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Impact of the host stage on the pear psyllid parasitoid Trechnites insidious, behaviour and fitness

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Acknowledgment

This research was supported by the grant 12/1/7798 of the PPP call of the Walloon Region, by an additional grant from Viridaxis S.A. and by the Interreg-Proverbio project funded by SPW-Feder-Interreg (https://www.interreg-fwvl.eu).

Abstract

The pear tree is a main economical orchard crops under temperate climate with more than twenty-five million tons of fruit produced each year. The psylla *Cacopsylla pyri* is the most important pest, as it infests all commercial pear tree varieties, it sucks the phloem sap and it cause damages to the trees by nutrient subtraction and disease transmission (sooty molds and phytoplasm). The management of this insect is mainly based on integrated pest management with a mix of natural and chemical controls. However, with the ban of a growing number of plant protection products and the increasing public interest in an "organic" mode of consumption, it is important to develop innovative ways of pest managements respectful of the environment. *Trechnites insidiosus* is the most abundant parasitoid of *C. pyri* but it has been poorly studied. The aim of this study is to evaluate the parasitism behaviour of *T. insidiosus* toward the different larval stages of *C. pyri,* and to evaluate the quality of the next generation individuals. We observed that stage 3 and 4 larvae are the most interesting hosts for *T. insidiosus* in order to produce individuals in quantity and in quality. This provides a basis for further studies and fulfill the lack of data concerning this insect in the literature, particularly with regard to its biology, its behaviour and its use in biological management.

Key words: *Trechnites insidiosus, Cacopsylla* pyri, Host stage, parasitoid behaviour, parasitoid fitness

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Introduction

Fruit crops represent a major part of agricultural production with more than 865 millions tons of fruit produced each year in the world. The pear tree is one of the main cash crops under temperate climate with 1381923 hectares and a bit less than twenty-four million tons of pear produced in 2018 ("FAOSTAT," 2020)). The European pear psylla *Cacopsylla pyri* L. (Hemiptera Psyllidae) infests all commercial pear tree varieties. It presents five developmental stages in addition to the adults, and all of them (especially the larval stages) suck sap and cause direct damages to pear trees (*Pyrus communis* L.), by nutrient substraction and therefore by weakening the trees and reducing their production. *C. pyri* also produces indirect damages caused by a high honeydew excretion on which the sooty molds can develop (Civolani, 2012). Moreover, they transmit various pathogens such as the phytoplasm *Candidatus Phytoplasma pyri* (Seemüller and Schneider, 2004) which is responsible of the pear decline disease by reducing tree vigour (Civolani, 2012). All these damages makes *C. pyri* the most important pests of European pear trees, which can cause heavy economic losses to most pear tree growing regions (Civolani, 2012).

Presently, *C. pyri* management is mainly based on integrated pest management with a mix of natural and chemical controls (Civolani, 2012). Indeed, an excessive use of non-selective toxic chemicals alone, decreases the effectiveness of these treatments over time because of a resistance that can appear in the treated populations (Buès et al., 2003; Civolani et al., 2007; Erler, 2004)(Buès et al., 2003; Civolani et al., 2007; Erler, 2004a). In addition, with the ban of a growing number of plant protection products and the increasing public interest in an "organic" mode of consumption, it is important to develop innovative ways of pest management respectful of the environment. Biological control and the use of beneficial insects could be then a solution for pest management in pear orchards. The bug *Anthochoris nemoralis* is known to be the main predator of the pear psylla. It is a generalist predator that may also feed on other arthropods, such as aphid mites, and lepidopteran eggs (Emami et al., 2014). However, their presence in pear orchards is generally not sufficient to control psyllid populations, especially at the beginning of the season (Erler, 2004). Indeed, the result can be variable and even in orchards without heavy chemical treatments, the predator population may not develop and the artificial introduction of this predator may represent a relatively high cost (Civolani, 2012). That is why it seems important to find other biological control

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solutions to offer a real alternative to growers wishing to turn towards a more extensive way of production.

Parasitoids because of their specificity, foraging capacity, high fecundity relative to host and their potential absence of negative effect on environment can make them a promising alternative or complement to the use of *A. nemoralis*. The parasitifaune of *C. pyri* is quite diversified and several species are currently mentioned such as *Trechnites insidiosus, Prionomitus mitratus* (Dalman), *P. tiliaris* (Dalma,), *Endopsylla sp*., *Psyllaephagus procerus* Marcet*, Syrphophagus ariantes* (Walker)*, Syrphophagus taeniatus* (Förster) and *Tamarixia* sp (Armand et al., 1990, 1991; Erler, 2004; Guerrieri and Noyes, 2009; Jerinic-Prodanovic et al., 2010). However, these species are difficult to rear and little information are available on their biology and on their potential use in biological control. Among these parasitoids of pear psyllids, *T. insidiosus,* even it is rarely found in pear orchards probably because of a high susceptibility to chemical treatments (Burts, 1983; Lacey et al., 2005; Sanchez, 2012) and a high level of hyperparasitism (Armand et al., 1991, 1990; McMullen, 1966; Sanchez, 2012), this species is regularly cited as the most abundant (Armand et al., 1991, 1990; Avilla and Artigues, 1992; Booth, 1992; Bufaur et al., 2010; Erler, 2004b; Herard, 1985; Miliczky and Horton, 2005; Sanchez, 2012). It is a koinobiont parasitoid that present several interesting characteristics in the biological management of pear psyllids such as a long period of activity as it can be present very early (April) and very late (November) in the season, meaning that it can be active at quite low temperatures (Armand et al., 1991, 1990; Bufaur et al., 2010; DuPont and John Strohm, 2020; Herard, 1985; Oudeh et al., 2013) and a first generation free of hyperparasitism (Armand et al., 1991, 1990). *T. insidiosus* is also the only species that has been used in a biocontrol program against a psyllid pest. It has been introduced in California for the biocontrol of *Cacopsylla pyricola* and while no data is available on its establishment and ability to control the psyllid for this study (Guerrieri and Noyes, 2009), several field studies revealed pics of parasitism that vary between 30 to 100% according to the location (Bufaur et al., 2010; Erler, 2004b; Jaworska et al., 1998; Oudeh et al., 2013), that may lead to an effective control of the psyllid populations (Talitski, 1996 in Unruh et al., 1994).

