

# Hungarian University of Agriculture and Life Sciences

# Biological and morphological aspects of the onion thrips (*Thrips tabaci* Lindeman, 1889) species complex

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#### **1. INTRODUCTION AND AIMS**

Onion thrips (*Thrips tabaci* Lindeman, 1889) is a cosmopolitan insect pest of economic importance, causing significant damage to dozens of cultivated plant species, including alliaceous, cabbage and tobacco crops (JENSER and SZENASI 2004, TRDAN et al. 2006, DIAZ-MONTANO et al. 2011, LI et al. 2014, LOREDO VARELA and FAIL 2022). The pest status of *T. tabaci* is attributed to its polyphagous nature, high reproductive rate, short generation time, transmission of plant viruses, ability to reproduce without mating and development of resistance to insecticides (MORSE and HODDLE 2006, DIAZ-MONTANO et al. 2011).

*T. tabaci* has received considerable attention due to its cryptic life habit and mode of reproduction. DNA sequences of the mitochondrial COI gene have confirmed that *T. tabaci* is a species complex, and it has been divided into three lineages: L1, L2 (leek-associated), and T (tobacco-associated) (BRUNNER et al. 2004). L1 leek-associated and T tobacco-associated lineages have arrhenotokous reproduction (TODA and MURAI 2007, FARKAS et al. 2020), while the L2 leek-associated lineage has thelytokous reproduction (KOBAYASHI and HASEGAWA 2012). Nevertheless, adults of the three lineages are considered indistinguishable based on their morphological features (JENSER and SZENASI 2004, KOBAYASHI and HASEGAWA 2012). Size differences have been observed in some populations colonizing tobacco and onion plants in Iran (FEKRAT et al. 2014), but a detailed morphometric analysis of the three identified lineages has not been carried out so far.

*T. tabaci* has been one of the most intensively studied thrips species, though some of the biological aspects of this species remain unknown (FAIL 2016). Two lineages of *T. tabaci* exhibit a haplodiploid sex-determination system that allows parents to control their offspring's sex by different fertilization

mechanisms. In two other haplodiploid species, the two spotted spider mite (*Tetranychus urticae* Koch) (MACKE et al. 2011) and Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (KATLAV et al. 2020), sex allocation is mediated by egg size. In these two species, egg size determines the probability of fertilization, with female (fertilized) eggs being larger than male (unfertilized) eggs. Since there are no further reports available for other haplodiploid arthropods, we intended to study this mechanism in the two arrhenotokous lineages of onion thrips.

The main aims of this Ph.D. project were to:

-Investigate the role of egg size on sex allocation in two arrhenotokous lineages of the *T. tabaci*.

- Study the effect of maternal age on male and female egg size of virgin and mated mothers of the L1, L2 and T lineages of *T. tabaci*.

- Examine morphometric variability in the size of eggs, first instar larvae and adults of the *T. tabaci* cryptic species complex, to identify characters that may be useful in distinguishing between L1, L2, and T lineages.

- Study the development of sexual size dimorphism in the ontogeny of both L1 and T lineages of *T. tabaci* to identify if sexual size dimorphism is present in eggs, first instar larvae, or develop by adult emergence.

### 2. MATERIALS AND METHODS

#### 2.1. Experimental design

To carry out the experiments, the thrips individuals were collected from the stock colonies of the three lineages. Bean leaves (*Phaseolus vulgaris* L) were used for all the treatments to facilitate the careful excavation of the eggs. Five groups were organized to test the morphometric variability of egg size, newly hatched first instar larvae and newly emerged adults among the L1, L2, and T lineages. The groups correspond to a) L1 virgin female; b) L1 mated female; c) L2 virgin female; d) T virgin female; and e) T mated female. The morphometric measurements of the eggs, first instar larvae and adults were performed on the progeny of every single group. The molecular method of FARKAS et al. (2020) was used to identify all of the females and males whose progeny was used in this study. All the rearing processes were performed in a growth chamber under controlled conditions at  $23^\circ$  C with a light and dark photoperiod regime of L16:D8h.

