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# Dispersal ability of *Barbitistes vicetinus* Galvagni & Fontana, 1993 (Orthoptera, Tettigoniidae) among different habitats including a morphometric analyses of the instars

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## Abstract

Habitats surrounding crop fields are often considered important for regulating agricultural pests and supporting their natural enemies; however, the presence of natural habitats can occasionally serve as reservoirs for outbreaks of species confined to these environments. *Barbitistes vicetinus*, an endemic bush-cricket native to northeast Italy, has become a major concern for agriculture and forestry due to the severe damage to broadleaf trees and crops such as vineyards and olive groves. During outbreaks, this species can move from natural habitats to adjacent crops, thus, studying its dispersal abilities is a key factor for understanding species habitat use and for developing control measures. In two experimental capture-mark-resight studies, we investigated movements and dispersal patterns of this flightless bush-cricket. In the first experiment, 100 *B. vicetinus* adults were simultaneously released in a vineyard and its adjacent natural habitat (hedgerow) and then sampled over an 11-day period. In the second experiment, 100 *B. vicetinus* individuals were released and subsequently sampled 24 h later, over the course of nine consecutive dates, covering the period from the nymph stage to adulthood. Moreover, to clarify the number of juvenile stages, a morphometric analysis was performed on 1347 individuals from the hatching period till the end of the adult stage. Similar movement rates were found between the two habitats, suggesting a comparable behavior to persist within them. However, movement rates were slightly higher within vineyards and changed depending on developmental stage. We found that this species had six nymphal instars and the adult stage. It was concluded that the higher dispersal ability exhibited by *B. vicetinus* during outbreaks allows individuals to colonize neighboring crops from adjacent habitats, thus management of the reservoir habitats should be considered to avoid spillover of individuals.

**Keywords:** Movement pattern, insect dispersal, *Ensifera*, vineyard, bush-cricket

## 1. Introduction

In the last decades, changes in land use across Mediterranean landscapes have interested more frequently marginal and peripheral rural areas. Natural and semi-natural habitats are often replaced by perennial crops such as vineyards and olive groves, forming mixed agro-ecosystems where agricultural fields are interspersed with forest patches and urban elements. While the presence of non-crop vegetation is often considered valuable to contain agricultural pests, promoting

the activity of natural enemies and other beneficial invertebrates (Thomson & Hoffmann 2009), the presence of natural habitats such as forests and hedgerows can occasionally be the reservoir of outbreaks of species relegated to these habitats (Jeger 1999; Lieutier et al. 2004; Simon et al. 2010).

Detailed knowledge about the spatial activities and the dispersal patterns of animals is very important for many ecological questions. Furthermore, movement parameters provide a basis for many

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behavioral and ecological interpretations (Samietz & Berger 1997). Movement and dispersal from natural and semi-natural habitats allow pest species to colonize agricultural fields, potentially causing economic losses. Therefore, comprehending the movement patterns of pests is crucial for effective species management and appropriate landscape planning strategies aimed at mitigating spillover from reservoir habitats (Mazzi & Dorn 2012). Dispersal abilities are affected by several habitat and landscape characteristics as habitat quality, size, shape, fragmentation (Kindvall 1999; Leisnham & Jamieson 2002; Diekötter et al. 2007) and could vary between and within species. Species-specific dispersal abilities could be influenced by morphological and behavioral differences (Samways 1989) as well as variations in the movement of developmental stages and between sexes (Diekötter et al. 2005). However, the majority of studies on the movement and dispersal of agricultural insect pests focused on winged species, while less attention has been paid to flightless species inhabiting forest habitats.

