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Barbitistes vicetinus outbreaks in forest ecosystems (Orthoptera, Tettigoniidae)

Coordinatore: Prof. Sergio Casella

Supervisore: Prof. Massimo Faccoli

Dottorando: Dr. Giacomo Cavaletto

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Padova, 14 November 2017 Giacomo Cavaletto

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Riassunto

Barbitistes vicetinus (Galvagni & Fontana, 1993) (Orthoptera, Tettigoniidae) è un ortottero forestale endemico dell'Italia nord-orientale descritto nei primi anni '90 e considerato specie rara per almeno 10 anni dopo il suo primo rinvenimento. Dal 2008, intense pullulazioni si sono ripetute nel suo areale collinare, causando gravi defogliazioni ai boschi e alle colture limitrofe. Al fine di approfondire le scarse conoscenze attualmente disponibili e fornire un primo approccio alla gestione delle esplosioni demografiche, il presente lavoro si focalizza principalmente su indagini biologiche ed ecologiche inerenti a questa specie.

In particolare, la riproduzione di *B. vicetinus* è stata studiata in 18 siti nel corso di 3 anni consecutivi (2013-2015), testando l'influenza della tipologia di vegetazione e della copertura del suolo sulla scelta dei siti di deposizione e sulla schiusa delle uova. Per la prima volta la densità di popolazione è stata indagata durante una pullulazione, facendo registrare valori medi superiori a 1 milione di individui/ha. I risultati ottenuti col presente lavoro indicano che, nonostante gli individui adulti di questa specie possano frequentemente alimentarsi sulle coltivazioni agrarie causando danni anche gravi, queste aree non costituiscono siti preferenziali per l'ovideposizione. Infatti, le più elevate densità di neanidi sono state rinvenute in aree boscate e con terreno coperto la lettiera di latifoglie, senza che, peraltro, venisse evidenziato un gradiente di densità progredendo dal margine del bosco verso il suo interno.

Negli stessi siti, in 4 anni consecutivi (2013-2016), il presente lavoro ha inoltre considerato la fenologia delle schiuse con l'obiettivo di incrementare le conoscenze sulla dinamica di popolazione delle neanidi e chiarire il ruolo di alcuni fattori ambientali nei riguardi di questi aspetti. Un effetto significativo della temperatura è stato riscontato sulle tempistiche di schiusa, evidenziando che la specie è in grado di schiudere anche a temperature basse (6°C). E' stato inoltre dimostrato un ruolo fondamentale dell'altitudine e dell'esposizione sulle schiuse, ancora una volta in relazione al fattore temperatura.

Un'indagine specifica ha preso in considerazione l'ecologia spaziale della specie, studiando come diverse variabili, su differenti scale spaziali, influenzano la densità di popolazione. In due anni consecutivi (2014-2015) lo studio ha indagato 200 siti ricadenti entro l'area coinvolta dalle pullulazioni. Su una scala spaziale relativamente piccola (250-500 m) è stato riscontrato un effetto negativo della frammentazione forestale sulla densità di popolazione dell'insetto. Inoltre, sulla stessa scala spaziale, la presenza di specie

forestali alloctone e sgradite all'insetto, in particolare la robinia, è corrisposta ad un'ulteriore riduzione della presenza di *B. vicetinus*, indipendentemente da quale fosse la composizione forestale su scala locale (10 m). Proprio su una scala locale, invece, si evince come l'insetto sia in grado di alimentarsi su un'ampia gamma di latifoglie forestali, causando intense defogliazioni, con danni medi fino al 40% nelle specie più colpite. Su una scala di paesaggio, la popolazione è globalmente diminuita tra il 2014 e il 2015, con un maggiore effetto dove la defogliazione dell'anno precedente era stata più intensa.

Il presente lavoro fornisce le prime conoscenze di base per questa specie riguardo la bio-ecologia di popolazioni in fase di pullulazione. Le informazioni acquisite riguardo la fenologia di schiusa e la scelta dei siti di ovideposizione rappresentano il primo passo per una gestione razionale delle esplosioni demografiche. Inoltre, l'analisi previsionale delle pullulazioni, basata sulla conoscenza della composizione del paesaggio nelle aree potenzialmente a rischio, potrà fornire un ulteriore contributo in tal senso. Infine, la comparazione di queste conoscenze con quelle relative ad altri ortotteri forestali potrà condurre a una maggiore e migliore comprensione del rapporto fitofago - pianta ospite anche in altri contesti modello.

Summary

Barbitistes vicetinus (Galvagni & Fontana, 1993) (Orthoptera, Tettigoniidae) is a bush-cricket endemic of north-east Italy, described in the early 1990s and considered a rare species for the following ten years. Since 2008 repeated and destructive outbreaks occurred in hilly areas causing heavy defoliations to forests and close crops. In order to improve the little knowledge currently available on this species, the present PhD project focused mainly on biological and ecological aspects concerning reproduction, site selection and population dynamics.

Reproduction was studied over 18 sites in 3 consecutive years (2013-2015), testing the influence of both vegetation type and soil cover on the maternal laying preference and egg hatching of the species. Nymph density was assessed for the first time during outbreaks, showing mean values of over 1 million individuals/ha. In this respect, the highest nymph density was recorded in forest with soil covered by broadleaf litter, without a gradient from inner forest to edge. Our results indicated, however, that cultivated habitats did not offer suitable oviposition sites to *B. vicetinus*, although the adults are commonly found also in crops where they can cause severe damage.

In the same sites, during 4 consecutive years (2013-2016), we also investigated the hatching phenology in order to better understand the nymph's population dynamics and to clarify the role of the environmental factors affecting these aspects. A strong positive effect of temperature on the hatching start as well as on the hatching duration was found, although the species is however able to hatch also at low temperature (6°C). Elevation and exposure, affecting temperature, were demonstrated to regulate phenology and duration of the hatching period.

Moreover, the spatial ecology of *B. vicetinus* was investigated over the whole outbreak area (200 sites in 2014 and 2015) assessing local and landscape variables driving population density and defoliation severity. We found a strong negative effect of forest fragmentation on the pest population density at relatively small spatial scale (250-500 m). In addition, the presence of a non-host alien tree species (i.e., the black locust) across the landscape further reduced the population density irrespective of local (10 m) tree composition. At local scale, *B. vicetinus* was however able to feed on a wide array of broadleaves tree species, causing severe defoliation with leaf damage up to 40% of the

canopy. At regional scale, instead, the population declined from 2014 to 2015 mainly in sites where the defoliation has been more severe in the previous year.

Above all, this work provides the first basal knowledge about bio-ecology of outbreaking populations of this species. The new insights on the hatching phenology and oviposition site preference, for instance, represent crucial information to predispose an effective outbreak management. In addition, the forecasting of the outbreak risk based on landscape composition and structure of areas potentially exposed to infestations will be furtherly useful. Finally, the comparison of our results to those concerning other outbreaking bush-cricket species, could lead to a better understanding of the pest - host plants relationships occurring in other model species.

Chapter 1

Introduction

Orthopteran outbreaks

Outbreaks of insect populations are common in the pest species (Hunter, 1995). Destructive consequences for human's economies and ecological communities are frequent in both agricultural and forest ecosystems and may arise, particularly, in Lepidoptera, Coleoptera and Orthoptera orders (Lorch et al., 2005). During these demographic fluctuations, the population density rises to values much higher than those usually presented by the species. The environmental factors and the increment of resources abundance can lead to insect outbreak (Gherlenda et al., 2016) as well as their depletion may correspond to immediate decrease in population density (Hall et al., 2015; Hall et al., 2017). In the same way, an important role seems to be represented by the weather and by biological balance breakage (Feng et al., 2012). In addiction there are evidence showing genetic differences in mitochondrial DNA between solitary and gregarious populations of the same species (Bailey et al., 2005).

The phenomenon of outbreak in Orthoptera is the subject of several studies and well known worldwide especially for Caelifera suborder and, in particular, for the superfamily of Acrididae (Showler, 1995). Every year some species of Caelifera pass from a lone to a gregarious phase becoming responsible for extensive damage mostly in Africa but also in Asia, Australia and the Americas (Fontana et al., 2002).

This group is usually characterized by its notorious voracity, high fecundity, wide polyphagy, and considerable dispersal ability (Rong et al., 2006). For these reasons, many of this species are considered among to the most dangerous and harmful pests for wild and cultivated plants (Guevara et al., 2009). Periodically their communities show a huge increasing of their abundance (Cigliano et al., 2002), resulting in considerable damage of human activities. On the other hand, important benefits come to many Orthoptera species from human-induced climate and landscape changes (Sergeev, 1998) which allow or favor their outbreaks. In fact, disturbed ecosystems can be decisive for leaving and spreading of native and invasive species (Samways & Sergeev, 1997; Latchininsky et al., 2011). Despite this, little information is available regarding mechanisms and causes that underlie population dynamics (Joern & Gaines, 1990; Lockwood, 1997; Joern, 2000) and Orthoptera phase transformation (Latchininsky et al., 2011). In addition, too little effort has be done in order to understand how natural communities drive the variations from non-outbreak to outbreak densities (Cigliano et al., 2002) with dramatic spread from the native areas to the neighboring ones (Todd et al., 2002). Therefore, so far, outbreaks predictions are not so reliable and this entail very often an intensive use of insecticides over large outbreak areas (Nailand & Hanrahan, 1993) with strong impacts on human health and non-target species (Samways, 2000).

Even in the recent past, intense outbreaks were caused, mostly in North Africa and Asia, by acridid species (Fam. Acrididae) such as *Schistocerca gregaria* (Forskål, 1775) (Latchininsky et al., 2011), *Locusta migratoria* (Linnaeus, 1758) (Tanaka & Zhu, 2005; Rong, 2006) and *Dociostaurus maroccanus* (Thunberg, 1815) (Benfekih et al., 2002). In north Italy, the outbreaks of the acridid *Calliptamus italicus* (Linnaeus, 1758) and *D. maroccanus* are well known. Recent outbreaks of *C. italicus* occurred in Emilia Romagna(2002-2005) and in Piemonte (2004-2008), which was also added to high density of *Kisella irena* (Fruhstorfer, 1921) (Massobrio et al., 2010). In Veneto, *C. italicus* outbreaks were recorded in the Lessinia area (Buzzetti et al., 2005) and in 2013-15 also in the province of Vicenza (Berici Hills) and Padova (Euganean Hills). For the Centre-South Italy, and in particular Sardegna and Puglia, several outbreaks of *D. maroccanus* were recorded (Melis, 1934; Baldacchino et al., 2012).

Among the Ensifera suborder the presence of gregarious phase is instead rare and little studied worldwide. The most important case in the last twenty years is probably represented by the Mormon cricket *Anabrus simplex* Haldeman, 1852 affecting wide Western North American areas with huge migratory bands (Lorch & Gwynne, 2000; Lorch et al., 2005; Sword et al., 2005; Bailey et al., 2007a, b; Srygley, 2014) with important damage to crops. In the last decades, other relevant katydid (Ensifera) infestations causing agricultural damage were reported in Southern Africa, by the Armored bush crickets of the genus *Acanthoplus* Stål, 1873 (Wohlleber, 1996; Minja et al., 1999) and in South Korea, by the Ussur brown katydid *Paratlanticus ussuriensis* (Uvarov, 1926) (Na et al., 2007; Moon et al., 2009; Bang et al., 2011; Jung et al., 2011). In Italy, *Decticus albifrons* (Fabricius, 1775), whose demographic explosions were recorded in the recent past in the South, caused large damage to crops (Massa et al., 2012). In Ensifera some species are also well known for damage caused to forests. In this context, over Europe, occasional outbreaks were reported in some species of bush-cricket genus *Barbitistes* (Charpentier, 1825) (Fam. Tettigoniidae).

The genus *Barbitistes* (Charpentier, 1825)

This genus includes only 9 West-Palaearctic species (Fig. 1.1), although the current taxonomic status of some of them is under discussion (Baur & Coray, 2004; Fontana & Buzzetti, 2004). *Barbitistes* can be distinguished by the most similar genera for the S-shaped male cerci and for the subgenital plate of female devoid of processes (Massa et al., 2012). The genus is close to the genus *Isophya* Brunner, 1878, but is distinguished by the poorly marked rib of the short and globular tegmina. The femurs are relatively short. The ovipositor is curved only in the final part and is strongly dented near the apex.

B. constrictus Brunner, 1878 is the only species never reported to Italy. It feeds on coniferous, mainly *Pinus* L. spp. and *Larix* Mill. spp., growing in Central and Eastern Europe where may cause occasional forest outbreak, as reported for Czech Republic and Poland (Escherich, 1928; Laussmann, 1994; Holuša et al., 2006).

B. alpinus Fruhstorfer, 1921 is frequently found in South-East France, Southern Switzerland, and Italian Alps (Fontana & Buzzetti, 2004; Massa et al., 2012). It is also regularly found in the Apennines, up to the Abruzzo region. The species feeds on both herbaceous plants (in particular *Petasites* Mill. spp.) and coniferous trees. It is a strictly mountain species living up to 2000 m.

B. ocskayi (Charpentier, 1850) is spread in the whole Balkan region, from Trieste (NE Italy) to Greece. The species often affects deciduous forests occurring in the Karst of Trieste with periodic outbreaks. In the last, occurred in 2004, the Forest Station of Duino-Aurisina recorded intense defoliations on *Fraxinus ornus* L. forests growing along the Italian-Slovenian border (Stergulc et al., 2005). In this context the species was observed to feed also on lepidopteran larvae (*Lymantria dispar* Linnaeus, 1758).

B. serricauda (Fabricius, 1794) is widely spread in Central and Eastern Europe, up to Ukraine, in the Northern part of the Balkan Peninsula as well as in the Italian Alpine foothills. Recently the species was detected also in Corsica. It feeds in mesophilic forests, in particular on *Quercus* L. spp., *Ostrya carpinifolia* Scop. and *Corylus avellana* L.. During the night it can be found also on herbaceous plants.

Barbitistes fischeri (Yersin, 1854) is an Iberian species, spread from Portugal to Southern France, up to Western Italy. It has been occasionally reported damaging crops in France, in particular vineyards.

Barbitistes yersini Brunner, 1878 is a trans-Adriatic species, widely distributed along the Balkan coasts and recorded for few Italian localities in Abruzzo and Marche regions (Central Italy). It is a thermophilic species developing on Mediterranean bushes and trees (*Spartium junceum* L., *Pistacia lentiscus* L., *Fraxinus* L. *sp*., *O. carpinifolia*). It was observed to feed also on aphids.

Barbitistes kaltenbachi Harz, 1965 is a bush species of Croatia, where typically lives in the forest edge areas. Although an old observation was reported to Italy (Massa et al., 2012), the presence of this species in Italy needs confirmation.

Barbitistes obtusus Targioni-Tozzetti, 1881 shows a taxonomic status still under discussion. Only a male of this species was found and described from Sardegna, but later the specimen was lost. As no additional individual has never been found, the description of *B. obtusus* could be based on an identification mistake, maybe with *Odontura* Rambur, 1839, a very similar genus occurring in the same area (Fontana & Buzzetti, 2004).

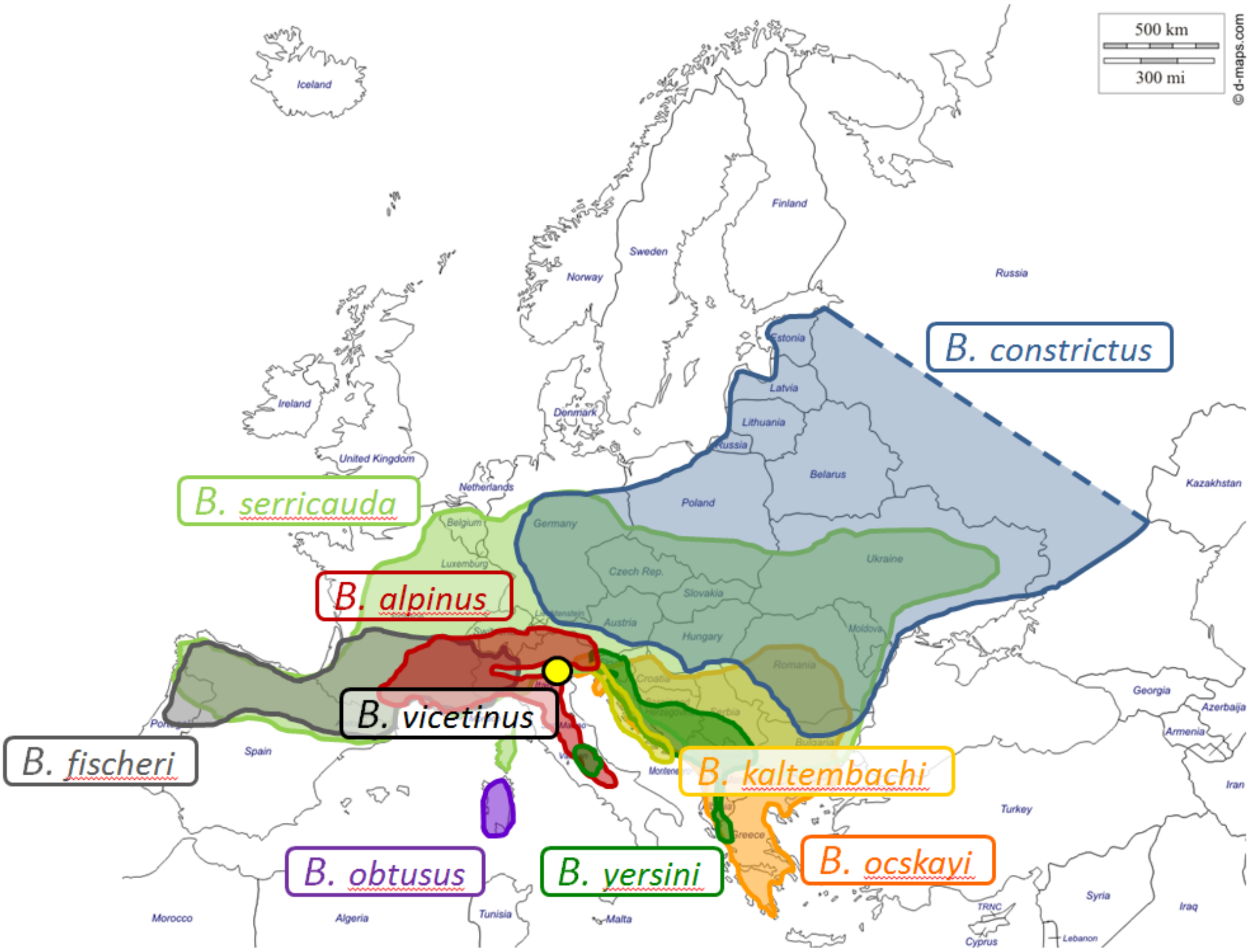

Figure 1.1 Map representing the distribution area of the 9 *Barbitistes* species over Europe

Barbitistes vicetinus Galvagni & Fontana, 1993

Figure 1.2 Different instars of *Barbitistes vicetinus*.

Barbitistes vicetinus (Fig. 1.2) is an Italian endemic forest pest species described very recently and spread almost exclusively in the Veneto region (NE Italy), where lives in hilly areas between sea level to about 600 m a.s.l.. The name of the species refers to the area in which the first specimen was found, close to the city of Vicenza (Vicetia in Latin). So far, individuals belonging to this species have been collected only in the Lessinia and Berici hills (Vicenza province) and in the Euganean Hills (Padova province) (Fontana et al., 2002) as well as in a valley of the Trentino region (Val d'Adige) (Agabiti et al., 2006; Mazzon et al., 2011; Fontana, 2012) (Fig. 1.3).

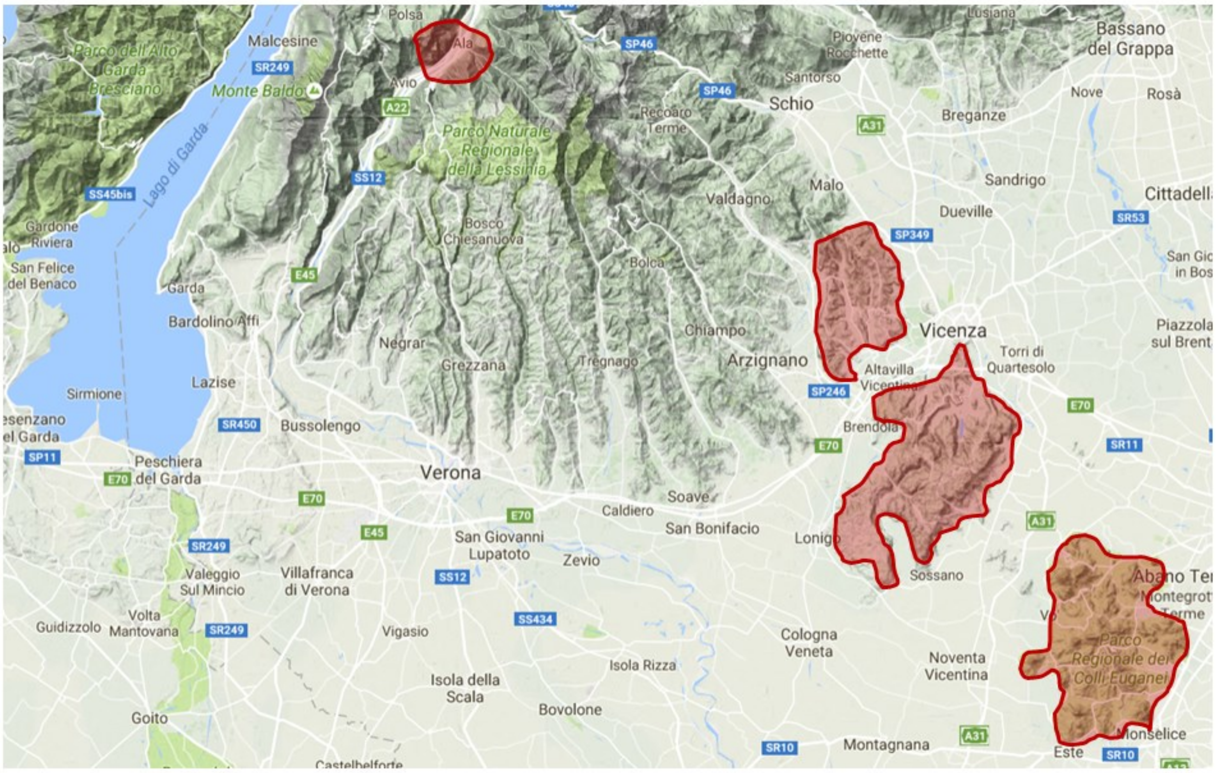

Figure 1.3 Distribution map of *Barbitistes vicetinus* in the endemic areas of North East Italy, in red.

The species lives mainly in forests and ecotone areas feeding principally on leaves of trees and shrubs (Galvagni & Fontana, 1999; Magello et al., 2003). For about two decades after its description, the species was considered rare and, only in the recent years, increasing population density was recorded in the Berici Hills.

