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ENVIRONMENTAL FACTORS IMPACT AND INCIDENCE OF PARASITISM OF *PSYLLAEPHAGUS BLITEUS* RIEK (HYMENOPTERA ENCYRTIDAE) ON POPULATIONS OF *GLYCASPIS BRIMBLECOMBEI* MOORE (HEMIPTERA APHALARIDAE) IN MEDITERRANEAN CLIMATIC AREAS ([1])

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Caleca V., Bella S., La Pergola A., Lombardo A., Lo Verde G., Maltese M., Nucifora S., Rizzo R., Tortorici F., Suma P., Rapisarda C. – Environmental factors impact and incidence of parasitism of *Psyllaephagus bliteus* Riek (Hymenoptera Encyrtidae) on populations of *Glycaspis brimblecombei* Moore (Hemiptera Aphalaridae) in Mediterranean climatic areas.

The red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera, Aphalaridae), is an Australian native sap-sucking insect pest of eucalypts that has been first reported for the West Palaearctic Region in 2008 and, in 2010, it has been found also in Italy. Subsequently its primary parasitoid, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), was also detected within the main European and North African infested areas, where no release of the parasitoid was ever performed. This study, carried out in 30 *Eucalyptus camaldulensis* plantations located along the coast, on the hills and the mountains in Mediterranean climatic areas of Sicily (Italy), aimed to determine the influence of environmental parameters on the incidence of both, the psyllid infestation level and the parasitization activity. *P. bliteus* reached highest average levels in summer samplings and resulted widespread in Sicily at all detected altitudes without statistically significant differences. *P. bliteus* parasitization is the main factor lowering *G. brimblecombei* infestation; this result, together with the accidental and contemporaneous arrival of the host and its parasitoid, could explain the absence of high damage level on eucalypts in Sicily. The most significant metric factors positively influencing *G. brimblecombei* infestation are the percentage of daily hours above 80% of relative humidity and the average maximum temperature, obviously related to other, but less significant climatic factors. The altitude affects both infestation and parasitization, but single sites could explain significantly more, so that the local conditions where the samplings were carried out have to be considered as the main responsibles for the variability in the obtained results. In any sampled Sicilian site, from sea level to 540 m a.s.l., both the psyllid and its parasitoids show a good adaptation to climatic conditions, confirming that areas fitting for *E. camaldulensis* growth fit also for *P. bliteus* activity, and proving that Mediterranean climate, differently from some inland areas of California, does not obstacle its parasitic activity.

KEY WORDS: Red gum lerp psyllid, Sicily, General Linear Model, Relative Humidity, Temperature.

INTRODUCTION

Glycaspis brimblecombei Moore (Hemiptera, Aphalaridae), commonly known as the red gum lerp psyllid, is an Australian native pest of *Eucalyptus* trees, which in the last years spread in many other regions.

It was detected outside Australia for the first time in California (USA), in 1998 (GILL, 1998; BRENNAN *et al.*, 1999); afterwards it was found in Hawaii (NAGAMINE and HEU, 2001), Central and South America (Mexico, CIBRIÁN *et al.,* 2001), Chile (SANDOVAL and ROTHMANN, 2002), Brazil (WICKEN *et al.*, 2003), Argentina (BOUVET *et al.*, 2005), Ecuador (ONORE and GARA, 2007), Venezuela (ROSALES *et al.*, 2008), Peru (BURCKHARDT *et al.*, 2008), El Salvador (JIMENEZ, 2013), Colombia (RODAS *et al.*, 2014) Uruguay (BALDINI *et al.*, 2006), Canary Islands (MALUMPHY, 2010), Morocco (BAMI, 2011, IBNELAZYZ, 2011), Algeria (REGUIA and PERIS-FELIPO, 2013), Tunisia (BEN ATTIA and RAPISARDA, 2014; DHAHRI *et al.*, 2014), Mauritius (SOOKAR *et al.*, 2013), and Madagascar (HOLLIS, 2004). From 2008 the psyllid was found in some European countries: Spain and Portugal (HURTADO and REINA, 2008;), France (COCQUEMPOT

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et al., 2012), Montenegro (MALUMPHY *et al.*, 2013), Greece (BELLA and RAPISARDA, 2013; REGUIA and PERIS-FELIPO, 2013). In Italy it has been recorded for the first time in Southern and Central regions in 2010 (LAUDONIA and GARONNA, 2010) and new information about pest spread were given by GARONNA *et al.* (2011) and PERIS-FELIPO *et al.* (2011). In 2011 it was recorded in Sicily (LO VERDE *et al.*, 2011), and few months after it was also detected in Sardinia (EPPO, 2011); now it is widespread in the whole country in areas where *Eucalyptus camaldulensis* Dehnh. is planted.

The host plant, like other species of the genus *Eucalyptus*, is very common in Italy as ornamental and forest species. As a consequence, serious damage was recorded on eucalyptus trees in parks, urban areas and plantations, and also the Italian beekeepers worried because of the dramatically lower quality and quantity of eucalyptus honey productions (GARONNA *et al.*, 2011; PIBIRI, 2011).

G. brimblecombei is a very aggressive exploiter of resources and for this reason its damage is important; outbreaks have been reported from many countries of South America were the species can cause the death of infested trees, resulting in serious timber production losses (DE QUEIROZ *et al.*, 2012).

The psyllid can be easily detected observing the presence on leaf surfaces of the characteristic white cones (lerps) produced by the nymphs which also serve as shelter until they reach adulthood (HALBERT *et al.*, 2001; BELLA, 2013). In California high population levels are reported to cause leaf fall, a decrease in growth rate, and in some cases death of the infested plant (GILL, 1998; DAHLSTEN *et al.,* 2005); eucalypt mortality rates can reach 15% in the first year of attack and up to 40% in the second year if efforts are not made to control the pest (GILL, 1998).

