This is the author's final version of the contribution published as:


The publisher's version is available at:
[https://www.sciencedirect.com/science/article/pii/S1049964419300921]

When citing, please refer to the published version.

This full text was downloaded from iris-Aperto: https://iris.unito.it/
Effect of different feeding sources on lifespan and fecundity in the biocontrol agent *Torymus sinensis*

Picciau L., Alma A., Ferracini, C.*

**Affiliation:**
University of Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy.

*Corresponding author: Chiara Ferracini, e-mail: chiara.ferracini@unito.it

**Compliance with ethical standards**
All the insect rearing and experiments were conducted in accordance with the legislation and guidelines of the European Union for the protection of animals used for scientific purposes (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). All experimental protocols using insects were approved by the *ad-hoc* Committee of DISAFA of the University of Torino.

**Conflict of interest**
The authors declare that they have no conflict of interest.

**Author contribution statement**
CF, LP, and AA conceived and designed research. CF and LP carried out field and laboratory assays. All authors contributed to the writing of the manuscript and approved the final manuscript.
Abstract

*Torymus sinensis* Kamijo (Hymenoptera, Torymidae) is a biocontrol agent released to control outbreaks of the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae). This wasp is mass reared in controlled conditions in order to be released on a large scale in chestnut orchards and coppices, thus factors such as food quality may deeply influence the effectiveness of parasitoids. To identify different diets that could be used to increase mass rearing, performance, longevity and fecundity of *T. sinensis* were assessed using honey, honey plus pollen, pollen, and water as diet, compared to unfed wasps in laboratory experiments.

In the present study diet quality greatly influenced survival and reproductive output in *T. sinensis*. Adults using honey plus pollen as food had the longest life span, and significantly increased fecundity compared to pollen alone, water, and unfed wasps. Median lifespan (day at which 50% of the initial number of wasps are still alive) was 31, 31, 2.5, 4, 1.5 days, in the honey, honey plus pollen, pollen, water, control, respectively.

The egg production in wasps fed with honey (diet 1) and honey plus pollen (diet 2) observed over lifetime showed the newly emerged specimens with an average of 2.40 ± 0.21 and 0.14 ± 0.12 respectively at day 0. The number of mature eggs increased rapidly reaching in diet 1 the highest average number, 26.60 ± 2.48, at day 6, and 30.2 ± 1.59 at day 7 in diet 2. No significant difference in the mean number of mature eggs between diets 1 and 2 was observed during all 13 weeks except for week 11. The mean number of reabsorbed eggs was 0 both in diet 1 and 2 at day 0. The mean number increased till 2.80 ± 0.66 at day 10 and 1.40 ± 0.24 at day 14 in diet 1 and 2 respectively.

The cumulative lifetime mean egg load was 1012.67 and 1095.82 when feeding honey and honey plus pollen, respectively, which was significantly higher than 32.40 for pollen, 58.40 for water and 43.57 for control.

Based on our studies, feeding the female wasps with honey and pollen increased lifespan by 33-fold, and fecundity by 2.3-fold at day 4 over unfed wasps.
This work contributes to a better understanding of the influence of different diets on lifespan and fecundity in the parasitoid wasp *T. sinensis*, to the refinement of mass rearing in controlled conditions, and to the optimization of classical biocontrol programs. Prior to the field release, it seems advisable to supply insects with sugar-rich diets to improve their performances in fields and to enhance parasitoid egg expenditure.

Key words: parasitoid feeding; honey-based diets; oosorption; mass rearing; Asian chestnut gall wasp.
Life history traits of insects in nature, namely survival, development, and fecundity, are deeply influenced by food quality. Food sources of adult parasitoids include host food, host hemolymph and tissues. However, host-feeding female parasitoids can also use non-host foods to prolong their lifespan and increase their reproductive potential, as they usually contain carbohydrates, proteins, and other trace elements (Liu et al., 2015).

