HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

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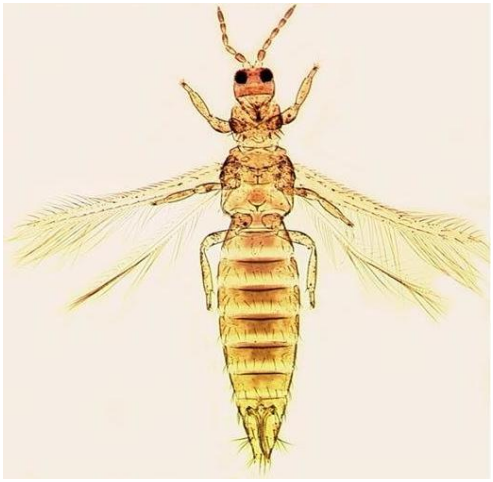
Biological, Ecological and Behavioural Aspects of Onion thrips (*Thrips tabaci* Lindeman, 1889) Species Complex

DOI: 10.54598/001740

Thesis of Doctoral (PhD) Dissertation



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BUDAPEST

2021

Ph.D. School

Name: MATE Doctoral School of Horticultural Sciences

Discipline: Plant Protection and Horticultural science,
Department of Entomology

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The applicant met the requirement of the PhD regulations of the Hungarian University of Agriculture and Life Sciences of Budapest and the thesis is accepted for the defence process.

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1. INTRODUCTIONS AND AIMS

Thrips tabaci belongs to the order Thysanoptera, suborder Terebrantia, family Thripidae, subfamily Thripinae and genus thrips. Since it has been first described by a Russian entomologist, Karl Eduard Lindeman in 1888, this species arose as one of the most important pests of horticultural and several agronomic crops worldwide. *T. tabaci* was recognized as a single thrips species before ZAWIRSKA (1976) described different biological types. Based on reproductive modes, host adaptations, virus transmission efficiency (TSWV), and morphological differences on the abdominal tergites of second-stage larvae she has proposed two distinctly different forms of *T. tabaci* types, namely, communis and tabaci types. Communis type had arrhenotokous reproductive mode (males produced from unfertilized eggs and females produced from fertilized eggs), and thelytokous reproductive mode (females produced from unfertilized eggs), broad host plants, less virus transmission efficiency, whereas tabaci type had only arrhenotokous reproductive mode, a small group of host plants and high virus transmission efficiency. Since then, based on DNA sequences of the Mitochondrial Cytochrome c oxidase I (mt COI) genes, *T. tabaci* has been cleavaged into three lineages: two leek-(L1, L2) and tobacco-associated (T)(Brunner et al., 2004; Fekrat et al., 2009; Jacobson et al., 2013; Kobayashi et al., 2013; Kobayashi & Hasegawa, 2012; Toda & Murai, 2007). The leek-associated (L1) is the ancient form of *T. tabaci*. It is believed that tobacco-associated (T) type diverged from the ancient form of L1 and since then adapted to solanaceous host plants (Brunner et al., 2004).

Both adults and larvae of *T. tabaci* cause damage to their hosts by piercing the surface tissues and sucking the contents of plant cells, which results in small silvery patches that turn into white blotches. Economic damage results directly through feeding and oviposition. Reliable estimates of economic losses are scarce and difficult to determine, due to its polyphagous nature and vector of several plant viruses, its direct damage has been estimated to cause more than U.S. $1 billion in crop losses annually worldwide (Balan et al., 2018). The latter pathogen is estimated to cause annual losses of U.S. $90 million to onion production in the USA alone (Gent et al., 2006) while the former can cause over U.S. $1 billion in crop losses annually worldwide (Goldbach & Peters, 1994). Subsequent studies have reported that the spread

of the *Tomato spotted wilt virus* on tobacco fields of East-Europe was caused by *T. tabaci*. (Chatzivassiliou et al., 1999).

Recent research in Europe and North America has concentrated on understanding the biological, behavioural and ecological aspects of *T. tabaci* so that any chosen control method can be targeted more efficiently against the pest. At this time, very little research has been done on *T. tabaci* as cryptic species complex worldwide. Scientific researchers have focused almost exclusively on the different biological, ecological and behavioural aspects of the L1- and L2-biotypes, which were approached from different disciplines. This is partly due to the fact that the leek-associated biotypes can be characterized by a broader host plant range and larger geographical distribution area. Studying the researches will quickly realize that our knowledge regarding the species complex is incomplete, which is especially relevant to the T-biotype. This Ph.D project combines researches regarding the ecological, biological, and behavioural aspects of the different *T. tabaci* biotypes in the laboratory in order to develop a sustainable and long term effective pest management strategy to control *T. tabaci* lineages outbreaks.

The aims of my study were the following:

1. Study the reproductive diapause of *T. tabaci* lineages at different temperature and photoperiod ranges. The aim of this research was to measure the effect of temperature and photoperiod on the pre-oviposition, reproductive diapause, oviposition, longevity and fecundity of *T. tabaci* lineages.
2. Study the inbreeding depression and its effects on the different lifetable parameters and sex ratio of the arrhenotokous lineages of the *T. tabaci* lineages. The aim of this research was to compare the lifetable parameters (fecundity, egg hatchability rate, longevity and sex ratio) of L1 and T lineages that underwent brother and sister for the consecutive generations.
3. Examine the influence of temperature on the sex ratio of the leek- (L1) and tobacco-associated (T) lineages. The aim of this research was to investigate the effect of temperature on the sex ratio and life table parameters of leek - (L1) and tobacco – associated (T) lineages. The study of sex ratio and their sympatric variation is necessary for realizing of causes and

results in the variations of population structures and mating systems.

4. The existence of deuterotokous reproduction mode in the *T. Tabaci* lineages. The aim of this work was to reveal if adult males could fertilize immature female pupae in the arrhenotokous L1 and T lineages and to identify if the mother to son inbreeding could induced deuterotokous reproductive mode.
5. Test the male fighting behaviours of L1 and T lineages. The aim of this paper was to characterize the male fighting behaviour and to compare the fighting performance at different ages in L1 and T lineages.

2. MATERIALS AND METHODS

2.1. Establishing colonies of the three lineages

To ensure a regular supply of individuals for the experiments, pure stock colonies of the *T. tabaci* lineages were maintained on different plant sources in the Department of Entomology, Szent István University in Budapest, Hungary. The arrhenotokous leek-(L1), thelytokous leek -(L2) and arrhenotokous tobacco-associated (T) lineages were maintained on leek (*Allium porrum* L.), cabbage (*Brassica oleracea* L. convar. *capitata* var. *alba*) and tobacco (*Nicotiana tabacum* L.) leaves, respectively. The colonies were maintained at 23°C under long daylight (16L: 8D).

Host plants: Cabbage and tobacco plants were used in this research. Cabbage plants were grown at the Experimental and Research Farm of the Horticultural Science Faculty of Szent István University, and tobacco plants were grown in the plant growing room of the Department of Entomology at Szent István University. Cabbage leaf discs dissected from head forming leaves were used in the bioassays of the two leek-associated (L1 and L2) *T. tabaci* lineages; because these two lineages perform well on this common host plant and tobacco leaf discs dissected from middle-aged leaves were used in the bioassays of the tobacco-associated (T) *T. tabaci* lineage. The plant parts were carefully examined before introduced to the culture in order to prevent unwanted thrips contamination (thrips-free plant material).

