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New bioethological observations on *Brahmaea (Acanthobrahmaea) europaea* and its host plants in Special Area of Conservation “Grotticelle di Monticchio” (Basilicata, Southern Italy) (Lepidoptera: Brahmaeidae)

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Abstract

Brahmaea (Acanthobrahmaea) europaea Hartig, 1963, is an Italian endemic exhibiting an exceedingly restricted geographic distribution. In 1971, a protected site was established for the species: the Nature Reserve of “Grotticelle” (Basilicata Region). Then, the Reserve area was incorporated into the perimeter of the Special Area of Conservation “Grotticelle di Monticchio”. In 1997, for the first time, a group of *B. (A.) europaea* caterpillars was found in nature. During research conducted in 2013-2014, an egg cluster of *B. europaea* was observed for the first time, and other groups of larvae were found inside the Reserve. Additional information was acquired about mobility of the caterpillars during their development. Palatability tests allowed to attest *Fraxinus angustifolia* subsp. *oxycarpa*, *Ligustrum vulgare* and *Phyllirea latifolia* as food plants of the moth among those present in the Reserve. From spring 2015 to spring 2017 other bioethological observations on *B. europaea* were conducted, as well as surveys aimed to study the distribution of its host plants in the habitats of SAC “Grotticelle di Monticchio”. During 2016, ten egg clusters and numerous groups of caterpillars have been identified on 12 different trees. In 2017, an egg cluster was observed, and other groups of larvae were found. These findings allowed to observe other details on spawning and behaviour of the caterpillars, until to pupate. In addition, a map of local habitat 91B0 “Thermophilous *Fraxinus angustifolia* woods” is also presented.

Key words: *Brahmaea (Acanthobrahmaea) europaea*, Brahmaeidae, Lepidoptera, ethology, host-plants distribution, egg clusters, Italy.

Introduction

Brahmaea (Acanthobrahmaea) europaea Hartig, 1963, commonly known as European Owl Moth or European Bramea, was discovered by Federico Hartig in 1963 in Basilicata, Southern Italy (Hartig 1963, 1966, 1997). Dufay (1970) reported a first bioethological account on this moth, an Italian endemic species with an extremely limited range of distribution (van Schepdael 1967; Rougeot 1971, 1975). In fact, the species occurs only in a very small area, mainly along Ofanto river (localities around Mt Vulture) and in a few localities along the valleys of Basento and Cavone rivers, in the provinces of Potenza and Matera [Basilicata Region] and Avellino [Campania Region] (Parenzan 1978; Bertaccini et al. 1995; Spicciarelli 2004, 2013, 2014; Mosconi et al. 2015).

The moth lives in mixed broadleaved forests dominated by deciduous oaks (in particular *Quercus cerris* L., in addition *Q. pubescens* Willd.), accompanied by leaved evergreens such as *Q. ilex* L., with flowering ash (*Fraxinus ornus* L.) and narrow-leaved ash (*F. angustifolia* subsp. *oxycarpa* (Willd.)) trees, hornbeams (*Carpinus orientalis* Mill. and *C. betulus* L.), wild privet (*Ligustrum vul-*

gare L.), hawthorn (*Crataegus monogyna* Jacq.), maples (*Acer monspessulanum* L., *A. campestre* L. and *A. opalus* Mill.), and broad-leaved phillyrea (*Phillyrea latifolia* L.), at an altitude between 200 and 800 m (Spicciarelli, 2013). As many other Palearctic species of *Brahmaea* (Nässig 1980; Freina 1982, 1985; Konno et al. 2001), also *B. (A.) europaea* feeds on Oleaceae (Bilek 1965, 1967; Stella 1992).

The European Bramea is on the wing only for a short period during the year, three or four weeks, rarely up to 5 or 6, between late March and early May, with a peak of activity in the first half of April (Laplanche 1973; Spicciarelli & Fimiani 2004). Adults are active during the early hours of the night, just after sunset, and they are also capable of flying at very low temperatures, even during snowfall (Dufay 1970; Spicciarelli & Fimiani 2004).