# 2.2. Egg collection from virgin and mated mothers of L1, L2, and T lineages

To measure the size of the eggs produced by virgin and mated mothers of L1, L2, and T lineages, pupae were collected from the stock colonies of *T. tabaci* and isolated separately in a 2 ml Eppendorf tube containing a bean leaf disk to ensure the virginity of the emerging females. Pupae were observed every twelve hours to record the time of adult emergence. From 77 pupae collected from the L1 lineage, 42 females and 15 males emerged. In the case of the T lineage, out of 69 pupae, 45 females and 15 males emerged. In the case of the L2 lineage, 23 females emerged from the 30 pupae collected. Right after adult emergence, 16 females from the L1 lineage and 19 females from the T lineage were accompanied by the emerged males (15 and 21 for the L1 and T lineages, respectively) for mating, one female and one male in each Eppendorf tube containing a bean leaf

disk. Each couple was kept together for 48 hours to ensure mating. Then males were removed and preserved in 96% ethanol for DNA-based identification. Meanwhile, the remaining females, of which there were 26 for each of the L1 and T lineages and 23 emerging females from the L2, were kept as virgins. All the virgin and mated females of the three lineages were isolated in a separate Eppendorf tube provided with the bean leaf disk less than 1 cm in diameter for oviposition. Bean leaf disks were changed every twelve hours until the given females died. Under a stereomicroscope, eggs laid in a bean leaf disk were carefully excavated with the help of a dissecting needle. In the morphometric analysis of the L1 and T mated groups, only the progeny of those mated females that consisted of both males and females were considered.

#### 2.3. Egg size measurements

The excavated eggs were placed on a microscopic glass slide under a calibrated compound light microscope with an ocular graticule for the size measurement. Under 600x magnification, eggs were measured for width and length. Egg volume was calculated according to the formula: V=width<sup>2</sup>\*length\* $\pi/6$  taken from CHURCH et al. (2019). After the measurements, the eggs were placed back on a new bean leaf disk inside the Eppendorf tube to facilitate hatching and subsequent juvenile development. Eggs were maintained in the controlled environmental conditions described above. As the morphological identification of males and females is not possible in the egg stage, the sex of the resulting offspring was recorded on the slide-mounted specimens of either first instar larvae or newly emerged adults. The eggs were checked every twelve hours for newly hatched larvae. The first instar larvae were sexed according to (VIERBERGEN et al. 2010). Immediately after hatching (maximum 12 hours), about half of the newly hatched larvae was placed in 75% alcohol and preserved at 4°C until the morphometric measurements were processed. While the other portion of the newly hatched first instar larvae were

reared on bean leaf disks until they reached the adult stage. The newly emerged adults were also placed in 75% alcohol and preserved at 4°C until they were measured.

# 2.4. Morphometric measurements of first instar larvae and adults of *T.tabaci* lineages

A total of 642 specimens of first instar larvae of the three lineages of *T. tabaci* (212 L1, 88 L2, and 342 T) and 808 specimens of the adult (253 L1, 150 L2, and 405 T) were slide mounted in drops of Berlese mounting medium. The slide-mounted specimens were kept in the oven at 50°C for two days. Then the slide-mounted specimens of first-instar larvae produced by mated mothers of L1 and T lineages were sexed according to VIERBERGEN et al. (2010). All the specimens were measured at 400x and 600x magnification under a calibrated compound microscope, with an eyepiece graticule, where the distance between the base of the setae was measured in nine chosen characters for the first instar larvae and eight chosen characters for the adults. The measured character state and chaetotaxy are adapted from VIERBERGEN et al. (2010) and SPEYER and PARR (1941). The morphological terminology of the adults follows PALMER et al. (1992).



Figure 1. Morphometric measurements of first instar larvae. (A) D3 and D4 head, the distance between dorsal setae three and four of the head; D3 and D7 pronotum, the distance between dorsal setae three and four of the pronotum. (B) D3 and D4 metanotum, the distance between dorsal setae three and four of metanotum. (C) D2 tergite II, the distance between dorsal setae two of abdominal tergite II (D) D2 tergite VIII, the distance between dorsal setae two of abdominal tergite VIII. (E) and (F) V3 sternite IX, the distance between ventral setae three of abdominal sternite IX on a male (E) and a female (F).



**Figure 2.** Morphometric measurements of adults. (A) Oc.s.2 and Oc.s.3, the distance between the second and third ocellar setae. (B) Meta.s.1 and Meta.s.2, the distance between lateral and median metanotal setae. (C) P.a.s., the distance

between inner posteroangular setae on the pronotum. (D) S3 tergite IV, the distance between dorsal setae three of tergite IV. (E) Discal.s.3, the distance between discal setae three of tergite VII; S2 tergite VIII, the distance between dorsal setae two of tergite VIII.

## 2.5. Effect of mating and egg size on the hatching probability of the eggs

To investigate whether egg size is an appropriate predictor of energy content in the L1 and T lineages of *T. tabaci*, we assessed the effect of mating

and egg size on the hatching probability of the eggs in the progeny of virgin and mated mothers of the L1 and T lineages.