The bush cricket *Barbitistes vicetinus* Galvagni and Fontana (1993) is endemic to northeastern Italy, with its distribution restricted to small, isolated hilly regions (Galvagni & Fontana 1993, 1999; Martínez-Sañudo et al. 2021). The first recorded outbreak occurred in the spring of 2008 in the Euganean Hills, with subsequent infestations affecting multiple areas. These outbreaks caused significant damage to forests and adjacent crops and posed a nuisance to nearby residents (Cavaletto et al. 2015). *B. vicetinus* has since become a significant threat to agriculture and forestry, particularly affecting vineyards and olive groves located near its natural habitat. The species is known to cause severe defoliation, with damage rates reaching up to 80% in several forest tree species (Cavaletto et al. 2019). Forests and hedgerows provide the primary habitats for oviposition and feeding, although during outbreaks, *B. vicetinus* can spread beyond its native environment, invading neighboring areas (Cavaletto et al. 2018). The species is polyphagous, feeding predominantly on plants such as *Rubus* L. spp., *Ostrya carpinifolia* Scop., *Fraxinus ornus* L., and *Castanea sativa* Mill. Its reproductive cycle includes a facultative diapause (Magello et al. 2003; Ortis et al. 2022); eggs are laid in the soil and may hatch after one summer from oviposition depending on summer temperatures experienced by the embryo or can remain in the ground for several years before resuming development (Cavaletto et al. 2020; Ortis et al. 2020, 2023). Egg hatching begins in late March, with adults appearing by the end of May and reproducing until early July, after which

the population dies off. The species is flightless and tends to ascend to the upper canopy, where it primarily consumes leaves, flowers, and fruits. During outbreaks, the appearance of a melanic form has been observed in the population (Cavaletto et al. 2019).

This species represents a valuable model for studying dispersal dynamics in flightless bush crickets during outbreaks. Unlike other species studied to date (e.g. Kindvall 1999; Berggren et al. 2001), *B. vicetinus* is a well-established species capable of exploiting both natural and agricultural environments for feeding and reproduction.

In this study, capture-mark-resight experiments were conducted to investigate the movement and dispersal abilities of *B. vicetinus*. We tested for differences in basic mobility, dispersal range, and developmental stages of *B. vicetinus* by comparing means of movement parameters after releasing individuals in both vineyard and hedgerow patches. In addition, morphometric analyses of the instars were carried out to elucidate phenology and development.

## 2. Materials and methods

### 2.1. Movement behavior depending on habitat type

To assess dispersal ability and movement patterns of *B. vicetinus*, we conducted two capture-mark-resight experiments in two sites in the Euganean Hills which included untreated vineyards within a forested and rural landscape. The Euganean Hills are characterized by about 100 volcanic hills covering an elliptical area of approximately 22,000 ha and with elevation ranges from sea level to 600 m a.s.l. The landscape is fragmented and consists of forest patches, interspersed with crop fields and rural elements (Kaltenrieder et al. 2009).

The experiment was performed in mid-June of 2012 and was designed to assess the movement pattern and dispersal behavior of *B. vicetinus* adults within a vineyard and its adjacent natural habitat, characteristic of this species. Individuals, manually collected from the vegetation, were kept in net boxes (containing small branches of host plants) in the study site (45°20'50.92"N 11°42'16.73"E) before and after marking. On the same day, bush-crickets were carefully marked by covering the back of the abdomen with acrylic marker (Uni Posca® M PC5M) and fitted with a single numbered tag on the upper part of the abdomen using a small drop of Loctite Super Attack adhesive® (Figure 1). This procedure has been employed in other mark-recapture studies without influencing insect



Figure 1. Example of marked adults of *B. vicetinus*.

behavior (Heller & Helversen 1990; Kindvall 1999; Brouwers & Newton 2010). In our previous studies, no significant differences in fitness were observed between marked individuals and unmarked ones, regardless of the type of marker used.

At the same site, 100 marked individuals were released in each of two distinct habitats within a 2 m<sup>2</sup> area surrounding the release point. The first habitat was a hedgerow dominated by *Rubus* spp., (40 m long, 5 m deep, max 2 m high), situated between a vineyard and a forest patch. The second habitat was a vineyard (1 ha) and the release point was in the middle of the crop. For four consecutive days after release, the habitats and the surrounding matrix were carefully searched for marked animals for approximately 10 h per day within a radius of 150 m around release points. Two additional observations were made during seventh and eleventh day after release. Positions of resighted individuals were recorded with the aid of measuring tapes and suitable landmarks.

## 2.2. Movement behavior depending on developmental stages

We conducted a capture-mark-resight experiment in a vineyard (45°17'12.1"N – 11°41'02.1"E) with the aim to understand movement rates of individuals of *B. vicetinus* during nymphal and adult stages. Individuals were collected and marked as described above and sex was recorded only during the appearance of the adult stage.

