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Growth and Development of *Acanthiophilus helianthi* (Diptera: Tephritidae) Feeding on Safflower, *Carthamus tinctorius*

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Abstract

Safflower fly, *Acanthiophilus helianthi* Rossi (Diptera: Tephritidae), undergoes four stages (egg, larva, pupa and adult) during its growth and development. In this study, observation showed that the egg's stage took 1.16 ± 0.00, larva's stage took 12.02 ± 0.13 and pupa's stage took 7.03 ± 0.08 days before the emergence of adults. The male adult survived for 21.97 ± 2.69 days, while the female lived 19.19 ± 1.50 days. It was observed that the eggs were laid in a cluster, with a range between 10 – 50 eggs per cluster. The length and width of the individual egg were 1.12 ± 0.03 mm and 0.20 ± 0.00 mm respectively. The percentages of the survived individual larva decreased from the first instar until third instar. In the experiment, the length and width of the larva reached 7.77 ± 0.08 mm and 1.84 ± 0.03 mm respectively. Pupae were observed changing in colour from pale white to dark brown. The length and the width of the pupae observed were 6.78 ± 0.16 mm and 2.90 ± 0.02 mm. The longevity of the adults *Acanthiophilus helianthi* Rossi was influenced by the diets they consumed, the presence of other individuals, wideness of the areas, differences in time taken within the life cycle (between different stages) and temperature in the laboratory.

Keywords: *A. helianthi* Rossi growth, hatchability, larvae, longevity, oilseed crop, safflower fly

Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop and an essential component of cropping systems in the dry regions and marginal areas of the world (Sabzalian *et al*., 2008). Like other crops, safflower suffers from various diseases and insects (Weiss, 2000). The most serious safflower pest in Asia and Europe is the safflower fly, *Acanthiophilus helianthi* Rossi (Tephritidae), also called the shoot fly or capsule fly (Saeidi and Adam, 2011;Talpur *et al*., 1995; Zandigiacomo and Iob, 1991). In Asia, the safflower fly devastates most production areas in Iraq (Al-Ali *et al*., 1977), Iran (Saeidi *et al*., 2012), Pakistan (Talpur *et al*., 1995) and India (Vaishampayan and Kapoor, 1970; Verma *et al*., 1974). In Iran, seed-yield loss due to the safflower fly is estimated to be 30-70% for different safflower cultivars (Sabzalian *et al*., 2010; Saeidi *et al*., 2013). Infestation of the adults and larvae directly reduces the quantity and quality of the safflower seeds (Saeidi *et al*., 2011). The safflower fly is a polyphagous insect belonging to the Tephritidae family (Ashri, 1971). Adult flies lay eggs on the inner side of involucral bracts of safflower green heads (Ashri and Knowles, 1960; Narayanan, 1961).

However, the studies on the growth and development of *A. helianthi* on safflower are still lacking. Understanding the growth and development aspects of this insect is important in predicting its development, emergence, distribution and abundance in the field. Due to this reason, the current study was conducted with the objective to obtain information on the growth and development of *A. helianthi* feeding on safflower seeds.

Materials and methods

Insect rearing

Colonies of *A. helianthi* were reared by using techniques adopted and modified from Chua (1991), Vargas *et al*. (2000), Kaspi *et al*. (2001), Carey *et al*. (2005), Hee and Tan (2006), Chuang and Hou (2008) and Wang *et al*. (2009). Fifty rotten flower heads of safflower were collected randomly from the farm of Agricultural Research Station in Gachsaran (N 50 30' E 100 50'). Rearing of *A. helianthi* was conducted under laboratory conditions at 23.92 ± 0.16 °C (Min: 21 °C; Max: 29 °C) and 61.14 ± 0.33% (Min: 51%; Max: 70%) relative humidity (RH) at the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, University Yasooj, Iran. Each infested flower head was kept individually in 24.5 × 13.5 × 13.0 cm plastic containers lined with 4.0 cm thick of sterilized vermiculite until the emergence of the adults. Emerged adults were collected and placed into 30.0 × 30.0 × 30.0 cm rearing cage lined with 4.0 cm thick of sterilized vermiculite.

