Journal of Plant Diseases and Protection (2019) 126:55–66
https://doi.org/10.1007/s41348-018-0196-y

ORIGINAL ARTICLE

New findings on infestation and phenology of *Dasineura oleae* Angelini (Diptera, Cecidomyiidae): an emerging pest on olive trees in the Palestinian Territories

Yacoub A. Batta[1]

Received: 12 September 2018 / Accepted: 22 October 2018 / Published online: 27 October 2018
© Deutsche Phytomedizinische Gesellschaft 2018

Abstract

Dasineura oleae is a gall midge on leaves and branches of olive trees. Due to the scarcity of information regarding to the infestation and phenology of *D. oleae* on olive trees, the objectives of this research were: (1) to study the pest status of *D. oleae* and its damage on olive trees, (2) to describe the life stages: egg, larva, pupa and adult, (3) to determine the life cycle duration and number of generations per year and (4) to describe the egg laying, larval development, pupation and adult emergence. Results indicated that there was an outbreak of *D. oleae* in the Palestinian Territories where the average rate of infestation on olive trees was 51.46% and the damage caused by the insect on leaves, branches and inflorescence of infested trees reached at an average infestation rate of 35.88, 22.70 and 26.54%, respectively. Eggs of *D. oleae* were laid on the lower leaf surface, and the hatched larvae penetrated the tissues underneath and induced galls in which they live and develop until the emergence of adults. *D. oleae* developed one generation per year in the hilly regions of Palestinian Territories and two generations in the coastal regions of these territories. A full description of the life stages has been provided for the insect. In conclusion, this study provides with new information regarding to the pest status of *D. oleae*, infestation and development of the insect on the different organs of olive trees. These findings are necessary for the insect management and its control.

Keywords Olive leaf gall midge · *Olea europaea* · Life cycle duration · Egg laying · Pupation · Adult emergence · Number of generations per year

Introduction

Dasineura oleae Angelini 1831 is one of the gall midges that belong to family Cecidomyiidae and order Diptera. This family contains over 6000 species of gall midges described Worldwide and half of them occurs in the Palaearctic Region (Gagné and Jaschhof 2014; Haarder et al. 2016). *Dasineura oleae* is a Mediterranean species and known from several countries of the Mediterranean basin which cultivate olive trees. For examples, it is known from Israel (Rivnay 1962; Gerson and Harpaz 1968; Avidov and Harpaz 1969), Jordan (Al-Tamimi 1997), Montenegro (former Yugoslavia) (Hrncic 1998), Syria (Darvas et al. 2000), Turkey (Doganlar et al. 2011) and Greece (Simoglou et al. 2012; Perdikis et al. 2015). Although *D. oleae* is an old pest and known from many Med-countries, it was never a threat to olive production except in the last years in certain Med-regions. Also, the pest status of *D. oleae* was varied across the countries concerned since, in some Med-regions in 2010–2015, there was an outbreak of the pest, but it can be controlled by the native biocontrol agents such as native parasitoids in these regions such as Hatay Province in Turkey (Doganlar et al. 2011) and Crete in Greece (Perdikis et al. 2015). The level of parasitism by the native parasitoids on the larvae and pupae of *D. oleae* amounted to 66.2 and 89.1%, respectively, in both counties. To date, the outbreak of *D. oleae* population in many Med-countries was thus regulated by

✉ Yacoub A. Batta
yabatta@najah.edu

[1] Department of Plant Production and Protection, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus, West Bank, Palestine

native parasitoids and no chemical control measures were needed to be applied.

To date, there were no previous records on *D. oleae* infestation on olive trees in the Palestinian Territories. In general, the available information on the various aspects of *D. oleae* infestation and development on olive trees are so little since it is considered as an emerging pest on olive trees in these territories and in other Med-regions. For example, the number of generations per year developed by the insect was varied across the countries concerned, it may be one generation per year in Jordan and Turkey (Al-Tamimi 1997; Doganlar et al. 2011) or two generations per year in Syria (Darvas et al. 2000). Also, the time of emergence of adult flies is not well-defined where some researchers reported that they appear in March through May and the insect lays its eggs on young leaves of olive trees during that period (Al-Tamimi 1997; Darvas et al. 2000; Doganlar et al. 2011). Also, the pest status of *D. oleae* has not been fully assessed in many countries that are subjected to the infestation by the insect. Moreover, some of the biological aspects in life history of *D. oleae* on olive trees were not clearly investigated: Where the insect lays its eggs (on or inside the tissues)? Where the pupation takes place (on or inside the tissues)? And how adult insect emerges from pupa if pupation takes place inside the tissues? Also, the description of life stages including egg, larva, pupa and adult has not fully performed. Therefore, the purpose of the present research was to study the pest status of *D. oleae* and the damage caused by the insect on olive trees in the Palestinian Territories, in addition to study, in depth, the following aspects of infestation and phenology of *D. oleae* on olive trees: (1) to describe and measure the different life stages of *D. oleae* including eggs, larvae, pupae and adults as well as to provide with colored photographs for these stages, (2) to determine the life cycle duration and the number of generations per year developed by *D. oleae* under local conditions of olive production in the Palestinian Territories, (3) to evaluate its damage potential on leaves and inflorescence and (4) to investigate other biological aspects of *D. oleae* infestation on olive trees such as the place of egg laying (on or inside tissues), pupation of the insect (how and where it pupates), how the hatched larvae enter the plant tissues and induce galls, how adult flies emerge from pupa formed inside the galls, etc.

Materials and methods

Identification of the insect

Specimens of the insect (male and female adults preserved in 70% ethanol), in addition to infested leaves and branches of olive trees with *D. oleae*, were sent to Dr. Marcela Skuhrava (Czech Zoological Society) as a specialist in identification of gall midges belonging to the family Cecidomyiidae. The insect was then identified by Dr. Skuhrava as *Dasineura oleae* Angelini, 1831 (family Cecidomyiidae, order Diptera). Voucher specimens of the insect were deposited in the collection of Czech Zoological Society, Vinicna 7, 128 00 Praha 2, Czech Republic.

