Final report – NKFIH (OTKA) K 109594

Reproductive biology of onion thrips

The onion thrips (*Thrips tabaci* LINDEMAN 1889) had been already considered a significant pest even before it was first described by a Russian entomologist in 1889. This thrips species has received considerable attention ever since due to its importance as a polyphagous, cosmopolitan pest of several crop plants. Despite being one of the most studied thrips species, several aspects of its biology remain unknown. A Polish thysanopterologist proposed in the 1970's that onion thrips has two distinct biotypes. Since 2004 it has been commonly accepted that *T. tabaci* forms a cryptic complex of 3 (sub)species: 2 leek-associated lineages (L1 and L2) that differ in their reproductive modes (one being arrhenotokous and the other one thelytokous) and an arrhenotokous tobacco-associated lineage (T). The primary purpose of this project was to examine the reproductive biology of arrhenotokous and thelytokous onion thrips.

1. Objectives

1.1. Establish onion thrips colonies of different reproductive modes and geographical origins

We have been collecting *T. tabaci* samples from several locations in Hungary throughout this project. After examining these samples, we have successfully identified thelytokous and arrhenotokous *T. tabaci* individuals. Up until now, we have not encountered a single deuterotokous *T. tabaci* individual in the field-collected samples, yet. For the identification of the reproductive mode of a living thrips specimen, we kept virgin females (selected as larvae from the field sample) isolated in microcentrifuge tubes and sexed their progeny when they matured to adults. Virgin arrhenotokous females produce only male progeny, whereas virgin thelytokous females produce only female progeny. Then in case of the arrhenotokous individuals the isolated female was mated with a male (produced by a different virgin female of the same sample) in order to allow the production of female progeny before the adult female was slide mounted for morphological identification. Total DNA was extracted from some of the *T. tabaci* individuals of each isoline and the cytochrome C oxidase subunit I (COI) region of the mitochondrial DNA was amplified with the use of a specific sense (5' ATTAATTATAGGRCTTTAYAAAGAAGG 3') and an antisense primer (5' GTAGTGAAAGTGAGCTACAACATAATA 3'). These primers were designed by using the available *T. tabaci* sequences in GenBank. The progeny of isolines that were grouped into one of the known lineages of onion thrips based on the sequenced PCR products were used to establish a pure culture of the respective lineages. This is how we have established thelytokous and arrhenotokous colonies from the progeny of the field collected *T. tabaci* specimen. These colonies were maintained and used in our experiments at the Department of Entomology, Szent István University in the entire duration of this project.

The sheer volume of *T. tabaci* individuals used in our consecutive experiments and the need to confirm the lineage of these individuals encouraged us to develop an identification tool, which does not

require the sequencing of the PCR products mentioned above. The PCR products were then digested with two restriction endonucleases (PsuI, PsyI). The restriction fragments obtained from the digestion were separated electrophoretically. In the T lineage there is no cleavage resulting in the original 780 bp fragment, in the L2 lineage there is one cleavage site resulting in 2 fragments (619bp + 161bp), and finally in the L1 lineage there are two cleavage sites resulting in 3 fragments (345bp + 274bp + 161bp). The identity of all specimen used in our consecutive experiments was confirmed by this method.

We have developed a thrips rearing method for the maintenance of our colonies. We discarded the use of artificial food and egg laying substrate due to its extreme labour requirement. However, we made steps to avoid any contamination with the use of plant leaves in culture maintenance. The L1 and L2 lineages of onion thrips were maintained in plastic containers containing leek and cabbage leaf sections, respectively. The needed leek and cabbage plants were produce at our experimental and research farm or obtained from the market. The leaves that were damaged by thrips were discarded and only symptom free, etiolated plant parts were used from both leek and cabbage heads. The leaves from both plants were cut into smaller leaf sections (about 3 by 5 cm in size) and examined for the presence of thrips under a stereo microscope before introducing them into the culture. These durable leek and cabbage leaf sections served as food and egg laying substrate for onion thrips. A piece of double-layered paper towel served as pupation site at the bottom of the container. For the maintenance of the tobacco-associated lineage we used tobacco leaves. As tobacco leaves were not as durable as leek or cabbage leaves, we followed this procedure: Detached leaves from young tobacco plants were transported to a small vial containing agar gel. The water uptake from the agar gel through the leaf stalk of tobacco contributed to increase the durability of the leaves despite supporting a thrips colony. The plastic containers were kept in our growth chambers under constant temperature and long daylight conditions. Tobacco plants were grown in a plant growth room to provide uninfested plants needed for colony maintenance or experiments.

