Bulletin of Insectology **72** *(1): 93-102, 2019*
ISSN 1721-8861 eISSN 2283-0332

First observations on the parasitoid complex and on the biology of *Dasineura oleae* during an outbreak in Tuscany, Italy

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Abstract

Dasineura oleae (Angelini) (Diptera Cecidomyiidae) was generally considered as a minor pest in Italy, but in 2013 an outbreak of this species was registered in the South - West of Tuscany. In the current work, we investigated several aspects of *D. oleae* outbreak in Tuscany: the life cycle, the pest status, variations in the infestation rate due to the first generation of 2018 and the role of parasitoids in this pest control. We observed that in 2017 *D. oleae* had one generation in Spring and one generation in Autumn. Our results proved that the infestation rate increased after the occurrence of *D. oleae* first generation of 2018 and also that *D. oleae* infestation rate differs across the outbreak area. Parasitism was lacking in most of the monitored sites, however where parasitization was present, a negative correlation between parasitism rate and infestation rate was evidenced. We detected three new species for Italy as parasitoids of *D. oleae*: *Platygaster demades* Walker, *Platygaster oleae* Szelenyi (Hymenoptera Platygastridae) and *Mesopolobus aspilus* (Walker) (Hymenoptera Pteromalidae).

Key words: olive leaf gall midge, *Platygaster oleae*, *Platygaster demades*, *Mesopolobus mediterraneus*, *Mesopolobus aspilus*, infestation, parasitization, olive tree.

Introduction

The olive leaf midge *Dasineura oleae* (Angelini) (Diptera Cecidomyiidae) is autochthonous in Italy (the type locality is Verona, but it has a Mediterranean distribution). As all the members of the Cecidomyiidae family, *D. oleae* first instar larvae stimulate the production of abnormal tissue formations (elongate galls, 3-5 mm long) by the host plant, *Olea europaea* L. The life cycle, from first instar larvae to pupae, takes place inside the galls (Arambourg, 1986). While low gall density lead to negligible damage, massive attacks of galling insects may cause severe damage (Skuhravý *et al*., 1980) by affecting photosynthesis (Huang *et al*., 2014) and nutrient transport (Motta *et al*., 2005). These effects may cause defoliation (Skuhravý *et al*., 1980) and led to yield losses (Simoglou *et al*., 2012) especially in open field, where resources may be limited (Fay *et al*., 1996).

Literature concerning the biology, the potential damage and the controlling factors of this pest is scarce, since it is generally considered a minor pest (Giraldi, 1952; Darvas *et al*., 2000). On the other hand, in recent years several outbreaks of *D. oleae* have been reported from Turkey, Syria, Greece, Montenegro and Palestinian Territories (Doğanlar *et al*., 2011; Simoglou *et al*., 2012; Baidaq *et al*., 2015; Batta, 2019). Since 2012, several foci of *D. oleae* high infestation have been also recorded in Italy (Boselli and Bariselli, 2015). First records of *D. oleae* outbreaks in Tuscany were registered in 2013 from a field in Gavorrano district (Grosseto province) (Picchi *et al*., 2017). From then on, the occurrence of *D. oleae* new foci has been reported from the entire Grosseto area, showing a fast, patchy spreading of this pest.

Chemical control strategies of galligenous insect are critical since insecticide effectiveness may be limited due to larvae protection by galls (Lyrene and Payne, 1995). The use of biological control agent may be an environmentally friendly alternative. Conservation biological control aims at natural enemies' conservation and enhancement (Barbosa, 2003). Several studies have been carried out in olive orchards addressing the role of parasitoids (Boccaccio and Petacchi, 2009), soil (Dinis *et al*., 2016; Albertini *et al.,* 2018) and canopy predators (Cardenas *et al*., 2006; Picchi *et al*., 2016) as natural enemies of the key pests. Galling insect populations are mainly controlled by parasitoids (Hawkins *et al*., 1997) that usually stabilize phytophagous populations and could play a major role in outbreaks ceasing (Sunose, 1984; Rhodes *et al*., 2014). Species belonging to Platygastridae (Hymenoptera) are proved to be very efficient in biological control of gall midges (Sampson *et al*., 2006; Ogah *et al*., 2011; Roubos and Liburd, 2013), especially if they do not compete with other parasitoids (Sunose *et al*., 1984). Sixteen species from 5 families (Platygastridae, Pteromalidae, Eulophidae, Eupelmidae and Torymidae) have been reported as parasitoids of *D. oleae*, reaching more than 65% of parasitization rate overall (Doganlar, 2011; Simoglou *et al*., 2012). In particular *Platygaster oleae* Szelenyi, *Platygaster demades* Walker (Platygastridae), *Eupelmus urozonus* Dalman (Eupelmidae) and *Quadrastichus dasineurae* Doganlar, LaSalle, Sertkaya et Doganlar (Eulophidae) have been addressed as the main natural controlling factors for this pest (Doğanlar *et al*., 2011; Baidaq *et al*., 2015). The infestation of *D. oleae* is increasing in southern Tuscany, raising concern from olive oil producers. The goal of this work is to investigate several aspects concerning this emerging pest and to lay the foundations for further investigations. Therefore, the aims of this study are: 1) to study *D. oleae* biology in southern Tuscany coastal area and assess the number of generations. 2) evaluate the extent of the outbreak area and estimate the pest status in selected orchards. Causes of this outbreak may be related to the lack of parasitoids, indeed there is a strong and urgent need to understand the role of natural enemies and how to enhance their role in pest control. As some parasitoids of gall midges may show density dependent responses (Sunose *et al*., 1984; Lill, 1998; He and Wang, 2014), we hypothesize that olive orchards that show high levels of *D. oleae* infestations, also display higher parasitism rates. We therefore aimed at 3) identifying the species involved in *D. oleae* control and test the relationship between the host density and the parasitization rate.