Adults of *B. europaea* can be attracted and monitored by artificial light, which is extremely effective for assessing the presence of the moth. However, despite unconfirmed rumors that caterpillars of the species had been found on *Fraxinus angustifolia* subsp. *oxycarpa* by Hartig himself, the observation of caterpillars in the wild has occurred with certainty only once a few years ago, on

Broad-leaved phyllirea (Spicciarelli 1997), a plant which is also known to occasionally host *Brahmaea* (*Brahmaea ledereri* Rogenhofer, 1873 in the Near East (Korb 1899; Freina 1985).

In order to preserve the European Bramea, the former Italian Ministry of Agriculture and Forestry established a National Reserve, of approximately 219 hectares, which probably represented the first Nature Reserve in the World to be specifically designed to protect an insect species (Audisio et al. 2012; Spicciarelli 2013). Today, this area is part of the Special Area of Conservation “Grotticelle di Monticchio” (SAC IT9210140), of approximately 342 hectares (Spicciarelli et al. 2011).

Even though concern about the species long-term survival was often raised (Zilli 1991; Spicciarelli 2000, 2004, 2006) and it was explicitly recorded in some red lists (e.g. Spicciarelli 2002), at present the species is not protected by law. *Brahmaea* (*Acanthobrahmaea*) *europaea* is not included in any of the Annexes of the “Habitats Directive” (92/43/EEC), nor in CITES, or in other international conventions. At the broadest level, habitat loss and fragmentation undoubtedly represents the most worrying threatening factor. At a local scale, the natural behaviour and activities are evidently disrupted by light pollution when this extends over the home range of the adults. In fact, the species is markedly phototropic and comes readily to artificial light. A traditional threat for *B. europaea* is also represented by collectors: this is a localized, handsome species that is highly coveted by amateur entomologists.

In a recent research, carried out between 2013 and 2014 (Spicciarelli 2018), palatability tests have established the acceptability of *Fraxinus angustifolia* subsp. *oxycarpa*, *Ligustrum vulgare* and *Phyllirea latifolia* leaves, while the flowering ash (*F. ornus*) is not accepted by the larvae. For the first time, and for two consecutive years (2013, 2014), the caterpillars of European Bramea were found inside the Reserve and on plants of narrow-leaved ash, in both cases, on isolated and not very high trees. Also, the discover of an egg cluster, for the first time, made it possible to highlight a specific behaviour of the species: the freshly hatched larvae reach an apical bud of the tree and, after consumption, switch to other buds below. The larvae of the third and fourth ages are very mobile on the plant, while the fourth and fifth instars move on the ground in search of new plants.

In the same search, a fragment of chrysalis attributable to our Brahmaeid moth, found in the excrements of a wild boar (*Sus scrofa* L.), demonstrates the negative impact that these animals may have during their foraging behaviour on the persistence of the populations of the moth at a local scale.

The scarcity of quantitative information on the population density at the larval stage does not prevent us from expressing concern about real consistency of the species population in the Reserve.

Also, the inability to feed on the flowering ash, the most

common species in Grotticelle, raises questions about the limited habitat range.

Between the spring of 2015 and the spring of 2017 other surveys were conducted with two objectives: first, to detect other groups of caterpillars and egg clusters, in order to obtain more data on the eggs deposition and on the larval behaviour on the plants and on the ground, until the pupation; the second, to evaluate the distribution of the Bramea host plants, in particular the area occupied by the narrow-leaved ash wood and the phytosociological aspects of the moth habitat.

Material and methods

The mapping of the area was done using both ground surveys and aerial photographs. After an assessment carried out in site, transects were placed inside the habitat 91B0 “Thermophilous *Fraxinus angustifolia* woods” representative area, phytosociological surveys were done along them. The site’s vegetation study was designed according to the phytosociological method (Braun-Blanquet 1964), that is, detecting of plant communities with floristic-statistical criteria, and used syntaxonomic nomenclature in according with the “Flora d’Italia” (Pignatti 1982) and the “Flora Europaea” (Tutin et al. 1993).