#### 2.6. Egg size and sex allocation

# 2.6.1. Comparison of male and female egg size produced by mated mothers of the L1 and T lineages

To quantify maternal investment in eggs, we compared the size of eggs in male and female offspring produced by mated mothers during their entire lifespan. In two studied species, *T. urticae* (MACKE et al. 2011) and *P. kellyanus* (KATLAV et al. 2020) egg size and gender were found to be correlated with the egg mass produced by young arrhenotokous females. Corresponding to these approaches, we compared male and female egg sizes in different age groups of the mothers; 3-5 days old (just like *T. urticae*) and 7-10 days old (similar to *P. kellyanus*) and above 10 days old.

#### **2.6.2.** Testing the model of egg size and sex allocation

We investigated whether the asymmetric allocation of male and female eggs by mothers could occur before fertilization and whether egg size influences the probability of an egg being fertilized (a female fertilized egg being larger than a male unfertilized egg) (Figure 3A, scenario 1).

Second, we tested whether mating affects the female resource allocation strategy if eggs draw more resources once they are fertilized (Figure 3B, scenario 2). To discriminate between these two scenarios, we compared the mean and the distribution of the egg size produced by virgin and mated mothers. Using only male eggs, we have also compared the size of the eggs of males produced by virgin and mated mothers for the entire lifespan of the mothers and in the abovementioned age groups.



**Figure 3.** Hypothetical mechanisms of sex-specific egg size in haplodiploid arrhenotokous lineages of *T. tabaci*. Fertilized and unfertilized eggs are presented in brown and blue, respectively

# 2.7. Effect of maternal age on the egg size

To investigate the effect of maternal age on egg size independently of the offspring sex, we used eggs produced by mated and virgin mothers of the L1, L2 and T lineages. Egg volumes were modelled depending on the maternal age for their entire lifespan and age groups: 3–5 days, 7–10 days and above 10 days. In the case of the L2 lineages, egg volume was modelled throughout the maternal age, 1-10 days old and above 10 days old.

### 2.8. Morphometric analysis of the *T. tabaci* species complex

### 2.8.1. Morphometric analysis of the eggs

Morphometric analyses of the eggs were performed to obtain information on the morphological variations among the L1, L2 and T lineages of *T. tabaci*.

### 2.8.2. Morphometric analysis of first instar larvae and adults

Principal Component Analysis (PCA) was used to find traits responsible for the variation amongst first instar larvae and adults of the three lineages.

#### 3. RESULTS AND DISCUSSION

#### 3.1. *Thrips tabaci* lineage identification

Based on the analysis of the mtCOI region, 87 mated and virgin females of the L1, L2, and T lineages and 36 males of both the L1 and T lineages, which were mated with their conspecific females (whose progeny were used in this study), proved to belong to the lineage as expected.

#### **3.2.** Effect of mating and egg size on the hatching probability of the eggs

Maternal mating status significantly influenced the probability of hatching in the L1 lineage. The overall number of oviposited eggs was 1305; 67% of 598 eggs and 44% of 707 eggs laid by virgin and mated mothers, respectively, hatched. Therefore, virgin mothers had a significantly higher probability of having eggs that hatched, whereas mated mothers had a significantly higher probability of having eggs that did not hatch. In the T lineage, the overall number of oviposited eggs was 2660; 61% of 1558 and 58% of 1102 eggs laid by virgin and mated mothers, respectively, hatched. Mating status did not influence the probability of hatching. Moreover, egg size did not influence the probability of hatching. Moreover, egg size did not influence the probability of hatching. Moreover, egg size did not influence the probability of hatching in the virgin mothers of the L1 lineage, whereas in the mated mothers of the L1 lineage, eggs that did not hatch. For both virgin and mated mothers of the T lineage, egg size significantly influenced the probability of hatching. Eggs that hatched were significantly larger than eggs that did not hatch.

The fact that larger eggs are generally more likely to hatch and produce fast-developing juveniles with higher survivorship than smaller eggs in the spider mite *T. urticae* indicates that egg size might be a predictor of energy content in some cases (MACKE et al. 2011), and our results confirm this: hatched eggs were significantly larger than the eggs that did not hatch in the L1 lineage laid by mated mothers and in the T lineage of *T. tabaci* for all eggs, independently of mating

status. Mating (once or multiple times) had no discernible effect on the hatchability of eggs in the bruchid beetle *Callosobruchus maculatus* (Fabricius) (FOX 1993).

