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LASIUS BRUNNEUS (FORMICIDAE FORMICINAE) AND *STOMAPHIS QUERCUS* (APHIDOIDEA APHIDIDAE): TROPHOBIONTS HARMFUL TO CORK OAK FOREST IN SARDINIA (ITALY) ([1])

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Loi A., Luciano P., Gilioli G., Bodini A. – *Lasius brunneus* (Formicidae Formicinae) and *Stomaphis quercus* (Aphidoidea Aphididae): trophobionts harmful to cork oak forests in Sardinia (Italy).

In 2010 and 2011, we studied the biology, behaviour, harmfulness and distribution of the population of *L. brunneus* and *S. quercus* in cork oak forests in Sardinia. During their life cycle, the two trophobionts benefit from the protection provided by the cork bark. In particular, the aphid undergoes a monoic holocycle, spending much of the year inside the ant nest. Our investigations on the distribution of infested trees suggested that they are grouped into clusters, even though we occasionally found cork oaks (especially large ones) colonized by the formicid without any attacks on the surrounding trees. The ant also digs its nests in the female cork, damaging the production, and its infestations involved up to 20% of the cork oaks. The distribution of the two species was limited to some cork oak forests in the central and north-eastern parts of Sardinia between 450 and 780 m a.s.l. where the climatic conditions are typical of the cold sub-zone of *Lauretum* type II with summer drought, according to Pavari's phytoclimatic classification.

KEY WORDS: Biological cycle, behaviour, harmfulness, nest distribution, species clustering.

INTRODUCTION

The cork oak (*Quercus suber* Linnaeus) is more or less abundantly distributed in all the siliceous areas of Sardinia, from the lowland up to the medium mountain zones, forming the best stands in the inland and rainier areas of the island (CAMARDA & VALSECCHI, 2008). The insect fauna of these forest formations has been studied in the past by extensive investigations of its composition (MARTELLI & ARRU, 1957; PROTA, 1962, 1963, 1973; BARBAGALLO, 1985; LUCIANO & ROVERSI, 2001) and by evaluations of the harmfulness and population dynamics of some species of defoliator lepidopterans (PROTA, 1970; LUCIANO *et al.*, 1982; LUCIANO & PROTA 1983, 1986; PROTA *et al.*, 1991), xylophagous coleopterans (CAO & LUCIANO, 2005; LUCIANO *et al.*, 2008) and the ant *Crematogaster scutellaris* Olivier (Formicidae Myrmicinae) (CASEVITZ-WEULERSSE, 1972, 1973).

Observations on the ant fauna were performed from September 2009 in the municipality of Alà dei Sardi, one of the most important in terms of the quantity and quality of female (or cultivated) cork harvested (VERDINELLI *et al.*, 2011). This research revealed the presence of four known arboreal species (the aforesaid *C. scutellaris* and the formicines *Camponotus vagus* Scopoli, *C. fallax* Nylander and *C. truncatus* Spinola) to which was unexpectedly added *Lasius brunneus* Latreille (Formicidae Formicinae), reported in Catalonia as an agent of direct damage to female cork (ESPADALER & ROJO, 2002).

Previous reports regarding the distribution of *L. brunneus* in Sardinia, indicated its presence in holm oak (*Quercus ilex* Linnaeus) forests in the central part of the island (GRANDI, 1935; RIGATO & TONI, 2011). It is a xylolytic and corticicolous ant living in all broadleaf habitats and is typically linked to the genera *Quercus*, *Tilia* and *Acer* (SEIFERT 1992, 2007). *L. brunneus* presents a broad distribution range from Western Europe to northern Africa as far as Israel, Crimea and Pakistan (WILSON, 1955; PETROV, 2005; PAKNIA *et al.*, 2008; VONSHAK & IONESCU-HIRSCH, 2009). It is a very fugitive and non-aggressive species with cryptic habits (WILSON, 1955; COLLINGWOOD, 1979; SEIFERT, 1992). In the cork oak ecosystems of Spain, its presence is limited to the cooler, more humid zones (ESPADALER *et al.*, 2006a, b). On the cork oak, this formicid's diet is based mainly on the honeydew produced by *Stomaphis quercus* Linnaeus (Aphidoidea Aphididae) (ESPADALER *et al.*, 2006a, b). Hence, our field observations conducted in spring 2010 allowed us to record the presence of this aphid in the cork oak stands of Alà dei Sardi, adding it to the known aphid fauna of Sardinia (BARBAGALLO, 1985; BARBAGALLO & ORTU, 2009). This aphid, of exceptional size between Aphidoidea (BLACKMAN & EASTOP, 1994; HOPKINS & THACKER, 1999), is an arboreal species with Palaearctic distribution, characterized by a very long stylet able to reach the phloem vessels of the trunks of *Q. suber*, *Q. petrea* Mattuschka Lieblein and *Q. robur* Linnaeus, as well as *Betula pendula* Roth and *Alnus glutinosa* Linnaeus Gaertn (BLACKMAN & EASTOP, 1994; ESPADALER *et al.*, 2006a). As far as is known (GOIDANICH, 1957; LORENZ &

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SCHEURER, 1998; ESPADALER *et al.*, 2006b), it undergoes a monoic holocycle on the host trees, although it cannot be excluded that in some particular environments it could undergo an anholocycle, as hypothesized for the congeneric *S. acquerinoi* Binazzi on *Acer pseudoplatanus* Linnaeus (BINAZZI & PENNACCHIO, 2002), or present a heteroic cycle in mixed populations of oaks, as is known for *S. japonica* Takahashi (TAKADA, 2008). All *Stomaphis* species live with the constant presence of ants of the genera *Lasius* and *Formica*, which in exchange for the honeydew establish an obligatory trophobiotic relationship with the ant (GOIDANICH, 1957; PONTIN, 1983; LORENZ & SCHEURER, 1998; MATSUURA & YASHIRO, 2006; TAKADA, 2008).