1.2.Measure complete life table parameters of onion thrips

The life table experiment was carried out at 21 degrees of centigrade and 16:8 hours of light and dark period with the L1 and L2 lineages of onion thrips. We have completed this test using cabbage leaf discs confined into 2 ml microcentrifuge tubes serving as food source and oviposition substrate as well. Eggs were obtained by allowing adult females to oviposit on the leaves for a 12hr period. Upon which, the adults were transferred to another similarly prepared tube and this was repeated until the desired number of eggs were deposited by each reproductive mode. The development of the eggs in the leaf discs were monitored at 12hr interval using the bottom light of a stereomicroscope. The duration of the egg stage was recorded as the time between when the given leaf disc was taken from the gravid female until the emergence of 1st instar larva. Newly emerged larvae were transferred to new tubes individually with a fresh leaf disc provided and monitored on a 12hr interval until they moulted to the second instar stage. The transition from first to second instar larvae was determined by the presence of a shed skin in the tubes, hence on living individuals there are no detectable morphological differences between the two larval stages. Prepupae were

identified by their short wing sheaths and erect antennae, while pupae were identified by long wing sheaths and antennae that are folded on the head. Adults were identified by their well-developed wings. The males and females of the arrhenotokous line were distinguished morphologically by the size of the abdomen, which is relatively bigger and darker in females. When arrhenotokous females emerged from pupae, a single male was immediately introduced for a period of 48hrs for mating but thelytokous females were kept in isolation individually. The male was then removed and kept in a separate tube. The longevity of males and females was recorded. In addition, the preoviposition period for the female was measured as the period from the emergence of the adult to the observation of the first egg laid. The length of the egg laying period (herein oviposition period) was determined by the total duration of days that the female was able to lay eggs before it died. Fecundity was determined by the overall egg production of the female in its entire lifespan. Daily fecundity on the other hand was determined as lifetime fecundity divided by oviposition period.

There was no significant difference between the total development time of females in the two lines of *T. tabaci*: it was 17.2 days and 17.8 days for the arrhenotokous and thelytokous lines, respectively. Development time of arrhenotokous males was 17.4 days. There was no significant difference detected in any juvenile stage between the two lines of arrhenotokous males and females. The lifespan of arrhenotokous males and thelytokous females was statistically equal, 25.8 and 27.0 days, respectively. However, arrhenotokous females seemed to live longer (32.4 days) and their oviposition period was also longer (29.3 days) than that of thelytokous females (24.9 days). There was no significant difference in daily and lifetime fecundity between the two lines. Lifetime fecundity of arrhenotokous and thelytokous females was 92 and 97 eggs, respectively. Daily fecundity of arrhenotokous and thelytokous females was 3.6 and 4.0 eggs, respectively. The preoviposition period of thelytokous females (3.2 days) was almost 2 days shorter than that of arrhenotokous females (5.1 days).

The manuscript about this experiment is still being prepared.

1.3.Study the reproductive diapause of onion thrips

Twenty onion thrips adult females from each lineage were isolated from our stock colonies. These adult females were reared in 2 ml microcentrifuge tubes containing tobacco leaf discs for T and cabbage leaf discs for L1 and L2 lineages as a food and oviposition substrate as well as for humidity control within the closed tubes. The leaf discs were replaced every 48 hours until enough eggs were collected. The eggs in the leaf discs were counted with the use of the bottom light of a stereomicroscope.

The leaf discs with eggs were split up into two groups and kept in a growth chamber at 23 ºC under long daylight (16L:8D) and short daylight (8L:16D) photoperiod conditions and checked every other 48 hours. The newly hatched first instar larvae were collected daily and kept isolated individually under similar conditions. The newly emerged virgin adult females were also kept individually isolated in order to observe egg laying. In the mated mother's treatment of the arrhenotokous lineages, a newly emerged adult female

was exposed to a single adult male for 48 hours. The females in both photoperiod treatments were observed twice a week and new leaf discs were provided until they died naturally.