The aim of this study is to evaluate for the first time in lab conditions the ability of *T. insidiosus* to control *C. pyri*. We first analysed its parasitism behaviour toward the different stages of *C. pyri* and evaluate the quality of the individuals of the following generation by measuring its developmental time, fecundity, size and sex-ratio generally used as proxy to measure the parasitoid fitness (Colinet et al., 2005). The main consensus about the stage preference of *T. insidiosus* for oviposition is that the female lays its eggs in stages 4 and 5 of pear psyllids(Armand et al., 1991, 1990; Booth, 1992) , however, this parasitoid has also been observed to oviposit mainly in larval stages 1, 2, and 3 of

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pear psyllids (McMullen, 1966). We therefore hypothesize, *T. insidiosus* female will be able to lay its eggs in all larval stages of *C. pyri* with a preference for the elder one that probably constitute the best hosts for the parasitoid from a nutritive point of view. We also hypothesize that the adults emerging from the lder stages will also present higher fitnesses than individuals developing in the other stages.

Material and methods

Biological models

The individual used for the experiments were initially collected from populations sampled in 2013 for *Cacopsylla pyri* and in 2016 for *Trechnites insidiosus* in the experimental pear orchard of Proefcentrum voor Fruitteelt, Sint-Truiden-Belgium. The populations were maintained in the laboratory on pear trees in standardized rearing that allow us to know the stage and the age of individuals, with the following climatic conditions: 24°C, 60% RH, and L16D8.

Parasitoid behaviour in relation with host stage

To detect in which stages the parasitoid *T. insidiosus* prefers to lay eggs and the consequences on further development, a group of twenty psyllid larvae of the same stage were placed on an artificial diet and were let for settle during two hours. The differentiation of the larval stages was based on the following descriptions: the three first larval stages are creamy yellow, while the fourth and the fifth stages transit between greenish-brown to dark brown (Chang, 1977). Moreover, first larval stages are the same size of a psyllid eggs, second larval stage are twice bigger and third, fourth and fifth instars have progressively larger wing pads (Chang, 1977). A fertilized *T. insidiosus* female of less than 48 hours was then placed at the center of the set up and its behaviour was recorded for thirty minutes with a Sony handycam (HDR XR200VE) during the afternoon. The use of an artificial diet in the experiment allow to standardize the environment and to minimize its influence on the parasitoid behaviour. One replicate consisted in five set-up (one for each larval stage) and ten replicates were performed. Using the event recorder software ODRec 3.0 (© Samuel Péan), the following behaviours were recorded and quantified: the number of host feeding, the time spent for

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grooming, the time walking, the time resting, the number of antennal contacts, the number of ovipositor insertions.

Parasitoid quality in relation with host stage

After the behavioural bioassays, all the psyllid larvae of a same replicate were placed on a same pear tree for fourteen days. We used in vitro cultivated pear trees (*Pyrus communis*) of the Williams cultivar (between 1 and 2 years old and 0.75–1 m high). Plants were obtained from Battistini Vivai (www.battisti-rebschule.it) and stored in individual cages in a climatic room at a controlled temperature of 24°C. After fourteen days the pear trees were daily checked for mummies and adult psyllids. Each mummy was then isolated in a falcon tube with a drop of honeydew until the emergence of parasitoids. Three days after emergence, parasitoids (male and female) were stored in a freezer at -20°C until size and egg load measurements.

Tibia measurement was used as a proxy for individual body size. The left hind tibia of each emerging individual was measured using the ImageJ 1.440 software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/, 1997–2008)

To estimate their egg load, each emerging female has been dissected as followed: The female was placed on an object blade with a small amount of water and crushed with a coverslip. To better extract the eggs from the abdomen, the pressure exerted on the coverslip started from the head towards the abdomen of the female. Only mature eggs (Figure 1) were counted.

Data analysis

To estimate if the parasitoid accepts to lay eggs in the stages that has been presented to the female, the host acceptance has been calculated as followed: number of ovipositor insertions divided by the number of antennal contacts. The host suitability (number of mummies divided by the number of ovipositor insertions) will then allow us to verify what host stage allows the best development of the parasitoid eggs until the adult stage. The emergence rate has also been calculated as the number of emerging adult parasitoid divided by the total number of mummies. Finally, the sex-ratio was calculated by dividing the number of males by the total number of emerging individuals.