Proteins are often regarded as important nutritional elements for insect reproduction, enhancing offspring production (Bong et al., 2014). Conversely, the energy obtained from a carbohydrate-rich diet is mainly focused on insect life maintenance, thus prolonging life span. Potential sugar sources for parasitoids include nectar and homopteran honeydew. These sugar-meals carried over from juvenile stage can be used immediately to generate energy for metabolic purposes and even stored (Olson et al., 2000). Furthermore, pollens contain a number of nutrients and often high levels of free amino acids, instead of most nectars, there included all 10 essential amino acids. Compared to nectars, pollens have higher level of protein, lipid and polysaccharides (Thompson et al., 1999; Zhang et al., 2004).

Insects, like many animals, have the ability to respond plastically to environmental stress, resorbing oocytes that are not oviposited (Moore and Attisano, 2011). Sugar-feeding may help synovigenic species to mature additional eggs, and can prevent parasitoids from resorbing eggs (Lee et al., 2004).

Honey has long been used as an alternate food source to sucrose solution for insects such as parasitoids mass reared in controlled conditions, but little is known about its actual properties in influencing reproduction and lifespan (Harvey et al., 2012). Honey is presumably the most studied artificial diet for parasitoids since it is palatable and cheap. Provision of honey solutions boosts mymarid and trichogrammatid parasitoid lifespan similarly to floral and extrafloral nectars, and even more than honeydew (Benelli et al., 2017). A lack of sugar availability may temporarily or permanently limit the reproductive success of parasitoids in agricultural system (Heimpel et al., 1997).
Pollen is a food for numerous insects, including many species of natural enemies, and parasitic Hymenoptera feed on pollen to varying degrees (Lundgren and Wiedenmann, 2004). It is one of the most nutritious non-prey food sources for parasitoids based on its protein levels, but pollen-feeding by parasitoids has been studied much less frequently than sugar-feeding (Lundgren, 2009).

*Torymus sinensis* Kamijo (Hymenoptera: Torymidae) is the biocontrol agent of the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) (Moriya et al., 2003), and is considered as one of the most successful examples of recent European classical biocontrol programs (Avtzis et al., 2018; Ferracini et al., 2018a; Matošević et al., 2017). Although research on the reproductive biology and fecundity of this synovigenic parasitoid was already carried out (Picciau et al., 2017), the impact of diet on its life history has never been studied. In the present work, we investigated if lifespan and fecundity of *T. sinensis* are affected by different sugar diets. Since factors such as food quality are also likely to determine the effectiveness of parasitoids, wasps were allowed to feed on honey, honey plus pollen, pollen, and water. Depending on the different diet, lifespan, egg load and egg resorption of *T. sinensis* individuals were compared with unfed wasps in laboratory experiments. The results were expected to deepen knowledge about the factors affecting the biocontrol efficacy of this species in controlled conditions, thus improving mass rearing by insectaries and private companies.

**Material and Methods**

**Insect culture**

Galls were randomly collected during winter 2016-2017 according to the method described by Ferracini et al. (2015; 2018b) in chestnut-growing areas of NW Italy with a stable population of *T. sinensis*. Galls were individually isolated in plastic vials (120mm in length by 25mm in diameter), and kept outdoor until *T. sinensis* emergence.

**Food sources**

Insects were provided *ad libitum* one of the following different diets: 1) undiluted honey, 2) undiluted honey plus organic grinded pollen grains, 3) organic grinded pollen grains, 4) distilled water. The
diet have been compared to a control, with no food source provided. For the first diet we used a total of 0.02 g of honeydew honey, disposing few droplets on a cardboard, using a pipette. For the second diet, a stock mixture was prepared adding 1 g of organic pollen to 30 g of honeydew honey; few droplets were placed on a cardboard, for a total of 0.02 g. For the third diet, 0.02 g of organic pollen grain was weighted, grinded with a pestle and directly inserted in the plastic tube. For the fourth diet, the cotton plug of the tube was imbedded with 3 ml of distilled water. In the control, no source of food and water was provided. Each sugar source was replaced once a week, while the moistened cotton plug was changed twice a week.

Parasitoid lifespan

Three newly emerged wasps for each sex, for a total of six individuals, were placed into plastic vials (120 mm in length by 25 mm in diameter), and provided with one of four different sugar diets throughout their adult life. Twenty replications were performed for each food source. The vials were maintained in climatic chamber under controlled conditions [15 ± 1 °C, 60 ± 5 % RH, and a photoperiod of 16:8 (L:D) h], and checked twice a day for dead wasps every 10 a.m. and 16 p.m. each day until all individuals had died.