Moreover, since 2008, severe outbreaks occurred in Euganean Hills, originally in restricted zones but then, in the subsequent years, affecting the whole hillside area (Fig 1.4).

Figure 1.4 *Barbitistes vicetinus* in chestnut during outbreak.

During outbreak *B. vicetinus* causes severe defoliations especially to forest species although able to affect also the crops cultivated close to the infested wooded areas, such as vineyards, olive grove and cherry orchards (Mazzon et al., 2011; Cavaletto et al., 2014, 2015) (Figs. 1.5-1.6). In addition, the outbreak was also a source of annoyance to people living close to the outbreaking areas, as the bush crickets invade streets and gardens (Cavaletto et al., 2015, 2016).

Figure 1.5 Typical crop areas cultivated close to the forest edge and damaged by *Barbitistes vicetinus* during outbreak.

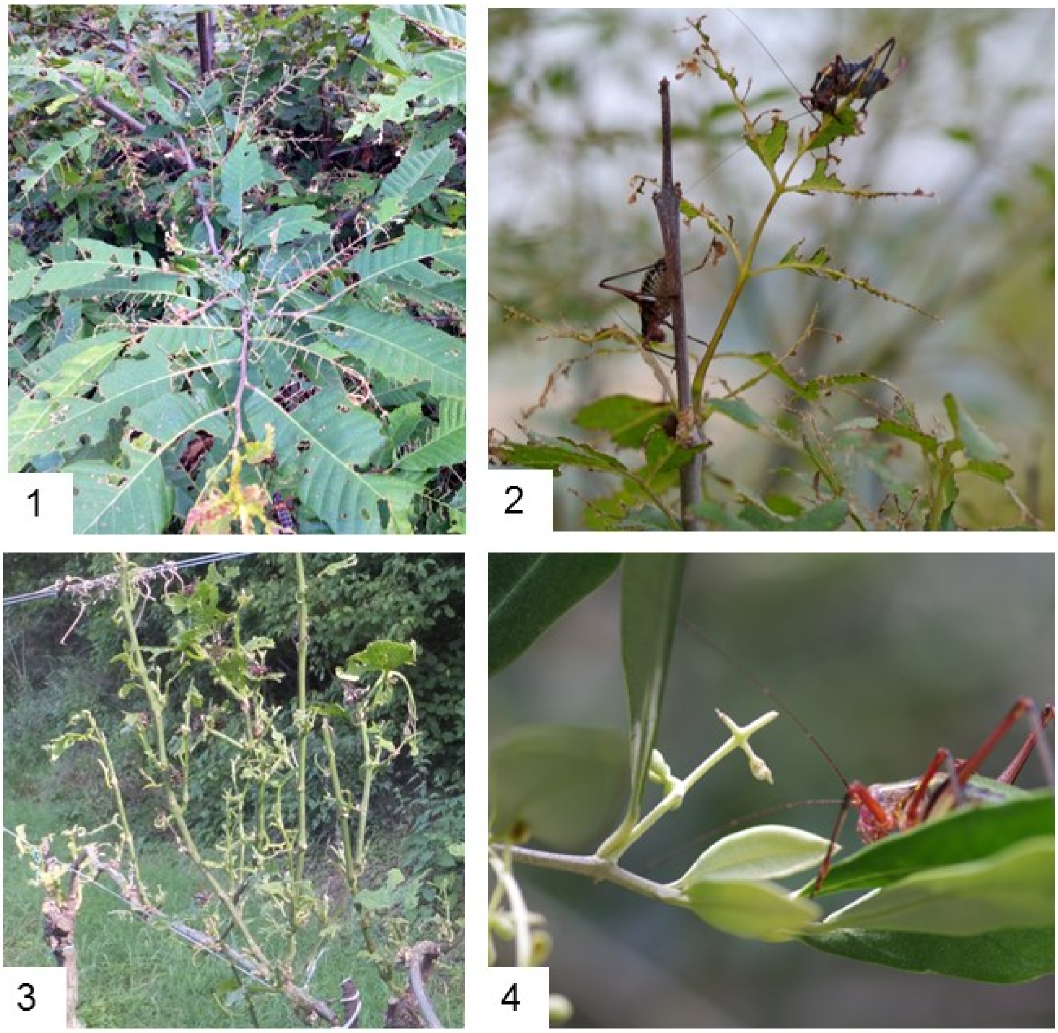

Figure 1.6 Leaf damage on chestnut (1), South European flowering ash (2) grape (3) and olive (4).

During outbreaks, *B. vicetinus* changes its ethology showing a tendency to gather in large numbers in small spaces. In addition, morphological features are modified too, with the appearance of a melanic form, with aposematic coloration (black with red on the joints and appendixes) (Fig. 1.7). Little is known about the biological or ecological significance of this phenomenon, although reported also in other Ensifera (Lorch & Gwynne, 2000; Lorch et al., 2005; Sword et al., 2005; Bailey et al., 2007a, b); some research carried out on outbreaking populations, however, show a correlation between the melanin content and the increased immune response (Bailey, 2011; Barbosa et al., 2012).

Figure 1.7 Typical green form (left) and melanic form (right) of *Barbitistes vicetinus*.

Like many other Tettigoniidae, *B. vicetinus* is a univoltine species overwintering as egg laid in the soil (Cavaletto et al., 2014). Egg hatching generally begins at the end of March, and the life-cycle lasts until the half of July when the last adults die after oviposition. The first adults appear in May while mating and oviposition last for the entire June (Fig. 1.8). As many other katydids, the eggs of *B. vicetinus* do not hatch in the year following the oviposition, but they remain diapausing into the soil at least for two winters.

The first instars nymphs feed mainly on bush and shrubs (in particular *Rubus* L. spp.) while, in subsequent weeks, the individuals climb up to the trees where they spend most of the time eating leaves. In addition, laboratory analysis conducted on the adult frass, confirmed the presence of animals in the diet, in particular moths, aphids and mites (Magello, 2001).

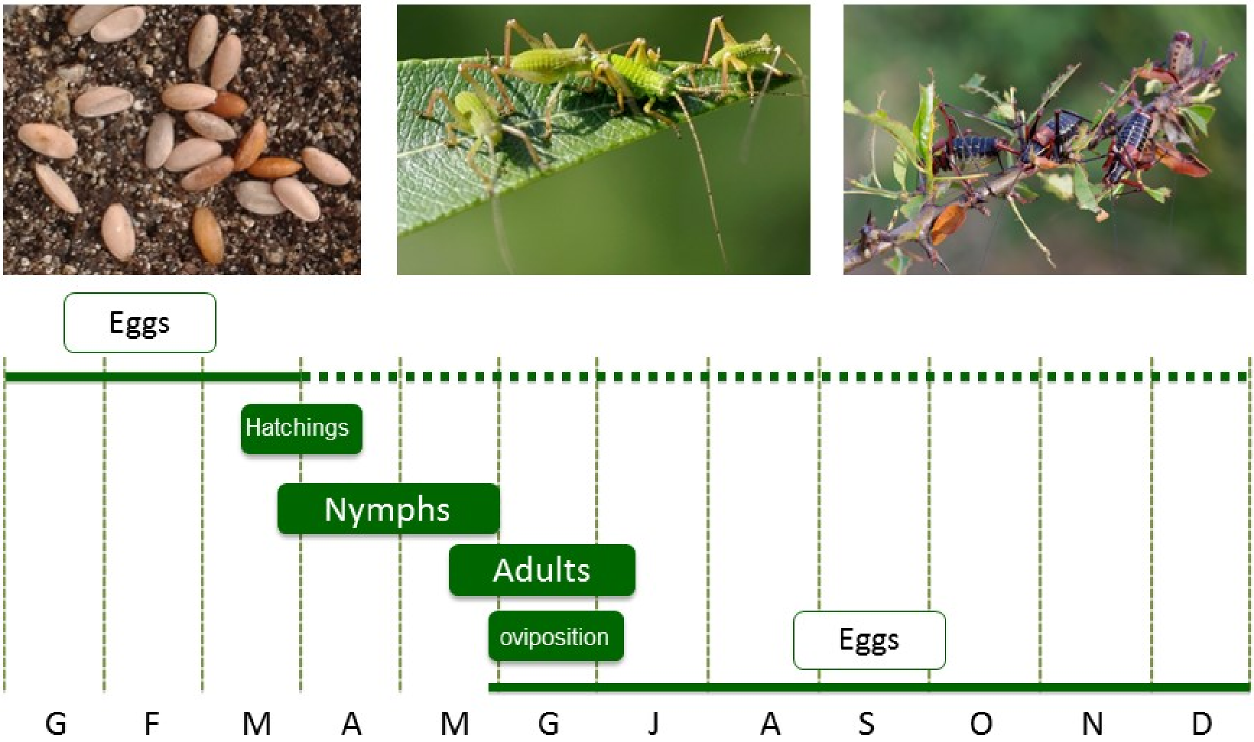

Figure 1.8 Life-cycle in *Barbitistes vicetinus*.

Aims

Barbitistes vicetinus is an endemic bush cricket currently occurring only in a few localities of north-east Italy. Since 2008 destructive and repeated outbreaks caused heavy damage to forests and crops. As the species was described just in 1993 and considered rare at least for the subsequent ten years, few data are currently available on pest biology and ecology. In this respect, the present PhD project focused mainly on 3 major aspects of the *B. vicetinus* bio-ecology, from adult reproduction (maternal behavior and egg hatching) to population dynamics (variables driving population density), as follows:

1) **Evaluate the oviposition site preference in the outbreak areas, and test the influence of soil and vegetation cover on the maternal behavior**

   We investigated the oviposition site preference recording the amount of hatched nymphs in different vegetation type (vineyard, hedgerow and positions from forest edge to inner forest) and with different soil cover (grass and litter).

2) **Investigate the hatching phenology of *B. vicetinus***

   We studied the egg hatching in 4 consecutive years in sites with different elevation and exposure; in laboratory the effect of temperature on the hatching phenology was also tested in controlled conditions.

3) **Study the local and landscape factors affecting both adult and nymphs population density**

   Monitoring the whole outbreak area, the spatial ecology of *B. vicetinus* was investigated assessing variables driving population density and defoliation severity at different scales. Effects of forest abundance and composition, landscape fragmentation and host-plants were explored at local and regional scale.

Chapter 2

Oviposition site preference of *Barbitistes vicetinus* (Orthoptera, Tettigoniidae) during Outbreaks

Giacomo Cavaletto*, Massimo Faccoli, Lorenzo Marini, Isabel Martinez-Sañudo and Luca Mazzon

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Running title: Oviposition preference of *Barbitistes vicetinus*

*Corresponding author:

Giacomo Cavaletto

Viale dell'Università 16,

35020 Legnaro (PD), Italy

E-mail address: giacomo.cavaletto@gmail.com

Tel. +39 049 8272875

Fax. +39 049 8272810

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Introduction

Locust outbreaks (Orthoptera, Caelifera) are a very well-known phenomenon affecting many African and Asian regions (Showler, 1995). Katydid (Orthoptera, Ensifera) infestations occur less frequently, although they can cause serious local damage (Gwynne, 2001; Lorch et al., 2005). The bush-cricket *Barbitistes* Charpentier, 1825 (Ensifera, Tettigonidae) includes only nine Palaearctic species, although the current taxonomic status of some of them is under discussion (Baur & Coray, 2004; Fontana & Buzzetti, 2004). To date, occasional *Barbitistes* outbreaks have been reported only for *Barbitistes constrictus* in conifer forests of Central and Eastern Europe and for *Barbitistes ocskayi* in broadleaf forests of north-east Italy (Escherich, 1928; Laussmann, 1994; Holuša et al., 2006; Massa et al., 2012).

In 1993, *Barbitistes vicetinus* Galvagni & Fontana was described as an endemic species of north-east Italy, being found only in small confined hilly areas (Galvagni & Fontana, 1993, 1999). The first outbreak of *B. vicetinus* occurred in spring 2008 in a restricted area of north-east Italy (Euganean Hills). Subsequently, the outbreak areas have enlarged, causing heavy damage to forests and neighbouring crops (mainly grape and olive), with severe defoliations rising up to approaching 90% in the most serious infestations (Mazzon et al., 2011; Cavaletto et al., 2014, 2015). Moreover, the outbreaks are also a source of annoyance to people living close to the attacked areas because the katydid invades streets and gardens (Cavaletto et al., 2015, 2016).

Because the outbreaks occurred only in the last decade, no data are currently available on pest biology and ecology. Similar to many species of this family, *B. vicetinus* overwinters as an egg in the soil (Cavaletto et al., 2014). However, little is known about the reproductive behaviour and maternal oviposition preferences, which are crucial factors for understanding the species habitat use. Although most studies concerning the selection of oviposition sites have focused on locusts inhabiting grasslands, meadows or fallows (i.e. Cherrill & Brown, 1992; Rong et al., 2006), specific studies on bush-crickets inhabiting forests are still very rare (Petit et al., 2007).

Knowledge about oviposition preferences is a crucial factor for explaining the distribution of the species in the landscape and developing rational pest-management strategies when needed. We conducted a 3-year field study investigating the oviposition preference of *B. vicetinus*. Specifically, we tested whether vegetation type and soil cover affects the oviposition preference. We studied whether, during outbreaks, *B. vicetinus* laid eggs in non-forest habitats, especially crop areas close to the forests. Once the preferred habitats were identified, we further tested whether small-scale differences in soil cover (broadleaf litter and grass) can affect oviposition preference. The present study reports, for the first time, data concerning the emergence density of *B. vicetinus* populations during outbreaks.

Materials and methods

The study area

The study area was situated in the Euganean Hills (Veneto Region, north eastern Italy), comprising approximately 100 hills of volcanic origin emerging from the alluvial sedimentary plain, with a highest elevation of approximately 600 m a.s.l. The hills cover an elliptical area of approximately 22 000 ha. The climate is characterized by an annual mean temperature of approximately 12°C and precipitation in the range 700-900mm (Kaltenrieder et al., 2009). Because of the numerous narrow and deep valleys, steep hills and different sun expositions, many microclimatic conditions influence the vegetation. A sub-Mediterranean flora occurs on the south-facing slopes with warmer and drier climatic conditions, whereas temperate deciduous vegetation grows on cooler and moister north-facing aspects (Del Favero, 2001). The vegetated landscape consists of forest patches, mixed with various types of crop fields and rural settlements.

Field trials

The present study was conducted in 18 sites selected across the outbreak area (Fig. 1 and Table 1). All of the selected sites contained broadleaf forests dominated by *Castanea sativa* Mill., *Fraxinus ornus* L., *Ostrya carpinifolia* Scop. and *Quercus pubescens* Willd. growing adjacent to grass-covered vineyards or meadows. Each forest edge consisted of shrubs including mainly *Rubus* L. spp., *Cornus* L. spp. and *Acer campestre* L.. The influence of vegetation type and soil cover on the oviposition site preference of *B. vicetinus* was assessed by 96 emergence traps set out in different numbers at 18 sites (for details, see below), in three consecutive years (2013–2015). Emergence traps have been used fairly extensively in agroecological studies to estimate population density of arthropods

(Sunderland et al., 1995), as well as to assess their habitat preference (Marrec et al., 2014).

The emergence traps consisted of a pyramidal wooden frame (50 cm high) with a square base of 0.25m^2. The frame was closed by a mosquito net with an opening top that allowed collection of *B. vicetinus* nymphs emerging from the soil. Traps were set out on the ground in relatively flat sites, devoid of obstacles such as roots or rocks, and far away from walking trails. None of the selected sites had experienced disturbances such as chemical treatments (herbicides or insecticides) or tillage operations in the previous years. The emergence traps were placed in the field in late winter (February) and insect sampling was carried out every 48 h from mid-March until the end of April, covering the whole hatching period of *B. vicetinus* (Cavaletto et al., 2015). At each sampling, all newly-emerged nymphs were collected from the traps and moved to the laboratory for identification, according to Massa et al. (2012), and counting.

Site	Sampling							Position	
	2013			2014		2015		Latitude N	Longitude E
	Vegetation type			Forest soil cover					
	Forest	Hedgerow	Vineyard	Edge	Inner	Edge	Inner		
1	-	-	-	(1) G	(1) L	(1) G	(1) L	45°15'45"	11°39'59"
2	-	-	-	(1) G	(1) L	-	-	45°15'34"	11°40'43"
3	-	-	-	(1) G	(1) L	(1) G	(1) L	45°16'24"	11°40'20"
4	-	-	-	(1) L	(1) L	-	-	45°15'40"	11°41'21"
5	-	-	-	(1) L	(1) L	(1) L	(1) L	45°16'08"	11°40'22"
6	-	-	-	(1) L	(1) L	-	-	45°16'44"	11°40'48"
7	-	-	-	(1) L	(1) L	(1) L	(1) L	45°17'20"	11°41'29"
8	-	-	-	(1) L	(1) L	-	-	45°17'22"	11°41'59"
9	(15)	(3)	(3)	(1) L	(1) L	(1) L	(1) L	45°17'12"	11°40'51"
10	-	-	-	(1) L	(1) L	-	-	45°17'40"	11°41'49"
11	(15)	(3)	(3)	(1) L	(1) L	(1) L	(1) L	45°17'00"	11°40'26"
12	-	-	-	(1) L	(1) L	-	-	45°18'33"	11°41'28"
13	-	-	-	(1) G	(1) L	(1) G	(1) L	45°18'03"	11°41'38"
14	-	-	-	(1) G	(1) L	-	-	45°18'18"	11°41'55"
15	-	-	-	(1) G	(1) L	(1) G	(1) L	45°18'54"	11°42'08"
16	-	-	-	(1) G	(1) L	-	-	45°18'32"	11°42'06"
17	-	-	-	(1) G	(1) L	(1) G	(1) L	45°19'42"	11°42'04"
18	-	-	-	(1) G	(1) L	-	-	45°19'15"	11°41'33"

Table 2.1 Characteristics of sampling sites and year of study. The number of emergence traps used every year is indicated in brackets. For 2013, in the forest, we considered different distances from the edge (0.5, 10, 30, 50, 200 m) with three emergence traps at each distance. For 2014 and 2015, in forest soil cover, G and L represent grass and leaf litter respectively.

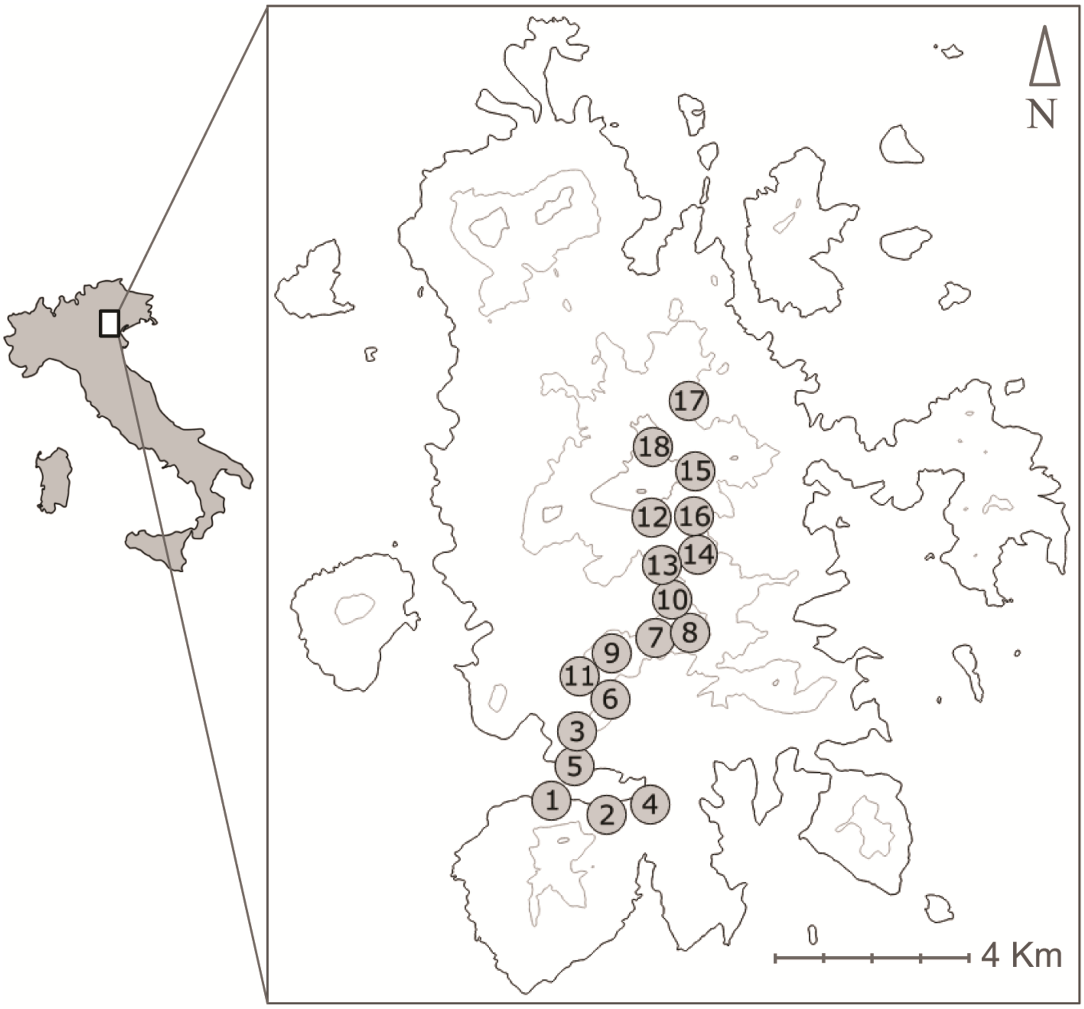


Figure 2.1 Map showing the Euganean Hills with the study sites represented by circles.

Effect of vegetation type

During spring 2013, the oviposition preference was tested in different contiguous vegetation types, namely woody vegetation (mixed broadleaf forest and narrow strips of woodland) and crops (grass-covered vineyard). Vineyards were 25m wide, whereas the woodland strips (i.e. hedgerows) were 10m wide and dominated by the same botanical species as the previously described forest. The study was conducted in two different sites (Fig. 1 and Table 1). In each site, a total of 21 emergence traps were placed: three in the middle row of the vineyard, three in the middle of the hedgerow and 15 in the forest. Within the forest area, we located traps at five distances from the edge (0.5, 10, 30, 50 and 200 m). Three traps were placed at each distance.

Effect of soil cover

To test the influence of soil cover on the *B. vicetinus* oviposition preference, forest litter and grass were compared. During spring 2014, at 18 sites, two emergence traps per site were set up in the forest, at 50 and 0.5m from the forest edge. Thus, a total of 36 traps were used (Table 1). The traps far from the edge (inner forest) were always placed on broadleaf litter, whereas those at the edge were either on broadleaf litter (nine sites) or on grass (nine sites). The same experimental design was repeated at nine sites in spring 2015 (Table 1), when, in four sites, the traps close to the forest edge were placed on leaf litter, whereas, in the other five sites, they were placed on grass, for a total of 18 emergence traps.

