Following the outbreak: preliminary findings on the landscape effect on *Dasineura oleae* and its parasitoids in Central Italy

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Abstract

Dasineura oleae (Angelini, 1831) (Diptera: Cecidomyiidae), the olive leaf gall midge, is a pest of olive crops that has never been problematic in Italy since 2016, when a massive infestation of this pest was reported in a small region of Central Italy. We selected infested olive orchards through farmers' reports aiming at quantifying the pest infestation level and the parasitism rate in each site. Also, we aimed at exploring the landscape effect in both pest and parasitoids, using proportion of olive crops and semi-natural habitats, as well as the Shannon index as a measurement of the landscape diversity, estimated at four different scales (250m, 500m, 750m and 1000m buffers around the sampling points).

Results showed different landscape effect depending on the organism and on the scale. We underlined a small-scale effect on the parasitism rate and a large-scale effect on the olive leaf midge mediated by the Shannon Index.

Moreover, some preliminary results showed that the parasitism rate was high in sites where plants associated with *D. oleae* parasitoids were present in the adjacent semi-natural habitat. Further study should deepen and validate our findings on the effect of landscape and of the vegetation on natural enemies of *D.oleae*. These results should stimulate new approaches in the studying of the olive gall leaf midge as well as new suppression strategies.

1. Introduction

Dasineura oleae (Angelini, 1831) (Diptera: Cecidomyiidae), commonly known as the olive leaf gall midge, has always been considered as a minor pest of the olive tree (*Olea europaea* L.) in several Mediterranean countries. Hence, the traits of this pest have never been deeply investigated and literature on this topic is scarce. In Italy, this pest has never been problematic since 2016, when some outbreaks were officially recorded, despite infestation reports from olive growers started in 2014 (Picchi et al., 2017). The larva causes damage to the young leaves and flower buds by feeding on the mesophyll and causing the formation of bulges on the leaves (Barnes 1948).

The infestation traced out a patchy distribution in the following years, in the Southern part of Tuscany (Central Italy), in the entire province of Grosseto (Petacchi et al. 2019; Picchi et al. 2017; Tondini and Petacchi 2019).

Specifically, the pest has been reported to be abundant in the countryside through field observations by farmers and technicians. In the latest years, outbreak have become common in different parts of the Mediterranean basin, leading to an increase in olive leaf gall midge case studies (Batta 2019; Batta and Doganlar 2020). In Italy, due to the renewed agronomic interest in this pest, its life cycle and the parasitoid complex of *D. oleae* were studied (Tondini and Petacchi, 2019). In addition, these authors hypothesized that the shortage of parasitization could have promoted *D. oleae* outbreak in Italy.

The natural enemies' complex of *D. oleae* in Italy consists of four species of koinobiont endoparasitoids. Twospecies belong to the family of Pteromalidae (*Mesopolobus aspilus* (Walker, 1835) and *Mesopolobus mediterraneus* (Mayr, 1903)), whereas the other two are Platygastridae wasps (*Platygaster demades* Walker 1835 and *Platygaster oleae* Szelenyi 1940). Only the latter species is a specific parasitoid of *D. oleae*.Both Pteromalidae and Platygastridae include species that are effective control agents of many Cecidomyiidae species. (He and Wang 2011; Roubos and Liburd 2013; Sampson et al. 2002).

Besides these recent studies, the knowledge about *D.oleae* is scanty and there are no studies on the effect of this phytophagous on the olive yield. On the other hand, results of Caselli et al. (2020) suggest that the pest may alter leaf morphology and influence some physiological activities of olive leaves related to photosynthesis performance.

While increasing knowledge about the pest and its enemies is an important step for researchers and future control strategies, one useful approach to deal with pests is to follow the infestation level throughout the area of interest, providing guidelines to farmers to help them make informed management decisions, as planned by the Integrated Pest Management (IPM) strategy (Barzman et al. 2015).

In this study, we aimed at quantifying pest populations to estimate the infestation level and, in gall-inducing insects; we evaluated the parasitism rate through the abundance of parasitoids wasps in galls. Taking into account both infestation and parasitism rates, prevention actions (proactive measures) and appropriate control measures (reactive measures) could be better applied (Mueller et al. 2005).

In addition, these data could contribute to the definition of the economic injury level and the economic threshold (Stern et al., 1959) both necessary to improve the effectiveness of IPM strategies and to set proper Conservation Biological Control (CBC) strategies.

A survey on the current pest status in the outbreak area may provide useful information to settle management strategies for farmers. It supplies data on both the pest population and its natural enemies, offering the chance to investigate not only the species distribution or the effect of the landscape composition and diversity but also the climate change effect on crop-pest synchrony over a long time (Haynes et al., 2014) or the dispersal probability, temperature tolerance and preference of its parasitoids (Chaianunporn and Hovestadt, 2015).

Pest outbreaks and pest control by natural enemies indeed have strong links to landscape patterns and they do not only depend on local in-field conditions (With et al. 2002). Arthropods of crop fields are influenced by the nearby habitats; indeed favourable local and landscape conditions with peculiar ecological traits can enhance predators and parasitoids and can lead to higher rates of pest suppression (Bengtsson et al. 2005; Bianchi et al. 2006; Haan et al. 2020; Happe et al. 2019; Tschumi et al. 2016). Usually, a high proportion of semi-natural habitats (hereafter "SNH") benefits natural enemies (Chaplin-Kramer and Kremen 2012 but see Tscharntke et al. 2005; Hawro et al. 2015) and an increase of the landscape complexity corresponds to higher parasitism in the crop (Cronin and Reeve 2005) but the biological control could depend also on taxon-specific mobility and dispersal capacity of pest and natural enemies (Gallé et al. 2019; Happe et al. 2019; Schweiger et al. 2005).

