AMERICAN UNIVERSITY OF BEIRUT

BIOLOGY AND ECOLOGY OF *TEPHRITOMYIA LAUTA* (DIPTERA: TEPHRITIDAE) IN LEBANON

by
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AN ABSTRACT OF THE THESIS OF

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The flower-head infesting tephritid, *Tephritomyia lauta*, is a specialist on globe thistle, *Echinops* species. The fly emerges from May to October at different elevations in Lebanon. The fly is closely associated with the phenology of its host plant reducing their host reproductive capacity as larvae feed and destroy seeds. The biology of this species is not well documented, and the immature stages have not been described. In this study, we aim to shed light on the life cycle, resource utilization, and ecology of *T. lauta*, as well as determining the extent of intraspecific variations within its different populations in Lebanon.

Flower head dissection of *Echinops* spp. showed that the eggs are deposited singly on the inner side of the bracts surrounding the seeds. As the larvae hatch, they tunnel and feed inside one achene each. The pupa occupies all of the inner space of the achene while tightly adhering to the inner seed coat. Because larvae and pupae are concealed within individual achenes, detection requires dissection of individual achenes. Third instar larvae from *E. viscosus* are significantly larger than those associated with the relatively smaller flower heads of *E. gaillardotii*. The larger flower heads of *E. viscosus* can sustain more larvae per flower head (average of 13 vs. 6) than those of *E. gaillardotii*. The larvae suffer mortality by the ectoparasitoid, *Pteromalus* sp. and the observed percent parasitism was around 42% in all collected samples. Morphometric studies on *T. lauta* adults, using two head and four wing measurements, revealed that the length of dm vein, second radial vein R_{2+3} and length of third radial vein R_{4+5} are significantly longer in adults reared from *E. viscosus*. This reflects that *T. lauta* flies can reach a larger size when reared in larger flower heads, as the length of dm vein is often correlated to body size in the Tephritidae. Nevertheless, the ovipositor length do not differ in females reared from both *Echinops* species. Females of *T. lauta* do not have mature ovaries upon emergence and need to feed on an extrinsic source of proteins to support egg production. Courtship behavior was described; no post-mating behavior occurred in this species. Sequences of the mitochondrial ND1 gene of *T. lauta* flies coming from the two *Echinops* species and at different elevations show very little variations, reflecting little intra-specific variations in this species.

This is the first attempt at describing the basic biology, life history and ecology of *T. lauta*, an insect with a potential as biological control agent of the weedy *Echinops* species.

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CHAPTER I

INTRODUCTION

A. Tephritid Fruit Flies

Fruit flies belong to the Tephritidae, one of the largest families of the Diptera, including more than 4,200 species that exist in temperate, tropical and subtropical regions of the world, but more concentrated in the tropics (Headrick and Goeden 1998; White 1988). Fruit flies are not abundant in extremely dry areas because of the scarcity of host plants (Bateman 1972). Most tephritids are phytophagous and can be divided into two main groups: fruit infesting species that are well studied because they attack economically important crops; and non-fruit-infesting (non-frugivorous) species (Freidberg and Kugler 1989). The frugivorous fruit flies belong to the families Dacinae and Trypetinea, they attack fruit seeds or pulps. Species belonging to this group are of high economic importance due to the severe damage they cause to fleshy fruits and vegetables they attack. The non-frugivorous tephritids are mainly associated with the flower heads of the Asteraceae which does not include economically important crops. Non-frugivorous tephritids attack living, fleshy and dry plant tissues such as stems, roots and leaves; sometimes forming galls within vegetative plant tissue (Headrick and Goeden, 1998). These gall tissues are made up of nutrients pulled out of the host plant (Hartley and Lawton 1992). However, very few species form galls within flower heads (Headrick and Goeden, 1998).

The species infesting the flower heads mainly feeds on achenes, receptacles, or ovules. Although non-frugivorous fruit flies are not economically important, some species have been considered successful biological control agents of introduced weeds, because of their ability to cause substantial damage to their host plants (Zwolfer, 1983; and White, 1988). *Urophora cardui* (Linnaeus, 1758) and *Tephritis conura* (Loew, 1844) are examples of fruit flies that were used to control thistles belonging to *Cirsium* genus. Most temperate species are univoltine (Freidberg and Kugler 1989, Bateman 1972), passing through a winter diapause (Bateman 1972), whereas tropical species are mostly multivoltine (Freidberg and Kugler 1989, Bateman 1972) with no known diapause (Bateman 1972). Goeden (1985) divides non-frugivorous fruit flies into several categories relying on the variations of different selected host plants. Monophagous species are those feeding on one plant species, or nearly monophagous, feeding on more than one species in the same plant genus. Oligophagous species infest more than one genus in one subtribe or several subtribes belonging to one tribe. Species attacking many subtribes that belong to the same family are referred to as generalists, whereas species attacking several families are considered polyphagous. There are 23, 000 species belonging to family Asteraceae grouped into 17 tribes, thus fruit flies feeding on several tribes of this family are considered generalists and non-polyphagous (Goeden 1985).

Tephritids are characterized by their wing pattern, venation and ovipositor. Some tephritids have hyaline wings, others exhibit complex patterns. The ovipositor consists of an aculeus enclosed in a sheath. The aculues is only extruded to lay eggs during

oviposition (Freidberg and Kugler, 1989). Females oviposit their eggs in living plant tissues. The emerging larvae feed on stems, leaves, flower heads or seeds.

Fruit flies are used to study modes of animal speciation, particularly sympatric speciation, gene flow, host race formation and other aspects of evolutionary biology and animal ecology (Bush, 1969).

B. Life Cycle

The general fruit fly cycle begins after the female oviposits its eggs in the host's flower head using its ovipositor. In the case of fruit infesting tephritids, the female lays its eggs under the epidermis of ripe fruit or vegetable. The eggs hatch into a first larval instar that feeds on living tissues. The developing larvae pass through three larval stages (instars) as it continues to feed, thus molting twice before it becomes a puparium. The puparium protects an inactive fourth larva that eventually sheds its skin to become a pupa (Christenson and Foote, 1960). Pupation of non frugivorous tephritids occurs inside the flower heads of the host plant, unlike other fruit flies that pupate inside the plant tissues or in the soil. Pupation usually takes place directly after the last larval stage is completed, or might be delayed. In a delayed pupation, the larva enters into a pre-pupal stage that varies in duration depending on species and environmental conditions (Freidberg and Kugler, 1989). Mating occurs after the adults emerge, feed and become sexually mature and the cycle begins again (Christenson and Foote, 1960).

C. Morphology

1. Morphology of Eggs

The size of a tephritid egg ranges between 0.5 to 1 mm. It is usually white, elongated and cylindrical in shape. It has a posterior and an anterior end. The former bears the pedicel and the micropyle, through which the sperm enters. The micropyle is situated slightly above the surface of the egg, and in some species tends to form a long filament that could be longer than the egg body itself (Freidberg and Kugler, 1989). The micropyle can bear one or several openings. The end opposite to the pedicel is smooth and rounded bearing no opening. The egg is formed in the ovariole and gets fertilized in the oviduct. The posterior end exits the gonopore first at the end of the aculeus. The follicle cells present at the surface of the egg are responsible for producing chorion that providing nutrients for the egg (Headrick and Goeden, 1998).

2. Morphology of Larvae

The lavae of a tephritidae are maggot shaped, small, elongated or ovoid. They are covered by a soft and flexible cuticle, usually having no appendages. The larvae color ranges from white to yellow, but might have a black color at their caudal end (White 1988). A typical tephritidae larvae passes through three larval instars. The first larval instar differs from the rest of instars in its spiracles and cephalopharyngeal skeleton. The second and third larval instars varies in shape from elongated such in fruit infesting species to oval shaped in stationary species (Freidberg and Kugler, 1989).

3. Morphology of Pupae

The puparium of fruit flies can be whitish, black, yellow or even black, sometimes bi-colored. It is oval shaped, usually with a smooth surface and clear segmentation (Freidberg and Kugler, 1989).

The pupa develops inside the puparium, which is a hard larval integument. The pupa forms independently of the puparium. The developing pupae uses its larval trachea for respiration within the puparium. Later, it uses its thoracic spiracles for respiration after development (Headrick and Goeden, 1998).

4. Morphology of Adult

a. Head

Fruit flies exhibit a hypognathous head divided into distinct regions due to the presence of ridges (Freidberg and Kugler, 1989). The ventral side of the head contains the oral opening, through which the proboscis can be pulled back. The oval compound eyes are located on the sides of the head, shining and reflecting various colors as long as the fly is alive. In dead flies, the eyes turn black and dull. The ocellar plate with three ocelli is located at the frons, which is the region between the two compound eyes. Adult fruit flies have segmented hairy antennae of the aristate type. The third segment is longer than the first and the second segment together (Freidberg and Kugler, 1989).

Two types of setae are present on the head: the acuminate setae and the lanceolate setae. The acuminate setae are thin, black or dark brown in color and taper towards one

end. The lanceolate setae are wide with brighter colors, usually yellow or white and narrowed at both ends (Freidberg and Kugler, 1989). The setae vary in size and shape depending on the species. Taxonomically, they are very useful in identification (Foote et al., 1993).

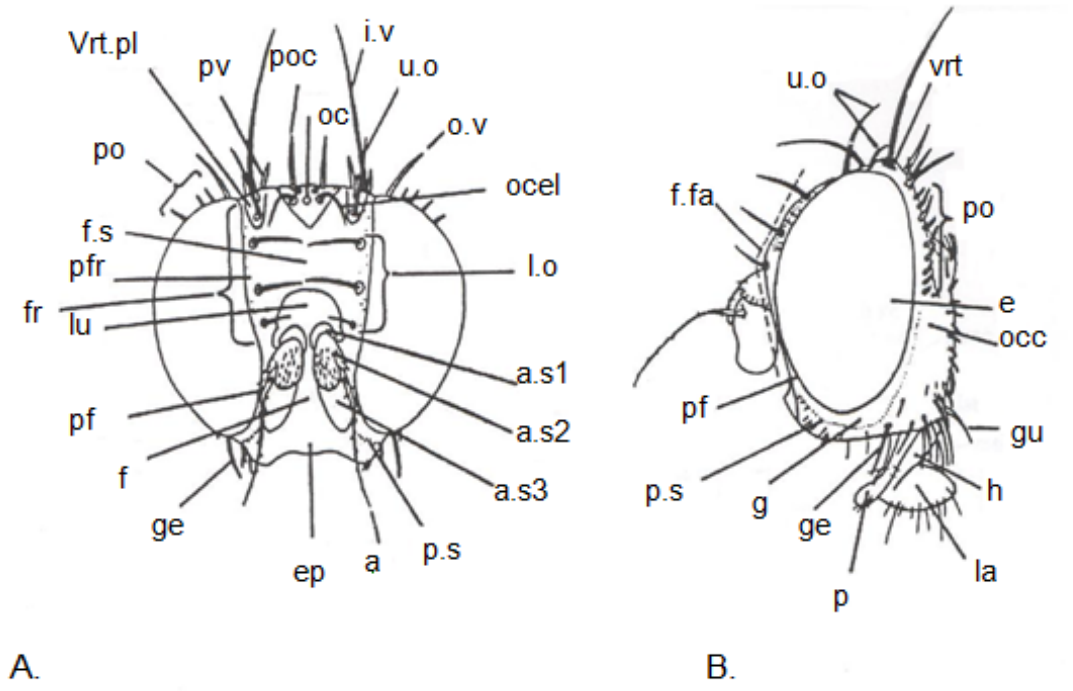

Fig. 1.1. A typical head of a fruit fly.
A: Front view; B: lateral view
Regions: a - arista; a.s1 - 1st antennal segment; a.s2 - 2nd antennal segment; a.s3 - 3rd antennal segment; e - eye; ep - epistome; f - face; f.f.a - fronto-facial angle; fr - frons; f.s - frontal stripe, g - gena; h - haustellum; la - labellum; lu - lunule; occ - occiput; p - palp; pf - parafacial; pfr - parafrontal; p.s - ptilinial suture; vrt - vetex; vrt.pl - vertical plate.
Setae: ge - genal; gu - gular; i.v - inner vertical; l.o - lower orbital; oc - ocellar; o.v - outer vertical, po - postorbital; poc - postocellar; pv - postvertical; u.o - upper orbital.

Source: Freidberg, A. and J. Kugler. 1989. Fauna Palaestina, Insecta IV - Diptera: Tephritidea, The Israel Academy of Sciences and Humanities, Jerusalem, p. 11.

b. Thorax

The thorax of fruit flies is divided into: prothorax, mesothorax and metathorax. The mesothorax is larger than the pro and metathorax, forming the largest part of the thoracic structure. The mesonotum is divided dorsally into the scutum and the scutellum which is important in Tephritdae identification (Foote et al. 1993). The setae on the thorax along with the pollinose areas, hairs and color patterns of the scutellum are useful in fruit fly taxonomy (Freidberg and Kugler, 1989).