Parasitoid fecundity

Emerging females only, used for egg load survey, were collected every day in number of 1,800 specimens originating 30 age-cohorts for each feed-test for a total of 180 cohorts. The insects were reared in plastic vials (120 mm in length by 25 mm in diameter) arranged in 5 groups each fed with different food combination: 1) honey, 2) honey plus pollen, 3) pollen, 4) water and 5) no food source (control). All of them were kept in a climatic chamber under controlled conditions (15 ± 1 °C, 60 ± 5% RH, and a photoperiod of 16:8 L:D) according to Picciau et al. (2017) and maintained till dissection.

Egg load and oosorption quantifying

Five females for each age cohort were collected daily and killed with ethyl acetate. The specimens were placed on a microscope slide (25 × 75 × 1 mm) into a phosphate buffered saline (PBS) solution
for ovary dissection. Egg load was assessed by dissecting each female under a stereomicroscope at 25 × magnification. The abdomen was separated from the rest of the body using a couple of needles. Ovaries were kindly removed from the abdomen by means of micro-pin needles and spread on a slide for egg load counts. Mature eggs were defined as described in Picciau et al. (2017). Egg resorption (oosorption) was also investigated by counting those eggs close to the lateral oviducts which displayed a collapsed pattern indicating a reabsorption process (Figure 1). The number of mature and reabsorbed eggs was counted under microscope at 80 × magnification.

Statistical analysis

The data on the female longevity of the different diets were log10 (x+1) transformed and transformed using analysis of variance (ANOVA). We used the Kaplan-Meier estimator (Kaplan and Meier, 1958) to visualize survivorship curves in each diet, and a log-rank test was used to determine differences in survival rates. The significance of each term in the model was analyzed using Wald v2 statistic. After testing for homogeneity of variance (Levene test), data were analyzed by T test independent samples (P < 0.05) to compare the egg load and the oosorption records comparing wasps fed with honey and with honey and pollen through 13 weeks. Furthermore, all data were analyzed within the first 8 days by a Generalized Linear Model (GLM) with Poisson distribution and Log link function. Averages among the diets were separated by a Bonferroni post hoc when significative difference were present. All analyses were performed using the software SPSS version 20.0 (SPSS, Chicago, IL, USA). The cumulative mean egg load was analyzed with a kruskal Wallis test, and averages were separated by a Bonferroni post hoc when significative difference were present.

Results

The results concerning the effects of different food sources on lifespan of the parasitoid *T. sinensis* are shown in Figure 2. Diet significantly affected lifespan, in particular wasps fed with honey (diet 1) and honey plus pollen (diet 2), increased lifespan to the greatest extent. This lifespan, in fact, was more than 20 times as long as that of individuals starved (control) (Figure 2). Furthermore, lifespan of the wasps was significantly reduced when they were fed with pollen (diet 3), and water (diet 4) as
well. Even if the average lifespan was higher when adults were provided with honey plus pollen, no significant difference was found between this source and honey alone.

Median lifespan (day at which 50% of the initial number of wasps are still alive) was 31, 31, 2.5, 4, 1.5 days, in the honey, honey plus pollen, pollen, water, and control, respectively. Furthermore, according to the diet all wasps were dead after 88, 108.5, 6, 7, 4 days in honey, honey plus pollen, pollen, water and control, respectively. The median survival time differed between female and male parasitoids, with females living longer (log rank test, \( \chi^2 = 5.42, \text{df} = 1, P = 0.0199 \)) (Figure 3).

The comparison of egg load and oosorption was performed through 13 weeks, considering only honey (diet 1) and honey plus pollen (diet 2), basing this choice on the diets with the longest lifespan. The egg production observed over lifetime showed that the newly emerged specimens had an average of 2.40 ± 0.21 mature eggs in specimens fed with diet 1 and 0.14 ± 0.12 mature eggs in specimens fed with diet 2 at day 0. In the following days the number of mature eggs increased rapidly reaching in diet 1 the highest average number, 26.60 ± 2.48, at day 6 with a maximum value per individual of 34 eggs. For this diet the highest number per individual was 40 and reached at day 17 when the average was 20.8 ± 5.21. The highest average number reached in diet 2 was 30.2 ± 1.59 at day 7 with a maximum value per individual of 34 eggs. For this diet the highest number per individual was 43 and reached at day 9 when the average was 26.5 ± 3.12. No significant difference in the mean number of mature eggs between diets 1 and 2 was observed during all 13 weeks except for week 11 (t = −0.452, df = 7.842, P = 0.663) (Figure 4).

