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Biology of the leafhopper, *Zyginidia sohrab* Zachvatkin, on corn under laboratory conditions

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Abstract
The life history of *Zygnidia sohrab* Zachvatkin was studied on potted corn at three constant temperatures (20, 25, 30± 1 °C) under laboratory conditions. The incubation periods of the eggs were 14.4, 11.1, 8.5 days and the mean development time of the five nymphal instars from hatching to adult emergence were 24.6, 15.7, 13.1 days at 20, 25, 30 °C respectively. Adult female longevity was the longest at 20 °C (77.1±23.7 days) and the maximum egg was laid at 25 °C (94.9 egg). The sex ratios (♂/♀) were about the same (1:1.2, 1:1.2 and 1:1.3) at three temperature respectively. The highest net reproductive rate "R_0" was 94.80 at 25 °C, the shortest mean generation time "T" was 31.08 days and the highest intrinsic rate of increase "r" was 0.134 at 30 °C. As high temperatures affected positively biology of *Z. sohrab*, population of the leafhopper may reach higher densities and create further damage, particularly on second crop maize.

Keywords: Corn, Leafhopper, *Zyginidia sohrab*, Biology, Life table

1. Introduction

The Leafhoppers have agronomic importance because of their particular ability to transmit virus and other pathogens and the damages they cause by feeding or egg-laying (Harris 1979; Nault and Ammar 1989; Virla and Paradell 2002) [9, 22, 33]. These characteristics are increased by their high reproductive potential, short life cycle and elevated dispersion and adaptability rates that is why they are important pests of agriculture.

The corn leafhopper, *Zyginidia sohrab* Zachvatkin, is a cicadellid and considered a serious pest of maize in maize growing regions of Turkey (Alaoğlu *et al.*, 2007; Ercan and Uysal 2007; Mutlu *et al.*, 2008; Mutlu and Sertkaya, 2015) [1, 28, 8, 20, 21]. The insect about 2.6 mm long and it is a multivoltine species, able to produce up to six generations a year in Southeast Anatolia region of Turkey, and feeds on maize and Johnson grass as a mesophyll sucker. *Z. sohrab* feeds mainly on the ventral surface of the leaf and causes direct damage to the corn plant by sucking especially bottom leaves (Mutlu and Sertkaya, 2015) [21]. The combination of sucking and punctures causes shrinking, browning and later drying of the early stage primary bottom leaves that were exposed to the pest and stripe-like purplish colors form and became brown (Alaoğlu *et al.*, 2007; Sade *et al.*, 2007) [1, 28]. The drying of these leaves causes economic loses in corn plants. Heavy infestations cause complete drying of corn plants a condition commonly known as "hopper-burn" (Moutous, 1979; Backus *et al.*, 2005) [18, 2].

Z. sohrab has received much attention during the last decade especially on second crop maize areas in Turkey. Since then the leafhopper damage has been reported on maize fields some part of Anatolia. It was stated that *Z.sohrab* was the most common and frequent harmful species, and its population density has significantly increased recently and became only dominated species by increasing over 90% among the other leafhopper species, *Empoasca decipiens* (Paoli), *Asymmetrasca decedens* (Paoli), *Psammotettix striatus* (Linnaeus 1758), feeding on the maize plant in Southeastern Anatolia Region of Turkey (Lodos, 1981; Şimşek, 1988; *Mutlu et al.*, 2008; Mutlu, 2013; Mutlu and Sertkaya, 2015) [14, 31, 20, 19, 21], as well as Central Anatolia Region (Ercan and Uysal 2007; Alaoğlu *et al.*, 2007; Sade *et al.*, 2007) [8, 1, 28,]. Besides, it was recorded that *Z. sohrab* gave five generations per year and entered to diapause period as adult depending on temperature (Mutlu and Sertkaya, 2015) [21] in Diyarbakir province, Southeast Anatolia Region. Ercan (2006) [7]. has shown that the population of *Z. sohrab* and its economic damage was rapidly increasing parallel with corn cultivated areas, because of the practice of commonly using unregistered pesticides on maize in Konya province located in Central Anatolia region of Turkey in recent years. However, economic losses caused by the insect