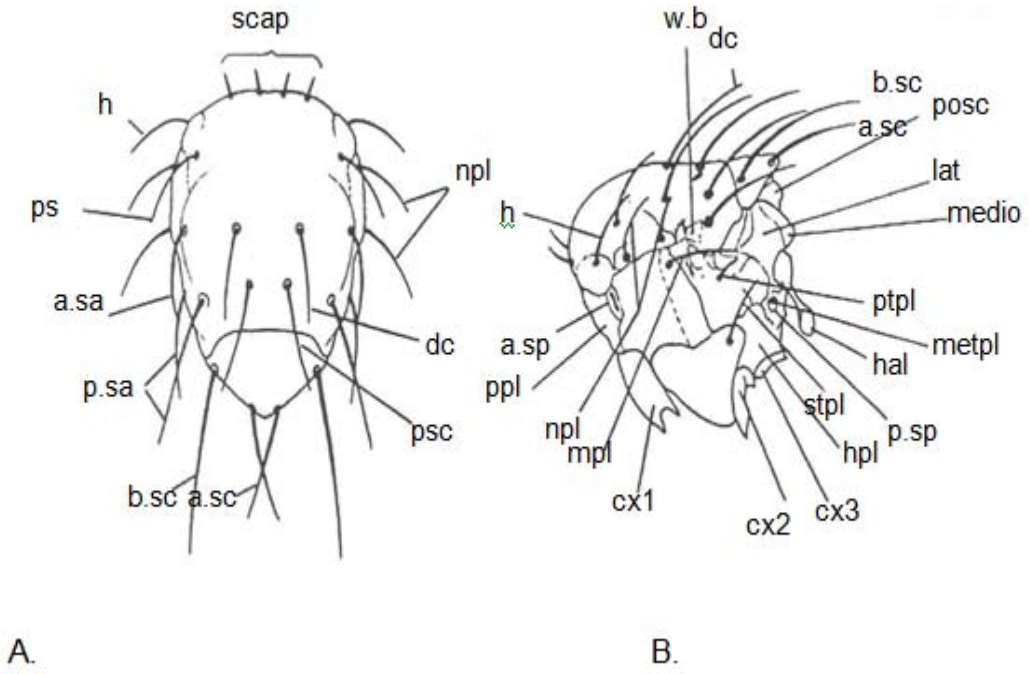

Fig. 1.2. A typical thorax of a fruit fly.
A: dorsal view; B: lateral view
Regions: a.sp - anterior spiracle; cx1 - forecoxa; cx2 - midcoxa; cx3 - hind coxa; hal - halter; hpl - hypopleuron; lat - laterotergite; medio - mediotergite; metpl - metapleuron; ppl - propleuron; posc - postscutellum; p.sp - posterior spiracle; w.b - wing base.
Setae: a.sa - anterior supra-alar; a.sc - apical scutellar; b.sc - basal scutellar; dc - dorsocentral; h - humeral; mpl - mesopleural; npl - notopleural; ps - presultural; p.sa - posterior supra-alar; psc - pescutellar; ptpl - pteropleural; scap - scapural; stpl – strenopleural.

Source: Freidberg, A. and J. Kugler. 1989. Fauna Palaestina, Insecta IV - Diptera: Tephritidea, The Israel Academy of Sciences and Humanities, Jerusalem, p. 13.

c. Wings

Fruit flies have one pairs of patterned wings on their mesothoracic segment. The length of the wing is usually 1.8 to 3 times the width with a round shape at the end. Different wing patterns are useful for identification of the species; they can be simple hyaline or vary from spots to bands. The wings are characterized by the arrangement of veins and shapes of cells. The stigma at the top of the subcostal cell is important in fruit fly taxonomy (Freidberg and Kugler, 1989).

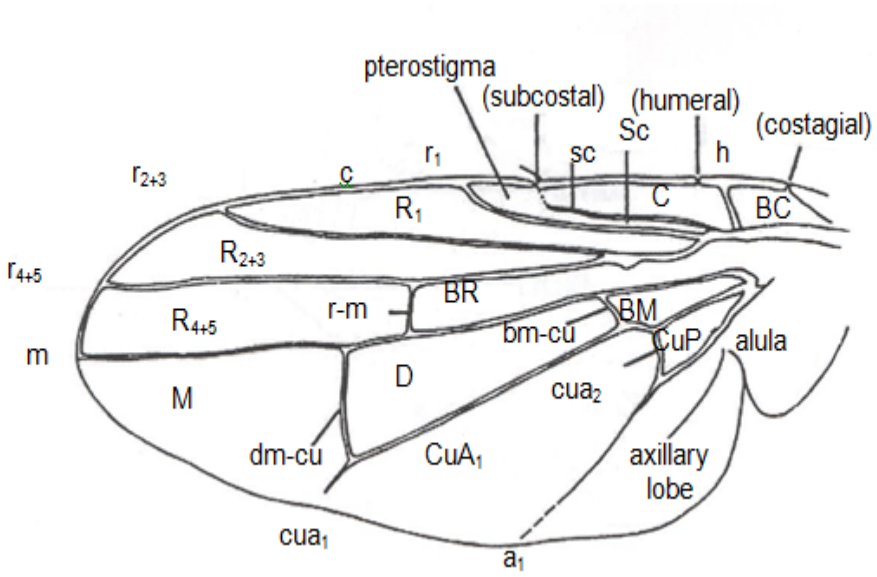

Fig. 1.3. A typical wing of a fruit fly.
Veins: a_1 - $anal_1$; bm-cu - basal medial-cubital; c - costa; cua_1 - 1st anterior cubitus; cua_2 - 2nd anterior cubitus; dm-cu - distal medial-cubital; h - humeral; m - media; r_1 - $radius_1$; r_{2+3} - $radius_{2+3}$; r_{4+5} - $radius_{4+5}$; r-m - radius-medial; sc – subcosta.
Cells: BC - basal costal; BM - basal medial; BR - basal radial; C - costal; CuA_1 - 1st anterior cubital; CuP - posterior cubital; D - discal; M - medial; R_1 - 1st radial; R_{2+3} - 2nd radial; R_{4+5} - 3rd radial; Sc – subcostal.
Names of costal breaks appear in parentheses.

Source: Freidberg, A. and J. Kugler. 1989. Fauna Palaestina, Insecta IV - Diptera: Tephritidea, The Israel Academy of Sciences and Humanities, Jerusalem, p. 15.

d. Legs

Fruit flies have three pairs of walking legs, each located on a thoracic segment. The three pairs of legs are similar in size and shape among different species of tephritidae (Foote et al., 1993). Some male fruit flies have rare sets of hair on their femora. Setae are also present on the fore femora and hind tibia (Freidberg and Kugler, 1989).

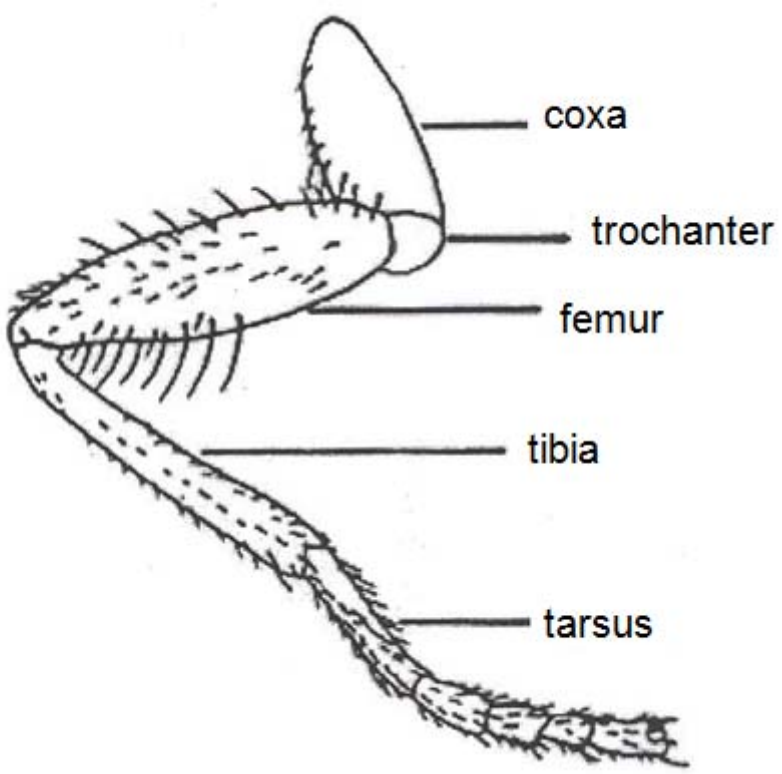

Fig. 1.4. Front leg of a fruit fly.

Source: Freidberg, A. and J. Kugler. 1989. Fauna Palaestina, Insecta IV - Diptera: Tephritidea, The Israel Academy of Sciences and Humanities, Jerusalem, p. 14.

e. Abdomen

Fruit flies have segmented abdomen that can be divided into two main parts: the pre-abdomen and the post-abdomen. The two abdominal regions vary among males and females. In males the pre-abdomen consists of five segments and the post-abdomen consists of four segments (6-10). In females the pre-abdomen consists of six segments while the post abdomen consists of three segments (7-9). In both cases the first two segments are fused together (Freidburg and Kugler, 1989).

The male genitalia (aedeagus) are usually located under the fifth abdominal segment. The oviscape which covers the ovipositor in females is located at the seventh segment and is useful in taxonomy. The aculeus (ovipositor tip) is connected to the oviscape by a membranous tube (inversion membrane) located at segment eight of the female's abdomen. The aculeus has a round pointed tip and is heavily scelerotized. It retracts back to the oviscape after oviposition (Freidberg and Kugler, 1989). Female fruit flies usually have two spermatheca in their abdomen to store sperms. The aculeus, shape of ovipositor and number of spermatheca in females, are of high taxonomic importance (Foote et al., 1993).

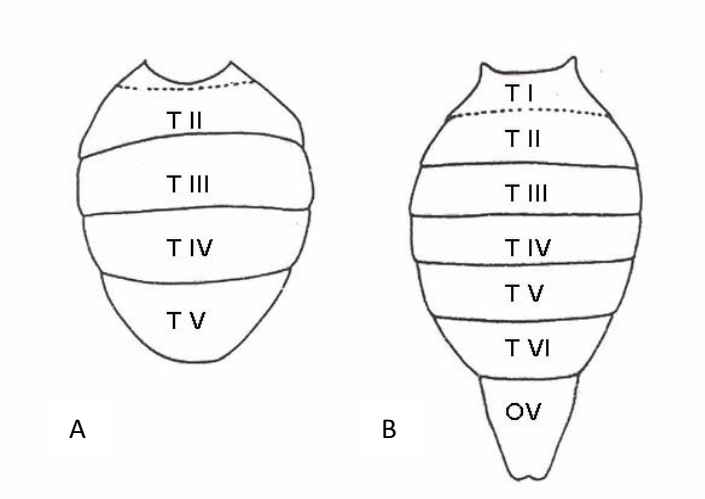

Fig. 1.5. The abdomen of male and female fruit flies.
A: male abdomen, T I - T V - 1st to 5th tergum;
B: female abdomen, T I - T VI - 1st to 6th tergum; ov – oviscape.

Source: Freidberg, A. and J. Kugler. 1989. Fauna Palaestina, Insecta IV - Diptera: Tephritidea, The Israel Academy of Sciences and Humanities, Jerusalem, p. 16.

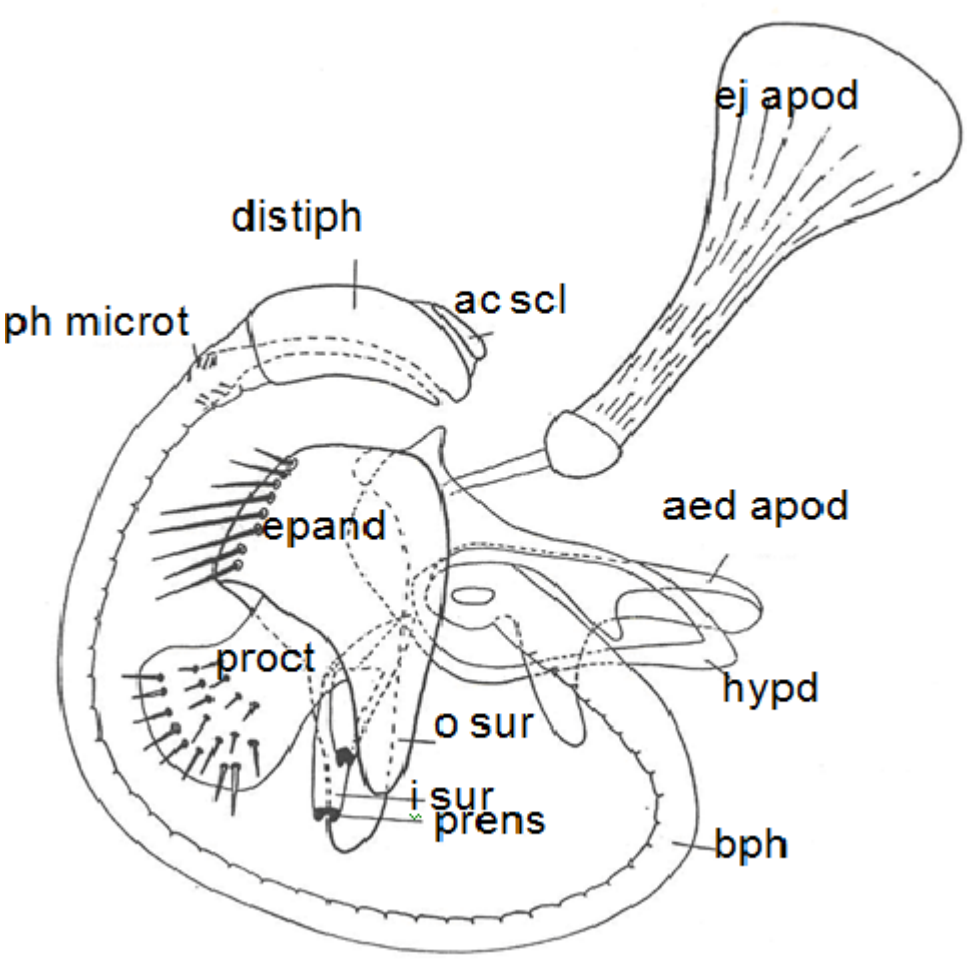

Fig. 1.6. A typical male fruit fly terminalia.
Ac.scl - accessory sclerite; aed.apod - aedeagal apodeme; bph - basiphallus; bph.microt - basiphallic microtrichia; distiph - distiphallus or aedeagus; ej.apod - ejaculatory apodeme; epand - epandrium; hypd - hypandrium; i.sur - inner surstylus; o.sur - outer surstylus; pens - prensiseta; proct - proctiger (cerci and anus).

Source: White, I. M. 1988. Tephritid Flies (Diptera: Tephritidea): Handbook for the Identification of British Insects, Vol. 10 (5a), Royal Entomological Society of London, London p. 82.

Fig. 1.7. A typical female fruit fly ovipositor.
Ac - aculeus; i.m - inversion membrane; ov – oviscape.

Source: Freidberg, A. and J. Kugler. 1989. Fauna Palaestina, Insecta IV - Diptera: Tephritidea, The Israel Academy of Sciences and Humanities, Jerusalem, p. 18

D. Life System Strategies of Fruit Flies

Fruit flies are classified into three general life history strategies depending on their host range (Zwölfer, 1983). The first group includes multivoline frugivorous fruit flies, with many generations per year. Fruit flies of this group exploit a wide range of host species. Adults are long lived. Females lay between 800 to 3000 eggs in host fruits. The larvae need 5 to 25 days to complete their development followed by pupation in the soil. This life strategy is usually common for polyphagous fruit flies.