Starting from the beginning of May 2013, we randomly marked and released 100 individuals in one point in the middle of the crop. Then, individuals were sampled for at least 8 h after 24 h from

the release date. We repeated this process for nine consecutive dates about every 10 days from the first date of release.

## 2.3. Morphometric analysis

To clarify the number of molts and thus the number of juvenile stages in *B. vicetinus*, we performed morphometric analyses on 1347 individuals (730 females and 617 males) collected in one site in the Euganean Hills. Sampling started from the hatching period (end of March) till the first week of June 2014, for a total of 18 sampling dates. Because the first instars can molt quickly, individuals were sampled about every 3 days during the first 2 weeks after hatching. After this period, we sampled individuals approximately every 10 days. At least 30 individuals were sampled for each date. Individuals were collected using a sweep net or small containers and subsequently stored in a freezer until measurements were taken. Measurements were made using a Stainless Hardened digital caliper with an LCD display and precision of 0.02 mm (Maurer, model 93110). As previously reported by Holuša et al. (2006) for *Barbitistes constrictus* Brunner von Wattenwyl, the following measurements were recorded for all captured specimens: the width of head, the length of pronotum, the length of hind femur and the length of hind tibia (Figure 2). Additionally, the different color morph (green or melanic) was recorded for each individual.

## 2.4. Analysis

To evaluate the effects of habitat type on movement behavior and dispersal, we calculated the following linear movement parameters (cf. Diekötter et al. 2005, 2007): the daily movement (DM), the mean daily movement (MDM) defined as the mean of all straight line distances between consecutive days observed for an individual, and the dispersal range (DR) representing the maximum distance between any two observations of one individual. We calculated these parameters considering all movements recorded within and between habitats and only movements recorded within a single habitat.

To evaluate the effect of developmental stages on movement rates, we built and validated a linear model (LM). The response variable was the daily movement (DM), representing the straight-line distance covered by an individual within 24 h, and the fixed effect was the date (the following day after release). The response variable was square root transformed to improve the linearity of the model.

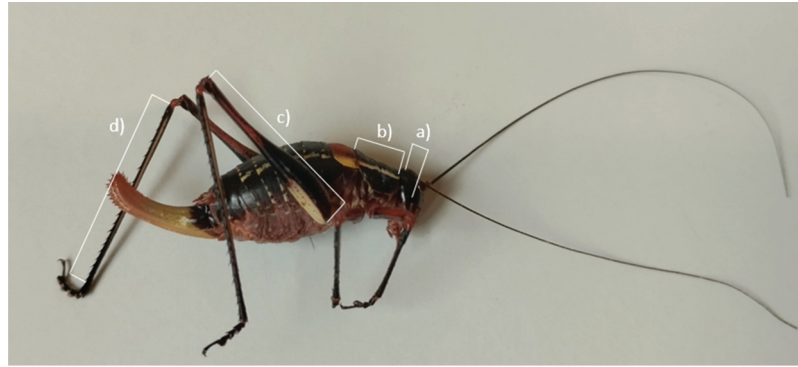


Figure 2. The measurements of *Barbitistes vicetinus*: (a) the width of head, (b) the length of pronotum, (c) the length of Hind femur, (d) the length of Hind tibia.

The mean DM for each sex was calculated considering all the resighted individuals from June 2013.

For both capture-mark-resight experiments, ANOVA tests were used for the comparison of the movement values. Point-to-point distances were calculated using AutoCAD 2008 Autodesk.

Morphometric data were analyzed using PCA method for both males and females, choosing PAM algorithm to find clusters. This technique was selected due to its ability to optimize the number of ideal clusters per dataset through silhouette width calculations. To determine the clustering effectiveness, we adopted previously described range conventions (Elleithy & Sobh 2013) of silhouette width: 0.7–1.0 indicates a strong grouping, 0.5–0.7 indicates a reasonable grouping, while 0.25–0.5 indicates a weak grouping.

All analyses were performed using R 4.2.1 (R Core Team 2020).