Statistical analysis

Generalized linear models were performed according to the data distribution , in order to test the potential influence of the host stage on the behaviours of the laying female parasitoids: the number of host feeding (Poisson), the time spent for grooming (Gaussian), the time walking (Gaussian), the

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time resting (Poisson), the host acceptance (number of antennal contacts/number of ovipositor insertions (Gaussian)) and on the quality of the emerging parasitoids (the number of mummies (Poisson), the host suitability (Poisson), the emergence rate (Binomial), the egg load (Poisson), and the developmental time (Gaussian)). We also used a generalized linear model (Gaussian distribution) to test the potential influence of the gender and of the host stage on the tibia size of emerging parasitoids. Moreover, Spearman correlations were made for each host stage, between the tibia size and the egg load at emergence of each female. Finally, using χ^2 tests, we compared the experimental results of sex ratio obtained for each larval stage to a 50/50 theoretical sex ratio.

Statistical analyses were performed using R version 3.3.3 Copyright (C) 2016 The R Foundation for Statistical Computing for Mac. All tests were applied under two-tailed hypotheses, and the significance level, P, was set at 0.05.

Results

a) Host feeding

The average number of host feeding observed in our experiments was very low, for each stage about 1 in 200 larvae were killed and then eaten by a parasitoid (X^2=0.19, DF=4, P=0.10) (Table 1).

b) Grooming

Grooming was an important part of the behaviour expressed by the parasitoid and was in equal proportion in all tested stages (42%) (F=0.90; DF=4; P=0.47) (Table 1).

c) Time walking

The host stage significantly influenced the walking time of the parasitoid (F=3.15, DF=4, P<0.05). Indeed, in the presence of stages 3, 4 and 5, the parasitoid spent between 33 and 42% of its time walking against 28 and 18% for stages 1 and 2 respectively (st1 VS. St5: t=1.98, P<0.05; st2 VS St3: t=2.48, P<0.05, st2 VS. St4: t=2.09, P<0.05, st2 VS. St5: t=3.36, P< 0.01) (Table 1).

d) Time resting

The host stage presented to the parasitoid had a significant impact on its time spent in resting (X^2=7.088, DF=4, P<0.001). Parasitoids with stages 3, 4 and 5 spent less time resting (2%) than

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those with stages 1 and 2 (17% and 32%, respectively) (st1 VS. St3: t=-1.99, P<0.05; st1 VS. St4: t=-2.04, P<0.05; st1 VS. St5: t=-1.98, P<0.05; st2 VS. St3: t=-2.99, P<0.01; st2 VS. St4: t=-2.51, P<0.05; st2 VS. St5: t=-2.41P<0.05) (Table 1).

g) Antennal contact, ovipositor insertion and host acceptance

The number of antennal contacts varied significantly with the developmental stage of the host ($\chi2$=800.30, DF=4, P<0.03). The minimum value was observed for the stage 2 while the maximum ones were observed in the stage 3 and 4 conditions (Table 1) (st2, VS. st3: t=2.48, P<0.05; st2, VS. st4: t=2.44, P<0.05)

The average number of ovipositor insertions was significantly different between stages ($\chi2$=443.92, DF=4, P<0.01) with stages 2 that received a significantly lower number of ovipositor insertions than stages 3, 4, and 5 (st2, VS. st3: t=2.15, P<0.05; st2, VS. st4: t=2.55, P<0.05; st2, VS. st4: t=2.30, P<0.05).

Moreover, acceptance was significantly different between stages (F=5.01, D=4, P<0.01). Indeed, the average ratio for stages 1, 2, 3 and 4 was around 50%, while for stage 5 it was around 20% (st1 VS. St5: t=3.70, P<0.01; st2 VS. St5: t=2.99, P<0.01; st3 VS. St5: t=3.16, P<0.01; st4 VS. St5: t=3.79, P<0.001) (Figure 2).

h) Number of mummies and host suitability

The average number of mummies was significantly different between stages (X^2=99.30, DF=3, P<0.001) with a higher mean number of mummies for the stages 3 and 4 than for stages 1 and 2 (st1 vs. st3: Z=5.03, P<0.001; st1 vs. St4: Z=4.58, P<0.001; st2 vs. St3: Z=4.94, P<0.001; st2 vs. St4: Z=4.47, P<0.001) (Figure 3).

The host suitability was significantly influenced by the host stage (X^2=4.45, DF=4, P<0.001). The highest ratio was observed for stages 2, 3 and 4 and was around 50% followed by stage 1 (15%) then stage 5 which had not generated any mummies (st1 vs. st2: t=2.67, P<0.01; st1 vs. St3: t=3.23, P<0.01; st1 vs. St4: 2.18, P<0.05) (Figure 4).

i) Emergence rate

The emergence rate did not vary significantly according to the stage (χ^2=44.463, DF=3, P>0.05). On the 162 mummies obtained, 155 emerged and 7 did not. For the stage 3, all mummies emerged

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(72/72), while for stages 1 and 4, two mummies did not emerge (2/14 and 2/60 respectively), and for stage 2, three mummies did not emerge (3/16).

j) Sex ratio

Individuals from stages 1, 2, and 3 had a balanced sex ratio (st1: χ^2=0.5, P>0.5, st2: χ^2=0.8, P>0.4, st3: χ^2=1.48, P>0.2), while individuals from stage 4 presented a sex ratio largely in favor of females (37 females vs. 8 males) (χ^2=8.52, P<0.01) (Table 2).