Based on previous experiences in 2013 and 2014 (Spicciarelli 2018), samplings in the SAC “Grotticelle di Monticchio” were repeated from the third week of April throughout May in spots where *B. europaea* caterpillars had been found.

Sprouts of narrow-leaved ash (*Fraxinus angustifolia* subsp. *oxycarpa*), wild privet (*Ligustrum vulgare*) and broad-leaved phyllirea (*Phyllirea latifolia*) were sampled, at various times of the day to take advantage of the different illumination of the potential guest buds of the larvae. Plants of all sizes (young, mature, old trees) were checked and sampled. On larger plants, the use of ladders and binoculars to watch the higher shoots were used. In the meantime, lower branches were beaten with a stick and the falling caterpillars collected on a large towel placed under the trees. Special attention has been devoted to the observation of higher apical shoots. The caterpillars were followed until pupation, and were video-recorded.

The phenological status of the trees, where the larvae were found, was checked later, after the collection of the caterpillars.

Results

In the first year of activity (2015), the inspections of plants resulted in no detection either larvae nor egg clusters. Also no defoliated apical buds or the presence of exuviae even was observed.

In the same year, a map of the habitat 91B0 “Thermo-

philous *Fraxinus angustifolia* woods” in the “Grotticelle di Monticchio” area was made. The area covered by this habitat was 72.5 hectares, equal to 21.2% of the SAC total area (342 hectares) (Fig. 1). Inside the Reserve, the habitat 91B0 covers 6.5 hectares, equal to 3.0% of the total area (219 hectares). In August 2015, 3 phytosociological surveys were done, allowing the identification of species associated to the food plants of the moth (Table 1).

Among samplings, differences in plant species assemblages and density were recorded. These surveys indicate evident differences in locations where it is sampled, as well as differences in both the composition and the density of the species. The density of European Bramea food plants is higher in “Grottarelle” and “Refezzella” localities, less in “Masseria Campo Santo” locality.

Other plants of narrow-leaved ash, broad-leaved phillyrea and wild privet, in small, very dense groups, are present in proximity to narrow natural channels and incisions of the soil, which can be filled with rain water for a few hours, at low altitudes especially.

A small group of plants of narrow-leaved ash is located inside the ruins of Castello di Monticchio (“Il Castello” on the map).

The following table shows an outline summary of the observed data.

During the winter 2015-2016, the rains were scarce and temperatures were mild resulting in an early sprouting (third week of March) of the narrow-leaved ash.

In the 2016, inspection of plants with larvae and egg clusters were repeated. Caterpillars were found detected (as of April 30, 2016), and ten egg clusters were recorded on nine of these plants (Fig. 2).

It is interesting the occurrence of two egg clusters on the same plant, where previously (in 2013, but not in 2014 and 2015) other caterpillars had been found. The two egg clusters, spaced one meter apart, were placed on two different branches, one below the other, in west toward Ofanto River. Other nearby narrow-leaved ash and broad-leaved phillyrea plants showed no egg clusters or caterpillars, also in later samplings.

The larvae were initially found as third and fourth instars (L3 and L4) and were very mobile on the plants moving frantically from on branch to another. The fourth and fifth instars move easily on the ground in search of new plants.

In the final period of the larval development (18–25 May 2016), the caterpillars fell from the trees, and sometime it was possible to follow them during their run on the ground. The caterpillars pupate, even 30-35 meters away from last their food plant, in small niches protected by veg-