have not been known Especially on second crop maize. The bio-ecology of the leafhopper was well studied (Mutlu and Sertkaya, 2015) [21], but the development of efficient pest management strategies to control this pest on maize in Turkey necessitates knowledge of its biology. Since knowledge of the biology and life cycle of *Z. sohrab* is lacking, the present study was conducted to determine some biological parameters (fertility, egg viability, duration of different developmental stages, sex ratio and longevity) and the effect of different temperature regimes on the biology of *Z. sohrab*. The construction of life tables under controlled conditions would provide useful information for characterizing population processes of the insect. The data obtained from the study has also aimed to increase the knowledge and to provide basic data for future studies to control of the leafhopper.

2. Material and Methods

All experiments were carried out at 20, 25 and 30 ±1 °C constant temperatures, 16:8 (L:D) h., 65 ± 5% RH under controlled conditions at Diyarbakir Plant Protection Research Institute during 2012. Adults of *Z. sohrab* was maintained on corn plants. Only insects with a uniform age structure were used in the experiments. Corn plants with 2-6 leaves stage were covered with a cylindrical cages (PET, 18x25 cm and 10x15 cm) with nylon mesh covering the top to prevent adults from escaping. Each experiment was repeated twenty times. All data collected from these experiments were calculated by analysis of variance (ANOVA), and means were compared through least significant test ($P < 0.05$).

2.1 Incubation Period and Nymphal Development

The incubation period of eggs was determined by placing pairs of newly emerged females and males in cages on single maize plant (4-6 leaves stage) and recording the dates on which the first nymph hatched (Marais, 1989) [17]. Eggs are inserted under leaf surface and are visually difficult to detect the naked eye (Mutlu and Sertkaya, 2015) [21].

Observations on hatching were recorded daily in the morning as well as in the evening. After hatching from the eggs, nymphs were gently caged individually on corn seedlings and observed daily, until they emerged as adults. On each day of molting, the exuvia were removed from the cages, and the number of days between molts was recorded (Stoner and Gustin, 1967) [30].

2.2. Preoviposition, oviposition, postoviposition, Adult Longevity

Potted corn plants (2-4 leaves stage) were covered with cages. Once freshly emerged, two adult male and one female of the insect were released on potted maize plants. They were allowed to remain twenty four hours on the plants and transferred daily to new plants by sucking tube. Every day the plants were changed for egg laying and continued till the death of all females. Preoviposition, oviposition and postoviposition periods were recorded for those adult females which completed all the phases. If the female died, the repeat was abandoned. Data were recorded from females living more than 10 days (Stoner and Gustin, 1967; Jabbar, 1974; Nordin and Ghani, 1995; Salim, 2002) [30, 11, 24, 29].

2.3. The sex ratios and nymphal mortality

The sex ratios of *Z. sohrab* at different temperatures were determined from the offspring of twelve adult female for the each temperature. Nymphs hatched from the total eggs of each female and were maintained for growing adults in cages. New generation adults were transferred to glass tubes and were put into deep freeze to eliminate the adults. Both male and female were counted under illuminated binocular microscope. The sex ratio were found out by dividing the total number adults by the sum of the total number female.

2.4. Fecundity and life table parameters

All data collected from the experiments were used to draw up life tables of *Z. sohrab* at each temperature. The life tables were drawn up according to using the formula:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

Where x: age in days, r: intrinsic rate of increase, l_x: age-specific survival, m_x: age-specific number of female offspring. The raw data was analyzed based on the theory of the age-stage, two-sex life table (Chi, 1988) [4] and using the program TWOSEX-MSChart (Chi, 2015) [5]. The age-specific survival rate (l_x) and the age-specific fecundity (m_x) were calculated from the daily records of survival and fecundity of all individuals in the cohort.

3. Results

3.1. Incubation Period and Nymphal Development

The development time of per adult periods of *Z. sohrab* at the three different temperature were given in table 1.