j) Parasitoid size

The measurement of the tibia, used here as a proxy for the size of the adult, differed significantly between the two sexes, males being smaller than females (F=43.35, DF=1, P<0.001) (Table 2). The measurement of the tibia also varied according to the host stages (F=3.33, DF=3, P<0.05). Individuals coming from a stage 2 larvae were in average smaller than the others (Table 2). No interaction was detected between sex and stage factors (F=0.96, DF=3, P=0.41).

k) The egg load

No impact of the host stage had been observed on the egg load of the female with a mean of around 11 mature eggs in the emerging females of all experimental condition (X^2=549.71, DF=3, P>0.2) (Table 2). However, a significant correlation between the tibia length and the female egg load was observed for females that developed from a stage 4 host (Spearman's R = 0.50, P <0.001, n=46), but not for females from the other stages (st1: Spearman's R = 0.38, P> 0.05, n=9, st2: Spearman's R = 0.66, P> 0.05, n=9, st3: Spearman's R = 0.12, P> 0.05, n=35) (Figure 5). The larger a female is, the more eggs she will have, but only if she has been laid in a stage 4 host.

l) The developmental time

The developmental time was significantly different between host stages (F = 34.35, DF = 3, P<0.001). Individuals laid in stage 1 host take about 31 days to emerge while the others took between 22 and 24 days before emergence (Table 2). There was no significant difference between sexes (F=0.008, DF=1, P=0.78), but the interaction between the stage factor and the sex factor was

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significant (F=2, 76, DF=3, P=0.04). Indeed, females that developed in stage 4 hosts seem to take more time to develop than males (Table 2).

Discussion

According to Armand et al. (Armand et al., 1991, 1990), and Booth, (Booth, 1992), *Trechnites insidiosus* lay their eggs in stages 4 and 5 of pear psyllids while for McMullen (McMullen, 1966) this parasitoid mainly oviposit in the three first larval stages.. In our study we observed that this parasitoid was able to lay eggs in the four first stages of *C. pyri,* with a higher production of mummies for eggs laid in stages 3 and 4. We also observed a lower attraction of the parasitoid toward the two first larval stages with less exploration and more time resting for these developmental stages than for the three others. Moreover, stage 2 received less antennal contact and ovipositor insertion, and stage 1 individuals present a lower suitability for *T. insidiosus* than the other stages. Stages 1 and 2 larvae also represent only 20 % of the total number of mummies produced in this experiment (10% each). In general, a lower parasitism rate of young stages is associated with a higher mortality of the larvae that are more susceptible to the injuries caused at oviposition (sting and/or venom) (Colinet et al., 2005). In addition, the mortality rate for young stages could also be higher because they have to go through at least three stages and thus they have more chance of dying between moults. On the contrary, *T. insidiosus* seems more motivated to find hosts in presence of psyllid stages 3, 4 and 5 as its time in movement is higher and its time in resting lower for these stages than for the two first ones. The clues left by the psyllids (honeydew, exuviae, volatiles...) could stimulate its locomotor behaviour and therefore increase the probability of the parasitoid to find hosts. This behaviour has already been observed in the parasitoid *Psyllaephagus pistaciae* whose searching time, locomotor behaviour, antennal drumming and ovipositor probing was increased by the presence of the pistachio psylla honeydew (Mehrnejad and Copland, 2006). The antochorid predator, *Orius sauteri*, forage more and lay more eggs in the presence of the pear psylla (*Cacopsylla chinensis)* honeydew (Ge et al., 2019). In our case, the fact that only the stages 3, 4 and 5 have this impact on the behaviour of *T. insidiosus* seem to indicate that the amount and/or the quality of the clues present in the environment are important to trigger this stimulation. Determining what are the clues influencing the exploratory behaviour of *T. insidiosus* could be an interesting next step of this study. Moreover, our results clearly showed a lower attraction to the fifth stage as few antennal contacts and ovipositors insertion were made and no mummy was obtained, with this stage which is probably too big and too advanced in its development to allow the development of *T. insidiosus*. Indeed, such as aphids facing a parasitoid, bigger psyllid larval stages are able to run away more easily than younger stages (Villagra et al., 2002). It has also been shown that the last instar of the aphid *Toxoptera citricida* present a greater immune response to parasitism (Walker and Hoy, 2003). The absence of mummy in the stage 5

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could be then explain by a combination between behavioural and immune response of the psyllid to the parasitoid attack (Colinet et al., 2005). This makes this larval stage to avoid absolutely for the breeding of the parasitoid, and for releasing periods.

From *T. insidiosus* point of view, stages 3 and 4 seem to represent the ideal host for laying eggs as 80 % of the mummies obtained in this experiment came from these two stages. Although they are larger and therefore more difficult to manipulate than stage 1 and 2, they seem to be the best candidates for the female parasitoid concerning the trade-off between the amount of food and the defense system. By parasitizing the stage 4 of *C. pyri, T. insidiosus* attack the psyllid population at the end of its developmental cycle. Such a characteristic confers a particularly important efficiency on the population dynamics of its host, and have an immediate repercussion on the resulting imaginal population and therefore on the next psyllid generation.