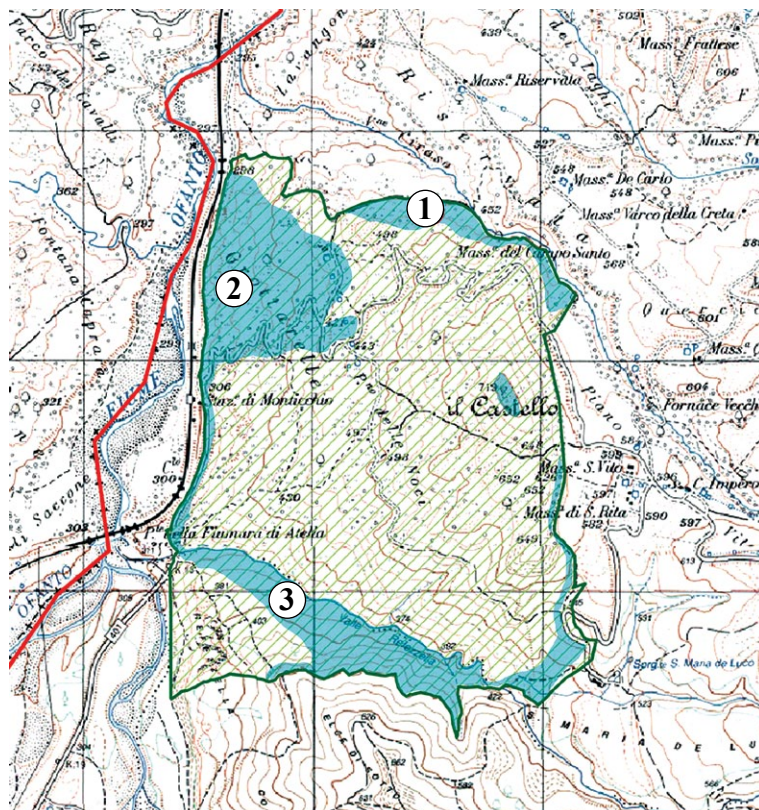


Fig. 1 – Habitat 91B0 “Thermophilous *Fraxinus angustifolia* woods” map, indicating the three areas where the phytosociological surveys were taken: Masseria Campo Santo (1); Grottarelle (2); Refezzella (3). The green line indicates the perimeter of the SAC, the red line indicates the regional border with Campania region.

Table 1 – Habitat 91B0 “Thermophilous *Fraxinus angustifolia* woods”: phytosociological survey, highlighting the European *Bramea* food plants.

Locality	Masseria Campo Santo (North-east)	Grottarelle (North-west)	Valle Refezzella (South)
Latitude	40.933165N	40.930377N	40.917925N
Longitude	15.551740E	15.542213E	15.543715E
Altitude (m)	480	360	330
Area (m ²)	225	225	225
Trees cover (%)	76	87	89
Herb-Shrubs layer cover (%)	76	65	50
Slope (°)	2	5	10
Aspect	N	W	SW
Tree layer			
<i>Acer monspessulanum</i> L. subsp. <i>monspessulanum</i>	-	1	-
<i>Acer neapolitanum</i> Ten.	1	-	-
<i>Acer opalus</i> Mill. subsp. <i>opalus</i>	-	-	1
<i>Carpinus orientalis</i> Mill. subsp. <i>orientalis</i>	-	1	-
<i>Fraxinus angustifolia</i> Vahl subsp. <i>oxycarpa</i> (Willd.) F. et R.	2	2	3
<i>Quercus cerris</i> L.	3	4	3
<i>Quercus ilex</i> L. subsp. <i>ilex</i>	-	-	1
<i>Quercus pubescens</i> Willd. subsp. <i>pubescens</i>	-	-	1
Shrubs layer			
<i>Acer opalus</i> Mill. subsp. <i>opalus</i>	2	-	1
<i>Carpinus orientalis</i> Mill. subsp. <i>orientalis</i>	-	3	-
<i>Cornus mas</i> L.	1	-	1
<i>Cornus sanguinea</i> L.	1	1	1
<i>Clematis vitalba</i> L.	1	1	1
<i>Cytisus scoparsi</i> (L.) Link subsp. <i>scoparius</i>	-	1	1
<i>Crataegus monogyna</i> Jacq.	-	-	1
<i>Fraxinus angustifolia</i> Vahl subsp. <i>oxycarpa</i> (Willd.) F. et R.	-	1	1
<i>Fraxinus ornus</i> L. subsp. <i>ornus</i>	1	1	-
<i>Genista tinctoria</i> L.	1	-	-
<i>Ligustrum vulgare</i> L.	-	1	-
<i>Phillyrea latifolia</i> L.	1	2	1
<i>Prunus spinosa</i> L.	-	1	-
<i>Pyrus pyraster</i> (L.) Burgsd.	1	1	-
<i>Ulmus minor</i> Mill.	1	1	-
Herb layer			
<i>Agrostis stolonifera</i> L.	1	-	-
<i>Asparagus acutifolius</i> L.	1	1	1
<i>Brachypodium sylvaticum</i> (Huds) P. Beauv.	1	1	1
<i>Carex pendula</i> Huds	1	-	-
<i>Dactylis glomerata</i> L.	1	1	1
<i>Galium aparine</i> L.	1	1	-
<i>Hedera helix</i> L.	1	1	1
<i>Helleborus foetidus</i> L. subsp. <i>foetidus</i>	-	1	-
<i>Phillyrea latifolia</i> L.	-	-	1
<i>Prunus spinosa</i> L.	-	1	-
<i>Ranunculus ficaria</i> L. subsp. <i>ficaria</i>	1	-	-
<i>Ruscus aculeatus</i> L.	2	1	1
<i>Urtica dioica</i> L. subsp. <i>dioica</i>	-	-	1