#### **3.3.** Egg size and sex allocation

# **3.3.1.** Comparison of male and female egg size produced by mated mothers of the L1 and T lineages

Sex allocation in haplodiploid *T. tabaci* is not mediated via egg size like in the two-spotted spider mite *T. urticae* (MACKE et al. 2011) and in Kelly's citrus thrips *P. kellyanus* (KATLAV et al. 2020). In both species, egg size and gender were found to be related with egg mass that was produced by young arrhenotokous females, 3-5 day old mothers in *T. urticae* and 7-10 day old mothers in *P. kellyanus*. The size of male and female eggs produced by mated mothers of the L1 lineage was not significantly different for the whole lifespan and at any age group, indicating that gender and egg size are independent of each other in this lineage regardless of maternal age. In the T lineage, gender seemed to be dependent on egg size, however, only in the maternal age group of 7-10 days, but gender was independent of egg size in the progeny of younger and older mothers.

# 3.3.2. Testing the model of egg size and sex allocation

By comparing the size of eggs produced by virgin and mated mothers, we showed that mated females laid significantly larger eggs throughout their lifespan and in all other age groups in the L1 lineage. The mean value of the egg size distribution of mated mothers in the L1 lineage was larger than that of virgins throughout their lifespan. Moreover, the size of male eggs produced by mated mothers was significantly larger than that produced by virgin mothers for the whole lifespan in the L1 lineage. This may suggest that there is no egg size that determines fertilization in this lineage, but rather it is the fertilization that may influence egg size. Eggs produced by mated mothers might receive more resources than those produced by virgins, and mating just increases egg size in general; leading to a larger size of male eggs produced by mated mothers than by virgins (Figure 3B, scenario 2). Contrary to the L1 lineage, in the T lineage, an entirely new scenario is needed to assess the obtained results; eggs laid throughout the lifespan of virgin mothers were marginally larger than those of mated mothers, and there were no significant differences in the size of male eggs produced by virgin or mated mothers throughout the lifespan.

Our findings indicate that these two lineages of *T. tabaci* have different resource allocation strategies in response to maternal mating status. In *P. kellyanus* (KATLAV et al. 2020), mating increases the early-life reproductive investment of females; therefore, mated females produce larger eggs than virgin females, just like our L1 lineage. However, male offspring of mated mothers had smaller eggs than those of virgins (KATLAV et al. 2020). In *Tetranychus ludeni* Zacher, virgin mothers laid significantly larger eggs than mated mothers, indicating a strategic resource allocation in response to mating status, with more resources being allocated to their male offspring when the mothers do not have the chance to produce female offspring (ZHOU et al. 2018).

#### **3.4.** Effect of maternal age on the egg size

In *T. tabaci*, maternal age overall affected the egg size of both males and females produced by mated mothers in the L1 and T lineages throughout their lifespans. The egg size of males and females of both lineages decreased with increasing maternal age. However, in the very young maternal age groups (3-5 day old mothers), 7-10 day old mothers, and older than 10 days, male and female egg size of mated mothers in both L1 and T lineages stayed at a constant level, without significant changes in egg size. While there is an increase in egg size at a very young maternal age (3-5 days) for male eggs produced by virgin mothers of the L1 and T lineages, this is not significant later in the age group of 7-10

days. Above 10 days old, the egg size of males produced by virgin mothers of the L1 lineage stayed at a constant level without changing egg size, and there is a decrease in egg size for male eggs produced by virgin mothers of the T lineage. In the case of the L2 lineage of the *T. tabaci* lineage, the egg size was linearly increasing with growing maternal age, but the rate of dependence (i.e., the slope of the trend) was decreasing up to day 10, and thereafter it did not change notably.

The most common explanation for this phenomenon is the resource depletion hypothesis, according to which it may be a physiological limitation of the females to produce offspring of the same size (BEGON and PARKER 1986, FOX 1993). The observed decrease in egg size with increasing maternal age has been proven in two related species of bruchid beetles, *Callosobruchus maculatus* (Fabricius) and *C. chinensis* (Fabricius) (Coleoptera) (FOX 1993, YANAGI and MIYATAKE 2002). Females with diminished resources for egg production laid smaller eggs with increasing maternal age, and those who were allowed to access food laid larger eggs even at later maternal ages. However, our experiment was not designed to test such a hypothesis. As long as the maternal diet was not manipulated in this study, our results are not consistent with the resource depletion hypothesis.

#### **3.5.** Morphometric analysis of *T. tabaci* species complex

#### **3.5.1.** Morphometric analysis of eggs

Overall, considering all the groups of the three lineages of *T. tabaci*, the female eggs of the L2 (virgin) mothers were significantly larger than all the other groups. These were followed by female and male eggs from mated mothers of the L1 lineage. Meanwhile, the egg size of the next group in magnitude, i.e., the male eggs of the virgin L1 mothers, is significantly bigger than the eggs of females and males of virgin and mated mothers of the T lineages, which are the three smallest groups among all groups of the three lineages.