Materials and methods

Study area

Field samplings were conducted in Gavorrano district (Grosseto province). This area plays a major role in Tuscan olive oil production, olive orchards of the Grosseto province representing almost 20% of the Tuscan olive groves and 50% of the woody crops in the Gavorrano district (Regione Toscana, 2010; Pasqual, 2012). Gavorrano district is a coastal area characterized by broadleaves forest and agricultural land, the climate is typically Mediterranean with annual mean temperature of 15.57 °C and annual average precipitation of 757.03 mm (data from the Braccagni weather station 42°52'22.08"N 11°04'32.57"E, source: Centro Funzionale Regionale, http://www.sir.toscana.it).

The number of sampling sites, the location, the sampling season and the sampling effort are described in table 1.

Life cycle

In order to describe *D. oleae* life cycle, a conventionally managed olive orchard (Gavorrano 42°52'51.73"N 10°56'46.7"E) was sampled nearly once a month from Autumn 2016 to Spring 2018. Samples consisted in randomly selected branches of infested olive trees. A mean of 71.2 galls (± 9.56 SE) were dissected monthly under a stereomicroscope (Leica DMC 4500) to expose *D. oleae* larvae and to identify larval stages according to Arambourg (1986). Two yellow sticky traps (40 × 24.5 cm, Serbios, Italy) were used to detect adult emergence. Traps were exposed at 1.40 to 1.80 m height on two olive trees placed at 230 m one from each other and with 56.4% and 44.4% of infested leaves respectively. Traps were replaced every 13 days on average. Mean catches of *D. oleae* females per day were calculated.

Table 1. Scheme of materials and methods.

	N° of sampling sites	Orchard	Geographic coordinates	Sampling period	Temporal sampling effort	Sample size
Life cycle	1		42°52'51.7"N 10°56'46.7"E	Autumn 2016-Spring 2018	Once a month	30 to 100 galls
Pest status	8	Site A	42°53'15.2"N 10°56'29.7"E	February 2017	Single sampling	5 branches from 5 trees
		Site B	42°52'47.2"N 10°56'20.7"E			
		Site C	42°52'58.0"N 10°56'29.7"E			
		Site D	42°53'31.0"N 10°55'41.9"E			
		Site E	42°36'06.9"N 11°15'02.0"E			
		Site F	42°33'30.3"N 11°20'12.2"E			
		Site G	42°53'28.6"N 10°55'27.3"E			
		Site H	42°43'27.1"N 11°13'05.1"E			
Infestation variation and parasitization rate	3 with low infestation	Site 1	42°54'25.7"N 11°00'10.8"E	March 2018-May 2018	Fortnightly	8 branches from 8 trees
		Site 2	42°54'31.1"N 10°59'50.1"E			
		Site 3	42°54'16.1"N 11°00'01.8"E			
	3 with high infestation	Site 4	42°53'15.2"N 10°56'29.7"E			
		Site 5	42°53'21.3"N 10°56'50.4"E			
		Site 6	42°53'33.2"N 10°58'47.5"E			

Infestation monitoring

Local extension services (OLMA, Terre dell'Etruria, Consorzio Agrario del Tirreno) provided us with the coordinates of olive orchards that presented *D. oleae* infestation in the Grosseto province. Therefore we evaluated the extension of the outbreak area as the area contained in the boundary that encompass all *D. oleae* infestation using the QGIS software (3.2.0- Bonn). Among these reports we selected eight olive orchards (Site A to Site H) according to their representativity of the area (coordinates are given in table 1). Pest management strategies in the selected sites followed P.A.N. (Piano Agricolo Nazionale) about sustainable use of pesticides (L.R. 25/99, Tuscany Region). Chemical compounds with larvicidal activity were used in July and September against *Bactrocera oleae* (Rossi) and copper sulfate against *Spilocea oleagina* (Cast.) Hugh in each orchard except Site A, that is organically managed. No pesticide was used against *D. oleae* since there is no registered chemical compound on this pest up to date. Samplings were carried out in February 2017 and March 2018 to evaluate the pest status along the study area. Samples from 5 random trees were picked up in each site. Samples consisted in the terminal five nodes of five branches that were randomly selected all around the tree canopy. We calculated the infestation rate and gall density as following:

$$infestation\ rate = \left(\frac{n^\circ\ leaves\ with\ galls}{total\ n^\circ\ of\ leaves}\right) * 100$$

$$gall\ density = \frac{total\ n^\circ\ of\ galls}{n^\circ\ of\ leaves\ with\ galls}$$