Collection of samples and life stages of the insect

During a period that extended over three successive years (2016–2018), samples of olive leaves, young branches and inflorescence that have shown infestation with *D. oleae* were collected from different localities of olive orchards in the northern part of the Palestinian Territories. Collected samples were characterized by having typical galls of the insect (see Fig. 1, 1–5) with variable number of galls according to the organ collected. The description of galls including shape, length, number of larvae hosted by gall chamber as well as the number of samples collected per inspected orchard and the time of collection is indicated in the different paragraphs of the study depending on the collection purpose. Localities involved in the collection of samples have the following coordinates: 32°11′52.5552″N, 35°17′6.072″E (Nablus area); 32°4′34.2228″N, 35°11′30.1596″E (Sulfeet area); 32°31′25″N, 35°02′11.11″E (Tulkarm area); 32°19′9.5844″N, 35°22′39.5508″E (Tobas area); 32°29′45.2688″N, 35°18′57.3696″E (Jenin area); and 32°11′54.8916″N, 34°58′25.392″E (Qalquilia area). The collected samples from these areas were used for studying the pest status of the insect and the damage caused on olive trees and other vegetative organs as well as the different aspects of the insect infestation and development under the local conditions of olive production. The galls induced by *D. oleae* in the collected samples were dissected to extract the immature stages of the insect that might be larva or pupa. The extracted larvae and pupae, in addition to adults emerged from galls were preserved in 70% ethanol for being morphologically described and photographed.

Pest status of the insect in the study area

A survey regarding to the level of infestation by insect on olive trees grown in the northern part of the Palestinian Territories was carried out during the time period extended from 15 March, 2017 to 15 April, 2017. The main objective was to assess the pest status and extent of damage that may be caused by *D. oleae* to infested olive trees. The survey includes the following steps and procedures: (1) taking random samples of infested trees as well as leaves, branches and stalks of flowers (inflorescence) in different localities within an extended area of olive orchards in six

Palestinian governorates having the following coordinates: Nablus (32°11′52.5552″N, 35°17′6.072″E); Sulfeet (32°4′34.2228″N, 35°11′30.1596″E); Tulkarm (32°31′25″N, 35°02′11.11″E); Tobas (32°19′9.5844″N, 35°22′39.5508″E); Jenin (32°29′45.2688″N, 35°18′57.3696″E); and Qalquilia (32°11′54.8916″N, 34°58′25.392″E); (2) counting the number of infested trees per 100 randomly chosen trees per locality/governorate as well as the number of infested leaves, branches and inflorescence per 100 randomly chosen organ per locality/governorate, four replicates (samples) were run per locality. We should observe that the tree was considered as infested if it had either one or more of its organs (leaves or branches or inflorescence) infested with one or more of typical galls of *D. oleae* (see Fig. 1, 1–5 for gall description); (3) calculating the percentage of infestation with the insect on the sampled trees as well as on the different vegetative organs of olive trees in the different localities and governorates, then comparing them after they have been statistically analyzed. For example, if the sample taken was an infested tree with leaves appearing *D. oleae* galls, then on which 100 leaves were randomly inspected in situ and found that 10 of them were infested with galls of *D. oleae*, the percentage of infestation was thus 10% and so forth for the other vegetative organs. Therefore, we applied the above procedure on randomly chosen olive orchards in 25 localities distributed over the survey areas in northern Palestinian Territories. For each locality, the infested trees, leaves, branches and flower stalks (inflorescence) were randomly chosen for calculating the percentage of infestation and four orchards (replicates) were used per locality. The survey was conducted during a period of 1 month (from 15 March, 2017 to 15 April, 2017). The olive cultivar and the altitude of each locality were also recorded during the survey.

Studying the various aspects of infestation and development of the insect

During three successive years (2016–2018), the various biological aspects of *D. oleae* were studied according to the following procedure: (1) collecting samples of young leaves, branches and inflorescence from olive orchards infested with *D. oleae*. The collection date started from early spring 2016 to late autumn 2017, this date should coincide with the presence of the adult insect and its immature stages throughout the year, then (2) inspecting the collected samples to check the presence of eggs (either on or in plant tissues of infested organs) using a dissecting microscope, then (3) dissecting the galls in the collected samples to check the presence of larvae and pupae of *D. oleae* using a dissecting microscope, (4) describing and photographing the different life stages of the insect (eggs, larvae, pupae and adults) in and on the collected organs, then following up the development of immature stages until the adult emergence, (5) following up the development of larvae (from first to third larval instar), then describing and photographing the instars developed inside the gall. Therefore, a detailed description and photographing of the life stages of *D. oleae* including the egg stage, larval instars, pupal and the adult stages was performed in the present study. Also, measuring the dimensions of these stages was done and the mean body length of these stages was calculated.

Studying the life cycle duration of the insect and number of generations per year

The determination of the life cycle duration (from egg laying to adult emergence) and the number of generations developed by *Dasineura oleae* per year was done in two separate localities in the survey area during a period of 1 year (from March, 15 2016 to March 30, 2017). The localities used during the study were: Aljarochia in Tulkarm governorate (32°31′25″N, 35°02′11.11″E) that represents the coastal region of olive production (olive cultivar Nabali-Mohassan, location at 120 m above sea level) and Seilet El-Dahr in Jenin governorate (32°29′45.2688″N, 35°18′57.3696″E) that represents the hilly region of olive production (olive cultivar Nabali, location at 503 m above sea level). In each locality, four separate olive orchards (No. 1–4, Table 2) were chosen for measuring the life cycle duration and number of generations per year according to the following method: one pair of newly emerged adults of *D. oleae* were put into small cage (3.5 cm diameter x 3.0 cm height) fixed onto healthy non-infested young olive leaves attached to living trees of the tested cultivars (Fig. 1, 9). Each cage consisted of two identical parts (top and bottom) which were made of transparent fiberglass with spongy margins to ensure a perfect fit when the two parts are assembled (Fig. 1, 8). Each part had a dish shape with a circular diameter of 3.5 cm and a height of 1.5 cm. The lower side of each part consisted of a tightly fixed fine muslin mesh to allow good aeration of the chamber when the leaf is confined with insects. The cages were fixed onto the leaves using a clamp so that the insects could access the confined part of the leaf (Fig. 1, 8). For each olive orchard in each locality, we used four young leaves together with one pair of *D. oleae* adults each. After egg laying by the confined females, the leaves were labeled and then checked regularly. The time passed from egg laying to the adult emergence was recorded to determine the life cycle duration and thus the number of generations per year developed by the insect.