Table 1: Immature stages of *Zyginida sohrab* at three temperatures

Duration of immature developmental stages (days)								
Temp. (°C)	**n**	**Egg**	**First instar nymph**	**Second instar nymph**	**Third instar nymph**	**Fourth instar nymph**	**Fifth instar nymph**	**Total (egg to adult emergence)**
20±1	20	14.43±0.08 a*	4.15±0.15 a	4.60±0.24 a	5.15±0.28 a	5.50±0.30 a	5.20±0.35 a	39.03±0.22 a
25±1	20	11.11±0.10 b	2.70±0.17 b	4.30±0.20 a	2.80±0.11 b	3.20±0.18 b	2.20±0.18 b	26.31±0.21 b
30±1	20	8.54±0.03 c	1.75±0.09 c	3.35±0.15 b	2.95±0.15 b	2.80±0.11 b	2.25±0.09 b	21.64±0.16 c

* Means in columns by different letters indicate significant differences among temperatures tested at p≤ 5 % (ANOVA)

The study clearly showed that the egg and nymph development time were strongly influenced by temperatures. The incubation period of eggs and nymphs were significantly shortened as the temperature increased up to 30 °C. It was recorded that the shortest incubation period and nymph development time were determined at 30 °C (8.5 and 13. 1 days respectively), while the longest time was recorded at 20 °C (14.4 and 24.6 days respectively) (Table 1). Five nymphal instars were recorded at three temperatures and the mean development time from hatching of the nymphs until adult emergence was 39.0, 26.3 and 21.6 days at 20, 25 and 30 °C respectively.

3.2. Preoviposition, oviposition, postoviposition, Adult Longevity

All these parameters of *Z. sohrab* obtained at the three temperature have been shown in Table 2.

Table 2: Mean longevity of *Zyginidia sohrab* females at three different temperatures

Longevity (days)					
Temp. (°C)	**n**	**Preoviposition**	**Oviposition**	**Postoviposition**	**Longevity**
20±1	20	6.80±1.23 **a***	59.65±19.66 **a**	10.65±10.85 **a**	77.10±23.72 **a**
25±1	20	5.90±1.29 **b**	45.75±16.82 **b**	8.30±11.33 **a**	59.95±19.60 **b**
30±1	20	5.70±1.26 **b**	32.40±15.25 **c**	7.95±6.96 **a**	46.05±18.24 **c**

*Means in columns by different letters indicate significant differences among temperatures tested at p≤ 5 % (ANOVA)

The results of this experiment indicated that while the temperatures did not have a significant effect on the preoviposition and post-oviposition periods, but oviposition periods were strongly affected by the temperatures and three different temperatures resulted in differences between groups in terms of statistical analysis (Table 2). It was observed that the newly emerged adult male and female were not ready for mating, as adult female and male ovarian maturity took place 5 to 6 days after emergence. The female insects did not begin to oviposit at once after copulation. The preoviposition period calculated ranged from five to six days (n = 20) after the emergence of adults. After one mating the female leafhopper continued to lay eggs for the rest of her life and on an average laid about 88.0, 94.9, 64.6 eggs for each temperatures 20, 25 and 30 ºC respectively. Females daily lay an irregular numbers of eggs and produced intermittently throughout the lifespan.

It was observed that mating usually takes place on the ventral surface of the leaf, away from direct light and during the day but there were not any data for copulation period. Different oviposition duration was observed among the females; considering only those that lived more than 10 days, we recorded that their oviposition period was very long and varied, from 22 days to 102 for 20 °C, 21 days to 83 days for 25 °C, 17 day to 71 days for 30 °C; in most cases it lasted from almost one month to three months, and one female continued to lay eggs at intervals for nearly four months.

The largest number of eggs laid by one female was 164, which occurred at a constant temperature of 20 °C, 158 egg at 25 °C and 97 egg at 30 °C. The longevity was very variable (table 2) and the females lived more than males. The observed longevity was77.1, 59.9 and 46.05 days respectively.

3.3. The sex ratios and nymphal mortality

The sex and nymphal mortality ratios of *Z. sohrab* determined at three different temperature were given in Figure 1 and Table 3.