Biological control is a major component of psyllid IPM; nowadays the red gum lerp psyllid seems to be under control after the introduction of the parasitoid wasp *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae). In the native areas the endemic *Psyllaephagus* species attack nymphs of Psylloidea, and a few are reported as hyperparasitoids attacking other *Psyllaephagus* species (RIEK, 1962; NOYES and HANSON, 1996). *P. bliteus* parasitizes *G. brimblecombei* and other psyllids as: *Boreioglycaspis melaleucae* Moore on *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae); *Creiis costatus* (Froggatt), *Ctenarytaina eucalypti* (Maskell), *Glycaspis granulata* (Froggatt) and *Glycaspis* sp. on *Eucalyptus* spp. (RIEK, 1962; HERTING, 1972; WITHERS, 2001; DAHLSTEN *et al.,* 2002; DAANE *et al.*, 2005; BERRY, 2007). The first classical biological control attempt against *G. brimblecombei* was performed in California collecting *P. bliteus* from its native area (i.e. Australia), then releasing it from the end of 1999 to 2003 (PAINE *et al.,* 2000; DAHLSTEN *et al.*, 2005). *P. bliteus* has also been deliberately introduced for classical biological control against *G. brimblecombei* into Mexico (PLASCENCIA *et al.,* 2005) and Chile (IDE *et al.*, 2006).

The arrival of *P. bliteus* in Italy (Sicily: CALECA *et al.*, 2011a; Italian peninsula: LAUDONIA *et al.*, 2014) is due to an accidental introduction, probably together with its host, as also happened in New Zealand, Brazil, Spain, Morocco, Greece, Tunisia, Portugal and Turkey (WITHERS, 2001; BERTI-FILHO *et al.*, 2003; BERRY, 2007; PEREZ-OTERO *et al.*, 2011; BAMI, 2011; BELLA and RAPISARDA, 2013; BELLA, 2014; DHAHRI *et al.*, 2014; KARACA *et al.*, 2015).

In the framework of an IPM programme, the monitoring activity results highly important to determine the moment of pest population peak, the occurrence of natural enemies and to estimate other factors that can affect the population dynamic.

As it is known, the climatic factors can affect both the pest population density and parasitization level (SANTANA *et al.*, 2003b; BELLA and RAPISARDA, 2014; FERREIRA FILHO *et al.*, 2015).

Studies conducted in California showed that the pest population suppression by the encyrtids performed better in coastal sites than in inner arid areas, due to the higher summer temperatures herein recorded that reduced the efficacy of the parasitoid activity; still, as psyllid numbers have dropped, the defoliation and death of E*ucalyptus* trees due to the psyllid have been reduced (DAANE *et al.*, 2005, 2012). In Brazil, a decrease of *G. brimblecombei* population was recorded in the late spring and early summer in function of the rainfall frequency or intensity (DE QUEIROZ *et al.*, 2012).

In Portugal DHAHRI *et al.* (2014) report how the infestation levels by *G. brimblecombei* were not significantly affected by the different latitudes and longitudes of the studied sites recording also a relatively low parasitism rate.

Recently in Italy, in areas where *P. bliteus* was absent*,* it has been suggested that *G. brimblecombei* population size in the new area of colonization is negatively affected by low winter temperatures, but also by high temperatures in the absence of rainfall (LAUDONIA *et al.*, 2014).

In the present paper the distribution and activity of *G. brimblecombei* and *P. bliteus* in a Mediterranean area, *E. camaldulensis* plantations distributed in all Sicily, were studied in function of some environmental factors (i.e. altitude and climatic conditions) discussing their importance from an applied point of view.

MATERIALS AND METHODS

PRELIMINARY SAMPLINGS ON *P. BLITEUS* DISTRIBUTION IN SICILY

A first preliminary survey on the distribution of *P. bliteus* in Sicily was performed from December 2011 to February 2012, in order to ascertain the presence of the parasitoid in the whole island. Totally 20 sites have been investigated, most of which along the coast due to the cold period of sampling. Each sample consisted of leaves of *E. camaldulensis* bearing a total number of at least 100 fully developed lerps. Lerps were analyzed to detect the exit hole of the parasitoid. In the laboratory lerps without a hole were removed counting the number of underlying clearly parasitized hosts (mummies). Psyllid nymphs found on the leaves have been reared at room temperature (for about two weeks) until adults emerged.

SEASONAL SAMPLINGS

After this preliminary survey that ascertained the presence of *P. bliteus* also in eastern Sicily (Fig. I), widening its distribution in the western areas of the island already stated by CALECA *et al.* (2011a), data regarding *G. brimblecombei* infestation level and *P. bliteus* parasitization level have been collected in Sicilian *E. camaldulensis* plantations located in areas that fits with the good development of this tree. Thirty sampling sites were chosen from the coast to the interior zones at three altitudinal ranges, as following: 3-52 m a.s.l. (Coast), 101-356 m a.s.l. (Hill) and 418-542 m a.s.l. (Mountain), corresponding to an average yearly temperature in 1965-1994 of 18-19°C, 17-18°C and 16-17°C respectively (Fig. II, Tab. 1).

Seven samplings were performed in each site: two in spring 2012 (18-28 Apr.; 30 May-6 Jun.), two in the following summer (12-24 Jul.; 28 Aug.-6 Sep.), two in the following autumn (11-18 Oct.; 27 Nov.-19 Dec.) and the last one in winter 2013 (1-19 Feb.).

