

Emergence phenology and temperature effect on the post-diapause egg development in the bush cricket *Barbitistes vicetinus* (Orthoptera, Tettigoniidae)

G. Cavaletto* , M. Faccoli, L. Marini and L. Mazzon 

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

Abstract

The tettigoniid *Barbitistes vicetinus* Galvagni & Fontana was described in 1993 as an endemic and rare bush-cricket of north-east Italy. Since 2008, this species has become a pest, causing repeated outbreaks with severe defoliations in broadleaf forests and neighbouring crops. Few data are currently available on ecology and life-cycle of this species, in particular about how temperature regulates egg-diapause and hatching phenology. The present work reports a field study regarding hatching phenology of *B. vicetinus*, surveyed with 84 emergence traps over four consecutive years (2013–2016). Moreover, the effect of temperature on the hatching was tested in the laboratory, exposing eggs to different temperatures. Field observations showed that hatching occurred between the end of March and beginning of April. In warmer years, hatching started early in the spring and lasted longer, while in colder years, hatching started later and was concentrated in a few days. Moreover, a significant effect of both elevation and exposure on the hatching start was observed. Results obtained from laboratory suggested the ability of the species to develop in the post-final diapause in a wide range of thermal conditions. After the diapause (terminated by a prolonged common exposure to low temperature) *B. vicetinus* was able to hatch from 6 to 23°C although, due to prolonged post-diapause development, hatching took place progressively later at colder temperatures. To manage *B. vicetinus* outbreaks, forecasting the seasonal phenology of egg hatching and its duration is important for an effective pest control.

Keywords: diapause, emergence traps, life-cycle, overwintering, pest, temperature

(Accepted 21 June 2019; First published online 24 July 2019)

Introduction

Several insect species of temperate regions escape adverse, cold seasons regulating 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, Tettigoniidae) of temperate climates overwinter as diapausing eggs laid as early as July or as late as October (Ingrisch, 1986a). For this reason, thermal conditions experienced by eggs before winter vary greatly depending upon the time of oviposition. It is known that embryonic development of most of the Western Palaearctic tettigoniids can last more than one year, at least optionally (Ingrisch, 1986a). In these species, embryogenesis is interrupted twice, by initial diapause in the very young embryo and by final diapause in the mature embryo. Whereas initial

*Author for correspondence
Phone: +39 049 8272875
Fax: +39.049.8272810
E-mail: giacomo.cavaletto@gmail.it

diapause can last for several years, final diapause is always terminated in one winter. The occurrence of initial diapause is supposed to be related to thermal imbalances such as low temperatures before overwintering or excessively warm temperatures in summer (Ingrisch, 1986b; Higaki & Ando, 2002). According to the different life-cycle, tettigoniid species can be divided in (i) annual (without initial embryonic dormancy), (ii) annual or biennial (depending on early or late laying time), (iii) biennial or longer (up to 8 years due to a prolonged initial diapause) (Ingrisch, 1986a). In some European species, however, the life cycle is even more variable, with the nymphs hatching from eggs after the first or the second winter independently, even within the same population (Ingrisch, 1984).

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). Due to its restricted geographic range, this species is assessed as Near Threatened in the IUCN Red List (Buzzetti *et al.*, 2016). However, since 2008, *B. vicetinus* has become a pest, causing repeated outbreaks occurring in ever-increasing areas. In forests and neighbouring crops (mainly vineyards and olive groves) were recorded heavy tree defoliations (Cavaletto *et al.*, 2019). Outbreaks were situated in a hilly area (Euganean Hills) of approximately 20,000 ha (Cavaletto *et al.*, 2015). Because *B. vicetinus* was described recently (1993) and its outbreaks occurred only in the last decade, few data are currently available on the ecology and life-cycle of this species. As several Western Palaearctic tettigoniids (Bailey & Rentz, 1990), *B. vicetinus* is a univoltine species overwintering as eggs in the soil (Cavaletto *et al.*, 2018). Moreover, there is evidence for the presence of a multi-year diapause of eggs (Magello *et al.*, 2003). In a recent study about oviposition site preference of *B. vicetinus*, Cavaletto *et al.* (2018) reported that – although during outbreaks the species is very common on crops – oviposition is mostly associated to a broadleaf litter of forest habitats. Although egg development of bush crickets is known to be affected by temperature (Behrens *et al.*, 1983), little is known about how this factor regulates the egg development in *B. vicetinus*. As oviposition of this species occurs mainly in the soil of sheltered forest areas, and because eggs are laid near the surface due to the short female ovipositor (1 cm), microclimatic conditions could be relevant factors in embryo development (Cavaletto *et al.*, 2018).

Knowledge of the factors influencing the post-diapause egg development is of crucial importance to manage insect pests producing harmful outbreaks. Moreover, the knowledge of the seasonal phenology of egg hatching and its duration is a major priority to set up an effective pest control. This study, carried out during four consecutive years in the outbreak area of north-eastern Italy, explores the *B. vicetinus* hatching phenology in relation to temperature and other environmental factors such as elevation and exposure of the oviposition sites. Effects of temperature on the hatching phenology and the estimation of thermal thresholds required for egg hatching were also tested under laboratory conditions.