1
2
3
4
5
6
7
8
9

◀**Fig. 1** Damage caused by *Dasineura oleae* on olive trees (*Olea europaea*), **1**–**2**. Galls induced by *D. oleae* on olive leaves (upper and lower views of galls on the infested leaves) (Scale bar = 5 mm each), **3**. Infestation with *D. oleae* on young olive branch (gall formation on young branch and leaves attached to it) (Scale bar = 5 mm), **4**. Heavy attack of *D. oleae* on the olive inflorescence (galls can be seen on the stem of the inflorescence and stalks of young fruits) (Scale bar = 5 mm), **5**. Enlarged view of galls formed on stalks of early set fruits and the inflorescence stem (Scale bar = 3 mm), **6**. Internal view of galls induced by *D. oleae* on young olive leaves or branches where the gall chamber contains one larva each (Scale bar = 500 µm), **7**. Opened gall in Fig. 5 showing young larva inside (one larva per gall chamber) (Scale bar = 1 mm), **8**. Main parts of the cage used for confining *D. oleae* adults (male and female) to olive leaves during the study of life cycle duration and number of generation per year (from left to right: top part, bottom part and clamp), **9**. Assembled cage that confines one pair of *D. oleae* adults and a part of olive leaf attaching to a living tree for conducting the study of life cycle duration and number of generation per year

Statistical analysis

The data collected during the survey were statistically analyzed by first calculating the mean % of infestation with the insect on olive trees in the surveyed areas. The same type of calculation was done on the leaves, branches and the inflorescence of infested trees in each locality/governorate. Analysis of variance (ANOVA) test and means' separation by Tukey's HSD test were run to determine whether there were significant differences in the infestation rates on the different vegetative organs of infested olive trees distributed over the localities/governorates of the surveyed areas, as well as to test whether there were significant differences in the infestation rates on vegetative organs in the different governorates of the survey areas.

Results

Pest status and infestation level with *Dasineura oleae* on olive trees and vegetative organs

The extent of damage and infestation rate with *D. oleae* on olive trees and vegetative organs of infested trees were assessed in the survey area. The global infestation rate with the insect on olive trees grown in the localities and governorates included in the survey was averaged to 51.46% with the highest infestation rate reached at 100% (Table 1). This would give a good indication on the outbreak of the insect in the survey region. Moreover, statistical analysis of the data obtained during the survey indicated that there were significant differences ($P = 0.05$) between the average mean % of infested leaves, branches and stalks of flowers of olive orchards in the region with the highest mean % of infestation on leaves (35.88%) compared to branches and stalks of flowers (22.70 and 26.54%, respectively) (Table 1). Similarly, significant differences ($P = 0.05$) were obtained between the average mean % of infested vegetative organs in the governorates included in the survey with the highest mean % of infestation in Sulfeet governorate (41.55%) and the lowest mean % of infestation in Nablus governorate (13.37%) (Table 1). However, there were no significant differences between the mean % of infestation in Sulfeet governorate and that of Tulkarm governorate (41.55% vs. 35.14%). The average mean % of infested vegetative organs of olive trees in other governorates was intermediate (28.63% in Qalquilia governorate; 22.22% in Tubas governorate and 18.87% in Jenin governorate) (Table 1). Therefore, all of the above-mentioned elements that were used for evaluation of infestation level with the insect on olive trees in the survey area confirmed the outbreak of *D. oleae* in the area and its pest status. It is noteworthy to mention that the infestation rate with *D. oleae* in the survey area was higher in the coastal region of olive production than that in the hilly region (35.14% in Tulkarm governorate with 120–310 m above sea level vs. 13.37% in Nablus governorate with 345–605 m above sea level) (Table 1).

Description of infestation and damage caused by *Dasineura oleae* on olive trees

The survey conducted on pest status of *D. oleae* indicated that the insect caused a serious damage to infested trees grown in the olive orchards in the northern part of the Palestinian Territories. This damage could be observed by inducing galls characteristic to the insect on young leaves (Fig. 1, 1–2). These galls had a mean length of 5.37 mm long and 2.85 mm wide (range: 5–6 and 2–3 mm, respectively, $n = 50$ specimens). Galls observed on young branches of infested trees were larger in size and more extensive than that observed on leaves (Fig. 1, 3). Almost similar type of galls was observed on the inflorescence of infested trees (Fig. 1, 4) where the stems of flowers and/or stalks of early set fruits are attacked by the insect by exhibiting large and extensive galls on these organs (Fig. 1, 5). Only one larva per gall chamber was observed when the galls on these organs were dissected under the dissecting microscope (Fig. 1, 6–7). Moreover, the infested leaves may drop prematurely and the infested branches may dry and then die during the growing season so there were no new leaves that can be developed during the growing season. Also, yield reduction as a result of infestation on the inflorescence may take place. This could be attributed to the failure of infested flowers to produce healthy, large fruits due to the attack of the insect on the stem of flowers and fruit stalk (Fig. 1, 4–5). This attack may cause redirection of the nutrient flow to the galls instead of

Table 1 Infestation rates with *Dasineura oleae* on leaves, branches and stalks of flowers of *Olea europaea* trees in 25 localities distributed in 6 governorates of northern West Bank of Palestinian Territories (data collected during the period 1 to July 15, 2017 and repeated during the same period in 2018)