The second group includes specialized frugivoroues fruit flies with a narrow host range. Adults do not live long, between 30 to 50 days. Females lay between 50 to 400 eggs. The larvae take 15 to 30 days to develop, followed by pupation in soil. In the presence of sufficient food supply, adults tend to emerge. The female lays few eggs in one host when the food supply is limited.

The third group includes non-frugivorous fruit flies that exploit different parts of their host. Species that belong to Trypetinae, Myopitinae and Tephritinae show this strategy (White, 1988). Non-frugivorous fruit flies can be univoltine or bivoltine. Adults are short lived and females oviposit 50 to 150 eggs. Larvae take 20 to 40 days to develop.

Life system strategies of fruit flies are subjected to change depending on ecological factors. Phenotypic plasticity is considered one of the most important factors that determine the life history strategy of fruit flies and enables them to change its life system, in response to resource availability and changing environments (Fletcher, 1989).

E. Mating

Adult males choose the flower head where mating will take place in case of non-frugivorous fruit flies. Seletion of the flower head is done based on its shape and odor. The male waits on the selected flower head for a female. The male defends its territory. The female locates this flower head used as rendezvous site for mating, while searching for its host to lay eggs. The male starts to dance. The dance is considered part of the courtship behavior and is specific to each genus (White 1988). In some species, males tend to exhibit a "kiss" strategy, which they touch the proboscis of females as a marital gift (Freidberg 1981). In other species, the males gather in swarms when ready to mate. Furthermore, males of some species produce sounds by a stridulatory strategy to attract females (Bateman, 1972).

Mating behaviors differ among univoltine and multivoltine species. Multivoltine species do not necessarily mate on the host plant unlike most species where mating occurs on host plants. Males attract females using chemical signals such as producing sex pheromones. Univoltine species do not use sex pheromones, they use visual signals such as their colorful body and banded wings instead (Bateman 1972).

F. Oviposition

Female fruit flies detect their host using visual and olfactory cues (Fletcher 1987). The oviposition behavior of fruit flies is similar among different species. It involves four significant steps: The female chooses an oviposition site and tests it. If the choosen site is suitable for oviposition, the female protrudes its ovipositor and punctures the host tissue to

break through the epidermis. The female moves its ovipositor under the skin to get a good space for its eggs (Christenson and Foote 1960). Some females mark their oviposition site with a deterrent pheromone to restrict other females from laying their eggs in the same area (Freidburg and Kugler, 1989).

G. Nutrition

The nutritional requirements of fruit fly larvae have not been determined. In several laboratory studies, the larvae were fed nutrient substances such as yeast and dried plant products. Nutrition during the larval stage is able to affect the longevity and fecundity of the coming adult stage (Bateman 1972).

However, more details are known about the nutritional requirements of adult Tephridae. A carbohydrate energy source and water are essential for adults' survival. Furthermore, sexual maturity can be attained with additional proteinaceous substances. Adults fed with a mixture of amino acids, vitamins, minerals and sucrose in the lab, laid more eggs and lived longer than those provided with nutrient substances only. In the field, adult flies were noticed to feed on natural products such as juices and tissues of decaying fruit, plant sap, nectar from flower and bird feces (Bateman 1972). Female longevity is higher with a decrease in oviposition sites, since most energy will be used by the female for survival instead of using it for oviposition. Adults might also feed on microorganisms present on the surface of flower heads, which are a good source for vitamins and nitrogen (Knio et al. 2007a). They usually get their water from dew or rain drops. Some tephritids feed on honeydew secreted by species that belong to order homoptera. The hydrolyzed

proteins, minerals and vitamins in honeydew enable adult flies to reach a normal status of fertility and fecundity (Christenson and Foote, 1960).

H. Movement

There are two types of movements in fruit flies; dispersive and nondispersive movements. Both movements are determined by the availability and accessibility of the host. Adults are associated with nondispersive movements when ample host fruits are available for oviposition, feeding and mating. Dispersive movement is a characteristic of individuals that did not locate suitable areas with abundant host or adults who decided to migrate from the area when the supply of host declined. Adults characterized with dispersive movements are able to travel long distances in a relatively short time searching for available and easily accessible host. Juveniles show significant dispersive movements before attaining sexual maturity (Bateman 1972). Adult flies showing nondispersive movements, restrict their flights in search for food, water and oviposition sites when the host is abundant. Adults may exhibit a daily pattern of movement around their host and nearby vegetation (Fletcher 1987).

I. Natural Enemies

Not all life stages of tephritids are equally subjected to the action of natural enemies. Egg and larvae, although present inside their host, they might be attacked by wasp parasites, mites and pathogenic microorganisms. Mature larvae, pupae and adult fruit flies are more subjected to the action of natural enemies.

Parasitism in fruit flies is controlled by many ecological conditions such as the season, parasite, host plant and climate. Hymenopterous parasitoids, specifically of the family Braconidae, attack the larvae of many tephritids. Larval parasites are much more common than egg and pupal parasites. Most of these parasites are present at low densities in nature, so they do not play a significant role in controlling the population of tephritids. This is mainly due to the fact that tephritids have a high reproductive and dispersal rates (Bateman 1972; Fletcher 1987). Non-frugivorous tephritids that attack flower heads of thistles are parasitized by species belonging to the family Pteromalidae. Pteromalidae parasitoids can be either endo or ectoparasitoids, sometimes causing the death of the larvae (Knio et al. 2007b).

Among insects, ants are considered one of the most important soil predators of fruit flies. They attack mature larvae, pupae and juvenile adults. Earwings and carabid beetles have been noticed to attack some larvae and pupae of fruit infesting tephritids. Spiders are considered major predators of adults' tephritids. Among vertebrates, birds are major predators of frugivorous fruit flies. Significant mortality rates of larvae and pupae have been recorded caused by microbial pathogens; fungi and bacteria (Fletcher, 1987).

J. Abiotic Factors

There exist many abiotic factors that affect the abundance of fruit flies, the most important of which are moisture, temperature and light.

Moisture plays an important role as a determinant of abundance of tephritids population. Populations of fruit flies do not exist in dry parts of the world, due to the

limited distribution of their host plants. Studies showed that there exists a positive correlation between moisture, measured by the amount of rainfall and population size of many species of tephritids. Dry periods affect the fecundity of adult females as well as immigration rates from other regions. Studies showed that mature larvae ready for pupation are highly sensitive to desiccation. Dry soil due to low humidity, leads to high mortality rates of newly emerged adults (Bateman 1972).

Temperature affects the rates of mortality, development and fecundity in fruit flies. Temperature plays a key role in determining the rates of development and synchronizatiom with environmental changes. The abundance of fruit flies is much higher in the summer than in winter. Univoltine species restrict oviposition to the summer season, whereas multivoltine species inhabiting tropical areas lay their eggs from spring to fall as long as the hosts are available. Development of the immature in fruit flies occurs between 10°C and 30°C. Maximum fecundity is attained at a temperature between 25°C and 30°C, whereas oviposition is preferred at lower temperatures. High temperature affects pupal survival and might lead to mortality due to prolonged pupal diapauses (Bateman 1972)

Light has a strong impact on the fecundity of female fruit flies by affecting several behaviors associated with female fecundity. Light affects the feeding, oviposition and rate of ovarian maturation of adult females and plays an important role in the mating activity of tephritids. Several studies showed that fruit flies subjected to bright light were able to attain sexual maturity earlier and oviposit sooner than those subjected to dim light. Dusk stimulates mating in many species of tephritidae (Bateman 1972).

K. Tephritids in Middle East

Very little research is conducted on fruit flies of the Middle East. Freidberg (1974) recorded thirty-five species of fruit flies from the southern shoulder of Mt. Hermon. The flies were collected during the summer starting May to early October and from different altitudes based on the altitudinal distribution of their host plants. Another study was done by Korneyev and Dirlbek (2000) on tephritidae of Syria, Jordan and Iraq. They presented a reviewed checklist of all species of tephritidae known from these countries with an addition of 28 new species recorded for the first time. A survey on the fruit flies of Crete was conducted by Neuenschwander and Freidberg (1983). They reported 47 different species of fruit flies, most of them occurred for the first time in Crete. Fruit flies were collected at all altitudes, starting from sea level up to 2400 m. However, flies were scarce at altitudes above than 1200 m because of the rarity of vegetation. They also reported 47 different species of host plants. A three- year survey was conducted in Lebanon between 1995 and 1998 on flowerhead-infesting fruit flies. The study was done on twenty thistle species that belong to family Asteraceae. The host plants yielded 18 different species of tephritids. Out of the 18 species, 15 were reported to occur for the first time in Lebanon. The study describes the host range and distribution non-frugivorous fruit flies associated with thistles in Lebanon. Anaylsis of the host range of fruit flies infesting flower head of thistles in Lebanon revealed that most of the flies are specialists attacking one genera of thistles. *Tephritomyia lauta* was reported to occur throughout the summer at all altitudes up to 1400 m. It was found in Lebanon in Beqaa Valley, Mount Lebanon and North Lebanon

(Knio et al.2002). This fly is a monophagous species that attacks only *Echinops* spp (Freidberg and Kugler 1989).

L. Genus *Tephritomyia* Hendel

The genus *Tephritomyia* belongs to the tribe Tephritini, subfamily Tephritinae of the family Tephritidae (Foote et al. 1993). The genus resembles *Acanthiophilus*, differing as follows: Head and eye are higher than long, frons showing dense hairs anteriorly, antennae with rounded apex, hyaline wing with uniform reticulation. Male aedeagus is sclerotized at the base.

Tephritomyia is characterized by having a yellow to brown head with yellowish to blackish hairs on antennae and glittering green to orange red eyes. Flies of this genus show a black thorax, white dense hairs and hyaline wings with black rounded spots. Abdomen varies from totally black to mainly yellow. The legs are yellow and hairy. Females that belong to this genus are larger than males. Females have a shiny black oviscape covered with whitish hairs.

The genus comprises 4 Afrotropical and 2 Palaearctic species. The larvae develop in the flower head of *Echinops* spp (Asteraceae). *Tephritomyia lauta* is a nonfrugivorous species that occurs in Asia Minor, Northern Africa, Greece, Turkey, Israel, Iran, Iraq and Syria (Freidberg and Kugler 1989).

M. Host-races and Speciation

There are many examples that illustrate co-evolution between fruit flies and their host plants. One of these strategies includes the development of spines and other defensive mechanisms against tephritids. Females developed specific adaptations to overcome these mechanisms such as having appropriate size of the ovipositor to lay eggs inside their host plants (Foote et al. 1993). Most phytophagous insects have shifted from their original host to a closely related host (Zwölfer 1982). Two isolated populations of tephritids might have a common ancestor, if their ancestor moved to a host plant that is similar to the original host ending up with two separate species (Zwölfer and Bush 1984).

Evolutionary biologists have always argued about the origin and evolution of species and host races. Some suggest that speciation is a resulted sympatrically, other biologists add geographical isolation as a factor that lead to a new species and host races. Many studied were done on insects to discover their mode of speciation. The most known example of sympatric speciation is that of the apple maggot fly *Rhagoletis pomonella.* *Rhagoletis pomonella* includes two races; one originally feeding on hawthorns and a second race that feeds on apples emerged after the introduction of apples to North America. Both races emerge as pupae when their corresponding host fruit is ready for oviposition. The two races are strictly associated with their host fruits; they do not feed on the host fruit of each other. Bush (1969) suggested a model based on several assumptions to explain the rapid formation of apple race in R. *pomonella.* He assumed that host selection has genetic basis. A fly chooses its host in response to chemical stimuli such as odors produced by plants. Any change in the olfactory system of the fly leads to the recognition of another compound

produced by a related plant. There exist several factors that enhance isolation and reduce gene flow. Factors include: disruptive selection when selection favors extreme phenotypes rather than average ones, conditioning where the animal learns to respond to a particular stimulus and magnitude of host shift which discusses how close or far the shift from one host to another (Bush 1969).

N. Aims of the Study

This study aims to describe the biology and ecology of *T.lauta* species that is not well documented in the Middle East, especially Lebanon. The study comprises the immature stages, resource utilization, behavior, courtship, mating and oviposition. The aims of this study are:

1. To describe the morphology of the immature stages of *T. lauta*.

2. To describe resource utilization and feeding behavior of the immature stages**.**

3. To compare the morphology and morphometry of adult flies reared from 2 different *Echinops* spp. at different elevation.

4. To describe the adult behavior, courtship, mating and oviposition behaviors of adults reared from different host species.

5. To determine the extent of intraspecific variations on adults associated with different *Echinops* spp and collected at different elevations through sequencing of mitochondrial genes.

Among its congeners, *Tephritomyia lauta* is poorly studied. Understanding its biology and ecology will enable us to assess the potential of this monophagous species as biological control agent of globe thistles, common weeds in Lebanon. The larvae of *T. lauta* destroy the seeds of their host plants and reduce their reproductive success. Therefore, they are important in keeping a balance in nature and preventing their host plants from becoming weedy.

CHAPTER II

MATERIALS AND METHODS

A. Flower Head Collection

Flower heads of globe thistles, *Echinops viscosus* and *E. gaillardotii* (Asteraceae), were collected haphazardly between 2012 and 2013 from various locations in Lebanon (Table 2.1), at different elevations, (Fig. 2.1), and at different stages of their phenology. They were identified using "Weeds of Lebanon" by W. Edgecombe (1970) Post (1932), 'Illustrated Flora of Lebanon' by Tohmé and Tohmé (2007) and by comparison with herbarium sheets at the Post Herbarium (BEI).

To compare the size of the flower heads of *Echinops viscosus* and *E. gaillardotii*, flower head samples of each species were dissected under a stereomicroscope. Using a dial caliper, the length and width of each flower head, the width of the receptacle, and the total number of seeds were recorded.

B. Rearing of Adult Flies

The samples collected from each location were divided into two smaller samples. The first sample consisting of 10 to 15 heads was placed in the fridge for subsequent measurement and dissection. The second sample comprising about 50 to 80 heads was placed in glass-topped, sleeve insectory cages (35x35x37cm) under fluorescent light

(12h/12 h cycle) and observed daily for fly emergence (Goeden, 1985). Adult flies emerging from flower heads, were fed with honey streaked on the inner glass top of the cages. Adults of *Tephritomyia lauta* emerging from *Echinops* spp were identified using keys by Freidberg and Kugler (1989) and White (1988). The emerging adult flies were collected and preserved for different purposes. Some adult flies were placed in separate cages for behavioral studies. Those used for morphological and morphometric measurements were collected in small plastic vials and stored in freezer at -20˚C to be mounted later. Other flies were stored at -70˚C in a deep-freezer (Fisher Scientific Isotemp Freezer) for subsequent molecular analysis.