Materials and methods

The study area

The study was carried out in the Euganean Hills (Veneto Region, north eastern Italy), a mountain area consisting of about 100 volcanic hills (of about 20,000 ha) with the highest elevation of 603 m a.s.l. The climate is characterized by an annual average temperature of 10–13°C and 700–900 mm

precipitation (Kaltenrieder *et al.*, 2009). The presence of valleys and steep hills generates different microclimatic conditions affecting the local vegetation composition. A sub-Mediterranean flora occurs on the southern slopes, whereas temperate deciduous trees and shrubs grow on cooler and moister northern ones (Del Favero, 2001). The landscape is characterized by the presence of broadleaf forest patches, mixed with various types of crop fields and rural settlements (Cavaletto *et al.*, 2018).

Field trials

To assess the hatching phenology of *B. vicetinus* under natural conditions, field trials were carried out during the spring of four consecutive years (2013–2016), at 18 sites selected in the outbreak area as described in Cavaletto *et al.* (2018) (table 1). All the sites were characterized by the presence of mixed broadleaf forests dominated by *Fraxinus ornus*, *Ostrya carpinifolia* and *Quercus pubescens* or *Castanea sativa*. The forest edge consisted mainly of *Rubus* spp., *Cornus* spp. and *Acer campestre*. None of the selected sites experienced anthropogenic 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) (table 1). The trap consisted of a pyramidal wooden frame (50 cm high) with a square base of 0.25 m² (0.5 × 0.5 m). The frame was closed by a white 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 also used in other ecological studies aimed to estimate the population density of arthropods (Sunderland *et al.*, 1995).

A total of 84 emergence traps were used in four consecutive years according to the experimental design reported in table 1. During the first year (2013), a preliminary study was carried out only in two sites by placing six traps per site, split into two habitats: three traps along the forest edge and three 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), nine of the 18 sites investigated in 2014 were again monitored, placing one trap per site and habitat, for a total of 18 emergence traps per year (table 1).

The emergence traps were placed in the field in late winter (February) while trap checking and insect collection were carried out every 48 h from mid-March to the end of April, covering the whole hatching period of *B. vicetinus* (Cavaletto *et al.*, 2015). At each trap check, all newly emerged nymphs were collected and identified according to Massa *et al.* (2012), counted and sexed (except for the first year). In each year, all the traps were removed from the field at the beginning of May.

To study the effect of temperature on hatching phenology, the mean daily temperatures recorded in each year by a weather station representative for the Euganean Hills (45°18'17"N, 11°41'52"E – Cinto Euganeo municipality, <http://www.arpa.veneto.it>), were related to data from the field trials. Moreover, elevation and exposure (northern or southern) of each monitored site were also recorded (table 1) to assess the influence of these factors on the hatching phenology.

Laboratory trials

In order to test the effect of temperature on egg-hatching phenology of *B. vicetinus*, a manipulative experiment was

Table 1. Sampling sites per year of study. Black dots indicate the number of emergence traps used every year and their position in the forest.

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

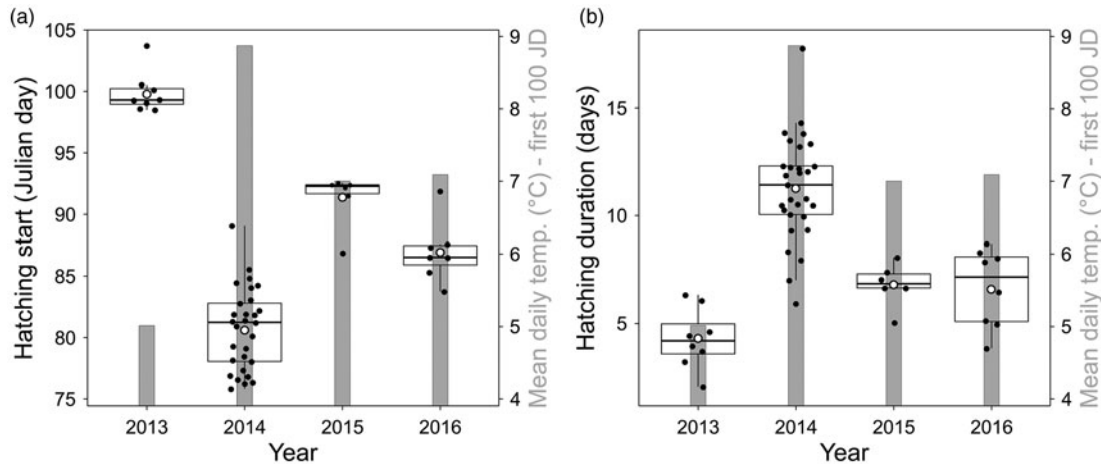


Fig. 1. (a) Hatching start (Julian days); (b) hatching duration (days) in the four sampling years. The thick lines of the box plots represent the medians, the black dots indicate every single data and the white dots show the means. The grey vertical bars represent the mean daily temperature (°C) recorded in the first 100 Julian days.