### 3. Results

#### 3.1. Movement behavior depending on habitat type

In both habitats, resight rates of individuals decreased over time. Individuals released in both habitats were found till the eleventh day: 73 individuals were resighted after release in the vineyard, while 52 individuals were resighted after release in the hedgerow. During the first four consecutive days, mean resight rates were higher in the vineyard (34%) compared to the hedgerow (19%). However, on the final two observation dates, resight rates declined in both habitats (<4%) (Figure 3).

Only four of the resighted individuals (0.05%) moved from the vineyard into forest, while 16 individuals (30%) moved from hedgerow to forest and 16 individuals (30%) moved from hedgerow to vineyard on at least one occasion (Figure 4).

The daily movement (DM) of individual *B. vicetinus* released in vineyard ranged from 0.49

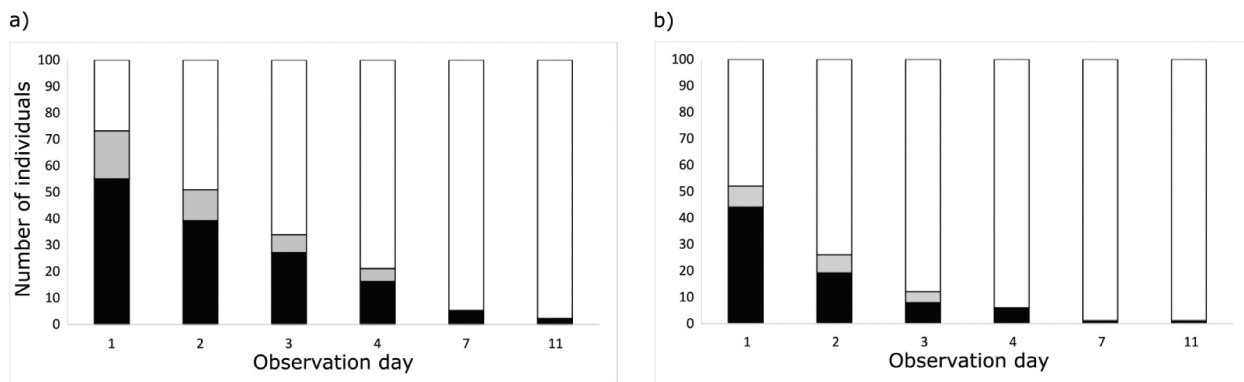


Figure 3. Resight rates (black), missing individuals found on following days (gray), and permanently lost individuals (white) of *Barbitistes vicetinus* on each observation day for release in (a) vineyard and (b) hedgerow. The number of released individuals was  $n = 100$  on both habitats.

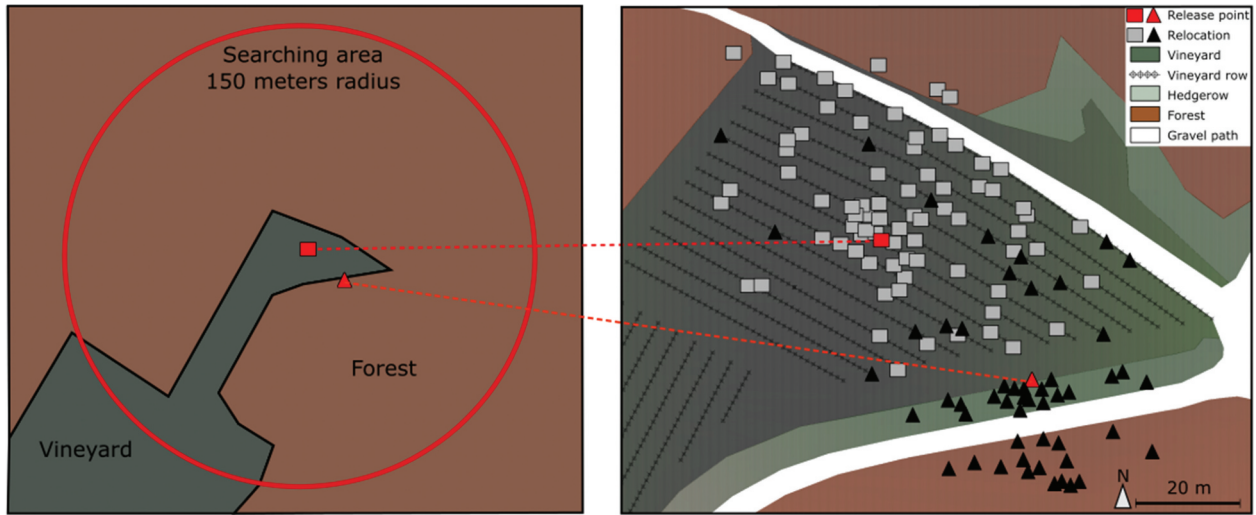


Figure 4. Landscape characteristics, observed movement and dispersal patterns of marked individuals of *Barbitistes vicetinus* in the two release habitats.