A mixture solution of honey and yeast extract in 3:1 ratio was prepared (Rattanapun *et al*., 2009). A piece of tissue was soaked in the solution and placed on the floor of the rearing cage. The diet was changed every two days.

Six non-infested flower heads of safflower placed individually on conical flasks in the cage were introduced to the cage as semi natural egging-devices for eggs laying. The egging-devices were kept for five days. These infested flower heads were then removed into 24.5 × 13.5 × 13.0 cm plastic containers lined with 4.0 cm thick of

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sterilized vermiculite to avoid contamination of microbes in the rearing cage.

Then, pupae found in the vermiculite were collected daily by sieving the vermiculite (Somta *et al.*, 2010). Collected pupae were placed back into the cage prepared earlier and kept until the emergence of the adults. Every five months, 50 rotten flower heads of safflower were obtained and kept in different cages until the emergence of the adults. These adults were then introduced into the established cage prepared to maintain the wilderness characteristics in the cage colony used in this study. Death bodies of the adults were removed from the cage every day and feeding devices (food container and tissues) were cleaned every two days to avoid fungal and bacteria contamination.

The artificial-egging-devices were prepared by adopting techniques established by Chua (1991) and Kaspi *et al.* (2001). Eggs were collected using a fine brush from the artificial-egging-device which was earlier put in established rearing cage for two hours. The eggs were soaked into distilled water to determine the viability of the eggs (Vargas *et al.*, 2000). The sanked eggs were viable, while the floated eggs were unviable. All the viable eggs were placed on a black fine mesh and kept in 90.0 mm diameter petri dishes. The petri dishes were sealed with parafilm to avoid larvae moving out of the dishes. After 24 hours, the petri dishes were observed and first instar larvae were collected and reared in the laboratory condition until the last larva moult (Godin *et al.*, 2002). Ten larvae were taken out daily and they were dipped into hot water (± 95 °C) for one minute. Then they were put on tissue paper for drying for two minutes before their bodies' morphometric measurement were taken. The media provided to the larvae were changed daily. This process was repeated daily until the last larva moulted.

The larvae that formed pupae were transferred to 3.0 × 3.0 cm small vials closed with fine muslin cloth tightened with rubber band. The pupae were kept individually until the adults' emergence. The males and females were kept separately in small container sized 15.0 × 20.0 × 10.0 cm covered with fine muslin cloth. The adults were also kept separately according to the day when they emerged. All the adults were fed and supplemented with honey and extracted yeast. All the dead individuals, in every stage, were removed to avoid contamination. The parameters recorded were as follows:

i. Durations taken in all stages; *ii*. Survived individuals in each stage; *iii*. Male and female longevity; *iv*. Length and width in each stage excluding the adult's stage (morphometric parameters)

The morphometric parameters measured were only for the eggs, larvae and pupae which were represented by the means of 40 individual eggs, 40 individual larvae from the first, second and third instars and 40 individual pupae. Other parameters such as sex and change in color of the adults were also recorded.

Statistical analysis

The comparison of longevity between the adult male and female was subjected to independent sample t-test performed by SPSS software (version 18).

Results and discussions

There were indicated four stages of *A. helianthi* during their life (Table 1); number of individuals, surviving percentages and the means of durations taken when the growth and development study was conducted.