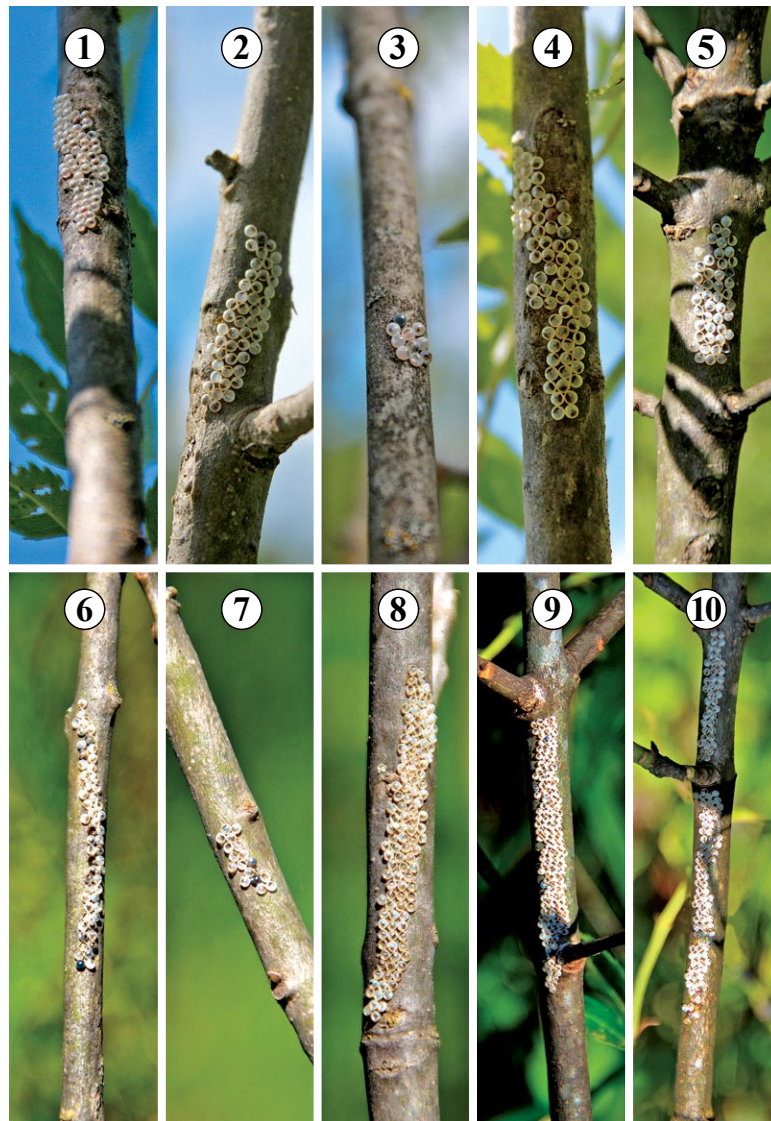


Fig. 2 – The ten egg clusters found in April-May 2016.

etation or rocks like those shown in Fig. 3. The movements of five caterpillars descended from trees to pupate were observed. In all five cases, in the ultimate part of the route the caterpillars were partly buried, remaining in the surface layer of the soil also as chrysalis.