Concerning the fitness of the parasitoids obtained in our experiments; early stages seem to be chosen to allocate males as a balanced sex ratio has been observed for the three first host stages, while the stage 4 was chosen to lay a majority of females. Indeed, it has already been proven that the host size/stage can influence the sex ratio of the offspring as female are generally laid in large hosts (Bernal et al., 1997; Jervis and Kidd, 1986; Van Den Assem et al., 1982). This strategy corresponds to the host size distribution model, which assume that the amount of resources available for the parasitoid development determines its fitness (Charnov, 1976; Charnov and Skinner, 1985). It is therefore more interesting for a female parasitoid to lay female eggs in big hosts that provide more resources (Jervis and Kidd, 1986) so that they have a high amount or reserve for egg load/production. Our experiments have been done with single females, it would be then interesting to test if this species in competition condition will produce more male, even in big hosts, as predicted by the local mate competition theory (Hamilton, 1967).

Stages 3 and 4 produced larger individuals than stage 2, probably because those stages have more abundant reserves that allow the parasitoid to grow further. More surprisingly, females that have been laid in a stage 1 host appeared to be as large as those, which developed in stage 3 and 4 host. One of the hypothetical mechanisms would be that when an egg is laid in a stage 1 larvae, the egg starts to develop only in specific conditions when the psyllid larvae has reached a specific level of development (Colinet et al., 2005). This hypothesis is supported by the fact that individuals coming from a stage 1 host take more time to develop than individuals from the other stages. It is also possible that the larvae of the parasitoid grow less rapidly in order to keep their host alive longer and thus allow a longer, but complete, development of the adult parasitoid. These hypotheses could be confirmed by the dissection of stage 2, 3 and 4 larvae that has been parasitized at stage 1 and the identification of the level of development of the parasitoid.

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We observed a quite low egg load in *T. insidiosus* female whatever the host stage they developed in, suggesting that this species is synovogenic and will produce eggs all along its life (around 20 days fed in lab conditions). Moreover, it is generally observed in parasitoids that the larger the female, the greater the egg load. In our study, this link is observed only for individuals issued from stage 4, confirming that this larval stage is the most suitable to lay female eggs. A question arises, anyway: Why do some large females have no or few mature eggs? The females just had honey and water before dissection and never met any psyllids. A stimulus such as mating, host feeding (Aung et al., 2012) or simply the presence of psyllids, is perhaps not necessary but would influence the production of eggs.

Finally, although the host-feeding and the grooming have not been impacted by the host stage they seem to play a great role in the ecology of *T. insidiosus*. Host-feeding is the consumption of host fluids exuding from oviposition wounds by the adult female parasitoid (Heimpel and Collier, 1996). This behaviour has already been described in other encyrtidae species (Aung et al., 2012) but never in *T. insidiosus.* The number of host-feeding events we observed in our experiments was very low, probably because the female we used were fed, hydrated and full of eggs therefore their only concern under these conditions was probably to lay a maximum of eggs. Host-feeding exist in *T. insidiosus* but to understand under which conditions this behaviour is expressed, a protocol should probably involve fertilized females but mostly hungry and/or with a low egg load. They would then have to make a choice between feeding to refill their reserve or their egg load or to lay eggs. It is also possible that *T. insidiosus* is able to discriminate between a parasitized and a non-parasitized larva. Therefore, a female arriving in a patch already visited by a conspecific would feed more easily on a host parasitized by a competitor and thus decrease the competition for his own descendants, but this hypothesis remains to be tested.

Grooming represents almost the half of *T. insidiosus* activity. Psyllids and especially the larvae, produce a lot of honeydew (Civolani, 2012), which is highly concentrated in sugar (Le Goff et al., 2019). Therefore, when this substance is on the parasitoid after an ovipositor insertion, it can be the site of a bacterial and/or fungal infection. This situation probably leaded to the selection of individuals that spent a lot of time cleaning themselves (legs, ovipositor antennae…). This behaviour probably helps individuals to live longer but it also contributes to maintain their locomotor activity and their ability to detect hosts. From the psyllid point of view this high honeydew production could also be a protection against parasitoids. Indeed, it has been observed that the honeydew of the pear psylla *Cacopsylla chinensis* limit the foraging behaviour of its predators and might form a defense for the psyllid (Ge et al., 2019). Moreover, such as aphid parasitoids that lost time manipulating and inserting its ovipositor in aphids exuviae, (Muratori et

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al., 2008), *T. insidiosus* has been observed trying to oviposit in drop of honeydew letting the time to psyllid larvae to run away. Finally, the time *T. insidiosus* spend grooming is a time that it does not spent looking for a host. An experiment analyzing the behaviour of the parasitoid with exuviae of the different stages and/or honeydew could be conducted to clarify the role that the psyllid wastes could play in its defense against parasitoids.

The purpose of this paper was to determine some of the biological characteristics of the pear psyllid parasitoid, *Trechnites insidiosus*, and to identify the most interesting stages for the development of this parasitoid. We showed for the first time that stage 3 and 4 larvae are the most interesting in order to produce individuals in quantity and quality. This provides a basis for further studies and fulfill the lack of data concerning this insect in the literature, particularly with regard to its biology, its behaviour and its use in biological management.

Conflict of interest statement

The authors of this article do not present any conflict of interest

Author contribution

GJLG, JB, TH designed the study, GJLG and JB, analyzed the data. BD, OL, GJLG caught the insect to start the rearing, maintained the rearing and the plant cultures. GJLG and TH wrote the manuscript. All authors contributed to manuscript improvement and gave their final approval for publication.