Table 1. Number of percentage (%) survival during the growth and development of *A. helianthi* Rossi

Stages	No. survival(s)	Survived (%)	Means ± S.E. (Days)
Collected eggs	504	100.00	-
Egg	484	96.03	1.16 ±0.00
Larvae 1st instar	373	74.01	2.52 ± 0.03
2nd instar	277	54.96	2.48 ± 0.03
3rd instar	133	26.39	7.02 ± 0.07
Pupa	87	17.26	7.03 ± 0.08
Adult male	34	6.75	35.74 ± 1.12
Adult female	32	6.35	28.19 ± 0.31

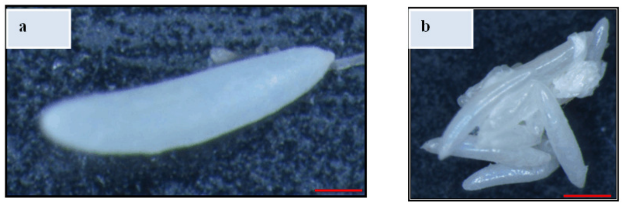

Fig. 1. a) Single egg of *A. helianthi* (Scale: 0.16 mm); b) a cluster of *A. helianthi* eggs (Scale: 0.42 mm)

Egg

It was observed that 96.03% of eggs hatched after 1.16 ± 0.00 days under laboratory conditions. The length and width of the eggs were 1.12 ± 0.03 mm and 0.20 ± 0.00 mm respectively. The eggs were transparent in color, cylindrical and tapers gently towards a narrower posterior end, banana shaped as shown in Fig. 1a (Headrick and Goeden, 1998; Pena *et al.*, 1998; White and Elson-Harris, 1992). It was observed that the eggs were laid in a cluster form ranging 10 – 50 eggs per cluster (Fig. 1b) even in artificially made egging-device once the oviposition took place (Pena *et al.*, 1998). According to Pena *et al.* (1998), females *of B. dorsalis* (Hendel) and *Anastrepha fraterculus* (Wiedemann) lay around 1,200-1,500 and 200-400 eggs respectively for their entire life in mango. It did not differ much for the numbers of eggs laid even in natural hosts or artificial egging- devices as the resources provided were sufficient enough for the larvae growth.

Observation showed that there was a difference in hatchability durations from *A. helianthi* as compared to other tephritids studied in the laboratory before. Results showed that the eggs of *A. helianthi* hatched earlier (1.16 ± 0.00 days) than *B. cacuminata* (Dhillon *et al.*, 2005; Raghu, 2002). Raghu (2002) and Dhillon *et al.* (2005) reported that *B. cacuminata* eggs hatched after 42 hours at 25 °C and the durations to hatch was between 1.0 to 5.1 days. The reasons are due to the different host types (pumpkin, bitter gourd, squash gourd, sponge gourd and cucumber), surrounding temperature where the studies were conducted and the species compared (Dhillon *et al.*, 2005; Pena *et al.*, 1998; Raghu, 2002). Pena *et al.* (1998) reported that the egg's stage of fruit flies last from 2-20 days. There were 3.97% of eggs failed to hatch and this was related to the temperature fluctuation in the laboratory even though it was under a controlled environment. Increment or decrement in temperature may affect the viability of the eggs. Golizadeh *et al.* (2009) reported that when temperature exceeds the tolerant limit of hatchability, the eggs of the insects will not hatch. In this study, the hatchability of *A. helianthi* eggs was observed at 23.92 ± 0.16 °C and this temperature was suitable for eggs to hatch.

Fig. 2. a) 1st instar larva; b) 2nd instar larva; c) 3rd instar larva

Fig. 3. Changes in coloration of pupa (from left to right); Scale: Bar 2.20 mm

Fig. 4. Larva and pupa of *Acanthiophilus helia*nthi Rossi

Fig. 5. Larva and pupa of *Acanthiophilus helianthi* Rossi inside flower head of safflower

Larva

Acanthiophilus helianthi underwent three larval instars (Fig. 2). According to Chang *et al*. (2007), a large majority of the larvae often died after reaching the third instar. Results obtained showed that the *A. helianthi* larval survivorship decreased as the time passed from one instar to another. The percentage of survived larvae decreased from 74.01% (first instar) to 54.96% (second instar). Even though the percentage of eggs hatched was high (96.03%), only a few (26.39%) of the larvae succeeded in reaching the third instar stage.