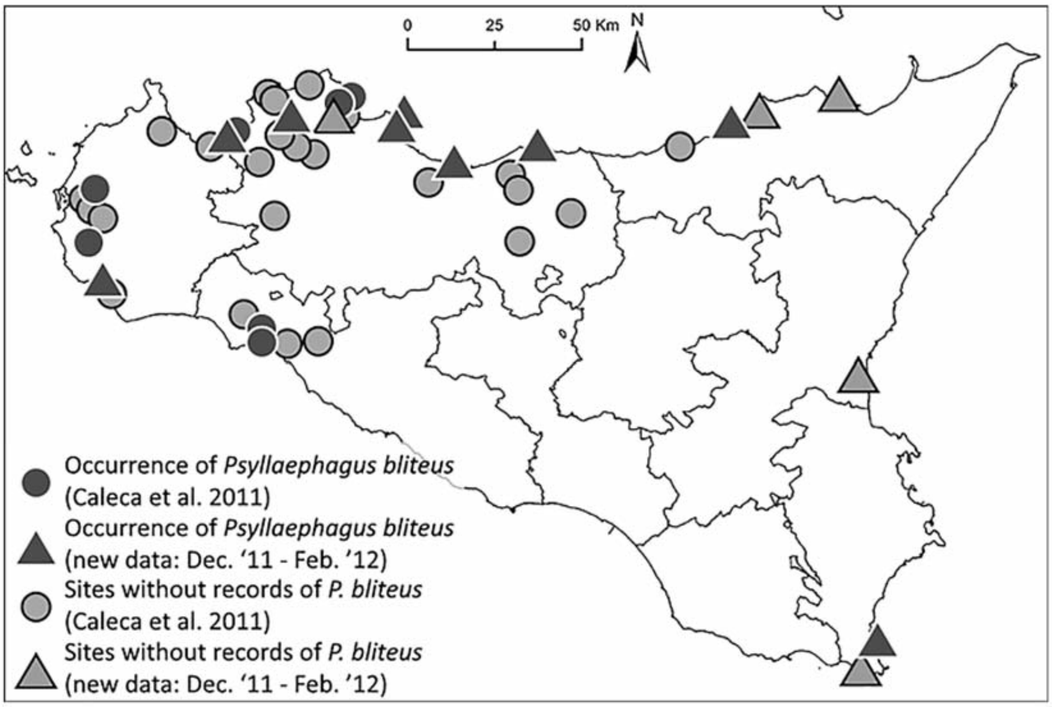


Fig. I – Occurrence of *Psyllaephagus bliteus* in Sicily, recorded from September 2011 to February 2012 (after Caleca *et al.*, 2011a, modified by addition of new data).

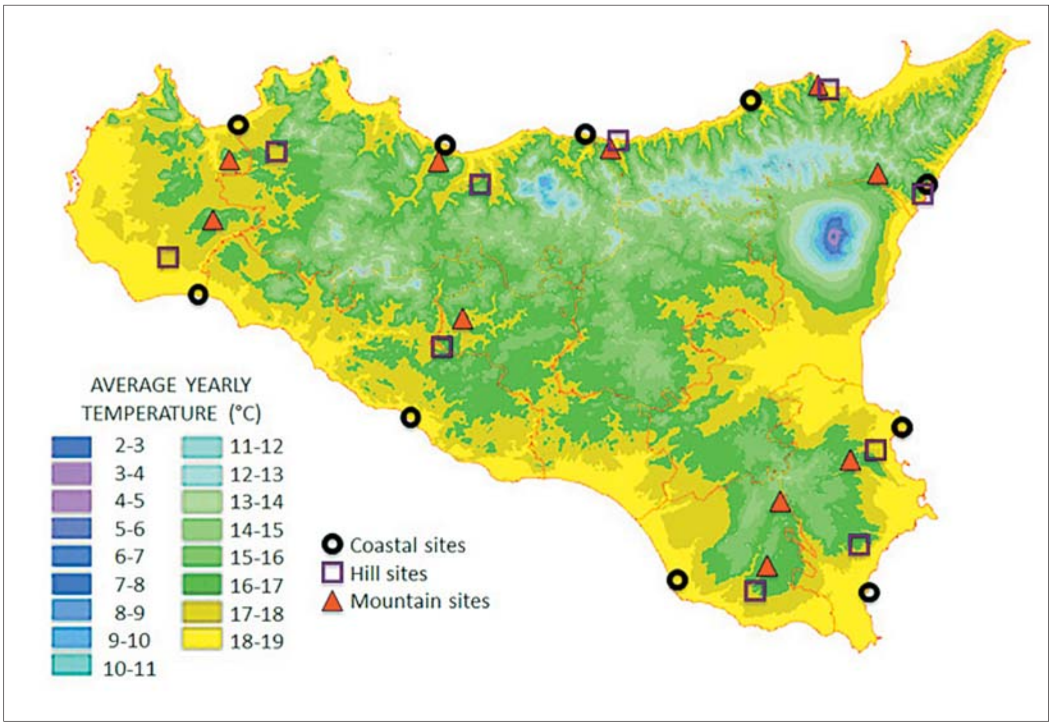


Fig. II – Average yearly air temperature in the period 1965-1994, based on data from the Sicilian Regional Hydrographic Service (after Drago, 2005), and localization of the 30 sampled sites.

At each sample date, in each site 20 leaves have been randomly collected from 5 trees, at man height in all four cardinal directions, reaching a total of 100 leaves. In this case the sampling time to collect 100 leaves has been set in 2 minutes. In order to have a larger set of data, if a total of 100 full mature lerps was not reached with the former sample, an additional sample was performed, collecting leaves bearing mature lerps directly from eucalyptus trees, recording the sampling time. In the laboratory the number of live 4^{th}-5^{th} instar nymphs, mummies and pierced mummies on each leaf has been counted. The final measure of the infestation was calculated as the number of live 4^{th}-5^{th} instar nymphs (including live mummies) collected per minute by one sampler in the field.

Regarding *P. bliteus* parasitization, all sampled leaves, after counting psyllid live instars, were put in glass jars,

Table 1 – List of all sampling sites, their latitude, longitude, altitude and cumulative rainfall (mm) recorded in 45 days preceding each sampling.