Data availability statement

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Table 1: Mean total number and mean total duration ± standard deviation of the different observed behaviours. Different letters indicate significant differences (n=10)

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Number of antennal contacts	17,50 ± 21,06 ab	9,60 ± 13,66 a	32,10 ± 30,19 b	31,70 ± 16,26 b	16,00 ± 10,19 ab
Number of ovipositor insertions	10,00 ± 13,33 abc	4,70 ± 7,90 ac	14,80 ± 11,72 b	17,80 ± 11,31 bc	4,00 ± 3,62 ac
Number of host feeding	0,00 ± 0,00 a	0,10 ± 0,32 a	0,10 ± 0,32 a	0,10 ± 0,32 a	0,00 ± 0,00 a
Host feeding duration (%)	0,00 ± 0,00 a	0,20 ± 0,62 a	0,01 ± 0,04 a	0,22 ± 0,70 a	0,00 ± 0,00 a
Time walking (%)	28,27 ± 21,72 a	18,49 ± 16,34 b	36,06 ± 18,77 c	33,32 ± 6,91 c	42,26 ± 10,73 c
Time resting (%)	16,73 ± 25,22 a	32,14 ± 31,67 a	3,76 ± 6,14 b	0,86 ± 1,53 b	0,71 ± 1,84 b
Grooming duration (%)	36,16 ± 19,97 a	40,19 ± 16,67 a	40,15 ± 21,65 a	41,81 ± 11,04 a	49,74 ± 11,01 a

Table 2: Mean ± standard deviation of the different measured parameters of emerging parasitoids quality, and number of replicates for each psyllid larval host stage

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	Stage 1	Stage 2	Stage 3	Stage 4
Sex ratio	0.33	0.30	0.49	0.21
Female tibia size (mm)	0.36 ± 0.02 a (n=9)	0.33 ± 0.03 b (n=9)	0.36 ± 0.02 b (n=35)	0.36 ± 0.02 b (n=46)
Male tibia size (mm)	0.33 ± 0.02 (n=3)	0.32 ± 0.02 (n=4)	0.33 ± 0.03 b (n=36)	0.34 ± 0.02 b (n=12)
Egg load	19.77 ± 10.50 (n=9)	8.48 ± 6.75 (n=9)	11.44 ± 7.45 (n=35)	11.30 ±10.70 (n=46)
Developmental time of females (days)	30.33 ± 2.65 (n=9)	22.33 ± 3.74 (n=9)	22.66 ± 2.83 (n=35)	21.89 ± 2.08 (n=46)
Developmental time of males (days)	31.33 ± 1.53 (n=3)	26.25 ± 7.23 (n=4)	22.86 ± 2.83 (n=36)	20.50 ± 1.93 (n=12)