Indeed, SNH provides natural enemies with resources such as alternative hosts or/and overwintering shelters. *D. oleae* parasitoids, except *P. oleae*, are oligophagous or polyphagous. Therefore, it is likely that vegetation adjacent to olive orchards hosts several plant species associated with alternative hosts (hereafter "associated plants"). Also, *M. mediterraneus* and *M. aspilus* are polyphagous parasitoids and hyperparasitoid that attack several species belonging to Coleoptera (Herting and Simmonds 1973; Vidal 1997) and Diptera, including numerous Cecidomyiidae (Askew et al. 2001; Del Bene and Landi 1993; Doğanlar 2011; Graham 1969). Moreover, it has been reported that such parasitoids attack some species of Hemiptera (Öncüer 1991), Hymenoptera (Askew et al. 2001; Askew et al. 2013; Gomez et al. 2006) and Lepidoptera (Herting and Simmonds1975; Viggiani 1967).

These hosts are associated with plants such as *Juniperus* spp. (Askew et al. 2001; Askew 1970), *Quercus* spp. (Askew et al. 2013; Gomez et al. 2006; Xiao et al. 2016), *Pinus* spp. (Askew et al. 2001) and *Cytisus scoparius* (Vidal, 1997).

Platygaster demades is an oligophagous parasitoid that attacks *D. oleae*, *Dasineura pyri* (Bouché, 1847), *Dasineura mali* (Kieffer, 1904) and *Wachtliella ericin*a (Low, 1885), that are respectively associated to *O.europaea*, *Malus domestica* (Suckow) Borkh (1803), *Pyrus communis* subsp. *pyraster* (L.) Ehrh. and *Erica* spp.

In this paper, we aimed at estimating the infestation level of *D. oleae* through the evaluation of the leaf infestation and the number of galls per infested leaf. Also, we estimated the effect of parasitoids by parasitism rate, to enable the definition of a risk assessment procedure and advice for farmers.

Moreover, we made an introductory investigation of the effect of landscape composition (olive orchard and SNH) and the Shannon index (hereafter "SH") was used as a measure of the richness of different land cover in the landscape- a synthetic description of the spatial diversity - at four different buffers (250 m, 500 m, 750 m, and 1000 m) for both pest and parasitoids. Finally, we present preliminary data on the effect of associated plants on the biological control of *D. oleae*.

2. Materials And Methods

2.1) The study area

The survey was carried out in the province of Grosseto, a coastal area of southern Tuscany (Central Italy). The area covers 5000 km^2 and it is relevant for the regional and national agricultural sector, as 25% of its territory is defined as "Utilised Agricultural Area" (UUA) (Regione Toscana 2012), and olive orchards occupy 14, 5 % of the land, which are generally small fields (Maselli et al. 2012). The Grosseto province produces 20% of the overall regional olive oil and has 19 % of the mills of the region (ISMEA 2018).

The climate is typically Mediterranean with dry and hot summers ("Csa" type according to the Köppen–Geiger classification (Peel et al. 2007)), mean annual precipitation of 730 mm/year and a minimum value of raining in July (5 mm). February is the coldest month whit a mean minimum of 0° C and July is the hottest one with a mean maximum of 32° C (Vallebona et al. 2015).

Grosseto is the driest and warmest area of Tuscany, with long summer dry periods and thus, more suitable to olive cultivation rather than other crops, due to some physiological mechanisms that confer resistance to drought stress (Sofo et al. 2008).

2.2) Monitoring of *Dasineura oleae* and its parasitoids

Sampling points were chosen in 2019 and they were selected from a database of local reports of *D. oleae* infested sites and considering the olive land-use. At the beginning, we collected information about infested fields in previous years from technicians of extension services and consortiums of the province (OLMA, Terre dell' Etruria, Consorzio Agrario del Tirreno). They periodically pointed out the presence of *D. oleae* infestations and provided GPS coordinates and an approximate estimation of the level of infestation through visual inspection of trees. Successively, we calculated the extension of the area dedicated to olive orchards through the "Utilised Agricultural Area" (UUA) value for each municipality and finally, we defined a list of 24 representatives infested olive orchards to include in our study (Fig. 1A).

Many of them used dimethoate application against the olive fruit fly (*Bactrocera oleae*, Rossi 1970; Diptera Tephritidae) in summer while no treatments against olive leaf gall midge were appliedduring the period of sampling and previous years.

In the proximity of the middle of each field, we performed a transect starting from the third row of the edge toward the centre of the field to minimize the edge effect and spillover from adjacent fields. We sampled six trees, choosing one tree every two, within the transect.

From each tree, four sprigs were picked up, one from each cardinal direction at circa 1.5 m height. The samples consisted of the apical eight nodes of the branch, to gather the latest growth of the plants and thus, the latest generation of *D. oleae*.

In the laboratory, we counted the number of leaves, the number of infested leaves and the number of galls in each sample. We estimated the leaf infestation level through the ratio between infested leaves and the total number of leaves in the sample (Tondini and Petacchi, 2019). We calculated the ratio between the number of galls and the number of infested leaves (hereafter "galls per infested leaf") and galls were dissected under the stereomicroscope. The number of galls dissected per sample ranged between a minimum of one gall or 10% of the galls found in the leaves of each sprig collected.