Site Nr.	Site name	Latitude N	Longitude E	Group of sites	Altitude (m a.s.l.)	Rainfall of 45 days preceding the sampling						
						Spring samples		Summer samples		Autumn samples		Winter sample
						1st	2nd	3rd	4th	5th	6th	7th
1	Augusta	37°14'36.35"	15°12'16.20"	Coast	3	111	13	0	48	19	139	31
2	Vendicari	36°48'15.70"	15°05'34.10"	Coast	5	61	2	1	0	22	101	75
3	Selinunte	37°35'11.26"	12°51'42.04"	Coast	11	55	18	5	7	115	194	125
4	Randello	36°50'25.00"	14°27'38.80"	Coast	15	113	3	0	0	119	137	74
5	Rocca di Caprileone	38°06'40.30"	14°42'33.46"	Coast	16	95	84	0	14	69	151	181
6	San Leone	37°16'08.92"	13°34'38.10"	Coast	17	82	5	0	15	59	166	81
7	Termini Imerese	37°59'19.10"	13°41'11.56"	Coast	29	50	16	1	24	49	151	169
8	Letojanni	37°52'55.25"	15°18'09.55"	Coast	30	107	32	6	9	38	145	48
9	Pollina	38°01'21.61"	14°09'11.77"	Coast	33	50	39	0	1	33	106	167
10	Balestrate	38° 02'25.80"	12°59'20.35"	Coast	52	104	28	6	12	81	159	262
11	Patti	38°08'47.34"	14°57'43.28"	Hill	101	69	38	0	6	46	84	236
12	Tusa bassa	38°00'08.56"	14°15'49.60"	Hill	125	40	25	0	21	30	111	162
13	Lago Trinità	37°41'08.33"	12°45'36.52"	Hill	168	79	27	4	13	90	202	169
14	Partinico	37°58'16.39"	13°07'04.59"	Hill	195	95	39	4	21	81	168	223
15	Scicli	36°48'45.00"	14°42'57.00"	Hill	200	142	1	0	6	92	131	104
16	Grotte	37°27'22.77"	13°40'43.54"	Hill	242	83	17	2	15	146	216	131
17	Taormina	37°51'42.00"	15°17'01.10"	Hill	250	No climatic datum						
18	Noto	36°55'55.00"	15°03'32.90"	Hill	320	155	12	0	6	52	130	104
19	Melilli	37°10'49.60"	15°06'49.60"	Hill	350	140	7	0	3	45	124	106
20	Cerda	37°53'13.63"	13°48'09.95"	Hill	356	57	27	2	19	53	212	227
21	Caccamo	37°57'02.70"	13°40'10.22"	Mountain	418	No climatic datum						
22	Francavilla di Sicilia	37°54'36.65"	15°07'42.60"	Mountain	425	178	92	0	12	53	122	131
23	Alcamo	37°56'45.46"	12°57'42.83"	Mountain	439	93	54	2	14	92	176	234
24	Sortino	37°09'35.80"	15°01'57.70"	Mountain	450	No climatic datum						
25	Modica	36°52'38.70"	14°45'12.70"	Mountain	460	No climatic datum						
26	Sorrentini	38°09'05.36"	14°56'20.77"	Mountain	478	74	64	0	16	78	176	334
27	Sutera	37°31'49.11"	13°44'42.34"	Mountain	501	135	30	2	11	47	116	147
28	S. Ninfa	37°47'05.12"	12°54'38.63"	Mountain	508	103	49	6	8	71	143	173
29	Giarratana	37°02'53.50"	14°47'58.00"	Mountain	520	309	13	0	38	83	76	105
30	Tusa	37°58'58.03"	14°14'20.21"	Mountain	542	63	42	0	27	16	114	179
Average coastal sites					21	83	24	2	13	60	145	121
Average hill sites					231	96	22	1	12	71	153	162
Average mountain sites					474	136	49	1	18	63	132	186

topped with a cotton cloth, to avoid excess of moisture, and maintained at room temperature for at least 15 days to allow the emergence of both adults of the psyllid and the parasitoid. Parasitism rate was calculated as ratio between emerged parasitoids and all emerged adults (parasitoids + psyllids).

The climatic data of each site refer to the ones deriving from the closest available fitting station of the Sicilian Agrometeorological Information Service (SIAS) of the Sicilian Region that kindly provided the following weather recordings: rainfall (total amount, number of events, intensity), relative humidity (daily percentage of RH>80% and of RH<40%), air temperature (daily maximum, minimum and average). Rainfall intensity rates refer to the classification by the World Meteorological Organization (WMO, 2014) for precipitation in the form of liquid water drops that have diameters greater than 0.5 mm:
– light rain, <0.41 mm/10 min;
– moderate rain, 0.41-1.6 mm/10 min;
– heavy rain, >1.6 mm/10 min.

These daily data have been grouped and referred to the 45 days before each sampling date. Climatic data were not available for four out of thirty sampling sites (one hill site and three mountain sites), because no SIAS station fits with them (Tabb. 1-2).

STATISTICAL ANALYSIS

Data were analysed using the General Linear Model, in which the response variable is "y" and the input variables are categorical and metric.

The first analysis regards *G. brimblecombei* infestation expressed as number of live 4^{th}-5^{th} nymphs collected per minute by one sampler (Inf/min). In order to apply the most common parametric statistics, the assumption of normality has to be satisfied. The original set of data was far from this distribution, but a simple transformation has been able to normalize data, y = log (1+Inf/min).

Input variables, categorical and metric:

– Sampling period, 1 (18-28 Apr. 2012), 2 (30 May-6 Jun. 2012), 3 (12-24 Jul. 2012), 4 (27 Aug.-6 Sep. 2012), 5 (11-18 Oct. 2012), 6 (27 Nov.-6 Dec. 2012), 7 (1-19 Feb. 2013).
– Site (see Tab. 1).
– Altitude, this metric variable has been used in alternative to Site.
– Parasitization (calculated as above described).
– Parasitization at the previous sampling.
– Light rain events (n) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
– Moderate rain events (n) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Heavy rain events (n) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Total light rain (mm) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
– Total moderate rain (mm) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Total heavy rain (mm) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Total rainfall (mm), recorded in 45 days preceding the sampling.
– Average maximum temperature of 45 days preceding the sampling.
– Average temperature of 45 days preceding the sampling.
– Average minimum temperature of 45 days preceding the sampling.
– Percentage of daily hours with Relative Humidity >80%, recorded in 45 days preceding the sampling.