performed. Eggs were collected in 2014 at a single site of the Euganean Hills (45°17'12"N; 11°40'51"E) known to have experienced severe outbreaks in the previous years. Egg collection was carried out on the 16th–17th of January by sampling soil in an area of about 5 m² free from branches, roots, stones and coarse litter. The soil sample (about 5 cm deep) was collected 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 isolate the eggs from the soil. Eggs were washed carefully from the remaining soil with running water. Then, eggs were collected using a soft forceps and a magnifying glass to detect only the eggs in final diapause when the embryo fills the shell, which were recognized according to their pre-hatching turgescence *sensu* Warne (1972). These eggs were divided into 25 groups of seven eggs each, for a total of 175 eggs. Every group was put in a

50 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). The eggs were buried 1 cm deep in the soil. All the beakers were kept outdoor in a sheltered shaded area until the beginning of the experimental laboratory trial. On the 3rd of February, five beakers were placed in each of five climatic chambers at 6, 10, 13, 16 and 23°C constant temperature, respectively. The soil in the beakers was sprayed with distilled water every 2–3 days to avoid dehydration. All the beakers were then inspected daily to detect the number of newly emerged nymphs. The observation ended 30 days after the last recorded emergence.

Statistical analysis

For both field and laboratory trials, the nymph emergence temporal trend was described as fitting a symmetrical sigmoid

function generated by MyCurveFit software (<https://mycurvefit.com/>). After estimating the parameters, we derived the Julian day (the continuous count of days since 1st of January – Julian Day 1) of the different proportions of emerged individuals (10, 50 and 90%). This analysis was carried out for each trap (in field trials) and for each group of eggs (in laboratory trials) both with total specimens and with the two sexes separately.

Field trials

The effect of trap position (forest edge or inner forest) and sampling year (2013–2016) on (I) the eggs hatching start (HS) (Julian day corresponding to the cumulated 10% of hatching) and on (II) the hatching duration (HD) (number of days elapsed from 10 to 90% of the hatching), were tested using general linear-mixed models (GLMMs). In both models, trap position and sampling year were included as fixed factors while the site (site ID) and, in each site, the replication number were included as random factors to account for spatial dependence in the sampling design.

Differences in male and female hatching phenology over three sampling years (2014–2016) were annually tested by different GLMMs for three cumulated hatching levels. The Julian day corresponding to the cumulated 10% (HS), 50 and 90% of hatching respectively was used as response variable while, sex and year, were used as fixed factors. Site ID was included as a random factor to account for spatial dependence in the sampling design.

The effect of elevation and exposure on the HS was evaluated by a GLMM considering elevation and exposure as fixed effects while the sampling year was included as a random factor to account for time dependence in the sampling design. Emergence traps catching less than four individuals per year were excluded because unsuitable for all the statistical analyses.

Laboratory trials

The effect of different temperatures on the number of days required to reach 50% of the total hatching was tested by one-way ANOVA. The same analysis was performed to evaluate also the effect of temperature on the HD and on amount of daily mean temperature required by eggs for the hatching at the same cumulated percentage (thermal sum) using the arbitrary threshold base temperature of 0°C. 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, main effect and their interactions were evaluated, when the latter were not significant the models were refitted without interactions. Assumptions of linear models were tested by inspecting diagnostic plots of model residuals. All analyses were run in R (R Core Team, 2016) using the nlme package (Pinheiro *et al.*, 2017) applying lme function.

Results

Field trials

In the four years (2013–2016), hatching of *B. vicetinus* always occurred between March and April. However, the HS (defined as the time needed to reach 10% of hatching) was significantly different among the four years (GLMM, $F = 118.05$,