Infestation variation and parasitization

Six additional organic olive orchards were selected in the study area based on *D. oleae* infestation rate, to assess the infestation variation due to *D. oleae* first generation of 2018 and the relationship between parasitization rate and infestation rate. We chose three olive orchards with low infestation (Site 1 42°54'25.7"N 11°00'10.8"E, Site 2 42°54'31.1"N 10°59'50.1"E and Site 3 42°54'16.1"N 11°00'01.8"E) and three olive orchards with high infestation (Site 4 42°53'17.3"N 10°56'28.4"E, Site 5 42°53'21.3"N 10°56'50.4"E and Site 6 42°53'33.2"N 10°58'47.5"E). Infestation was considered low when the rate of infested leaves was below 20% and gall density was below 2 gall for each leaf (Doganlar *et al.*, 2011, modified). The sampled sites lay in an area of 3.2 km^2, had an average area of 4.6 ha, with an altitude of 54 to 114 m above sea level and the predominant cultivars are Frantoio, Leccino and Moraiolo. All orchards were rainfed, subjected to weed mowing and tillage once a year, pest control strategies were not applied in previous years. Samplings were carried out every 14 days on average, in March 2018 and May 2018. Since Frantoio cv. has shown to be one of the most susceptible cultivars to *D. oleae* attack (Al-Tamimi, 1997), 5 olive trees of this cultivar were selected and marked with a tag along a transect from the core of the field to one edge confining with woods. Eight apical branches of 8 nodes were picked all around the canopy of each tree and stored in sealed plastic bags until examination. Every sample was subdivided in one subsample of 3 and one subsample of 5 branches. The first was used to rear parasitoids to adulthood from *D. oleae* galls. The branches were placed in plastic jars at laboratory condition (26 °C) for one week. Each container was checked daily and emerged parasitoids were freeze-killed and stored in 70° ethanol. Specimens were identified to species by taxonomists. Leaves from the remaining five branches were subdivided in mature and young leaves. The total number of leaves, the number of galled leaves and the number of galls was counted. The infestation rate and the gall density were calculated as described in the previous paragraph. Two samplings were carried out in March 2018 (March I, March II), before the occurrence of the first generation of *D. oleae* of 2018. These samplings accounted for *D. oleae* infestation of both generations of 2017. Two additional samplings were performed in May 2018 (May I, May II), to account for the infestation after the first generation of *D. oleae* of 2018. This was calculated by analysing young leaves, since *D. oleae* lay eggs exclusively on newly expanded leaves (Arambourg, 1986). Dissection of 10% of the galls was performed to assess parasitization rate (Sampson *et al.*, 2002):

$$parasitization\ rate = \left(\frac{n^\circ\ of\ parasitized\ galls}{n^\circ\ of\ analysed\ galls}\right) * 100$$

Data analysis

A goodness of fit test (Shapiro-Wilk test) and homoscedasticity test (Bartlett test) were performed before applying parametrical tests. We evaluated the infestation rate across the study area in 2017 and 2018 with Generalized Linear Model (GLM), with "Time" and "Site" as effects. We used the binomial distribution since data are proportions. Tukey post-hoc test was applied to identify variation of the infestation rate from 2017 to 2018. A preliminary test was performed on the selected sites on data of March 2018 to confirm whether the distinction between "High infested" and "Low infested" sites was effective (t-test). Variation of the infestation rate between March 2018 and May 2018 (effect "Time") was tested for both sites categories (effect "Infestation level") with a Generalized Linear Mixed Model (GLMER) (binomial distribution) with site and sampled olive tree as random effect. Gall density was tested with a Linear Mixed Model (LMM) with the same fixed and random effects. Homoscedasticity was tested by visual inspection of residual plots and Shapiro-Wilk test was applied to residuals. *P*-values were obtained by likelihood ratio tests of the full model with the effects "Time", "Category" and their interaction, against models without each one the effects in question. A detailed analyses was carried individually on each site throughout a two-sided pairwise test. We tested the significance of the infestation variation on both infestation rate (pairwise Wilcoxon test) and gall density (t-test). In sites where the parasitization was detected, the relationship between the parasitization rate and the infestation variation between March and May was tested by a Pearson correlation test. All the analyses were conducted in R environment (version 3.5.1, R Development Core Team, 2018). GLMER and LMM were carried out adopting the *lmerTest* package version 2.0-32.