Name of the locality/governorate in the survey areas	Altitude above sea level (in meters)/olive cultivar in the survey areas	Mean % of infested trees per locality/governorate (n = 100 samples, 4 repl.)	Mean % of infestation on vegetative organs of infested trees in the survey areas*			
			Mean % infested leaves/ infested tree (n = 100 samples, 4 repl.)	Mean % infested branches/ infested tree (n = 100 samples, 4 repl.)	Mean % infested flower stalks/ infested tree (n = 100 samples, 4 repl.)	Average mean % of infested vegetative organs/governorate**
Hawoz region/Tubas	374 m/Nabali	30.5	36.5	2.5	5.5	22.22 ab
Tayaseer/Tubas	370 m/Nabali	54.0	57.5	6.5	34.5	
Elfar'a/Tubas	182 m/Nabali	46.0	32.5	21.0	3.5	
Bal'a/Tulkarm	420 m/Nabali	86.5	66.0	50.5	32.5	35.14 cd
Aljarochia/Tulkarm	120 m/Nabali-Mohassan	100	83.0	18.5	23.0	
Almasqofa/Tulkarm	162 m/Nabali-Mohassan	100	63.5	52.0	28.5	
Kafr Elabad/ Tulkarm	310 m/Nabali	100	55.0	49.5	27.0	
Dinabeh/Tulkarm	130 m/Nabali	60.0	37.5	29.0	74.5	
Ektaba/Tulkarm	182 m/Nabali	50.0	20.5	10.0	7.0	
Baqa Hatub/ Qalquilia	458 m/Nabali	60.5	37.5	29.0	74.5	28.63 bc
Jaioss/Qalquilia	250 m/Nabali	34.0	40.5	15.0	74.5	
Azzoun/Qalquilia	258 m/Nabali-Mohassan	30.0	21.5	26.0	27.5	
Jeensafoot/Qalquilia	410 m/Nabali	50.0	20.5	4.5	0	
Kafr Lqef/Qalquilia	333 m/Nabali	20.0	40.5	18.0	0	
Wadi kana/Sulfeet	236 m/Nabali-Mohassan	100	64.5	58.0	65.5	41.55 d
Garb Sulfeet/Sulfeet	367 m/Nabali	84.0	44.5	41.0	42.0	
Sharq Sulfeet/ Sulfeet	404 m/Nabali-Mohassan	64.0	14.5	10.5	33.5	
Assira Shamalieh/ Nablus	605 m/Nabali	14.5	5.0	2.5	0	13.37 a
Sarra/Nablus	429 m/Nabali	10.0	40.0	27.0	0	
Beit Eiba/Nablus	345 m/Nabali	20.0	15.5	2.0	25.0	
Jet/Nablus	455 m/Nabali	8.0	18.5	35.0	0	
Seilet El-Dahr/Jenin	503 m/Nabali	42.5	24.5	5.0	9.5	18.87 a
Marj Bin-Amer/ Jenin	354 m/Nabali-Mohassan	14.5	24.0	7.5	41.5	
Al-Izbeh/Jenin	250 m/Nabali	49.0	1.0	30.0	15.5	
Tel Ta'nak/Jenin	180 m/Nabali	58.5	32.5	17.0	18.5	
Average mean % of infested trees or vegetative organ*	–	51.46	35.88 B	22.70 A	26.54 A	–

Selection of period was attributed to the presence of maximum number of insect galls

*Means followed by the same letter are not significantly different at $P = 0.05$ in average mean % of infested trees per vegetative organ

**Means followed by the same letter are not significantly different at $P = 0.05$ in average mean % of infestation on vegetative organs per governorate

developing fruits. The infested tissues with the insect may eventually wither and die.

Egg laying and larval development of *Dasineura oleae*

We observed that the females of *D. oleae* laid their eggs on the lower leaf surface in a scattered form. The eggs were very small and can't be seen by the naked eye but under the dissecting microscope (mean length: 0.36 mm, range: 0.31–0.43 mm, n = 100 specimens). Eggs have an elongate shape with brownish to reddish color (Fig. 2, 10–11). After egg hatching into the first larval instar (Fig. 2, 12), the neonate larvae penetrated into the leaf tissues then secreted large amount of saliva in the feeding sites. This secretion induced gall formation around the penetrating larvae so the young gall was formed. The enclosed larva then developed into the subsequent stages inside the gall (2nd and 3rd instars, Fig. 2, 13–14) so the host gall enlarged and increased in size accordingly.

Description of the life stages of *Dasineura oleae*

The dissection of galls containing the insect collected from infested leaves, branches and stalks of flowers revealed the presence of three distinct larval instars as well as pupa as follows:

- *First larval instar* resulting from egg hatching, it was characterized by having yellow-bright color, barrel shape and vermiform-type. It had chewing mouthparts by which it can penetrate the tissues underneath and then induce gall around the penetrating larva. It had a small size with a mean body length of 0.55 mm (range 0.40–0.75 mm, n = 100 specimens) (Fig. 2, 12).
- *Second larval instar* had almost similar characteristics as in the first instar but it was bigger in size with a mean body length of 1.17 mm (range 1.05–1.38 mm, n = 100 specimens) (Fig. 2, 13).
- *Third larval instar* had bigger size in comparison with the previous instars (mean length of full-grown third larval instars: 2.62 mm, range 1.96–3.02 mm, n = 100 specimens) (Fig. 2, 14), it had almost similar characteristics as in the previous instars. However, before molting into pupa, the full-grown larva contracted to give what is called prepupa which had yellow color but with smaller size than the full-grown third larval instar.
- *Pupa* had reddish-yellow to light-red body color. It was covered by a thin cover called puparium through which we can see the head of the insect with dark-brown compound eyes and antennae (Fig. 2, 15–17). The pupa had a mean length of 1.88 mm (range 1.68–2.18 mm, n = 100 specimens). Moreover, on the anterior part of pupal head, there was a sharp pointed process protruding anteriorly (Fig. 2, 16). This process was used for exiting of the pupa from the host gall prior to adult emergence. Emergence of the adult took place outside the gall and the emerged adults live freely.