Table 2.1. Dates and locations of collected *Echinops* samples.

Date of collection	Location
June 2012	Aaichiye (Jezzine Co.), South Lebanon
June 2012-2013	Ayteet (Sour Co.), South Lebanon
June 2012	Khaldeh (Baabda Co.), Mount Lebanon
June 2012	Al Nabi Shayth (Baalback Co.), Beqaa Valley
August 2012	Soghbine (West Beqaa Co.), Beqaa Valley
Sept. 2012	Faraya (Keserwan Co.), Mount Lebanon
Sept. 2012	Faqra (Keserwan Co.), Mount Lebanon
Sept. 2012	Batroun (Batroun Co.), North Lebanon
June-July 2013	Beqaata (Chouf Co.), Mount Lebanon
June 2013	Zawtar (Nabatieh Co.), South Lebanon
June 2013	Al Naame (Chouf Co.), Mount Lebanon
June 2013	Al Naqoura (Sour Co.), South Lebanon
June 2013	Qnaitra (Nabatieh Co.), South Lebanon
June 2013	Deir Mimas (Marjayoun Co.), South Lebanon

Date of collection	Location
July 2013	El Mansourieh (Metn Co.), Mount Lebanon
July 2013	Kfarsir (Nabatieh Co.), South Lebanon
July 2013	Choueifat (Aley Co.), Mount Lebanon
July 2013	Zahle (Zahle Co.), Beqaa Valley
July 2013	Fghal (Jbeil Co.), Mount Lebanon
July 2013	Baaqline (Chouf Co.), Mount Lebanon
July 2013	Deir Al Qamar (Chouf Co.), Mount Lebanon
July 2013	Niha (Chouf Co.), Mount Lebanon
July 2013	Dmit (Chouf Co.), Mount Lebanon
July 2013	Dedde (Koura Co.), North Lebanon
July 2013	Aasoun (Ed Donie Co.), North Lebanon
July 2013	Khirbet Kanafar (West Beqaa Co.), Beqaa Valley
July 2013	Beit Meri (Al-Metn Co.), Mount Lebanon
August 2013	Fghal (Jbeil Co.), Mount Lebanon
August 2013	Ehmej (Jbeil Co.), Mount Lebanon

Date of collection	Location
August 2013	Hammana (Baabda Co.), Mount Lebanon
August 2013	Baysour (Aley Co.), Mount Lebanon
Sept. 2013	Kfarnabrakh (Chouf Co.), Mount Lebanon
Sept. 2013	Batloun (Chouf Co.), Mount Lebanon
Oct. 2013	Ain Qni (Chouf Co.), Mount Lebanon

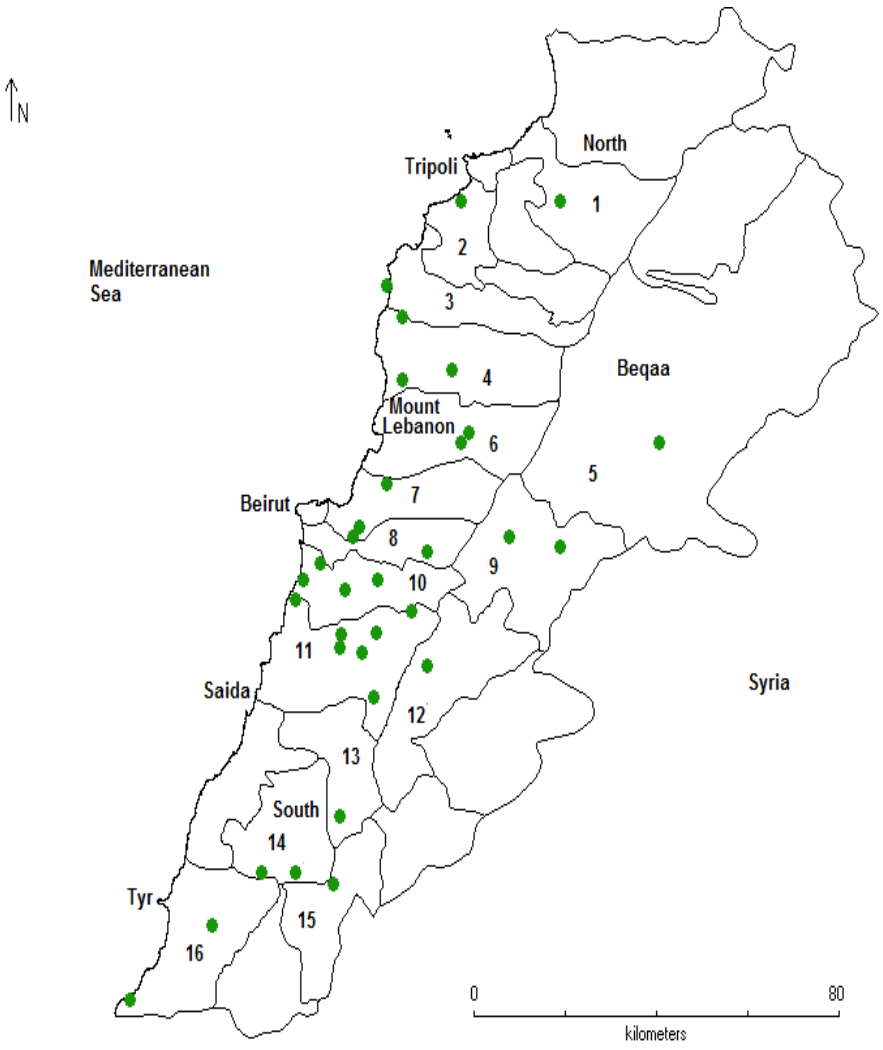

Fig 2.1: Map of Lebanon showing collection sites of *Echinops* species.
(1) Ed Donie Co. ; (2) Koura Co. ; (3) Batroun Co. ; (4) Jbeil Co. (5) Baalback Co. ; (6) Keserwan Co. ; (7) Metn Co. ; (8) Baabda Co. ; (9) Zahle Co. ; (10) Aley Co. ; (11) Chouf Co. ; (12) West Beqaa Co. ; (13) Jezzine Co. ; (14) Nabatieh Co. ; (15) Marjayoun Co. ; (16) Sour Co.

C. Flower Head Dissection

From every flower head sample, a small sample consisting of 15 flower heads was removed and placed in the refrigerator, to be used for dissection and studying of *T. lauta* immature stages. Prior to each dissection, the length of the flower head from the base of the receptacle to the tip of the florets was measured using a dial caliper along with flower head's width (Fig. 2.2). The flower heads were dissected under a Leica, Zoom 2000 stereomicroscope. The surface of the globe thistle and bracts were carefully observed under the microscope to check for eggs, after which each floret was removed and dissected separately by dissecting every single floret and achene to search for larvae. When larvae were found, their number, location, and type of damage they caused were recorded. Moreover, the stage and size (maximum length and width) of the immatures found were recorded. The immature stages (eggs, larvae and pupae) were either kept in 70% ethanol or placed in plastic tubes with few seeds for rearing. Finally, the total number of seeds in each flower head was reported.

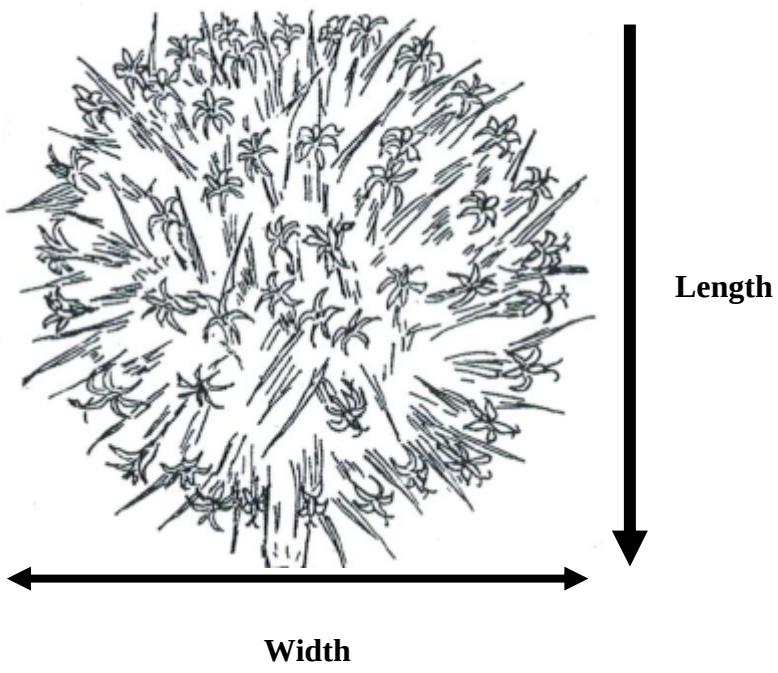

Fig. 2.2. *Echinops* flower head measurements.

Source: Edgecombe, W. S. 1970. Weeds of Lebanon, American University of Beirut, Beirut, page 393.

D. Comparative Studies on the Immature Stages

The immatures of *Tephritomyia lauta* were obtained by the dissection of flowers heads of *Echinops viscosus* and *E. gaillardotii*. The maximum length and width of the immature stages were measured using the ocular micrometer calibrated with a stage micrometer of the stereomicroscope. The external morphology of the immatures emerging from *E. viscosus*, mainly eggs, third instar and pupae were described and compared to the immatures emerging from *E. gaillardotii* followed by statistical tests to detect whether there were significant variations in the size of the flies emerging from each host plant.

Percent infestation per sample was also recorded by dividing the number of infested heads in a sample over the total number of dissected heads in the sample.

The cephalopharyngeal skeletons and spiracles of the third instars larvae of *T.lauta* emerging from both *Echinops* spp. were prepared according to Philips (1946) with some modifications. The larvae were cut crosswise using a razor blade near their anterior and caudal ends. The posterior and anterior parts were kept in a solution of 10% KOH. The next day, the bodies were washed twice with distilled water then, mounted on a slide over a drop of glycerol to be observed under the microscope. Slides of the posterior ends were used to describe the posterior spiracles of the larvae emerging from the two hosts, while slides of the anterior ends were used to observe the anterior spiracles and cephalopharyngeal skeletons.

E. Morphological and Morphometric Studies on Adult Flies

1. Morphology of Adults

Adults of *T. lauta* reared from *Echinops viscosus* (n = 15 females; 9 males), and from *E. gaillardotii* (n = 14 females; 8 males), were used to describe their external morphology. Using a stereomicroscope, the following measurements were taken: length and width of the head (Fig 2.3); the length of the distal medial vein (dm), the length of the third radial (R_{4+5}) vein, and the length of the fourth radial (R_{2+3}) vein and wing width (Fig 2.4). In case of *T. lauta* females, the length and width of the oviscape were also recorded (Fig 2.5).

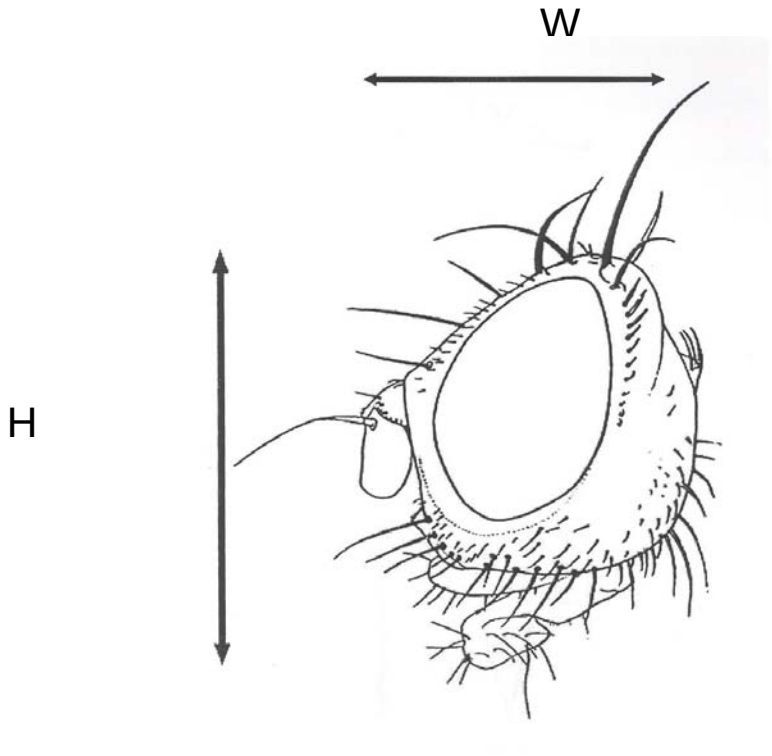

Fig. 2.3. Fruit fly head showing the measurements taken for morphometric studies.
H - height; W - width.

Source: Adopted with modification from: Freidberg, A. and J. Kugler. 1989. Fauna Palaestina, Insecta IV - Diptera: Tephritidea, The Israel Academy of Sciences and Humanities, Jerusalem, p. 154.

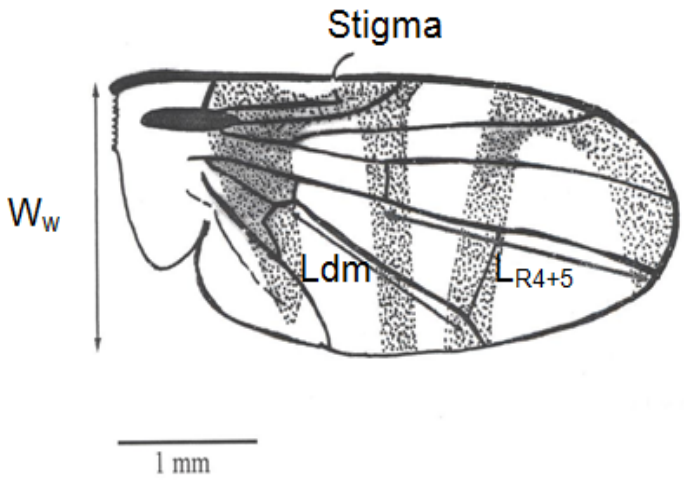

Fig. 2.4. Tephritid wing showing the measurements taken for morphometric studies.
W_w – wing width; L_{dm} - length of the distal medial vein; L_{R4+5} – length of the third radial cell.