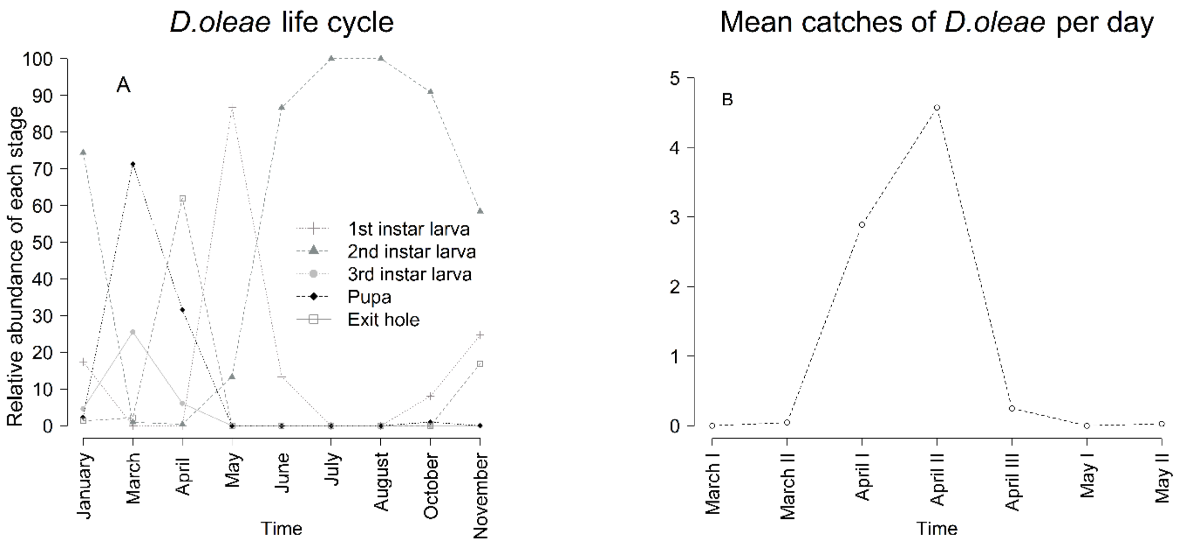

Figure 1. *D. oleae* life cycle in Tuscany **(A)** and mean catches of *D. oleae* females on yellow sticky traps **(B)**.

Results

Life cycle

Two generations of *D. oleae* develop each year, the first generation occurs in spring while the second was observed in autumn (figure 1A). The first catches of *D. oleae* females on yellow sticky traps were observed at the beginning of April and a peak of catches was detected between the 10th and the 26th of April (figure 1B). In the sampled site females were observed laying eggs on surface of olive tree young leaves. Fully developed galls were observed at the beginning of May and larvae reached the second stage at the end of May. Second instar larvae were detected until February of the next year, but we did not observe any diapause during winter. Samples collected at the end of November showed exit holes (16.9% of analysed galls) as well as young expanded leaves hosting first instar larvae (24.7% of analysed galls), showing the occurrence of a second generation.

Infestation monitoring

In the Grosseto province the extension of *D. oleae* infestation is 3258 km^2 on overall 4503 km^2 (72% of the area). We recorded an average infestation rate of 50.3%, with Site H (42°43'27.1"N 11°13'05.1"E) showing the highest rate of infested leaves (84.6%) and Site E (42°36'06.9"N 11°15'02.0"E) showing the lowest (23.2%) (figure 2). The infestation rate differed significantly across monitored sites according to GLM. Post hoc test revealed that the infestation rate is not related to geographical distance between monitored sites. Furthermore, Site A (organically managed) did not show significant difference from Site D and Site F (both conventionally managed). Site B and Site G showed a significant increase of the infestation rate between 2017 and 2018, while Site D, Site F and Site H showed a significant decrease of the infestation rate.

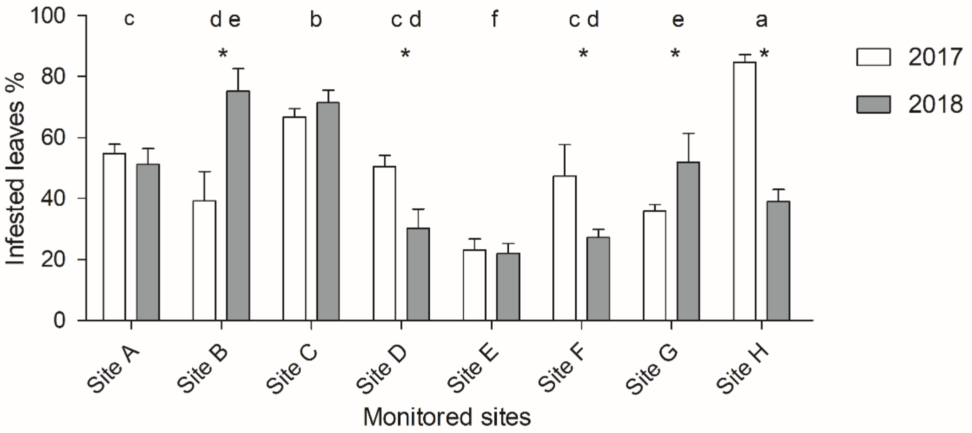

Figure 2. *D. oleae* infestation rate, expressed as the rate of infested leaves (Mean ± SEM) in eight monitored olive orchards (sites) in the Grosseto province in February 2017 (white) and March 2018 (grey). Significant differences between 2017 and 2018 were reported as * (p < 0.05). Letters show significant differences (p < 0.05) among sites in February 2017.

Infestation variation and parasitization rate

The difference between “High infested” and “Low infested” sites was significant at the beginning of the sampling season (March 2018) for both infestation rate ($p < 0.001$) and gall density ($p < 0.01$) according to t-test. The GLMM showed that both fixed effects (“Infestation level” and “Time”) and their interaction significantly influenced the infestation rate ($\chi^2_{(3)} = 142.119$, $p < 0.0001$), showing a steeper increase in Low infested sites. In particular March I and March II were not significantly different, while there was a significant increase in May I and May II. The LMM showed that both factors (“Gall density” and “Time”) and their interaction were significant ($\chi^2_{(3)} = 12.948$, $p = 0.004749$) (figure 3). Analyses of the infestation variation in each sampling site showed a significant increase in infestation rate ($p < 0.05$), except for Site 4 and 5. Gall density showed a significant increase in all “Low infested” sites (Site 1 to 3) but was not significantly different in “High infested” sites (Site 4 to 6) (figure 4). Parasitization was not detected in four sites out of six. Site 4 and 5 showed 31.9% and 15.3% of parasitized galls.

Correlation test revealed negative correlation (R = −0.63, $p < 0.05$) between parasitism rate and the infestation rate variation across *D. oleae* first generation of 2018. A stronger negative correlation was found on gall density variation (R = −0.72, $p < 0.05$) (figure 5).