Statistical analysis

To test the effect of vegetation type, distance from forest edge and soil cover, we used general linear-mixed models (GLMM). In the three models, the fixed effects were vegetation type, distance from the edge (continuous variable) and soil cover, respectively. In each model, we also included site ID as a random factor to account for spatial dependence in the sampling design. Tukey's honestly significant difference (HSD) post-hoc comparison test was applied when the fixed effects were significant ($P<0.05$). The response variable was the cumulative number of nymphs per trap collected during the whole sampling season (natural log-transformed to reduce variance heterogeneity). Assumptions of linear models were tested by inspecting diagnostic plots of model residuals. All analyses were run in r (R Core Team, 2014) using the nlme package (Pinheiro et al., 2017) applying the lme function.

Results

General results

During the hatching periods of the three sampling years (2013–2015), a total of 822, 1427 and 76 first-instar nymphs of *B. vicetinus* were collected, respectively. Maximum values were found in woody areas with the soil covered by leaf litter, with a mean±SE of 20.5±3.9, 51.6±7.7 and 5.5±1.1 nymphs per emergence trap for each year. The minimum values were registered in vineyards (1.8±1.3 nymphs per trap in 2013). Only 7.3% of traps did not capture any individuals.

Effect of vegetation type

The GLMM indicated that the vegetation type had a significant effect on the presence of *B. vicetinus* (F=22.04, d.f.=2, 38, P<0.001), with a higher nymph density in forest and hedgerows than in vineyards (Tukey's HSD test, P<0.01) (Fig. 2). Instead, when focusing only on forest, increasing distances from the forest edge did not show any statistical differences in nymph density (F =0.20, d.f.=1, 27, P=0.654) (Fig. 3).

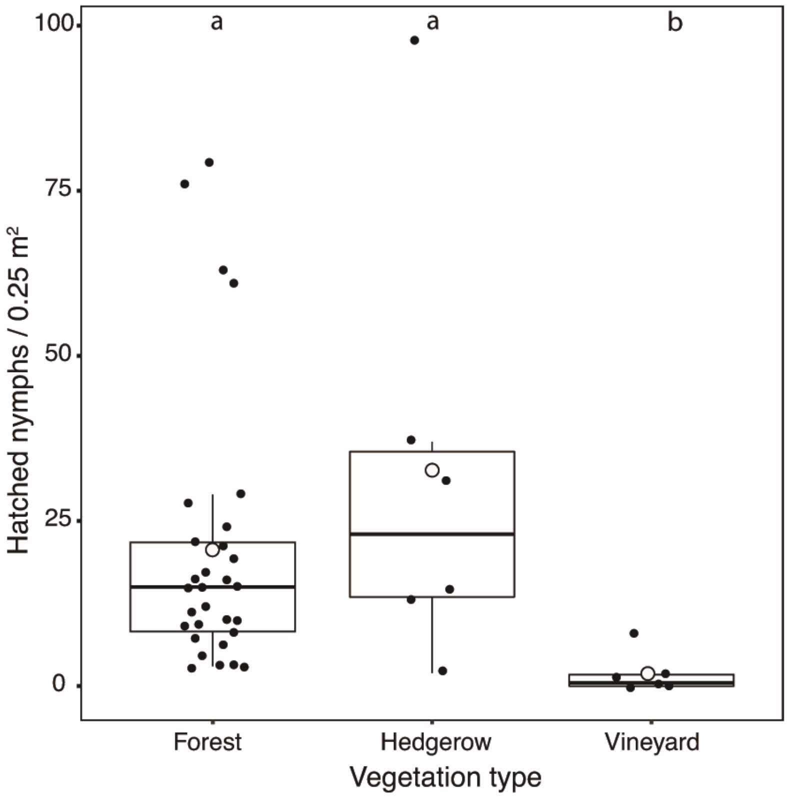


Figure 2.2 Effect of the three different vegetation types on the number of *Barbitistes vicetinus* nymphs hatched per 0.25 m^2. The thick lines represent the medians and the white dots indicate the means. Treatments marked with different lowercase letters indicate significant differences (Tukey HSD test P < 0.01).

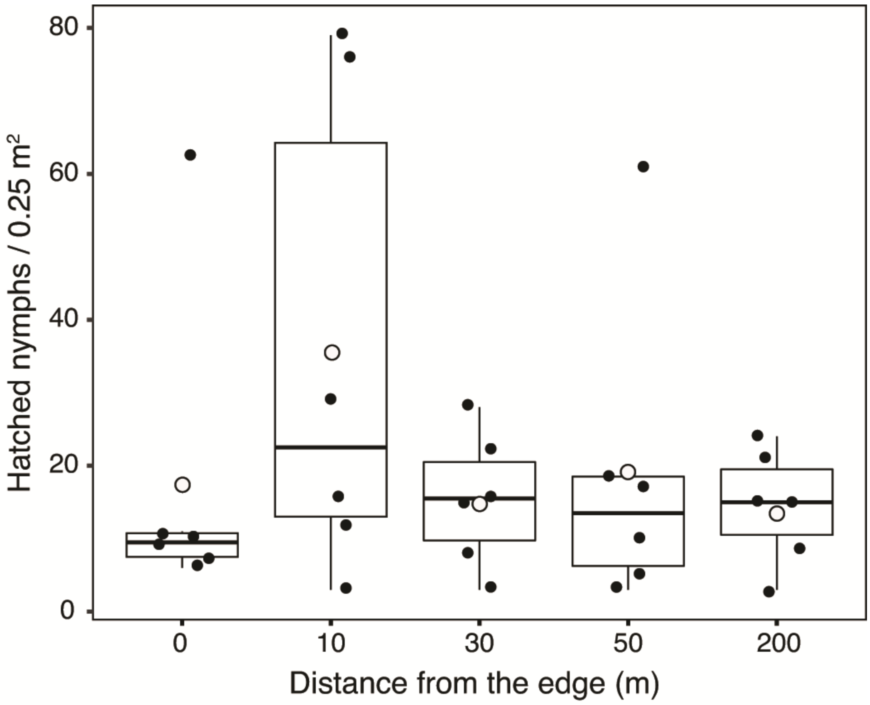


Figure 2.3 Effect of five different distances from the edge toward the inner forest on the number of *Barbitistes vicetinus* nymphs hatched per 0.25 m^2. The thick lines represent the medians and the white dots indicate the means.

Effect of soil cover

Studies conducted in 2014 and 2015 showed a significant effect of soil cover on nymph density (F=28.89, d.f.=2; 33, P<0.001). In particular, traps located on grass had a significantly lower density compared with traps set up out on broadleaf litter, both along the edge and in the inner forest. By contrast, both litter areas showed high and similar nymph densities with no significant differences (Tukey's HSD test) (Fig. 4).

As a result of the edge features, it was not possible to compare grass and litter on the edge directly at any site. Nevertheless, the similar insect density recorded in the two litter types and the clear difference between inner litter and grass provide indirect support for the hypothesis of a strong difference between the edge litter (higher) and the grass (lower) with respect to the number of emerging *B. vicetinus* nymphs.

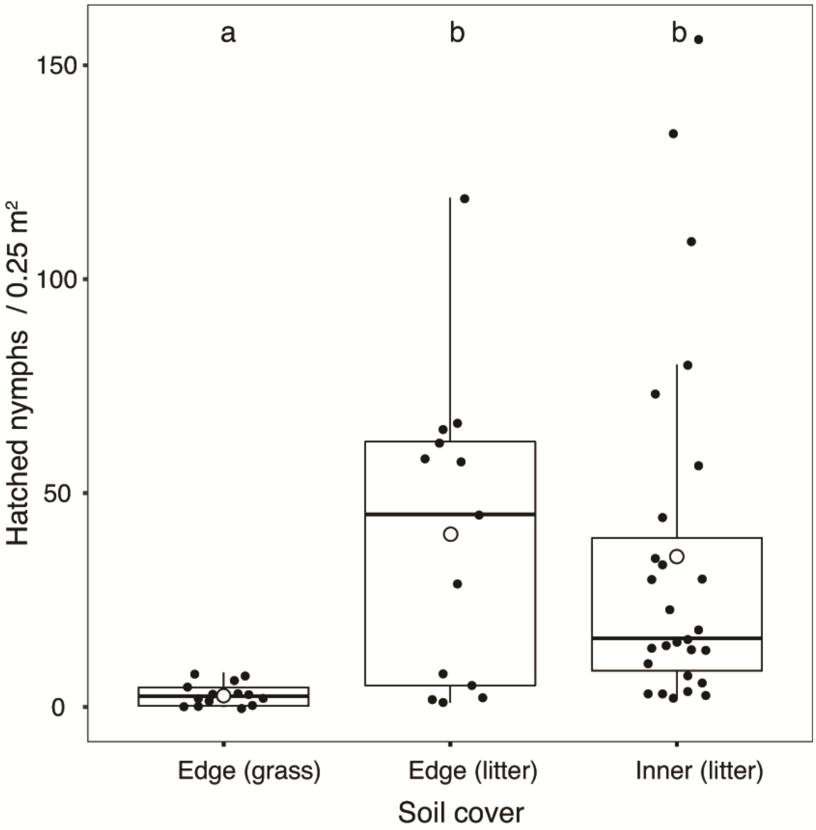


Figure 2.4 Effect of different soil cover on the number of *Barbitistes vicetinus* nymphs hatched per 0.25 m^2. The thick lines represent the medians and the white dots indicate the means. Treatments marked with different lowercase letters indicate significant differences (Tukey HSD test P < 0.01).

Discussion

The present study reports, for the first time, data regarding *B. vicetinus* oviposition site preference and nymph density recorded during three consecutive outbreak years. A significant effect of vegetation type and soil cover was found, with oviposition mainly being associated with woody vegetation (forest and hedgerows) and broadleaf litter, even if the species is abundant also in the neighbouring crops during outbreaks. A large number of tettigoniid species living in temperate climates spend most of their life-span in the egg stage (Bailey & Rentz, 1990). Indeed, the embryonic development of several Western Palaearctic species of tettigoniids can last for more than 1 year as a result of a prolonged diapause (Ingrisch, 1986). *B. vicetinus* spends several months as an egg, indeed a multi-year egg diapause has been reported (Magello et al., 2003; Cavaletto et al., 2014). The selection of the oviposition site is thus of crucial importance because local environmental conditions can affect egg survival, as well as influence growth and development of the resulting offspring. Although some tettigoniid can lay eggs in plant tissues (i.e. plant stems), according to Gwynne (2001), the probable ancestral oviposition site for these species is the soil.

Studies regarding hatch density of tettigoniids laying in the soil carried out by emergence traps are rare; but see Holuša et al. (2006). Despite this species being harmful to crops during outbreaks, the result of the present study show that *B. vicetinus* oviposition mainly occurs in forests and hedgerows. Similar to many other insects, egg development in bush-crickets is temperature-dependent and accelerates with fluctuating temperatures (Behrens et al., 1983). Moreover, a low temperature is usually recognized as the primary mortality factor for insects during overwintering (Haye et al., 2010; Rusch et al., 2011), with consequences for the density and structures of the subsequent generations (Faccoli & Buffo, 2004; Faccoli & Stergulc, 2006). However, the largest number of emergences recorded in the present study in sheltered areas does not appear to be linked to the overwintering survivorship (not considered here) but, instead, to the oviposition preference for these areas. Laying eggs in shelter conditions (forests and hedgerows) may be related to the reduced thermal fluctuations during winter (Ingrisch & Boekholt, 1983). This strategy has been recorded in many other insects (i.e. species within Chrysomelidae and Curculionidae families) (Ulmer & Dosdall, 2006a; b). The range of daily temperature variations decreases with increasing depth in the ground, thus providing a more constant temperature during winter. Among Orthoptera, several grasshoppers take advantage of this stable environment by laying eggs at a depth of approximately 5 cm (Leather et al., 1993). Because *B. vicetinus* has a short ovipositor (approximately 1 cm in length), oviposition in sheltered areas could help this species to avoid wide temperature fluctuations affecting very shallow eggs. A preference for oviposition in forest sites was reported also in *Pholidoptera griseoaptera* (De Geer, 1773), which has a similarly short ovipositor (Ingrisch, 1986). Soil cover was another important factor affecting selection of the oviposition site, with a strong positive effect of forest litter on nymph density. Broadleaf litter showed an insect density over ten-fold higher (>39 individuals/m^2) than areas covered by grass. The maternal preference for oviposition in broadleaf litter might be a result of the presence of a soft soil dominated by a large amount of organic material that facilitates oviposition with respect to the more compact soil covered by grass. The preference for a thick layer of leaf litter is known also for other insect species (e.g. Nitidulidae) because a deep layer provides more sheltered microclimatic conditions and good protection against lethal low temperatures (Ulmer & Dosdall, 2006b; Rusch et al., 2011).

On the basis of our findings, the soil tillage suggested as possible control practice against many orthopteran species (Hein & Campbell, 2004) is probably useless for the containment of *B. vicetinus* populations. Instead, during outbreaks, the management of the forest edges is of major importance for the control of this pest. In this context, where the habitat is sensitive and the conventional control programmes are not feasible, it will be important to develop innovative and environmental-friendly strategies. Biological control strategies such as the use of entomopathogenic fungi (e.g. *Metarhizium* Sorokīn genus) or insecticides with low environmental impact (e.g. natural pyrethrum) (Cavaletto et al., 2015) could be considered at the end of the hatching period and before the spread of the new bush-cricket nymphs from the forest to the crops.

In conclusion, although *B. vicetinus* is commonly found in open habitats where it can cause severe damage to crops, these habitats did not offer suitable oviposition sites. The results of the present study provide useful insights with respect to the management of *B. vicetinus* outbreaks, especially where crops are cultivated close to the woody vegetation. Unlike other Orthoptera species, which use crops as main habitat, *B. vicetinus* only becomes a pest in crops during outbreaks when it spreads out from its natural woodland habitat. As shown in the present study, this species rarely chooses vineyards as the oviposition site. Because oviposition can also take place in the inner forest, to improve the outbreak management, further studies will be required to determine the movement patterns and dispersal ability of the species from the forest hatching sites to the close crops.

Chapter 3

Hatching phenology in outbreak populations of the bush cricket *Barbitistes vicetinus* (Orthoptera, Tettigoniidae)

Giacomo Cavaletto, Massimo Faccoli, Isabel Martinez-Sañudo and Luca Mazzon

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell’Università 16, 35020 Legnaro (PD), Italy

Introduction

Many temperate species of insects escape adverse seasons by entering diapause (Tauber & Tauber, 1976). Diapause in insects is genetically controlled and regulated by environmental factors such as photoperiod and temperature (Denlinger, 2002).

Tettigoniids (Orthoptera, Ensifera) of temperate climates overwinter as diapausing eggs laid as early as July or as late as October (Ingrisch, 1986). For this reason, thermal conditions experienced by eggs before winter vary greatly depending upon the time of oviposition. In some tettigoniids the embryogenesis is interrupted by initial diapause in the very young embryo or by final diapause in the mature embryo. Whereas initial diapause can last for several years, final diapause persists only for one winter (Higaki & Ando, 2002). The occurrence of initial diapause could be related to cold conditions before overwintering or to excessively warm temperatures in summer (Higaki & Ando, 2002). Thus, the life-cycle of tettigoniids can be divided in i) annual, ii) annual and/or biennal, iii) biennal and/or longer (Ingrisch, 1986).

The tettigoniid *Barbitistes vicetinus* Galvagni & Fontana was described as an endemic and rare bush-cricket, confined only in small hilly areas of north-east Italy (Galvagni & Fontana, 1993; 1999). Since 2008, this species became a pest, when repeated outbreaks occurred in ever-increasing surface areas. In this context, heavy damage to forests and neighbouring crops (mainly vineyards and olive groves) as well as annoyance to people living close to the attacked areas were recorded. Outbreak areas are situated in a hilly area (Euganean Hills) of approximately 20.000 ha (Mazzon et al., 2011; Cavaletto et al., 2014, 2015, 2016). As the outbreaks started only in the last decade, few data are currently available on ecology and life-cycle of this species.

Like many tettigoniids, *B. vicetinus* overwintering as eggs in the soil (Cavaletto et al., 2014). In a recent study, we reported a significant effect of vegetation type and soil cover on the oviposition site preference. In particular, although during outbreaks the species is very common in crops, the oviposition is mainly associated to forest habitats and broadleaf litter (Cavaletto et al., 2017).

As several Western Palaearctic species of tettigoniids (Bailey & Rentz, 1990), *B. vicetinus* spends most of its life-cycle in the egg stage and a multi-year egg diapause was reported for this species (Magello et al., 2003; Cavaletto et al., 2014). Although little is known about the factors regulating the diapause of this species, egg development of bush crickets is temperature dependent (Behrens et al., 1983). As *B. vicetinus* prefers sheltered forest areas for oviposition (Cavaletto et al., 2017), thermic factors could be relevant because – due to the short ovipositor (1 cm) of the species – laid eggs are covered by just a shallow soil layer.

To manage insect species with harmful outbreaks such as *B. vicetinus*, knowledge of the factors influencing the interruption of the winter diapause is of crucial importance. Moreover, knowing the seasonal phenology of egg hatching and its duration is of major priority for an effective outbreak management.

This study, conducted during four consecutive years in an outbreak area, presents, for the first time, data on *B. vicetinus* hatching phenology, as well as on the effect of elevation and exposure. Moreover, the influence of temperature on the hatching phenology and the estimation of thermal thresholds for the egg development have been tested in laboratory conditions.

Materials and methods

The study area

The study area was situated in the Euganean Hills (Veneto Region, North Eastern Italy) consisting of about 100 volcanic hills (of about 22,000 ha) with the highest elevation of about 600 m a.s.l.. The climate is characterized by an annual average temperature of 10-13°C and 700-900 mm precipitations (Kaltenrieder et al., 2009). The presence of valleys and the steep hills generate different microclimatic conditions and influence the vegetation composition. A sub-Mediterranean flora occurs on the southern slopes, whereas temperate deciduous vegetation grows on cooler and moister northern ones (Del Favero, 2001). The landscape is characterized by the presence of broadleaves forest patches, mixed with various types of crop fields and rural settlements (Cavaletto et al., 2017).

Field trials

In order to assess the hatching phenology of *B. vicetinus* in natural conditions, field trials were carried out during the spring of four consecutive years (2013–2016), in 18 sites (replicates) selected across the outbreak area as described in Cavaletto et al. (2017) (Tab. 3.1). All the sites were characterized by the presence of broadleaf forest dominated by *C. sativa*, *F. ornus*, *O. carpinifolia* and *Q. pubescens*. The forest edge consisted mainly of *Rubus* spp., *Cornus* spp. and *A. campestre*. None of the selected sites experienced disturbances such as tillage operations or chemical treatments in the previous years.

Field trials were conducted using emergence traps placed in two different contiguous habitats (forest edge and inner forest, at 50 m from the edge) (Tab. 3.1). The trap consisted of a pyramidal wooden frame (50 cm high) with a square base of 0.25 m^2. The frame was closed by a mosquito net with an opening top allowing the collection of newly emerged *B. vicetinus* nymphs. Traps were set out on the ground in flat areas devoid of obstacles such as large stones or logs on the soil. Similar emergence traps were used also in previous *B. vicetinus* field trials (Cavaletto et al., 2017) and in other ecological studies aimed to estimate population density of arthropods (Sunderland et al., 1995).

A total of 84 emergence traps were used in four consecutive years in 18 sites according to the experimental design reported in Tab. 3.1. During the first year (2013), the study was carried out only in two sites placing 6 traps per site, split in two habitats: 3 traps along the forest edge and 3 in the inner forest. In the second year (2014), 18 sites were monitored placing one trap in both habitats per each site, for a total of 36 emergence traps. During the third and fourth years (2015 and 2016), 9 of the 18 sites investigated in 2014 were used, placing one trap per habitat, for a total of 18 emergence traps/year (Tab. 3.1). The second, third and fourth years included also the two sites considered in the first year (2013).

The emergence traps were placed in the field in late winter (February) and trap checking and insect collection were carried out every 48h from mid-March to the end of April, covering the whole hatching period of *B. vicetinus* (Cavaletto et al., 2015). At each trap checking all newly emerged nymphs were collected and taken to the laboratory for identification (according to Massa et al., 2012) and counting. In addition, the sex of specimens was determined, except for the first year of the trial (2013). In the four sampling years all the emergence traps were removed from the field at end of April. Moreover, in order to study the effect on the hatching phenology, the mean daily temperatures of Euganean Hills, recorded in each year by a barycentric weather station (Cinto Euganeo municipality, www.arpa.veneto.it), were overlapped with both hatching start and hatching duration data from field trials.

Elevation and exposure of every site were also recorded to assess the influence of these factors on the hatching phenology.

				2013		2014		2015		2016	
Site	Site position and characteristics			Trap position in the forest							
	Latitude – Longitude	Elevation	Exposure	Inner	Edge	Inner	Edge	Inner	Edge	Inner	Edge
1	45°15'45"N 11°39'59"E	65	Northern			●	●	●	●	●	●
2	45°15'45"N 11°39'59"E	28	Northern			●	●				
3	45°15'40"N 11°41'21"E	86	Southern			●	●	●	●	●	●
4	45°15'34"N 11°40'43"E	144	Southern			●	●				
5	45°17'22"N 11°41'59"E	303	Northern			●	●	●	●	●	●
6	45°16'24"N 11°40'20"E	85	Southern			●	●				
7	45°17'40"N 11°41'49"E	175	Southern			●	●	●	●	●	●
8	45°16'08"N 11°40'22"E	228	Northern			●	●				
9	45°17'00"N 11°40'26"E	340	Southern	●●●	●●●	●	●	●	●	●	●
10	45°16'44"N 11°40'48"E	287	Southern			●	●				
11	45°18'54"N 11°42'08"E	228	Southern	●●●	●●●	●	●	●	●	●	●
12	45°17'20"N 11°41'29"E	285	Southern			●	●				
13	45°17'12"N 11°40'51"E	129	Northern			●	●	●	●	●	●
14	45°18'33"N 11°41'28"E	171	Southern			●	●				
15	45°18'18"N 11°41'55"E	283	Northern			●	●	●	●	●	●
16	45°18'03"N 11°41'38"E	274	Southern			●	●				
17	45°18'32"N 11°42'06"E	213	Northern			●	●	●	●	●	●
18	45°19'42"N 11°42'04"E	443	Southern			●	●				

Table 3.1 Characteristics of sampling sites and year of study. The number of emergence traps used every year and their different position in the forest is indicated as a black dot.

Laboratory trials

In order to follow the egg-hatching phenology of *B. vicetinus* in standard conditions, a laboratory test was conducted under controlled temperatures. Eggs were collected in a single site of the Euganean Hills (45°17'12" N; 11°40'51"E) known to have experienced important outbreaks in the previous years (Cavaletto et al., 2017). Egg collection was carried out, on 16 January 2014, by sampling forest soil in an area of about 5 m^2 free from branches, roots, stones and coarse litter. The soil (about 5 cm deep) was picked up with a small shovel and carried to the laboratory by sturdy plastic bags.