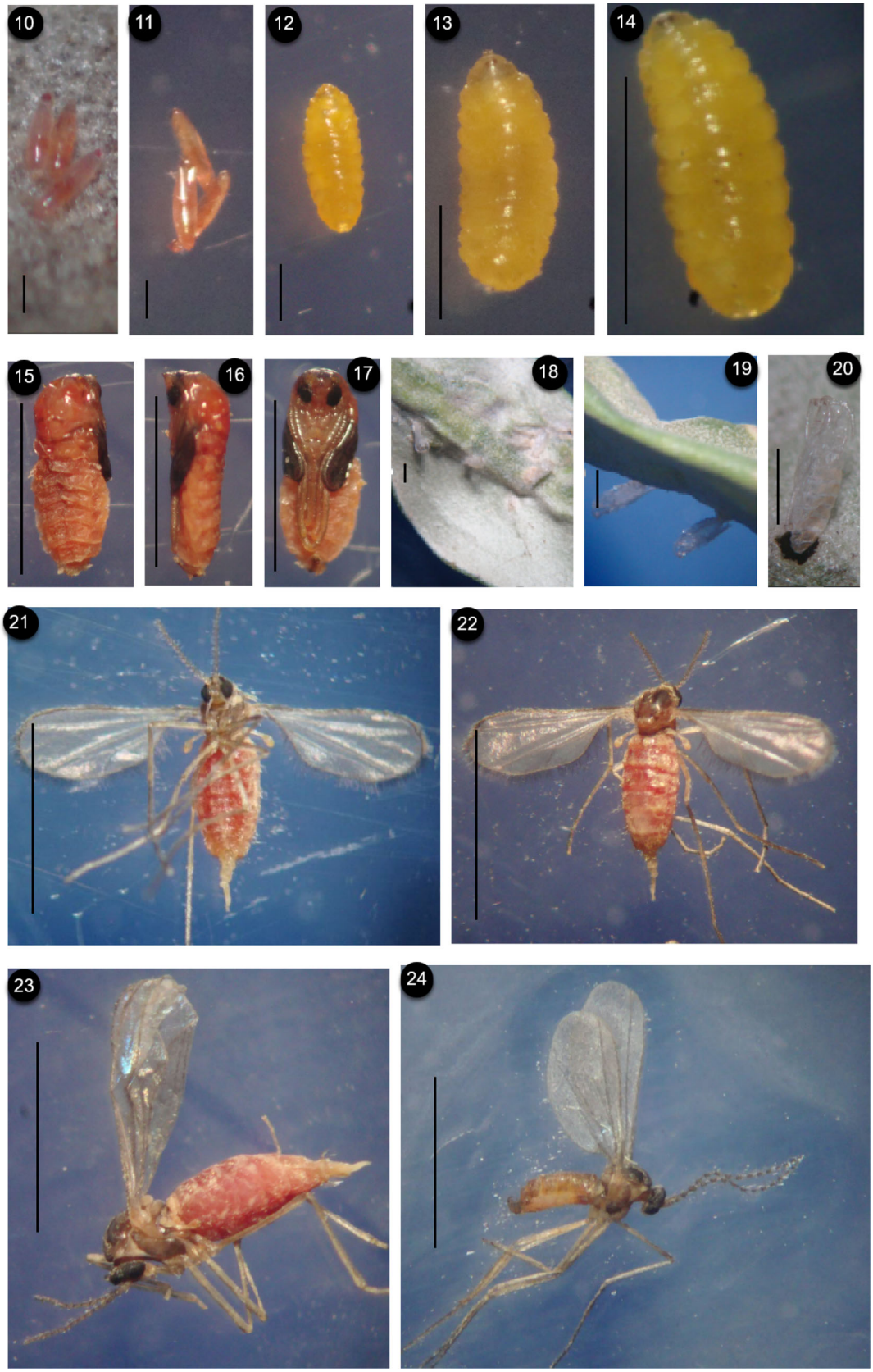
In addition to the above-mentioned life stages of *D. oleae*, there is an *adult stage* where the male and female adults were characterized by having three pairs of long legs as well as one pair of membranous wings which were slightly covered with scattered short black hairs with reduced wing venation (veins are light-brown in color). Also, they had one pair of antennae which were long, often moniliform (string-of-beads shape) but with whorls of long hair originating from each segment. In addition to that, female adults had characteristic reddish abdomen, brown thorax, dark-brown head with relatively large black compound eyes, they lived freely outside the gall (Fig. 2, 21–23). Also, the females had a prominent ovipositor appearing at the end their abdomen, whereas the males had an aedeagus at the end of abdomen (Fig. 2, 24). The mean body length for the males was 1.72 mm (range 1.55–1.98 mm, n = 100 specimens) and 1.93 mm (range 1.82–2.18 mm, n = 100 specimens) for the females.

Pupation and emergence of adults of *Dasineura oleae*

Pupation of the completely developed larvae of *D. oleae* took place in the host galls induced by the insect on the leaves and other vegetative organs of olive trees (Fig. 2, 15–17). Upon the dissection of galls, there was one larva or pupa per gall chamber. Adult emergence of *D. oleae* from pupa took place after exiting of the mature pupa from the host gall. Pupal exit was completed by making a small hole in the wall of gall chamber using the sharp pointed process existing on the anterior part of pupal head (Fig. 2, 16). After pupal exit from gall, the pupa remained attached to the leaf surface in an erect position (Fig. 2, 18–20) and it remained so even after the adult emergence from pupa leaving an empty skin called exuvia (Fig. 2, 20).