Olive orchards were visited between 26th March and 3rd April 2019, to catch the latest period before flicking of pest and parasitoids. During this period, *D. oleae* develops into the third instar stage and parasitization became evident.

To define levels of gall midge infestation we separated "low-infested" and "high-infested" olive orchards considering the average value of galls per infested leaf. The threshold between low and high infestation was defined through the observations made by Doganlar from infested olives in Turkey (2011) and by our experience from previous years of samplings.

We considered a low-infested olive orchard when the mean value of the number of galls per infested leaf was lower than 3 otherwise, the olive orchard was considered high- infested.

The parasitism rate was calculated as the relative abundance of parasitoids on the number of galls inspected. Eventually, we provided farmers with management guidelines by the number of galls per infested leaf in combination with the parasitism rate.

2.3) Landscape Metrics And Vegetation Sampling

The landscape analysis was performed using QGis 3.8.2 Zanzibar (QGIS Development Team 2019). We assessed the land-use of the twenty-four fields in four concentric buffers with radii of 1000 m, 750 m 500 m and 250 m buffer, nested around each olive orchard. Land-use classes were obtained from Geoscopio WebGIS Service of Tuscany region (Regione Toscana, 2020). The land-use types belong to five classes: excluding olive orchards and SNH we considered construction land, annual crop and other orchards.

Land-use percentage of olive orchards and SNH were calculated for each buffer and were used as estimators of landscape composition. Besides, we evaluated the spatial composition and diversity of each buffer through the Shannon Index. This index considers the proportional abundance of each habitat type with the highest value for the smallest amount of land-use dominance (McGarigal et al. 2012).

In addition, we collected some preliminary data about plant assemblages in the SNH nearby the olive orchards. To do so, we chose four fields to represent the combination of two levels of abundance of SNH in 250 m buffer (high and low) and two levels of parasitism (high and low).

The landscape patterns in 250 m buffers around these fields are shown in Fig. 1B. The spontaneous vegetation in SNHwas sampled with three transects of 100 m at five different distances (0 m, 25 m, 50 m,75 m, 100 m), starting from the edge with olive orchard towards the field. In each site, the transects were perpendicular to the olive orchards and parallel between them. The abundance of both associated plants and "no-associated plants" (plant species not associated with parasitoids) was evaluated by visually assessing each species cover (in percentage) in a 2.5 m buffer around each sampling point.

2.4) Statistical Analysis

All the analyses were performed with R software (R Development Core Team 2016). First, we ensured that 1000 m buffers did not overlap more than 10% in order to guarantee statistical independence (Steffan-Dewenter, 2002) and we checked the collinearity among landscape variables.

The effect of landscape factors (olive composition, SNH composition and SH index) on the leaf infestation and parasitism rate -as an average value for each olive field- were estimated through Generalized Linear Model using a binomial distribution. Since overdispersion was detected, we corrected the standard errors using a quasi-binomial model for each scale and validated. The interaction between the composition of olive, SNH and SH was evaluated in the models. Significative variables according to GLMs were also tested exploring landscape traits of sites by a K-means partitional clustering.Thus, partitional clustering was done using the values of the landscape indices described above.

The optimal number of clusters was assessed through the Elbow method based on the sum of the square for each cluster (Zhang et al. 2016) and characteristics of each cluster were graphically evaluated (package ggiraphExtra: Moon 2018).

We estimated differences in parasitism rate among clustered sampling sites applying Generalized Linear Models using parasitism rate as the response variable and landscape structure indices as explanatory ones and assuming a binomial distribution of the data.

The model was tested against a null model and χ2 and *p*- values were obtained by likelihood tests. Estimated marginal means and standard errors were obtained with the function "emmeans" (emmeans package: Lenth 2020) and post-hoc Tukey tests were carried out with "multcomp" package (Hothorn et al. 2008).

To visualize the association between plant species type ("associated" or "no-associated") and sampling sites we calculated the mean cover of each plant species for each sampling site (sites 18, 24, 25 and 26). Then we carried out a Correspondence Analysis (CA) using the packages "FactoMineR"(Lê et al. 2008) and" factoextra" (Kassambara and Mund 2019).

In the CA, *O.europaea* was not considered because it is not attacked by other insect species that are potential hosts for parasitoids, apart from *D. oleae*.

3. Results

3.1) Infestation and parasitism rates

We found that the mean number of infested leaves on the total leaves sampled was 23% with the highest value of 71%. Only three olive orchards out of24 had more than 50% of leaves infested. The mean number of galls per infested leaf was 1.77 and only two olive orchards exceeded the threshold of 3 galls per infested leaf (range of infestation rate from 1 to 3.24 galls per infested leaf). The parasitism rate exceeded 10% in 15 olive orchards (62.5%) and the mean value of parasitism was 15.8 (range of parasitism rate was 0.0%- 43.7%). Parasitoids were absent in samples from three olive orchards. The results of each olive orchard are reported in Table 1.

3.2) The Landscape Effect

GLMs showed significant effect at 250 m, 750 m and 1000 m buffer, as reported in Table 2. At the 250 m scale, the parasitism rate increased as the SH index increased. Then, this positive correlation was explored by multivariate analysis (Fig. 2) that divided sites into 4 clusters. They were renamed on their traits as in the legend in Fig. 2 and the GLM approach underlined a significant difference between clusters (p = 0,003). Then, through a post-hoc test, we explored the differences between clusters. The leaf infestation rate increased as the diversity increased in large buffers, at 750 and 1000 m, but it was not possible to explore in more detail the traits of sites due to problems in clustering. The sites are not enough to divide olive fields based on the chosen variables.