Sampled soil was then sifted with different sieves of mesh progressively smaller (weaves from 20 to 1 mm) in order to remove the extraneous materials from the soil containing eggs. The remaining soil was carefully washed with water running on the thickest sieve with the aim to easily detect only the eggs in final diapause. Then, eggs were collected using a magnifying glass and a soft forceps. The eggs at the final diapause stage were recognized according to their turgescence, since it is known that at this moment eggs are close to the pre-hatching (stage 23-24) *sensu* Warne (1972). Subsequently, they were divided in 25 groups of 7 eggs each. Every group was inserted (1 cm deep) in a 25 ml plastic beaker containing 1 cm of drainage material (gravel) and 3 cm of forest soil previously sterilized by autoclaving (120 °C for 15 min). All the beakers, covered by a net blocked on the top with a rubber band, were kept outdoor near the laboratory in a sheltered shaded area until the beginning of the experimental laboratory trials.

On 3 February, five beakers were placed in each of five climatic chambers at 6, 10, 13, 16 and 23°C, respectively, waiting for nymph emergence. From this moment on, all the beakers were inspected daily and the hatched nymphs were recorded and removed. The test ended 30 days after the last detected hatch. The soil in the beakers was slightly sprayed with water every 2-3 days to avoid dehydration.

With the aim to detect the relationship between thermal sum and hatching phenology, the mean daily temperatures achieved by the eggs were added from 1st January to hatching time (Julian day corresponding to the cumulated 50% of hatchings for each tested temperature) with the arbitrary base temperature of 0°C. Until the positioning of the eggs in the different climatic chambers, in which daily conditions were constant, the temperature near the eggs was also. A data logger (HOBO, U23 Pro V2, Onset Computer Corporation, Bourne, MA, USA) was indeed placed 2 cm deep in the ground, during the whole winter before sampling, as well as, in the laboratory during the processing period.

Statistical analysis

For both field and laboratory trials, the number of recorded hatchings for each trap, in function of the time was fitted in a sigmoid curve by using the online MyCurveFit software (https://mycurvefit.com/). Inserting the hatching percentage (in particular 10% and 50%) as value in the curve equations, allowed us to calculate the corresponding Julian day of the occurrence. This procedure was also carried out for males and females, separately.

Field trials

With the aim of reducing the error in the evaluation of factors affecting the hatching dynamics, field traps with less than 4 individuals per year were excluded from the analysis. Elevation and exposure (northern or southern) of each monitored site were assessed using Google Earth Pro software (https://www.google.com/intl/it/earth/desktop/).

The effect of habitat (edge or inner forest) and sampling year (2013-2016) on the egg hatching start, reported as the Julian day corresponding to the cumulated 10% of hatchings, were tested using a general linear-mixed model (GLMM).

Trap position and sampling year were also included as fixed factors in a GLMM to test their effect on the hatching duration in term of number of days elapsed from 10% to 90% of the hatchings.

In both models, site ID and replications were included as random factors to account for spatial dependence in the sampling design.

The relationship between hatching start and its duration was assessed using a correlation test.

The different male and female hatchings were tested by different GLMMs for three cumulated hatchings levels, in the sampling years 2014-2016. The Julian day corresponding to the cumulated 10%, 50% and 90% of hatchings respectively was used as response variable while sex and year were inserted as fixed effects. Site ID was included as random factor to account for spatial dependence in the sampling design.

The effect of elevation and exposure of monitored sites on the hatching start (10% of cumulated hatching), were evaluated by a GLMM considering elevation and exposure as fixed effects and the year of sampling as random factor, to account for time dependence in the sampling design.

<u>*Laboratory trials*</u>

The effect of different temperatures on the hatching time of eggs reared in lab, reported as the Julian day corresponding to the cumulated 50% of hatchings, was tested by one-way ANOVA. Moreover, the same test was performed to evaluate the effect of temperature on the hatching duration and on the thermal sum required by eggs for the hatching. A Tukey HSD post-hoc comparison test was applied when the fixed effects were significant ($P<0.01$).

In all performed GLMMs, for both field and laboratory trials, single factors and their interactions were evaluated, when the latter were not significant the models were reassessed without interactions. Assumptions of linear models were tested by inspecting diagnostic plots of model residuals.

All analyses were run in R (R Core Team, 2014) using the nlme package (Pinheiro et al., 2017) applying lme function.

Results

Field trials

<u>*Hatching phenology*</u>

In the four sampling years (2013-2016) the hatching period of *B. vicetinus* always occurred between March and April. The time needed to reach the 10% of hatching (hatching start) was however significantly different among the 4 monitored years (GLMM, F=118.05, d.f. = 3, 24, $P<0.001$). This time was significantly affected by the year factor, ranging from a minimum mean value of 80.70 ± 0.62 days (2014) to a maximum of 99.89 ± 0.60 (2013) (Fig. 3.1).

Considering all the sampling years, the mean hatching duration, (i.e. the mean time elapsed from 10% to 90% of the hatchings), lasted 8.94 ± 0.49 days, although with high significant differences among years (GLMM, F=27.73, d.f. = 3, 26, P=<0.001) ranging from a minimum of 4.30 ± 0.50 days (2013) to a maximum of 11.28 ± 0.45 days (2014) (Fig. 3.2).

Hatching start and hatching duration were significantly but negatively correlated (R^2 = 0.62, d.f.=1, 49, P< 0.001) (Fig. 3.3). Moreover, neither hatching start nor hatching duration were affected by the trap position (forest edge vs inner forest: F=1.16, d.f. = 1, 24, P=0.29 and F=0.39, d.f. = 1, 26, P=0.539 respectively).

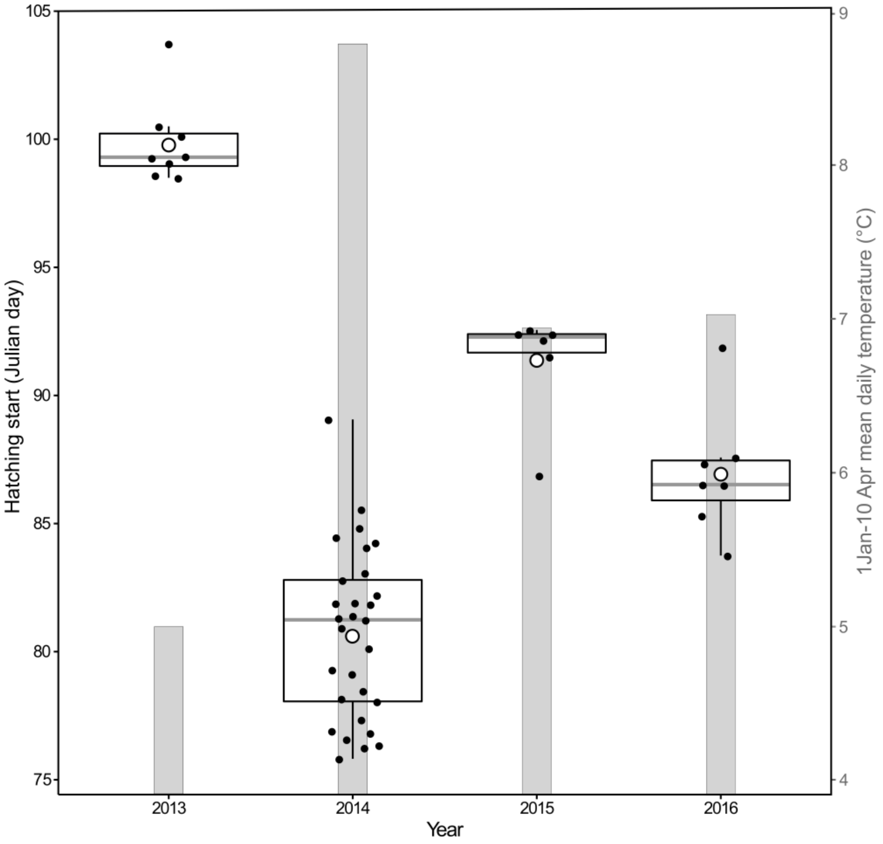


Figure 3.1 Time (Julian day) needed to reach the 10% of hatching (hatching start) in the four sampling years. The thick lines of the box plots represent the medians and the white dots indicate the means. The grey vertical bars represent the mean daily temperature (°C) between 1st January and 10th April for each year.

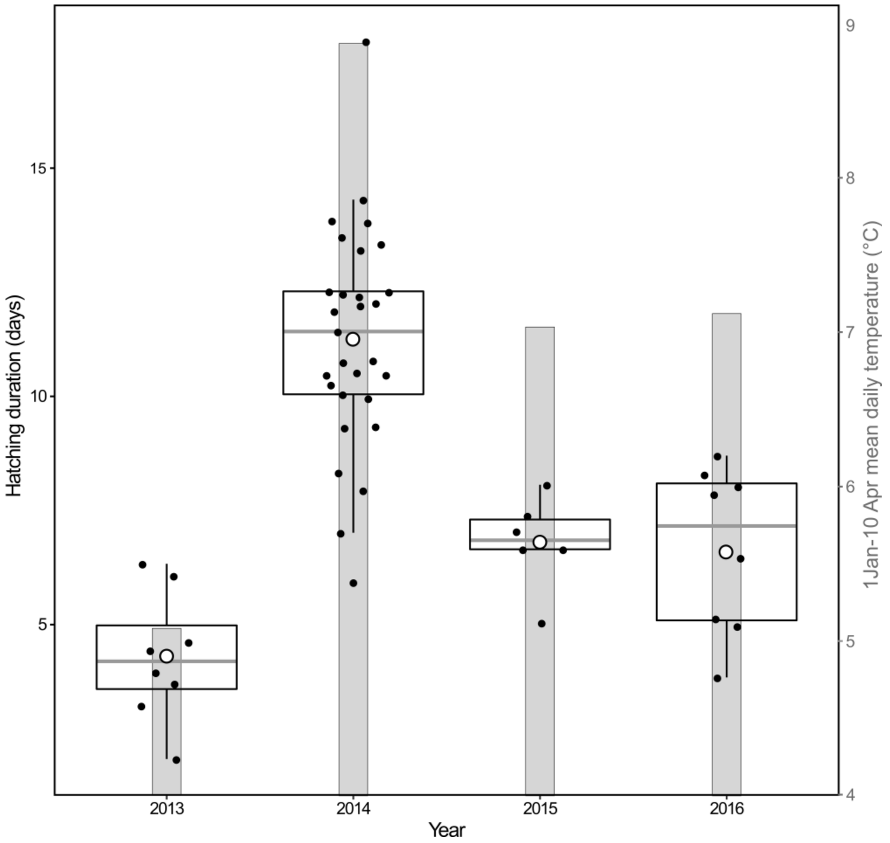


Figure 3.2 Hatching duration (days) in the four sampling years. The thick lines of the box plots represent the medians and the white dots indicate the means. The grey vertical bars represent the mean daily temperature (°C) between 1st January and 10th April for each year.

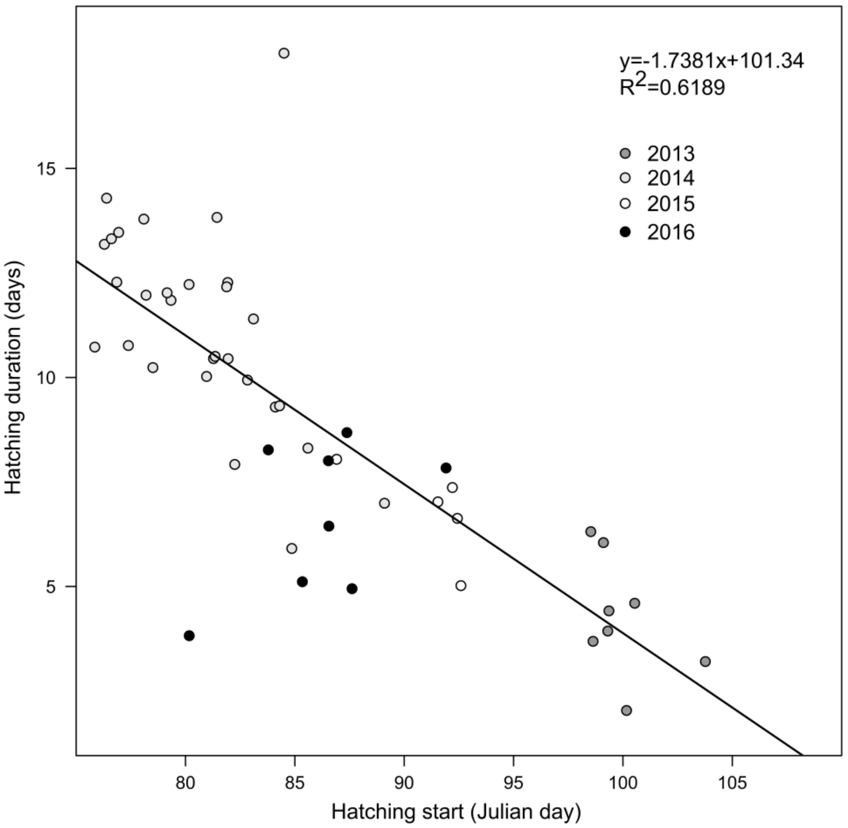


Figure 3.3 Relationship between hatching start and hatching duration. The colour of the points represents different sampling years.

For the subsequent analysis, the first sampling year (2013), in which data was collected just in two sites and the insects sex was not recognized, was not considered.

The GLMMs related to the cumulated hatching at different percentage (10%, 50% and 90%) of males and females showed that the two sexes hatched in a significantly different time in all the 3 sampling years (2014-2016) without any significant interaction between sex and year (Fig. 3.4). For all the cumulated percentage of hatchings considered (10%, 50% and 90%), males always occurred significantly earlier than females, 1.89 ± 0.32 days (F=19.69, d.f. = 1, 49, P<0.001), 1.87 ± 0.24 days (F=24.93, d.f. = 1, 49, P<0.001), and 2.36 ± 0.48 days (F=18.05, d.f. = 1, 49, P<0.001), respectively.

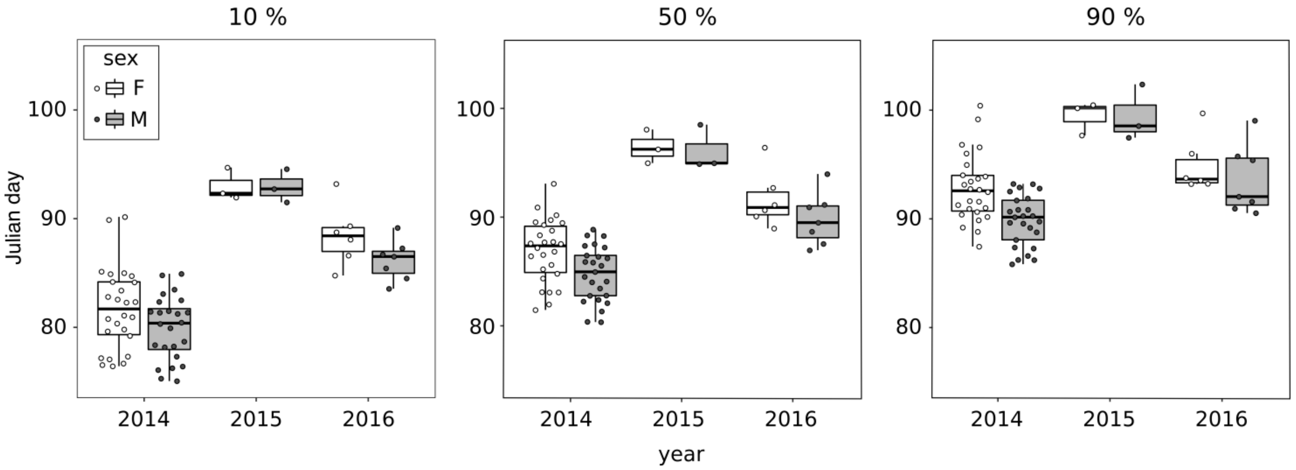


Figure 3.4 Time (Julian day) needed to reach the 10%, 50 % and 90% of hatching in the three sampling years and in the two sexes. The thick lines of the box plots represent the medians.

Effect of elevation and exposure

The model indicated significant effects of both elevation and exposure on the hatching start (reaching of 10% of the hatching). Furthermore, a significant interaction between the two factors was found (Tab. 3.2), i.e. when exposure was southern the elevation effect was stronger, while when exposure was northern the elevation effect became less apparent (Fig. 3.5).

	d.f.	F-value	p-value
Intercept	1, 37	644.17	<0.001
Elevation	1, 37	14.35	<0.001
Exposure	1, 37	25.18	<0.001
Elevation X Exposure	1, 37	7.62	<0.01

Table 3.2 Results from the mixed model testing the effects of elevation and exposure on the hatching start (reaching the 10% of hatching).

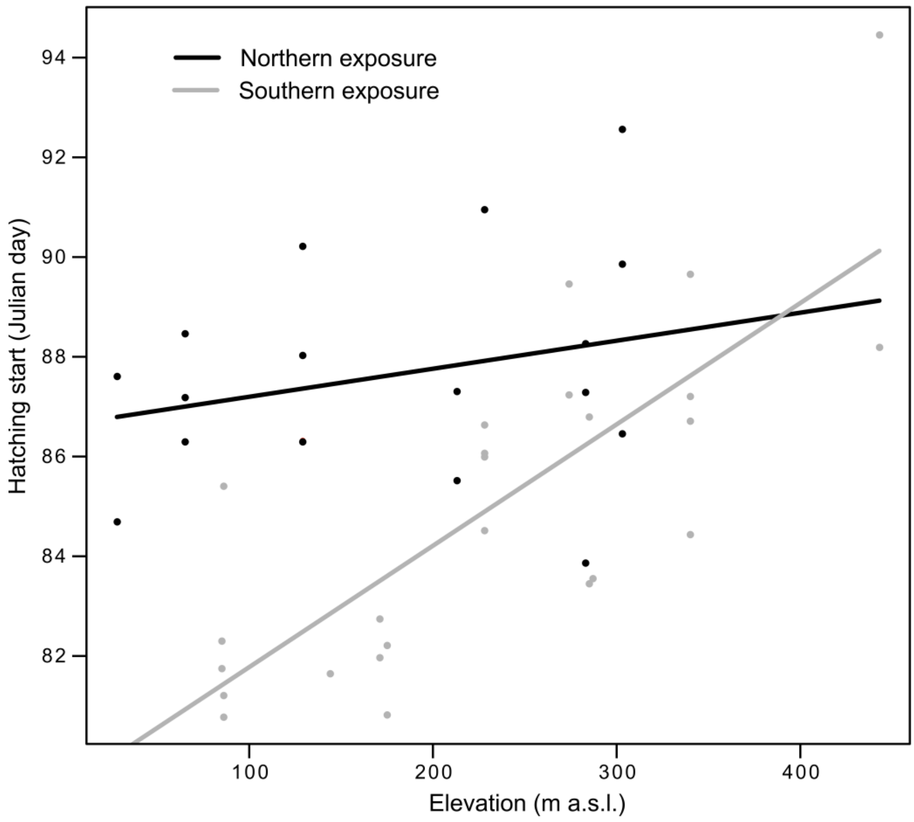


Figure 3.5 Interaction effect between elevation and exposure on the time (Julian day) needed to reach the 10% of hatching (hatching start).

Laboratory trials

General results

A total of 147 nymphs (70 males and 77 females) emerged from the 175 tested eggs (84%) (Fig. 3.6). In particular, the hatching percentage of eggs exposed at 6, 10, 13, 16 and 23 °C was 86%, 89%, 94%, 86% and 66% respectively. The first hatch was recorded on the 11th February in the hottest plot while the last hatch occurred in the coolest plot on the 24th April.

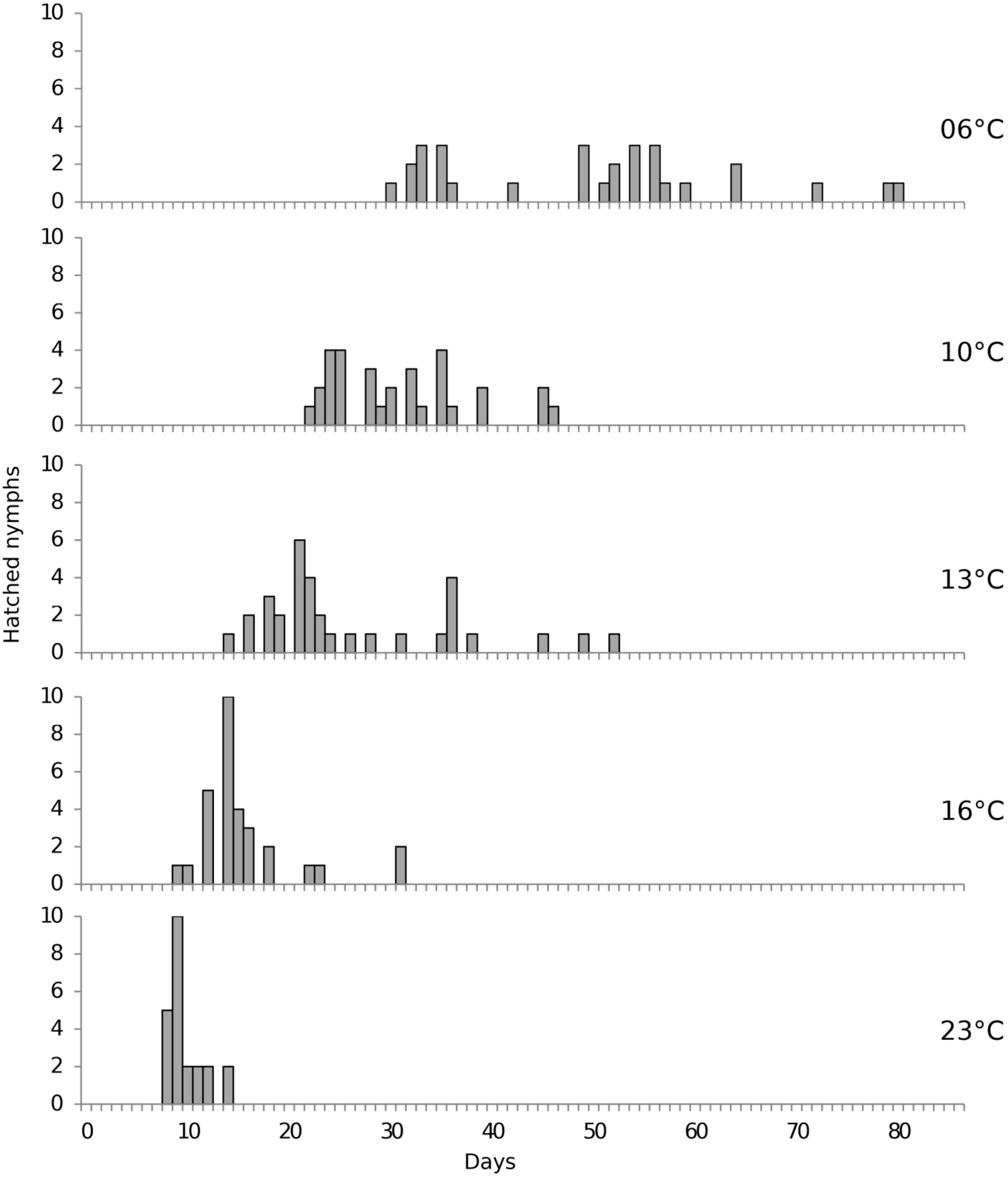


Figure 3.6 Distribution of the hatchings at different temperatures. Number of days corresponds to the time since the beginning of the exposure at each temperature.

