival rate even more.

Experiment 4. The field sampling of soil and litter, bark and winter hibernacula of *L. salicis* larvae carried out on 3 April 1984 (temp. = 6°C) yielded nine female *T. nitidulus* from the bark samples. In the laboratory the egg parasites were observed on 4 April (4 specimens), 5 April (4 specimens) and 7 April (1 specimen). Egg masses of the satin moth obtained from a rearing on poplar leaves, were being parasitized after 15 minutes of exposure to these females and resulted in a new generation of the egg parasites. No egg parasites emerged from soil and litter, nor from hibernacula of hibernating satin moth larvae. Since all 9 parasites were females it seems unlikely that many males hibernate. The progeny of the *T. nitidulus* consisted of both males and females: *T. nitidulus* is arrhenotokous, and therefore the females obtained from the bark samples had obviously mated before winter.

Experiment 5. From the batches of recently parasitized eggs that were placed under outdoor conditions on 24, 25 and 26 October 1983 no *Telenomus* adults emerged in May 1984. When the eggs were opened after having been in the climatic chamber for a period of 14 days, no living larvae, pupae nor adults were found. The contents of the eggs varied from a brown slime in eggs that had been parasitized most recently before they were placed outdoors, to greenish gelatinous or hardened contents that covered half of the egg in egg-

masses that had had a maximum parasite development of five days before being placed outdoors. Although all eggs were black before they were brought in the climate chamber on 2 May 1984, developing *T. nitidulus* had died during the winter.

No first instar larvae hatched from the unparasitized egg masses of mated *L. salicis* females that were placed outdoors on 18 October 1983. The eggs contained dead embryos and a brownish slime, indicating that under the conditions of this experiment *L. salicis* could not hibernate in the egg stage.

Experiment 6. Fresh eggs of *Mamestra brassicae* (Noctuidae) *Lymantria dispar* (Lymantriidae) and *Laotioë populi* (Sphingidae) offered to *T. nitidulus* females were inspected by the parasites but oviposition never occurred. *Mamestra brassicae* has been recorded by Bírová (1979) as a host of *T. nitidulus* in Slovakia. With respect to *Lymantria dispar*, Brown (1931) had already noted that parasitization by *T. nitidulus* "was only slight and not at all natural". In this experiment no oviposition took place. The parasites encountered great difficulties in walking over the hairy egg masses of this lepidopteran. In contrast, *T. nitidulus* appears to have adapted itself particularly to the egg masses of the satin moth, which are covered with a hardened spumilin. In order to reach the eggs underneath this cover, the female egg parasites bite tunnels through the spumilin and subsequently turn around to parasitize the eggs (Figs. 1-4). This behaviour has also been reported by Nef (1983). The polyphagous *Agrotis segetum* (Noctuidae), which is mentioned by Kozlov and Kononova (1983) as an autumn host for *T. nitidulus*, has been recorded as a defoliator of *Salix* sp., a tree that is also attacked by *L. salicis*. However, eggs of *A. segetum* are deposited singly (only very rarely in small groups) on the soil; they are not covered by spumilin. Moreover, in our trials, *T. nitidulus* could not survive in the egg or larval stage in the eggs of its host *L. salicis*. These differences make it unlikely that the noctuid *A. segetum* can be considered as a host. Kozlov in an earlier publication (1967) only mentioned *L. salicis* as a host for *T. nitidulus*. In a personal communication to the author, Huggert said that he once found overwintering *T. nitidulus* in the melting snow at the base of trees in Sweden.

As indicated earlier, the record of *Telenomus californicus* on eggs of the satin moth is believed to be erroneous (Torgersen and Ryan, 1981). These authors consider *T. californicus* to be host-specific for *Orgyia pseudotsugata*.

Summary

During an outbreak of the satin moth, which in 1983



Fig. 1 Selection of tunnelling site.



Fig. 2 Digging the tunnel through the spumilin cover.



Fig. 3 Oviposition in host egg.



Fig. 4 Marking spumilin in and near tunnel.

covered 258 ha of 11-year-old poplar and willow stands in the Flevopolder, The Netherlands, six experiments were carried out to investigate the host specificity of *Telenomus nitidulus*, an egg parasite of *Leucoma salicis*. The following results were obtained:

– Egg masses of the satin moth obtained from an artificial rearing and attached to poplar trees in the outbreak area on 10 May had been parasitized by *T. nitidulus* by 18 May, i.e. at a time when no eggs of the satin moth are naturally available. This finding indicated that if *T. nitidulus* were host specific, it must hibernate in the adult stage.

– From egg masses that had been heavily parasitized by *T. nitidulus* in the summer of 1983, no adults developed in spring 1984, indicating that the parasite does not hibernate in the egg or larval stage in egg masses oviposited by the satin moth during the summer.

– Female *T. nitidulus* emerging from naturally parasitized egg masses of the satin moth in June 1983 were kept in a climatic chamber at 24° C and placed in vials outdoors in July 1983. On 25 June 1984, the oldest group of *T. nitidulus* had survived for a period of 370 days and was still vital (87.5% survival). Seventy-five per cent of a group of female *T. nitidulus* that had received extra honey on 3 November 1983 were still alive on 27 April 1984, the day of inspection, notwithstanding 69 days of light frost, 13 days of moderate frost and one day of severe frost during that period.

– Samples of bark, soil, litter as well as hibernacula of hibernating *L. salicis* larvae were retrieved from the outbreak area on 3 April 1984. Nine female *T. nitidulus* emerged from the samples taken from the bark of poplars. No parasites emerged from any of the other samples.

– Developing eggs and larvae of *T. nitidulus* in host eggs placed under outdoor conditions in October 1983 did not survive winter conditions. Unparasitized *L. salicis* eggs from mated females were also killed during the winter.