Pupae and adults of parasitoids have been detected on *D. oleae* third instar in March and April 2018 (figure 6D). Usually, each larva hosted one parasitoid, but in few cases we observed two parasitoid pupae in one single larva.

Reared adult specimens of parasitoids were identified as *Platygaster demades* Walker, *Platygaster oleae* Szelenyi (Platygastridae), *Mesopolobus mediterraneus* (Mayr) and *Mesopolobus aspilus* (Walker) (Pteromalidae) (figure 6). A female of *P. demades* was observed by using a stereomicroscope while performing oviposition probing and oviposition drilling on *D. oleae* eggs under laboratory conditions, in an artificial arena.

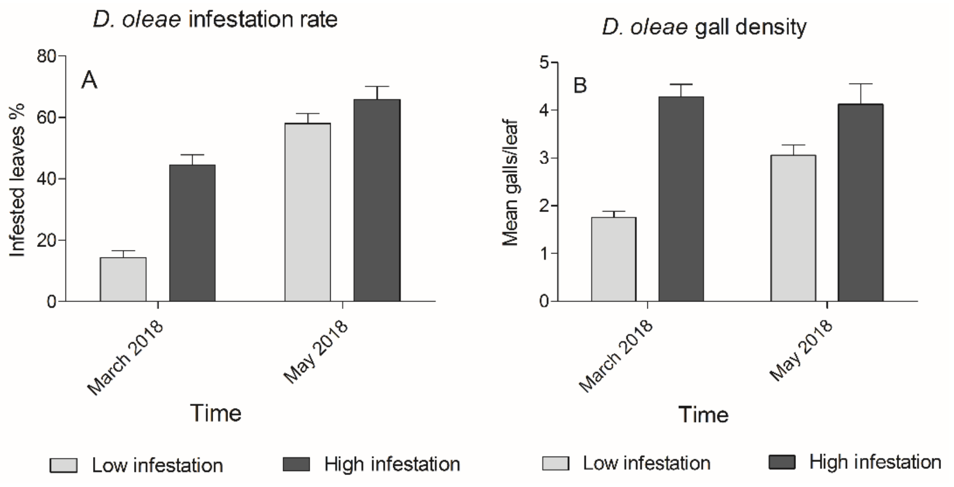

Figure 3. *D. oleae* infestation in low infested sites (light grey) and high infested sites (dark grey): **A)** Infestation rate is expressed as the percentage of infested leaves (Mean ± SEM); **B)** Gall density is expressed as the mean number of galls for each leaf (Mean ± SEM). The two samplings performed in the same month are merged (March 2018, May 2018).

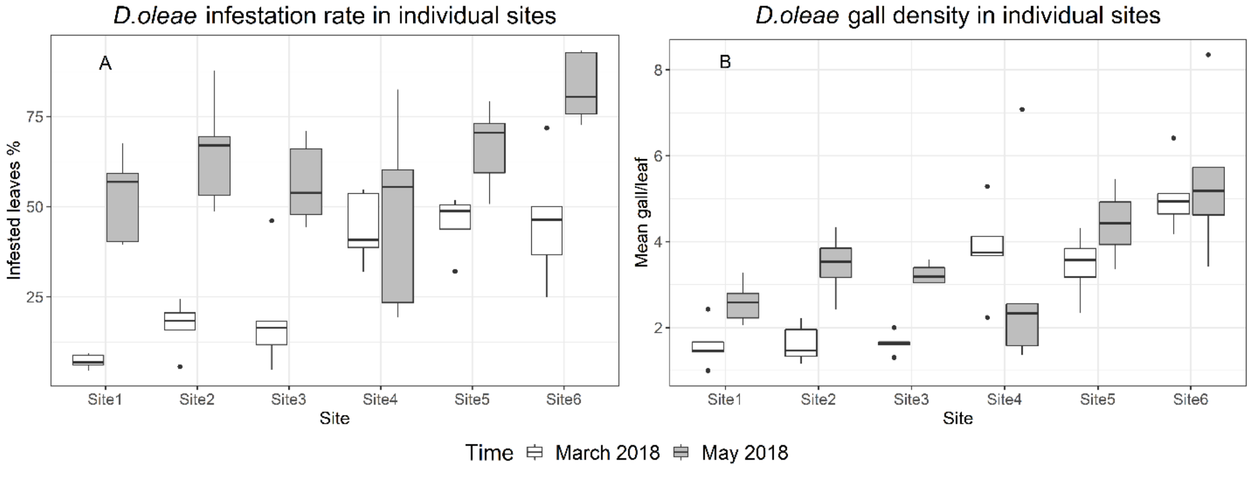

Figure 4. Boxplots of: **A)** Infestation rate, expressed as the rate of infested leaves; **B)** Gall density expressed as the mean number of galls for each leaf. In March 2018 (white) and May 2018 (grey).