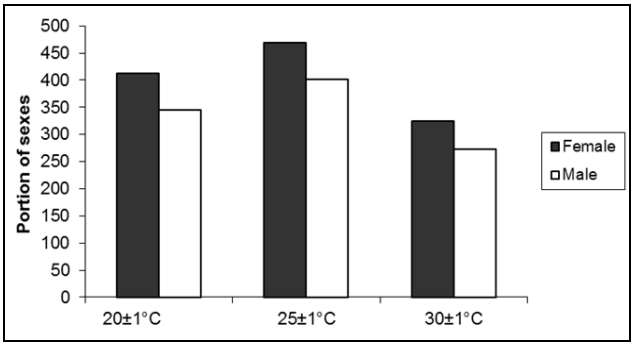

Fig 1: Percentage portion of sexes of *Zyginidia sohrab* at three temperatures.

The female insects slightly outnumbered the males and the ratio of males to females being approximately 1:1.2, 1:1.2 and 1:3 at the three temperature respectively (Figure 1). The study indicated that overall sex ratio of the leafhopper were in favor of the females.

The table showed that the highest nymphal mortality was found at 20 °C and the three temperatures resulted in separate groups in terms of statistical analysis.

Table 3: The nymphal mortality ratio of *Zyginidia sohrab* at different temperature

Temp. (°C)	n	Total Number of Nymph	The Number of Nymph Reached Adult Stage	Nymph Mortality Ratio (%)
20±1	20	1.115	758	30.5±6.0**a***
25±1	20	1.072	871	22.6±4.9**b**
30±1	20	782	597	19.1±4.5**b**

*Means in columns by different letters indicate significant differences among temperatures tested at p≤ 5 % (ANOVA)

While nymphal mortality increased with the lowering of temperature (30.5%), this ratio were determined as 19.1% at the higher temperature (30 °C).

3.4. Fecundity and life table parameters

Temperature had influenced significantly the total number of eggs laid by *Z. sohrab* females (Figure 2).

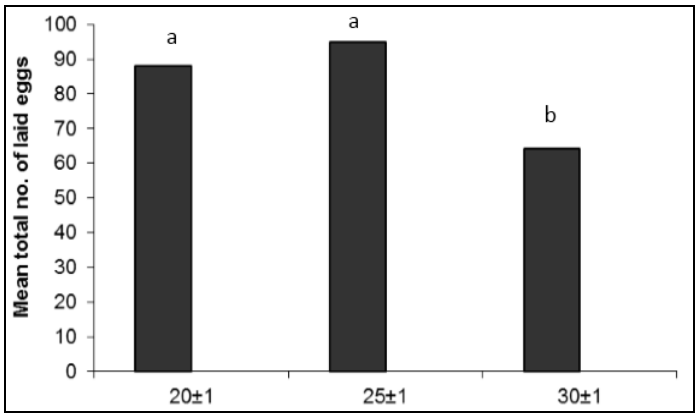

Fig 2: Mean total number of eggs laid by *Zyginidia sohrab* at three temperatures.

Mean total number of eggs laid by females at 25 °C was significantly higher with 94.9 eggs/♀ than at 20 °C with 88.0, 30 °C with 64.6 eggs/♀. The average daily number of eggs obtained were 1.53, 2.17 and 2.27 at the three temperatures (20, 25 and 30 ºC respectively).

Age-specific fecundity, m_x, and survival curve, l_x, are shown in Fig.3.