– Eggs of *Mamestra brassicae* (Noctuidae) and *Lymantria dispar* (Lymantriidae), which some authors have claimed are hosts for *T. nitidulus*, were refused by the egg parasite. As the eggs and larvae of *T. nitidulus* died in its host under winter conditions, Kozlov and Kononova's contention that the parasite would survive in eggs of *Agrotis segetum* oviposited during autumn seems unlikely.

From the above results it is concluded that the egg parasite *T. nitidulus* is host-specific for the satin moth (*Leucoma salicis*).

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Samenvatting

De gastheerspecificiteit van *Telenomus nitidulus* (Thomson), eiparasiet van de satijnvlinder

De gastheerspecificiteit of monofagie van een parasiet (dat wil zeggen dat de parasiet maar één andere insectesoort parasiteert), wordt als een belangrijke eigenschap beschouwd omdat dergelijke parasieten vaak in staat zijn om insectenplagen effectief te onderdrukken (DeBach, 1964; 1971).

Over de gastheerspecificiteit van *Telenomus nitidulus*, die de eitjes van de satijnvlinder parasiteert, bestaat in de literatuur de nodige verwarring. Verscheidene auteurs (Brown, 1983; Bírová, 1979; Kozlov, 1983; Muesebeck et al., 1951, Thompson, 1958) geven aan dat ook eitjes van andere vlindersoorten geparasiteerd worden.

Naar aanleiding van een satijnvlinderplaag in Oostelijk Flevoland die in 1981, 30 ha populier en wilg besloeg en zich in 1982 en 1983 tot respectievelijk 80 ha en 258 ha uitbreidde, werd een zestal experimenten met deze eiparasiet uitgevoerd, die het volgende uitwezen:

- Op eihooptjes van de satijnvlinder, die door middel van een kunstmatige kweek op populiereblad vroegtijdig verkregen waren en die op 10 mei 1983 uitgezet werden op populieren in het aantastingsgebied, werden bij controle op 18 mei van dat jaar parasiterende *T. nitidulus* aangetroffen. Hieruit kan worden geconcludeerd dat als deze eiparasiet gastheerspecifiek zou zijn, zij hoogst waarschijnlijk in het volwassen stadium zou overwinteren. De eitjes van de satijnvlinder zijn immers op zijn vroegst pas vanaf midden juni beschikbaar.
- Uit eihooptjes van de satijnvlinders, die in de zomer van 1983 geparasiteerd waren, ontwikkelden zich in het voorjaar van 1984 geen parasieten meer. "Overliggen" van de parasiet of diapauze van het ei of de larve

in de oude eihooptjes kon in Nederland dus uitgesloten worden.

- Vrouwelijke eiparasieten die in de winter van 1983/84 in buisjes in de buitenlucht bewaard werden, hadden op 25 juni 1984 een leeftijd van 366 dagen en waren (letterlijk) nog springlevend. Van 86 vrouwelijke *T. nitidulus* die extra honing in de buisjes hadden gekregen op 3 november 1983, waren er op 27 april 1984 nog 65 (75,6%) in leven ondanks het feit dat in de tussentijdse periode 74 dagen met lichte vorst, 13 dagen met matige en één dag met strenge vorst optraden.

- In het aantastingsgebied van de satijnvlinder werden op 3 april 1984 schors-, grond- en strooiselmonsters verzameld om de overwinteringsplaats van de parasiet te bepalen. Uit de schorsmonsters van populier kwamen negen vrouwelijke *T. nitidulus* te voorschijn. *T. nitidulus* overwintert dus in elk geval achter de schors van populieren in ons land. Uit de grond- en strooiselmonsters die direct aan de voet van in 1983 aangetaste populieren waren genomen kwamen geen eiparasieten te voorschijn; evenmin uit spinsels van overwinterende satijnvlinderlarven.

- Eieren en larven van de parasiet die op 21-25 oktober 1983 in eihooptjes van de satijnvlinder tot ontwikkeling kwamen en daarna onder buitenluchtomstandigheden in buisjes werden bewaard, bleken op 2 mei 1984 alle dood te zijn. Ook ongeparasiteerde, bevruchte eieren van de satijnvlinder van dezelfde data stierven in de winter af.

- Eitjes van *Mamestra brassicae* (Noctuidae) en *Lymantria dispar* (Lymantriidae) die in de literatuur worden genoemd, werden steeds door de eiparasiet geweigerd. *T. nitidulus* maakt in het dekschuim van de eihooptjes van de satijnvlinder verticale gangetjes, waarna zij zich om draait en de onder het schuim liggende eitjes parasiteert. Dit wijst op verregaand aangepast parasiteringsgedrag en dus op specificiteit. Eitjes van *Lothoë populi* (Sphingidae) werden eveneens geweigerd.

Uit de resultaten van deze proeven blijkt dat de eiparasiet van de satijnvlinder als imago overwintert, en dat haar een dusdanig lang leven is beschoren, dat zij in staat is de twaalf maanden te overbruggen die elk jaar tussen de eilegperiodes van de satijnvlinder liggen. De hieruit voortvloeiende conclusie is dat zij dan gastheerspecifiek moet zijn. Aangezien uit de bastmonsters van populier alleen negen vrouwtjes tevoorschijn kwamen en geen enkel mannetje lijkt het waarschijnlijk dat alleen of hoofdzakelijk de vrouwelijke eiparasieten overwinteren. Een van de mogelijke voedselbronnen voor de eiparasieten is de honingdauw die door luizen op populier wordt geproduceerd. Onder laboratoriumomstandigheden werd deze gretig als voedsel aanvaard.