During spring 2017, as of May 6, three other larvae groups were detected on *Fraxinus angustifolia* subsp. *oxycarpa* and *Ligustrum vulgare*. Only on one narrow-leaved ash plant one egg cluster was found. The tree was 3.5 m height and of 3.0 cm D.B.H. (Diameter at Breast Height). The egg cluster, with 193 eggs, was on branch of 6.5 mm diameter, away from the apical bud 75 cm and facing southeast, 4 unhatched eggs only. Many larvae moved from this plant to neighbouring narrow-leaved ash and privet plants (Fig. 4). In the plants that the previous year hosted egg clusters or larvae, no new eggs or caterpillars were found.

In a particular area of the SAC, where the largest number of the egg clusters and larvae has been found, the soils are rich in water in every season of the year, and are also frequented by wild boars, which determine the lifting and the disruption of the turf.

This is a part of forest populated by young plants of narrow-leaved ash, where a fire occurred a few years ago.¹ A large presence of thorny shrubs (*Prunus spinosa* L. and *Pyrus pyraeaster* Burgsd.) prevents the activity of the boars below the narrow-leaved ashes. Also, in this area the horn-beam is present with high density.

The presence of red deer (*Cervus elaphus* L.), is proven by the clear footprints left on the wet ground and the bites

¹ I do not identify this area with greater details, to avoid attracting the attention of “Bramea collectors”.

Table 2 – *B. europaea* egg clusters (April-May 2016). In three other plants of *F. angustifolia*, on which it was not possible to climb for search the egg clusters, caterpillars and small exuviae on end branches were spotted. Of these trees, the height (h) and the Diameter at Breast Height (D.B.H.) were detected: first plant, h = 7.0 m, D.B.H. = 9.4 cm; second plant, h = 6.5 m, D.B.H. = 8.8 cm; third plant, h = 5.5 m, D.B.H. = 7.7 cm.

ID (egg cluster)	egg numbers	unhatched egg	facing	diameter of the branch (mm)	exuviae presence on same branch	distance from the apical bud (cm)	height of the host plant (m)	host plant D.B.H. (cm)
1	70	2	East	9.5	si	85.0	3.5	2.7
2	49	1	East	9.5	si	75.0	3.0	2.4
3	10	1	East	7.0	si	70.0	4.0	3.4
4	70	2	East-Southeast	9.0	si	55.0	2.5	2.6
5	45	-	East	10.0	si	70.0	4.5	3.7
6	55	3	East	5.0	si	65.0	3.5	4.2
7	18	2	East	7.0	si	60.0	5.0	6.7
8	116	-	East	9.0	si	65.0	4.5	6.1
9	141	3	East-Southeast	7.0	si	60.0	5.5	8.6
10	128	3	East	6.0	si	55.0	same plant of the number 9	



Fig. 3 – Two different niches for *B. (A.) europaea* chrysalis.



Fig. 4 – In the left picture, 2017 egg cluster. First time caterpillars group on *L. vulgare* found in wild, on the right.

left on the bark of young narrow-leaved ash trees (Fig. 5). Damages by browsing were identified on wild privet, also. In a few weeks, the defoliated plants (as a result of the trophic activity by the caterpillars) recovered their vegetation, with new shoots. These trees did not produce any samaras, probably because of their young age.

Discussion

In 2015 no caterpillar and no egg cluster of *B. europaea* on food plants present in SAC “Grotticelle di Monticchio” were identified. However, in 2016, ten egg clusters and several groups of caterpillars were found. In 2017, only one egg cluster was detected.