Life cycle duration of *Dasineura oleae* and number of generations per year

Under the Palestinian conditions of olive production (average annual temperature and relative humidity in the survey area were 16.6 °C and 52.8%, respectively, PMA 2017), *D. oleae* developed one generation per year in the hilly region (study area: Seilet El-Dahr in Jenin governorate located at 503 m above sea level; olive cultivar Nabali), whereas it developed two generations per year in the coastal region of olive production (study area:


10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

◀**Fig. 2** Life stages of *Dasineura oleae* on the different organs of olive trees, **10–11**. Egg stage (Scale bar = 150 µm each), **12**. First instar larva (Scale bar = 200 µm), **13**. Second instar larva (Scale bar = 600 µm), **14**. Third instar larva (Scale bar = 2 mm), **15–17**. Pupa formed inside the gall (dorsal, lateral and ventral views, respectively) (Scale bar = 1.5 mm each), 18. Pupae exited from gall chambers seen on the leaf surface (Scale bar = 2 mm), **19–20**. Empty pupal skins (exuviae) left behind on leaf surface after the emergence of adults from pupae (Scale bars = 2 and 1 mm, respectively), **21–23**. Adult female (ventral, dorsal and lateral views) with long ovipositor at end of abdomen (Scale bar = 2; 2 and 1.5 mm, respectively), **24**. Adult male (lateral view) with aedeagus at end of abdomen (Scale bar = 2 mm)

Aljarochia in Tulkarm governorate located at 120 m above sea level; olive cultivar Nabali-Mohassan) (Table 2). Therefore, the average life cycle duration of *D. oleae* in the coastal region was 210.5 days (7.01 months) for the first generation and 148.0 days (4.93 Months) for the second generation. In the hilly region, the average life cycle duration of *D. oleae* was 357.75 days (11.92 Months) (Table 2). The insect of this single generation hibernated in pupal form in the galls during the winter time then developed to give adult in the beginning of the next spring (from early March to early April). Emergence of adults coming from the single generation of the insect in hilly regions or those coming from the second generation in the coastal regions coincided with the appearance of new flushes of leaves and shoots in the beginning of spring so that, after mating, the females began to lay eggs on young leaves, flowers and branches.

Discussion

We observed that the major type of damage that has been caused by *D. oleae* in the survey area was on the leaves and branches of olive trees. The occurrence of this type of damage was reported by many investigators in various Med-countries such as Jordan (Al-Tamimi 1997), Turkey (Doganlar et al. 2011) and Greece (Simoglou et al. 2012). We also observed another type of damage that has been caused by the insect on the inflorescence of olive trees. In the present study, the rate of infestation due to damage caused by the insect on the inflorescence was relatively high (26.54%). This damage is important because it caused induction of galls in the stems of flowers as well as in the stalks of early set fruits so the development of these fruits was impaired and thus yield reduction occurs. Few researchers have reported this type of damage without, however, indicating the rate of inflorescence infestation (Doganlar et al. 2011).

In the present study, we have declared the outbreak of *D. oleae* in the study area (6 governorates of the Palestinian

Table 2 Determination of the life cycle duration of *Dasineura oleae* and its number of generations per year under the Palestinian conditions of olive production (average annual temperature and relative humidity in the survey area were 16.6 °C and 52.8%, respectively, PMA 2017)

Locality/governorate	Olive orchards in each locality	Life cycle duration			Number of generations per year and its duration
		Start and end of the cycle		Duration of the cycle in days	
Aljarochia/Tulkarm (coastal region; 120 m above sea level; olive cultivar Nabali-Mohassan)	No. 1	15/03/2017–10/10/2017	15/10/2017–1/03/2018	209 and 137	2 Generations: first with 210.5 days (7.01 months) duration, and second with 148.0 days (4.93 months) duration
	No. 2	15/03/2017–1/10/2017	15/10/2017–22/02/2018	200 and 130	
	No. 3	15/03/2017–15/10/2017	15/10/2017–25/03/2018	214 and 161	
	No. 4	15/03/2017–20/10/2017	15/10/2017–28/03/2018	219 and 164	
	Average			210.5 and 148.0 days	
Seilet El-Dahr/Jenin (hilly region; 503 m above sea level; olive cultivar Nabali)	No. 1	15/03/2017–25/02/2018		347	1 Generation with 357.75 days (11.92 months) duration
	No. 2	15/03/2017–10/03/2018		360	
	No. 3	15/03/2017–21/03/2018		371	
	No. 4	15/03/2017–3/03/2018		353	
	Average			357.75 days	

Territories) where the average mean % of infestation of olive trees in the study area was 51.46% and the mean infestation rate on the different vegetative organs of olive tree subjected to the insect attack was varied according to the locality and the governorate. For example, the highest means of infestation rate were obtained in the localities of Tulkarm and Sulfeet governorates with no significant differences in mean % of infestation rates in these governorates, but the lowest means were obtained in the localities of Nablus governorate. We have also observed that the altitude of the locality plays an important role in the outbreak of *D. oleae*. For example, the highest mean infestation rate (86.5–100%) was obtained in the localities of Tulkarm governorate with 120–420 m above sea level, whereas the lowest mean infestation rate (8.0–20%) was obtained in the localities of Nablus governorate with 345–605 m above sea level. Very few studies have indicated the outbreak of *D. oleae* in certain Med-regions. For example, the outbreak of *D. oleae* in Hatay Province, Turkey by Doganlar et al. (2011). The authors reported that the highest and lowest infestation rates in this province were 78.2 and 2.0% at 162 and 618 m above sea level, respectively. This result almost agreed with our findings and may indicate that the altitude plays an important role in the level of infestation. Almost similar results on *D. oleae* outbreak were obtained in Crete, Greece with 89% of infestation rate (Perdikis et al. 2015). However, the outbreak of the insect in the above countries was controlled by the native parasitoids as recently reported by Doganlar et al. (2011) in Turkey and Perdikis et al. (2015) in Greece. In the present study, the outbreak of the insect was not yet controlled and the insect damage is still high especially in the coastal region of infested area (100% infestation rate in Aljarochia in Tulkarm governorate at 120 m above sea level). To date, we did not record any type of parasitism on the larvae or pupae of the insect in the areas of outbreak. The lack of native parasitoids may probably explain the widespread infestation with the insect in the coastal region of the study area. Further studies on parasitism of larvae and pupae of *D. oleae* by native parasitoids and their role in the insect control in the infested areas would be investigated in the future studies.