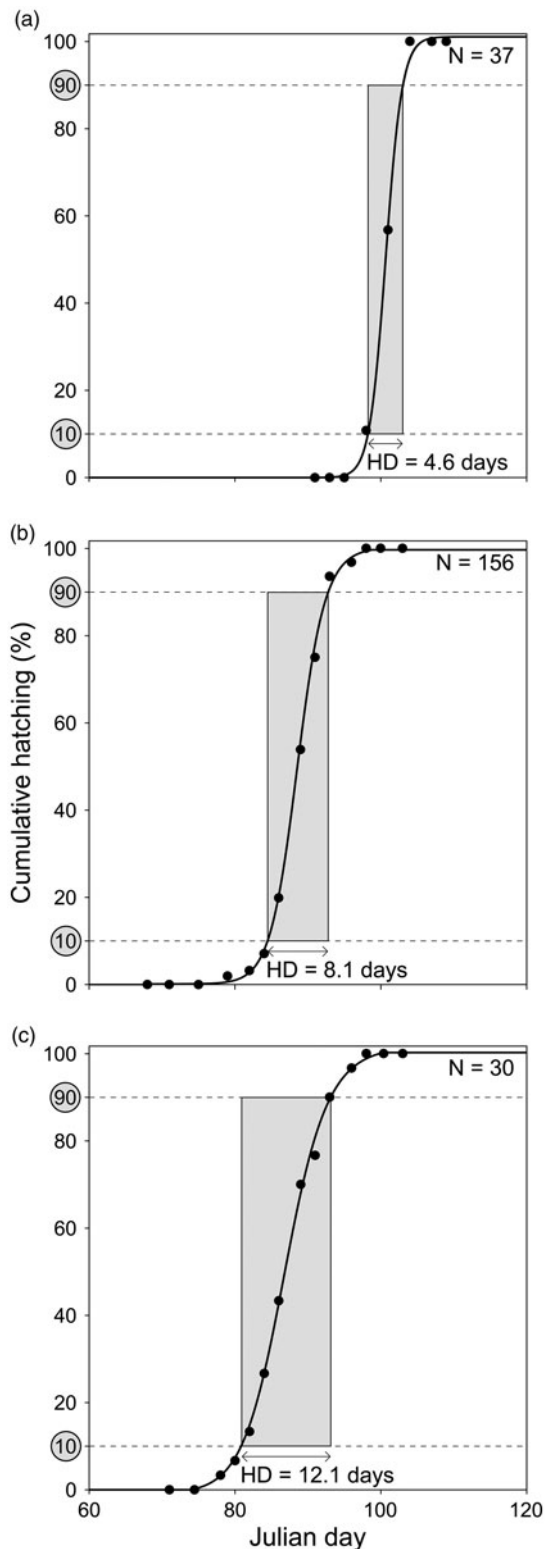


Fig. 2. Examples of sigmoid curves modelled on the hatching data for a short (a), medium (b) and long (c) hatching duration in three different emergence traps. The dots indicate the cumulated hatching percentage at each sampling time. The width of the grey columns corresponds to the hatching duration.

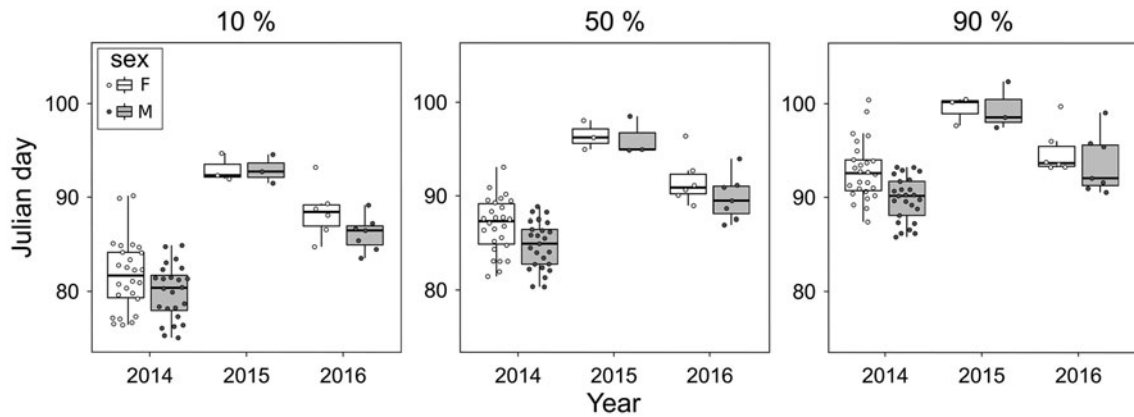


Fig. 3. Mean time (in Julian days) needed to reach the 10, 50 and 90% of hatching in the three sampling years by the two sexes. The dots indicate every single data and the thick lines of the box plots represent the medians.

d.f. = 3, 24, $P < 0.001$), ranging from (mean \pm standard error) 80.70 ± 0.62 (in 2014) to 99.89 ± 0.60 (in 2013) Julian days (fig. 1a).

The mean HD (defined as the mean time elapsed from 10 to 90% of the hatching) of all the sampling years lasted 8.94 ± 0.49 days, although with differences among years (GLMM, $F = 27.73$, d.f. = 3, 26, $P < 0.001$) ranging from 4.30 ± 0.50 days (in 2013) to 11.28 ± 0.45 days (in 2014) (figs 1b and 2). Comparing HS and HD with the mean air temperatures (January to April), we observed that in cool years hatching occurred later in the season and lasted for a shorter time, while in warm years hatching took place earlier and lasted longer (fig. 1a, b).

Moreover, neither HS nor HD 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, $P = 0.539$, for HS and HD respectively).

In the three sampling years (2014–2016) the GLMMs always showed a significant earlier male hatching of 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, $P < 0.001$), at the cumulated hatching percentages (10, 50 and 90% respectively), with no significant interaction between sex and year (fig. 3).

The GLMM indicated a significant effect of both elevation and exposure on the HS. Southern exposures, as well as lower elevation sites, showed an early hatching compared to northern exposures and higher elevation sites. Furthermore, a significant interaction between elevation and exposure was found (table 2), with the elevation having a greater effect on HS in southern exposure sites than in northern exposure ones (fig. 4).

Laboratory trials

A total of 147 nymphs (70 males and 77 females) emerged from the 175 tested eggs (84%). The proportions of hatching eggs exposed at 6, 10, 13, 16 and 23°C were 86, 89, 94, 86 and 66% respectively. We found a trend for a lower hatching percentage at 23°C compared to lower temperatures (ANOVA, $F = 2.47$, d.f. = 4, 20, $P = 0.08$). The first hatching was recorded on the 8th day of treatment (11th of February) in the warmest plot while the last one occurred in the coolest plot on the 80th day of treatment (24th of April) (fig. 5).