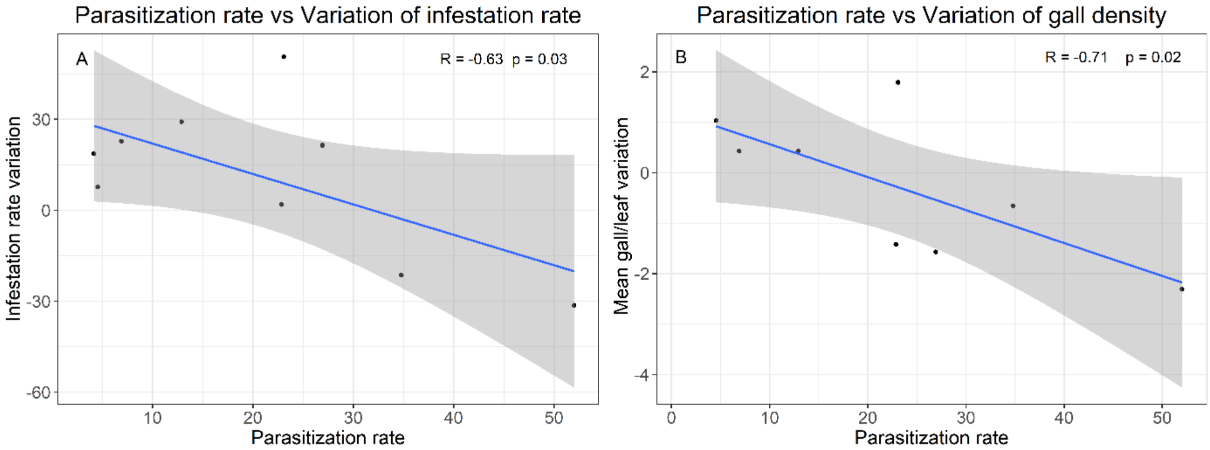

Figure 5. A) Correlation between parasitization rate and variation of infestation rate between March 2018 and May 2018; **B)** Correlation between parasitization rate and variation of gall density between March 2018 and May 2018.

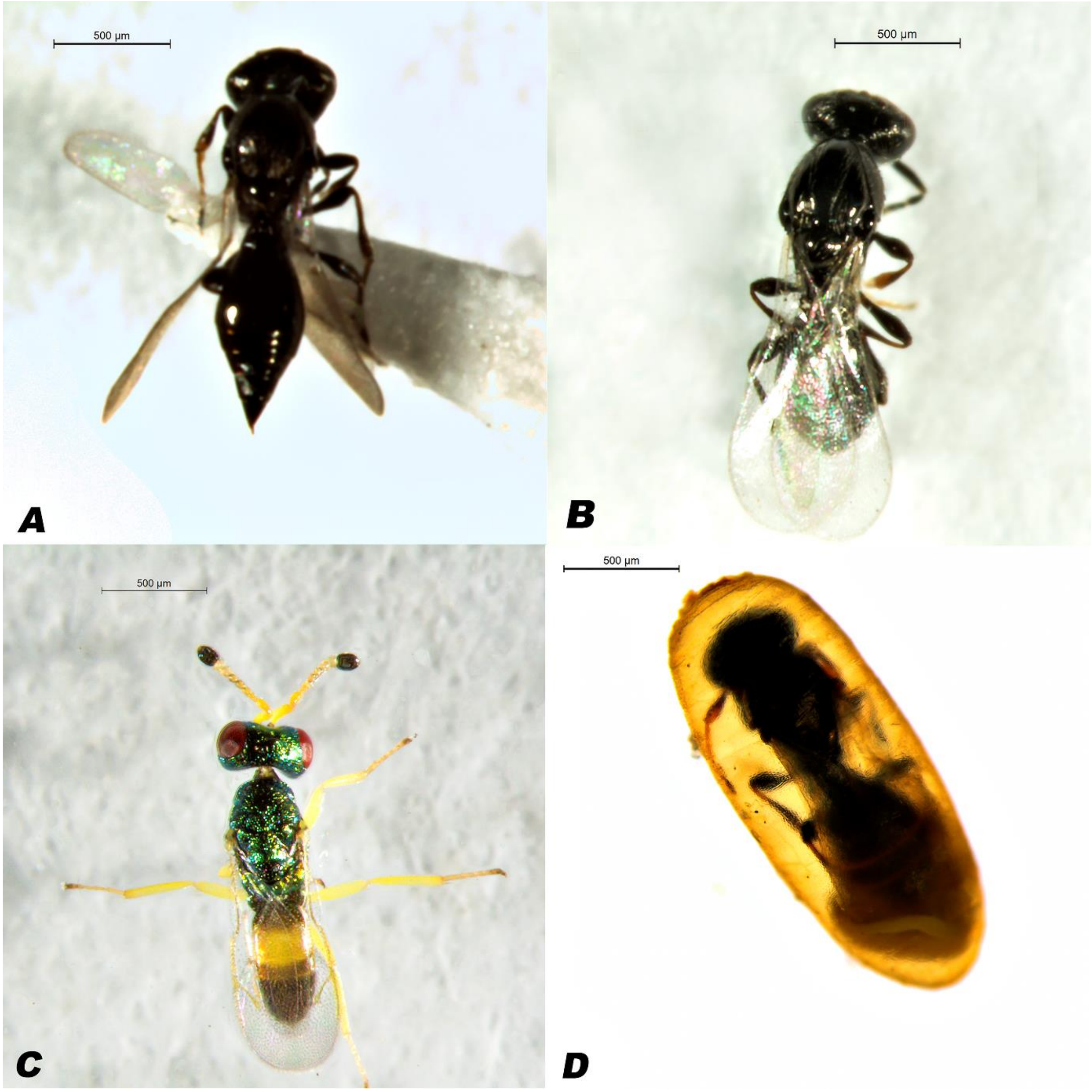

Figure 6. Parasitoids reared from *D. oleae* galls: **A)** *P. demades*; **B)** *P. oleae*; **C)** *Mesopolobus* sp.; **D)** Parasitoid pupae inside *D. oleae* third instar larvae (Leica DMC 4500). Scale bars 500 µm. Specimens deposited in the collection of the Biolabs, Scuola Superiore Sant'Anna Pisa (Italy).