#### 3.5.2. Morphometric analysis of first instar larvae and adults

The morphometric variation of the first instar larvae of different groups of *T. tabaci* lineages ordinated by PCA is shown in Figure 4. Along PC1 and PC2, the only clear partitions are shown between L2 females and L1 males produced by virgin mothers and between L1 females and L1 males produced by virgin mothers.



**Figure 4.** Principal Component Analysis biplot for the observed variables of larval size of different groups of *T. tabaci* lineages. Vector captions: D3 head, (transformed) D3 pronotum (trD3 pronotum), V3 stern.IX, D2 terg.VIII, D4 metanotum. Brown arrows represent the measured variables and ellipses of different colours represent the 95% confidence range around the sampling points relative to the lineage (L1, L2, T), mated status (V: virgin, M: mated), and gender (m: male, f: female).

The result of PCA conducted on adult measurements is shown in Figure 5. The visualization of the results of the PCA suggested a clear separation between females of T and L lineages, but L1 and L2 cannot be separated according to the measured characters. Regarding the males of the L1 and T lineages, we detected that it does not make a meaningful difference whether they are produced by virgin or mated mothers. However, a notable difference can be detected between L1 and T males. Moreover, a clear separation between males

and females is shown in the adult stage but not in the first instar larvae of both the L1 and T lineages.



**Figure 5.** Principal Component Analysis biplot for the observed variables of the adults of different *T. tabaci* lineages. Brown arrows captions: Oc.s.3, P.a.s, Meta.s.1, Meta.s.2, Oc.s.2, S2 terg.VIII. Brown arrows represent the measured variables, and ellipses of different colours represent sampling points relative to the lineage (L1, L2, T), mated status (V: virgin, M: mated), and gender (m: male, f: female).

The morphometric analysis method used in this study for discriminating between lineages of *T. tabaci* requires a prior species identification method, especially when *T. tabaci* specimens are collected from the field, which may include other thrips species. Other *Thrips* spp., such as the *T. hawaiiensis* species group (PALMER and WETTON 1987), *Thrips parvispinus* Karny (JOHARI et al. 2014), and two morphologically similar thrips species, *Thrips fuscipennis* Haliday and *Thrips sambuci* Hegger (FEDOR et al. 2014), exhibit morphometric variations as well. Therefore, the discrimination of *T. tabaci* lineages based only on morphometric analysis may lead to misidentification in potentially mixed field-collected samples.

Sexual size dimorphism is a common phenomenon in insect taxa where both sexes differ in body size (SHINE 1989). There are different explanations for how sexual size dimorphism arises in insects, and most of them rely on the ontogeny of males and females. In particular, it is often unclear if the individuals of the ultimately larger sex are already larger at hatching/birth if they grow faster, or if they grow for a longer time. BLANCKENHORN et al. (2007) found that in four out of seven studied arthropod taxa with female-biased sexual size dimorphism, the difference between growth rates in sexes is more important than the difference in developmental time. It has also been shown that the female larvae develop from larger eggs than male larvae do; thus, the female has a larger size from the very beginning of its development and maintains the size differences during the whole ontogeny (BUDRIENE et al. 2013).

In this study, there is no egg size sex difference in both the L1 and T lineages produced by mated mothers of *T. tabaci*. The differences were not significant, either, in our first study, where the sex allocation in arrhenotokous lineages of *T. tabaci* was not mediated by egg size. Moreover, this study shows that males and females do not exhibit any size differences in the first instar larvae. The differences became visible later in the adult stage. In this respect, we can presume that the formation of the sexual size dimorphism in both lineages of *T. tabaci* may be attributed to the different growth rates of males and females. To our knowledge, no studies of insect eggs have found differences in eggs that develop into different sexes.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