Table 2 – Average air temperature (°C) recorded in 45 days preceding each sampling.

Site number	Site name	Spring samples		Summer samples		Autumn samples		Winter sample	Average 1st-7th samples
		1st	2nd	3rd	4th	5th	6th	7th	
1	Augusta	12.7	17.1	25.5	26.7	23.5	17.9	10.5	19.1
2	Vendicari	14.0	17.9	24.5	27.4	24.7	19.2	12.4	20.0
3	Selinunte	13.0	17.3	25.2	26.1	21.7	17.7	11.3	18.9
4	Randello	15.2	18.1	24.9	27.7	24.0	18.6	11.6	20.0
5	Rocca di Caprileone	14.7	17.3	25.0	26.8	24.4	18.9	11.6	19.8
6	San Leone	13.4	17.3	24.6	26.0	22.9	18.3	11.5	19.2
7	Termini Imerese	14.2	18.2	24.5	25.4	22.7	14.5	10.4	18.6
8	Letojanni	12.9	16.4	23.2	26.4	22.8	17.7	11.6	18.7
9	Pollina	14.2	16.7	24.4	26.0	23.2	16.7	10.4	18.8
10	Balestrate	14.2	18.0	25.1	26.3	23.0	17.1	11.0	19.2
11	Patti	13.4	16.1	24.0	25.6	23.1	16.2	9.9	18.3
12	Tusa bassa	14.8	16.9	25.0	26.5	23.7	17.0	10.3	19.2
13	Lago Trinità	12.8	17.2	25.6	26.4	21.9	16.9	9.9	18.7
14	Partinico	13.4	17.5	24.2	25.1	21.9	15.2	9.7	18.2
15	Scicli	13.2	17.1	25.2	27.7	23.0	18.1	10.4	19.2
16	Grotte	11.7	18.2	27.4	27.9	22.7	16.2	9.0	19.0
18	Noto	12.4	17.1	26.5	27.7	23.5	18.1	10.2	19.4
19	Melilli	12.4	17.1	25.2	27.7	23.0	18.1	10.6	19.2
20	Cerda	13.1	18.0	24.2	25.2	22.4	13.7	9.4	18.0
22	Francavilla di Sicilia	12.1	15.9	24.0	26.9	22.4	13.9	9.4	17.8
23	Alcamo	11.3	15.3	24.7	24.8	20.5	13.2	7.6	16.8
26	Sorrentini	13.9	15.9	24.6	25.9	22.6	15.4	9.1	18.2
27	Sutera	10.6	15.1	24.4	24.9	20.0	14.6	7.4	16.7
28	S. Ninfa	11.9	16.0	25.7	26.0	21.7	16.3	8.8	18.1
29	Giarratana	11.7	15.4	24.2	26.7	21.6	15.1	8.0	17.5
30	Tusa	13.5	15.5	24.0	25.5	22.4	15.9	8.6	17.9
Average coastal sites		13.9	17.4	24.7	26.5	23.3	17.6	11.2	19.2
Average hill sites		13.0	17.3	25.3	26.7	22.8	16.6	9.9	18.8
Average mountain sites		12.2	15.6	24.5	25.8	21.6	14.9	8.4	17.6

– Percentage of daily hours with Relative Humidity <40%, recorded in 45 days preceding the sampling.

Output variable:
y= log (1+Inf/min)

In order to single out factors influencing *P. bliteus* parasitization, all variables have been taken into account, including the infestation level (this time used as input variable) and the lagged infestation level referred to the previous period.

Input variables, categorical and metric:
– Sampling period, 1 (18-28 Apr. 2012), 2 (30 May-6 Jun. 2012), 3 (12-24 Jul. 2012), 4 (27Aug.-6 Sep. 2012), 5 (11-18 Oct. 2012), 6 (27 Nov.-6 Dec. 2012), 7 (1-19 Feb. 2013).
– Site (see Tab. 1).
– Altitude, this metric variable has been used in alternative to Site.
– Infestation= log (1+Inf/min).
– Infestation of the previous sampling.
– Light rain events (n) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
– Moderate rain events (n) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Heavy rain events (n) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Total light rain (mm) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
– Total moderate rain (mm) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Total heavy rain (mm) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
– Total rainfall (mm), recorded in 45 days preceding the sampling.
– Average maximum temperature of 45 days preceding the sampling.
– Average temperature of 45 days preceding the sampling.
– Average minimum temperature of 45 days preceding the sampling.
– Percentage of daily hours with Relative Humidity >80%, recorded in 45 days preceding the sampling.
– Percentage of daily hours with Relative Humidity <40%, recorded in 45 days preceding the sampling.

Output variable:
y= Parasitization

In order to single out the best model, a progressive elimination of variables has been performed, dropping out variables one by one as they show the lowest significance level (higher P-value). In this way we can obtain the model showing the highest global significance with the lowest number of variables.

For what concerns weather conditions, as the variables are closely correlated with each other, it is reasonable that only a few of them have been selected in the final model.

As regards the influence of altitude, an alternative model has been tested; here only the altitude of the site is taken into account instead of the different sites. This model is more parsimonious, because it uses just one degree of freedom instead of 25.

RESULTS

Rainfall and average temperature recorded in the sampling period are shown in Fig. III and Tabb. 1, 2; these records are close to those recorded in 1965-1994 (DRAGO, 2005).

P. bliteus was the only parasitoid emerged from *G. brimblecombei*; it has been recovered in all 30 sampled sites, confirming data from our preliminary survey performed in winter 2011-12 and stating that the parasitoid is widespread all over Sicily.