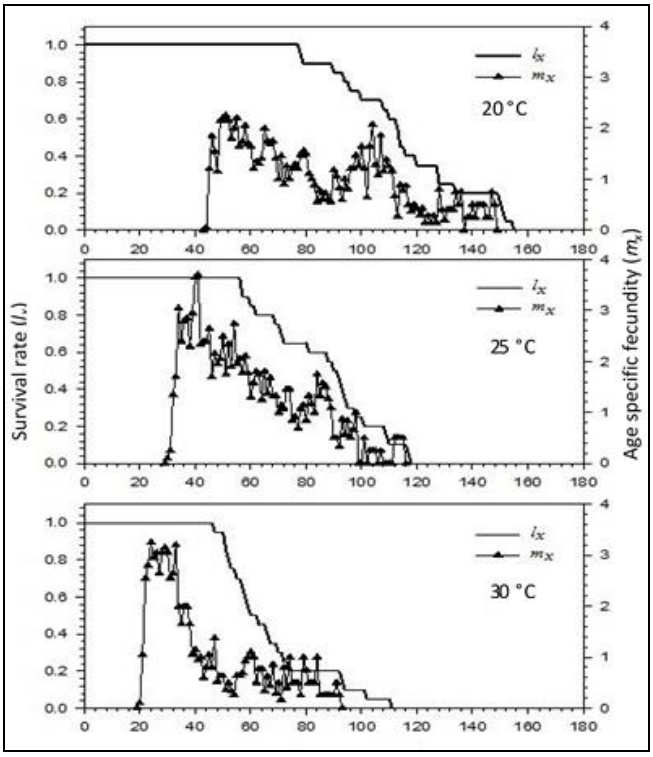

Fig 3: Age-specific fecundity, m_x, and survival curve, l_x of *Zyginidia sohrab* at different temperatures in the laboratory.

The survival of females at 20 °C was longer than that of obtained at 25 °C and 30 °C, and become shorter with increase in temperature. Age-specific fecundity of *Z. sohrab* reached a peak and declined rapidly at 30 °C. The shape of m_x curve at 25 °C was similar to that of 30 °C. There occurred a weak peak of m_x at 20 °C, and production of offspring was distributed over a longer time period (Fig. 3).

Life table parameters of *Z. sohrab* adult females at 20, 25 and 30 °C temperatures are reported in table 4.

Table 4: Net reproductive rate (R_0), intrinsic rate of increase (r), and mean generation time (T) of Zyginidia *sohrab* at different temperatures

Temperature (°C)	(R_0)	(r)	T (days)
20±1	87.28±7.90	0.069±0.013	64.57±1.24
25±1	94.80±7.41	0.097±0.001	46.20±0.86
30±1	64.66±4.29	0.134±0.002	31.08±0.61

The intrinsic rate of increase (r) was calculated as 0.069, 0.097 and 0.134 respectively. Net reproductive rate (R_0) was 87.28, 94.80, and, 64.66 and mean length of a generation (T) was 64.57, 46.20 and, 31.08 days, at temperatures tested, respectively.

4. Discussion

It is well known that the temperature- dependent development rate of an insect is a fundamental feature of its life history. Although three temperatures were tested in this study, the results suggest that pre adult stages of *Z. sohrab* are very sensitive to temperature changes, and that an increase in temperatures within the tolerance limits of the leafhopper would favour the development rate. These results clearly showed that development from egg to adult emergence decreased as temperature increased. Similarly, Tokuda and Matsumura (2005) [32] also demonstrated that the developmental periods of eggs and nymphs of *Cicadulina bipunctata* (Melichar) were significantly shortened as the temperature increased up to 34 °C. Additionally, Raupachet *et al.,* (2002) [26] indicated the incubation period of *E.decipiens* was strongly affected by temperature and the shortest egg development time at 28 °C. Likewise results were recorded by researchers who studied different leafhopper species (Lenicov and Virla, 1993; Salim, 2002; Özgen *et al.,* 2009) [13, 29, 27].

Five nymphal instars were recorded at three temperature tested. Various other leafhopper species in Europe, North America, Asia and Africa have also been found to moult five times before the adult stage was stated (Delong, 1971) [6], although some leafhopper species have been known pass through only four or as many as six nymphal stage.

The results also showed that the preovipoisiton period lasted at least five to six days after the emergence of adults at tested temperatures. Salim (2002) [29] reported that the mango leafhoppers, *Idioscopus nitidulus* Walker 1870, begin to mate 4.7+1.6 days after adult emergence. It was determined that mating takes place normally within 2 to 5 days of emergence of adults of *Z. quyumi* and for ovarian maturity of adult female takes 1 to 4 day after emergence and female usually lay eggs on the third day after mating (Jabbar, 1974) [11].

The outcomes indicate that as temperature was increased, the oviposition period was decreased (Table 2). Also higher temperatures had partly negative effects on egg producing, combined with high humidity levels. Naseri *et al.,* (2008) [23]. stated that the density of *E. decipiens* was increased with increasing temperature and decreasing relative humidity on bean. Witt and Edvards (2000) [34]. Results displayed the high temperature negatively influenced cicadellid fecundity, Madden *et al.,* (1986) [16] also illustrated fewer eggs being laid at lower temperature. According to recent findings, oviposition durations can be changed according to leafhopper species, host plants, temperature and humidity conditions (Jabbar, 1974; Salim, 2002; Chen *et al.,* 2010) [11, 29, 3].