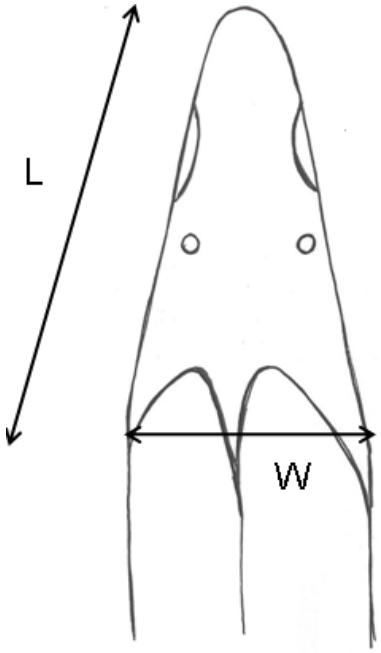

Fig. 2.5. Aculeus tip of tephritid female showing the measurements taken for morphometric studies.
L - length; W – width

2. *Morphology and Morphometry of the Female Ovipositor*

To describe the morphology of the female ovipositor, the abdomen was cut using a razor blade and kept overnight in a solution of 10% KOH for clearing. The next day, the ovipositor tip was washed in distilled water then mounted over a drop of glycerol on a slide for observation under a stereomicroscope. A slight pressure was applied to the oviscape on one side, in order to pull the aculeus outside the oviscape using a pin (White 1988). Then, the maximum length and width of the aculeus were measured using a calibrated ocular micrometer. The measurements were compared among *T. lauta* females emerging from both *Echinops* species.

3. Statistical Analysis

Statistical analysis was conducted to check for morphometric differences between males and females emerging from the same *Echinops* sp, between males and females emerging from the two *Echinops* spp., and/or between the two species regardless of gender. A two tailed t-tests were performed for samples with a normal distribution, as well as Mann-Whitney tests for variables deviate from normality. All variables measured were tested for normality using Shapiro-Wilk test. All statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences).

F. Behavioral Studies of Adults

The pre-mating, mating and post mating behaviors of adult *T. lauta* emerging from both *Echinops viscosus* and *E. gaillardotii* were studied in the lab. Adult males and

females were placed in transparent plastic vials supplied with water, honey and sometimes a small fresh flower head for oviposition. All behaviors exhibited by the flies during this period were recorded including body positions, wing movement, duration of mating and post mating behavior.

G. Ovarian Maturation

To determine the effect of diet on egg maturation, adult females newly emerging from both *Echinops* spp. were collected and placed in separate cages and supplied with different diets. Females were fed with one of the two treatments: the first treatment, adults were supplied with honey and water. The second treatment, females were supplied with honey, water and yeast hydrolysate (yeast hydrolysate: sucrose: water) (4: 7: 10) (Tsiropoulos 1978). The yeast solution was provided through cotton wicks dipped daily in the solution and hung from the glass top of the cage. Females were dissected at days 0, 1, 3,5,7,10,15 and 20 days after keeping them on one of the two treatments. The presence of mature eggs per female, their number and size were reported.

H. Parasitism

As for the flower head samples placed in insectary cages, they were used to monitor the emergence of *T. lauta* adults. The following data was recorded: the number of flower heads per sample, number and gender of *T. lauta* adults, number and identity of other emerging insects including parasitoids.

Percent parasitism was calculated as total number of emerged parasitoids divided by total number of emerged *T. lauta* plus adult parasitoids, and then multiplied by 100.

I. Molecular Studies

1. DNA Extraction

DNA was extracted from *T. lauta* flies that were stored at -70˚C upon emerging from the two *Echninops* hosts. Each fly was placed in a separate 1.5 ml microcentrifuge tube and crushed vigorously with a glass rod. A total of 200 µl of grinding buffer (10 mM Tris-HCl pH 7.8, 60 mM sodium chloride (NaCl), 300 mM sucrose, 10 mM ethylenediamine tetraacetic acid (EDTA) pH 8) was added to the tubes while grinding. Then, 200 µl of lysis buffer (300 mM Tris-HCl pH 7.8, 1% sodium dodecyl sulfate, 20 mM EDTA pH 8) was added to the tubes and then were kept on ice for 30 min. The tubes were centrifuged at 15000 rpm for 5 min to pellet debris and 300 µl of the supernatant was transferred to a new tube. A total of 400 µl of 4 M NaCl was added to each tube and mixed.

The tubes were centrifuged again at 15 000 rpm for 5 min and 650 µl of the supernatant was transferred to a new tube. One milliliter of absolute ethanol was added to each tube and kept on ice for 30 min. The tubes were centrifuged 15 min at 15 000 rpm, the supernatant was discarded, and 500 µl of 75% ethanol was added. The tubes were centrifuged 5 min at 15 000 rpm, the supernatant was discarded, and 100 µl of 75% ethanol was added. After a final centrifugation 5 min at 15 000 rpm, discard the supernatant without dislodging the pellet. The pellet was allowed to dry in air, and resuspended in 200

µl TE (10 mM Tris-HCl pH 7.8, 1 mM EDTA pH 8). The DNA extracts were stored at -20ºC.

2. Polymerase Chain Reaction and Sequencing

To determine the genetic variations if exists among the two *T. lauta* flies emerging from *E. gaillardotii* and *E. viscosus*, a 680 bp fragment of the mitochondrial gene ND1 (Nicotinamide adenine dinucleotide dehydrogenase subunit 1) was amplified and sequenced. The primers used were of Smith et al. (2002): ND1-F1, 2 (degenerate pair: 5'-ATCATAACGAAACCGAGGTAA and 5'-ATCATAACGAAATCGAGGTAA), and ND1-R1 (5'-CAACCTTTTAGTGATGC), with the modification of including another reverse primer ND1-R2 (5'-CAACCTTTTTGTGATGC).

Each amplification was done using a total volume of 50 µL containing 2 µL of DNA template, 5 µL of PCR buffer (50 mM KCL, 10 mM Tris-HCL pH 8.3 at 25˚C), 2.5 µL of 500 mM forward primer mix, 2.5 µL of 500 mM reverse primer mix, 5 µL of 200 µM each dNTP mix, 3 µL of 1.5 mM $MgCl_2$, 25 µL of distilled water, and 5 µL of 1.25 units Taq polymerase diluted 1/20. The amplification reaction was conducted in a Bio-Rad DNA engine thermocycler with the following program: one cycle at 94ºC for 4 minutes; 35 cycles at 93ºC for 30 seconds, 50 ºC for 30 seconds, and 72ºC for 45 seconds; and one cycle at 72ºC for 10 minutes. After the amplification reaction was complete, the PCR products were run on a 1% agarose gel to check for DNA bands. A volume of 50 µl PCR product was purified on a column using a commercial kit (Illustra DNA and Gel Band Purification Kit, GE Healthcare). The samples used for sequencing contained 20 ng/ul of

purified product sent along with 10 pmole of primer ND1-F mix. The samples were sequenced at Macrogen Sequencing service at Mcrogen Inc. (South Korea). The sequences were analyzed using ChromasLite program and edited manually. Clustal Omega was used online to detect any variations between two sequenced samples.

CHAPTER III

RESULTS

A. Comparative Morphometric Studies of the Flower Heads of *Echinops* spp.

Echinops gaillardotii was collected from coastal areas in Lebanon and low altitude mountains (up to 600 m) while *E. viscosus* occurred at low-high altitude regions (above 500 m) and from the Bekaa valley.

In order to compare the two host plants exploited by *T. lauta*, four flower head parameters were measured. These were flower head length and width, receptacle width, and total number of seeds (Table 3.1.). The flower heads of *E. gaillardotii* were relatively smaller (mean width = 5.4 cm), globular but conical with dark blue florets. On the other hand, those of *E. viscosus* were relatively larger (mean width = 6.2 cm), globular with light blue florets and usually with longer spines sticking out from the flower heads. Among the four parameters tested, head length and head width differed significantly between the two species. Receptacle diameter and total number of seeds were not significantly different in both spp.

Table 3.1. Descriptive statistics of the flower head parameters of the two host plants of *T. lauta*, *Echinops viscosus* and *E. gaillardotii*.

Host Plant	Flower Head Parameters	Mean	SE	Range
Echinops viscosus	**Flower Head Length (cm)**	5.90 [A]	0.15	3.2-10
(N=84)	**Flower Head Width (cm)**	6.20 [C]	0.15	3.6-11
	Receptacle (cm)	0.75 [E]	0.05	0.4-1.7
	Number of Seeds	156 [F]	4.5	72-285
Echinops gaillardotii	**Flower Head Length (cm)**	5.10 [B]	0.10	2.9-6.6
(N=46)	**Flower Head Width (cm)**	5.40 [D]	0.10	3-6.8
	Receptacle (cm)	0.70 [E]	0.05	0.4-1.2
	Number of Seeds	151 [F]	5	82-271

Means followed by different letters were found to be statistically different ($p < 0.05$) using Mann-Whitney test.

B. Studies on the Immature Stages of *Tephritomyia lauta*

1. Morphology and Morphometry of the Immatures

a. The Eggs

Eggs of *T. lauta* were obtained from the dissection of *Echinops viscosus* flower heads in advanced bud stage. They are white, shiny and smooth with a knob-like at the end. They have an average length of 1.20 ± 0.02 mm (mean ± SE) (n = 59; range: 0.98-1.27) and an average diameter of 0.24 ± 0.005 mm (n = 59; range: 0.19-0.34). The average number of eggs per flower head was found to be 9 ± 3.50 (n = 6 heads).

The eggs were laid singly, glued at one end to the interior of the bract surrounding the floret, but not inserted in the plant tissues. They were oriented in a parallel direction with the bracts. They were found upon removal of one or two bracts surrounding the seed. No eggs were obtained from the dissection of *E. gaillardotii* flower heads, and thus the eggs of the two populations were not compared.

b. The larvae

The total number of larvae collected from both *Echinops* spp. was 498 larvae from 47 infested heads. A total of 112 second instar larvae were obtained upon the dissection of flower heads of both species (86 from *E. viscosus* and 26 from *E. gaillardotii*). The second instars were white to yellowish in color, elongated, segmented and with brown posterior spiracles. Second instars collected from *E. viscosus* had an average length of 1.5 ± 0.04 mm (mean ± SE) (n = 86; range 0.98-2.2) and an average width 0.7 ± 0.03 mm (mean ± SE) (n = 86; range 0.39-1.96) while those collected from *E. gaillardotii* had an average length of 1.75 ± 0.06 mm (mean ± SE) (n = 26; range 1.03-2.21) and an average width 0.93 ± 0.04 mm (mean ± SE) (n = 26; range 0.49-1.23). The measurements are summarized in Table 3.2.

Table 3.2. Descriptive statistics of the length and width of second instar larvae of *T. lauta* found in *Echinops viscous* and *Echinops gaillardotii*.

Host	N	Mean Length (mm)	SE	Range	Mean Width (mm)	SE	Range
Echinops viscosus	86	1.50 [A]	0.05	0.98-2.2	0.70 [C]	0.05	0.39-1.96
Echinops gaillardotii	26	1.75 [B]	0.05	1.03-2.21	0.90 [D]	0.05	0.49-1.23
Both *Echinops* spp.	112	1.60	0.05	0.98-2.21	0.75	0.05	0.39-1.96

Means followed by different letters were found to be different significantly using Mann-Whitney Test ($p < 0.05$).

Statistical testing for the distribution of the length and maximum width of the second instars emerging from both *Echinops* spp. using Shapiro-Wilk tests showed that both distribution are not normal ($p < 0.05$). Hence, the length and maximum width of the second instars were compared using Mann-Whitney Test and proved to be statistically different in larvae associated with different *Echinops* spp

The third instars of *T. lauta* emerging from *Echinops viscosus* and *E. gaillardotii* were found to be yellowish, elongated and segmented. Third instars reared from *E. viscosus* had an average length of 3.76 ± 0.14 mm (mean ± SE) (n = 106; range 1.96-7.4) and an average width 1.61 ± 0.04 mm (mean ± SE) (n = 106; range 0.78-2.59) while those reared from *E. gaillardotii* had an average length of 2.68 ± 0.12 mm (mean ± SE) (n = 31; range 1.96-4.9) and an average width 1.26 ± 0.05 mm (mean ± SE) (n = 31; range 0.98-1.96). The measurements are summarized in Table 3.3.

Table 3.3. Descriptive statistics of the length and width of *T. lauta* third instar larvae found in *Echinops viscosus* and *E. gaillardotii*.

Host	N	Mean Length (mm)	SE	Range	Mean Width (mm)	SE	Range
Echinops viscosus	106	3.80 [A]	0.15	0.96-7.4	1.60 [C]	0.05	0.78-2.59
Echinops gaillardotii	31	2.70 [B]	0.15	1.96-4.9	1.30 [D]	0.05	0.98-1.96
Both *Echinops* spp.	137	3.50	0.15	1.96-7.40	1.50	0.05	0.78-2.59

Means followed by different letters were found to be different significantly using Mann-Whitney Test ($p < 0.05$).

Statistical testing for the distribution of the length and maximum width of the third instars emerging from both *Echinops* spp. using Shapiro-Wilk tests showed that both distribution are not normal ($p < 0.05$). Therefore, the length and maximum width of the third instars were compared using Mann-Whitney Test and proved to be statistically different among larvae associated with the two host plants.

c. The pupae

Eight pupae were obtained from the dissection of *Echinops viscosus* and seven from the dissection of *E. gaillardotii* flower heads. The pupa of *T. lauta* was dark brown to black in color, shiny and barrel-shaped. The lower part of the puparium was dark brown while the rest was shiny black. The average length of the pupae ($n = 15$) associated with *Echinops* spp. was 5.4 mm and the average width was 1.37 mm (Table 3.4). The pupa associated with the larger flower heads of *E. viscosus* were larger than those associated

with *E. gaillardotii* flower heads (mean length of 5.74 mm vs. 5.01 mm, and mean width of 1.87 mm vs. 0.8 mm, respectively). However, statistical tests conducted using Mann-Whitney shows that there is a significant difference in width of pupae associated with both spp. Larger samples of pupae must be further analyzed to confirm the statistical results as the sample size from each host plant was small (8 and 7, respectively) (Table 3.4).