Here we report the results of two years of field observations aimed at investigating some aspects of the biological cycle and behaviour of the two trophobionts in order to assess the harmfulness of the formicid when the cork planks are harvested, to study the distribution of the ant's colonies within the sample plots and to verify the distribution of its population in the main cork oak areas of Sardinia.

MATERIALS AND METHODS

Observations on the biology of the ant and aphid symbionts were conducted from April 2010 to August 2011 in Alà dei Sardi in the cork oak forest at the locality Pedrutocchis (526428E, 4496509N, 660 m a.s.l.). The cork oak forest contains trees of different ages, and the canopy cover is partly discontinuous due to the presence of pastures; it lies along a hilly slope that reaches a vast valley with the S'Ena Boltutto stream flowing at its bottom. Cork extraction is practised in a scalar manner, since the trees reach the end of the debarking interval in different years, an interval that lasts from 12 to 16 years in this territory.

During spring-summer, surveys were carried out in this area every 15 days to record the presence of the two trophobionts on the trunk and at the collar of about ten infested cork oaks as well as in the thickness of their bark by means of its partial removal. In July, the observations were extended to all the debarked trees at the locality in order to record the developmental stages under the freshly harvested cork bark to estimate the level of damage to the planks caused by the excavation of nests. During autumn-winter, the surveys were monthly; to investigate the time of appearance of the different developmental stages of the two trophobionts, we progressively extracted the bark from an infested 7-8 m high cork oak with trunk section of 76 cm at breast height.

To study the spatial distribution of the two trophobionts in July 2011, we delimited plots within the cork oak stands where a high abundance of infested trees had been recorded. Two rectangular plots (60 x 80 m), ca. 300 m apart, were set up at Pedrutocchis. A third plot of the same size was set up ca. 1,200 m away from the first two at the locality Sa tanca e s'ena (526685E, 4498021N, 648 m a.s.l.). Within each plot, we recorded the distance of all the trees from each other, the respective diameters at breast height and the presence of an active or previous *L. brunneus* infestation, the latter marked by the persistence of cork plaques of the preceding extraction on the newly deposited cork (ESPADALER *et al.*, 2006a). Statistical tests were applied to the data on active or previous infestations to identify spatial clusters (KULLDORFF, 2006) in order to evaluate if the infested cork oaks were nearer to each other than would be expected as a consequence of the natural distribution of all cork oaks growing in the plots. For this purpose, we used both the Cuzick-Edwards test (CUZICK & EDWARDS, 1990) and the test based on the spatial scan statistic (KULLDORFF & NAGARWALLA, 1995; KULLDORFF, 1997). The Cuzick-Edwards test, based on the concept of *k*-nearest neighbour, was conducted for different values of *k* (i.e. for different hypotheses about the size of the clusters of infested trees) and the significance was adjusted according to the Holm method for multiple testing (LEHMAN & ROMANO, 2005) to take account of dependence between the related statistics. Since this test is not able to indicate the position of the clusters, we then considered the test based on the spatial scan statistic, which allows identification of a circular region in which the probability that a tree is infested is significantly higher than for trees outside the region.

The comparison between the diameters of the infested and non-infested trees was carried out with the non-parametric Wilcoxon test (HOLLANDER & WOLFE, 1999) because of the strongly asymmetric distribution of the diameters in the samples. We used this test to determine if the mean tree diameter differed between trees occupied by a *L. brunneus* nest and unoccupied trees, as performed by other authors for this and other formicid species (VILLAGRAN & OCETE, 1990; DOLEK *et al.*, 2009; SANTINI *et al.*, 2011).

Finally, to investigate the distribution of the two symbionts in Sardinia, we checked 40 randomly selected cork oaks over an area of 2-3 ha between April and June 2011 at 40 localities within the main cork oak areas. We interrupted this type of sampling when we recorded a cork oak infested by *L. brunneus* and we proceeded to evaluate the infestation on 100 randomly selected cork oaks in the surrounding area, not counting the trees examined to that point. The sample size was equal to that used in Spain (ESPADALER *et al.*, 2006a); for the 100 trees examined at each infested locality, we recorded the diameters at breast height and we applied once again the Wilcoxon test to these values to compare the distributions of the diameters between infested and non-infested trees.

Available climatic data (ARRIGONI, 1968; DETTORI *et al.*, 2001) were used to perform a qualitative comparison between the climatic conditions of the infested and non-infested localities to determine possible factors that might explain the distribution of the ant.