4. Discussion

Dasineura oleae was always considered a minor pest in olive orchards compared to the olive fruit fly, and for this reason, the influence of biotic and abiotic factors on the pest and its natural enemies as well as the effects on plant physiology and yield have never been deepened. In this study, we tried to unravel the significance of different environmental factors adding some preliminary findings of the effect of landscape on both leaf infestation by the olive leaf gall midge and parasitism rate. In addition, we have introduced the importance of the vegetation assemblages in nearby SNH to improve the biocontrol of *D.oleae*. This approach gathers the need for IPM guidelines and the hunger for knowledge about the traits of this pest and its natural enemies, nowadays a cause of increasing concern in olive orchards, in order to provide successful control strategies and appropriate landscape management.

From our results, it turned out preliminary findings of the landscape effect mediated by the diversity of the land cover. The parasitism rate was significantly influenced by the SH index only at the smallest scale and, through the exploration by the multivariate analysis and post-hoc approach, we confirmed the result. These findings support the hypothesis that parasitoids, with their limited body size, often show dispersal limitation (Ritchie & Olff 1999, Kruess & Tscharntke 1994, 2000).

In addition, we observed that the parasitism is high even in clusters characterized by a high abundance of olive orchards, where parasitoids may find the olive leaf gall midge in this season. The other two clusters with the lowest values of parasitism rate consisted of land use that did not provide alternative resources to parasitoids ("other land-use" cluster) or by high abundance of SNH and olive orchards but a low value of land diversity ("low landscape diversity" cluster).

Diversity of the landscape is the main driver of the natural enemy's effectiveness because diverse habitats could sustain different natural enemy's assemblages (Bianchi et al. 2006). Indeed, this could lead to an increasing rate of pest suppression (Greenop et al. 2018; Griffin et al. 2013; Letourneau et al. 2009). Diverse habitat types provide different resources and, in the case of parasitoids with multiple hosts that live in different plant species, the availability of food can be a key factor in the population dynamics of a parasitoid-host system. (Wackers 2002).

Moreover, our preliminary results on the composition of adjacent vegetation suggested that, by the parasitoids perspective, the quality of the landscape seems more relevant than the amount of SNH. The four fields consisted of different vegetation assemblages, as shown in the CA, and we noticed that where *Erica* sp. was sampled. and, to a lesser extent *P.communis* subsp. *pyraster*, the parasitism rate was high, regardless of the composition of the landscape. *Erica* sp. was present in both fields with high abundance of parasitoids, and where the parasitism rate reached 44% (site 26, Table 2) we found the highest cover of *Erica* sp.

These plants are both attacked by alternative hosts of *P. demades*, such as *Wachtliella ericina* (Low, 1885), a gall-inducing insect of Ericaceae (Ghahari & Buhl, 2011; Skuhrava e Skuhravy, 1994) and *Dasineura pyri* (Bouchè, 1847), the pear leaf midge.

Tondini et al. (unpublished results) used Malaise traps to evaluate the species and the abundance of parasitoids of *D. oleae* in olive orchards of the same province. These authors highlighted that *P. demades* was the most abundant olive leaf gall midge parasitoid and that its peak of abundance was in the same period as the *D. oleae* adults' peak (March - April).

We supposed that the presence of *Erica* sp. in the surroundings of the olive field is essential to support the population of *P.demades*, and thus, the role of this parasitoid deserves an appropriate deepening and further dedicated experimental approaches. So far, we could hypothesize that populations of *P. demades* hosted in *Erica* sp. in the adjoining vegetation, may quickly colonize olive orchards when *D. oleae* population increased.

In addition, parasitoids can find chemical cues to the host location. First, they use general cues emitted by different plant species that compose the host habitat and then intercept specific cues to locate accurately the host (Meiners et al., 2015, Silva and Clarke, 2020). Thus, different habitats with wild or cultivated species could promote the abundance of parasitoids and influence their effectiveness on *D. oleae* galls.

The evaluation of the efficacy of parasitoids performance should assess not only the overall landscape effect and the scale of observation but also the peculiarity of species considered. As Roland and Taylor (1997) suggest, the spatial structure of the habitat combined with animal movement strongly influence the host-parasitoid dynamics; therefore, in our opinion further studies should be addressed to clarify the complexity of trophic stimuli on *D. oleae*'s parasitoids, especially considering the ecology of *P.demades*.

Unexpectedly, we did not find a significant effect of the SNH abundance on the pest, even if usually is an important factor for pest control: the review made by Veres et al. (2013) summarizes that high proportions of semi-natural areas exhibited low pest abundance in adjoining cultivated fields.

Perennial non-crop habitats could provide nectar, pollen and alternative resources such as overwintering sites and refugees for antagonists. Specifically, the proportion of non-crop area in the landscape has been positively related to species richness (Schmidt et al. 2005), predators' abundance (Ali et al. 2020; Gardiner et al. 2009a) and subsequent suppression of pests as demonstrated in soybean (Gardiner, et al. 2009b) and oilseed rape (Thies and Tscharntke 2010). Further case studies should provide a reliable conclusion about this issue.