Table 3.4. Descriptive statistics of the length and width of pupae of *T. lauta* found in *Echinops viscosus* and *Echinops gaillardotii*.

Host Plant	**N**	**Mean Length (mm)**	**SE**	**Range**	**Mean Width (mm)**	**SE**	**Range**
Echinops viscosus	8	5.70 ^{A}	0.70	1.37-7.4	1.90 ^{B}	0.20	0.73-2.36
Echinops gaillardotii	7	5.00 ^{A}	0.20	4.56-5.9	0.80 ^{C}	0.40	0.22-2.36
Both *Echinops* spp.	15	5.40	0.40	1.37-7.4	1.40	0.20	0.22-2.36

Means followed by different letters were found to be different significantly using Mann-Whitney Test ($p < 0.05$).

Morphometric studies on the immature stages of *T. lauta* showed that it is difficult to differentiate between the larvae of *T. lauta* emerging from both *Echinops* spp. based on size; however, the pupa associated with *E. viscosus* seem larger than those associated with *E. gaillardotii*. Larger samples of pupae from both *Echinops* spp. must be studied to get more precise statistical results.

2. Cephalopharyngeal Skeleton and Anterior and Posterior Spiracles of the Third Instar Larvae

The cephalopharyngeal skeleton of *T. lauta* third instars (length = 0.95 mm) show two stout and strongly sclerotized and pigmented mouth hooks. Each hook consists of a long, curved and pointed apical tooth and a subapical tooth (Fig. 3.1).

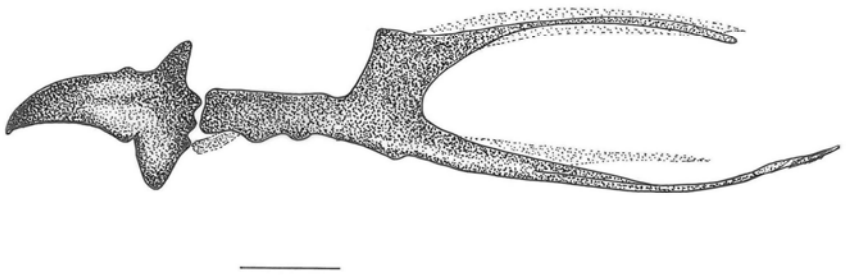

Figure 3.1. Cephalopharyngeal skeleton of *Tephritomyia lauta* third instar larva (bar scale = 0.1 mm).

The anterior spiracles (length: 0.18 mm; width 0.08 mm) of *T. lauta* larvae had 5 round papillae (Fig. 3.2). The posterior spiracular were elongated and light brown with 12 thick internal trabeculae (Fig. 3.3). Their average length was 0.06 mm $\pm$ 0.0004 (0.066-0.74; n = 24) and average width was 0.02 mm $\pm$ 0.0005 (0.021-0.028; n = 24).

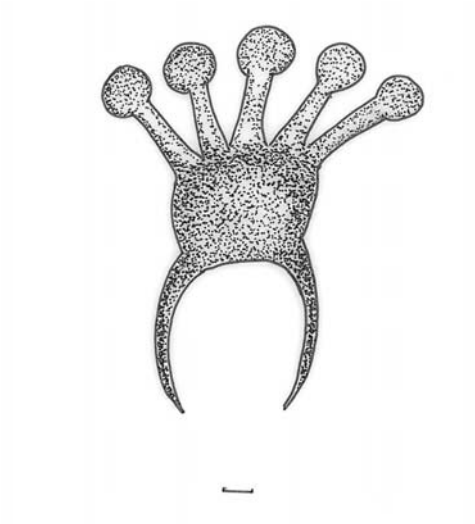

Figure 3.2. Anterior spiracles of *Tephritomyia lauta* larva (bar scale = 0.01 mm).

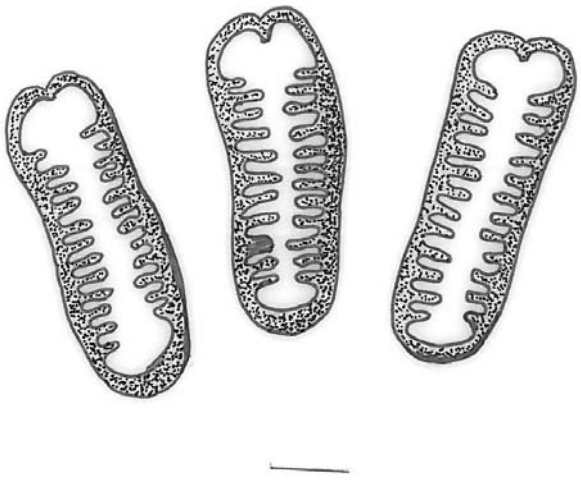

Figure 3.3. Posterior spiracles of *Tephritomyia lauta* larva (bar scale = 0.01 mm).

C. Morphometric Studies on Adult Flies

1. *Head and Wing Measurements*

Morphometric studies, using four wing and two head measurement,s were conducted on a total of 124 *T. lauta* adults reared from *Echinops* species. The measurements were statistically compared among flies emerging from *E. viscosus* (37 males and 45 females) and adults emerging from *E. gaillardotii* (18 females and 24 males).

Statistical analysis using Mann-Whitney test showed that males and females emerging from *E. viscosus* differed significantly in length of the third radial vein (R_{4+5}) while no statistical difference existed between males and females emerging from *E. gaillardotii*. Statistical studies comparing adults emerging from different *Echinops* spp. showed that females reared from *E. viscosus* and *E. gaillardotii* did not differ significantly ($p > 0.05$) in any of the studied parameters except for the second radial vein (R_{2+3}). On the other hand, males associated with these two host plants differed in the length of the distal medial vein (Ldm), length of third radial vein (R_{4+5}) and in the wing width ($p < 0.05$), reflecting the larger size of males reared from the larger flower heads of *E. viscosus* (Table 3.5).

The means of each variable between adult flies of the two host associated-populations, regardless of gender, were significantly different ($p > 0.05$) for the length of the distal medial vein (Ldm), the length of the second radial vein (R_{2+3}) and the length of third radial vein (R_{4+5}). This also reflects that adults reared from the larger flower heads of *E. viscosus* are usually bigger than those reared from the relatively smaller flower heads of *E. gaillardotii* (Table 3).

Table 3.5a. Morphometric characteristics of the adults of the *Echinops viscosus* –associated population of *Tephritomyia lauta.*

Host race	*Echinops viscosus* -associated race					
Variable (mm)	Males (N=37)		Females (N=45)		Both (N=82)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Head width	1.40 ±0.05	0.98-1.86	1.40 ±0.03	0.98-1.96	1.40 ±0.02	0.98-1.96
Head height	1.60 ±0.05	1.37-1.91	1.60 ±0.01	1.42-1.86	1.60 ±0.01	1.37-1.91
Wing width	1.95 ±0.05	1.67-2.16	1.90 ±0.04	1.57-2.16	1.90 ±0.02	1.57-2.16
Length of R2+3cell	3.10 ±0.05	2.64-3.3	3.15 ±0.02	2.73-3.3	3.10 ±0.01	2.63-3.33
Length of R4+5cell	1.85 ±0.05	1.61-1.98	1.90 ±0.01	1.6-2.0	1.90 ±0.01	1.60-2.05
Length of dm vein	1.85 ±0.05	1.65-1.98	1.90 ±0.01	1.66-2.0	1.90 ±0.01	1.65-2.0

Table 3.5b. Morphometric characteristics of the adults of the *Echinops gaillardotii* –associated population of *Tephritomyia lauta.*

Host race	*Echinops gaillardotii*-associated race					
Variable (mm)	Males (N=24)		Females (N=18)		Both (N=42)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Head width	1.40 ±0.04	0.98-1.91	1.40 ±0.05	0.98-1.86	1.40 ±0.03	0.98-1.91
Head height	1.60 ±0.03	1.32-1.86	1.60 ±0.03	1.42-1.81	1.60±0.02	1.32-1.86
Wing width	1.80 ±0.04	1.53-2.03	1.90 ±0.04	1.37-2.06	1.90 ±0.03	1.37-2.06
Length of R2+3cell	2.95 ±0.05	2.47-3.36	3.00 ±0.06	2.16-3.3	3.00 ±0.03	2.17-3.37
Length of R4+5cell	1.80 ±0.03	1.38-1.98	1.85 ±0.04	1.33-2.26	1.80 ±0.02	1.33-2.27
Length of dm vein	1.80 ±0.03	1.48-1.98	1.80 ±0.04	1.33-2.0	1.80 ±0.02	1.33-2.0

2. Morphololology and Morphometry of the Female Ovipositor

The length and width of the ovipositor of 45 females from *E. viscosus* and 18 females from *E. gaillardotii* were measured. The distribution of these two parameters was found to be not normal ($p < 0.05$) in both species. Hence, non- parametric tests were performed and showed that there was no significant difference in the means of ovipositor length and width ($p > 0.05$) of females reared from *E. viscosus* and *E. gaillardotii* (Table 3.6).

Table 3.6. Mean ovipositor length and width of *T. lauta* females reared from different *Echinops* species.

Host	**N**	**Mean Length (mm)**	**SE**	**Range**	**Mean Width (mm)**	**SE**	**Range**
Echinops viscosus	45	1.52 **A**	0.04	1.18-2.1	0.28 **B**	0.004	0.23-0.33
Echinops gaillardotii	18	1.70 **A**	0.09	1.16-2.2	0.27 **B**	0.01	0.170.33

Means followed by the same letter were not found to be different significantly using Mann-Whitney Test ($p > 0.05$).

The long aculeus (ovipositor tip) of females of *T. lauta* was gradually tapering with a narrow but not pointed tip (Fig. 3.3). The tip of the aculeus was suited for deep penetration between the florets without piercing the plant tissues. Usually, female tephritids need a sharper and narrower aculeus to oviposit its eggs in the flower head they infest (Zwölfer 1972). The headlets of both *Echinops* spp. are large and compact, and thus the females exploiting such flower heads need a long and a wide ovipositor to penetrate the bracts and lay their eggs.

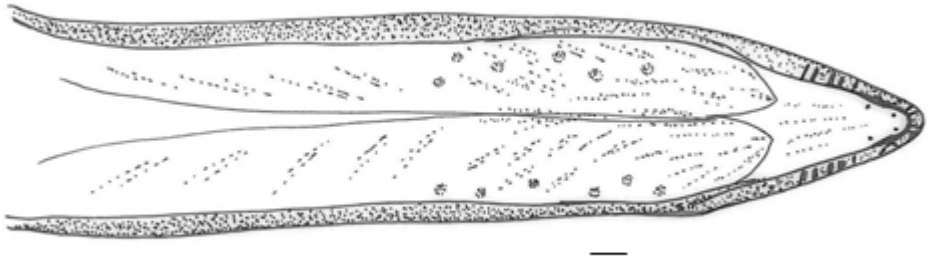

Fig.3.4. Female *Tephritomyia lauta* ovipositor (bar scale = 0.1 mm)

D. Biology of *Tephritomyia lauta*

The study showed that *Tephritomyia lauta* was found at different elevations in Lebanon, from the coastal areas to high altitude mountains (up to 1400 meters). Monitoring adult emergence from collected flower head samples showed that *T. lauta* was reared from *E. viscosus* from late June until mid October, and from *E. gaillardotii* from early June until August.

Dissection of flower head samples showed that the eggs are laid in the 'open bud' or 'early blossom' stages of *Echinops*. They are deposited singly, with their longitudinal axis either parallel or slightly diagonal with respect to the vertical axis of the floret. They were found between the second or third bract surrounding the floret. Each egg hatched into a first instar larva which bore inside the soft tissues of an immature achene (seed). As the larva feed and grow, it molts into a second, then into a third instar larva while remaining totally concealed within its achene. The area tunneled inside the achene becomes larger as the larva increases in size. Eventually, the third instar larva forms a puparium and the puparial case adheres tightly to the inner achene coat, in a way that the puparium occupies all of the inner space of the achene. Pupae were detected in flower heads at the post-blooming stage. What was peculiar in this species was the fact that it was impossible to detect the presence of larvae inside the flower heads as in the case with other tephritid species which clearly show damaged seeds. Since each *T. lauta* larva fed individually inside its 'own' achene and since there was no sign of outside damage to the achene, it was impossible to detect the presence of larvae unless all achenes in a flower head were removed and dissected one by one. Finding the larvae of *T. lauta* turned out to be a tedious job as each flower head contains about 150 seeds, and 'uninfested' seeds look exactly like 'infested' ones. This may be the reason why the immature stages of this species were not previously studied.

E. Resource Utilization

Tephritomyia lauta females oviposited more than one egg per flower head. The total number of eggs found in *Echinops viscosus* flower head ranged from 2-25, with an average of 9 ± 3.5 eggs per head (n = 6 flower heads).

Flower head dissection showed that the number of larvae found within a flower head of *Echinop*s spp. ranged from 1-53 larvae per flower head with a mean of 10.6 ± 2 larvae per head (n = 498 larvae from 47 flower heads). More specifically, in *E. viscosus*, a mean of 13.41 ± 2.33 (range: 1-53; n = 389 larvae from 29 flower heads) larvae were found per flower head; while, in *E. gaillardotii*, a mean of 6 ± 1.0 (range: 1-13; n = 104 larvae from 18 flower heads) larvae were found per flower head. The results show that the relatively larger flower heads of *E. viscosus* could sustain a higher number of larvae per flower head than those of *E. gaillardotii*.

The larvae were found inside flower heads at the ‘ blooming stage’ with average flower head length of 5.5 ± 0.12 cm (n = 98), and average maximum head diameter of 5.77 ± 0.13 cm (n = 98). Table 3.7 summarizes the flower head samples with negative records.

Table 3.7. Samples of *Echinops* flower heads with negative records, not yielding dipterous or parasitoid adults.

Location	County	Date
E. viscosus		
Beqa'ata	Chouf	5-6-2013
Deir Mimas	Maarjyoun	16-6-2013
Al Mansorieh	Metn	2-7-2013
Zahle	Zahle	5-7-2013
Khirbet Kanafar	West Beqaa	7-7-2013
Ketran	El Doniyyeh	9-7-2013
E.gaillardotii		
Qnaitra	Nabatieh	21-6-2013
Haret Aloumara	Aley	2-7-2013
Ayteet	Sour	7-7-2013
Dedde	Al koura	7-7-2013
Kawneen	Bint Jbeil	25-8-2013

Infestation of *Echinops* flower head samples by *T. lauta* flies was moderate. It was calculated by determining the number of flower heads infested with *T. lauta* immatures out of the total number of dissected flower head per sample (containing about 10-15 heads). Percent infestation was found to be 34.8% $\pm$ 8.18 (range: 0-90%; n = 13 samples) in samples collected from different locations (Table 3.8).