- 1. The fact that larger eggs are more likely to hatch than smaller eggs in the L1 lineage laid by mated mothers and in the T lineage of *T. tabaci* for all eggs, independently of mating status, indicates that egg size might be a predictor of energy content in some cases. Mating also affected the hatching probability of the eggs in the L1 lineage, with more eggs hatching from virgins than from mated mothers; and in the T lineage, mating did not affect the hatching probability of the eggs.
- 2. Sex allocation in haplodiploid *T. tabaci* is not mediated by egg size like in *Tetranychus urticae* and *Pezothrips kellyanus*. Egg size and gender were independent of maternal age in the L1 lineage, while in the T lineage, the observed egg size difference between males and females was only present in the progeny of young females (7–10-day-old mothers). Our results demonstrate that studying the relationship between egg size and gender in a narrow age group of mothers might lead to misconclusions unless different mechanisms are working in mated females depending on their age.
- 3. Eggs laid by mated mothers were significantly larger than eggs laid by virgin mothers throughout their lifespan and in all other age groups in the L1 lineage. The mean value of the egg size distribution of mated mothers in the L1 lineage was larger than that of virgins throughout their lifespan. This suggests that no egg size determines fertilization in this lineage, rather, it is fertilization that may influence egg size. Eggs produced by mated mothers receive more resources than those produced by virgins, and mating just increases egg size in general, thus leading to a larger size of male eggs produced by mated mothers than by virgins (Figure 2B, scenario 2). Contrary to the L1 lineage, in the T lineage, an entirely new scenario is needed to assess the obtained results; eggs laid throughout the lifespan of virgin mothers were significantly larger than those of mated mothers.

- 4. Egg size decreases with increasing maternal age in the L1 and T lineages of *T. tabaci* and this cannot be attributed to the resource depletion hypothesis. In the case of the L2 lineage of *T. tabaci* lineage, we can conclude that egg size was linearly increasing with growing maternal age, but the rate of dependence (i.e., the slope of the trend) was decreasing up to day 10, and thereafter it did not change notably.
- 5. Morphometric measurements were used to discriminate between lineages of *T. tabaci*. The results revealed significant differences in egg size between the three lineages. Female eggs of the L2 (virgin) mothers were larger than all the other groups. These were followed by female and male eggs from mated mothers of the L1 lineage. Meanwhile, the size of the male eggs of the virgin L1 mothers is bigger than the eggs of females and males of virgin and mated mothers of the T lineages, which are the three smallest groups among all groups of the three lineages.
- 6. Females of the L and T lineages are different in size from each other in the adult stage but not in the first instar larval stage. The morphometric analysis used in this study for discriminating between lineages of *T. tabaci* requires a prior species identification method, especially when thrips specimens are collected from the field, which plausibly may include other thrips species that exhibit morphometric variation as well. Therefore, the discrimination of *T. tabaci* lineages based only on morphometric variation may lead to misidentification in potentially mixed field collected samples.
- 7. Adult sexual size dimorphism in L1 and T lineages is not mediated by the size of the eggs and newly hatched first instar larvae, but the different growth rates of males and females results in sexual dimorphism in the adult stage.

# 5. NEW SCIENTIFIC RESULTS

- 1. Larger eggs are more likely to hatch than smaller eggs in the L1 lineage laid by mated mothers and in the T lineage of *T. tabaci* for all eggs independently from mating status.
- 2. Our results provide the first empirical evidence that egg size has no influence on sex allocation in this haplodiploid species and urges for further testing of this process in other haplodiploid species.
- 3. Eggs produced by mated mothers were larger than eggs produced by virgin mothers in the L1 lineage. In the T lineage, eggs produced by virgin mothers were larger than those of mated mothers. Our findings indicate that these two subspecies of *T. tabaci* have different resource allocation strategies in response to maternal mating status.
- 4. Male eggs produced by mated mothers were larger than those produced by virgin mothers in the L1 lineage, but in the T lineage, there were no differences in the size of male eggs produced by mated and virgin mothers.
- 5. The overall decreasing effect of increasing maternal age on egg size within both genders of the L1 and T lineages cannot be attributed to the resource depletion hypothesis, whereas in the L2 lineage, there is an increase in egg size with increasing maternal age.
- The female eggs of the L2 lineage were larger than those of the two other lineages.
- 7. The size of females of the L and T lineages is well separated from each other in the adult stage but not in the first instar larva.
- Adult sexual size dimorphism in L1 and T lineages is not mediated by the size of eggs and first instar larvae but by different growth rates of males and females.