Hatching phenology

A significant effect of temperature on the hatching time (recorded at 50% of the total hatching) was found (ANOVA, F= 97.56, d.f. = 4, 20, P<0.001), with warmer temperatures corresponding to earlier hatching; significant differences in the hatching time occurred between all the tested temperatures, except for 16°C vs 23°C (Tukey HSD test, P<0.05) (Fig. 3.7).

Hatching duration varied significantly between high and low temperatures (ANOVA, F= 6.47, d.f. = 4, 20, P<0.002) with shorter times at higher temperatures (Tukey HSD test) (Fig. 3.8).

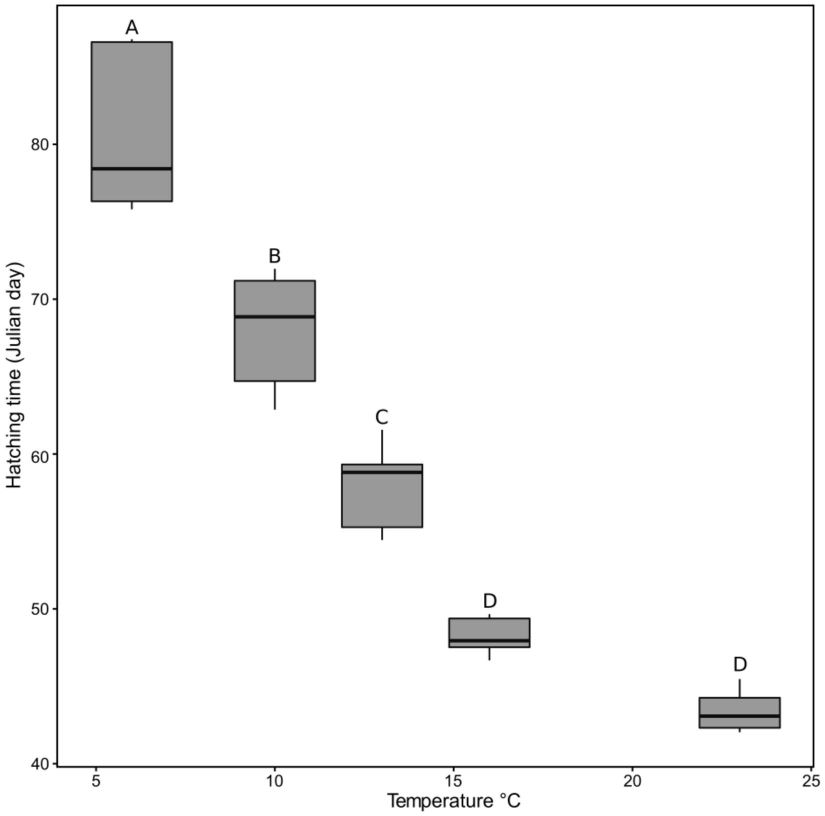


Figure 3.7 Time (Julian day) needed to reach the 50% of hatching (hatching time) at different temperatures. The thick lines of the box plots represent the medians.

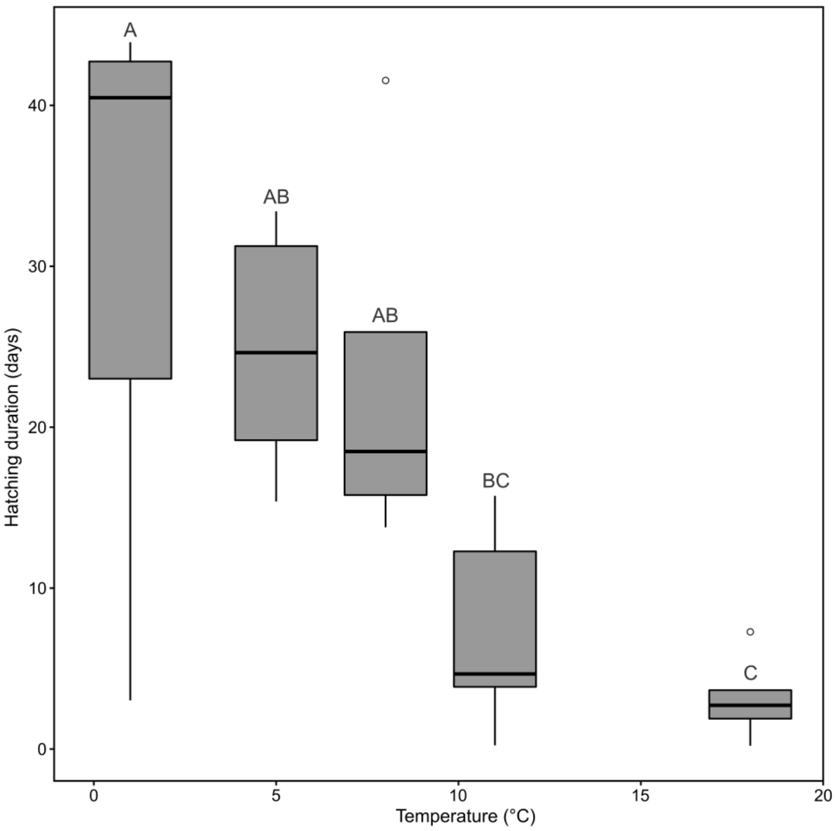


Figure 3.8 Hatching duration (days) at different temperatures. The thick lines of the box plots represent the medians.

Thermal sum

Significant differences emerged between the sum of daily mean temperatures achieved by eggs (Thermal sum), recorded until 50% of the total hatching, at different tested temperatures (ANOVA, F= 10.27, d.f. = 4, 20, P<0.001). A significantly lower thermal sum was required by eggs exposed to warm temperatures (16 and 23°C) compared to eggs exposed to mild ones (10 and 13°C). Interestingly, at the coolest temperature (6°C), the thermal sum showed a middle value without any significant difference with those recorded at the other temperatures (Fig. 3.9).

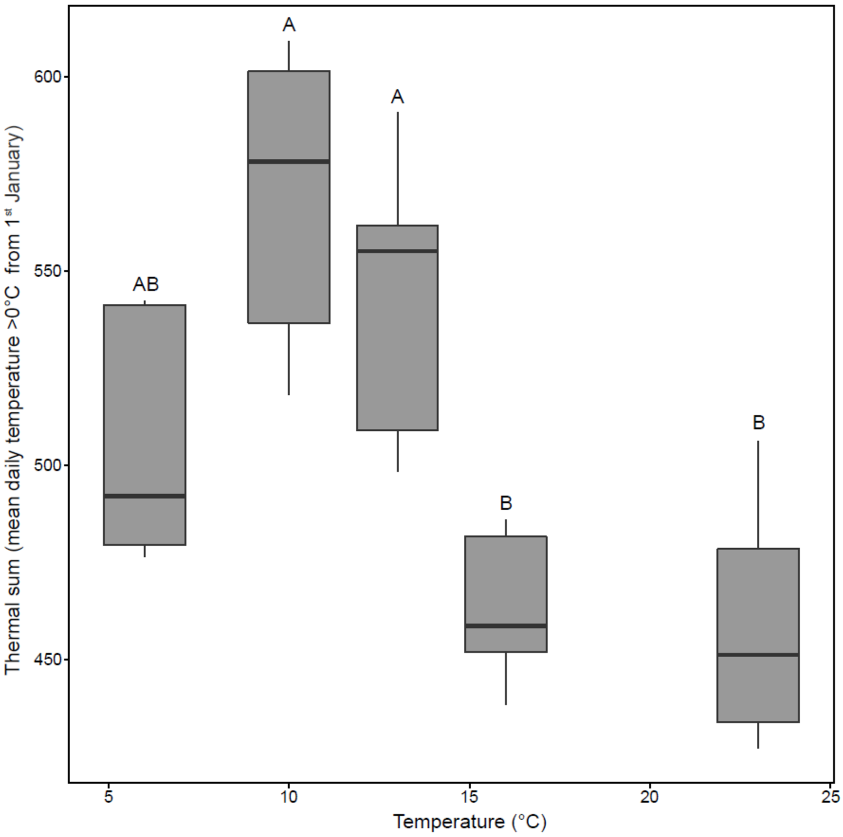


Figure 3.9 Sum of daily mean temperatures achieved by eggs (Thermal sum) recorded until 50% of the total hatching in different temperatures.

Discussion

Field trials

For the first time, the present study reports 4-year field data regarding hatching phenology of the outbreak bush-cricket *B. vicetinus*. Results obtained here allowed drawing some aspects of the emergence pattern of the species*.*

In most of the Western Palearctic tettigoniid species, the embryonic development is interrupted by two diapauses. The first diapause (initial diapause) requires at least one winter to be broken, while the second one (final diapause) is always terminated by a single winter (Ingrish, 1986). Preliminary studies reported that the embryonic development of *B. vicetinus* can last for more than one year according to a prolonged initial diapause (Magello et al., 2003; Cavaletto et al., 2014). For this reason, it's noteworthy that all our field and laboratory observations regarded a group of eggs with an advanced embryonic development being in final diapause. To evaluate how long this diapause can be prolonged, a long-term study monitoring populations of *B. vicetinus* is now in progress.

Overall, our field results show that hatching phenology significantly differs in the investigated years, in relation to both the beginning of the hatching (hatching start) and the number of days needed to complete it (hatching duration). During the four years considered, hatching usually occurred early in the spring, between end of March and beginning of April. Interestingly, hatching start and duration were significantly but negatively correlated each-to-other. Years with early hatching start (i.e., 2014) presented long hatching duration, while years with late hatching start (i.e., 2013) had a short and concentrated hatching duration. Overlapping both hatching start and hatching duration data with the mean air temperatures (January to April) we can notice that in cool years hatching occurs later and concentrated in a few days, while in warm years hatching takes place earlier and last longer (Figs. 3.1 and 3.2). Thus, although this observation is not supported by direct soil temperature of the sampling sites, we can assume that temperature strongly affects the hatching phenology in the 4 monitored years.

The embryonic development of tettigoniids is composed of 26 stages (Warne, 1972). In temperate regions the initial diapause takes place in the very young embryo, while during the final diapause embryos are fully-developed and almost ready for hatching (stage 23/24) (Ingrish, 1986). In this respect, prolonged low temperatures occurring in spring probably delay the hatching start and lead to its synchronization, shortening the hatching duration as observed in 2013 and 2015. A short hatching duration allows the first instar nymphs to find quickly in spring, the resources required for their development. Indeed, the newly hatched nymphs climb bushes and trees eating the young leaves, which are available only for a short period in the early spring. The effect of chilling on the termination of final diapause was investigated also in the tettigoniid *Eobiana engelhardti* (Uvarov, 1926), pointing out that hatching was clearly synchronized by prolonging the chilling period of the eggs chilled for different periods at 3°C before transfer to 15°C (Higaki & Ando, 2002). A reduction of hatching duration in relation to long exposition to cold periods during diapause is known in many winter diapausing insects of temperate regions (e.g. Collier & Finch, 1983; Wipking, 1995).

Regardless of hatching phenology, in all the study years, males hatched earlier than females. This behaviour was reported in many insect species as an adaptive response of females to minimize the time-lag between eclosion and mating (Wedell, 1992).

Our results pointed out that both altitude and exposure play a significant role in the hatching phenology. The interaction between these two factors showed that in sites having a south exposure, decreasing in altitude leads to a hatching anticipation, while in the northern sites the altitude does not affect the hatching start (Fig. 3.5). It seems that, once again, temperature plays an important role in anticipating or postponing the hatching start. Because of the numerous narrow and deep valleys and steep hills of the Euganean Hills, the microclimate conditions occurring in this area are considerably variable. In the northern slopes, the effect of altitude is mitigated by the cooler conditions due to the reduced solar irradiance; the opposite occurs along the southern slopes where daily and seasonal thermal excursions are more pronounced (Susmel & Famiglietti, 1968). These results suggest that *B. vicetinus* responds promptly to local micro-environmental climatic conditions.

Laboratory trials

Results obtained in laboratory by placing overwintering eggs at different constant temperatures support the hypothesis of a strong influence of temperature in the *B. vicetinus* hatching phenology. Interestingly, more than 65% of the eggs incubated hatched at all the tested temperatures, suggesting the ability of the species to interrupt the final diapause in a wide range of thermal conditions. Indeed, *B. vicetinus* was able to hatch from 6 to 23 °C although the highest hatch performance was recorded within a range from 6 to 16°C. At warmer temperatures (up to 16°C) the hatching of 50% (hatching time) took place progressively earlier in a significant way, but at the highest tested temperature (23°C), the hatching time did not significantly decrease further (Fig. 3.7).

Similarly, the hatching duration significantly decreased with increasing temperatures. It is noteworthy that, even the lowest temperature (6°C) did not prevent the termination of final diapause, but led to an extended hatching period 7 times longer than that observed at the highest temperature (Fig. 3.6). Observations from other tettigoniid species, such as *P. griseoaptera* and *E. engelhardti*, gave 11°C and 9°C respectively as the lowest temperature thresholds for the egg development (Hartley & Warne, 1973; Higaki & Ando, 2002).

In addition, laboratory data showed that the different tested temperatures induce significantly different thermal sums of hatching. In particular, at cool temperatures hatching took place at significantly different and increasing thermal sums. However, eggs exposed at the lowest temperature (6° C) neither followed this trend nor showed a different thermal sum than eggs exposed at higher temperatures. These results suggest that the termination of the final diapause can also occur at low temperatures, even if requiring a longer time for hatching. In case of hatching delay caused by low temperatures embryonic maturation – and the subsequent egg hatching – would occur with low thermal thresholds compensated by the prolongation of the period the embryo has to conclude development.

Because other factors, besides temperature, such as moisture and photoperiod experienced maternally can influence hatching phenology, as reported in other tettigoniids (Ingrisch, 1984), future research focused on these abiotic parameters of environment will be useful to improve the knowledge of *B. vicetinus*.

Chapter 4

Habitat fragmentation and invasion of an alien tree reduce outbreak severity of an eruptive forest pest

Giacomo Cavaletto, Luca Mazzon, Massimo Faccoli and Lorenzo Marini

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE),
University of Padova, Viale dell’Università 16, 35020 Legnaro (PD), Italy

Introduction

Orthopteran outbreaks can play a key role in the dynamics of both natural and managed ecosystems. Unlike the more common outbreaks caused by grasshopper species (Orthoptera, Caelifera) (Showler, 1995), infestations caused by bush crickets (Orthoptera, Ensifera) occur much less frequently, despite they can be as much severe (Gwynne, 2001; Lorch et al., 2005). In forest or shrubland habitats, bush-crickets species often occur at low density for very long periods until their first outbreak (Lorch & Gwynne 2000; Kim, 2001; Lorch et al., 2005, Sword et al., 2005; Bailey et al., 2007a, b; Srygley, 2014). In Europe, isolated but severe forest outbreaks were reported for several bush-cricket species belonging to the genus *Barbitistes*, which caused severe defoliations in both forests and neighbouring agricultural areas (Escherich, 1928; Laussmann, 1994; Holuša et al., 2006). However, the local and large-scale drivers of these relatively rare but severe bush-cricket outbreaks are still unknown.

Bush-crickets are considered highly generalist polyphagous species, but their individual fitness and population dynamics can be strongly affected by host plant quality (Joern & Gaines, 1990; Jones & Coleman, 1991; Joern & Behmer, 1997). Although multiple host plant species are potentially able to sustain the development of generalist insects, these may be adapted to consume mainly the regionally most abundant host plants (Mitter et al., 1979; Kerslake & Hartley, 1997). Specific plant characteristics such as primary and secondary metabolites, mechanical defences or phenology may limit their palatability (Ehrlich & Raven, 1964; Strong et al., 1984; Schoonhoven et al., 1988; Bernays & Chapman, 2000; Frago et al., 2010). In this context, alien invasive plant species may present chemical and mechanical defences that did not co-evolve with the native herbivores. The enemy release hypothesis predicts that there will be few specialist herbivores attacking the alien plant and host switching by native specialists are usually rare (Keane & Crawley 2002). Several European forests are now dominated by alien tree species that have replaced native tree species presenting novel host for local native herbivores. In this context, understanding plant host selection and local habitat requirements of outbreaking species is crucial to predict pest damage and to understand the tree composition that can be more susceptible to insect defoliation.

Beside the local host quality and availability, landscape processes may also play a key role in driving insect outbreaks (Peltonen et al., 2002; Johnson et al., 2005; Liebhold et al., 2006). Insect species can experience the landscape at different spatial scales depending on their dispersal ability (Holt, 1996; Debinsky & Holt, 2000). Due to their low mobility, bush cricket species are expected to strongly respond to landscape processes such as habitat loss and fragmentation at relatively small-spatial scales (Marini et al., 2009; 2010), compared to forest pests characterized by larger mobility (e.g. Raffa et al., 2008). In this context, forest habitat fragmentation can pose a substantial barrier to the development of local outbreaks of bush-crickets. Several forest pests even with large mobility are known to respond positively to landscape connectivity (Safranyik et al., 2010; James et al., 2011). In particular, viability of local pest populations depends on the degree of habitat loss and isolation at regional scale, as well as on pest traits such as niche breadth and dispersal ability (Ewers & Didham, 2006; Bailey, 2007). Although most of the forest regions historically attacked by bush-cricket species are subjected to a certain degree of habitat loss and fragmentation, the role of these drivers in limiting bush-cricket outbreak is currently unknown.

In this study, we considered *Barbitistes vicetinus* (Orthoptera, Tettigoniidae), a destructive bush-cricket species, as model system. The species has been causing severe defoliation across Sub-Mediterranean fragmented forests (Cavaletto et al., 2014). We sampled the pest for two years across over 200 sites across fragmented forest landscapes in North-East Italy. In the region, a large proportion of the native forests have been historically invaded by the black locust (*Robinia pseudoacacia* L.), a north-American alien tree species. In the introduced range, black locust tends to live longer than in its native range becoming invasive probably due to a lower herbivore pressure (Cierjacks et al., 2013). The general aim of the study was to evaluate the role of local tree composition and forest fragmentation on *B. vicetinus* defoliation severity and on the spatio-temporal dynamics of its local outbreaks. In particular, we expect that *B. vicetinus* defoliation is negatively affected by the large presence of black locust at the local and landscape scale and by strong habitat fragmentation due to its low dispersal ability.

Materials and methods

Model species

Barbitistes vicetinus is a large (25 mm) flightless bush cricket, endemic for NE Italy. Since 2008, this polyphagous species produced severe outbreaks causing intense defoliation on broadleaves forests grooving in hilly areas. *Barbitistes vicetinus* overwinters as eggs laid in the ground, and the whole biological cycle lasts from end of March to the half of July with producing only one generation per year.

The study area

The study area was the Euganean Hills, which covers 22,000 ha in the Veneto Region (North Eastern Italy). The area is characterized by about 100 volcanic hills and elevation ranges from see level to 600 m a.s.l.. The climate is characterized by an annual average temperature of about 12°C and 700-900 mm precipitation (Kaltenrieder et al., 2009). Because of the numerous narrow and deep valleys and the steep hills, different microclimatic conditions influence the vegetation. A sub-Mediterranean flora occurs on the south facing slopes with warmer and drier climatic conditions, whereas temperate deciduous vegetation grows on cooler and moister north-facing aspects (Del Favero, 2001). The landscape is characterized by the presence of forest patches, mixed with various types of crop fields and urban areas.

Site selection

In order to study the *B. vicetinus* ecology in the outbreak area, during springs 2014 and 2015, a sampling method was planned with the aim to monitoring both adult and nymph population density, as well as the damage caused by the species. We created a 750 x 750 m grid on a digitized map of the study area obtaining 480 square cells (each cell was 56.25 ha wide) using Qgis (QGIS Development Team, 2014. QGIS Geographic Information System. Open Source Geospatial Foundation Project, http://qgis.osgeo.org). Applying local detailed land-use maps (Quadro conoscitivo – Regione Veneto), the whole area was characterized in different land cover categories (urban areas, forest, annual crops, orchards, wetlands, etc.). As reported in a previous study, *B. vicetinus* prefers forest habitats both for feeding and overwintering (Cavaletto et al., 2017). In order to focus the survey on areas with a presence of the insect, we chose only cells with at least 15% of area covered by forest. We obtained, in this way, 203 cells within which we randomly select a point within the forest class (Fig. 4.1).

Host preference and damage

In September of both sampling years (2014 and 2015), all the sites were visited in order to assess visually the leaf damage for each forest species present. The mean percentage of the leaf area consumed by *B. vicetinus* was estimated carefully observing all the plants for each species in a radius of 5 m around the sampling point. This defoliator left a typical frayed margin on the consumed leaves (Mazzon et al., 2011), allowing an easy identification of its damage. The same two operators conducted together the estimation in all the sites, in order to avoid the risk of operator bias.

Adult density

In order to evaluate the adult density of *B. vicetinus*, in each sampling point, during both sampling years (2014 and 2015) we performed a frass collection method (Morris, 1949; Timbergen, 1960; Zandt 1994; Fischbacher et al., 1998; Mizutani & Hijii, 2001; Wesołowski & Rowinski, 2008; Sweetapple & Barron, 2016). We placed a 2 x 1 m (Connola et al., 1966) thick plastic nets, erected in hammock fashion and fixed to four trees with nylon wires and suspended (Clout & Gaze, 1984; Effler et al., 2006) at least 1.5 m above the ground (Fig. 4.2A) to avoid wild animals disturbance. A plastic mash was chosen for the nets preparation in order to allow rain water to move through the material, but keep frass in the trap. Each net was stretched and weighted by a 1 kg stone tied into it, so that frass would drop in the center and not be blown out by wind (Timbergen, 1960; Connola et al., 1966; Hinks et al., 2015). Despite adverse weather conditions are generally known to be the major cause of frass pellet destruction (Kamata & Igarashi, 1994; Zandt, 1994; Seki & Takano, 1998; Mizutani & Hijii 2001; Lambrechts et al., 2008) causing critical errors in estimates (Southwood, 1978; Zandt, 1994), control observations confirmed that strong wind and heavy rain did not occur during the study. In order to obtain enough material to provide meaningful information (Casey et al., 2009), the nets were left exposed for 20 days, in both years. All the nets were installed between the end of May and the beginning of June and were removed between the end of June and the beginning of July of the two sampling year. In this way, frass were collected during the period when all the insects had become adults (Galvagni & Fontana 1999). At the end of the collecting period the frass accumulated in the nets were cleaned by leaves, twigs and other materials. Frass samples were stored in paper bags, carried in laboratory and dried for 48 h in a 65 °C oven (Effler et al., 2006). After that, sample material was sorted using a sieve (Kamata & Igarashi, 1994) to remove fine debris, and weighed to the nearest 0.01 g using a WTC 2000 precision balance (Radwag Balances and Scales). The clear preponderance of *B. vicetinus* during outbreaks compared to other Orthoptera species and the specific form of the frass produced by this species, allows to consider irrelevant the presence of other insect excrements after cleaning and sorting of the samples.