As for percent infestation of *Echinops* flower heads by *T. lauta* and other insects, it was found to be 42.6% $\pm$ 8.7 (range: 0-100%; n = 13 samples) (Table 3.8).

Table 3.8 Number of flower heads infested with immatures of *T. lauta* (percent infestation with *T. lauta*), and number of heads infested with *T. lauta* and other insects (total percent infestation) in dissected *Echinops* samples.

Sample location	No. of heads with *T. lauta* larvae	Total no. of heads per sample	**Percent infestation with *T. lauta***	Total no. of heads with insects	**Total percent infestation**
Ketran, Ed Donie Co.	6	12	**50**	8	**66.7**
Aassoun, Ed Donie Co.	9	10	**90**	10	**100**
Beit, Mery*Al-Matn Co.	3	12	**25**	7	**58.3**
Fghal, *Jbeil Co.	7	12	**58**	8	**66.7**
Ain Zhalta, Chouf Co.	2	8	**25**	8	**25**
Ehmej, Jbeil Co.	6	12	**50**	7	**58.3**
Batloun, Chouf Co.	5	9	**55.6**	5	**55.6**
Fghal*, Jbeil Co.	2	11	**18.2**	2	**18.2**
Dedde*, Koura Co.	7	10	**70**	7	**70**
Hammana, Baabda Co.	1	10	**10**	1	**10**
Khar Nabrakh, Chouf Co.	0	8	**0**	2	**25**
Zahleh, Zahle Co.	0	8	**0**	0	**0**
Khirbet Kanafar, West Beqaa Co.	0	8	**0**	0	**0**

Plants collected from sites with a star were *E. gaillardotii*; while all the rest were *E. viscosus*

F. Courtship Behavior and Mating of Adults *T. lauta*

Adults of *T. lauta* were observed *in copula* several times in the lab. The male approached the female at close proximity (1-2 cm apart), then raised its wings 45° and started to vibrate them with 2 vibrations per second walking in a full circle or more frequently in a semi circle around the female. Afterward, wing vibrations increased to 3 to 5 vibrations per second before the male attempted to mount the female. The female stood motionless in its place or responded with few wing vibrations.

During mating, the wings of the male were held at 10°, while the wings of the female are held at 45° with respect to the longitudinal body axis. The male's head was positioned between the thorax and abdomen of the female's body. The female sometimes walked around few centimeters, but most of the time stayed motionless in its place. No post-mating behavior was observed in this species.

Mating took place between 11:00 am and 7:00 pm. The average duration of mating was 320 min ± 24 min (n = 18).

G. Predation and Parasitism

In the laboratory and in the field, adults of *T. lauta* were preyed upon by different species of spiders, mainly crab spiders, upon emergence from the flower heads.

The immature stages of *T. lauta* suffered mortality due to parasitism by *Pteromalus* sp. (order Hymenotera; family Pteromalidae). This was the only observed parasitoid of the larvae observed upon dissection of *Echinops* flower heads. *Pteromalus* sp. was identified as larvae-larvae ectoparasites of *T. lauta*. Its larvae were found feeding

externally on their host. The parasite did not change the shape of the larvae or its behavior. Parasitized *T. lauta* larvae continued to feed and grow. The presence of the parasite can only be identified upon dissection of flower heads looking for larvae.

Percent parasitism in all collected *Echinops* samples was moderate to high as summarized in Table 3.8. Average percent parasitism in *Echinops* species was 42.5% ± 7.01 (range: 0-100; n = 27 samples). More particularly, it was 31.5% ± 8.6 (n = 12 samples) in *E. viscosus* and 51.3% ± 10.3 (n = 15 samples) in *E. gaillardotii*.

Table 3.9 Percent parasitism by the ectoparasitoid, *Pteromalus* sp. (Hymenoptera: Pteromalidae), in flower head samples of *Echinops* species.

Sample location	Date of collection	No. of flower heads	No. of emerged *T. lauta*	No. of emerged *Pteromalus*	Percent parasitism*
E. viscosus					
Zahle, Zahle Co.	5-7-2013	55	0	7	100
Khirbet Kanafar, West Beqaa Co.	7-7-2013	23	2	3	60
Ketran, Ed Donie Co.	7-7-2013	45	24	4	14
Aasoun, Ed Donie Co.	8-7-2013	80	13	8	38
Aasoun, Ed Donie Co.	24-7-2013	75	11	10	48
Baysour, Aley Co.	19-8-2013	53	5	3	38
Ehmej, Jbeil Co.	24-8-2013	37	100	0	0
Hammana, Baabda Co.	26-8-2013	27	10	1	9
Batloun, Chouf Co.	1-9-2013	48	6	3	33
Kfar Nabrakh, Chouf Co.	1-9-2013	18	5	3	38

Sample location	Date of collection	No. of flower heads	No. of emerged *T. lauta*	No. of emerged *Pteromalus*	Percent parasitism*
Ain Qni, Chouf Co.	7-10-2013	35	25	0	0
E. gaillardotii					
Ayteet, Sour Co.	2-6-2013	137	1	8	89
Zawtar, Nabatieh Co.	11-7-2013	84	0	10	100
Al Naame, Chouf Co.	14-6-2013	41	0	2	100
Al Naqoura, Sour Co.	16-6-2013	25	0	1	100
Aaisheye, Jezzine Co.	1-7-2013	83	1	4	80
Fghal, Jbeil Co.	5-7-2013	50	9	4	31
Aisheye, Jezzine Co.	30-7-2013	23	1	0	0
Beit Mery, Al Metn Co.	30-7-2013	100	23	14	38
Beit Mery, Al Metn Co.	30-7-2013	120	15	11	42
Beit Mery, Al Metn Co.	30-7-2013	100	24	33	58

Sample location	Date of collection	No. of flower heads	No. of emerged *T. lauta*	No. of emerged *Pteromalus*	Percent parasitism*
Beit Mery, Al Metn Co.	30-7-2013	80	12	19	61
Fghal, Jbeil Co.	13-8-2013	20	14	0	0
Fghal, Jbeil Co.	22-8-2013	80	16	0	0
Fghal, Jbeil Co.	23-8-2013	50	6	0	0
Dedde, Al Koura Co.	25-8-2013	38	2	2	50

*Percent parasitism was calculated as total number of emerged *Pteromalus* divided by total number of emerged *T. lauta* plus *Pteromalus* then multiplied by 100.

H. **Ovarian Maturation**

To test whether *T. lauta* females need to feed and accumulate proteins before maturing eggs, females were placed on different diets, then taken and dissected at different days after emergence to check for the presence of mature eggs.

The effects on a carbohydrate (water and honey) and protein (water, honey and yeast hydrolysate) diets on ovarian maturation are summarized in Table 3.10. Honey which was included in both diets is made up of high proportion of sugars, sucrose, water and traces of proteins (Chapman, 1982). It was mainly a source of carbohydrates.

In all treatments, newly emerged *T. lauta* females (days 0 and 1) did not show any mature eggs. Therefore, the females do not transfer proteins from the larval stage and do not have mature ovaries upon emergence. They need to feed to attain reproductive maturity.

When females were kept on a carbohydrate diet (honey and water), they showed mature eggs in their ovaries starting day twenty, while females kept on a protein diet (yeast hydrolysate diet) developed mature eggs much earlier, starting day 7 after emergence. The carbohydrate diet supported some egg production in *T.lauta* females after a long pre-oviposition period. On the other hand, all females kept on the yeast hydrolysate diet developed mature eggs after a pre-oviposition period of 7 days. Therefore, proteins are essential for *T. lauta* to have mature ovaries, and a protein diet shortens the pre-oviposition period of adult females.

Table 3.10. Pre-oviposition period, ovarian maturation and size of *Tephritomyia lauta* females kept on a honey and water diet (diet H) or on a yeast hydrolysate diet (diet Y).

Days after emergence	Diet H		Diet Y	
	Length of ovary: mean ± SE (range)	Width of ovary: mean ± SE (range)	Length of ovary: mean ± SE (range)	Width of ovary: mean ± SE (range)
0	0.60 ± 0.01 (0.59-0.65)	0.20 ± 0.01 (0.17-0.24)	0.60 ± 0.02 (0.53-0.65)	0.18 ± 0.00 (0.18-0.18)
1	0.40 ± 0.01 (0.41-0.47)	0.13 ± 0.01 (0.12-0.15)	0.47 ± 0.00 (0.47-0.47)	0.15± 0.00 (0.15-0.15)
3	0.70 ± 0.01 (0.59-0.77)	0.30 ± 0.01 (0.21-0.35)	0.90 ± 0.05 (0.71-1.18)	0.01 ± 0.02 (0.24-0.47)
5	0.60 ± 0.02 (0.53-0.71)	0.30 ± 0.02 (0.24-0.35)	0.70 ± 0.01 (0.65-0.77)	0.30 ± 0.06 (0.24-0.30)
7	0.70 ± 0.01 (0.71-0.77)	0.30 ± 0.01 (0.30-0.35)	**1.00 ± 0.09** (0.71-1.53)	**0.40 ± 0.05** (0.30-0.71)
10	0.80 ± 0.03 (0.71-0.94)	0.30 ± 0.03 (0.24-0.41)	**0.70± 0.01*** (0.65-0.71)	**0.50 ± 0.02*** (0.41-0.53)
15	0.80 ± 0.01 (0.71-0.83)	0.30 ± 0.01 (0.30-0.35)	**1.30 ± 0.09** (1.18-1.48)	**0.70 ± 0.07** (0.53-0.77)
20	**1.40 ± 0.1** (1.18-1.53)	**0.50 ± 0.1** (0.24-0.71)	**1.40 ± 0.03** (1.30-1.42)	**0.70 ± 0.05** (0.59-0.89)

In bold are marked mature ovaries; *mature ovaries but with resorbed eggs

Table 3. 11 summarize the average size of eggs found in mature ovaries of females kept on the two different diets. Dissection of mature *T. lauta* females feeding on water and honey at day 20 after emergence showed 6 eggs (n = 4 ovaries) in their ovaries. On the other hand, females feeding on yeast hydrolysate diet, which started having mature ovaries from day 7 and on after emergence, had 3-10 eggs/ovary (mean number of eggs/ovary: 5.8 ± 0.8; n = 12 ovaries).

Table 3.11. Egg size of *Tephritomyia lauta* females with honey and water diet (diet H) or with yeast hydrolysate diet (diet Y).

Days after emergence	Diet H			Diet Y		
	Length of egg: mean ± SE (range)	Width of egg: mean ± SE (range)	proportion of females with mature ovaries	Length of egg: mean ± SE (range)	Width of egg: mean ± SE (range)	proportion of females with mature ovaries
0	-	-	-	-	-	-
1	-	-	-	-	-	-
3	-	-	-	-	-	-
5	-	-	-	-	-	-
7	-	-	-	1.15 ±0.01 (0.1-1.19) n = 5	0.30 ±0.01 (0.26-0.31) n = 5	1/5
10	-	-	-	Eggs resorbed	Eggs resorbed	3/3 but resorbed eggs
15	-	-	-	1.20 ±0.02 (1.06-1.23) n = 11	0.25 ±0.01 (0.22-0.26) n = 11	2/2
20	1.70 ±0.20 (1.12-1.90) n= 4	0.40 ±0.04 (0.27-0.44) n= 4	2/2	1.20 ±0.02 (1.10-1.28) n = 11	0.25 ±0.01 (0.22-0.31) n = 11	3/3

Similar to *T. lauta*, newly emerged females of *T. nigricornis* and *T. bisetosa* are not sexually mature. They must feed on a protein hydrolysate diet to maturate eggs in their ovaries. Protein hydrolysate diet contains vitamins and peptides required for ovarian maturation of fruit flies (Fytizas 1973). An extrinsic source of proteins is significant for egg production by *Bactrocera tyroni* as carbohydrate diets alone did not support egg production in these species (Drew 1987), *Rhagoletis completa* (Tsiropoulos 1978) and *R. pomonella* (Hendrichs et al. 1993). However, females of *Anastrepha serpentina* and *Ceratitis capitata* produce a small number of eggs when kept on a carbohydrate diet. The females of *Bactrocera oleae* and some other species can attain a low fertility when fed a sucrose diet (Tsiropoulos 1977).

The pre-oviposition period of *T. lauta* kept on a carbohydrate diet (honey + water) is similar to the pre-oviposition period for *T. nigricornis* and *T. bisetosa* (20 days) (Knio et al. 2007a); but longer than reported for *C. capitata* (4 days).

I. Molecular Studies on Adult Flies

Table 3.12 summarizes the number of sequences obtained per plant host and per location.

Table 3.12. Number of flies whose ND1 gene fragments were sequenced per host plant.

Location	Host	ND1 Gene
Sour	***Echinops gaillardotii***	**3**
Baalback	***Echinops viscosus***	**3**
Khaldeh	***Echinops gaillardotii***	**2**
Jezzine	***Echinops gaillardotii***	**6**
Faraya	***Echinops viscosus***	**2**
Jbeil	***Echinops gaillardotii***	**4**
Hammana	***Echinops viscosus***	**2**
Ehmej	***Echinops viscosus***	**3**
Kfar-Nabrakh	***Echinops viscosus***	**3**
Ain Qni	***Echinops viscosus***	**1**
Beit-meri	***Echinops gaillardotii***	**2**
TOTAL		**31**

1. DNA Sequences of the Mitochondrial ND1 Gene Region of Echinops viscosus associated T. lauta and Echinops gaillardotii associated T. lauta

In order to examine possible genetic differences between the two populations, the mtDNA of 31 flies (14 from *E. viscosus* and 17 from *E. gaillardotii*) was extracted and the ND1 gene was amplified and sequenced from 4 elevations across Lebanon (0-500m, 500-1000m, >1000m and Beqaa region). The obtained sequences were aligned by hand to the *Drosophila melanogaster* mitochondrial DNA (RefSeq NC_001709). A region of 680 bp (correponding to RefSeq NC_001709 11728-12408) could be read from 20 specimens. The sequences of the two host-associated populations, presented in Fig.3.2, were identical when compared using Clustal Omega program (Goujon et al., 2010). The results suggest adults *T. lauta* emerging from both hosts are identical. More recently, more flies were reared from new locations (Ehmej, Jbeil Co. and Beit Meri, Metn Co., Mount Lebanon; Nabi Sheet, Baalback Co., Beqaa) and their ND1 gene was sequenced. Preliminary analysis of this data suggests some intraspecific variation. Further work will be needed to assess the true extent of gene flow among different populations of this fly.