2.2. Effects of temperature and photoperiod on lifetable parameters

Conducting bioassays: To initiate the experiments, random sampled female individuals (20 individuals) were isolated from the stock colonies of each lineages, and reared individually in 2 ml microcentrifuge tubes on leaf discs of their preferable host plant and held at 23°C under long daylight (16L: 8D) conditions. To ensure the production of enough eggs for the treatments, these adult females were transferred to new tubes daily until their death. Then the females were preserved in 96% ethanol individually until the lineage was confirmed based on sequences of a mitochondrial COI (mtCOI) product (Farkas et al., 2020)

Treatments: Three treatments were set up in environmental growth chambers: (1) 23°C under long daylight (16L: 8D), (2) 23 °C under short

daylight (8L: 16D), and (3) 15 °C under short daylight (8L: 16D). Leaf discs confined into 2 ml microcentrifuge tubes individually and containing eggs of maximum 24 h of age and were exposed to the above treatments because eggs are more sensitive to photophase (NAKAO 1998). Thus, exposure of adults to short daylight is not enough to induce reproductive diapause. The newly hatched first instar larvae were isolated individually in a microcentrifuge tube containing a leaf disc dissected from the lineages' host plant and reared until they became adults. The newly emerged virgin male and female adults were collected daily and kept under the same conditions until they died.

2.2.1. Effect of temperature and photoperiod on preoviposition period and reproductive diapause

Newly emerged virgin female adults of the L1, L2 and T lineages were kept isolated individually and were transferred to a new microcentrifuge tube containing a leaf discs of their preferred host plant every 24 h intervals. The preoviposition period was calculated as the time from adult emergence to the beginning of oviposition. Leaf discs were changed daily until the observation of the first egg using the bottom light of a stereomicroscope (Alpha, NSZ-606, Novel optics, Ningo Yongxin, China). When females began laying eggs, leaf discs were changed regularly at 48 h intervals and diapausing females were provided new leaf discs in a similar way until their death.

To measure the incidence of reproductive diapause, the oviposition of females was monitored in their entire lifetime. The criteria employed to categorize females in reproductive diapause was the failure to oviposit during their lifetime. Females that did not lay a single egg during their lifetime were considered being in a reproductive diapause and females that laid eggs during their lifetime were considered reproducing females. However, some females died within a relatively short period of time without laying a single egg. Those females that died before reaching the age of the upper bound of the 95 percent confidence interval of average preoviposition time were excluded from this test. Therefore, the females that lived longer than the upper bound of the 95 percent confidence interval of average preoviposition time and produced some eggs were considered reproducing and those that did not lay a single egg as being in reproductive diapause.

2.2.2. Effects of temperature and photoperiod on oviposition period, longevity and fecundity

The length of the oviposition period (i.e., the period between the first and the last egg laid, measured in days), longevity (i.e., the period between the emergence and the death of the adult, measured in days), and fecundity (i.e., total number of eggs laid) were calculated for each female. Females that escaped or those that were accidentally hurt during handling were excluded.

2.3. Inbreeding depression and its effects on the different lifetable parameters

To initiate the experiment, 24 female adults were isolated from the stock colonies of L1 and T lineages, and reared individually in 2 ml microcentrifuge tubes on cabbage and tobacco leaf discs, respectively, as a food source and oviposition substrate and held at 23 °C under long daylight (16L: 8D) conditions. To ensure the production of enough eggs for the treatments, these adult females were transferred to new tubes daily until their death. Then, females were preserved in 96% ethanol individually until the lineage was confirmed based on sequences of a mitochondrial COI (mtCOI) product (FARKAS et al. 2020). The newly hatched progeny from these mothers were raised individually to adulthood. These progenies used as a parental generation (F_1) for the subsequent inbred line, and assumed that inbreeding coefficient was equal to zero, which was used as a control to compare F_2 and F_3 generations. Thus, F_2 and F_3 generation assumed that inbreeding coefficient was greater than one. The inbreeding coefficient for female haplodiploids that are the product of full sib mating is equal to that of diploids (WRIGHT 1969). Thus, full sibling mating created an inbreeding coefficient (F) for inbred haplodiploid females of 0.25 and 0.375 for generation F_2 and F_3, respectively. To measure the intensity of inbreeding depression in the L1 and T lineages, we performed crosses with brother and sister, and mother and son relatedness.

2.3.1. Brother and sister Inbreeding depression

Treatments: Brother and sister inbreeding line was performed for three subsequent generations. Females were randomly chosen from the progeny of the previous generation. Thus, first-generation (F_1) females were

daughters of females taken from stock culture and used as a parental generation in the experiment, second-generation (F_2) females were daughters of F_1 females and third-generation (F_3) females were daughters of F_2 females, and therefore females of all generations were assumed to be related.

In order to ensure mating between brother and sister of the L1 and T lineages: a newly emerged F_1 brother and sister produced from the same mother were confined into the same microcentrifuge tube for 48 h. Then the male was removed and its sister was kept isolated individually for the rest of its lifetime. Leaf discs were changed every 48 h and eggs in the leaf discs were counted using the bottom light of a stereomicroscope. Fecundity, egg hatchability (in %), longevity (in days), sex ratio of the progeny was measured.

2.4. Effect of temperature on sex ratio and lifetable parameters

Rearing: The arrhenotokous L1 and T lineages were used for this experiment. To produce the known age females from known lineages the following procedures were used: 12 unknown age females were randomly isolated from the stock culture of arrhenotokous L1 and T lineages and reared individually in 2-ml microcentrifuge tubes on cabbage head and tobacco leaf discs, respectively. Eggs in the leaf discs were counted by using the bottom light of a stereomicroscope. The progeny was raised to adulthood in isolation since the newly emerged larvae. In order to ensure mating in the L1 and T lineages a newly emerged female and male adult was confined into the same microcentrifuge tube for 24 h. Then the male was removed and the female adult was kept isolated individually for the rest of its life.

Treatments: In the laboratory, three temperature ranges such as 15, 23, and 30 °C were established to test the effects of temperature on the sex ratio and lifetable parameters of the arrhenotokous leek- (L1) and tobacco-associated (T) *T. tabaci* lineages and the test was performed under controlled environmental growth chamber with long daylight (16L: 8D).

2.4.1. Effect of temperature on the pre-oviposition, fecundity, egg hatchability and longevity

The newly emerged female adults of the L1 and T lineages were exposed to 15, 23, and 30°C. Female adults were transferred to a new microcentrifuge tube containing a leaf discs of their preferred host plant in

every 24 h intervals for their entire lifetime. The eggs in the leaf tissue were counted using the bottom light of a stereomicroscope. The pre-oviposition period of the L1 and T mothers (n=17, 19) at 15°C, (n=38, 35) at 23°C, and (n=37, 45) at 30°C, respectively, was calculated as the time between adult emergence to the observation of the first egg, measured in days. When females began laying eggs, leaf discs were changed regularly at 24 h intervals. Fecundity (i.e., the total number of eggs laid) of the L1 and T mothers (n=17, 39) at 15°C, (n=38, 35) at 23°C and (n=37, 45) at 30°C, respectively, was calculated for each female. Egg hatchability of the L1 and T mothers (n=17, 19) at 15°C, (n=38, 35) at 23°C and (n=37, 45) at 30°C, respectively, was calculated as the ratio of the hatched eggs and total number of eggs per female, expressed in percent. Longevity (i.e., the period between the emergence and the death of the adult) of the L1 and T mothers (n=46, 45) at 15°C, (n=39, 35) at 23°C and (n=38, 45) at 30°C, respectively, was measured in days.

2.4.2. Effect of temperature on the sex ratio

The development of the progeny produced by mated females was terminated in either the first or second instar stage and all of the larvae were preserved in 75% ethanol. At a later period, the preserved larvae were slide mounted in drops of Berlese mounting medium. To dry the specimens, the slides were kept in an oven at 50°C for 2 days. The nymphal progenies were collected from the mothers of the L1 and T lineages (n=17, 15) at 15°C, (n=39, 34) at 23°C, (n=38, 45) at 30°C, respectively. The number of larvae identified as male and female from each temperature treatments are presented in Table 1.