Nymph density

With the aim of study the hatching density of *Barbitistes vicetinus*, in 2015, the number of nymphs was assessed using the sticky traps method, in a subset of the same sites in which the adult population density was evaluated through the frass collection (40 of 203). The sites chosen to study the nymph population were selected among the frass collection sites considering the logarithmic distribution of the collection (more rarefied at higher densities) in order to survey sites in the entire variability of adult density. Sticky traps have been used in other field trials conducted on Orthoptera to estimate population density (Lambrinos, 2000; Panzer, 2002; Gardiner et al. 2005) and the method seems to fit well to capture nymphs of species with eggs laid into the ground. In each of the 40 sites, 3 sticky traps were placed in the ground in an area of few meters around the net used for frass collection; in the total 120 traps were used. The traps consisted of a mosquito net box (25 cm high) with an open square base of 0.25 m^2 (Fig. 4.2B), fixed to the ground with four metal rods in the corners; it were set out on the ground in flat and devoid of obstacles areas. The internal side of the net was sprinkled with a thin layer of entomological glue (Temo-O-Cid, Kollant) in order to block the hatched nymphs. In fact, the first instars of this species climb quickly up the vertical structures, immediately after the hatching, attempting to reach the tree branches (Magello et al., 2003). A plastic net was fixed to the top of each trap to avoid animal predation inside the sampling areas. All the traps were placed in the field between 10 to 15 March 2015, and removed between 20 to 25 April 2015 capturing insects for the whole hatching period (Cavaletto et al., 2014). At the end of the collection, *B. vicetinus* and other Orthoptera nymphs were identified (according to Massa et al., 2012) and counted directly in the field.

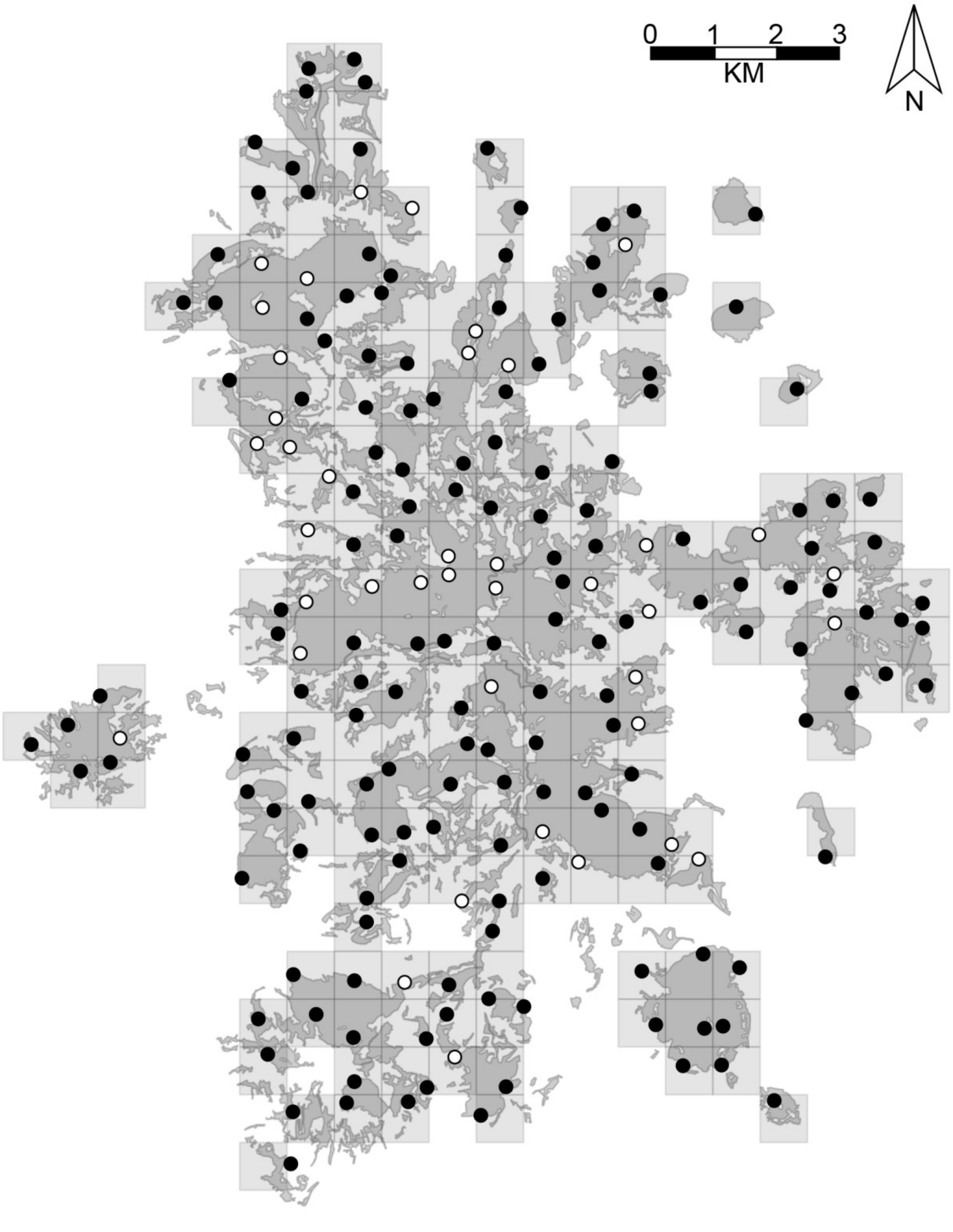


Figure 4.1 Map showing the forest in the Euganean Hills (deep grey) and the study sites represented by the 750x750m cells (light grey) with the frass collection - damage assessment points (black circles) and the sites where the nymphs density was also assessed (white circles).

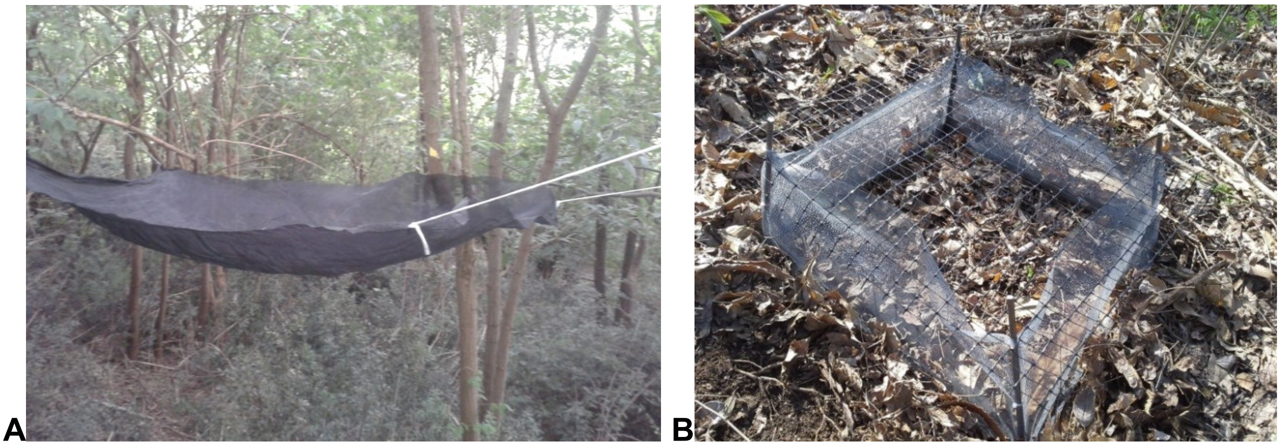


Figure 4.2 Net used for frass collection in the adult density assessment (A), and sticky traps set up to evaluate the nymph population (B).

Local environmental variables

In order to investigate the local scale forest composition, during September 2014, in all the sites studied by frass collection, the basal area for each forest species was assessed using a Mantax Blue Caliper (Haglöf, Sweden). All trees and shrubs in an area of 78.5 square meters around the nets (radius of 5 m), were measured: the correct position of the plants were evaluated using a Vertex IV ultrasound instrument (Haglöf, Sweden). At the local scale, since the basal area of different forest species were correlated, we reduced the number of predictor variables using principal component analysis (PCA) on the main forest species (present at least in 30 sites). High value of the first factor (PCA1) defined greater basal area of thermophilic species (*Ostrya carpinifolia, Fraxinus ornus and Quercus* spp.), as well as lower amount of *Robinia pseudoacacia*. Increasing value of PCA2 defined, progressively, forest with lower level of chestnut (*Castanea sativa*) and higher level of *Robinia pseudoacacia* and conversely (Supplementary material).

Landscape variables

The landscape scale forest composition was explored, instead, with the same Qgis software and digital maps detailed above, in order to quantify the amount of the different forest type in each of 100, 250, 500, 750 and 1000 m buffers around the nets. First, we quantified the total cover of forest. Second, we considered three categories: chestnut forest, black locust forest and thermophilic forest expressed as the proportion of the total forest area at the five scales.

Statistical analysis

Host preference

In order to recognize the forest species ranking for the damage, we calculated the mean value for the percentage of leaf consumed in each species in both sampling year. The corresponding standard errors were also evaluated. Analysis of the results allows us to better understanding the host preference of *B. vicetinus*.

Population density in relation to local and landscape factors

As the duration of the frass collection periods differed by a few days between some sites, the data were standardized dividing the total weight by the number of exposure days (Hinks et al., 2015).

To test the effect on the population density of the sampling year, of the total basal area and relative species abundance at local scale, as well as the effect of forest area and the forest type composition at landscape scale, five different general linear-mixed models (GLMMs) were performed at five scales (100, 250, 500, 750 and 1000 m buffers around the sampling sites). In each model we included site ID as a random factor to account for spatial dependence in the sampling design. From the initial models including all variables, we removed with a backward deletion all the not significant, starting from the interactions. The final general linear-mixed models (GLMMs) had the log-transformed frass abundance + 0.01 as response variable. Six predictors were used in the full models, one categorical fixed factor (the sampling year) and five continuous fixed factors (PCA1 and PCA2 at the local scale, the total forest area at the five scales and, on it, the relative percentage of *R. preudoacacia*. We also included the quadratic term of forest area in the landscape. Moreover, the interaction between the sampling year and the percentage of *R. pseudoacacia* on the total forest area was included in the full models. Site ID was also included. For each scale, we computed Akaike's Information Criterion (AICc) with the aim to detect the scale to which the different factor better explained the population density variation.

Assumptions of linear models were tested by inspecting diagnostic plots of model residuals.

Temporal dynamics

To better understand the link between feeding and oviposition site preference, we tested whether adult density in 2014 and nymph density in 2015 explained the *B. vicetinus* population in 2015 using linear regression. To test for potential density dependence we tested whether the population growth from 2014 to 2015 was affected by the density in 2014. R was defined as R=log(density2015/density2014).

All model residuals did not present spatial autocorrelation. All analyses were run in R (R Core Team, 2014). GLMMs were performed using the nlme package (Pinheiro et al., 2017) applying lme function.

Results

Host preference and damage

Considering all the forest species monitored in all the sampling sites, the mean damage, expressed as a percentage of leaf area consumed by *B. vicetinus*, was 16.52±0.88% in 2014, and 7.81±0.39% in 2015. The peak value in a single site was recorded, both in 2014 and 2015, on *F. ornus* (with a damage of 95% and 75%, respectively). Considering all the sites, species with the highest percentage of leaf area consumed were *F. ornus*, *O. carpinifolia and Quercus* spp. in the first sampling year and *F. ornus*, *C. avellana* and *O. carpinifolia* in the second year. Moreover, in both years, the species with the lower damage was *R. pseudoacacia* (Fig. 4.3).

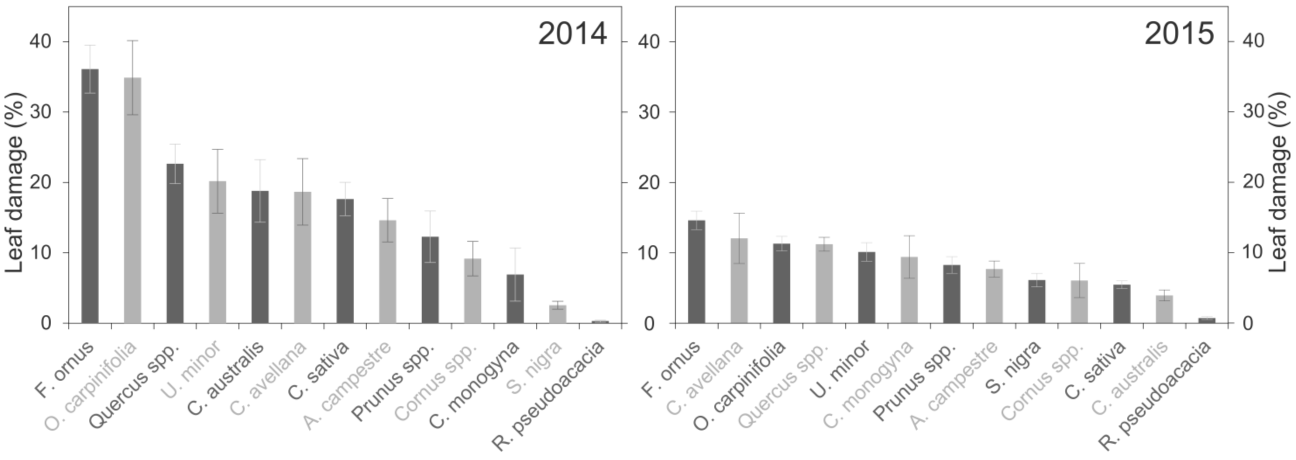


Figure 4.3 Mean percentage of leaf damage assessed for the main forest species in the two sampling year. Error bars represent standard error of the mean.

Adult density

With the frass collection we obtained data by 165 and 194 nets respectively in 2014 and 2015. The mean frass weight was 0.68±0.06 g/day/net in 2014 and 0.33±0.03 in 2015; the highest values was 4.82 and 4.18 g/day/net in the two sampling year respectively (Fig. 4.4).

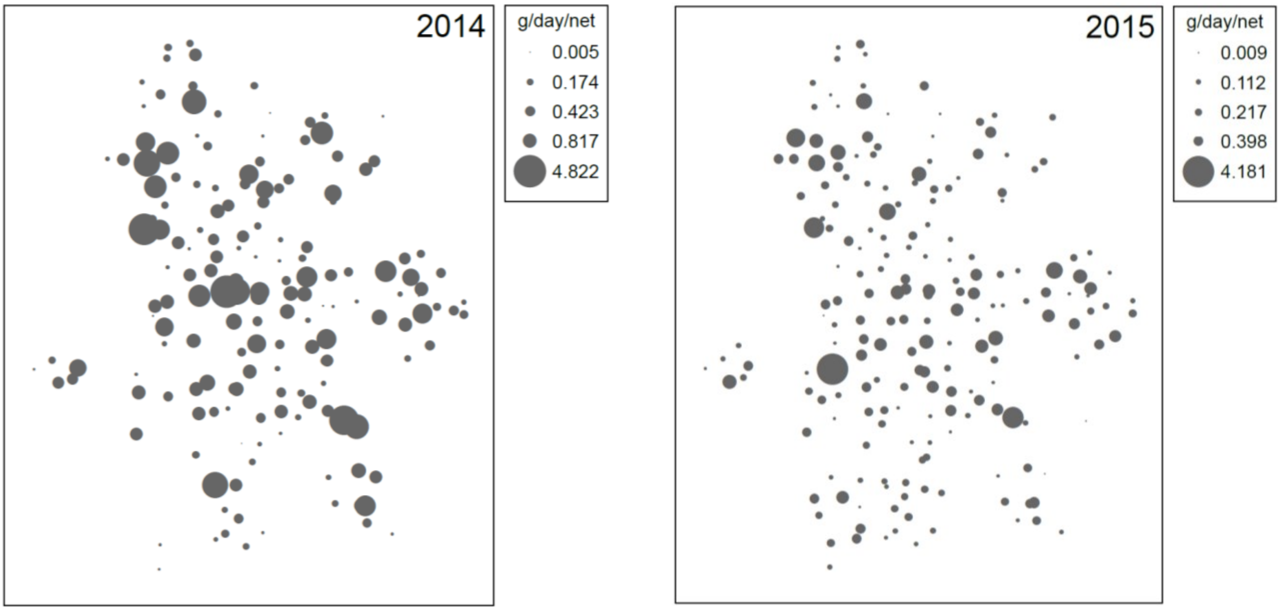


Figure 4.4 Bubble plot maps of frass collection in the two sampling years: bubble sizes correspond to the abundance (g/day/traps) recorded in each site. In the legends, the smallest and the largest circles correspond to the minimum and maximum value while the other circles indicate the quartiles

Regarding models conducted to test the effect of both local and landscape variables on the adult population density at different scale, the Akaike's Information Criterion (AICc) (Supplementary material) showed a lower value in the model considering landscape factors at 250 m around a sapling sites. We reported only the analysis conducted at this scale but not dissimilar values were observed in the model considering landscape factors at 500 m. The Anova (Type II) on the final general linear-mixed model (GLMM) indicated no effect of the variables at the local scale (Tab. 4.1). Conversely, a significant effect was found at landscape scale, in particular, the frass abundance increased in function of higher percentage of forest in the 250 m buffer around the net, as well as a significant interaction between this factor and the sampling year was shown (Fig. 4.5A). We found that forest cover exhibited a quadratic effect. Furthermore, a significant and negative effect of the proportion *Robinia pseudoacacia* over the total forest area in the 250 m buffer, was found (Fig. 4.5B). In addiction significant effect in the year factor was found, with a significantly greater population density recorded in 2014 compared to 2015.

	Chi sq	**Pr (>Chi sq)**
(Intercept)		
PCA1	2.459	0.219
PCA2	1.513	0.117
Year	69.263	<0.001
Forest % in a 250m buffer	14.537	<0.001
I(Forest % in a 250m buffer^2)	3.948	0.047
R. pseudoacacia % in a 250m buffer	8.762	0.003
Year x Forest % in a 250m buffer	11.428	<0.001

Table 4.1 Results from the linear model testing the effects of sampling year, local and landscape factors on the *Barbitistes vicetinus* collected frass.

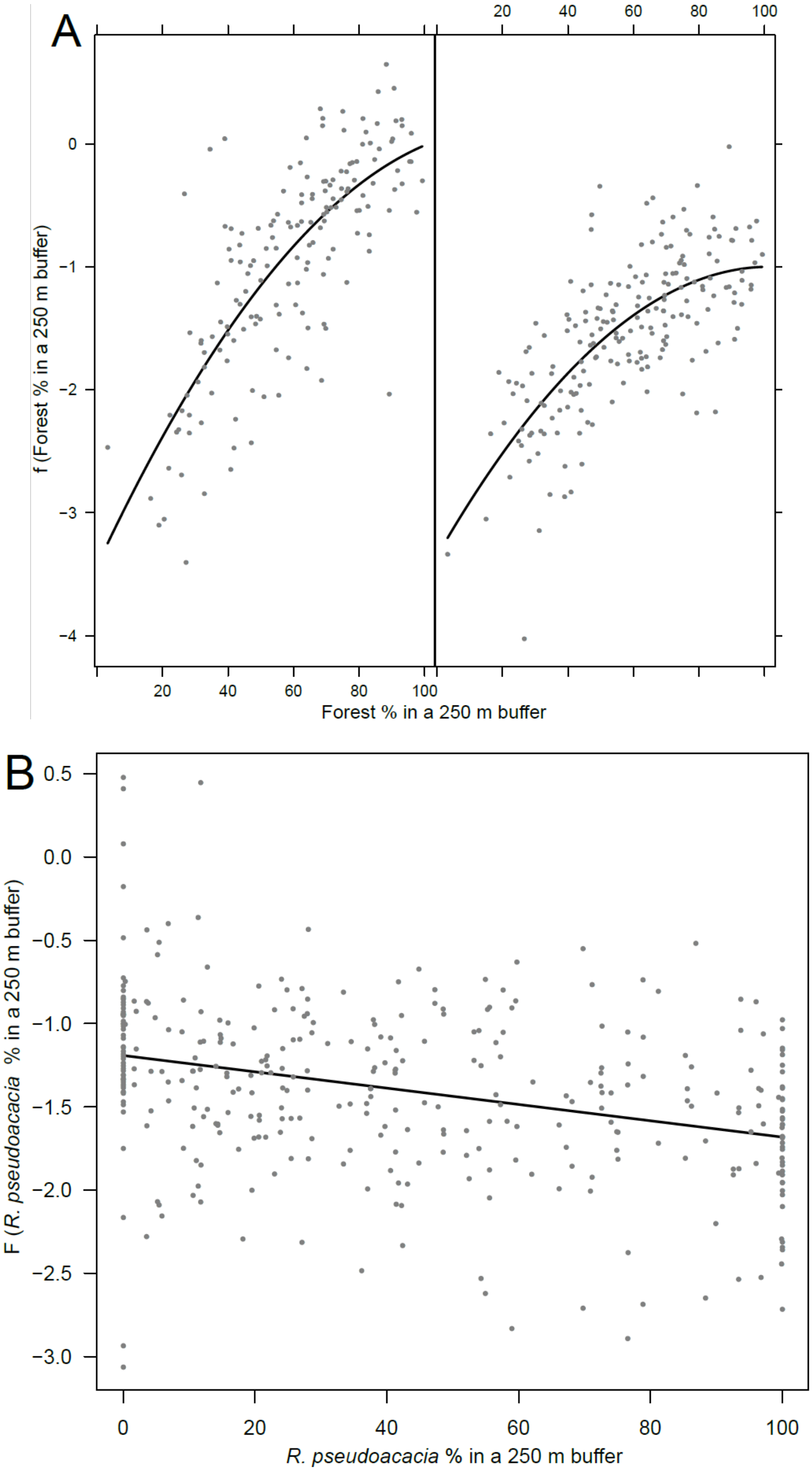


Figure 4.5 Interaction effect between the percentage of forest in the 250 m buffer and the sampling year (A) and the effect of the percentage of *Robinia pseudoacacia* compared to the total forest area in the 250 m buffer (B) on the *Barbitistes vicetinus* collected frass.