RESULTS AND DISCUSSION

OBSERVATIONS ON THE BIOLOGY AND BEHAVIOUR OF *LASIUS BRUNNEUS* AND *STOMAPHIS QUERCUS*

The field surveys showed that *L. brunneus* workers were active on the bark all year long, although their number was significantly reduced in winter and in the hottest hours of summer (Fig. I, 1). Especially in spring (the season of maximum activity of the colonies), the infested trees presented accumulations of brownish frass at the base of the trunk or in the crevices of the cork bark. All year round, the bark presented canopy-shaped shelters above the trenches external to the nest and their entrances; they were built by the workers, mixing cork frass and other deposits with their saliva.

By removing the bark, it was possible to observe that the ant's brood chambers were excavated at different heights above ground in the female cork and male cork overlying it,

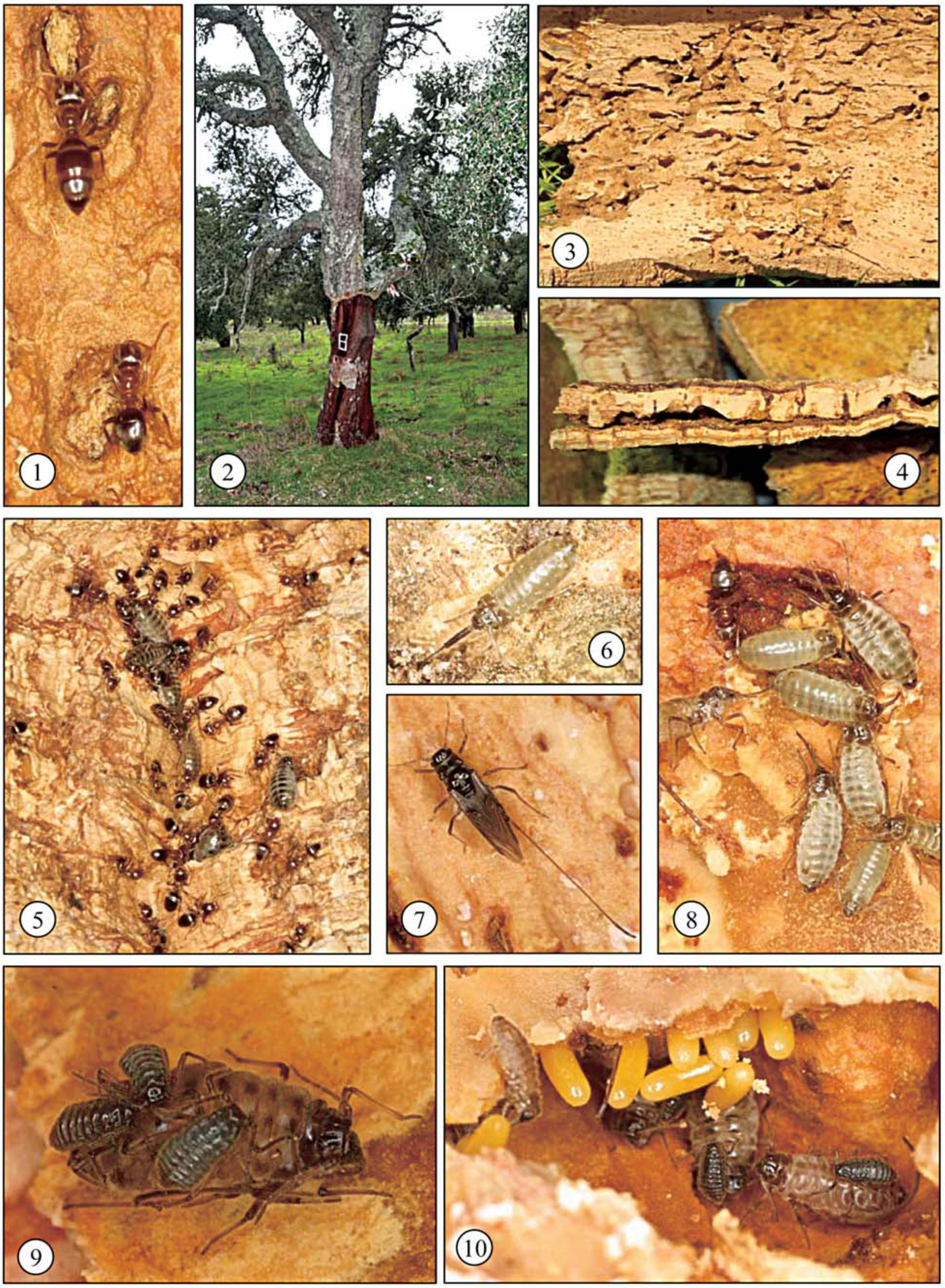

Fig. I – Photos of *Lasius brunneus* and *Stomaphis quercus* taken during 2010 at the cork oak stand in Alà dei Sardi: workers of *L. brunneus* (1); oak with cork plaque of the 2008 extraction on the newly deposited bark (2); cork plank damaged by ants excavations (3); trasversal view of cork plank excaved by the ants (4); old nymphs of *S. quercus* feeding in the cork cervices attended by ant workers (20 May) (5); young nymph feeding at the cork oak collar (27 May) (6); winged viviparous female inside the ant nest (10 June) (7); group of old nymphs inside the feeding chamber (20 July) (8); oviparous female mounted by three neotenic males (9) and newly-laid yellow-gold eggs (10) (27 November).

confirming what was observed by ESPADALER *et al.* (2006b) concerning the distribution of the ant society in cork oak trees. Larvae of the formicid were observed from April to September. Two winged queens were found inside the same nest on 7 July 2010 at Alà dei Sardi, and three were recorded on 26 May 2011 at Tempio Pausania. These discoveries seem to suggest a wider period of nuptial flights, compared with what observed in central Europe for this species (SEIFERT, 2007).