At 750 and 1000 m buffer scale, the leaf infestation increase as the diversity of the landscape increase. We expected that the pest increase where olive orchards are more abundant since *D. oleae* does not have any alternative host plant and spends the entire life cycle in the crop, to our knowledge. In addition, currently there is no specific pesticide formula against olive leaf gall midge. Therefore, the relationship between olive orchards abundance and the pest should have been logically positive. Moreover, olive orchards could be considered as a stable and structurally complex agroecosystem and the pest may not need to move over a field to find potential alternative food resources or a particular niche to complete its life cycle, except the olive canopy. In olive, there is also the key pest, the olive fruit fly, which increases its activity- density moving towards olive fields and in dominated-olive landscapes (Picchi et al. 2016). The availability of food resources is one of the main drivers for many pests (Power 1992). However, we found that the diversity of land cover supported a high level of infestation of olive leaves. A complex landscape may favour not only natural enemies but also pest population as found by in aphid in cereal crop by Thies et al., (2005) leading to an overall neutral effect of landscape on biological control.

Beyond the fact that data should be validated, this occurrence could be linked to certain factors. One hypothesis is that the increase of the land diversity, the natural enemies population may direct to other organisms, or rather become prey or hyperparasitized, thus less effective at large scale and this situation lead to vantages to the olive leaf galls midge (Roseheim, 1998).

Frere et al. (2007) call this situation “apparent mutualism”: if predators become satiated by higher densities of one alternative prey species, then a second prey species may benefit from the presence of the first, therefore the pest may benefit by the lack of spillover inside cultivated fields. Besides, by its peculiar structure, olive orchards may not be affected by spillover of functional diversity from the outside (Albertini et al. 2017; Picchi et al. 2019).

We do not want to exclude other factors that could mask some important environmental variables, so further analysis is needed to understand better how the landscape affects the ecology of such wasps and pests, beyond other abiotic factors such as temperature, rainfall photoperiod or other variables connected to the development of the olive tree.

More environmental variables of the landscape need to be taken into account such as the spatial configuration (Haan et al. 2020). Boccaccio and Petacchi (2009) investigated the relationship between landscape configuration and parasitoids of the olive fruit fly and the study revealed that parasitism is significantly affected by the presence of natural and semi-natural woodland in the landscape surrounding the orchard. The composition of the landscape affected on a medium scale (750 m) while fragmentation acted at a large scale (1 to 2 km buffer).

The landscape effect is a relevant factor to understand spatial dynamics and population trends for both pest and parasitoids.

In conclusion, our preliminary results suggest that both pest leaf infestation and parasitism rate are influenced by the diversity of the landscape.

In addition, a preliminary experiment in specific fields suggested that some plant species associated with alternative hosts of *D. oleae* parasitoids are related to high parasitism rates.

Such results came from the farmers' reports and the establishment of a local survey, a useful tool of IPM that allows knowing the current trends in pest and provide management guidelines to farmers. Together with sensible landscape management, it may help to preserve natural enemies, improve the reliability and effectiveness of the pest suppression service and prevent or reduce the severity of pest outbreaks.

Declarations

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

MSP, RP designed the survey. MSP, GM collect leaves and inspected the galls, NA made vegetation samplings and landscape analysis. ET analysed data. MSP wrote the manuscript. All authors read and approved the manuscript.

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Tables

Tab.1 Characteristics of the sampling sites, with geographical coordinates and meters above sea level (m.a.s.l.). For each site, the leaf infestation rate and parasitism rate were reported as the average of the sampling replication. In addition, we reported the sampling results through the number of parasitoids sampled, the total number of galls and the number of galls inspected and the percentage estimated on the total. The protocol consisted of the opening of at least one gall in each sprig. Moreover, the ratio between the number of galls per infested leaves was reported.

ID	*latitude*	*longitude*	*m.a.s.l.*	*Leaf infestation rate*	*Parasitism rate*	*Total Parasitoids*	*Total galls*	*Total opened galls*	*% of analysed galls*	*Galls/ infested leaf*
1	42.4303306	11.4337197	40	0.714	0.096	8	819	83	10.13	3.10
2	42.7885200	11.2292240	40	0.204	0.250	6	92	24	26.09	1.21
3	42.8928080	11.2170970	165	0.091	0.125	2	52	16	30.77	1.63
5	42.6452386	11.5062583	190	0.158	0.222	6	75	27	36.00	1.29
6	42.6576772	11.3991171	210	0.138	0.421	8	78	19	24.36	1.53
8	42.8032499	11.1496279	50	0.455	0.108	4	362	37	10.22	2.00
9	42.5564064	11.4117549	120	0.594	0.324	24	551	74	13.43	2.67
10	42.4368320	11.3756149	25	0.511	0.278	15	527	54	10.25	2.61
11	42.7606829	11.2556789	120	0.080	0.286	4	39	14	35.90	1.39
12	42.8033059	11.2074170	60	0.088	0.143	3	56	21	37.50	1.60
13	42.8301040	11.3064839	130	0.066	0.077	1	25	13	52.00	1.00
14	42.6393049	11.1280320	30	0.474	0.118	4	278	34	12.23	2.16
15	42.7939090	11.2768309	100	0.135	0.050	1	71	20	28.17	1.34
17	42.8677419	11.1483059	110	0.140	0.048	1	84	21	25.00	1.65
18	42.8919520	10.9283089	110	0.293	0.037	4	957	107	11.18	3.24
20	42.9852684	10.9389858	100	0.085	0.000	0	44	13	29.55	1.42
21	42.7160279	11.1733059	80	0.043	0.154	2	24	13	54.17	1.14
23	42.5447332	11.4365112	100	0.178	0.143	2	105	14	13.33	1.54
24	42.6018329	11.2507330	45	0.028	0.333	2	14	6	42.86	1.40
25	42.5661800	11.3767946	50	0.039	0.000	0	20	11	55.00	1.43
26	42.8503419	11.1089940	65	0.091	0.438	7	41	16	39.02	1.24
27	42.8276909	11.1889829	125	0.384	0.025	1	317	40	12.62	2.13
29	42.9166555	11.0178649	50	0.192	0.000	0	115	21	18.26	1.67
30	42.5733380	11.4680560	200	0.469	0.125	5	364	40	10.99	2.09