## 6. REFERENCES

- 1. BEGON, M., PARKER, G.A. (1986): Should Egg Size and Clutch Size Decrease with Age? *Oikos*, 47(3): 293-302.
- BLANCKENHORN, W.U., DIXON, A.F.G., FAIRBAIRN, D.J., FOELLMER, M.W., GIBERT, P., VAN DER LINDE, K., MEIER, R., NYLIN, S., PITNICK, S., SCHOFF, C., SIGNORELLI, M., TEDER, T., WIKLUND, C. (2007): Proximate causes of Rensch's rule; does sexual size dimorphism in arthropods result from sex differences in development time. *American Naturalist*, 169(2): 245-257.
- 3. BRUNNER, P.C., CHATZIVASSILIOU, E.K., KATIS, N.I., FREY, J.E. (2004): Host associated genetic differentiation in *Thrips tabaci* (Insecta: Thysanoptera), as determined from mt DNA sequence data. *Heredity*, 93(4): 364-700.
- 4. BUDRIENE, A., EDUARDAS, B., NEVRONYTE, Z. (2013): Sexual size dimorphism in the ontogeny of the solitary predatory wasp *Symmorphus allobrogus* (Hymenoptera: Vespidae). *Comptes Rendus Biologies*, 336(2): 57-64.
- 5. CHURCH, S.H., DONOUGHE, S., DE MEDEIROS, B.A.S., EXTAVOUR, C.G. (2019): Insect Egg Size and Shape Evolve with Ecology but Not Developmental Rate. *Nature*, 571(7763): 58–62.
- DIAZ-MONTANO, J., FUCHS, M., NAULT, B.A., FAIL, J., SHELTON, A.M. (2011): Onion thrips (Thysanoptera: Thripidae): A global pest of increasing concern in onion. *Journal of Economic Entomology*, 104(1): 1–13.
- FAIL, J. (2016): Speciation in *Thrips tabaci* Lindeman, 1889 (Thysanoptera): The Current State of Knowledge and Its Consequences. *Polish Journal of Entomology*, 85: 93–104.
- FARKAS, P., GYÖRGY, Z., TÓTH, A., SOJNÓCZKI, A., FAIL, J. (2020): A Simple Molecular Identification Method of the *Thrips Tabaci* (Thysanoptera: Thripidae) Cryptic Species Complex. *Bulletin of Entomological Research*, 110(3): 397–405.
- FEDOR, P., PEÑA-MÉNDEZ, E.M., KUCHARCZYK, H., VAŇHARA, J., HAVEL, J., DORIČOVÁ, M. AND PROKOP, P. (2014): Artificial neural networks in online semiautomated pest discriminability: an applied case with 2 Thrips species. *Turkish Journal of Agriculture and Forestry*, 38(1): 111-124.
- 10. FEKRAT, L., MANZARI, S., SHISHEHBOR, P. (2014): Morphometric and molecular variation in *Thrips tabaci* Lindeman (Thysanoptera Thripidae)

populations on onion and tobacco in Iran. *Journal of Agricultural Science and Technology*, 16: 1505–1516.

- 11. FOX, C.W. (1993): The Influence of Maternal Age and Mating Frequency on Egg Size and Offspring Performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia*, 96: 139–146.
- 12. JENSER, G., SZÉNÁSI, Á. (2004): Review of the biology and vector capability of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). *Acta Phytopathologica et Entomologica Hungarica*, 39(1-3): 137–155.
- JOHARI, A., HERLINDA, S., PUJIASTUTI, Y., IRSAN, C., SARTIAMI, D. (2014): Morphological and genetic variation of *Thrips parvispinus* (Thysanoptera: Thripidae) in chili plantation (*Capsicum annuum* L.) in the lowland and highland of Jambi Province, Indonesia. *American Journal of Bioscience*, 2(6-1): 17-21.
- KATLAV, A., COOK, J.M., RIEGLER, M. (2020): Egg Size-mediated Sex Allocation and Mating-regulated Reproductive Investment in a Haplodiploid Thrips Species. *Functional Ecology*, 35(2): 485–498.
- 15. KOBAYASHI, K., HASEGAWA, E. (2012): Discrimination of reproductive forms of *Thrips tabaci* (Thysanoptera: Thripidae) by PCR with sequence specific primers. *Journal of Economic Entomology*, 105(2): 555–559.
- 16. LI, X.W., FAIL, J., WANG, P., FENG, J.N., SHELTON. A.M. (2014): Performance of arrhenotokous and thelytokous *Thrips tabaci* (Thysanoptera: Thripidae) on onion and cabbage and its implications on evolution and pest management. *Journal of Economic Entomology*, 107(4): 1526–1534.
- 17. LOREDO VARELA, R.C., FAIL, J. (2022): Host Plant Association and Distribution of the Onion Thrips, *Thrips tabaci* Cryptic Species Complex. *Insects*, 13: 298.
- MACKE, E., MAGALHÃES, S., KHAN, H.D.T., LUCIANO, A., FRANTZ, A., FACON, B., OLIVIERI, I. (2011): Sex Allocation in Haplodiploids Is Mediated by Egg Size: Evidence in the Spider Mite *Tetranychus Urticae* Koch. *Proceedings of the Royal Society. Biological Sciences*, 278(1708): 1054–1063.
- 19. MORSE, J.G., HODDLE, M.S. (2006): Invasion biology of thrips. *Annual Review of Entomology*, 51: 67–89.
- PALMER, J.M., WETTON, M.N. (1987): A morphometric analysis of the *Thrips hawaiiensis* (Morgan) species-group (Thysanoptera: Thripidae). *Bulletin of Entomological Research*, 77(3): 397-406.
- PALMER, J.M., MOUND, L.A., HEAUME, G.J. (1992): In: BETTS, C.R. (Ed.): IIE guides to insects of importance to man 2, Thysanoptera. Wallingford: CAB International, 73 p.