Our observations showed that the aphid, like other congeners (MATSUURA & YASHIRO, 2006), undergoes its cycle almost exclusively inside the nest of the tending ant. In fact, only in the months of April and May was it possible to find individuals, isolated or in groups of 5-6, feeding in the crevices of the cork bark both directly in contact with the external environment or protected by the shelters built by the ants (Fig. I, 5). When these aphids were disturbed, the ant workers that attended them quickly pulled them by the cuticle of the body so that they extracted their stylet from the plant tissues as rapidly as possible. This operation required from 2 to 10 minutes according to whether there were I-II instar nymphs or III-IV instar nymphs and mature apterous viviparous females. Once the stylet was free, the ants urged the aphids to move away quickly, pulling them by the legs or, if young nymphs, snatching them with their mandibles by the rostrum and hauling them away toward other shelters.

During spring 2010 and 2011, juvenile forms of *S. quercus* were also observed at the collar of infested cork oaks (Fig. I, 6), up to 5 cm deep in the litter, always attended by a large number of symbiont ants, confirming that the collar zone is a preferred sucking site (GOIDANICH, 1957).

After removal of the cork bark, we observed that the sucking activity took place mainly inside chambers purposely excavated in the female and male cork of the trunk and main branches by the ant workers. During spring 2011, different-aged nymphs were found up to 6.5 m above ground in niches dug in the male cork, especially at the axil of primary and secondary branches. Each of these chambers often contained groups of aphids, up to 8-12 individuals, feeding next each other (Fig. I, 8). In making these niches, the ants generally respect the cork of the last 2 years in more direct contact with the phellogen. This forms a kind of buffer zone 2-3 mm thick that separates the aphids from the subero-phellodermic cambium and from the underlying vascular tissues.

Among the many observed aphids (of which 109 were collected), only a single winged viviparous female was recorded inside the ant nests observed during removal of the infested planks (Fig. I, 7). The specimen was observed walking in the galleries of the bark, except when it halted periodically to secrete honeydew, which was immediately taken by the ants constantly following it. This single discovery seems to confirm that the care given by the ants limits or sometimes inhibits the formation of alates (JOHNSON, 1959; ROBERTI, 1962; KLEINJAN & MITTLER, 1975), even via cutting of the wings (KUNKEL, 1973), as is known to be practised by ants of the genus *Lasius* (TREMBLAY, 1995). Therefore, the shortage of alates would help explain the limited possibility of dispersion of this aphid, which is thus relegated to the territory of the formicid (HOPKINS & THACKER, 1999).

In summer and autumn, the activity of the two symbionts is concentrated inside the bark and the active and passive transfers of the nymphs in the protected shelters on the bark become progressively rarer until they disappear beginning from the first decade of October.

Removal of the bark from an infested cork oak allowed us to observe *in situ* the evolution of the oviparous generation. On 27 November 2010, we recorded oviparous females, apterous like the parthenogenetic ones and of similar size, at 50-70 cm above ground; they had an evidently swollen abdomen and carried the long rostrum turned posteriorly below the body, a sign that they had stopped feeding. Each female was mounted by 2-3 neotenic males, one of which intent on mating (Fig. I, 9); however, these males were also observed trying to mate with the older nymphs, confirming what is known among the genus *Stomaphis* (GOIDANICH, 1957; HEIE, 1995; MATSUURA & YASHIRO, 2006; TAKADA, 2008). The oviparous females were generally found in small groups of no more than 5 individuals inside the previously described feeding chambers. The newly-laid yellow-gold eggs were found in the same cavities; they were combined in groups of 2-8 eggs, arranged one next to the other and adhering to the cork walls furthest from the zone of nymph and female feeding (Fig. I, 10).

On 15 January 2011, we found nymphs of various ages, still belonging to the oviparous generation, newly-laid eggs and others of a brownish colour marking advanced embryonic development. The males mounted not only the mature females but also those with a swollen abdomen and visibly exhausted. This observation of the contemporary presence of different developmental stages of the aphid during winter contrasts with what was reported for the cork oak forest of Sant Hilari Sacalm (Catalonia, Spain), in which only wintering eggs were found even as early as November (ESPADALER *et al.*, 2006b); however, it agrees with what has been observed for other species of *Stomaphis* living in Japan (MATSUURA & YASHIRO, 2006; TAKADA, 2008).

As with the parthenogenetic generations, during the cold season the aphids live with the constant presence of a group of *L. brunneus* workers, which never cease their activity inside their nest. If disturbed, the ants try to carry the oviparous females away, ignoring the males or nymphs which independently flee shortly thereafter.

On 5 April 2011, we recorded the beginning of hatching of the wintered eggs under the cork bark. The I instar nymphs were carried directly by the ants, even though they were able to walk independently. On 15 April, their diffusion already involved the external part of the bark of the host trees.