Results obtained on egg laying by *D. oleae* and its larval development are new and reported here for the first time. They may contribute to understanding how the galls of *D. oleae* are induced and how the larva develops after the gall induction. The fact that larva develops inside the gall was reported by many investigators who worked on this subject (Darvas et al. 2000; Doganlar et al. 2011; Simoglou et al. 2012), but no reports are available on mechanism of gall induction by the insect. Further investigations in this respect are recommended to be carried out to specify the mechanism of gall induction by this insect. However, in other gall-inducing insects, certain researchers have investigated into the mechanism of gall induction by these insects. For example, in psyllid insects that induce galls on their host plants, Raman (1991) stated that the first instar nymphs of *Trioza jambolanae* (Triozidae) insert their relatively short stylets into mesophyll layer through stomata then inject large amounts of saliva that stimulates division activity in the metaplasied cell(s). The injected saliva of *T. jambolanae* contains high-molecular weight proteins (Carango et al. 1988), mitogenic lipids (Farmer 2000) and high concentrations of phytohormones (Straka et al. 2010). The injected saliva activates and regulates growth by triggering novel patterns of differentiation, which follow at the site of metaplasied cells so gall formation is initiated by an increase in the thickness of leaf tissues in this site (Harper et al. 2004).

The detailed information obtained on the life stages of *D. oleae* especially the colored photographs and associated measurements are new and reported here for the first time. No previous detailed illustrations and measurements of these stages were reported. It is expected that such information would help in a good management of this insect and/or in its control. Moreover, the results obtained on pupation and emergence of adults of *D. oleae* are new and have not been investigated before. One important result that has been obtained in this research is the observation of empty pupal skins (exuviae) that have been left after emergence of the adults and these exuviae remain attached to the leaf surface even after the emergence of adults. Observation of these exuviae on the leaf surface could be used as an indication for the adults' emergence and then timing of the chemical control of the insect by applying contact insecticides against the emerged adults.

Results obtained on the life cycle of *D. oleae* may contribute to understanding the sequence of events in the insect life cycle and may help in timing of its control by knowing the start and end of the cycle. In the present research, we observed that *D. oleae* developed one generation per year on olive trees cultivated in the hilly regions of the Palestinian Territories, whereas two generations per year were developed on olive trees cultivated in the coastal regions of these territories. However, there is uncertainty of number of generations per year that the insect develops in the different regions of olive production in the Med-countries. For example, only one generation per year was developed in Jordan and Turkey (Al-Tamimi 1997; Doganlar et al. 2011, respectively) whereas, two generations per year were developed in Syria (Darvas et al. 2000). The altitude and location of the area affect greatly the number of generations per year developed by the insect, for example, Doganlar et al. (2011) reported the development of one generation per year in the locality of Dokuzdal in Hatay Province, Turkey, at 618 m above sea level (rate of

infestation with *D. oleae* was 2.0%). This result agreed with our findings on development of one generation per year in the locality of Seilet El-Dahr in Jenin governorate at 503 m above sea level (rate of infestation with *D. oleae* was 42.5%).

According to certain researchers, the native parasitoids which appear along with the pest outbreak were enough to suppress the increase in pest population, thus the level of damage caused by this pest always remained low (Doganlar et al. 2011; Perdikis et al., 2015). In spite of the important role of native parasitoids in regulation of *D. oleae* population, certain control measures could be suggested against the insect when the suppressing effect of native parasitoids on *D. oleae* population is not high. For example, i) applying the chemical control of the insect should be optimized and adjusted to coincide with the emergence of the adults after the completion of life cycle in early spring. The objective of this type of control measure is mainly to decrease the insect population and to prevent the emerged females to lay their eggs on the susceptible organs of olive tree especially on the stalks of olive flowers. Effective and contact insecticides should be tested and optimized for achieving a good control. In this respect, we propose to test the efficacy of certain insecticides at a large scale under field conditions especially during the period of adult emergence (March–April) by spraying infested trees with insecticides to protect the new shoots and young flowers from infestation by the emerging adults, so the yield loss could be reduced. Another spray with insecticides would be practiced against the emerging adults of the second generation (October–November) in the coastal regions and ii) using pheromone traps to detect the low densities of the insect in olive orchards. We suggest this method for monitoring the insect population because it is used against other cecidomyiids such as entrapment of males of the hessian fly (*Mayetiola destructor*; Diptera: Cecidomtiidae) by the female sex pheromone of this insect (Schmid et al. 2018) and capturing of males of the saddle gall midge (*Haplodiplosis marginata*, Diptera: Cecidomyiidae) by the female sex pheromone of this insect (Rowley et al. 2018). So far, some natural enemies of the *D. oleae* especially the parasitoids were identified in certain Med-regions. For example, in Turkey, Doganlar et al. (2011) reported the occurrence of 12 species of native parasitic Hymenoptera belonging to five families of which *Platygaster oleae* Szelenyi is the most effective. The same authors stated that all of these native parasitoids are parasitizing on the larvae and pupae of this insect but not yet used commercially as biocontrol agent of the insect. As a future study, we suggest identifying the native parasitoids of *D. oleae* in our region then choosing and rearing the most effective species of these parasitoids, and then applying them against the larvae and pupae of the insect especially during the fall season and before the emergence of *D. oleae* adults in the beginning of spring.