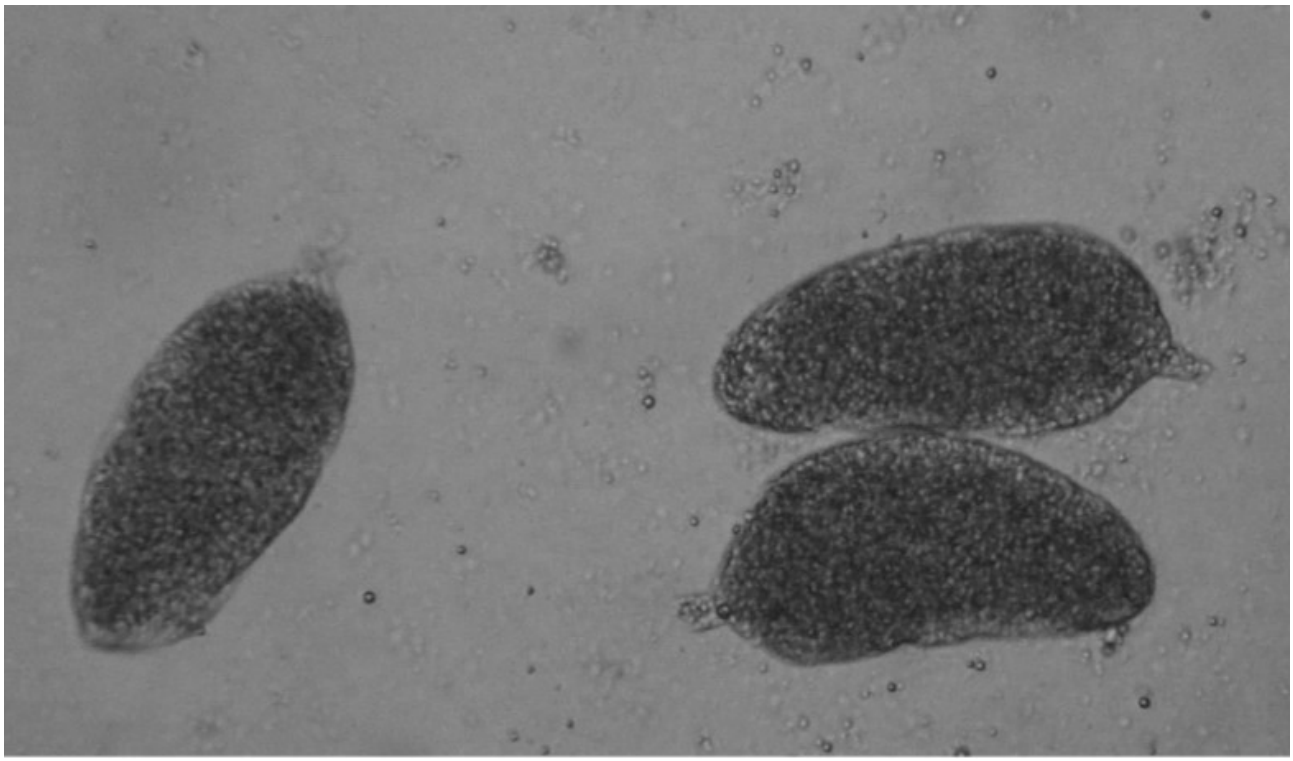

Figure 1 : mature eggs of *Trechnites insidiosus*

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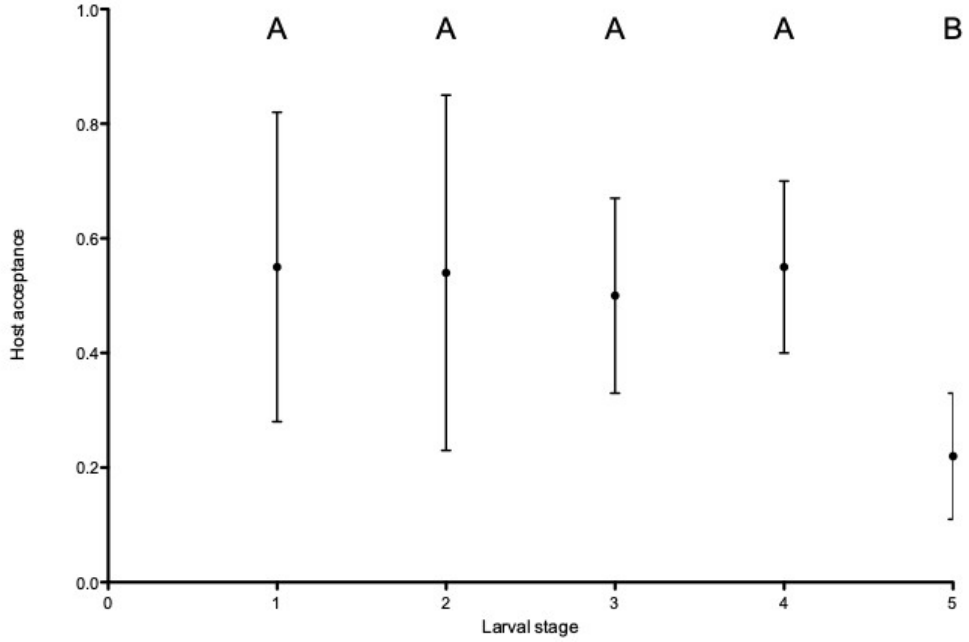

Figure 2: Mean host acceptance (number of ovipositor insertion/number of antennal contacts) according to the developmental stage of the host ± standard deviation. Different letters indicate significant differences (stage 1, n = 6; stage 2, n = 5; stage 3, n = 9; stages 4, n=10; stage 5, n = 10)

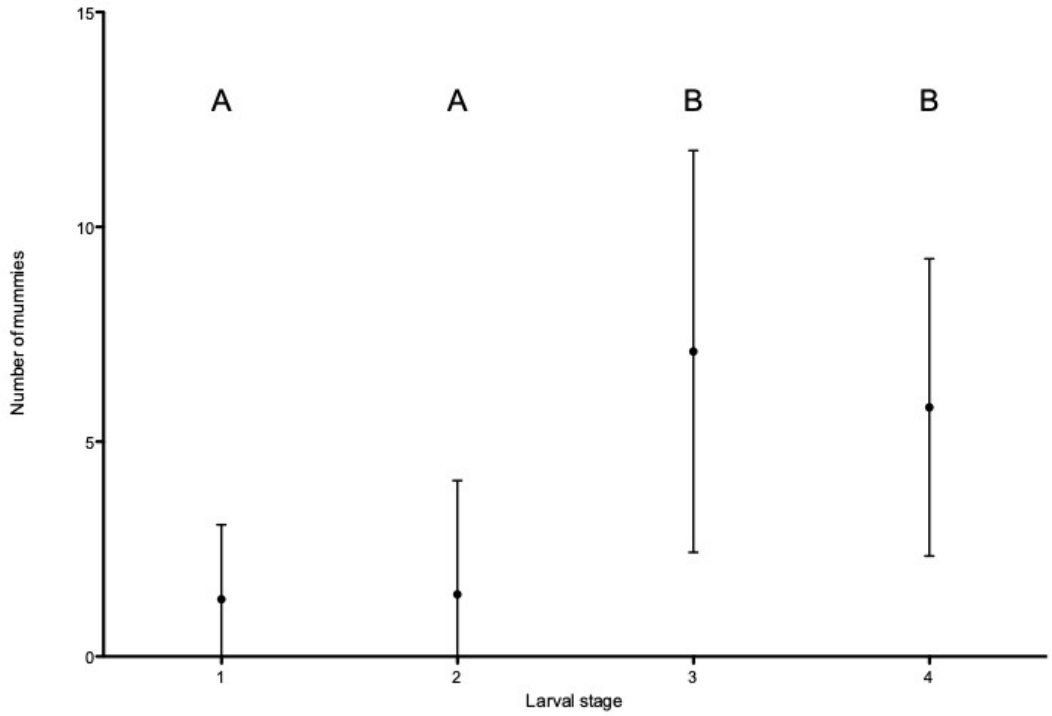

Figure 3: Mean number of mummies according to the developmental stage of the host ± standard deviation. Different letters indicate significant differences (n=10)