LEVEL AND CHARACTERISTICS OF THE *L. BRUNNEUS* INFESTATION

In July 2010, in the cork oak stand of Pedrutocchis (ca. 60 ha) there were only 4 infested trees among 100 newly debarked ones chosen at random from the ca. 400 trees debarked in that period. The excavations produced by the formicid involved 50-70% of the surface of the infested planks (on average, 154.2 ± 49.0 cm long and 3.5 ± 0.3 cm thick), causing them to crumble in several tracts during their removal and thus their incomplete separation from the phellogen. These portions of cork, representing the most internal part of the nest, remain closely attached to the phellogen and remain as plaques on the surface of the newly formed bark in the following years (Fig. I, 2). This represents the most evident symptom of a previous infestation, as also observed by ESPADALER *et al.* (2006a) in the cork oak forests of Catalonia. In all cases, the cork obtained from the infested trees was discarded or only partly used as ground cork (Fig. I, 3 and 4).

On samples of infested planks placed in plastic bags immediately after extraction and taken to the laboratory, we recorded a mean of 425 *L. brunneus* workers and 19 individuals apterous morphs (mostly nymphs) of *S. quercus* per m².

During our observations, we noticed that this ant species also attacks particularly young cork oaks with a diameter less than 20 cm, confirming what was observed in Spain (ESPADALER *et al.*, 2006a). Its colonies were recorded not only on trees debarked many years earlier but also on trees in which cork harvesting had been practised only a couple of years before; in this case, the nest entrances were most often situated along the cortical incisions made to facilitate the next cork extraction, which have become preferential entrance routes for the ant workers, similar to what has been observed for other insects that damage the cork oak (CAO & LUCIANO, 2005). This suggests that debarking does not compromise the survival of the ant colony, which progressively re-colonizes the newly deposited cork.

DISTRIBUTION OF THE FORMICID

The surveys carried out in the three 4,800 m² plots revealed an active or previous infestation in 15-18% of the trees. The Cuzick-Edwards test led to reject the null hypothesis of random distribution of the ant's presence on the cork oaks in the Pedrutocchis (B) and Sa tanca e s'ena plots ($p < 10^{-4}$ in both cases) but not in the Pedrutocchis (A) plot ($p > 0.40$). The test based on the spatial scan statistic confirmed these results and identified a significant primary cluster at both Pedrutocchis (B) and Sa tanca e s'ena (as suggested by the distribution of the cork oaks reported in figure II). Nevertheless, the real size of the cluster could be smaller (KULLDORFF, 2006), as indicated by the Cuzick-Edwards test which indicates strong significance already for $k = 1$ ($p \sim 10^{-5}$).

Therefore, the test results suggest that, with a non-sporadic infestation, the presence of the formicid generally involves groups of neighbouring trees, probably due to the symbiont aphid's low capacity for diffusion. Indeed, because of its large size, the location of its colonies inside the cork bark and the negligible number of alate morphs observed, this aphid does not seem able to cover large distances, thus also indirectly determining the location of the ant nests. In his study on the biology of this aphid, GOIDANICH (1957) mentioned that the colonies remain year in and year out on the same trees, whether the trees are close to each other or very distant from one another.

The mean diameter of the infested trees was always greater than that of the non-infested ones (Table 1). The two-sided Wilcoxon test led to rejection of the hypothesis of equal distribution of the diameters in the two groups in both Pedrutocchis plots [$p = 0.02$ and 0.04 respectively for (A) and (B)] but not in the Sa tanca e s'ena plot. However, for the latter the p value of the test was not very high (0.08) and thus the result could have been influenced by the lower number of in-