2.5. THE EXISTENCE OF DEUTEROTOKOUS REPRODUCTION MODE IN THE *T. TABACI* (THYSANOPTERA: THRIPIDAE) LINEAGES

Mother-son inbreeding: To test whether a mother and son inbreeding is the factor to induce a deuterotokous reproductive mode of the L1 and T lineages: 10 female adults were isolated randomly from each stock colonies of the L1 and T lineages, and reared individually in 2 ml micro-centrifuge tubes. Cabbage and tobacco leaf discs were provided for L1 and T lineages, respectively, to serve as a food source and oviposition site. Although, before the cabbage and tobacco leaf discs provided as a food and oviposition site: the dissected cabbage head and tobacco leaves were checked using the bottom light of a stereomicroscope to ensure that the leaf discs were not contaminated

by other thrips species before experimentation. To ensure the production of ample eggs for the treatments, these adult females were transferred to new tubes daily until their death. Then the females were preserved in 96% ethanol individually until the lineage was confirmed based on sequences of a mitochondrial COI (mt COI) product (FARKAS et al. 2020). The newly hatched progeny from these mothers were raised to adulthood. These progenies were considered as F_1 generation and used as a parental generation for the subsequent inbred line. Thus, all the tested inbred generations were assumed to be related.

To ensure a son for virgin female: 30 virgin single adult females were isolated from the F_1 females and reared individually on their preferable food leaf discs for 2 days at 23 °C. As they were virgins, their eggs were unfertilized and assume to be developed into sons. Then the reproducing females were placed at 15 °C. At this low temperature, their longevity is increased which increases their chance for mating with their own son. When the son became adults, single mother and son were confined into the same microcentrifuge tube for 48 h. Then the male was removed and the female adult was kept isolated individually in the rest of its lifetime. The progeny produced from these mothers were considered as F_1 mother and son inbred line and their subsequent daughters were collected for F_2 mother and son inbreeding. Mother and son inbreeding has tested for two subsequent generations such as (F_1) females were daughters of females taken from stock culture and used as a parental generation in the experiment and (F_2) females were daughters of F_1 females.

2.6. Fighting behaviour of male

Rearing: To initiate the experiment, twenty female adults of unknown age were isolated from the stock colonies of each lineage and reared individually in 2 ml microcentrifuge tubes on leaf discs of their preferable host plant. L1 females were given cabbage and T female's tobacco. To ensure the production of ample male progeny for the experiment, these adult females were transferred to new tubes daily until their death. Then the females were preserved in 96% ethanol individually until the lineage was confirmed based on sequences of a mitochondrial COI (mt COI) product (FARKAS et al. 2020). The newly hatched first instar larvae were individually transferred to new microcentrifuge tubes and kept at 23 °C under a long photoperiod (16L: 8D). Upon reaching maturity, adult males were collected daily for the purposes of

the experiment. These males were isolated and kept individually in a separate micro-centrifuge tube. This way ensured that no males had any contact with other males.

Bioassay: Arena technique as described by KIRK (1987) was adapted for this experiment. Transparent PCR tube caps were used to form the arena. A total of 80 males (40 belonged to L1 and 40 belonged to T lineages) were used for this experiment, and the pairs of males were 2, 5, 8, 10, 12 days old in four replications. Two males with no previous fighting experience were transferred into the PCR tube cap, and a microscopic glass coverslip was immediately sealed onto the PCR tube caps to form the roof of the arena and this way was used to prevent males from escaping.

Video recording: A Euromex VC.3036 video camera was placed above the arena on a stereomicroscope at a height of 15 cm that enabled it to view the whole arena. This was connected to a video recorder hp computer and each experiment was recorded with a resolution of 1920X1080 for a duration of 10 min. Data was obtained from the recorded video. Each recording had a different arena.

Observations: A total of 40 video recordings (20-20 for L1 and T, with 40 individuals for each lineages) were observed. Fighting was defined as observations of contact between two males with antennal bouts, abdominal flicking, grabbing and flipping, where these actions took more than 1 second. The percentage of fighting males, fighting frequency (number of fights recorded in the arena during the observation period), pre-fight period (the time before the first fight), duration of fight and re-fighting time interval (the time between two consecutive fights) were recorded during each 10-minute observation.

5. RESULT AND DISCUSSION

5.1. Effects of temperature and photoperiod on the lifetable parameters

5.1.1. Effects of temperature and photoperiod on the preoviposition period

The preoviposition periods of L2 lineage were longer under 8L: 16D at 23 °C as compare with preoviposition periods of females of the L2 lineage reared under 16L: 8D at 23 °C. But there was no significant difference in the L1 and T lineages. Additionally, the preoviposition periods of all three lineages were longer with decreasing temperature (15 °C) and lengthening dark period settings (8L: 16D). Longer preoviposition periods were observed in the L1 lineage (27 days) under 8L: 16D at 15 °C and that was about ten times as long as that of those females reared under 8L: 16D at 23°C.

In this work, the preoviposition periods of females in the L2 lineage were significantly longer when the females were exposed to 8L: 16D at 23 °C, but there was no significant difference in the L1 and T lineages. Brodsgaard (1994) found no difference in preoviposition period of *F. occidentalis* between 4L: 20D, 8L: 18D and 16L: 8D at 25 °C. The variation in photoperiod response among *T. tabaci* lineages suggests that adaptation in different photo-regime might be another variability between the lineages. Thus, L2 lineage may have experienced a physiological adaptation to long daylight only whereas; L1 and T lineages may have experienced a physiological adaptation to both short and long daylight at optimum temperatures of 23 °C. On the other hand, a significant difference in preoviposition period of of females in all lineages was observed between 15 and 23 °C under 8L: 16D. It indicates that short daylight does not act in isolation to influence the length of the preoviposition period of females in the L1 and T lineages. More specifically, we suggest that the preoviposition period's females in the L1 and T lineages determined by the effect of both low temperature and short daylight. (T Murai, 2000; Sakimura, 1937a) found longer preoviposition period of females in the L2 lineage at 15 °C. Longer preoviposition period under 10L: 14D at 20 °C, but no difference between 10L: 14D and 16L: 8D at 15 °C has been reported in *F. occidentalis* (Ishida et al., 2003). Long daylight (L16:D8) and high temperature (29 °C) increased the preoviposition periods of *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) and absence of egg laying at these conditions

indicates some evidence of reproductive diapause (EKESI et al. 1999). Longer preoviposition periods (19 days) have also been reported in *Heliothrips haemorrhoida* (Bouché) (Thysanoptera: Thripidae) at 15 °C (Rivnay, 1935). In this result, shortest preoviposition periods were 9.03 days for T lineage and longest preoviposition periods were 27.7 days for L1 lineage under 8L: 16D at 15°C. (Murai, 1987) has found longer preoviposition period in *F. intonsa* under 13L: l1D (2.8 days) than 14L: 10D (3.5 days) at 20 °C.

5.1.2. Effects of temperature and photoperiod on reproductive diapause

Reproductive diapause was detected for 40 % of the tested females in the T lineage under 8L: 16D at 23 °C, however, reproductive diapause was not detected in the L1 and T lineages under 8L: 16D at 23 °C. Reproductive diapause in all three lineages was detected under 8L: 16D at 15 °C). At this condition, the incidence of reproductive diapause in the L1, L2, and T lineages was about 50, 42, and 28 %, respectively.