GGGAGCAACTCAATAAATAGGAAAGAACAAAACTTAATTTTAAATAAAAA

ATTAAATTAAATAAATCACAACCTAAAAAAATTACTCTAAATAATATTCT

CATAAATAAAATTCTTGAATATTCAGCTATAAAAATCAAAGCAAAACCTC

CTCTTCTATATTCAACATTAAATCCAGAAACTAATTCAGATTCACCTTCA

GCAAAATCAAAAGGAGTACGATTTGTTTCAGCTAAACAAATACAAAATCA

AACTAATATAAGAGGAAAAAAAATAATTAAAAATCAAAAAAATTTTTGAT

AATAATAAAAATCTATTATATTATATCTTCCAATTAAAAAAACAAAAGAT

AATAAAATTAAAGCTATTCTAACTTCATAAGAAATTGTTTGAGCAACAGA

ACGTAATCCTCCTAATAAAGAATAATTTGAATTAGAAGACCAACCAGCAA

TTATAACAGTATAAACTCCTAAACTTGTACATCTTAAAAAAAACAACAAT

CTTAAATTAAAAGAATATAATTTTACAAAAAAAGGTATACATAATCAAAC

AACTAAAGAAAAAAATAAAGAAAAAACAGGAGAAAAATAATAAGAAATAT

AATTTGACAATAAAGGATAAGTTTGTTCCTTAGAAAATTTTAATTTGGTA

CCAAAAGGGGGGGGGGGGGCTATTTTTTT

Fig. 3.5 *Tephritomyia lauta* ND1 region

2. DNA Sequences of the Mitochondrial COXI, COXII and ND6 Gene Region of Echinops viscosus associated T. lauta and Echinops gaillardotii associated T. lauta

The amplification of other genes such as *COXI*, *COXII* and *ND6* using primers from the literature (Smith et al., 2003; Barr and McPheron, 2006) was attempted repetitively using whole genome DNA extracted from adult *T. lauta* flies emerging from both hosts. No PCR products were obtained, suggesting that *T. lauta* has mutations at the primer binding site of the three loci, which necessitates primer design for these regions.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Tephritomyia lauta is a flower head infesting tephritid that is specialist on Echinops species. The biology, ecology, and morphology of the immature stages of *T. lauta* are poorly described in the literature compared to other species of fruit flies.

This study revealed that the immature and adult stages of *T. lauta* emerging from *Echinops viscosus* and *E. gaillardotii* cannot be differentiated morphologically. Morphometric studies revealed that the third instar larvae and pupae associated with *E. viscosus* are significantly larger, reflecting the larger size of the exploited host plant. The study describes the life cycle of *T. lauta* including resource utilization and feeding behavior. It reveals for the first time how *T. lauta* larvae feed and how they are concealed in individual achenes, which do not show any sign of damage unlike other flower head tephritids species. The ovipositor tip of the females was similar in morphology and size between the two host associated populations although the flower heads of *E. viscosus* were larger. This may be attributed to the fact that regardless the size of the flower head, the florets were rather lax in the globular head and therefore easily accessible to the flies. Therefore this did not place any selective pressure on the shape of the ovipositor's tip. Eggs were deposited in the open bud stage and early bloom stages of the flower heads. The aculeus tip was narrow, pointed but not sharp, and not suited for piercing the plant tissues.

The eggs were carefully placed vertically on the inner bracts covering the seeds without piercing the host tissues.

Comparative morphometric studies conducted on *T. lauta* adults emerging from both *Echinops* spp. and using 2 head and 4 wing measurements showed significant differences in the length of the distal vein (Ldm), the second radial vein (R_{2+3}) and the third radial vein (R_{4+5}) between the two host associated populations. This shows that like the larval stages, adults emerging from the relatively larger flower heads of *E. viscosus* were bigger in size. Similarly, the body size of many non-frugivorous tephritids is positively correlated with the size of the flower head of the host plant (Zwölfer 1988).

Females *T. lauta* proved that they need an extrinsic source of proteins to mature their ovaries and produce eggs when kept on different diets. The same case was observed in R. *pomonella* (Hendriches et al. 1993), *R. completa* (Tsiropoulos 1978) where carbohydrate diets were not enough to lead to egg production. Diets contained different proteins and carbohydrates ratios, because the main components of a diet needed for development are proteins and carbohydrates. Insufficient amounts of amino acids in a diet might affect development and fitness of female fruit flies (Chapman & Nash, 2014). The fecundity assay showed that females feeding on a protein diet matured their ovaries earlier than females feeding on a carbohydrate diet only and produced larger number of eggs. Females kept on a protein diet had eggs in their ovaries at day 7 after emergence while females kept on a carbohydrate diet showed eggs in their ovaries at day 20 after their emergence. Moreover, diets with low protein quantity had significant effects on larval development in medfly larvae (Chapman & Nash, 2014).

Molecular studies using sequencing of a mitochondrial gene (ND1) revealed no differences in the sequences between the adults emerging from both *Echinops* spp. The results were expected, because when two host plants belong to the same family, even same genus then, the hosts associated with them are not expected to show high genetic variations (Craig et al. 1993), which is the case of *Echinops viscosus* and *Echinops gaillardotii*.

In conclusion, we have described the full biology and ecology of *Tephritomyia lauta* in Lebanon and described its courtship and mating behavior. More studies at the molecular level are needed to elucidate the low level of intraspecific genetic variation. This may be achieved by sequencing more genes and more individuals reared from different species of *Echinops* at different elevations. More sampling should specifically be done in the northern part of the Bekaa Valley and the extreme South and North of the country.

To detect ultra-microscopic differences among immatures and among ovipositor tips of female flies from different host plants, scanning electron micrographs will be needed.

Starch gel electrophoresis of isozymes, complemented with AFLP or microsatellite analysis should be undertaken to assess the extent of gene flow between different populations of *T. lauta* populations in Lebanon.

BIBLIOGRAPHY

Barr, N. B., & McPheron, B. A. (2006). Molecular phylogenetics of the genus Ceratitis (Diptera: Tephritidae). *Molecular phylogenetics and evolution*, *38*(1), 216-230.

Bateman, M.A. 1972. The Ecology of Fruit Flies. *Annual Review of Entomology* 17, no. 1: 493-518.

Bush, G.L. 1969. Sympatric Host Race Formation and Speciation in Frugivorous Flies of the Genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* 23, no. 2: 237-251.

Chapman, R.F. 1982. *The insects: structure and function*, Third edition, Harvard University Press, Cambridge. 919 pp.

Chapman, T. and W. J. Nash, 2014. Effect of Dietary Components on Larval Life History Characteristics in the Medfly (*Ceratitis capitata*: Diptera,Tephritidae). *Plos One* 9, no. 1: 1-9: e86029. doi:10.137.

Christenson, L.D. and R. H. Foote. 1960. Biology of Fruit Flies. *Annual Review of Entomology* 5, no. 1: 171-192.

Craig, T.P., J.K. Itami, W.G. Abrahamson and J.D. Horner. 1993. Behavioral Evidence for Host-Race Formation in *Eurosta solidaginis*. *Evolution* 47, no. 6: 1696-1710.

Drew, R.A.I. 1987. Behavioural strategies of fruit flies of the Genus *Dacus* (Diptera, Tephritidae) singificant in mating and host-plant relationships. *Bulletin of entomological research* 77, no. 1: 73-81.

Edgecombe, W. S. 1970. *Weeds of Lebanon*, American University of Beirut, Beirut, 447 pp.

Fletcher, B.S. 1987. The Biology of Dacine Fruit Flies. *Annual Review of Entomology* 32, no. 1: 115-144.

Fletcher, B.S. 1989. Life history strategies of tephritid fruit flies. In: A.S. Robinson and G. Hooper, *Fruit Flies: Their Biology, Natural Enemies and Control*, Volume 3B, Elsevier Science Publishers, Amsterdam, pp: 195-208.

Foote, R.H., F.L. Blanc and A.L. Norrbom. 1993. *Handbook of the fruit flies (Diptera: Tephritidae) of America North of Mexico*. Cornell University Press, Ithaca. 571 pp.

Freidberg, A. 1974. Tephritidae (Diptera) from Mt. Hermon. *Israel Journal of Entomology*. 9: 133-39.

Freidberg, A. 1981. Mating behaviour of *Schistopterum moebiusi* Becker (Diptera: Tephritidae). *Israel Journal of Entomology* 15: 89-95.

Freidberg, A. and J. Kugler. 1989. *Fauna Palaestina, Insecta IV- Diptera: Tephritidae*, Israel Academy of Sciences and Humanities, Jerusalem, 212 pp.

Fytizas, E. 1973. Influence d'une seule administration de levure hydrolysée sur la fécondité des femelles de Dacus oleae Gmel. (Diptera : Trypetidae). *Annales de la Zoologie-Ecologie Animale* 5 : 39-44.

Goeden, R.D. 1985. Host-plant relations of *Trupanea* spp. (Diptera: Tephritidae) in Southern California. *Proceedings of the Entomological Society of Washington* 87, no. 3: 564-571.

Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J, Lopez R. 2010. A new bioinformatics analysis tools framework at EMBL-EBI**.** *Nucleic acids research 2010 Jul, 38 Suppl: W695-9* doi:10.1093/nar/gkq313

Hartley, S.E. and J.H. Lawton. 1992. Host-Plant Manipulation by Gall-Insects: A Test of the Nutrition Hypothesis. *The Journal of Animal Ecology* 61, no. 1: 113-119.

Headrick, D.H. and R.D. Goeden. 1998. The biology of nonfrugivorous tephritid fruit flies. *Annual Review of Entomology* 43: 217-241.

Hendrichs, J., C.R. Lauzon, S.S. Cooley and R.J. Prokopy. 1993. Contribution of natural food sources to adult longevity and fecundity of *Rhagoletis pomonella*(Diptera: Tephritidae). *Annals of the Entomological Society of America* 86, no. 3: 250-264.

Knio, K.M., S.H. Kalash and I.M. White. 2002. Flowerhead-infesting fruit flies (Diptera: Tephritidae) on thistles (Asteraceae), in Lebanon. *Journal of Natural History* 36, no. 5: 617-629.

Knio, K.M., R.D. Goeden and D.H. Headrick. 2007a. Fecundity and longevity of the sibling and sympatric species, *Trupanea nigricornis* (Coquillett), a polyphage, and the the narrowly oligophagous *T. bisetosa* (Coquillett) (Diptera: Tephritidae). *Proceedings of the Entomological Society of Washington* 109, no. 3: 579–595.

Knio, K.M., R.D. Goeden and D.H. Headrick. 2007b. Natural enemies of the cryptic and sympatric species, *Trupanea nigricornis* (Coquillett), a polyphage, and the narrowly oligophagous *T. bisetosa* (Coquillett) (Diptera: Tephritidae). *Proceedings of the Entomological Society of Washington* 109, no. 1: 187-197.

Korneyev, V. and Dirlbek, J. The Fruit Flies (Diptera: Tephritidae) of Syria, Jordan and Iraq. 2000. *Studia Dipterologica*. 7. no. 2: 463-482

Neuenschwander, P. and A Freidberg. 1983. The fruit flies of Crete (Diptera: Tephritidae). *Israel Journal of Entomology* 17: 81-94.

Phillips, V.T. 1946. The biology and identification of trypetid larvae (Diptera: Tephritidae). *Memoirs of the Entomological Society.* Number 12.

Post, G. E. 1932. *Flora of Syria, Palestine and Sinai*, Volume II, American University of Beirut, Beirut, 928pp.

Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins D. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega**.** *Molecular Systems Biology 7 Article number: 539* doi:10.1038/msb.2011.75

Smith, P.T., B.A. Mcpheron and S. Kambhampati. 2002. Phylogenetic Analysis of Mitochondrial DNA Supports the Monophyly of Dacini Fruit Flies (Diptera: Tephritidae). *Annals of the Entomological Society of America* 95, no. 6: 658-664

Smith, P. T., Kambhampati, S., & Armstrong, K. A. (2003). Phylogenetic relationships among Bactrocera species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, *26*(1), 8-17.

Tohmé, G., & Tohmé, H. S. (2007). *Illustrated flora of Lebanon*. Beirut: National Council for Scientific Research.

Tsiropoulos, G.J. 1977. Survival and reproduction of Dacus oleae (Gmelin) fed on chemically defined diets. *Zeitschrift für Agewandte Entomologie* 84: 192-197.

Tsiropoulos, G.J. 1978. Holidic diets and nutritional requirements for survival and reproduction of the walnut husk fly. *Journal of Insect Physiology* 24: 239-242.

White, I.M. 1988. Tephritid Flies (Diptera: Tephritidae): *Handbook for the Identification of British Insects*, Volume 10 (5a), Royal Entomological Society of London, London, 134 pp.

Zwölfer, H. 1972. Investigations on *Chaetorellia sp*. associated with *C. solstitialis*. In: Weed project for the University of California, Division of Biological Control. Delémont, Switzerland: Commonwealth Institute of Biological Control. Report nr 7. 21 pp.

Zwölfer, H. 1982. Patterns and driving forces in the evolution of plant-insect systems. *Proceedings of the Fifth International Symposium in Insect-Plant Relationships, Wageningen,* pp. 287-296.

Zwölfer, H. 1983. Life systems and strategies of resource exploitation in tephritids. In: R. Cavallero (editor). *Fruit flies of economic importance*: Proceedings of the CEC/IOBC International Symposium, Athens, Rotterdam, pp. 16-32.

Zwölfer, H. 1988. Evolutionary and ecological relationships among the insect fauna of thistles. *Annual Review of Entomology* 33: 103-122.

Zwölfer, H. and G.L. Bush. 1984. Sympatrische und parapatrische Artbildung. *Zeitschrift für Zoologische Systematik Evolutionsforschung* 22: 211-223.