G. brimblecombei infestation and *P. bliteus* parasitization levels recorded in all seasonal samplings are shown in Figs IV-VII, while the average levels recorded in each altitudinal

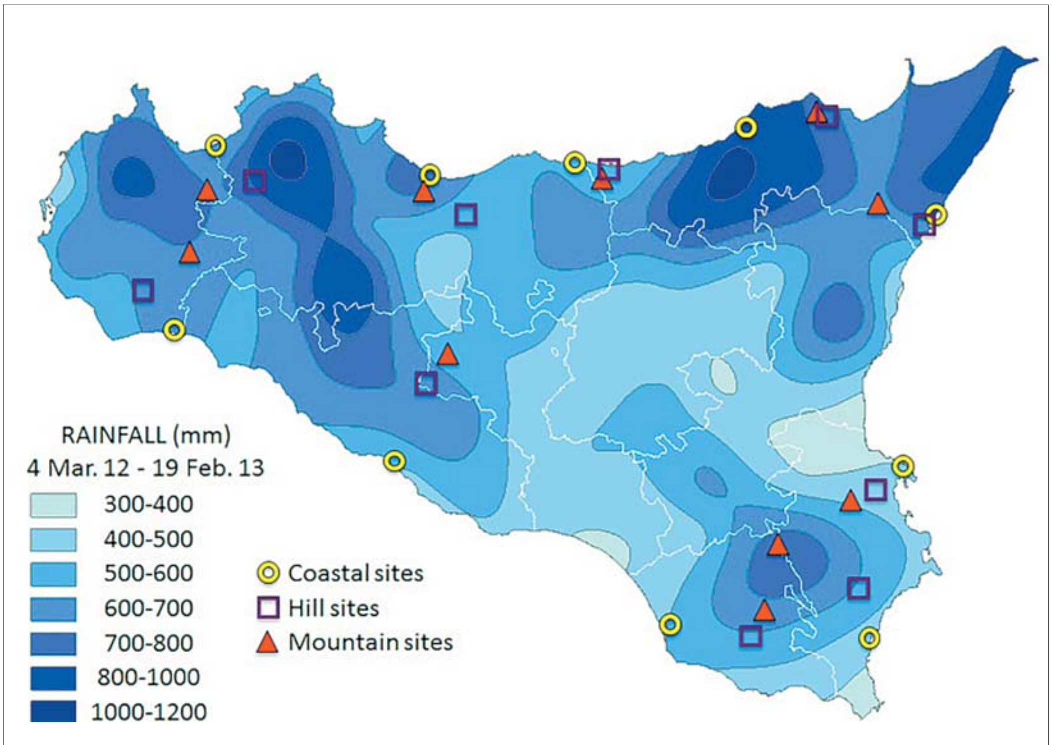
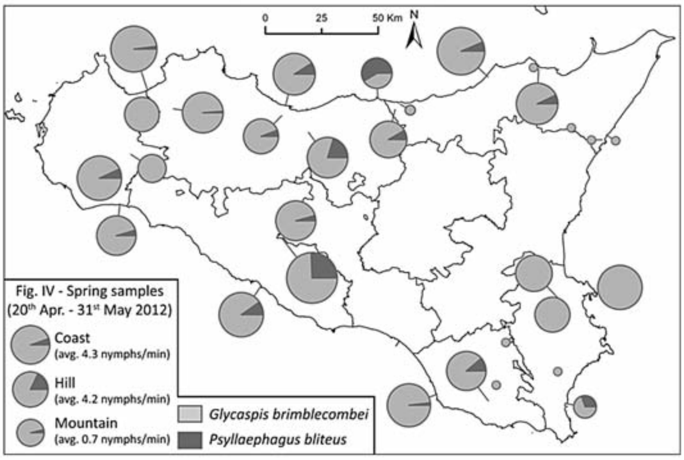
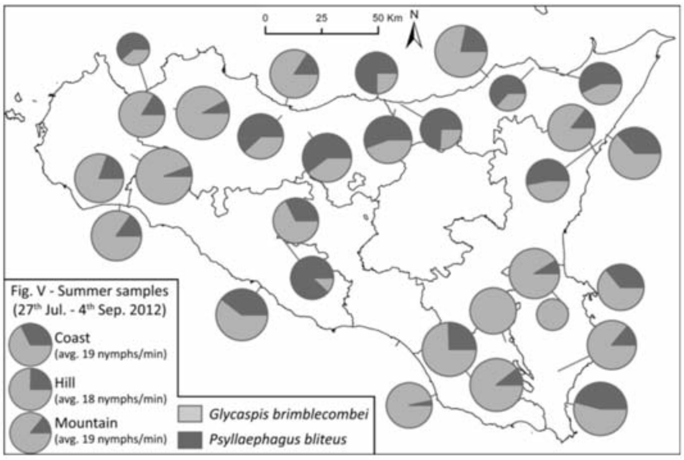
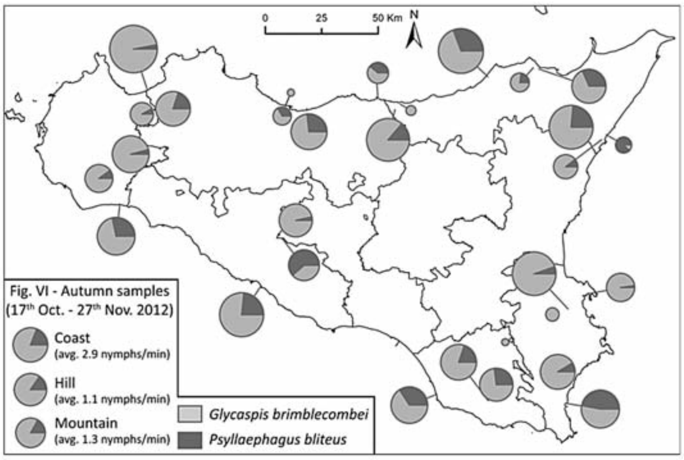
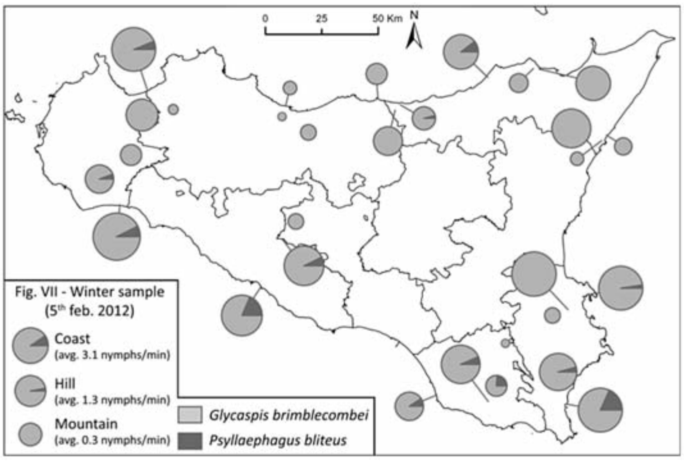