- 22. SHINE, R. (1989): Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *The Quarterly Review of Biology*, 64(4): 419-461.
- 23. SPEYER, E.R., PARR, W.J. (1941): The external structure of some thysanopterous larvae. *Transactions of the Royal Entomological Society of London*, 91: 559–635.
- TODA, S., MURAI, T. (2007): Phylogenetic analysis based on mitochondrial COI gene sequences in *Thrips tabaci* (Thysanoptera: Thripidae) in relation to reproductive forms and geographic distribution. *Applied Entomology Zoology*. 42(2): 309–316.
- 25. TRDAN, S., ŽNIDAR, D., VALI, N., ROZMAN, L., VIDRIDH, M. (2006): Intercropping against onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in onion production: on the suitability of orchard grass, lacy phacelia, and buckwheat as alternatives for white clover. *Journal of Plant Diseases and Protection*, 113(1): 24–30.
- 26. VIERBERGEN, G, KUCHARCZYK, H., KIRK, W.D.J. (2010): A key to the Second Instar Larvae of the Thripidae of the Western Palaearctic region (Thysanoptera). *Tijdschrift Voor Entomologie*, 153: 141–160.
- 27. YANAGI, S.-I., MIYATAKE, T. (2002): Effects of maternal age on reproductive traits and fitness components of the offspring in the bruchid beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Physiological Entomology*, 27(4): 261-266.
- ZHOU, P., HE., X.Z., WANG, Q. (2018): Sons from Virgin Mothers Produce More Daughters in a Haplodiploid Mite. *Systematic and Applied Acarology*, 23(9): 1869-1878.

# LIST OF PUBLICATIONS

# PUBLICATIONS RELATED TO THE TOPIC OF THE THESIS

# Journal articles with IF:

**MUSA, S.**, LADÁNYI, M., FAIL, J. (2022): There is no influence of egg size on sex allocation in arrhenotokous lineages of *Thrips tabaci* Lindeman. *Insects*. 13(5): 408. <u>https://doi.org/10.3390/insects13050408</u> (IF=2.769) Q1

**MUSA, S.**, LADÁNYI, M., LOREDO VARELA, R.C., FAIL, J. (2023): A morphometric analysis of *Thrips tabaci* Lindeman species complex (Thysanoptera: Thripidae). *Arthropod Structure & Development*. 72: 101228. https://doi.org/10.1016/j.asd.2022.101228 (IF=2.075) Q1

# Abstracts of the conferences:

**MUSA, S.**, LADÁNYI, M., FAIL, J. (2021): Egg size increases with maternal age in leek associated thelytokous (L2) *Thrips tabaci* lineage. 6th symposium on Palaearctic Thysanoptera, P.28. (Zamárdi, 2021. September 13-17).

# PUBLICATIONS UNRELATED TO OR NOT DIRECTLY RELATED TO THE TOPIC OF THE THESIS

**MUSA, S.**, MUSA, F. (2023). Incidence of onion thrips *Thrips tabaci* Lindeman in some cabbage varieties in Kosovo. *Indian Journal of Entomology*. Online published Ref No. e23480 <u>https://doi.org/10.55446/IJE.2023.840</u> (IF = 0.18) Q4

**MUSA, S.**, MUSA, F. (2023). Evaluation of insecticides efficacy against *Thrips tabaci* Lindeman on onion. International Journal of Entomology Research. Volume 8, Issue 4, 2023, Pages 1-5. ISSN: 2455-4758. <u>https://www.entomologyjournals.com/archives/2023/vol8/issue4</u>

MUSA, F., BERISHA, D., **MUSA, S**. (2023). Aphids (Hemiptera: Aphididae) on plum in Kosovo. International Journal of Entomology Research. Volume 8, Issue 5, 2023, Pages 10-13. ISSN: 2455-4758. <u>https://www.entomologyjournals.com/archives/2023/vol8/issue5</u>

# Abstracts of the conferences:

WOLDEMELAK, W.A., FARKAS P., **MUSA S.**, LOREDO VARELA R.C., FAIL. J. (2018): Do adult males inseminate female pupae of arrhenotokous onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae)? 60<sup>th</sup> Georgikon Scientific Conference, P. 49. (Keszthey, 2018. Oktober 4-5).