In this experiment, 8L: 16D at 23 °C induced reproductive diapause in the T lineage but neither the L1 nor the L2 lineages entered reproductive diapause (all females continued ovipositing). This result has been supported by Nakao (1994) where 10L: 14D at 25 °C induced reproductive diapause in *Thrips nigropilosus* (Uzel) (Thysanoptera: Thripidae). In addition, reproductive diapause was detected in all three lineages when the temperature was dropped to 15 °C under 8L: 16D. Nakao (1998) found reproductive diapause in *Thrips setosus* (Moulton) (Thysanoptera: Thripidae) under 10L: 14D at 18 °C. Therefore, reproductive diapause seems to occur in the T lineage likely due to the effect of short daylight, and in the L1 and in the L2 lineages likely due to low temperature. Interactions between low temperature and short photoperiod are known to induce reproductive diapause (Murai, 1987). Murai (1987) has also reported that *F. intonsa* females under 10L: 14D at 23 °C produce eggs, but 100 % of these females entered reproductive diapause when the temperature was dropped to 20, 16 and 12 °C under 10 h daylight. Kamm (1972) reported reproduction diapause induced in the *Anaphothrips obscurus* (Müller) (Thysanoptera: Thripidae) by exposing larvae to short days (10L: 14D) and Lewis (1973) observed it in *Limothrips cerealium (Haliday)* (Thysanoptera: Thripidae). The incidence of reproductive diapause varied across different geographical locations in the *Haplothrips brevitubus* (Karny) (Thysanoptera: Phlaeothripinae) (Fujimoto et al., 2014).

5.1.3. Effects of temperature and photoperiod on oviposition period

Length of oviposition periods of the L1 and T lineages were shorter under 8L: 16D at 23 °C, as compared with oviposition periods of females reared under 16L: 8D at 23 °C. Females of the L2 lineage laid eggs for 14.9 and 20.5 days under 8L: 16D and 16L: 8D at 23 °C, respectively.

Our result indicates that the oviposition periods of all lineages were significantly decreased under 8L: 16D at 23 °C). Females of *H. brevitubus* exposed to 16L: 8D at 20 °C initiated oviposition within 15 days after adult emergence at 20 °C, while those exposed to short-day conditions entered reproductive diapause (Fujimoto et al. 2014). In addition to decreasing oviposition period, all three lineages were still able to produce fewer eggs under short daylight. Irregular and sporadic egg production under long daylight (16L: 8D) at 29 °C was reported in *M. sjostedti* (Ekesi et al., 1999). In this result, the egg production in all lineages under a short daylight (8L: 16D) at 15 °C was also irregular and sporadic and to lay the next egg females had 5 to 8-day oviposition intervals. Females under these conditions laid very few eggs.

5.1.4. Effects of temperature and photoperiod on the longevity

The average longevity of reproducing females of the T lineage were longer under 8L: 16D at 23 °C than those females reared under 16L: 8D. Nevertheless, there was no difference in the L1 and L2 lineages between 8L: 16D and 16L: 8D at 23 °C. Furthermore, all three lineages had significantly longer longevity under 8L: 16D at 15 °C than under 8L: 16D at 23 °C. All reproducing females in the L1 and L2 lineages reared under 8L: 16D at 15 °C died within 48 days whereas, reproducing females in the T lineage died within 76 days.

Longevity of reproducing females in the T lineage was significantly affected by 8L: 16D at 23 °C. However, the longevity of reproducing females in the L1 and L2 lineages was not affected by 8L: 16D at 23 °C. Adult longevity in all three lineages were increased under short daylight (8L: 16D) at 15 °C than those females reared under short daylight (8L: 16D) at 23 °C. Longest longevity under short daylight (4L:20D) at 25 °C has been reported in *F. occidentalis* (Brodsgaard 1994) and adult longevity increased with decreasing temperature (14 °C) under 12L:12D in *M. sjostedti* (Trybom) (Ekesi et al. 1999) and (10 °C*)* under 16L:8D in *Thrips obscuratus* (Crawford)

(Thysanoptera: Thripidae) (Teulon and Penman, 1991). In this result, there was longevity difference between reproducing and diapausing females, where reproducing females showed shorter longevity than diapausing females and it could be due to the direct relationship between fecundity and longevity. Because fecundity is a key factor in terms of reducing longevity. Therefore, diapausing females could have longer longevity due to the trade-off of lower investment in producing eggs and reproducing females could have shorter longevity due to the overall cost of investment in producing eggs. Having longer longevity during an unfavourable season would probably help them to build up their population immediately for the next favoured season. The possible reasons for the variations of longevity among the lineages might be due to the geographic origin resulting from local adaptation to ambient climatic conditions and it might be one of the differences within the *T. tabaci* lineages.

5.1.5. Effects of temperature and photoperiod on fecundity

In all three lineages, the lowest fecundity rates were recorded under 8L: 16D at 23 °C as compared with the fecundity rates under 16L: 8D at 23 °C. Similarly, in all three lineages the lowest fecundity rates were recorded under 8L: 16D at 15 °C as compared with the fecundity rates under 8L: 16D at 23 °C. This indicates that the responses of the three lineages to temperature and photoperiod are indistinguishable.

In all lineages, fecundity was decreased under 8L: 16D at 23°C. Thus, a short daylight period is likely to have a direct negative effect on the fecundity of all *T. tabaci* lineages. Furthermore, sharp fecundity reduction was recorded due to low temperature (15 °C) under 8L: 16D. Murai (2000) and Sakimura (1937) found that temperature is a factor to increase and decrease the fecundity of L2 lineage. Fecundity decreased in *T. nigropilosus* (Nakao, 1994), where fecundity was dropped under short daylight and low temperature. Fewer eggs were laid per female per day under short photoperiod than long photoperiod at 15°C, but no significant difference in the total number of eggs was observed between the two photoperiods (Ishida et al. 2003). During this experiment, there were few symptoms of damage on the leaf discs under 8L: 16D at 15 °C than under 16L: 8D 23 °C. This has implied that the feeding level of these lineages is directly interlinked with the temperature and length of the photo phase. (Murai 1987) has been reported that the general activity, including

feeding activity of thrips, is known to be higher in long daylight than in short daylight.

5.2. Inbreeding depression and its effects on different life table parameters

5.2.1. Effects of brother and sister inbreeding on longevity

The average longevity of L1 and T lineages were 27 and 30.6 days in the F_1 generation, respectively, while it was 15.7 and 17.28 days in the F_3 generation, respectively. There was 27 and 43 % longevity reduction for L1 lineage in the F_2 and F_3 generation, respectively, and it was 30 and 44 % for T lineage in the F_2 and F_3 generations, respectively, as compared with the longevity of females in the F_1 generation. This indicates that continued brother and sister inbreeding increase the level of inbreeding depression on longevity of females.

5.2.2. Effects of brother and sister inbreeding on fecundity

In both lineages, fecundity reduction was started from F_2 generation and greatly reduced in the F_3 generation. The fecundity rates of females of the L1 and T lineages were 42.43 and 39.33 eggs in the F_3 generation, respectively, while it was 82.2 and 76.68 eggs in the F_1 generation, respectively. In both lineages, the inbred females laid 59 % fewer eggs in the F_3 gener0ation than as compared with the inbred females in the F_2 generation. This indicates that subsequent brother and sister inbreeding induced higher depression on the fecundity of L1 and T lineages.

5.2.3. Effect of brother and sister inbreeding on egg hatchability

Egg hatchability rate started to decrease from F_2 generation for both lineages. There inbreeding depression detected in egg hatchability rates between F_2 and F_3 for the L1 lineage but it was no inbreeding depression detected for T lineage. In both lineages, more than 82% of eggs hatched in the F_2 generation, although the egg hatchability rates were less than 72 and 80 % in the F_3 generation for L1 and T lineages, respectively.

5.2.4. Effect of brother and sister inbreeding on sex ratio of the progeny

In both lineages, there were significant differences in the male and female ratio between F_1 and F_3, and between F_2 and F_3, generations, and there was no significant difference in the male and female ratios in the F_2 generation compared with the F_2 generation. The proportion of male and female of the L1 and T lineages was about 1:4.5 and 1: 4 in the F_3, respectively.