Fig. III – Sampled sites and map of rainfall recorded in Sicily during the sampling period, 4 Mar. 2012-19 Feb. 2013 (map kindly drawn by Luigi Pasotti using all daily data coming from SIAS stations).









Figs – IV-VII – *Glycaspis brimblecombei* infestation (4th-5th nymphs collected per minute per person) and *P. bliteus* parasitization (%) recorded by seasonal samplings in all 30 Sicilian sites; pie chart variation size is normalized by log.

group of sites is shown in Fig. VIII. In spite of some differences detectable in these figures, infestation and parasitization levels recorded at three different ranges of altitude resulted not statistically different; as shown in Figs IV-VII the variability of both infestation and parasitization levels is very high.

In all three altitudinal ranges infestation by *G. brimblecombei* reached a clear peak during the first summer sampling performed in July (Fig. VIII, 1), while the peak of parasitization due to *P. bliteus* was a little delayed maintaining high percentages also in the second summer sampling and the first one of autumn (Fig. VIII, 2).

The results of statistical analysis related to the infestation, measured as number of live 4^{th}-5^{th} nymphs collected per minute per person, are reported in the final model (Tab. 3).

As already described in materials and methods, Tab. 3 shows the input variables resulted as the most significant ones, obtaining the model having the highest global significance with the lowest number of variables.

The variable with the highest impact on infestation levels is *P. bliteus* parasitization (see Adjusted MS and F in Tab. 3). Although theoretical model contemplates that the present infestation depends on the previous parasitization, at unknown lag, evidently such lag is closer to the present date of sampling than to the previous sampling, which is around 45 days before the present date. This could be the reason why between the two covariate, parasitization and parasitization at the previous sampling period, the first one results significant, while the second one can be eliminated. Obviously, the parasitization enters the model with a negative slope (see coefficient in Tab. 3) that is an increment of the parasitization lowers the infestation.

As the variables of weather conditions are closely correlated one to each other, the average percentage of daily hours with RH > 80% was selected in the final model as the most significant one; for temperature, the chosen one is the average maximum temperature of the 45 preceding days, while rainfall is not present among the most significant variables, probably because it is linked to the relative humidity. Both of selected climatic variables (RH> 80% and average maximum temperature) enter the model with a positive slope (see coefficient in Tab. 3).

Beside the weather conditions, also the sampling period is significant; the site, although significant, has lower impact (Tab. 3).

An alternative model has been tested; there, in place of the different sites, only the altitude of the site was taken into account. This model is more parsimonious because it uses just one degree of freedom instead of 25; unfortunately, it explains a significantly smaller part of variance; this means that the altitude is an important factor, although many other local factors, typical of the site, are also influent, but the variability of the sites is very high.

Also in the statistical analysis regarding the factors influencing the parasitization, the lagged variable (infestation of the previous period) has resulted to be not significant, probably due to the same reasons expressed in the previous analysis regarding the infestation. The infestation has a negative impact, and this could be considered reasonable, because when there is an outbreak of infestation, the parasitization has a delay in the rise. In Tab. 4, the sampling period is the main factor influencing the parasitization, followed by the infestation level (with negative influence, as already written) and the percentage of daily hours with relative humidity> 80% (positively influencing). The altitude is an important factor too, but single sites explain significantly