Tab.2 Effect of olive composition (OL), semi-natural habitats (SNH) composition and Shannon index (SH) A) on the leaf infestation made by the olive leaf gall midge *Dasineura oleae* and B) on the parasitism rate on the olive leaf gall midge *D. oleae.*

Buffer (m)	Outcome	Predictor	df	F value	*p* value
250	Parasitism rate	SH	1	7.6535	0.01126
500	//	//	//	//	//
750	Leaf infestation rate	SH	1	5.1812	0.03292
1000	Leaf infestation rate	SH	1	11.9310	0.00226

Tab. 3 List of plant species sampled in sites 18, 24, 25 and 26 of the survey on *Dasineura oleae* in Tuscany. Species that are known to be plants associated with alternative hosts of parasitoids from literature are crossed. Indeed, we reported for each sampling point plants that were sampled in 250 m buffer. Besides, some notes and the code associated with the CA (fig.3) are reported. At the bottom, the percentage of the abundance of olive and semi-natural habitats (SNH) with the parasitism rate and cover of associated plants are reported. The cover by *Erica* sp. and *Pyrus communis* subsp. *pyraster* is pointed out as they are host plants of alternative hosts of *Platygaster demades.*

	Families	Plants	Alternative host plants species (from literature)	Parasitoids (from literature)	Presence of associated plant in each sampling point				Notes	CA code
					ID 18	ID 24	ID 25	ID 26		
	Anacardiaceae	*Pistacia lentiscus* L.								Pi_len
	Asparagaceae	*Asparagus acutifolius* L.							Naturalized	As_acu
		Ruscus aculeatus L.								Ru_acu
	Cistaceae	*Cistus* sp.								Ci_sp
	Ericaceae	*Arbutus unedo*								Ar_une
		Erica sp.	X	*Platygaster demades* (Walker, 1835)		x		x		Er_sp
	Fabaceae	*Robinia pseudoacacia* L.							Invasive	Ro_pse
		Spartium junceum L.							Naturalized	Sp_jun
		Cytisus spinosus (L.)								Cy_spi
	Fagaceae	*Quercus cerris* L.	X	*Mesopolobus mediterraneus* (Mayr, 1903)		x				Qu_cer
		Quercus ilex L. subsp. *ilex*	X	*Mesopolobus mediterraneus* (Mayr, 1903)		x				Qu_ile
		Quercus sp.	X	*Mesopolobus mediterraneus* (Mayr, 1903)	x	x	x			Qu_sp
		Quercus suber L.	X	*Mesopolobus mediterraneus* (Mayr, 1903)				x	Alien	Qu_sub
	Lamiaceae	*Lavandula stoechas L.* subsp. *stoechas*								La_sto
	Lauraceae	*Laurus nobilis* L.							Naturalized	La_nob
	Myrtaceae	*Myrtus communis* L.								My_com
	Oleaceae	*Fraxinus ornus L.* subsp. *ornus*								Fr_orn
		Phillyrea sp.								Ph_sp
	Poaceae	*Arundo donax* L.							Invasive	Ar_don
	Ranunculaceae	*Clematis vitalba* L.	X	*Mesopolobus mediterraneus* (Mayr, 1903)			x			Cl_vit
	Rhamnaceae	*Paliurus spina-christi* Mill.							Naturalized	Pa_spi
	Rosaceae	*Crataegus monogyna* Jacq.								Cr_mon
		Pyrus communis subsp. *pyraster (L.) Ehrh.*	X	*Platygaster demades* (Walker, 1835) *Mesopolobus mediterraneus* (Mayr, 1903)		x		x		Py_pyr
		Rosa canina L.								Ro_can
		Rubus ulmifolius Schott								Ru_ulm
	Santalaceae	*Osyris alba* L.								Os_alb
	Smilacaceae	*Smilax aspera* L.								Sm_asp
	Ulmaceae	*Ulmus* sp.								Ulm_sp
	Viburnaceae	*Sambucus* sp.								Sam_sp
	-	-	-	-	-	-	-	-		

Total	*18*	*30*	*7*	_	1	5	2	3		
% Olive orchards					56,31%	9,59%	19,03%	43,81%		
% SNH					32,35%	16,58%	17,58%	37,67%		
Parasitism Rate					5%	17%	0%	44%		
Host Plant Cover					22,46%	63,2%	2,3%	82,13%		
Cover Pyrus communis subsp. *pyraster*					0%	1%	0%	0%		
Cover Erica sp.					0%	4,6%	0%	75,3%		