Nymph density

Regarding sticky traps, the nymph density corresponded to a total of 560 *B. vicetinus* captured in the 120 traps, with a mean value of 14±3.85 nymphs per site and 4.67±0.93 nymphs per trap. Only 32/120 traps (26.67%) and 3/40 sites (7.50%) did not capture any individual. In addition, 159 nymphs of other Orthoptera species were collected (22.11% of the total specimens), of which 72 nymphs in only one site, with a mean of 3.98±1.79 nymphs per site and 1.33±0.41 nymphs per trap.

Temporal dynamics

The correlation test performed to study the feeding and the oviposition site preference indicated a direct relationship between the log-transformed total nymphs + 1, collected in 2015, in function of frass abundance recorded in 2014 (R^2=0.193; P value=0.005; cor=0.439) (Fig. 4.6A). In the same way, the frass abundance evaluated in 2015 was directly affected by the number of nymphs recorded few months before, in the early spring of the same year (R^2=0.160; P value=0.012; cor=0.400) (Fig. 4.6B). Furthermore, a negative effect of the density in 2014, on the population growth from 2014 to 2015 was found (R^2=0.195; P value=0.005; cor=0.441) (Fig. 4.7).

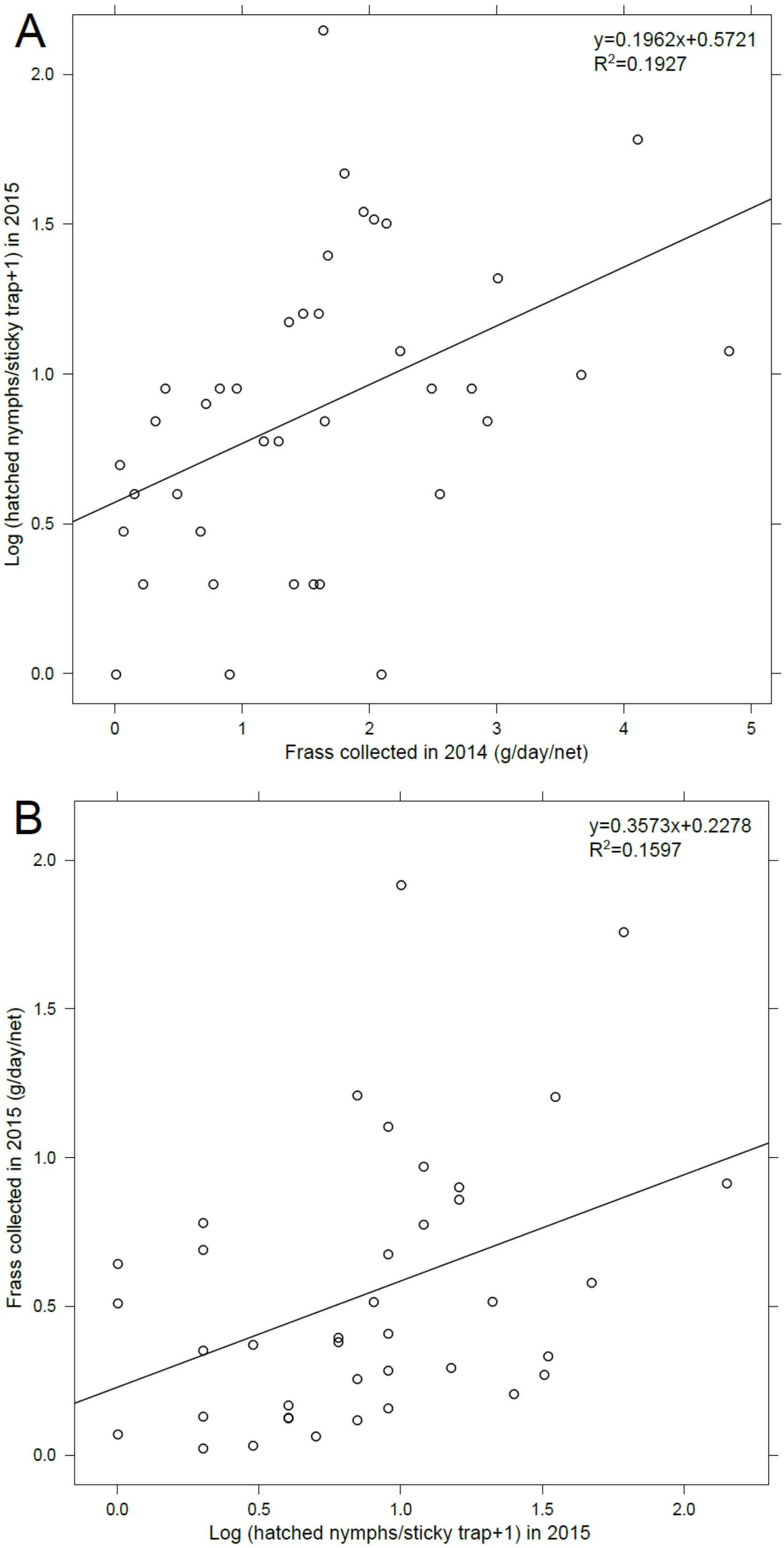


Figure 4.6 Relationship between the log-transformed sum of the nymphs recorded in 2015 in function of the abundance of frass collected in 2014 (A); relationship between frass collected in 2014 and the log-transformed sum of the nymphs recorded in 2015 (B).

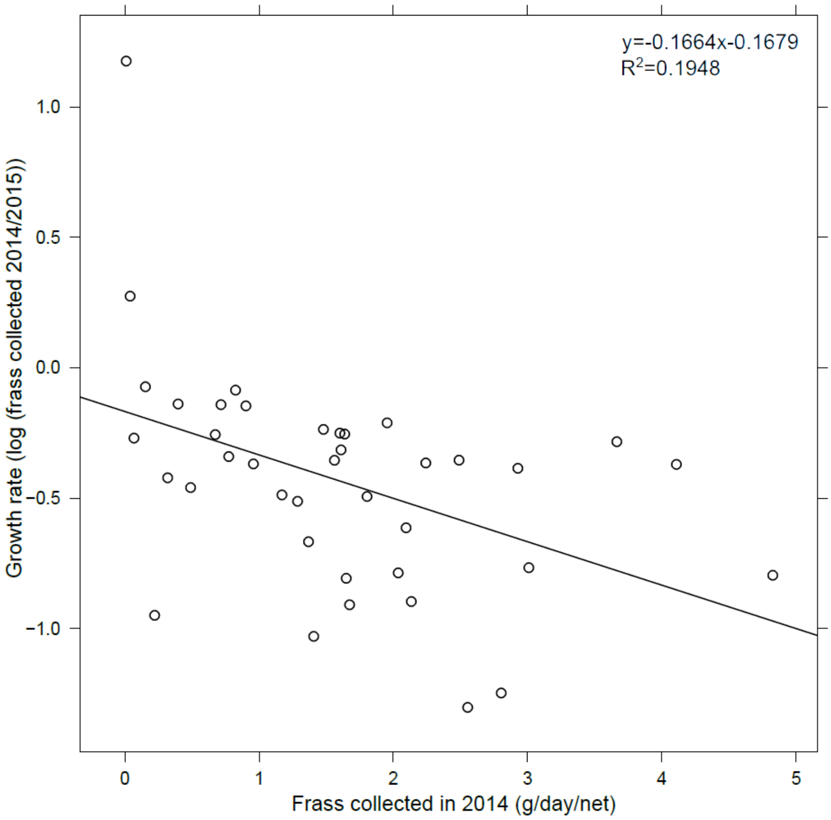


Figure 4.7 Relationship between log-transformed 2014-2015 frass collection growth rate and frass collected in 2014.

Discussion

A number of abiotic and biotic landscape features are expected to control forest-pest dynamics (Holdenrieder et al., 2004). Considered from small to large scales, these factors range from variation in host-tree composition, forest stand attributes (e.g., tree species composition, density, stand size, fragmentation, isolation, ratio of edge to interior), topographical characteristics up to regional processes such as landscape composition and configuration patterns. Here, we analyzed the spatial ecology of a low mobile forest pest in order to evaluate local and landscape processes driving population density and defoliation severity. We found a strong negative effect of forest fragmentation on the pest population density at relatively small spatial scale (250-500 m). Beside the fragmentation effect, the presence of a non-host, alien tree species across the landscape further reduced the population density irrespective of local tree composition.

At the local scale, we observed that *B. vicetinus* was able to feed on a wide array of broadleaf tree species in both years, causing severe defoliation with leaf damage up to 40% of the canopy. Among the most abundant tree species, the only species that was clearly avoided was black locust. In a mixed plant community, each particular tree species is relatively less abundant and its distribution is likely to be more patchy, making it less available to specialist herbivores (Yamamura, 2002). We did not find a relationship between tree diversity or forest basal area and local defoliation. Although local tree composition did not affect defoliation severity, the pest strongly responded to processes working at larger spatial scales.

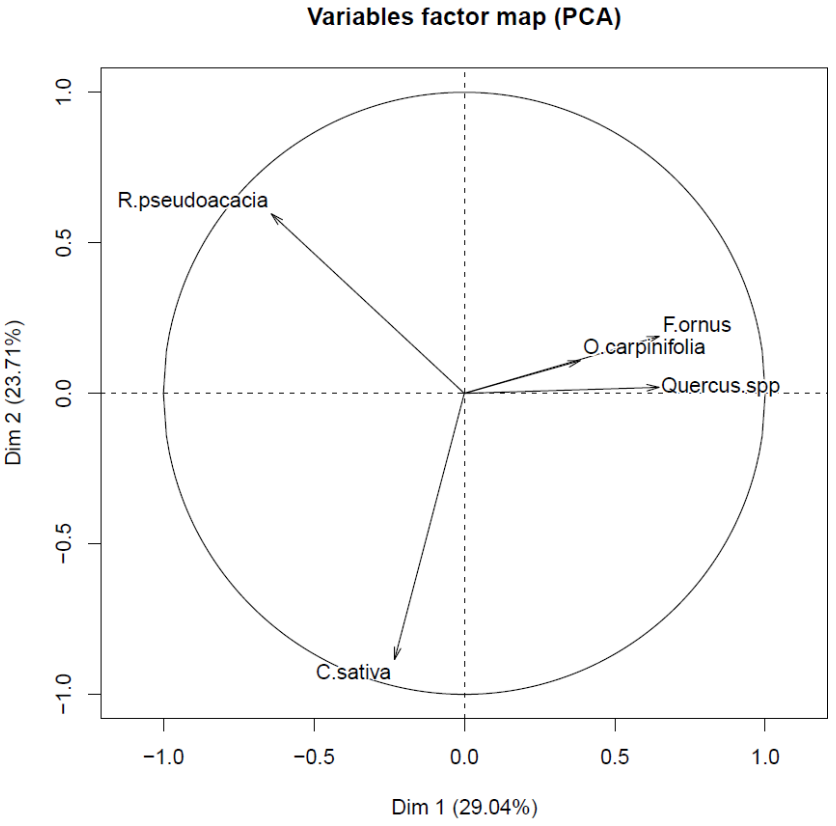
The stronger driver of local defoliation was the proportion of forest in the surrounding landscape (radii 250-500 m). Based on dispersal data recorded on similar flightless species (e.g. *Pholidoptera griseoaptera*, Diekötter et al., 2005), *B. vicetinus* is expected to move less than 100-200 m during its whole life. Hence, the species is expected to disperse at relatively small spatial scale. By disrupting dispersal, fragmentation probably altered a critical component of meta-population dynamics and population persistence (Hanski, 2011). In this respect, our result is similar to other pests that have been demonstrated to respond to forest fragmentation due to their dispersal limitation (Robert et al., 2012). On the other hand, pests with higher dispersal ability are more likely to establish across fragmented landscapes, reducing the relative influence of landscape features on their dynamics (Robinet & Liebhold, 2009). Landscape connectivity can also affects herbivory by altering the movement and interactions among herbivores, their natural enemies, and host plants (Tscharntke & Brandl, 2004). In our model system the role of natural enemies attacking *B. vicetinus* should be low, and the negative effect of low connectivity on the herbivores was likely related to their reduced ability to find resources across the forest mosaics (De La Vega et al., 2012).

The positive effect of host concentration in the landscape was reduced by the presence on black locust. Black locust in the landscape occurred in small homogenous monospecific stands interspersed with native forest at relatively small spatial scale (GIS data). The association of less palatable host trees in the landscape likely decreased damage from our polyphagous herbivore in our mixed forests. Apart from this host concentration effect, a landscape mosaic with non-host species can also provide physical or chemical barriers to host location by foraging or dispersing herbivores (Jactel & Brockerhoff, 2007). For instance, a native potentially suitable forest embedded in a relatively impermeable matrix of black locust would be easily isolated from neighbouring pest population. Dispersal across an unsuitable matrix usually induces a higher mortality risk.

Due to the high density of the species during outbreaks, we expected to find a negative density-dependence in the population dynamics. Although we sampled only two years we could use a space-by-time substitution to test the effect of density dependence processes on population dynamics. The regional population generally declined from 2014 to 2015, but this decline was more pronounced in sites where the defoliation has been more severe in the previous year. This signal indicated that competition for host plants due to crowding is probably a key driver of population dynamics. Other potential mechanisms explaining the observed negative density feedback could also include deterioration of host quality, increased mortality caused by pathogens, and increase of prolonged egg diapause as an adaptive mechanism to escape adverse ecological conditions (Marini et al., 2015).

Although pest occurrence patterns are often dictated by landscape features, yet much of the foundational knowledge of forest pest dynamics is derived from studies of localized interactions among trees and pests (Ferrenberg, 2016). Our contribution elucidates the role of factors at multiple spatial scales to explain local defoliation by an eruptive bush-cricket species. Pest dispersal limitation due to habitat fragmentation and the invasion of a non-host alien tree resulted as the key determinants of local defoliation severity. Our results will help predicting spatial patterns of forest defoliation according to landscape composition and configuration.

Supplementary material



Principal component analysis (PCA) performed, on the local scale, on the main forest species (present at least in 30 sites).

Buffer scale in the GLMM (m radius)	AICc value
100	978.3438
250	940.8147
500	940.8401
750	966.2817
1000	986.4921

Akaike's Information Criterion (AICc) performed on the general linear-mixed models at different scale.

Chapter 5

Conclusions

Orthopteran outbreaks can play a key role in the dynamics of both natural and managed ecosystems. Unlike the more common outbreaks caused by grasshopper species (Orthoptera, Caelifera) (Showler, 1995), infestations caused by bush-crickets (Orthoptera, Ensifera) occur much less frequently, despite they can be as much severe (Gwynne, 2001; Lorch et al., 2005). Until 2008, the bush-cricket *Barbitistes vicetinus* caused heavy defoliation to broadleaves forests and close crops during repeated outbreaks. From an ecological point of view, the infestations of *B. vicetinus* are a very interesting phenomenon because outbreaks occurred in an endemic species unknown until 1993 (Galvagni & Fontana, 1993).

The main aim of this study was to improve biological and ecological knowledge and to develop a strategy to manage outbreaks of this new pest. In particular, biological and ecological investigations help to highlight key issues linked to life-history and behavior of the species and try to explain some of the major outbreak dynamics.

An effective management of an unbalanced population density needs firstly a good knowledge of both maternal behavior and oviposition site preference. In this study nymph population density was assessed for the first time for this species, showing mean value of over 1 million individuals/ha. Results indicated also a wide and remarkable diffusion of the insects over the outbreak area. According to our survey, we demonstrated that cultivated habitats did not offer suitable oviposition sites to *B. vicetinus*, although the species is commonly found also in crops where the adults can cause severe damage. In this respect, a higher nymph density was recorded on woody vegetation with soil covered by broadleaf litter, without a gradient from the inner forest to the edge.

We also investigated the egg hatching phenology in order to better understand the nymph’s population dynamics and to clarify the role of the environmental factors affecting these aspects. Our field and laboratory trials revealed a strong effect of temperature on the hatching start as well as on the hatching duration. The species is able to hatch also at low temperature (6°C). Elevation and exposure, affecting temperature, regulate the hatching period; their analysis can help in outbreak monitoring and forecasting.

Moreover, the spatial ecology of *B. vicetinus* was investigated assessing local and landscape variables driving population density and defoliation severity. We found a strong negative effect of forest fragmentation on the pest population density at relatively small spatial scale (250-500 m). Beside fragmentation, the presence of a non-host alien tree species (i.e., the black locust) across the landscape further reduced the population density irrespective of local tree composition. At local scale, we observed that *B. vicetinus* was able to feed on a wide array of broadleaves tree species, causing severe defoliation with leaf damage up to 40% of the canopy. At regional scale, the population declined from 2014 to 2015 mainly in sites where the defoliation has been more severe in the previous year.

Barbitistes vicetinus was chosen as model species especially because it is an endemism producing severe outbreaks. This work provides, above all, the first basal knowledge about biology and ecology of outbreaking populations of this species. The new insights on the hatching phenology and oviposition site preference represent the first step of the outbreak management. So far without specific knowledge about hatching site and period, the applied control strategies rarely resulted really effective. In addition *B. vicetinus* hatched in a very sensitive period, during bloom, with a large amount of other insect species, including pollinators. For this reason, a clever management of the control protocols (e.g., insecticide treatments) is required. In addition, the forecasting of the outbreak risk based on landscape composition of the potentially infested areas will be furtherly useful. Moreover, the comparison of our results to other outbreaking bush-cricket species, could lead to a better understanding the relationships between pest and host plants also in other model contexts.

The following future perspectives would be addressed to improve the knowledge of outbreaks and their drivers:

- genetic studies based on mitochondrial analysis of the *Barbitistes* populations (population genetics and phylogeny of the genus *Barbitistes*);

- dispersal ability and movement patterns of *B. vicetinus*;
- species fecundity and multi-year diapause regulation during outbreaks;
- natural enemies role in the outbreak dynamics.

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Annexes

Information of the experiments performed during my PhD were also disseminated at the national level to technicians and farmers through a specialized magazine.

Cavaletto G., Giannone F., Mazzon L., 2015: Forti presenze nei Colli Euganei di *Barbitistes vicetinus*. L'informatore Agrario, 22/2015: 50-54.

NOTE BIOLOGICHE E POSSIBILITÀ DI CONTROLLO

Forti presenze nei Colli Euganei di *Barbitistes vicetinus*

Dal 2008 sono state segnalate importanti pullulazioni di *Barbitistes vicetinus* nell'areale dei Colli Euganei, con il coinvolgimento anche di colture agrarie (vite, ciliegio e olivo). Il monitoraggio del territorio e la tempestiva segnalazione dell'avvio delle schiuse nelle diverse aree sono fondamentali per impostare una efficace lotta precoce all'insetto

Foto 1 Giovani esemplari di *Barbitistes vicetinus* a poche ore dalla schiusa (5 mm)

Foto 2 Femmina adulta di *Barbitistes vicetinus* (21-25 mm)

Foto 3 Maschio adulto di *Barbitistes vicetinus* (forma melanica) (18-23 mm)

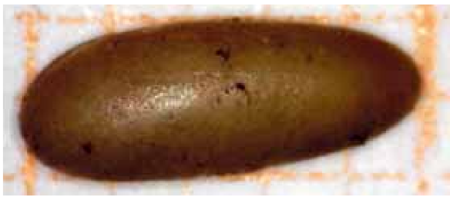


Foto 4 Uovo di *Barbitistes vicetinus* su reticolo millimetrato (lunghezza 5 mm)

di **Giacomo Cavaletto, Filippo Giannone, Luca Mazzon**

Barbitistes vicetinus Galvagni & Fontana, 1993 è un ortottero di recente descrizione, endemico del Veneto (Lessini Orientali, Monti Berici e Colli Euganei) e della bassa Val d'Adige trentina. Frequenta ambienti boscosi, preferendo le parti sommitali delle chiome di alberi e arbusti; questo rende la specie piuttosto elusiva e di difficile studio (Galvagni e Fontana, 1993;1999; Magello *et al.*, 2003).

Come riconoscere la specie

L'**individuo neoschiuso** (lunghezza 5 mm) si presenta di color verde chiaro, opaco, con gli arti che gradualmente imbruniscono a partire dalla parte distale (*foto 1*).

La colorazione di fondo dell'**insetto adulto** (il cui corpo misura tra i 18 e i 23 cm nel maschio e tra i 21 e i 25 cm nella femmina) è di un verde brillante, con minutissima punteggiatura bruno-ocra. Le antenne sono rosso-brune, le zampe hanno femori color rosso vivo e tibie giallo-ocra.

Le tegmine sono di color bruno-rossastro (*foto 2*), molto corte; l'insetto non è provvisto di ali sviluppate. In condizioni demografiche di elevata densità la specie manifesta il fenomeno del melanismo, sviluppando popolazioni di individui con intensa e diffusa pigmentazione scura. In tali casi il colore diventa nero brillante sul pronoto e sulla parte superiore dell'addome. Su entrambi i lati, la banda gialla che percorre il corpo dal pronoto fino al settimo segmento addominale ha colore molto intenso.

I femori delle zampe anteriori e mediane sono rossastri; quelli delle posteriori sono rossi e neri. Tutte le tibie e i tarsi sono neri (*foto 3*).

Le **uova** di *B. vicetinus* (*foto 4*) hanno forma ellittica, sono leggermente bombate e schiacciate, di color bianco sporco, rosa-grigio o tortora chiaro, a seconda del livello esterno di umidità al quale vengono osservate. Hanno lunghezza massima pari a 5 mm e larghezza fino a 2,5 mm, con uno spessore di 1,2 mm. Vengono deposte singolarmente nel terreno nei primi 2-3 cm di suolo.

Ciclo biologico

L'insetto presenta un'unica generazione annua e sverna come uovo nel terreno. Le schiuse di regola iniziano a partire dalla seconda metà di marzo. **I primi adulti compaiono nel mese di maggio e tra la fine del mese e l'inizio di giugno si hanno i primi accoppiamenti e, a seguire, le prime deposizioni.** Gli adulti si rinvengono sulla vegetazione normalmente sino ai primi di agosto. Il ciclo biologico subisce delle variazioni temporali in funzione delle temperature delle diverse località e delle diverse annate.