Concerning the oosorption, the number of reabsorbed eggs observed over lifetime showed that the newly emerged specimens had an average of 0 collapsed eggs both in diet 1 and 2. Subsequently the number increased, reaching in diet 1 the highest average number, 2.80 ± 0.66, at day 10 with a maximum value per individual of 5. For this diet the highest number per individual was 6 and reached only at day 14 when the average was 2.60 ± 0.87. The highest average number reached in diet 2 was 1.40 ± 0.24 at day 14 with a maximum value per individual of 2 reabsorbed eggs. For this diet the highest number per individual was 2 and reached for the first time at day 11 when the average was
0.40 ± 0.40. Significant differences were found only in weeks 1, 3, 12 and 13 (week 1: \( t = -1.364, df = 8.053, P = 0.209 \); week 3: \( t = -4.418, df = 6.075, P = 0.004 \); week 12: \( t = -0.926, df = 1.010, P = 0.523 \); week 13: \( t = -3.000, df = 3.000, P = 0.058 \)) (Figure 5).

The comparison among all the diets was performed only through the first 8 days when only the specimens provided with diet 1 and 2 remained alive (Figure 6). The unfed wasp, with the shortest lifespan, showed a peak at the third day with an egg load mean of 17.70. The specimens fed with pollen had the higher egg load during the last three days before dying reaching an average egg load of 8.53. In case of water, the egg load followed a normal distribution with the highest mean value of 18.20 eggs at day 2. The mean egg load for wasps provided with honey and honey plus pollen showed a positive trend with lower values until day 3 with a subsequent increase in the following days overcoming the other feeding sources (Figure 6). The cumulative lifetime mean egg load was 1012.67 and 1095.82 when feeding honey and honey plus pollen, respectively, which was significantly higher than 32.40 for pollen, 58.40 for water and 43.57 for control.

**Discussion**

Egg maturation in parasitoids depends on the diet provided to adult females (Onagbola et al., 2007). It is considered a fitness-related parameter, which may influence the reproductive potential of biocontrol agents and thus the population dynamics of host. However, different diets may have an effect on the egg maturation patterns through lifetime of synovigenic parasitoids species (Jervis et al., 2001). In fact, lipids and proteins, from either the diet and teneral reserves, are important for production of the eggs (Ellers and Van Alpen, 1997), and the balanced acquisition of nutrients has great impact on the size and number of eggs produced (Harvey et al., 2012; Wheeler 1996). However, egg production is also improved by various artificial diets based on carbohydrate solutions, and exploitation of sugar sources or pollen is widespread in a broad range of orders, including Hymenoptera (Wackers et al., 2008). Food quality greatly influences life history traits such as survival, development, and fecundity of insects. Its availability over time can affect fitness-related traits and may alter egg maturation (Benelli et al., 2017) in synovigenic species, there included *T.*
Carbohydrate-rich diet access, even as intermittent feeding and without additional proteins, has a strong effect on life span being able to increase longevity of about 5 times compared to water alone as demonstrated by Ellers et al. (2011). Among the different sugar source, honey is considered the most effective food to promote egg maturation (Hogervorst et al., 2007), and improve fecundity by prolonging parasitoid lifespan (Mutitu et al., 2013). Moreover, pollen can also be exploited as food source to improve fecundity when used in addition to honey (Zhang et al., 2004).