All of the tested L2 females (N=98) produced eggs under both short and long daylight conditions. The behaviour of arrhenotokous L1 females was identical under short and long daylight conditions (only 1 out of 103 tested females did not lay eggs). The T lineage of onion thrips did produce eggs under long daylight conditions (all of the tested females, N=104), however, under short daylight conditions 44 % of virgin and 32 % of mated T type females did not produce a single egg before they died. As there was no statistical difference between the percentages of females in reproductive diapause, we concluded that regardless of mating status (whether the female is virgin or mated) about 30-40 % of the tobacco associated onion thrips population entered reproductive diapause under these conditions. We are currently working on a similar experiment regarding the light conditions but at lower constant temperature (15 ºC). The manuscript about this experiment is still being prepared.

1.4.Study the response of arrhenotokous onion thrips to inbreeding

We can report some interesting observations in our inbreeding trials. We exposed the L1 arrhenotokous lineage of *T. tabaci* to two different inbreeding treatments: in one hand a newly emerged virgin female adult was coupled with one of its virgin brothers for 48 hours; on the other hand a newly emerged virgin female was kept isolated until its first son emerged as adult and they were housed together in an Eppendorf tube for 48 hours before the male was removed. We measured the fecundity and lifespan of a cohort in the progeny as well as the sex ratio. Regarding the latter, a larger proportion of males were produced by the mated females in the mother and son treatment (68:71=males:females) than in the sister and brother treatment (37:112=males:females). Since females can only be produced from fertilized eggs in the arrhenotokous lineages, it indicates lower fertilization success when the only mate is a son compared to a brother. The hatchability of the eggs and proportion of female progeny was not affected by either type of inbreeding treatments but fecundity and lifespan was reduced in both and in the brother and sister treatment, respectively.

To our greatest surprise, we did observe the appearance of deuterotoky in one females produced in the mother and son inbreeding treatment. This female was kept isolated in an Eppendorf tube and despite being virgin, it produced sons and daughters. We were running follow up tests and molecular identification of this unique female adult and its progeny in order to exclude that it was the result of undetected contamination in the trial. Since the outcome of these tests confirms this as genuine result, it means the second report of deuterotoky in *T. tabaci*. Deuterotoky did not seem to be a stable trait in our T type onion thrips but after rearing 3 follow-up generations of the female progeny it completely disappeared, all the females in the last generation produced exclusively male progeny. We suspect that the mother and son inbreeding treatment played a role in the appearance of these rare, deuterotokous females but we need to confirm it in further experiments. To our knowledge, this is the first report of linking deuterotoky to inbreeding in Thysanoptera.

1.5.Study the isolation between onion thrips of different reproductive forms

The mating behaviour between males and females of different *T. tabaci* lineages was also studied in the project. We coupled a 2-7 days old single adult female from each of the 3 known lineages with a 2-7 days old single adult male of the L1 and T lineages. This resulted in 6 treatments and the number of couples in each treatment varied between 25 and 30. The behaviour of the couples was recorded for 10 minutes and the sequences of the mating process noted. Couples from the same arrhenotokous lineages (L1 female and L1 male, T female and T male) usually mated with each other, 25 out of 26 and 24 out of 25 couples for the T and L1 lineages, respectively. However, couples from different arrhenotokous lineages never mated with each other, regardless of which lineage the male or the female belonged to. There were no mating observed in 26 couples of T females and L1 males, and similarly no matings between 29 T males and L1 females. We concluded that despite the same reproductive mode in the L1 and T lineages (i.e. arrhenotoky), there is a strong reproductive isolation between these two lineages of *T. tabaci*. To the contrary, there seems to be no reproductive isolation between the L1 and L2 lineages of *T. tabaci*, despite the difference in their reproductive mode: arrhenotoky and thelytoky in the L1 and L2 lineages, respectively. Mating was recorded in 22 out of 27 couples of L1 males and L2 females. Whereas mating was recorded in only 2 out of 29 couples of T males and L2 females. This confirms our findings in earlier studies about the mating behaviour between individuals of L1 and L2 lineages but provides new evidence of the reproductive isolation between the T and the other 2 leek-associated lineages. It has been demonstrated in our earlier lab study that the ratio of successful mating between individuals of the L1 and L2 lineages is rather low, about 2%. Nonetheless, gene transfer between these two lineages has been confirmed and it cannot be ruled out between the T and L2 lineages either, but requires follow-up studies. Some of our preliminary studies in this field have been published but a manuscript is in preparation describing the mating behaviour within and between all 3 different lineages of onion thrips.