In this result, brother and sister inbreeding of the L1 and T lineages caused greater longevity reduction. The negative effect of inbreeding on the longevity was expressed after a single round of inbreeding. High inbreeding effect on the longevity (38 percent longevity reduction) has been reported in *Uscana semifumipennis* (Girault) (Hymenoptera: Trichogrammatidae) (Henter, 2003). We found that the effect of brother and sister inbreeding on longevity was the same among lineages and this indicates that the longevity of these two lineages are likely similar in their suscebtibility to inbreeding depression. As longevity is not a female trait only: it is surprising to detect sever inbreeding depression a trait is not obviously sex limited. Henter (2003) reasoned out that it could be that longevity for males and females is under different genetic control. As the inbreeding effect continued for third generation, the inbreeding depression may be due to over dominance. Because, when overdominance is the cause a continuously inbred line will never be as fit as an outcrossed line once variation is lost. Finally, it could be that haplodiploidy does not purge genetic load effectively enough to prevent inbreeding depression (Henter, 2003).

We tested the effects of brother and sister inbreeding on fecundity for three subsequent generations and the result has shown that the inbreeding depression detected since from F_2 generation. In this result, brother and sister inbreeding of the L1 and T lineages caused approximately 11 and 38 % fecundity reduction in the F_1 and F_2 generations, respectively. Thus, the magnitude of inbreeding depression between the two lineages seemed similar. The ovipositing inbred females in the L1 and T lineages were less likely to lay eggs in the F_2 and F_3 generations than outbred females in the F_1 generation. Life- time productivity show high levels of inbreeding depression in *Drosophila simulans* (Sturtevant) (Diptera: Drosophilidae) (Wright et al., 2008). It is possibly less surprising to see inbreeding depression in fecundity, given that this is clearly a female trait. The fecundity reduction for inbred

females in both lineages may be interlinked with the mating ability of inbred males. Mating with inbred males has significant direct effects for the fitness of female in the *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), likely mediated by effects of inbreeding status on the number of sperm in male ejaculates. Fecundity reduction due to the reduced number of sperm in the ejaculates of inbred males has been reported in the *C. maculatus* females (Fox et al. 2012). Inbreeding generally reduces male mating activity such that inbred males can also have smaller accessory glands, transfer less sperm and produce sperm that are less motile, less viable or have a greater frequency of abnormalities, all of which can reduce the fertilization success and fitness of inbred males relative to outbred males (Fox et al., 2012). The reduced fecundity of *C. maculatus* females mated to inbred males is likely due at least in part to the reduced number of sperm in the ejaculates of inbred males. That inbred males produce fewer sperm and high levels of inbreeding reduce male fertilization success under sperm competition in guppies *Poecilia reticulate* (Zajitschek et al., 2009). The other possible reason may be related to the vulnerability to inbreeding depression and could thus mediate the male-specific effect of inbreeding. The negative effect of inbreeding known to affect spermatogenesis is to decrease the number of sperm in the ejaculates of inbred males in *Forficula auricularia* (Linnaeus) (Dermaptera: Forficulidae) and lead to reduce the number of eggs produced by the mating partners (Fox et al., 2012; Okada et al., 2011). The other potential negative effects of inbreeding is to reduce the quality of sperm in the ejaculates of inbred males, which hampers embryonic development, lowers the hatching success of the eggs, as well as reduces the number of eggs that females are able to produce after having stored the sperm for a relatively long time (Mehlis et al., 2012). The fecundity of female in *C. maculatus* was sperm limited, and this was primarily occured when females mate with inbred males (Fox et al. 2012).

The results of this study show that subsequent brother and sister mating led to decrease the egg hatchability rate in the L1 and T lineages. The egg hatchability percentage in the L1 and T lineages started to decrease from the F_2 generation. Brother and sister inbreeding caused 11 and 23 % egg hatchability rate for L1 lineage in the F_2 and F_3 generations, respectively, and 11 and 26 % hatchability rate for T lineage in the F_2 and F_3 generations, respectively. These indicats that the inbred females in the L1 and T lineages were less likely to hatch in the F_1 and F_2 generations. The egg hatchability rates

in the L1 were significantly different between F_2 and F_3 generations. However, there was no significant different in the T lineage between F_2 and F_3 generations. Sever inbreeding depression in egg hatchability rates have been reported in *Lymantria dispar* (Linnaeus) (Lepidoptera: Erebidae) (Higashiura et al., 1999).

In this study brother and sister inbreeding has a direct effect on the variation of proportions of male and female. The proportion of male and female was about 1:3 in the F_1 generation, which is the expected proportion in the arrhenotokous thrips species (Lewis, 1973). However, the proportion of females in both lineages was significantly higher in the F_3 generation than in the F_1 and F_2 generations. Thus, frequent brother and sister mating led to female biased progeny in both lineages. In this treatment, brother and sister inbreeding negatively affected the lifetable parameters of the inbred mothers, this indicates that the mothers were in poor condition and this may lead to female biased progeny at F_3 generation.

5.3. Effect of temperature on sex ratio and lifetable parameters

5.3.1. Effect of temperature on pre-oviposition

The pre-oviposition periods of L1 and T lineages were longer with decreasing temperature settings. The average pre-oviposition periods of females of the L1 and T lineages at 15 °C were 5.47 and 11.31 days, respectively, that were about 5.5 and 8.5 times as long as of that of those at 30°C for L1 and T lineages, respectively.

The results from this experiment has shown that the mean preoviposition period of the L1 and T lineages was extended with decreasing temperature. Fekrat et al. (2009) reported that the preoviposition period of the L1 and T lineages was 2.35 and 2.5 days at 25 °C on onion and tobacco, respectively. Murai (2000) reported that the preoviposition period of the L2 lineage was 3.41 days at 23 °C on honey and pollen, which seems to be higher in comparison to the preoviposition period of the L1 and T lineages. In this study, females of the L1 and T lineages have a similar preoviposition period at 23 °C, but have a different preoviposition period at 15 °C. The preoviposition period of the L1 and T lineages was 5.47 and 11.31 days at 15°C, respectively. Murai (2000) has stated that the preoviposition period of the L2 lineage was 8.64 at 15 °C on honey and pollen, which is quite different to the preoviposition

of the L1 lineage in this study. However, females of the T lineage in our study seem to have a relatively longer preoviposition period at 15 °C than females of the L2 lineage in his study. Sakimura (1937) has reported that the preoviposition of the L2 lineage was 3.9 days at 15 °C on *Allium cepa*, which is relatively shorter as compared to our results and that of reported by Murai (2000). In this study, the preoviposition period of the L1 and T lineages was little longer than one day at 30 °C and it seems to be shorter than the preoviposition period (of the L2 lineage at 30 °C (1.96 days) on honey and pollen (Murai, 2000).

5.3.2. Effect of temperature on fecundity

The lowest fecundity of the L1 lineage was recorded at 15°C, and greatest fecundity rates were observed at 23°C and 30°C, whereas the fecundity rates of the T lineage were not significantly different at 23°C and 15°C and it was significantly lower at 30°C than that of at 23°C. This indicates that the responses of the two lineages to temperature are clearly distinguishable at 15°C. Reproductive diapause was detected for 29 and 7 females in the L1 and T lineage at 15°C, respectively. This indicates that the incidence of reproductive diapause is higher in the L1 lineage at 15°C.

Our results indicate that among the tested temperature levels 23 °C is the optimal temperature for the reproduction of *T. tabaci* lineages. Based on the observed fecundity, the optimum temperature for maximum egg laying was at 23 °C. Murai (2000) has reported that the fecundity of the L2 lineage was 270 eggs at 23 °C on honey and pollen, however, the fecundity started to decrease at 25°C and dropped drastically to 62 eggs per female at 30°C. Thus, food source also affects lifetable parameters of *T. tabaci* (Moraiet et al., 2017). Sakimura (1937) presented also that *T. tabaci* deposited 80 eggs on allium cepa at 18°C. In this study, the fecundity of the L1 lineage was significantly decreased at 15 °C than at the other two temperature levels, but the fecundity of the T lineage at 15°C was statistically non-significant difference to that of at 23 °C, which indicates that the T lineage could better tolerate lower temperatures than the L1 (this study). The fecundity reduction was observed for both lineages at 30 °C and it seems to be close to the upper threshold in the entire *T. tabaci* cryptic species complex.