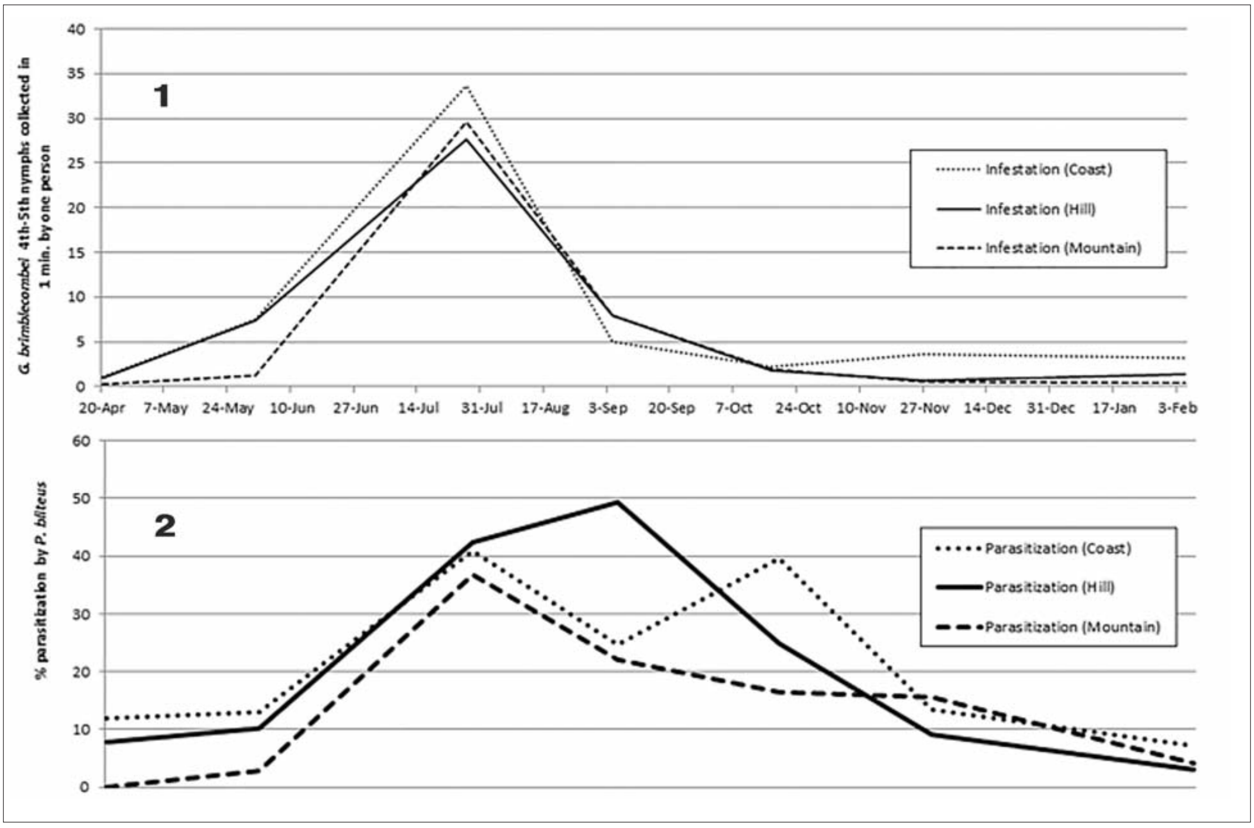


Fig. VIII – Trend of average infestation level of *G. brimblecombei* (1) and percentage of parasitization by *P. bliteus* (2) recorded in all groups of sites (Coast, Hill and Mountain).

Table 3 – Analysis of Variance for *G. brimblecombei* infestation, log(1+Infestation/min), using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P	Coefficient (metric variable)
P. bliteus parasitization	1	0.051	8.940	8.940	28.21	0.000	-1.317
Percentage of daily hours with Relative Humidity >80%	1	2.544	4.121	4.121	13.01	0.000	0.020
Average maximum temperature	1	9.197	2.944	2.944	9.29	0.003	0.144
Sampling period	6	17.143	17.965	2.994	9.45	0.000	
Site	25	37.090	37.090	2.994	4.68	0.000	
Error	118	37.390	37.390	0.317			
Total	152	103.415					
S = 0.562908, R-Sq = 63.84%, R-Sq(adj) = 53.43%							

Table 4 – Analysis of Variance for *P. bliteus* parasitization, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P	Coefficient (metric variables)
Sampling period	6	7.370	9.396	1.566	44.32	0.000	
log(1+Infestation/min)	1	0.009	0.952	0.952	26.95	0.000	-0.138
Percentage of daily hours with Relative Humidity >80%	1	1.170	0.561	0.561	15.88	0.000	0.007
Site	25	6.387	6.387	0.255	7.23	0.000	
Error	119	4.204	4.204	0.035			
Total	152	19.140					
S = 0.187961, R-Sq = 78.04%, R-Sq(adj) = 71.94%							

more; so that we can say – as previously seen – that there are local conditions that cannot be reduced to a simple altitudinal factor.

DISCUSSION

After the first record of *P. bliteus* in Sicily, its spread all over the region occurred in a short time, showing the high dispersal capacity. Similarly, *Closterocerus chamaeleon* (Girault), a parasitoid released in Sicily to control the eucalypt gall wasp *Ophelimus maskelli* (Ashmead) (Hymenoptera, Eulophidae) (Rizzo *et al.*, 2015), rapidly spread through short and long distance dispersal mechanism, being widely distributed in the region after 18 months from its release in 2006 (Caleca *et al.*, 2011b).

At present, *Psyllaephagus bliteus* is widespread in Sicily and has been recorded at all detected altitudes without statistically significant differences.

Mediterranean areas fits with the good development of *Eucalyptus camaldulensis*, *Glycaspis brimblecombei* and its specific parasitoid *P. bliteus*, differently from what recorded in the arid climatic areas of inner California, where *E. camaldulensis* and *P. bliteus* showed clear difficulties in their development (Daane *et al.,* 2012).

P. bliteus parasitization is the main factor negatively influencing (=lowering) *G. brimblecombei* infestation; this result, together with the accidental and contemporaneous introduction of the psyllid and its parasitoid (Caleca *et al.*, 2011a), could explain the absence of high damage level on eucalypts in Sicily, confirming results achieved by Margiotta *et al.* (2017) which state that *P. bliteus* parasitization causes a 64% reduction of the host population. In this context, it is to further investigate the influence that a strong attack of the invasive bronze bug *Thaumastocoris peregrinus* Carpintero & Dellapé (Heteroptera, Thaumastocoridae) can have against both the host and the parasitoid (Suma *et al.*, 2014, 2018).

The most significant metric factors positively influencing *G. brimblecombei* infestation are daily hours above 80% of relative humidity and the average maximum temperature, obviously related to other, but less significant climatic factors.

Sampled sites with their particular characteristics are factors influencing infestation and parasitization more than their altitude; therefore local conditions of each site cannot be reduced to a simple altitudinal factor.

P. bliteus parasitization on *G. brimblecombei* showed to be influenced by the sampling period, as well as by the host infestation level (negatively) and by the percentage of daily hours of relative humidity >80% (positively), recording the highest average levels in summer samplings.

The time interval of about 45 days between one sample and the next was not able to confirm that both *G. brimble-*

combei infestation and *P. bliteus* parasitization were influenced by previous recorded levels as stated by Margiotta *et al.* (2017), because current levels are likely affected by previous levels reached in a moment closer to current sampling than to the previous one.

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