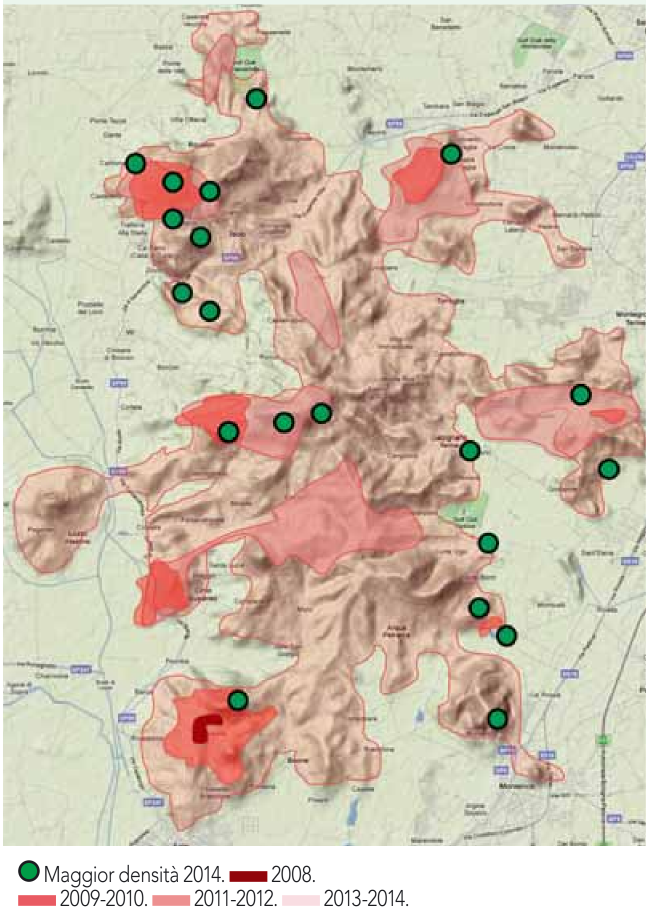
Alimentazione

B. *vicetinus* è una specie prevalentemente fillofaga che si alimenta a spese di piante forestali arboree e arbustive come carpino nero, olmo campestre, roverella, orniello, nocciolo, bagolaro, castagno, acero, barba di capra, sanguinella, corniolo scotano, e molte altre, manifestando una notevole polifagia. Gli esemplari giovani, invece, si trovano con estrema frequenza su piante del genere *Rubus*.

Il margine dell'erosione fogliare si presenta tipicamente sfilacciato, elemento distintivo degli attacchi di questa specie.

Con le pullulazioni degli ultimi anni, la specie ha interessato spesso le colture agrarie più prossime alla vegetazione forestale, arrecando danni soprattutto su vite, ciliegio e olivo.

FIGURA 1 - Diffusione delle pullulazioni di *Barbitistes vicetinus* nel comprensorio dei Colli Euganei (PD)



● Maggior densità 2014. ■ 2008.
■ 2009-2010. ■ 2011-2012. ■ 2013-2014.

Pullulazioni nel Padovano

Nel 2008, sui Colli Euganei, nel comune di Baone (Padova), località Calaone, (Monte Cero, alto versante nord-occidentale), è stato segnalato il primo focolaio. L'anno successivo, si sono avute pullulazioni più importanti, con coinvolgimento di colture agrarie (*foto 5*).

Infine, nel corso della primavera-estate 2010 le segnalazioni si sono estese a quasi tutti i comuni appartenenti all'area euganea (*figura 1*). Attualmente il fenomeno interessa la quasi totalità del comprensorio collinare (Mazzon *et al.*, 2011). Nel corso degli anni si è assistito all'incremento demografico anche di diverse altre specie di ortotteri ensiferi, tra le quali alcune hanno evidenziato attività predatoria nei confronti di *B. vicetinus* (*foto 6*).

Danni

Gli individui neoschiusi acquisiscono in poco tempo buona capacità di spostamento e tendono a portarsi rapidamente verso la parte alta della chioma.Nelle aree più colpite dalle pullulazioni, le piante spontanee subiscono defogliazioni prossime e talvolta superiori all'80-90%.

Con l'aumentare delle dimensioni, l'insetto è in grado di compiere notevoli spostamenti e invade anche le piante coltivate limitrofe alle aree boscate, in particolare vite, olivo e ciliegio.

Sulla vite, l'attacco è precoce e si sviluppa a carico dei germogli e delle foglie in distensione, quando ancora la massa fogliare è piuttosto limitata. In alcuni casi è possibile osservare anche 3-4 individui per foglia. La defogliazione è rapida e si allarga velocemente lungo la fila e poi ai filari contigui (*foto 7*). **Nel ciliegio, l'azione di B. *vicetinus* è a carico soprattutto delle foglie e dei frutti, dato che la precoce fioritura generalmente sfugge all'attacco dell'insetto. Nell'olivo si notano solo sporadiche erosioni all'apice dei germogli e il danno è concentrato soprattutto sulle mignole, che vengono in alcuni casi completamente asportate** (*foto 8*).

Foto 5 Pullulazione di *Barbitistes vicetinus* su vite

Foto 6 Adulto di *Tettigonia viridissima* mentre preda un maschio di *Barbitistes vicetinus* in vigneto

Foto 7 Danno precoce su vite Chardonnay causato dagli stadi giovanili

Studi sulla densità di popolazione

Al fine di determinare la densità di popolazione di *B. vicetinus* in ambiente naturale, nel corso del 2013 è stata condotta una sperimentazione volta a rilevare il numero di individui/m², durante tutto il ciclo biologico dell'insetto. A intervalli regolari di 20 giorni, tra marzo e luglio, la vegetazione spontanea del Monte Cero (area di prima pullulazione) è stata monitorata con il metodo degli *open quadrats* (Richards e Waloff 1954; Gardiner *et al.*, 2002; Gardiner *et al.*, 2005). Sono state individuate 14 aree coperte da vegetazione spontanea arborea e arbustiva, all'interno delle quali sono state delimitate altrettante parcelle di 9 m².

In ciascuna data di campionamento, in ogni parcella, venivano rilevati tutti gli esemplari di *B. vicetinus*, conteggiando accuratamente gli insetti sulla vegetazione, «a vista».

Tale approccio è reso possibile dall'atteggiamento piuttosto confidente della specie.

Il picco demografico è stato raggiunto in meno di un mese dalle prime schiuse e la pullulazione ha fatto registrare valori medi superiori ai 12 individui/m².

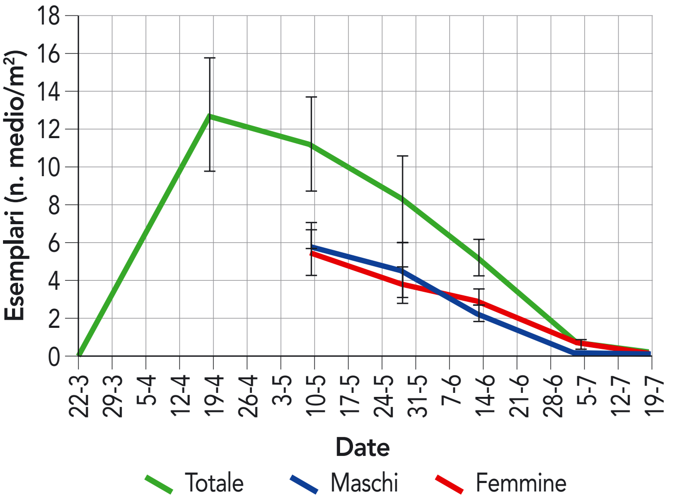
Anche se in progressivo calo, la densità si mantiene stabilmente oltre i 5 individui/m² fino alla metà di giugno, per poi azzerarsi quasi totalmente intorno alla metà di luglio. La *sex ratio* (rapporto dei sessi) complessiva non evidenzia differenze significative tra il numero di maschi e femmine (rapporto 1:1), ma va sottolineata una maggior longevità delle femmine alla fine del ciclo (*grafico 1*).

Una seconda prova è stata condotta sugli adulti il 4 giugno 2013, con il metodo dei *box quadrat* (Bridle *et al.*, 2002; Virdee e Hewitt, 1990), confrontando vegetazione spontanea arbustiva e vigneti limitrofi (10-15 m dal margine boschivo - Monte Cero).

Con un retino quadrato di 1 m di lato, che veniva addossato alla vegetazione per raccogliere tutti gli esemplari presenti nell'unità di superficie, il monitoraggio è stato eseguito 20 volte sulla vegetazione spontanea e 20 volte su vigneto, lungo un transetto ideale corrispondente al margine del bosco e al primo filare di vite a esso parallelo, campionando ogni 10 m.

Pur non presentando differenze significative (*grafico 2*) la maggiore densità media rilevata in vigneto potrebbe essere causato da un effetto di concentrazione (Cavaletto *et al.*, 2014).

GRAFICO 1 - Dinamica della popolazione di *B. vicetinus* a Monte Cero (PD)



Le barre verticali rappresenano ± errore standard.

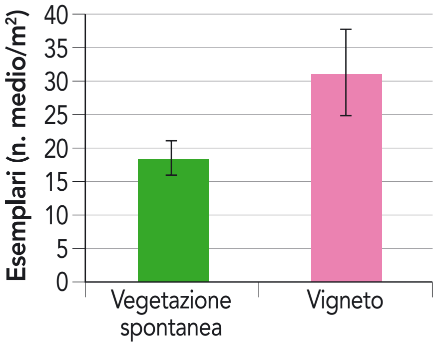
Densità e dinamica delle schiuse

Nel corso della primavera 2013, sul Monte Rusta (Cinto Euganeo - Padova) sono state monitorate le schiuse di *B. vicetinus*, per determinarne il periodo e la dinamica. È stato inoltre possibile indagare i siti preferenziali per l'ovideposizione e la densità della popolazione al momento della schiusa. A tal scopo sono state posizionate 90 *emergence traps* con fondo aperto.

Le trappole sono state posizionate in bosco (a diverse distanze dal margine, 200, 50, 30 e 10 m), al margine del bosco e nella successione siepi spontanee-vigneti che si alternavano. Le trappole sono state ispezionate a intervalli di 48 ore, dall'inizio delle schiuse fino alla loro conclusione.

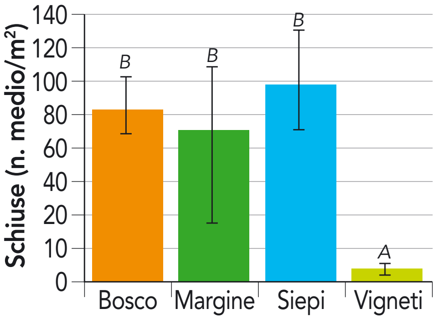
Foto 8 Erosione delle mignole su olivo

GRAFICO 2 - Densità media di *B. vicetinus* su vegetazione spontanea e vigneto a Monte Cero (PD)



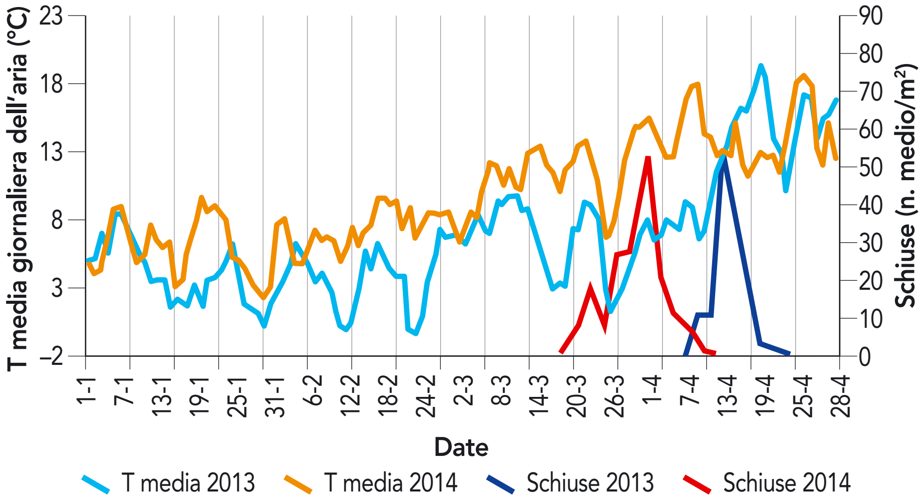
Dati sottoposti all'Analisi della varianza (ANOVA). Le barre verticali rappresentano ± errore standard.

GRAFICO 3 - Densità media delle schiuse di *B. vicetinus* in diversi ambienti del Monte Rusta (PD)



I dati sono stati accorpati per ambienti omogenei.
Le barre verticali rappresentano ± errore standard. Dati sottoposti all'Analisi della varianza e al Tukey's HSD test.
A lettere diverse corrispondono differenze statisticamente significative per P < 0,01.

GRAFICO 4 - Confronto tra dinamiche di schiusa e temperature medie giornaliere presso il Monte Rusta (PD) nel biennio 2013-2014



Le barre verticali rappresenano ±errore standard.

Le temperature più alte del 2014 probabilmente hanno favorito un anticipo delle schiuse di 15 giorni.

Come si può osservare dal *grafico 3*, la densità delle schiuse è risultata significativamente inferiore nei vigneti rispetto agli ambienti di bosco, margine di bosco e siepe.

I risultati mostrano dunque come la specie prediliga deporre in ambiente coperto da vegetazione spontanea arborea e arbustiva.

Apparentemente non vi sembra essere una preferenza per il sito di deposizione delle uova in termini di distanza dal margine del bosco. Le schiuse sono iniziate il 4 aprile e sono terminate il 23 aprile. Il periodo di schiusa risulta dunque concentrato in un intervallo di soli 20 giorni.

I dati raccolti permettono di stimare, per gli ambienti boschivi e di siepe, una densità di schiusa media superiore agli 850.000 esemplari/ha.

Nella primavera 2014 un'ulteriore prova di stima della densità di schiusa è stata condotta nello stesso sito con i medesimi metodi. Come visibile nel *grafico 4*, nel secondo anno di indagine si è verificato un anticipo nelle schiuse di circa 15 giorni, probabilmente dovuto a temperature medie maggiori, relative al periodo precedente la schiusa. Quest'anno (2015) le schiuse sono iniziate il 30 marzo.

Prove di controllo in semicampo

Nel biennio 2012-2013, contemporaneamente agli studi sulla biologia, sono state condotte delle prove per valutare l'efficacia di alcuni insetticidi, molti dei quali di origine naturale, nel controllo dell'insetto.

Per i test sono stati utilizzati dei manicotti di tulle, sostenuti da un telaio rigido, lunghi 50 cm e con diametro di 20 cm, posizionati in modo da isolare una porzione di un tralcio di vite. Ogni tesi era strutturata su quattro ripetizioni.

Al momento della prova, sono stati introdotti in ciascun manicotto 10 insetti; la loro vitalità è stata poi monitorata dopo 1 giorno (T + 1), 3 giorni (T + 3) e dopo 7 giorn dal trattamento (T + 7), per evidenziare la diversa efficacia degli insetticidi. In *tabella 1* sono riportate le diverse tesi a confronto. Tutti i prodotti sono stati utilizzati alla dose di etichetta.

Come evidenziato in *tabella 1*, la deltametrina consente di ottenere un rapido abbattimento della popolazione e la sua completa eliminazione a 3 giorni. Spinosad e *Metarhizium* ottengono buoni risultati (10 e 4% di sopravvivenza) a 7 giorni dal trattamento.

Ulteriori approfondimenti hanno valutato l'efficacia di due prodotti (deltametrina e piretro) in diversi dosaggi e modalità distributive.

In particolare, soluzioni con diverse concentrazioni di deltametrina sono state applicate sulla vegetazione inserita nei manicotti. Il 50% di questi manicotti conteneva gli insetti mentre nel rimanente 50% gli insetti sono stati introdotti solo una volta che la vegetazione trattata si era asciugata.

In *tabella 2* si evidenzia come il trattamento all'insetto con deltametrina risulti pienamente efficace a T + 3 anche a metà e 1/4 della dose di etichetta.

Per quanto riguarda invece la persistenza, deltametrina sulla vegetazione è in grado di dare risultati interessanti (numero di individui sopravvissuti inferiore al 20%) a 3 giorni dal trattamento. La risposta del piretro (*tabella 3*) è risultata debole in tutte le date e a quasi tutti i dosaggi. Solo il doppio dosaggio è stato in grado di ottenere un risultato statisticamente differente dal testimone (17% di individui sopravvissuti dopo 7 giorni).

TABELLA 1 - Efficacia di alcuni prodotti insetticidi testati nel controllo di *B. vicetinus*

Sostanza attiva	Dose f.c. in etichetta (mL o g/hL)	Individui sopravvissuti (%)			
		T + 0 gg	T + 1 gg	T + 3 gg	T + 7 gg
Testimone non trattato		100	100 *A*	91 *A*	66 *A*
Azadiractina	300 mL/hL	100	100 *A*	88 *AB*	70 *A*
Piretro naturale	120 mL/hL	100	95 *A*	80 *AB*	51 *AB*
Deltametrina	50 mL/hL	100	30 *B*	0 *C*	0 *C*
Metarhizium sp.	200 g/hL	100	92 *A*	51 *B*	4 *C*
Spinosad	20 mL/hL	100	84 *A*	59 *AB*	10 *BC*
Thiamethoxam	20 g/hL	100	93 *A*	70 *AB*	53 *AB*

Dati sottoposti all'Analisi della varianza e al Tukey's HSD test. A lettere diverse corrispondono differenze statisticamente significative per $P < 0{,}01$.

Spinosad e *Metarhizium* consentono di ottenere buoni risultati a 7 giorni dal trattamento.

Fondamentale ruolo del monitoraggio

La recente descrizione della specie *B. vicetinus* ha anticipato solo di pochi anni il fenomeno delle sue pullulazioni nei Colli Euganei. **Le esplosioni demografiche hanno comportato pesanti defogliazioni in ambito boschivo, immediatamente accompagnate da importanti danni nelle coltivazioni limitro-**

TABELLA 2 - Efficacia di deltametrina nel controllo di *B. vicetinus*

Sostanza attiva ([1])	Individui sopravvissuti (%)		
	T + 0 gg	T + 1 gg	T + 3 gg
Inserimento degli insetti nel manicotto prima del trattamento			
Testimone non trattato	100	100 *A*	100 *A*
Deltametrina (1/64 N)	100	100 *A*	60 *AB*
Deltametrina (1/32 N)	100	98 *A*	48 *AB*
Deltametrina (1/16 N)	100	83 *BC*	33 *BC*
Deltametrina (1/8 N)	100	93 *AB*	30 *BC*
Deltametrina (1/4 N)	100	63 *DE*	0 *C*
Deltametrina (1/2 N)	100	75 *CD*	0 *C*
Deltametrina (N)	100	60 *E*	0 *C*
Inserimento degli insetti nel manicotto dopo il trattamento			
Testimone non trattato	100	100	100 *A*
Deltametrina (1/64 N)	100	90	50 *AB*
Deltametrina (1/32 N)	100	93	43 *BC*
Deltametrina (1/16 N)	100	90	25 *BCD*
Deltametrina (1/8 N)	100	90	40 *BC*
Deltametrina (1/4 N)	100	98	15 *BCD*
Deltametrina (1/2 N)	100	83	8 *CD*
Deltametrina (N)	100	88	0 *D*

([1]) Dose impiegata rispetto a quella riportata in etichetta (50 mL/hL). Dati sottoposti all'Analisi della varianza e al Tukey's HSD test. A lettere diverse corrispondono differenze statisticamente significative per $P < 0,01$.

La persistenza di deltametrina garantisce buoni risultati a 3 giorni dal trattamento, anche a dosi inferiori a quelle di etichetta.

TABELLA 3 - Efficacia di piretro naturale nel controllo di *B. vicetinus*

Sostanza attiva (*)	Individui sopravvissuti (%)			
	T + 0 gg	T + 1 gg	T + 3 gg	T + 7 gg
Testimone non trattato	100	100	91	66 A
Piretro naturale (1/4 N)	100	100	95	83 A
Piretro naturale (1/2 N)	100	98	85	60 AB
Piretro naturale (N)	100	95	80	51 AB
Piretro naturale (2 N)	100	95	61	17 B

(*) Dose impiegato rispetto a quella riportata in etichetta (120 mL/hL). Dati sottoposti all'Analisi della varianza e al Tukey's HSD test. A lettere diverse corrispondono differenze statisticamente significative per $P < 0,01$.

Il piretro naturale risulta efficace solo a dose doppia rispetto a quella riportata in etichetta.

fe, in particolare vite e olivo, ma anche a orti e giardini. Inoltre spesso si è registrato disagio alla popolazione, in prossimità delle aree soggette a pullulazione per l'invadenza della specie.

La stima della densità delle popolazioni, sia in ambito forestale sia nei contesti agrari, ha evidenziato livelli di presenza molto elevati, che superano i 31 individui/m^2 in vigneto.

Il monitoraggio delle schiuse nel terreno ha messo in luce che *B. vicetinus* è un insetto tipicamente legato ad ambienti forestali; la maggior parte delle ovideposizioni si verifica, infatti, in terreno con copertura arborea o arbustiva spontanea, con densità medie prossime al milione di individui per ettaro.

La comparsa degli insetti è precoce e segue di pochi giorni il germogliamento delle piante ospiti; negli ultimi tre anni le schiuse sono iniziate nel periodo compreso tra la seconda metà di marzo e l'inizio di aprile, con una durata media di circa 20 giorni. Dopo la schiusa gli esemplari si portano rapidamente sulla chioma causando le tipiche erosioni fogliari.

Nei contesti agrari la conoscenza della morfologia dell'insetto è fondamentale per il riconoscimento della specie, al fine di non confonderla con i numerosi altri ortotteri presenti ma non dannosi. La conoscenza della biologia della specie, in particolare, l'epoca e la durata del periodo di schiusa nonché le preferenze nei confronti dei siti di ovideposizione sono presupposti fondamentali per l'impostazione di razionali ed efficaci programmi di contenimento delle pullulazioni.

La lotta è fortemente condizionata dal particolare ambiente interessato dalle pullulazioni; la continua migrazione di insetti dalle fasce boscate confinanti con i vigneti o gli oliveti costringe a un costante controllo della vegetazione.

Nell'olivo e in altri alberi da frutto o di importanza ornamentale, la lotta chimica può essere facilmente evitata posizionando precocemente, ai primi di aprile, delle bande trattate con vischio entomologico, sul tronco delle piante (a 50-80 cm dal suolo), tali da impedire la risalita degli insetti

Su vite i trattamenti precoci, sui primi stadi giovanili, possono essere effettuati con prodotti naturali a bassa persistenza e a basso impatto. A questo proposito buona efficacia ha evidenziato lo Spinosad (*tabella 1*). L'impiego di prodotti di questo tipo in un contesto, come quello euganeo, caratterizzato da elevata presenza di aree naturali, diventa ancora più importante soprattutto alla luce della diffusa presenza di fioriture e di insetti pronubi che caratterizzano questo momento della stagione. Oltretutto, la precocità dell'intervento in vigneto, quando il cordone presenta solo germogli di lunghezza limitata, consente di utilizzare bassi volumi di acqua.

L'impiego di prodotti a base di deltametrina si è rilevato particolarmente efficace e di rapido effetto anche a dosi inferiori a quelle di etichetta (*tabella 2*). Tuttavia, si consiglia il ricorso a questo insetticida esclusivamente in casi particolari di pesanti infestazioni e dove sia richiesta una pronta azione. L'impiego di prodotti di sintesi e a largo spettro, infatti, potrebbe nel lungo periodo diventare controproducente per l'effetto dannoso che esercita sugli insetti utili e sui nemici naturali in particolare.

Giacomo Cavaletto, Filippo Giannone
Luca Mazzon
Dipartimento Dafnae, Università di Padova

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