In conclusion, the pest status of *D. oleae* on olive trees and its damage on the different vegetative organs of infested trees was assessed in the Palestinian Territories. Due to the high mean rates of infestation with the insect, we were able to declare the outbreak of the insect in the study area. This outbreak is still uncontrolled and occurs in high densities. Also, the role of native parasitoids in the control of this insect was not yet assessed. The present study proved that the females of *D. oleae* laid their eggs on the lower leaf surface and/or young shoots, stem and stalks of olive flowers. The hatched larvae penetrated the tissues underneath and induced galls in which they lived and developed until the adult emergence. Pupation of the insect took place in the galls but before the adult emergence, the mature pupa exited outside the gall then the adult emerged from pupa. The insect developed one generation per year in the hilly region of olive production in the Palestinian Territories (life cycle duration was 357.75 days or 11.82 months) or two generations per year in the coastal region of these territories (life cycle duration was 210.5 days or 7.01 months and 148.0 days or 4.93 months for the first and second generations, respectively). Galls induced by *D. oleae* on young leaves, branches and stem of the inflorescence, as well as stalks of the fruits set early in the growing season were described and photographed in the present study. This description included gall shape, gall length and the number of larvae hosted by gall chamber. Therefore, the present research provided detailed information on the life stages of *D. oleae* since until present such information are not available or scarce and superficial. Moreover, the probable control measures that could be undertaken against this pest were discussed in this paper.

Acknowledgements I would like to thank Dr. Marcela Skuhrava from Czech Zoological Society for identification of the insect as *Dasineura oleae*. Many thanks for my colleagues in the Department of Plant Production and Protection, An-Najah National University who helped in copyediting the manuscript. I would not forget to thank the anonymous reviewers who revised the manuscript and provided useful comments.

Authors' contribution The authors collected the samples of insect then carried out the assessment of its pest status and the damage caused by the insect. They carried out the description of life stages and other aspects of infestation and development of the insect. They prepared the manuscript and revised it.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors comply with ethical standards.

References

Al-Tamimi MMA (1997) Population trends of olive leaf midge *Dasineura oleae* Loew (Diptera: Cecidomyiidae) and the effect of some insecticides on the midge and its parasites in Amman district. NCARE, Jordan, p 77

Avidov Z, Harpaz I (1969) Plant pest of Israel. Israel University Press, Jerusalem, p 549

Carango P, McCrea KD, Abrahamson WG, Chernin MI (1988) Induction of a 58,000 Dalton protein during goldenrod gall formation. Biochem Biophys Res Commun 152:1348–1358

Darvas B, Skuhravá M, Andersen A (2000) Agricultural Dipteran pests of the Palaearctic region. In: Darvas B (ed) Contributions to a manual of Palaearctic Diptera, general and applied dipterology. Science Herald, Budapest, pp 565–650

Doğanlar M, Sertkaya E, Skuhrava M (2011) Pest status of olive leaf gall midge *Dasineura oleae* (Angelini, 1831), description of *Lasioptera oleicola* Skuhravá sp. new (Diptera: Cecidomyiidae) and effectiveness of parasitoids on their populations in Hatay, Turkey. Turk Entomol Derg-TU 35:265–284

Farmer EF (2000) Potent mitogenic lipids from gall-inducing insects. Trends Plant Sci 5:359–360

Gagné RJ, Jaschhof M (2014) A catalog of the Cecidomyiidae (Diptera) of the World, 3rd ed., Digital version 2

Gerson V, Harpaz I (1968) Notes on gall-midges from Israel and their parasites. Entomol Bericht Amst 28:144–148

Haarder S, Bruun HH, Harris KM, Skuhravá M (2016) Gall midges (Diptera: Cecidomyiidae) new to the Danish fauna. Ent Tidskr 137:79–98

Harper LJ, Schönrogge K, Lim KY, Francis P, Lichtenstein CP (2004) Cynipid galls: insect-induced modifications of plant development create novel plant organs. Plant Cell Environ 27:327–335

Hrncic S (1998) A survey of olive pests in Montenegro. IV International symposium on olive growing. ISHS Acta Hortic 586:23–28

Perdikis D, Arvaniti K, Malliaraki S, Angelak A (2015) The outbreak of the olive leaf gall midge populations and the importance of indigenous natural enemies in its control. In: lOBC/wprs WG (eds) Integrated protection of olive crops. 7th Meeting, Kalamata, Greece 11–14 May 2015

PMA (Palestinian Ministry of Agriculture) (2017) Department of Statistics. Palestinian Authority, Ramallah, p 50

Raman A (1991) Cecidogenesis of leaf galls on *Syzygium cumini* (L.) Skeels (Myrtaceae) induced by *Trioza jambolanae* Crawford (Homoptera: Psylloidea). J Nat Hist 25:653–663

Rivnay D (1962) Field crop pests in the Near East, vol 10. Springer, Dordrecht, p 450. https://doi.org/10.1007/978-94-017-1544-7

Rowley C, Cherrill AJ, Leather SR, Hall DR, Rope TW (2018) Factors affecting trap catch in pheromone-based monitoring of saddle gall midge *Haplodiplosis marginata* (Diptera: Cecidomyiidae). Pest Manag Sci 74(2):406–412. https://doi.org/10.1002/ps.4721 **Epub 2017 Oct 16**

Schmid RB, Knutson A, Giles KL, McCornack BP (2018) Hessian Fly (Diptera: Cecidomyiidae) biology and management in wheat. J Integr Pest Manag 9(1):1–12. https://doi.org/10.1093/jipm/pmy008

Simoglou KB, Karataraki A, Roditakis NE, Roditakis E (2012) *Euzophera bigella* (Zeller) (Lepidoptera: Pyralidae) and *Dasineura oleae* (F. Low) (Diptera: Cecidomyiidae): emerging olive crop pests in the Mediterranean. J Pest Sci 85:169–177

Straka JR, Hayward AR, Neil-Emery RJ (2010) Gall-inducing *Pachypsylla celtidis* (Psyllidae) infiltrate hackberry trees with high concentrations of phytohormones. J Plant Interact 5:197–203