This seems to highlight a great difference of the population dynamics of our moth in three consecutive years, both in numbers and in spatial distribution. A result that

resembles differences in adult sampling with lamp traps: some authors even with strong sampling efforts failed in same years to collect European Bramea with the lamp (e.g. Laplanche 1973). This population dynamics should be better investigated linking it to the environmental conditions, especially the winter microclimatic parameters in the natural site (Howicki et al. 2009).

Fraxinus angustifolia results to be the main host of *B. europaea*, while both wild privet and broad-leaved phillyrea plants have a secondary role or a replacement one. This outcome is confirmed by what occurred in 2013 and 2014 (Spicciarelli 2018).

The comparison of the egg clusters found reveals interesting common characteristics (see table 2): the size of the branch on which they were laid, their distance from the apical bud, their exposure, the age and measures of the host plant, always not old enough to produce samaras. In egg clusters, unhatched eggs were very few (from 0 to 4).



Fig. 5 – Two young narrow-leaved ash trees damaged by red deer bites (2016). In the Monticchio Regional Forest, red deer were introduced between the 2006 and the 2010. A group stands permanently in “Grotticelle di Monticchio”. On the plant in the left picture, an egg cluster of *B. europaea* was found in 2014. In 2017, the main trunk was completely dried, even if new sprouts emerged lower, close to the roots.

These data confirm and add details to the *B. europaea* deposition features. The presence of the smaller exuviae on the same branch of the egg cluster upholds what was observed in 2014: the larvae reach an apical bud of the tree as soon as they hatch and, after its consumption, switch to the buds below (Spicciarelli 2018). The size of the egg clusters found in this study has a greater range than in previous observations: low numbers are probably due to a partial or disturbed deposition, while the greatest egg clusters have many more eggs than those laid in lab conditions (Stella 1992; Spicciarelli 2013). Then, in one case, two egg clusters were found on the same plant, despite the availability of other young of narrow-leaved ash plants nearby.

It's not possible on the base of these data to reach a conclusion about a preferred size or age of the plants to lay eggs. European Owl Moth seems to prefer young plants to oviposit, but these data need to be confirmed by other observations. In this work a pattern of mobility is confirmed that can be considered as “nomadic foraging” (Spicciarelli 2018), as defined by Fitzgerald & Peterson (1988).

The mature *B. europaea* caterpillar goes down the tree and moves above the ground for 20-30 meters before moulting into a chrysalis, burrowing superficially in the soil.

Habitat 91B0 “Thermophilous *Fraxinus angustifolia* woods” occupies the 21.2% of the total surface of SAC “Grotticelle di Monticchio” corresponding to about 72.5 hectares, an area partially covered by the European *Braemia* food plants, as evidenced by the phytosociological surveys (Fig. 1). The forestry and phytosociological features of the SAC shows a widespread presence of the eastern hornbeam (*Carpinus orientalis* Mill. subsp. *orientalis*) that prevents the narrow-leaved ash renewal in many areas (Spicciarelli 2013), and the co-occurrence of the alien plant *Robinia pseudoacacia* L. The moth host plants have their highest concentration at the lower altitudes of the SAC, close to the Ofanto River, an area whose vegetation in the near past was more continuous and where numerous catches with lamp were obtained (Spicciarelli 2014).

The environment of “Grotticelle di Monticchio” is interested by a large population of wild boars, which feeds

also on chrysalis found in the ground. Probably they contribute to a reduction of the *Braema* population (Spicciarelli 2018), while the presence of red deer is linked to the bark of young narrow-leaved ash trees they feed on.

In the main area where the egg clusters were found, the presence of dense arboreal and shrubby vegetation, as well as prickly plants in herb layer, make the site almost inaccessible to these mammals.

Then data show clearly that the managing body of the SAC, Carabinieri Forestry, should be involved in modulating appropriate measures for the protection and conservation of the southern ash, the main food plant of *B. europaea*, to dissemination and renewal. As well as promote its environment outside and contiguous to the SAC observed in the last years. The protection of *B. europaea* requires also a significant increase of the area populated by the narrow-leaved ash, reversing the degradation of the protected area.

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