Discussion

In 2017 according to our observations, *D. oleae* developed two generations in the study area. This pest usually develops one generation each year, but a second generation may be observed under favourable climatic conditions (Doganlar *et al.*, 2011; Baidaq *et al.*, 2015) and in coastal areas (Batta, 2019). Gall midges' populations rely on the synchronization between imagines emergence and availability of target host organs, since the adult lifespan is within one or few days (Yukava, 2000). We observed that the entire population reached imaginal stage in spring during the first generation, when the olive trees were developing new foliage. The second generation was observed in November 2016, when favourable weather conditions induced shoot extension and new foliage, providing suitable sites for galling. Batta (2019) reported the occurrence of two generations each year in coastal areas of Palestinian territories, while found low numbers of second generation exit holes, similarly to the findings of Graora *et al.* (2015) on *Dasineura brassicae* Winnert. Adults emergence was registered over a month, but most of the catches were observed between the 10th and the 26th of April, with a considerable delay if compared to results of Batta (2019) in Palestinian territories. Differences between our results and the research of Batta (2019) may be explained by different climatic conditions of Italy and Palestine.

Tremblay (1991) reported that *D. oleae* overwinters as a second instar diapausing larvae. This author states that extreme environmental conditions may induce summer diapause in *D. oleae*. Our results did not show a defined diapausing period, since we observed that second instar larvae of *D. oleae* were actively moving.

D. oleae infestation reports are widely spread over the Grosseto province, occurring on 72% of the area and are mainly distributed in the coastal area. We observed an average infestation rate of 50.3% in monitored sites. This value exceeds three times the average value reported by Doganlar *et al.* (2011). Post hoc test revealed significant differences between sites but did not show specifics differences between the organically managed olive orchard (Site A) and conventionally managed olive orchards. These results are consistent with current observations in organic orchards that exhibit high infestation rates. This suggests that the management may be not a critical factor in determining the pest density. The rate of infestation of *D. oleae* does not seem to follow a geographical gradient, showing a patchy distribution. This is a common feature in gall midges outbreaks (Skuhravý *et al.*, 1983; Ukwungwu and Joshi, 1992). Differences in this pest density across the region may be due to several factors such as different susceptibilities of olive tree variety to *D. oleae* attack (Al-Tamimi, 1997) and microclimatic variations (Ukwungwu and Joshi, 1992).

The economic threshold of *D. oleae* is not known, nevertheless high infestation rates led to yield losses due to defoliation, deficiency in nutrient assimilation (Doganlar *et al.*, 2011) and presence of galls on flower stalks (Doganlar *et al.*, 2011; Batta, 2019). It is known that galls may affect distribution of chemical elements in the leaf tissue and that high gall density may greatly increase leaf weight, causing precocious leaf fall (Skuhrava *et al.*, 1980). Several gall midges are responsible for severe yield losses at high density (Sampson *et al.*, 2002; Censier, 2015) due to delayed plant growth (Censier, 2015), bud necrosis and abortion (Gagnè, 1989). There is no actual assessment of yield losses due to *D. oleae* attack. Since the relevance of this pest is rising, a quantification of production losses should be assessed.

D. oleae infestation rate has increased significantly from March 2018 to May 2018, especially in Low infested sites. The further increases in this pest attack, after first *D. oleae* generation suggests that the current outbreak of this pest is still ongoing. The analyses of the infestation variation in each site showed a significant increase in the infestation rates where parasitization was low or not detected. On the other hand, Site 4 (42°53'17.3"N 10°56'28.4"E) and Site 5 (42°53'21.3"N 10°56'50.4"E) showed respectively 31.9% and 15.3% of parasitized galls on average and non-significant variations in both infestation rate and gall density. These results suggest that this rate of parasitism could be enough to stabilize *D. oleae* populations and slow the outbreak down. Indeed, the correlation test showed that high parasitization rate led to a decrease in the infestation rate and gall density across *D. olae* first generation. These results suggest an association between the deficiency of parasitoids and this pest outbreak. Parasitoids lack may be due to several factors such as different effects of climate changes on host and parasitoids (Chen *et al.*, 2018) and asynchronization with their host (Grabenweger *et al.*, 2007; Hance *et al.*, 2007). Nevertheless parasitoids seem to play a major role in the reduction of several cecidomyiids infestations (Sampson *et al.*, 2002; He and Wang, 2011). The lack of collection of parasitoids in Low infested sites and the subsequent steeper increase of the infestation rate therein, may be due to delayed-density dependent response of parasitoids. Indeed *P. demades* showed type III functional response toward *Dasineura mali* (Kieffer), showing strong density dependent response (He and Wang, 2014). This response may be delayed, resulting in belated control of the pest (Sunose, 1985).