Table 2. Results from the mixed model testing the effect of elevation and exposure on the hatching start – HS (reaching 10% of hatching).

Factor	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 \times exposure	1, 37	7.62	<0.01

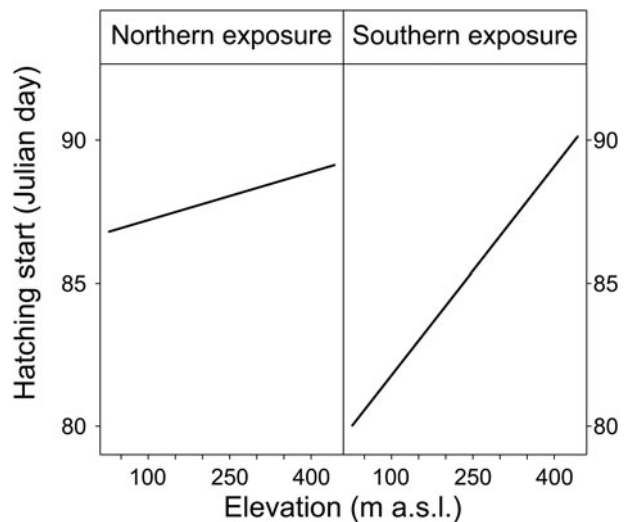


Fig. 4. Interaction between elevation and exposure on the hatching start.

Temperature produced a significant effect also on the number of days required to reach 50% of the total hatching (ANOVA, $F = 97.56$, d.f. = 4, 20, $P < 0.001$), with the earlier hatchings recorded at warmer temperatures. Significant differences occurred between all the tested temperatures, except for 16 vs. 23°C (Tukey HSD test, $P < 0.05$) (fig. 6a). Also HD varied significantly between temperatures (ANOVA, $F = 6.47$, d.f. = 4,

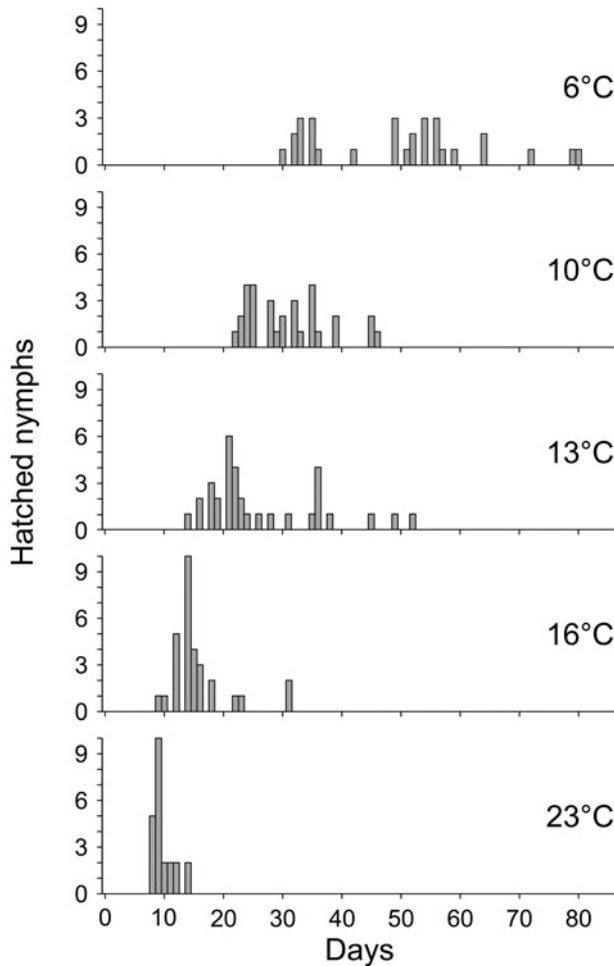


Fig. 5. Hatched nymphs at different temperatures. The number of days corresponds to the time since the beginning of the exposure at each temperature.

20, $P < 0.002$) with shorter duration at warmer conditions (Tukey HSD test, $P < 0.05$) (fig. 6b).

Significant differences in the amount of the mean daily temperature (thermal sum), required by eggs to reach 50% of total hatching, occurred among the different tested temperatures (ANOVA, $F = 10.27$, d.f. = 4, 20, $P < 0.001$). In particular, 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. 6c).