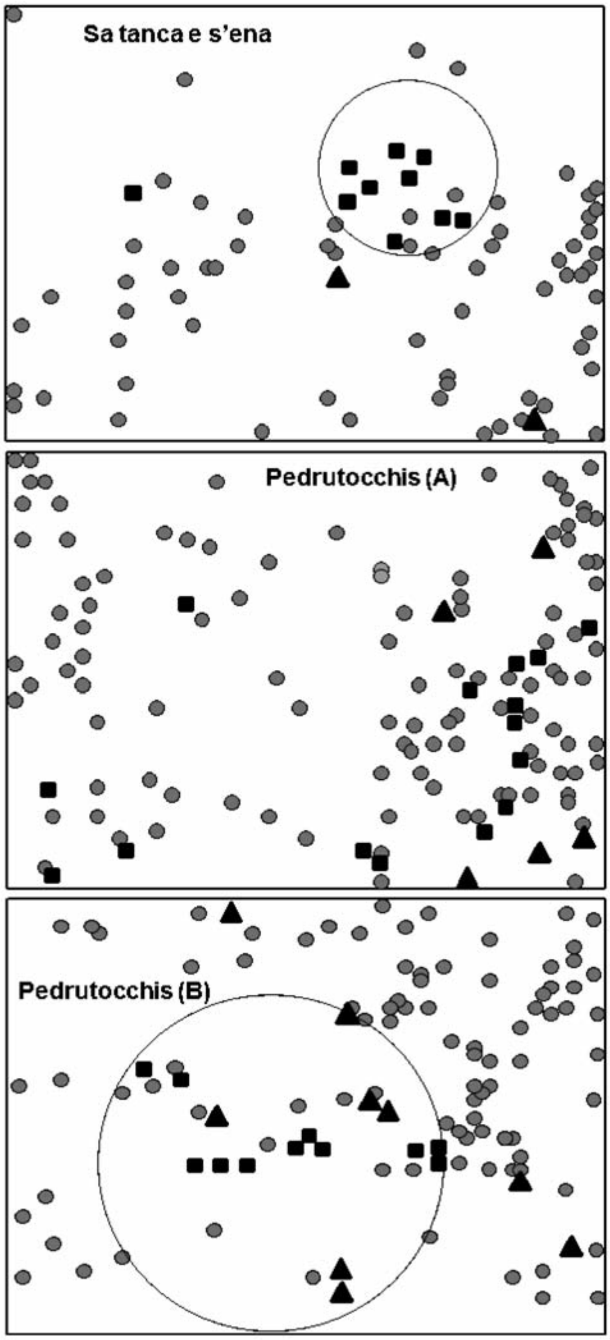

Fig. II – Distribution of the cork oaks in the three 4,800 m² plots in which *L. brunneus* infestations were surveyed (circles = non-infested cork oaks; squares = infested cork oaks; triangles = cork oaks with symptoms of previous infestation) (Alà dei Sardi, July 2011). Represented in two of the three plots is the size of the circular area in which the probability of infestation is highest, as indicated by the Cuzick-Edwards test (the circular area has a radius of 12.01 m at Sa tanca e s'ena and 25.58 m at Pedrutocchis (B)).

Table 1 – Mean diameters of all the non-infested cork oaks and those infested by *L. brunneus* in three 4,800 m² plots (Alà dei Sardi, July 2011).

Locality	Trees examined	Trees infested by *L. brunneus*		Diameter non-infested trees	Diameter infested trees
	(no.)	(no.)	(%)	(cm) $\bar{x} \pm s.d.$	(cm) $\bar{x} \pm s.d.$
Sa tanca e s'ena	79	12	15.2	34.1 ± 11.8	40.0 ± 13.6
Pedrutocchis (A)	129	20	15.5	34.0 ± 30.6	41.6 ± 29.6
Pedrutocchis (B)	109	20	18.3	29.3 ± 17.8	40.3 ± 25.9

fested trees at that locality. Therefore, the results suggest that the size of the host trees could help determine the distribution of the infestation.

PRESENCE OF *L. BRUNNEUS* AND *S. QUERCUS* IN OTHER PLACES IN SARDINIA

During surveys conducted from April to June 2011 in 40 Sardinian localities (Table 2 and Fig. III), we recorded the presence of *L. brunneus* and *S. quercus* not only at Alà dei Sardi but also in other cork oak forests in the municipalities of Aggius, Tempio Pausania, Buddusò, Orune and Nuoro. We found a single cork oak hosting *L. brunneus* at both Aggius (locality Santa Degna), where there is a mixed wood of cork oak and holm oak, and in the pure cork oak stand at Buddusò (locality Santa Reparata); the two trees were of markedly different size, with diameters of 25 and 105 cm respectively.

The surveys carried out at the other infested localities revealed a level of infestation varying from 7 to 20% (Table 3). However, the percentage of trees occupied by the formicid in the Sardinian cork oak forests was much less than that observed in Spain, where 19 plots at 4 different localities showed very high variability between 4 and 92%, with a mean infestation rate for localities varying from 12.4 to 56.5% (ESPADALER *et al*., 2006a).

The Wilcoxon test showed a significant difference between the mean diameter of the infested and non-infested trees at four of the eight localities (Table 3). At Maria Naspa, in particular, there was strong significance despite the low number of infested trees (7), since 6 of them had diameters over 100 cm (up to 192 cm), which placed them within the 25% of highest values in this plot. These results suggest a relationship between the presence of the formicid and its symbiont and the size of the host trees, as also observed in the 4,800 m^2 plots. Nevertheless, as observed in Sardinia and in Catalonia (ESPADALER *et al*., 2006a), the thick bark of the cork oak assures conditions favourable to the establishment and permanence of *L.*

Table 2 – List of the 40 localities (the bold numbers indicate localities where *L. brunneus* was recorded).