The statistical analysis of data indicated that longevity of *Z. sohrab* varied significantly depending on temperature (Table 2). This study's results agree with Yiğit and Erkılıç (1987) [35], for life span of female *A. adanae* at 25 °C and 30 °C (31-116, 7-82 days respectively). Similarly Salim (2002) [29] noted that life span of *Nephotettix virescens* (Distant, 1908) was 2-60, 4-45, 4-35, 2-30 days at 24, 28, 32, 34 °C, respectively.

Mean fecundity of *Z.sohrab* females was also significantly affected by temperature (Fig. 2). Salim (2002) [29] has shown egg laying capacity of the female insect decreased with further increase in temperature. Moreover, the present study showed the highest daily egg laying capacity was obtained at 30 °C (average 2.27). However the lowest total number of eggs was recorded at 30 °C. The daily egg laying capacity appears to be more directly affected by temperature and high temperature also contributed positively to the female's daily egg laying capacity as determined by other biologic parameters. Furthermore, Macgill (1932) [15]. Has shown the daily egg laying capacity of *E.* (*Zygina*) *pallidifrons* was 1-3 eggs, Klein (1948) [12] recorded that *Empoasca lybica*, Berg. Laid 1-7 eggs, Jabbar (1974) [11] determined that *Z. quyumi* laid 3.39 eggs on maize, 4.0-4.08 eggs on wheat.

Laboratory study presented that there seems to be no correlation between sex ratio and the temperature regimes.

The percentage of female emerged adults varied from 53.1% to 54.5% at temperatures tested. Likewise, Madden *et al*., (1986) [16] also recorded that the sex ratio of three different Dalbulus species, *Dalbulus maidis* (Delong and Wolcott), *Dalbulus gelbus* DeLong, 1950 and *Dalbulus elimatus* (Ball) at four temperature regimes (20, 23, 26, 29 °C) were 1:1 and they reached to conclusion that the sex ratio were not affected by temperatures or species.

Temperature had influenced the total mortality percentage of *Z.sohrab* during development from egg to adult emergence. Nymph mortality increased with the lowering of temperature in our study. In mortality test, total mortality occurred at 20 °C was higher than both 25 °C and 30 °C. It is concluded that temperatures lover than 20 °C are not suitable for development of *Z.sohrab*. In the results of the present study, the nymphal mortality ratio of *Z. sohrab* agrees with Pitre (1970) [25] who recorded the nymph mortality rate of *D. maidis* was 36% between 22.2 - 31.1 °C and Jabbar (1974) [11] stated that total mortality of *Z. quyumi* on maize was 24.4-56.2 on natural and laboratory conditions, respectively.

The obtained *r* value at 30 °C was the highest than the values obtained at other temperatures tested. However, the maximum R_0 value per *Z. sohrab* female was obtained at 25 °C, compared with the other temperatures (Table 4). R_0 value did not reflect the highest *r*, because of the longer generation time (*T*) at 20 °C, compared with 25 °C and 30 °C. These results suggested that increasing temperature shortened generation time (*T*) as well as increased intrinsic rate of increase (*r*). Hogg (1985) [10] studied life tables of *E. fabae* and concluded that intrinsic and finite rates of increase were largest, and mean generation time shortest, at the regime with highest temperatures for this leafhopper.

5. Conclusions

On the basis of the obtained results, the following conclusions may be drawn and used for further research on *Z. sohrab*. Temperature influenced significantly development duration of all stages of *Z. sohrab* on corn. Development from egg to adult emergence was decreased as temperature increased. In addition to, high temperature contributed positively to the female's daily egg laying capacity as determined by other biologic parameters. Considering all these findings, if the air temperature increase a few degrees Celsius because of global warming in the long term in Turkey, the population of *Z. sohrab* would reach higher densities compared with the past and create further damage, particularly on second crop maize with 2-4 leave stage. Further studies especially economic losses caused by the pest, economic threshold on second crop maize and enhanced study of the activity of egg parasitoids and other natural enemies for the pest should be done.

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