Discussion

The present work reports 4-year data of hatching phenology occurring in outbreak populations of the bush-cricket *B. vicetinus* and the effect of temperature on egg hatching in the laboratory. Overall, our field results show that hatching phenology significantly differed among years, in relation to

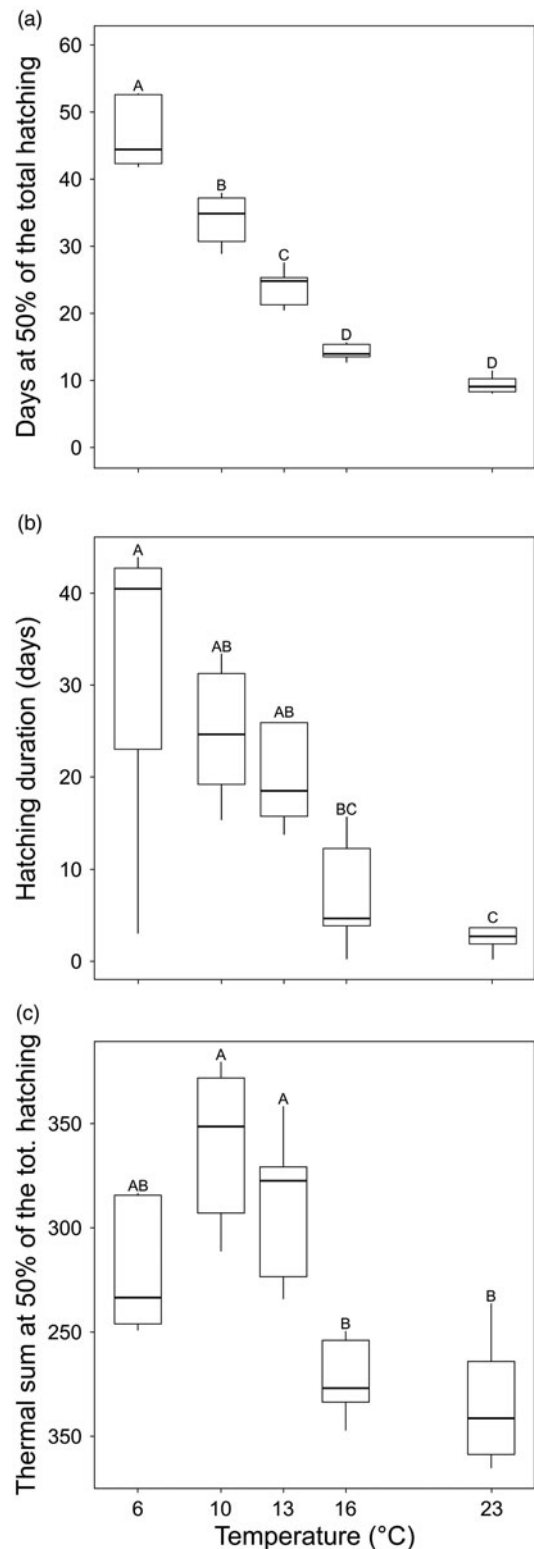


Fig. 6. (a) Number of days required to reach 50% of hatching, (b) hatching duration (days) and (c) sum of mean daily temperatures required (thermal sum) to reach 50% of the total hatching, at the five different tested temperatures. The horizontal lines of the box plots represent the mean.

both the beginning of hatching (HS) and the number of days needed to complete it (HD). During the four years considered, hatching usually occurred early in the spring, between the end of March and beginning of April. Interestingly, warm years with early hatching (i.e. 2014) had a longer duration, while cold years had an opposite pattern.

Preliminary studies report that the embryonic development of *B. vicetinus* can last for more than one year due to a prolonged initial diapause (Magello *et al.*, 2003). For this reason, it is noteworthy that all our field and laboratory observations regarded a group of eggs with an advanced embryonic development being in final diapause.

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 almost fully-developed (stage 23/24) (Ingrisch, 1986a). In this respect, our data show that prolonged low temperatures delay the HS and probably lead to its synchronization, shortening the HD as observed in 2013 and 2015. A short HD allows the first instar nymphs to emerge gregariously and to quickly find the food resources required for their development. Indeed, the newly hatched nymphs climb bushes and trees feeding on the young leaves, which are available only for a short period in early spring. The effect of chilling on the termination of final diapause was investigated also in the tettigoniid *Eobiana engelhardti* (Uvarov), pointing out that hatching became clearly simultaneous by prolonging the chilling period of the eggs at 3°C before to be transferred to 15°C (Higaki & Ando, 2002). A reduction of HD in relation to longer exposure to colder periods during diapause is known in many winter diapausing insects of temperate regions (e.g. Collier & Finch, 1983; Wipking, 1995).

Regardless of hatching phenology, males always hatched earlier than females. This behaviour was reported in many insect species as an adaptive response of females to minimize the time-lag between hatching and mating (Wedell, 1992). In many Orthoptera species, sexual differences in the length of post-embryonic development could be a strategy whereby males have sufficient time to attain sexual maturity and are ready to mate with females when they become fertile (Lopez *et al.*, 2007).

Both elevation and exposure played a significant role in the hatching phenology of *B. vicetinus* suggesting that the species responds to local micro-environmental climatic conditions. Sites with southern exposure and low elevations led to earlier hatching, while in the northern exposure sites elevation did not affect the HS. Elevation and exposure, influencing temperature, hence play an important role in pre- or postponing the HS. 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. On the northern slopes, the effect of elevation is mitigated by the cooler conditions due to the reduced solar radiation; the opposite occurs along the southern slopes where daily and seasonal thermal variation is more pronounced (Susmel & Famiglietti, 1968).