Fig. 6. Larva of *Acanthiophilus helianthi* Rossi inside flower head of safflower

Fig. 7. Infested flower head including pupa of *Acanthiophilus helianthi* Rossi

Fig. 8. Pupa of *Acanthiophilus helianthi* Rossi

Fig. 9. Pupa of *Acanthiophilus helianthi* Rossi

Fig. 10. Pupa of *Acanthiophilus helianthi* Rossi

Fig. 11. Adult of *Acanthiophilus helianthi*

Fig. 12. Exit hole of *Acanthiophilus helianthi* Rossi adults on flower head of safflower

The larvae survived for 12.02 ± 0.13 days before pupation took place and this period was shorter compared to what has been described by Pena *et al.* (1998) where the larval stage of fruit flies was between 2-4 weeks. Within this duration, it was observed that the larvae reached 7.77 ± 0.08 mm length and 1.84 ± 0.03 mm width. According to Pena *et al.* (1998), full grown larvae measures approximately 7.00 mm in length, but they did not mention the body width. White and Elson-Harris (1992) reported that third instar larva of the fruit flies average size was 6.50-10.00 mm in length 1.00-1.50 mm in width. Only 17.26% larvae survived to pupation.

Pupa

Pupation starts from the prepuparial stage in which the mouthparts are invaginated and the integument take on a waxy appearance (Headrick and Goeden, 1998). The duration of the prepupa within the puparium is unknown. The prepupal integument is shed and adheres to the innerwall of the puparium. The pupa forms within the puparium after the prepupal moult.

During the observation, the pupae took 7.03 ± 0.08 days before the adult emerged. The duration taken in this stage was shorter than for other species, which generally takes 2 – 4 weeks. Differences in durations taken to form pupa were mainly due to temperature and relative humidity. Stresses due to environmental changes in most cases hasten the growth of the insects for survival (White and Elson-Harris, 1992; Pena *et al.*, 1998).

On the average, the pupae size were 6.78 ± 0.16 mm in length and 2.90 ± 0.02 mm in width. The colour of pupae gradually changed from pale yellow to dark brown as the times changed for pupae to develop (Figs. 2 and 3). According to Headrick and Goeden (1998), the processes of hardening and darkening of the integument during the pupae development were within certain time frame. Other aspects of larva and pupa development are presented in Figs. 4, 5, 6 and 7, 8, 9, 10 respectively.

Adult

Fig. 11 shows the adult female of *A. helianthi*. Adults emerged (Fig. 12) after eight days of pupation at temperatures of 23.92 ± 0.16 °C. The emergence of the *A. helianthi* Rossi adults in this study was faster compared to *B. cacuminata* (Hering) as described by Raghu (2002). He reported that, at 25 °C, the pupae of *B. cacuminata* (Hering) took approximately 12 days before the emergence of the adults.

Morphologically, according to White and Elson-Harris (1992), the scutum of adult *B. papaya* was predominantly black with lateral yellow stripes, a black T-shaped mark on both males and females abdomen and typical dacine wing pattern. The males posses pecten. There were yellow marks on the thorax and made the *A. helianthi* wasp-like appearances (Fletcher, 1987).

In this study, it was observed that the longevity of the male was 21.97 ± 2.69 days, while female lived for 19.19 ± 1.50 days. Statistically, the longevity of the male was observed not significantly (P>0.05) longer than the females'. There were no indication as to which a gender might survive longer compared to the other and yet this can be further discussed since the longevity might be influenced by a vast number of factors.

The growth of fruit flies and longevity of the tephritids adults depends on the diet consumed (Vargas *et al.*, 2000; Zur *et al.*, 2009). The diets, either natural, semi-natural or artificially made, which are provided during the rearing, may contribute to longevity periods. In this study, concentrated honey as sugar sources enriched with carbohydrates and concentrated yeast extract as protein sources were provided for the adults. Besides protein, concentrated yeast extract also provides the vitamins and minerals needed by the adults. The nutrients in the diets play important role and their functions is crucial for insects to grow and develop. For instant, carbohydrate provided energy for routine life activities such as flight (Zur *et al.*, 2009; Wang *et al.*, 2009).