5.3.3. Effect of temperature on egg hatchability

There were significant differences in egg hatchability rates under 15°C and 30 °C for the L1 lineage but there were not significantly different under 15 and 23 °C. The egg hatchability rates were pairwisely significantly different under 15, 23 and 30°C for the T lineage. The egg hatchability rates for both lineages were higher at 15°C and 23°C than at 30°C. In both lineages, more than 85% of eggs hatched at 15°C and 23°C, while the rates were less than 78% at 30 °C.

In both lineages 76-78% of the eggs hatched at 30 °C and about 91-93% of the eggs hatched at 15 °C and 86-95 % of the eggs hatched 23 °C. This suggests that high temperature is likely to have a direct effect on the egg hatchability rate. Murai (2000) has reported that only 10% of the eggs hatched in L2 lineage at 30 °C, which is an extremely high percentage of egg mortality as compared to this study. However, in this result the egg hatchability of L1 and T lineages is mild drop at 30°C, while the egg hatchability of the L2 was drastically dropped at 30 (Murai, 2000). Thus, the L1 and T lineages fares better at 30 than L2 lineage. Therefore, it suggests that L1 and T *T. tabaci* lineages have a better capacity to survive at high temperature as that of the L2 lineage. Jiang et al. (2016) reported that 100 percent egg mortality of *A. obscurus* was observed at 35 °C. Sakimura (1937) reported a summer decline in the density of Japanes population of *T. tabaci*, a trend that may be indicated by the high egg mortality at high temperatures. Ekesi et al. (1999) described that the population of *M. sjostedti* decreased at 30 °C due to less fecundity and high egg mortality rate.

5.3.4. Effect of temperature on longevity

Longevity of the L1 and T lineages decreased with raised temperature conditions. Maximum longevities of the L1 and T lineages were 34.22 and 81.82 days at 15°C, respectively.

Adult longevity significantly affected by temperature and shorter (13.63-13.91 days) at the upper temperature of 30 °C for L1 and T lineages, respectively. Murai (2000) also reported a decrease in the longevity of L2 lineage as temperature increases (12.8 days), which means the effect of higher temperature on longevity is similar in the entire *T. tabaci* cryptic species complex. Teulon and Penman (1991) also presented a decrease in longevity of *T. obscuratus* as temperature increases. This result suggests that the

temperature at 30 °C have an inhibitory effect on the longevity of the L1 and T lineages. There were 24 females of the T lineage at 15 °C that produced only male offspring and they were considered as virgin females. Although, in contrast, mated or virgin females did not differ in probability of longevity. Therefore, mating at 15 °C does not have a significant effect on longevity, which is also known from L1 lineage at 20 °C (Li et al., 2015), and *Franklinothrips* n. sp. at 20 and 25 °C (Hoddle, 2002), *Echinothrips americanus* (Morgan) (Thysanoptera: Thripidae) at 23 °C (Krueger et al., 2016). The longer longevity of the L1 and T lineages were observed at 15 °C (34.22 and 81.82 days), respectively, which means that T lineage fares better at low temperature than L2 lineages.

5.3.5. Effects of temperature on the sex ratio

The male: female ratios in the progeny of females of the L1 and T lineages were significantly influenced by temperature treatment. In both lineages, there were significant differences in the male and female ratio between conditions under 15°C and 23°C, and between the ones under 15°C and 30°C, and there was no significant difference in male and female ratios compared conditions under 23°C and 30°C. The proportion of male's log-linearly decreased as temperature increased. This shows that under 15°C, male ratio was notably higher than under warmer conditions for both lineages, L1 and T. All females of the L1 lineage were successfully mated (females produced both male and female progenies), while 24 females of the T lineage were not successfully mated (females produced only male progenies) at 15°C.

Temperature has a direct effect on the variation of proportions of male and female. In this study, the proportion of females in both lineages was significantly less at 15 °C and higher at 30 °C. The sex ratio (female/male) of *Thrips palmi* (Karny) (Thysanoptera: Thripidae) adults on eggplant was highest (2.03) at 31° C and the lowest (1.22) at 16° C (Yadav and Chang, 2014). The observed natural proportion of males was highest in June until the beginning of July and was subsequently decreasing in summer becoming the lowest at the end of August on potato (Jenser et al., 2006). Producing significantly higher proportion of females at 23 and 30°C reveals that the arrhenotokous *T. tabaci* lineages can easly build-up its population, while producing significantly lower proportion of females at 15 °C reveals that the arrhenotokous *T. tabaci* lineages can slowly build-up its population.

5.4. The existence of deuterotokous reproduction mode

5.4.1. Pupal insemination

In this result, adult males have attempted to mate with female pupa, but pupal insemination is not possible in the *T. tabaci* lineages within 24 hours of mating. Thus, all the tested females produced only male progeny in the arrhenotokous leek-(L1) and tobacco associated- (T) *T. tabaci* lineages.

5.4.2. Mother to son inbreeding

Deuterotokous reproductive mode was detected during mother to son inbreeding test. At F_2 generation, the inbred virgin female taken from F_1 generation produced a combination of male and female progenies, and this female was considered as a deuterotoky ones.

Three virgin females produced a total of 20 females and 75 males in two consecutive generations. F_1 female has produced 10 deuterotoky females, and 2 of them died at the pupal stage; of the 8 deuterotoky females, only 1 was continued to produce both males and females and the rest 7 females were turned to their original arrhenotokous mode of reproduction. Similarly, at F_2 generation, of the eight deuterotoky females, only one virgin female yielded both male and female progenies and the rest seven females were turned to their original arrhenotokous reproduction mode. After three generations, all the deuterotoky females turned to their original arrhenotokous mode of reproduction.

This result has shown that mother and son inbreeding has induced deuterotokous reproductive mode in the T lineage (the virgin inbred female produced the combination of male and female progeny). Therefore, the mating between mother and son potentially changes the arrhenotoky female to deuterotokous reproductive mode in the T lineage. However, deuterotokous reproduction mode was not detected in the L1 mother and son inbreeding for the tested generations. The deuterotokous females of T were reared for three subsequent generations, and their progeny sex ratio was female biased at F_0, and F_1 generations and during the F_2 generation the proportion of males were higher than females. Lastly, the deuterotokous females turned back to their original arrhenotokous reproduction mode. F_3 deuterotokous females produced all male progeny. Therefore, this result indicated that deuterotokous

reproductive mode is not a persistence reproductive mode in the T type other than it is irregularity reproductive mode.

Reproductive mode determination in *T. tabaci* lineage based on the sex of progeny takes more time. A time saving technique for differentiation of the reproductive mode using the COI sequences was reported (Toda and Murai, 2007). However, sometimes the lineages showing arrhenotokous were reported wrongly as showing thelytoky according to the COI based technique (Aizawa et al., 2016). This might be due to the interbreeding between arrhenotokous and thelytokous *T. tabaci* lineages is resulting the thelytokous female carry arrhenotokous male-originated nuclear gene (LI et al. 2015). Heteroplasmy where females carry multiple mitochondrial DNA genes within the cell has been reported in *T. tabaci* population (Gawande et al., 2017).