Results obtained from laboratory tests support the hypothesis of a strong influence of temperature on the *B. vicetinus* hatching phenology. A mean of 84% of the total eggs hatched during the test (at least 65% at each temperature), suggesting the ability of the species to develop in the post-final diapause in a wide range of thermal conditions. Indeed, *B. vicetinus* was able to hatch from 6 to 23°C although the highest emergence was recorded within a range from 6 to 16°C. At warmer temperatures (up to 16°C) the 50% of hatching took place

progressively earlier, but at the highest tested temperature (23°C), did not further accelerate development. Similarly, HD significantly increased with decreasing temperatures. The persistence of low temperatures during the whole hatching period could be the cause of the extension over time. Though the lowest temperature (6°C) did not prevent the development in the post-final diapause, this led to an extended hatching period, seven times longer than that observed at the highest temperature. Low thermal thresholds are common in many tettigoniid species hatching early in spring (Ingrisch, 1985; Hartley, 1990). Observations on other tettigoniid species, such as *Pholidoptera griseoptera* (De Geer) and *E. engelhardti*, report respectively 11 and 9°C as the lowest temperature thresholds required for egg development (Hartley & Warne, 1973; Higaki & Ando, 2002).

In addition, our laboratory trials show that the different tested temperatures induced significantly different thermal sums of hatching. In particular, when exposed to cool temperatures in the final part of the development, eggs usually hatched at significantly higher thermal sums. Nevertheless, eggs exposed to the lowest temperature (6°C) did not require a thermal sum different than eggs exposed to all the other tested temperatures. These results suggest that the development in the post-final diapause may also occur at low temperatures, even if requiring a longer time. In case of low temperatures, egg hatching would occur even at lower thermal thresholds, however compensated by a prolonged period of embryo maturation inducing a hatching delay.

Knowledge on hatching phenology may be a useful tool for setting up monitoring and control of the nymphs before they spread extensively. Our results allow to draw the first description of the emergence pattern of *B. vicetinus*. Better understanding of this species' life history traits and behaviour will help to improve the ability to forecast the occurrence of outbreaks and the effectiveness of control strategies. Besides temperature, other factors such as moisture, nutrition and photoperiod experienced maternally may influence hatching phenology of *B. vicetinus*, as reported in other tettigoniid species (Ingrisch, 1984). Future research focused on the thermal conditions experienced by the eggs before winter may highlight their effects on the hatching phenology and, even more, on the possibility of a multi-year embryo development. In fact, *B. vicetinus* lays their eggs very early compared to other Tettigoniids of temperate climates (Ingrisch, 1986a) and the conditions experienced in this long period prior to overwintering may strongly influence diapause. These environmental parameters would be useful to improve both the knowledge about biology and ecology of the species and thus the management of outbreaks.

Acknowledgments

We are grateful to Isabel Martínez-Sañudo for her support in the writing and to Filippo Giannone for help during field work, and to Fabio Trivellato and Stefano Marangoni for assistance in field and laboratory trials. We are grateful to the farmers who provided sites for sampling and field work. The present study was partially supported by ex-60% project [Mazzon 60A08-0839/14] of the University of Padua, by a grant under [Prin GEISCA 2010-2011] (MIUR) and by a scholarship under a partnership between University of Padua and ERSA.

References

- Bailey, W.J. & Rentz, D.C.F. (1990) *The Tettigoniidae: Biology, Systematics and Evolution*. Hong Kong, Springer.