Viable protein source will extend the longevity, but if the source is provided early, the flies will utilize it, reproduced and died earlier (Wang *et al.*, 2009). In contrast, Canato and Zucoloto (1997) stated that sugars source were important for the *C. capitata* female adult since the insect was successfully producing eggs without ingesting protein. Tsiropoulos (1977) stated that some of the *Rhagoletis* species such as *R. complete* Cresson, *R. pomonella* (Walsh) and *R. cingulata* (Loew) can survive and are able to produce eggs on carbohydrate and water alone.

Other factors which affect the growth, development and longevity were the presence of other individuals in the surrounding areas. In the study, males and females were kept separately, therefore the males and females could only lived for 21.97 ± 2.69 days and 19.19 ± 1. 50 days respectively. With the presence of other individuals of either the same or different gender in the optimum density, the flies may potentially survive longer than the results revealed and this was reported by Meksonngsee *et al.* (1988) who reported that *B. tau* can survive up to 148 days. Dhillon *et al.* (2005) reported that the longevity of *B. cucurbitae* can last from 21-179 days.

The extents of the area where the flies can mobilize to fulfill their requirements and needs can influence the growth, development and longevity (Zur *et al.*, 2009). The flies need shelter, sufficient area foraging for foods, find mates and other life routines for their survival. Big areas usually provide all the requirements of the flies. However, if the area is big but too dense it may affect the life of the flies. In this study, *A. helianthi* adults were kept in the small container sized 15.0 × 20.0 × 10.0 cm covered with fine muslin cloth. Even though, the containers were not dense with flies, food and supplements, but the areas were probably not enough for the flies to move freely. Polyphagous and multivoltine tephritids are known for their high mobility thus the distribution wide across the region (White and Elson-Harris, 1992).

The different time taken for each stage from the egg to adult may also affect the life cycle and the longevity (Pena *et al.*, 1998). Different stages face and experience different needs. For example, larvae need to feed more during growth stage so that they can develop well to adulthood. Fernandes-Da-Silva and Zoculoto (1993) reported that *C. capitata* larvae utilized the nutrients of the oranges mainly from the lower part of the fruits where the nutrients are denser. They also found that the longevity was shorter when compared to papaya, but higher in the emergence of the adults.

The longevity of *A. helianthi* in this study was also influenced by the temperature inside the laboratory. Mean temperature in the laboratory was 23.92 ± 0.16 °C and fluctuated with very minimal changes. There were reports that stated the temperature was very crucial in the life cycle longevity of the fruit flies (Dhillon *et al.*, 2005; Golizadeh *et al.*, 2009; Nyamukondiwa and Terblanche, 2009; Pena *et al.*, 1998; Tsiropoulos, 1977; Vargas *et al.*, 2000). The temperature was also reported to influence the maturation and sexual behaviour of the males of *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha serpentina* and *Anastrepha striata* (Aluja and Mangan, 2008). According to Vargas *et al.* (2000), both adults male and female of *B. cucurbitae*, *B. dorsalis* and *C. capitata* survived in different durations at different temperature. Golizadeh *et al.* (2009) reported that temperature affected the specific rate functions of survival, reproduction, population growth and development of many insects and this influence directly the life cycle and the longevity.

Conclusions

Acanthiophilus helianthi is a relatively less studied species in Iran specifically on the growth, development and longevity. As other tephritid fruit flies, *A. helianthi* underwent four stages namely egg, larva, pupa and adult in their life. The eggs, larvae and pupae took 1.16 ± 0.00, 12.02 ± 0.13 and 7.03 ± 0.08 days respectively. *Acanthiophilus helianthi* completed all the stages within 20.21 ± 0.21 days. Statistically there were no differences of the longevity between the male (21.97 ± 2.69 days) and the female (19.19 ± 1.50 days). The longevity was influenced by several factors such as the different stages within the life cycle, laboratory temperature, relative humidity and supplements provided for the adults.

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