No.	Municipality	Locality	Altitude (m a.s.l.)	UTM Coordinates East	UTM Coordinates North
1	Aggius	Abbafritta	436	507378	4532667
2	Aggius	Santa Degna	530	504935	4530792
3	Tempio Pausania	Cusseddu	441	511350	4529540
4	Tempio Pausania	Caniffa	451	511466	4529028
5	Tempio Pausania	Parapinta	447	511819	4528444
6	Calangianus	Puzzu di rana	524	516765	4528532
7	Calangianus	Venapiccina	541	517787	4527958
8	Bortigiadas	Conca manna	199	503475	4525311
9	Berchidda	Sutturu e Concas	215	509774	4514486
10	Berchidda	Sa Mudditta	219	508150	4513888
11	Alà dei Sardi	Madrone	510	532167	4500563
12	Alà dei Sardi	S'Enatu e su mele	590	530506	4498900
13	Alà dei Sardi	Sa tanca e s'ena	648	526685	4498021
14	Alà dei Sardi	Pedrutocchis	660	526428	4496509
15	Buddusò	Santa Reparata	623	523278	4495870
16	Buddusò	Tilibilche	787	525493	4491205
17	Buddusò	Sozzuighe	798	526811	4490698
18	Buddusò	Dispensa	770	528520	4489847
19	Buddusò	Sos Canales	754	527831	4489525
20	Putifigari	Faedda	270	455694	4488926
21	Villanova Monteleone	Sa Tanca de pottitu	321	455708	4486252
22	Villanova Monteleone	Lacianu	362	452354	4483943
23	Montresta	Turre	441	458318	4469985
24	Nughedu S. Nicolò	Badde de Mandras	693	502744	4487489
25	Bultei	Su preideru	750	504464	4485021
26	Burgos	Pedras rujas	812	497536	4477195
27	Orune	Fenazzu	799	527479	4474052
28	Orune	Sas tuppas	781	525878	4473923
29	Orune	Lusteddia	854	528491	4473418
30	Orune	Cant. S. Efisio	740	525679	4472962
31	Nuoro	Maria Naspa	729	525634	4472166
32	Nuoro	Sa fenargia	748	523865	4470367
33	Nuoro	Lardine	672	524282	4468466
34	Scano di Montiferro	S'alchimissa	481	467428	4455862
35	Mamoiada	Costa perdosa	528	523476	4454211
36	Abbasanta	Crastu de itumbaru	362	482665	4447188
37	Gesturi	Giara	584	500143	4399170
38	Fluminimaggiore	Conca moddizzi	257	454855	4362222
39	Iglesias	Bega sa carroccia	546	456400	4359700
40	Sinnai	Monte Crexia	629	533527	4348020

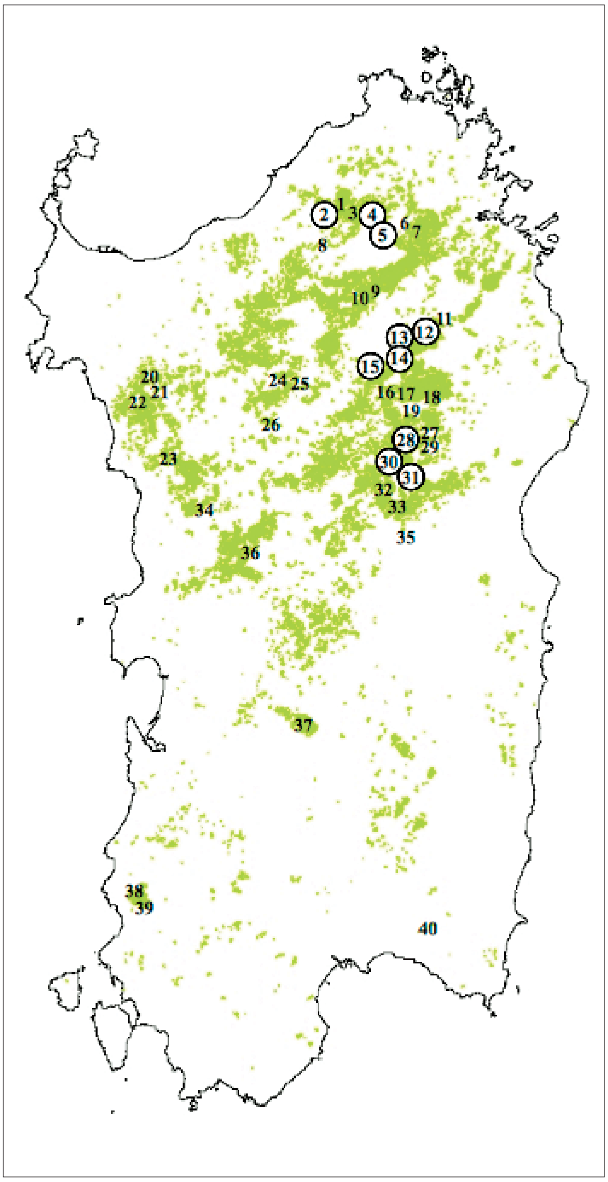

Fig. III – Map of Sardinia with the distribution of cork oak forests in green; the numbers indicate the position of the localities listed in Table 2 and those circled in black show the localities where *L. brunneus* was recorded.

brunneus colonies also in young trees of modest diameter. This contrasts with the report by COLLINGWOOD (1979) that the species builds nests in old trees, above all oaks; it also contrasts with what is known for the congeneric *L. fuliginosus* Latreille on *Q. robur* and *Q. petrea* in Piedmont, where colonies were only found on the larger trees (GOIDANICH, 1957). In both cases, the older trees had thicker bark that could be inhabited by the populous societies of the two formicids.