Figures

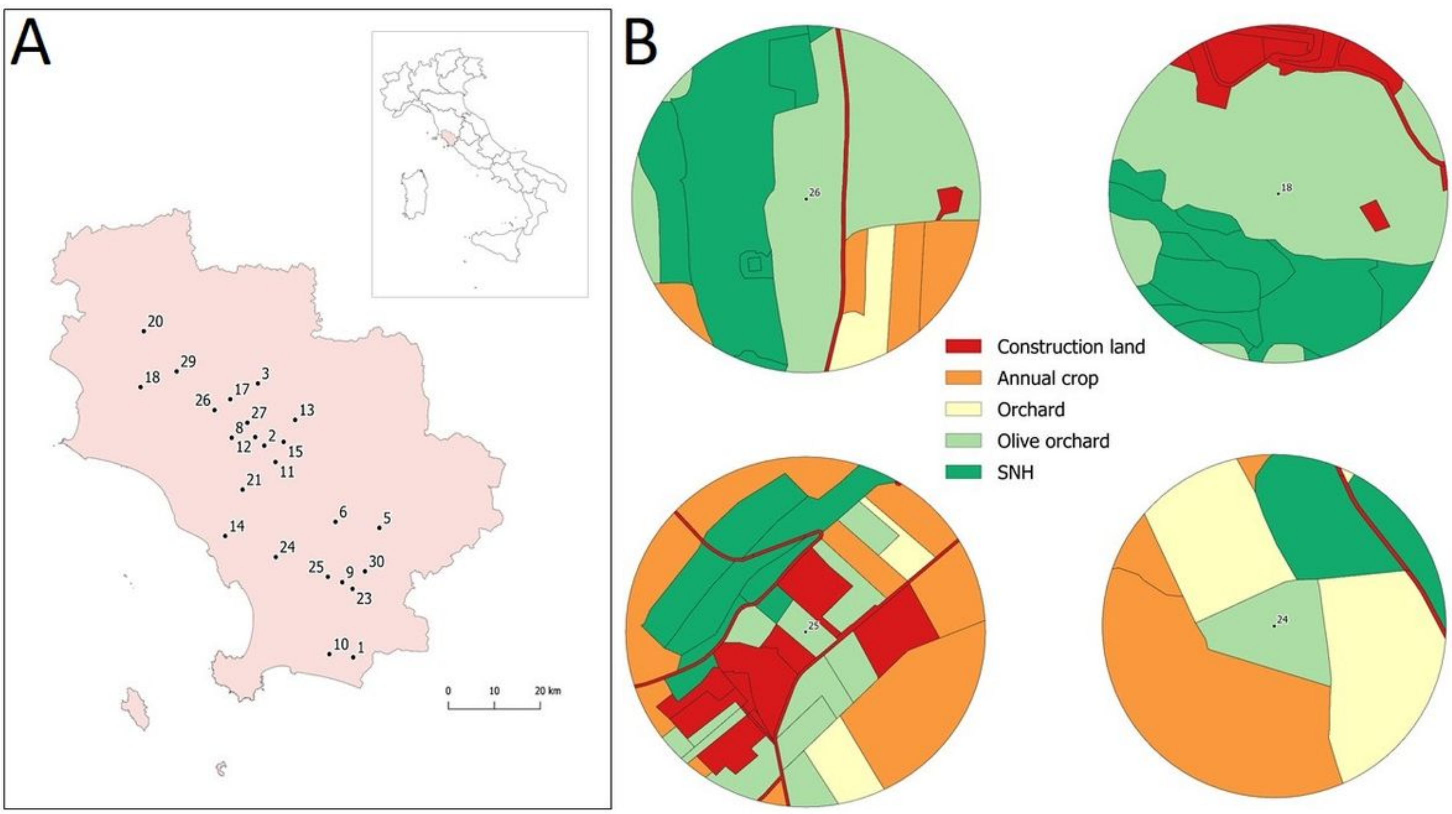

Figure 1

A) Map of the 24 olive orchards that have been part of the survey on Dasineura oleae. The olive orchards are located in the province of Grosseto (Central Italy). B) Landscape patterns, at 250 m buffer, of focal fields (18, 24, 25 and 26) where we evaluated the botanical composition in the adjacent SNH.