5.5. Fighting behaviour of male

5.5.1. Behavioural observations during fighting

Aggressive fighting interactions occurred in the small arena with different fighting movements. When the two contestants approached each other, they first contacted each other with their moveable antennae, and then chased each other around the arena. Occasionally, the contestants stopped to vibrate their wings and rub their hind legs. Antennal bouts, jumping flipping, stabbing and pitching are the most commonly observed fighting movements. These movements can be considered assessments: this is how an individual may determine the body size and fighting ability of its opponent to decide whether to continue or stop fighting. The outcomes of assessments between males in fights is that the greater male chases the smaller one until it flees rather than being engaged in the fight (Bernard J. Crespi, 1986). Fights often ranged from few initial abdominal bouts, antennal flicking to escalated aggressive interactions. Several prolonged fights with stabbing and pitching beyond the less aggressive abdominal and antennal bouts, abdominal flipping and flicking, were observed.

5.5.2. Fighting performance

Both lineages showed different fighting performance. L1 males exhibited more frequent fighting as 100 % of them fought, whereas T males

fight less frequently. By contrast, the L1 males had a longer duration of fight, shorter prefight period, and refighting interval than T males. The difference between fighting in the two lineages is likely due to the variation in type and quantity of male-produced pheromones. Because, males interact over extended periods, so a pheromone may be involved as were found with males of *F. occidentalis*, *F. intonsa*, *M. sjostedti* and *T. palmi* (Akella et al., 2014; Hamilton et al., 2005; Kirk, 2017; Niassy et al., 2019). It has been reported that a male *F. occidentalis* produces pheromone during aggregation. Akinyemi and Kirk (2019) found that an antiaphrodisiac pheromone was produced in *F. occidentalis* males but not in females. Thus, as L1 and T lineages have substantial variations genetically and in host adaptations, the type and quantity of male-produced pheromones may be varied too, and it may be the reason behind variations in the intensity of male fighting performance observed.

It is not clear what males gain from fighting in the absence of females and food in the arena. Terry and Gardner (1990) suggested that males might be defending a space. It is possible that chemical signaling controls fighting. Male-specific cuticular hydrocarbons and male-produced pheromones play a vital role in communication, particularly in mating and fighting. However, these chemicals have not been studied in *T. tabaci* lineages. These two lineages have shown different fighting performances, and it might be due to their body size differences and amount of male specific cuticular hydrocarbons. Thus, the body size, type and amount of male-specific cuticular hydrocarbons and male-produced aggregation pheromones in L1 and T lineages need further studies.

6. NEW SCIENTIFIC RESULTS

1. Reproductive diapause was detected in T lineage due to 8L: 16D at 23 °C and in all three lineages due to lower (15 °C) under 8L: 16D.
2. The subsequent mating between brother and sister caused inbreeding depression: the longevity, fecundity, egg hatchability rate of the inbred females were decreased in the L1 and T lineages.
3. The proportion of male decreased during subsequent brother and sister inbreeding. The successfully mated females with their brother produced notably higher female progeny in the F_2 and F_3 generations than F_1 generation.
4. The proportion of female increased as temperature increased and decreased as temperature decreased. The proportion of female

increased as temperature increased and decreased as temperature decreased.

5. Pupal insemination is not possible in the *T. tabaci* lineages.
6. Mother and son inbreeding has induced deuterotokous reproductive mode in the T lineage. Therefore, the mating between mother and son potentially changes the arrhenotoky female to deuterotokous reproductive mode in the T lineage.
7. Fighting interactions (parallel abdominal bouts, antennal bouts, flipping, stabbing, and jumping fighting tactics) were observed between the males in L1 and T lineages.

7. REFERENCES

1. Aizawa, M., Watanabe, T., Kumano, A., Miyatake, T., Sonoda, S. (2016): Cypermethrin resistance and reproductive types in onion thrips, *Thrips tabaci*; (Thysanoptera: Thripidae). Journal of Pesticide Science, 41(4): 167-170.
2. Akella, S. V., Kirk, W. D., Lu, Y., Murai, T., Walters, K. F., Hamilton, J. G. (2014): Identification of the aggregation pheromone of the melon thrips, Thrips palmi. PloS One, 9(8): e103315.
3. Akinyemi, A. O., Kirk, W. D. J. (2019). Experienced males recognise and avoid mating with non-virgin females in the western flower thrips. PLOS ONE, 14(10): e0224115.
4. Balan, R. K., Ramasamy, A., Hande, R. H., Gawande, S. J., Krishna Kumar, N. K. (2018): Genome-wide identification, expression profiling, and target gene analysis of micro RNA s in the Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), vectors of tospoviruses (Bunyaviridae). Ecology and Evolution, 8(13): 6399-6419.
5. Brodsgaard, H. F. (1994): Effect of photoperiod on the bionomics of *Frankliniella occidentalis* (Pergande) (Thysanoptera , Thripidae). Berlin, 117: 498-507.
6. 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 mtDNA sequence data. Heredity, 93(4): 364-370.
7. Crespi, Bernard J. (1986): Territoriality and fighting in a colonial thrips, Hoplothrips pedicularius, and sexual dimorphism in Thysanoptera. Ecological Entomology, 11(2): 119-130.
8. Ekesi, S., Maniania, N. K., & Onu, I. (1999): Effects of temperature and photoperiod on development and oviposition of the legume flower thrips, *Megalurothrips sjostedti.* Entomologia Experimentalis et Applicata, 93(2): 149-155.
9. 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

10. Fekrat, L., Shishehbor, P., Manzari, S., Nejadian, E. (2009): Comparative development, reproduction and life table parameters of three populations of *Thrips tabaci* (Thysanoptera: Thripidae) on onion and tobacco. Journal of Entomological Society of Iran, 29(1): 11-23.
11. Fox, C. W., Xu, J., Wallin, W. G., Curtis, C. L. (2012): Male inbreeding status affects female fitness in a seed-feeding beetle. Journal of Evolutionary Biology, *25*(1): 29-37.
12. Fujimoto, K., Sakurai, T., Nakao, S. (2014): Geographic variation in diapause induction and pre-oviposition period of adult females in *Haplothrips brevitubus* （Thysanoptera: Phlaeothripinae）. Journal of the Japanese Society of Applied Animal and Insects, 58: 47-54.
13. Gawande, S. J., Anandhan, S., Ingle, A. A., Jacobson, A., Asokan, R. (2017): Heteroplasmy due to coexistence of mtCOI haplotypes from different lineages of the Thrips tabaci cryptic species group. Bulletin of Entomological Research, 107(4): 107, 534-542.
14. Gent, D. H., du Toit, L. J., Fichtner, S. F., Mohan, S. K., Pappu, H. R., Schwartz, H. F. (2006): Iris yellow spot virus: An emerging threat to onion bulb and seed production. Plant Disease, 90(12): 1468-1480.
15. Goldbach, R., & Peters, D. (1994). Possible causes of the emergence of tospovirus diseases. Seminars in Virology, 5(2): 113-120.
16. Hamilton, J. G., Hall, D. R., Kirk, W. D. (2005): Identification of a male-produced aggregation pheromone in the western flower thrips Frankliniella occidentalis. Journal of Chemical Ecology, 31(6): 1369-1379.
17. Henter, H. J. (2003): Inbreeding depression and haplodiploidy: Experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. Evolution, 57(8), 1793-1803.
18. Higashiura, Y., Ishihara, M., & Schaefer, P. W. (1999): Sex ratio distortion and severe inbreeding depression in the gypsy moth *Lymantria dispar* L. in Hokkaido, Japan. Heredity, 83(3): 290-297.
19. Hoddle, M. S. (2002). Developmental and reproductive biology of *Scirtothrips perseae* (Thysanoptera: Thripidae): A new avocado pest in California. Bulletin of Entomological Research, 92(4): 279-286.
20. Ishida, H., Murai, T., Sonoda, S., Yoshia, H., Izumi, Y., Tsumuki, H. (2003): Effects of temperature and photoperiod on development and