All the cork oak areas infested by *L. brunneus* are concentrated in the central and north-eastern part of Sardinia and they fall between 447 m a.s.l. at Tempio Pausania (locality Parapinta) and 781 m at Orune (locality Sas tuppas). The climatic conditions of these localities are typical of the cold sub-zone of *Lauretum* type II with summer drought, according to Pavari's phytoclimatic classification (PAVARI, 1935; DE PHILIPPIS, 1937); they have a mean annual precipitation higher than or equal to 800 mm and a mean temperature in the hottest month of not more than 24 °C (DETTORI *et al.*, 2001). As reported by ARRIGONI (1968), the same localities have climatic conditions equivalent to those defined by Thornthwaite for humid climates falling within types B1 to B3 (first-third mesothermic). ESPADALER *et al.* (2006a), who used the latter climatic classification, also found that the diffusion of the species was limited to Catalonian cork oak zones with humid and sub-humid climate (from B1 to B3 and C2). Nevertheless, in our study it was not always possible to find the formicid in localities bordering on the infested ones, and thus with the same climatic conditions, as in the case of the two localities Cusseddu and Caniffa in the municipality of Tempio Pausania or of Fenazzu and Sas tuppas in the municipality of Orune. We can infer that the very circumscribed distribution of *L. brunneus* is conditioned both by large-scale climatic factors and by local characteristics such as the proximity to water courses (as in the case of Caniffa) or low soil permeability causing prolonged water stagnation (as at Sas tuppas), which favours particularly high air humidity.

Most of the non-infested cork oak stands are in the medium and hot sub-zones of *Lauretum* (DETTORI *et al.*, 2001), hence with drier and hotter conditions. This demonstrates that climate can be considered a factor limiting the distribution of the two symbionts, which seem to prefer cooler and wetter cork oak forests in Sardinia, as in Spain (ESPADALER *et al.*, 2006a).

Table 3 – Levels of infestation recorded at localities in the municipalities of Tempio Pausania, Alà dei Sardi, Orune and Nuoro in 100 randomly selected cork oaks and results of the Wilcoxon test of the difference between the diameters of infested and non-infested trees.

Municipality	Locality	Diameter non-infested trees (cm) $\bar{x}$ ± *s.d.*	Infested trees (no.)	Diameter infested trees (cm) $\bar{x}$ ± *s.d.*	*p* value Wilcoxon test
Tempio Pausania	Parapinta	80.5 ± 30.5	7	80.1 ± 23.4	0.836
Tempio Pausania	Caniffa	40.1 ± 18.4	16	50.6 ± 22.7	0.034
Alà dei Sardi	S'enatu e su mele	35.0 ± 13.0	17	39.2 ± 13.1	0.133
Alà dei Sardi	Sa tanca e s'ena (*)	46.7 ± 15.7	13	39.3 ± 14.5	0.203
Alà dei Sardi	Pedrutocchis (*)	34.0 ± 24.0	16	47.4 ± 30.5	0.008
Orune	Cant. S. Efisio	67.4 ± 43.7	20	89.4 ± 59.2	0.044
Orune	Sas Tuppas	62.3 ± 37.8	10	77.7 ± 53.7	0.322
Nuoro	Maria Naspa	74.7 ± 4.70	7	126.6 ± 42.0	0.004

(*) The 100 examined trees did not fall within the 4.800 m^2 plots considered in Table 1.

CONCLUSIONS

Our investigations have increased the number of species known to colonize the cork oak in Sardinia. Therefore, despite the studies already conducted, it must be inferred that the knowledge of the insect fauna linked to this tree is still incomplete. In particular, we have added information on the ant fauna, demonstrating that several species in addition to the already known *C. scutellaris* can find an ideal habitat between the cork bark and wood of this tree.

The observations carried out on *L. brunneus*, recorded for the first time in Sardinian cork oak forests, allowed us to obtain further knowledge of its biology and ethology and to study its relationships with its symbiont *S. quercus*. We also established that *S. quercus* has a monoic holocycle that depends on the attendance by the ant workers and on the protection offered by the formicid nest.

The surveys of the distribution of infested trees were limited by the small extension of the examined plots and by the small number of replications (3). Nevertheless, they suggest an aggregated distribution, even though we occasionally found cork oaks (especially large ones) colonized by the formicid without any attacks on the surrounding trees.

The field surveys showed that the distribution of *L. brunneus* and *S. quercus* is limited to cork oak stands growing in particularly humid habitats situated between 450 and 800 m a.s.l. and characterized by a mean annual precipitation of at least 800 mm, thus emphasizing a strongly stenoecious character expressed by both species.

The infestation levels of *L. brunneus* in Sardinia are much lower than those recorded in Spanish cork oak forests, even though the economic damage could be equivalent given the high quality of the Sardinian cork, largely destined to the production of bottle stoppers.

An assessment of the effective harmfulness of the formicid in all the infested cork oak stands might suggest interventions to limit its population, since debarking alone does not assure the extinction of its societies. However, insecticide control trials conducted in Spain via the application of poisoned baits proved ineffective (ESPADALER *et al*., 2006b). This indicates the necessity of techniques of integrated control, planning insecticide interventions at the time of debarking when the colonies are numerically reduced by removal of part of the nest and are also more exposed to the lethal action of the active principles.

Nevertheless, the naturalistic value of the two trophobionts, considered rare species in other European areas, should not be neglected (HOPKINS & THACKER, 1999; ROTHERHAM, 2011). They establish a close mutualistic symbiosis which, because of the size of the aphids (among the largest in the world; ESPADALER & BERNAL, 2008) and the constant attendance of numerous ant workers, is easily observable on the trunks of cork oaks in spring and thus could be exploited for educational purposes. In our opinion, this raises the question of conservation of the two species, at least in protected areas.

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