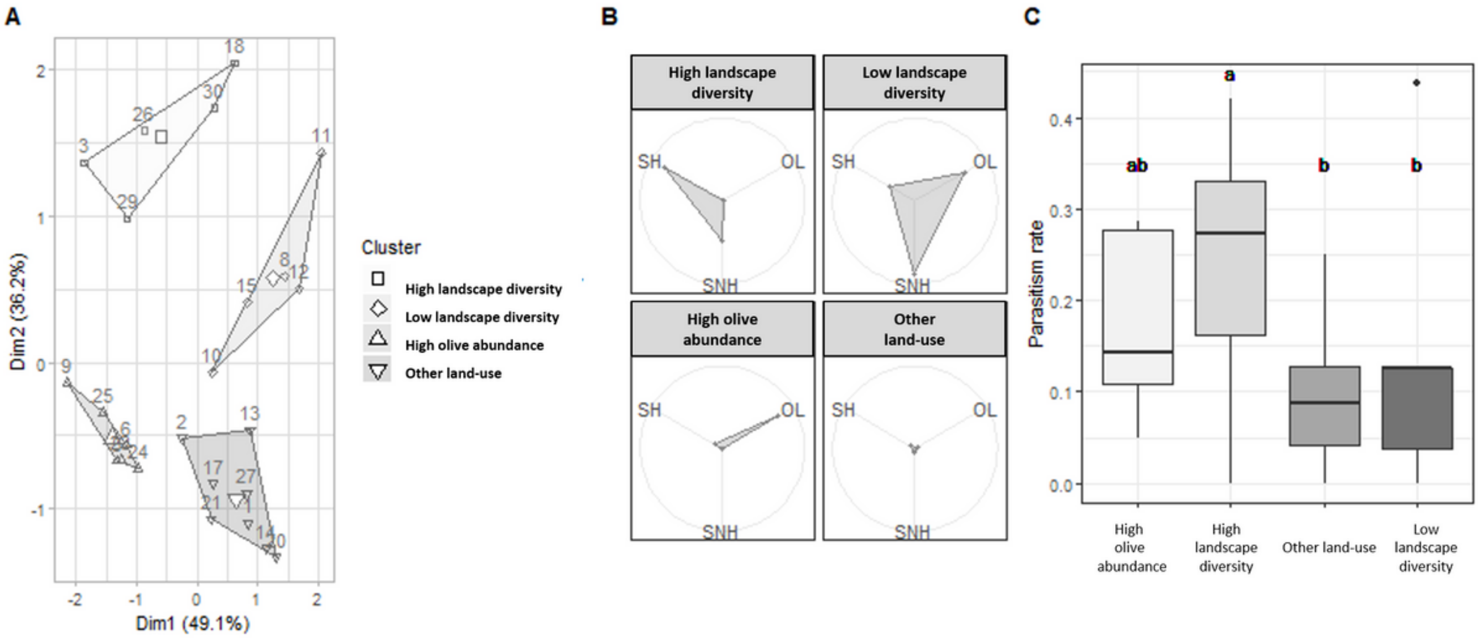

Figure 2

The cluster analysis of parasitism rate in 250 m buffer: A) the olive fields as clustered by environmental variables. B) the structure of each cluster through environmental variables: olive composition (OL), semi-natural habitat composition (SNH) and Shannon landscape diversity index (SH). C) The boxplot of the parasitism rate for cluster, considering the mean value. In addition, the Tukey post-hoc results are reported.

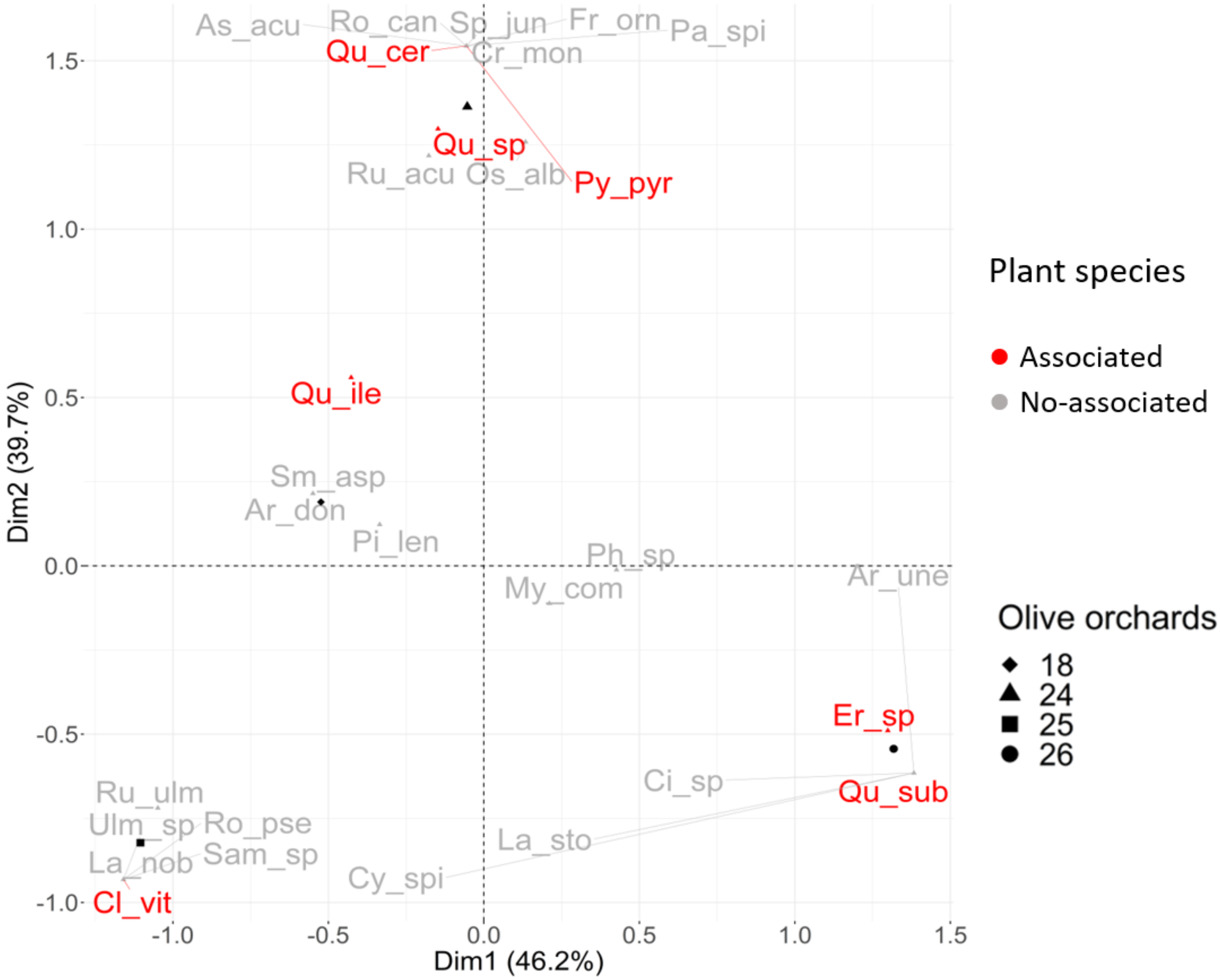

Figure 3

Correspondence Analysis of the vegetation composition of focal olive orchards. Plants associated with alternative hosts of Dasineura oleae parasitoids are reported in red. The legend is in the CA code column in Tab. 3.