oviposition of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Japan, 38(1): 65-68.
21. Jacobson, A. L., Johnston, J. S., Rotenberg, D., Whitfield, A. E., Booth, W., Vargo, E. L., Kennedy, G. G. (2013): Genome size and ploidy of Thysanoptera. Insect Molecular Biology, 22(1): 12-17.
22. Jenser, G., Lipcsei, S., Szenasi, A., Hudak, K. (2006): Host range of the arrhenotokous populations of *Thrips tabaci* (Thysanoptera: Thripidae). Acta Phytopathologica et Entomologica Hungarica, 41(3-4): 297-303.
23. Jiang, H. xue, Zhang, X. chen, Niu, S. huan, Feng, J. nian. (2016): Effects of temperature on the development, reproduction and population growth of Anaphothrips obscurus (Thysanoptera: Thripidae). Journal of Asia-Pacific Entomology, 19(4): 1175-1181.
24. Kamm, J. A. (1972). Environmental influence on reproduction diapause, and morph determination of *Anaphothrips obscurus* (Thysanoptera: Thripidae). Environmental Entomology, 1(1): 16-19.
25. Kirk, W. D. J. (2017): The aggregation pheromones of thrips (Thysanoptera) and their potential for pest management. International Journal of Tropical Insect Science, *37*(2): 41-49.
26. 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.
27. Kobayashi, K, Yoshimura, J., Hasegawa, E. (2013): Coexistence of sexual individuals and genetically isolated asexual counterparts in a thrips. Scientific reports, *3*(1): 1-4.
28. Krueger, S., Moritz, G., Lindemann, P., Radisch, D., & Tschuch, G. (2016). Male pheromones influence the mating behavior of *Echinothrips americanus*. Journal of Chemical Ecology, 42(4): 294-299.
29. Lewis, T. (1973). Thrips their biology, ecology and economic importance. Academic Press Inc. London, UK, 36-50
30. Li, X.-W., Fail, J., Shelton, A. M. (2015): Female multiple matings and male harassment and their effects on fitness of arrhenotokous *Thrips tabaci* (Thysanoptera: Thripidae). Behavioral Ecology and Sociobiology, 69(10): 1585-1595.

31. Mehlis, M., Frommen, J. G., Rahn, A. K., Bakker, T. C. (2012): Inbreeding in three-spined sticklebacks *Gasterosteus aculeatus* L.: Effects on testis and sperm traits. Biological Journal of the Linnean Society, 107(3): 510-520.
32. Moraiet, M. A., Ansari, M. S., Basri, R. (2017): Biological parameters of onion thrips, *Thrips tabaci* Lindeman on onion cultivars. Journal of Plant Protection Research, 57(2): 120-128.
33. Murai, T. (2000). Effetcs of temperature on developmet and reproduction of the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera:Thripidae), on pollen and honey solution. Applied Entomology and Zoology, 35(4): 499-504.
34. Murai, Tamotsu. (1987): Reproductive diapause of flower thrips, franklin/ella intonsa*:* Proceedings of the International Smyposia, held at Smolenice, Czechoslovakia, Sept. 9-14, 1985. SPB Academic Publishing, the Hauge, pp. 542.
35. Nakao, S. (1994): Photothermic control of wing form and reproductive diapause in female thrips nigropilosus uzel (thysanoptera:Thripidae). *Japan*)*189*): 183-189.
36. Nakao, S. (1998): Effects of photoperiod and temperature on induction and termination of reproductive diapause of *Thrips setosus* moulton (Thysanoptera: Thripidae). *Japan*, *42*: 172-173.
37. Niassy, S., Tamiru, A., Hamilton, J. G., Kirk, W. D., Mumm, R., Sims, C., de Kogel, W. J., Ekesi, S., Maniania, N. K., Bandi, K. (2019): Characterization of male-produced aggregation pheromone of the bean flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae). Journal of Chemical Ecology, 45(4): 348-355.
38. Okada, K., Blount, J. D., Sharma, M. D., Snook, R. R., & Hosken, D. J. (2011): Male attractiveness, fertility and susceptibility to oxidative stress are influenced by inbreeding in Drosophila simulans. Journal of Evolutionary Biology, 24(2): 363-371.
39. Rivnay, E. (1935): Ecological studies of the greenhouse thrips, *Heliothrips haemorrhoidalis*, in Palestine. Bulletin of Entomological Research, 26(2): 267-278.
40. Sakimura, K. (1937): The life and seasonal histories of *Thrips tabaci* LIND. In the vicinity of Tokyo, Japan. Oyodobutu Zasshi, 9: 1-24.

41. Terry, L. I., Gardner, D. (1990): Male mating swarms in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Journal of Insect Behavior, 3(1): 133-141.
42. Teulon, D. A. J., Penman, D. R. (1991): Effects of temperature and diet on oviposition rate and development time of the New Zealand flower thrips, *Thrips obscuratus*. Entomologia Experimentalis et Applicata, 60(2): 143-155.
43. Toda, S., Murai, T. (2007): Phylogenetic analysis based on mitochondrial COI gene sequences in Thrips tabaci Lindeman (Thysanoptera: Thripidae) in relation to reproductive forms and geographic distribution. Applied Entomology and Zoology, 42(2): 309-316.
44. Wright, S. I., Ness, R. W., Foxe, J. P., Barrett, S. C. (2008): Genomic consequences of outcrossing and selfing in plants. International Journal of Plant Sciences, 169(1): 105-118.
45. Yadav, R., Chang, N. (2014). Effects of temperature on the development and population growth of the melon thrips, *Thrips palmi*, on eggplant, Solanum m*elongena*. Journal of Insect Science, 14(1): 1-9.

PUBLICATIONS IN THE SUBJECT OF THIS STUDY

Journal articles with IF:

1. **Woldemelak,W.A.,** Ladányi, M., & Fail. J. (2021): Effect of temperature on the sex ratio and life table parameters of the leek- (L1) and tobacco- associated (T) *Thrips tabaci* lineages (Thysanoptera: Thripidae). Population Ecology (Q2, IF=2.10)
2. **Woldemelak, W.A**. (2020). The Existence of Deuterotokous Reproduction Mode in the *Thrips tabaci* (Thysanoptera: Thripidae) Cryptic Species Complex. Journal of Horticultural Research*, 28*, 21 - 28.
3. **Woldemelak, W.A**. (2020). Fighting Behaviour of Male Onion Thrips, *Thrips tabaci* (Thysanoptera: Thripidae) Lineages. Acta Phytopathologica et Entomologica Hungarica, *55*, 123-130.

Abstracts of conference:

1. **Woldemelak, W.A.,** Farkas P., Musa S., Varela R.C.L., & Fail. J. (2018). Do adult males inseminate female pupae of arrhenotokous onion thrips, *T. tabaci* (Thysanoptera: Thripidae)? 60th Georgikon Scientific Conference, P. 49. (Keszthey, 2018. Oktober 4-5).

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

Journal articles with IF:

1. **Woldemelak, W.A**. (2021): Reproductive biology of thrips insect species and their reproductive manipulators. Journal of the Entomological Research Society (Accepted on August 8. 2021) (IF= 0.55)
2. **Woldemelak, W.A**. (2020). Role of aromatic plants in the integrated pest management (IPM) of Thrips tabaci Lindeman (Thysanoptera: Thripidae). African Journal of Agricultural Research (Inpress)

3. **Woldemelak, W.A**. (2020). The Major Biological Approaches in the Integrated Pest Management of Onion Thrips, Thrips Tabaci (Thysanoptera: Thripidae). Journal of Horticultural Research*, 28*, 13 - 20.

Abstracts of conference:

1. Farkas P.; Tóth A., **Woldemalek W. A**., Pénzes B., Fail, J. (2018): A dohánytripsz (Thrips tabaci Lindeman) fajkomplex versenyképessége vöröshagymán. In: Haltrich, A.; Varga, Á. (szerk.) 64. Növényvédelmi Tudományos Napok Budapest, 2018. február 20-21. Előadások összefoglalói, p. 25.