In the present study diet quality equally influenced the survival and the reproductive output in T. sinensis. Adults fed with honey plus pollen had the longest life span, and this diet significantly increased fecundity compared to control. In particular, T. sinensis responded to a honey supplement, increasing the mean survival up to 49 days, considering female wasps provided with honey plus pollen, confirming previous data by Picciau et al. (2017). Conversely, when only pollen or water were provided similar effects to control were recorded. In fact, wasps reached a mean survival of 2.5, 4, and 1.5 days, respectively. Significant reduction in longevity, with no significant difference between sexes, was recorded highlighting how parasitoids wasps are especially sensitive to sugar deprivation as adults. As already pointed out by Olson et al. (2000), laboratory lifespan of many parasitoids species is typically less than 5 days in the absence of sugar but exceeds 2 to 3 weeks when sugar meals are provided, confirming our results.

Honey has long been used in controlled conditions as an alternate food source to sucrose solution for insects such as parasitoids, but its actual properties in influencing parasitoid longevity and reproduction have been little explored. The content of honey is dominated by sugars, although it also contains trace amounts of vitamins or minerals, with tiny amounts of several compounds thought to function as antioxidants (Harvey et al., 2012). Based on our studies, feeding the female wasps with honey plus pollen increased lifespan by 33-fold, and fecundity by 2.3-fold at day 4 over unfed wasps. Moreover, pollen of plants is known to be a source of food for a large number of insect species in nature, but pollen feeding by parasitoids has been studied less frequently than sugar feeding. Some groups of insects have been reported feeding on pollen (e.g., Bombyliidae, Eulophidae, Mutilidae,
Scoliidae, Trichogrammatidae) (Jervis et al., 2008; Patt et al., 1997; Zhang et al., 2004). In our experiment, pollen alone did not increase lifespan but a higher longevity was recorded when it was provided in addition to honey. In case of scarcity of food, insects, like many animals, have the ability to adapt to environmental stress, resorbing oocytes that are not oviposited (Moore and Attisano, 2011). In our experiments the presence of egg deterioration related to oosorption was verified in *T. sinensis*, as previously hypothesized in Picciau et al. (2017). The comparison of egg load and egg maturation trend recorded in the specimens fed with honey and honey plus pollen through their lifetime suggest that the addition of pollen in the diet does not heavily affect the number of egg production. On the contrary it seems to have a strong impact on egg resorption probably due to the increased nutritional value of the food provided. Compared to males, females were in fact able to reallocate resources to somatic maintenance as evidenced by no reduction in lifespan, especially in their first month of life. Comparing the mean cumulative egg load recorded with all different diets to the unfed wasps, a significant increase in fecundity was detected. In particular feeding with honey increased the total egg load by 23.2-fold, and with honey plus pollen by 25.1-fold, whilst with only water only 1.4-fold and pollen alone showed no significant difference. As already stated by Zhang et al. (2004) for *Trichogramma* spp., honey and pollen should be considered a complete diet for *T. sinensis* as well conversely to pollen and water. This knowledge about feeding sources influence on parasitoid life-history traits is essential to improve mass-rearing techniques. In particular, *T. sinensis*, being a biocontrol agents employed in classical biocontrol programs in Europe, may be positively affected by diet in laboratory conditions. A balanced acquisition of nutrients has indeed great influence on the size and number of eggs produced (Harvey et al., 2012; Wheeler 1996).

Modern agriculture has simplified the agro-ecosystem landscape, increasing the pest population density and reducing the alternative non-crop sources for natural enemies (Benelli et al., 2017). In a frame of a biocontrol programs based on conservative and augmentative actions, nectar and
honeydew accessibility in the field is very important to improve parasitoid effectiveness, as already pointed out in case of scarce flower resources (Pinheiro et al., 2015; Wäckers et al., 2008). Moreover, providing parasitoids with sugar-rich diets before their release to the field is recommendable to improve their performance and optimize the egg laying (Hougardy and Mills, 2007).

The current findings have offered some important insights into the life history traits of *T. sinensis*, providing new cues to enhance methods of mass rearing for biocontrol. To overcome occurring field limitations, the employment of artificial sugar sources, flowering cover crops and the management of natural food resources may be helpful to improve natural populations and to enhance the fitness of parasitic wasps in both conservative and augmentative biocontrol programs.

**Acknowledgements**

The authors would like to thank Ester Ferrari and Marianna Pontini for managing *T. sinensis* mass rearing, and Silvia T. Moraglio and Fabio Mazzetto for assistance with statistical analysis.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
References