- Behrens, W., Hoffmann, K.H., Kempa, S., Gessler, S. & Merkel-Wallner, G. (1983) Effect of diurnal photoperiods and quickly oscillating temperatures on the development and reproduction of the cricket, *Gryllus bimaculatus*. *Oecologia* **59**, 279–287.
- Buzzetti, F.M., Hochkirch, A., Massa, B., Fontana, P., Kleukers, R. & Odé, B. (2016) *Barbitistes vicetinus*. The IUCN Red List of Threatened Species 2016: e.T47697291A47715932.
- Cavaletto, G., Giannone, F. & Mazzon, L. (2015) Forti presenze nei Colli Euganei di *Barbitistes vicetinus*. *L'Informatore Agrario* **22**, 50–54.
- Cavaletto, G., Faccoli, M., Marini, L., Martinez-Sañudo, I. & Mazzon, L. (2018) Oviposition site preference of *Barbitistes vicetinus* (Orthoptera, Tettigoniidae) during outbreaks. *Agricultural and Forest Entomology* **20**, 414–419.
- Cavaletto, G., Mazzon, L., Faccoli, M. & Marini, L. (2019) Habitat loss and alien tree invasion reduce defoliation intensity of an eruptive forest pest. *Forest Ecology and Management* **433**, 497–503.
- Collier, R.H. & Finch, S. (1983) Effects of intensity and duration of low temperatures in regulating diapause development of the cabbage root fly (*Delia radicum*). *Entomologia Experimentalis et Applicata* **34**, 193–200.
- Denlinger, D.L. (2002) Regulation of diapause. *Annual Review of Entomology* **47**, 93–122.
- Del Favero, R. (2001) PBPRCE: Progetto Boschi del Parco Regionale dei Colli Euganei. Parco Regionale dei Colli Euganei, Este.
- Galvagni, A. & Fontana, P. (1993) *Barbitistes vicetinus* sp. n., dell'Italia Nord-orientale (Insecta, Orthoptera, Tettigoniidae). *Atti dell'Accademia Roveretana degli Agiati* **VII, II, B**, 199–206.
- Galvagni, A. & Fontana, P. (1999) Morfologia, biologia e bioacustica di *Barbitistes vicetinus* Galvagni and Fontana, 1993. *Atti dell'Accademia Roveretana degli Agiati* **VII, IX, B**, 37–56.
- Hartley, J.C. (1990) Egg biology of the Tettigoniidae. pp. 41–70 in Bailey, W.J. and Rentz, D.C.F. (Eds) *The Tettigoniidae: Biology, Systematics, and Evolution*. Hong Kong, Springer.
- Hartley, J.C. & Warne, A.C. (1973) The distribution of *Pholidoptera griseoaptera* (Degeer) (Orthoptera: Tettigoniidae) in England and Wales related to accumulated temperatures. *Journal of Animal Ecology* **42**, 531–537.
- Higaki, M. & Ando, Y. (2002) The effect of temperature on embryonic development and local adaptation in the life cycle of *Eobiana engelhardti* subtropica Bey-Bienko (Orthoptera: Tettigoniidae). *Applied Entomology and Zoology* **37**, 625–636.
- Ingrisch, S. (1984) The influence of environmental factors on dormancy and duration of egg development in *Metriopectera roeseli* (Orthoptera: Tettigoniidae). *Oecologia* **61**, 254–258.
- Ingrisch, S. (1985) Effect of hibernation length on termination of diapause in European Tettigoniidae (Insecta, Orthoptera). *Oecologia* **65**, 376–381.
- Ingrisch, S. (1986a) The plurennial life cycles of the European Tettigoniidae (Insecta: Orthoptera) – 1. The effect of temperature on embryonic development and hatching. *Oecologia* **70**, 606–616.
- Ingrisch, S. (1986b) The plurennial life cycles of the European Tettigoniidae (Insecta: Orthoptera) – 2. The effect of photoperiod on the induction of an initial diapause. *Oecologia* **70**, 617–623.
- Kaltenrieder, P., Belis, C.A., Hofstetter, S., Ammann, B., Ravazzi, C. & Tinner, W. (2009) Environmental and climatic conditions at a potential Glacial refugial site of tree species near the Southern Alpine glaciers. New insights from multiproxy sedimentary studies at Lago della Costa (Euganean Hills, Northeastern Italy). *Quaternary Science Reviews* **28**, 2647–2662.
- López, H., Nogales, M., Morales, E. & Oromí, P. (2007) Habitat use and phenology of the large insular endemic grasshopper *Acrostira euphorbiae* (Orthoptera: Pamphagidae). *Bulletin of Entomological Research* **97**, 117–127.
- Magello, E., Cogo, A. & Fontana, P. (2003) Nuove osservazioni sulla biologia di *Barbitistes vicetinus* Galvagni & Fontana, 1993, specie criptica, abitatrice delle chiome di boschi decidui (Italia Nord-orientale) (Insecta, Orthoptera, Ensifera, Phaneropteridae). *Bollettino del Museo Civico di Storia Naturale di Venezia* **54**, 45–55.
- Massa, B., Fontana, P., Buzzetti, F.M., Kleukers, R. & Odé, B. (2012) Fauna d'Italia – Vol. XLVIII: Orthoptera. Calderini, Bologna.
- Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. & R Core Team (2017) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131, Available online at <https://CRAN.R-project.org/package=nlme>.
- R Core Team (2016) R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing.
- Sunderland, K.D., De Snoo, G.R., Dinter, A., Hance, T., Helenius, J., Jepson, P., Kromp, B., Lys, J.A., Samu, F., Sotherton, N.W., Toft, S. & Ulber, B. (1995) Density estimation for invertebrate predators in agroecosystems. In *Arthropod Natural Enemies in Arable Land I: Density, Spatial Heterogeneity and Dispersal*. Toft, S. & Riedel, W. (eds). Acta Jutlandica. Aarhus University Press, Denmark. **70, 2**, 133–162.
- Susmel, L. & Famiglietti, A. (1968) Condizioni ecologiche ed attitudini culturali dei Colli Euganei. *Annali del Centro di Economia Montana delle Venezie*. Cedam, Padova.
- Tauber, M.J. & Tauber, C.A. (1976) Insect seasonality: diapause maintenance, termination, and postdiapause development. *Annual Review of Entomology* **21**, 81–107.
- Warne, A.C. (1972) Embryonic development and the systematics of the tettigoniidae (Orthoptera: Saltatoria). *International Journal of Insect Morphology and Embryology* **1**, 267–287.
- Wedell, N. (1992) Protandry and mate assessment in the wartbiter *Decticus verrucivorus* (Orthoptera: Tettigoniidae). *Behavioral Ecology and Sociobiology* **31**, 301–308.
- Wipking, W. (1995) Influences of daylength and temperature on the period of diapause and its ending process in dormant larvae of burnet moths (Lepidoptera, Zygaenidae). *Oecologia* **102**, 202–210.