Among the four parasitoid species that we detected inside *D. oleae* galls, we found 3 new records for Italy: *P. demades*, *P. oleae* and *M. aspilus* (Stoch, 2004; Norman, 2018; Noyes, 2018). Besides these, the facultative hyperparasitoid *M. mediterranus* was detected. The species richness of the observed parasitoid complex is lower than the one referred by Doganlar (2011), indeed this author reports 11 parasitoids species, 7 of which are not recorded in Italy. We did not observe *E. urozonus* as a parasitoid of *D. oleae*, contrary to Doganlar findings, that addressed this species as one of the main controlling factors of *D. oleae*.

Parasitic microhymenoptera such as Platygastridae and Chalcidoidea are one of the main controlling factors of several species of the Cecidomyiidae family (Cameron and Redfern, 1978; Sampson *et al.*, 2002; Roubos and Liburd, 2013). In particular *P. demades* is known to efficiently control *D. mali* and *Dasineura pyri* (Bouche) populations in New Zeland (He and Wang, 2014).

P. demades is known to parasitize both eggs and larvae of *D. mali* (He and Wang, 2015), but was observed as a second instar larval parasitoid on *D. oleae* (Baidaq *et al.*, 2015). However, we observed a female of *P. demades* performing oviposition drilling (*sensu* He and Wang, 2015) on *D. oleae* eggs for the first time. Further investigations on the interaction between *P. demades* and *D. oleae* are needed.

We detected both early and late pupae (*sensu* Sampson *et al.*, 2006) of parasitoids on *D. oleae* third instar larvae. Parasitism rate of koinobionts such as *Platygastridae* and *Mesopolobus* may not be evident in early parasitization stages (Hakins *et al.*, 1997). Earlier detection of parasitization may be assessed by larval anatomization (Baidaq *et al.*, 2015) or by molecular analyses (Gariepy *et al.*, 2008).

Results from Baidaq *et al.* (2015) showed that *P. demades* may reach 30% of parasitism on second instar larvae, while we only detected the presence of parasitoids on third instar larvae. Baidaq evidences are based on larvae anatomization, while we analysed live exposed larvae. Nevertheless, our finding is consistent with the synchronization of *Platygaster* sp. with *D. oleae* life cycle. *D. oleae* larvae usually hosted one parasitoid, but few cases showed multiple host for each larva as observed by Mouallem (1986) and Roubos and Liburd (2013).

While *P. demades* is known to attack several species of the genus *Dasineura*, *P. oleae* is a specific endoparasitoid of *D. oleae*. Literature concerning this species is scarce, but Doganlar (2011) and Al-Tamimi (1997) addressed *P. oleae* as one of the main mortality factors for this pest.

Both *M. mediterraneus* and *M. aspilus* are known as endoparasitoids of *D. oleae* and several others Diptera, Coleoptera, Hymenoptera and Lepidoptera (Vidal, 1997; Garrido Torres *et al.*, 1999; Askew *et al.*, 2001; Doganlar *et al.*, 2011; Toth *et al.*, 2011). Biological control efficacy of parasitoids may be influenced by availability of non-host food resources (Lewis *et al.*, 2002) or shelters (Griffith *et al.*, 2008). Since several parasitoids feed on flower nectar (Jerwis *et al.*, 1993), the study of plants-parasitoids associations have a crucial role into addressing agroecological practices. Studies on *P. demades* revealed a marked longevity increase when *Fagopyrum esculentum* (buckwheat) was available (Sandanayaka and Charles, 2006). Despite the lack of data on feeding behaviour of both *M. aspilus* and *M. mediterraneus*, some studies report that these species are associated with several hosts that live on plant of the Mediterranean maquis such as *Juniperus communis*, *Cytisus scoparius*, *Quercus cerris*, *Quercus ilex* and *Quercus suber* (Askew, 1970; Vidal, 1997; Gomez *et al.*, 2006; Askew *et al.*, 2013). These results suggest that the presence of hedgerows composed by these plant species may enhance the abundance of *D. oleae* parasitoids. Chemical control strategies have not been tested on this species yet. On the other hand, high infestation rates may be controlled by exceptional use of pesticides on early development stages. Indeed, synthetic systemic larvicides or alternative botanical insecticide that have proved to be effective on other gall midges (Pavela *et al.*, 2009).

Conclusions

D. oleae outbreak was first reported in Tuscany around 2013. Results of this study suggest that the outbreak is still ongoing and could be related to parasitoids deficiency. However, we observed that several parasitoids, previously unknown in Italy, seem able to keep *D. oleae* populations stable. We assessed the overall parasitization rate, but the relative contribution of each parasitoid species to *D. oleae* control is yet to be valued. Moreover, parasitization rate may be underestimated if evaluated on early-mid larvae. In this context, the development of molecular techniques would enable early detection and identification of parasitoids on second and third instar larvae of *D. oleae*.

Acknowledgements

We are particularly grateful to Peter Neerup Buhl for the identification of Platygastridae specimens and to Mircea-Dan Mitroiu (University of Iasi) for the identification of Chalcidoidea specimens. We would like to extend our sincere thanks to Susanna Marchi and Alice Albertini for reviewing a draft of this paper. We would like to thank Malayka Picchi, Gaia Monteforti, Michelangelo Benza and Cristina Ghelardi for field and laboratory assistance. We thank landowner for permission to access their olive orchards. We would like to thank the anonymous reviewers and the editor for their suggestions to improve this manuscript. This work has been realized with the financial support of Tuscany Region and Scuola Superiore Sant'Anna, PhD Programme in Agrobiosciences.

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Received November 15, 2018. Accepted March 20, 2019.