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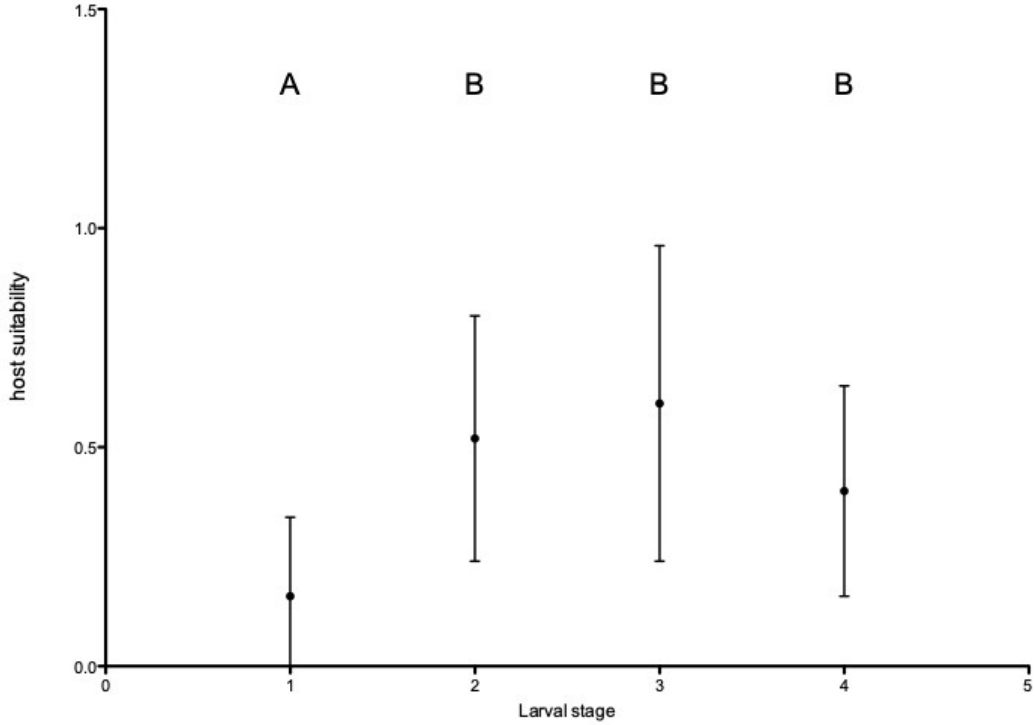

Figure 4: Mean host suitability (number of mummies/number of ovipositor insertions) according to the developmental stage of the host ± standard deviation. Different letters indicate significant differences (stage 1, n = 6; stage 2, n = 5; stage 3, n = 10; stages 4, n=10; stage 5, n = 9)

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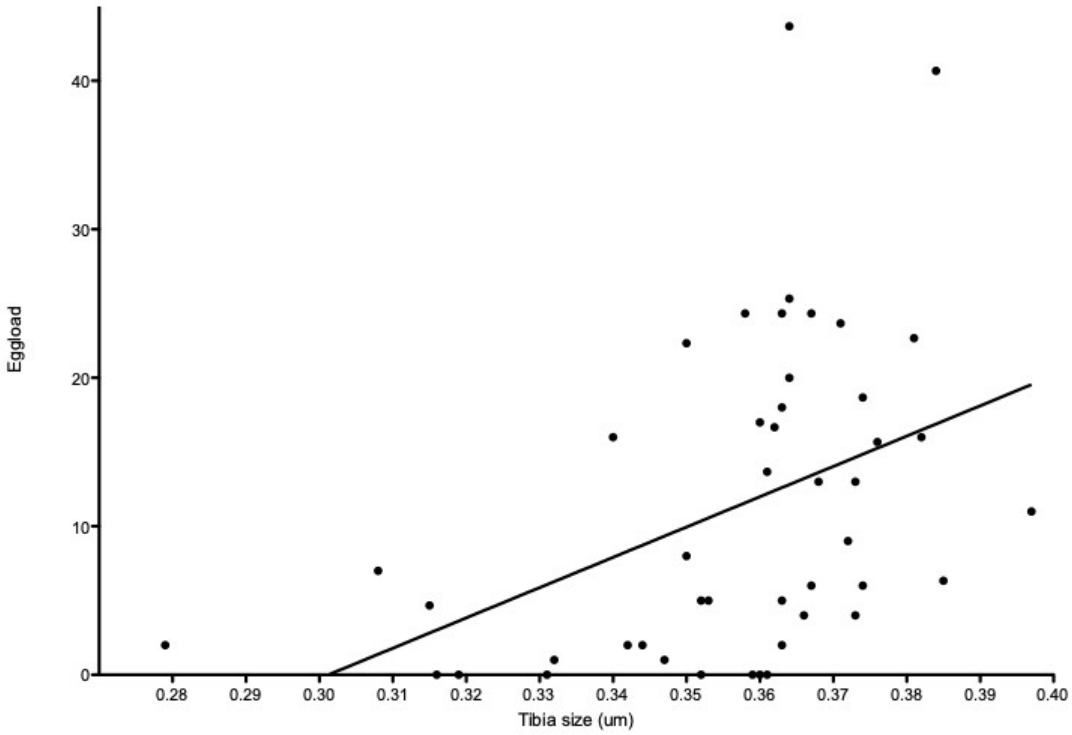

Figure 5: Correlation of the number of eggs per female from the stage 4 with the tibia size (n=46)