m to 24.34 m, the MDM ranged from a minimum of 1.2 m to a maximum of 14.93 m, while the DR ranged from a minimum of 1.87 m to a maximum of 33.70 m. The DM of individual *B. vicetinus* released in hedgerow ranged from 0.8 m to 31.74 m, the MDM ranged from a minimum of 1.49 m to a maximum of 17.83 m, while the DR ranged from a minimum of 1.40 m to a maximum of 54.74 m (Figure 5, Table I).

3.2. Movement behavior depending on developmental stages

From the 900 individuals released in the vineyard across the nine sampling dates, 574 were resighted (Figure 6). We observed limited movement rates during the initial sampling dates as the nymph stages

were undergoing development, while higher values were observed from June, after the appearance of the adult stage (df = 8, F = 19.66, p-value = 0.000). Adult movement rates decreased only on June 30th, likely due to unfavorable climatic conditions. During the first four dates, the daily movement (DM) ranged from 0 m to 8.54 m, while during the subsequent dates, the DM ranged from 0 m to 44.10 m. The male DM was  $6.9 \pm 1.2$  m (69 individuals), while female DM was  $3.9 \pm 0.3$  m (219 individuals).

3.3. Morphometric analysis

Using PCA of the data (730 total females and 617 total males), including all four morphometric traits, we found a clear separation of the instars (Figure 7, Table II). For both females and males, the first

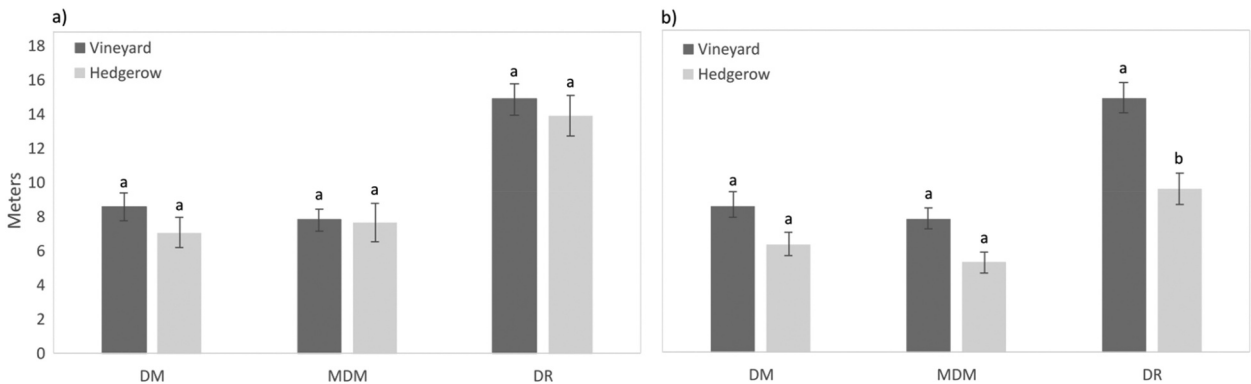


Figure 5. Means and SEs of the parameters DM, MDM and DR of *Barbitistes vicetinus* following release in a vineyard and an adjacent hedgerow: (a) movements within and between habitats; (b) movements within a single habitat. Different letters indicate significant differences according to Tukey's multiple comparison test ( $p < 0.05$ ).

Table I. ANOVA analysis of movement parameters of *Barbitistes vicetinus* following release in the two habitats.

	df	MS	F	P-value
a)				
DM	1	54.69	1.52	0.220
MDM	1	0.253	0.016	0.897
DR	1	45.99	0.576	0.448
b)				
DM	1	106.4	3.448	0.066
MDM	1	38.25	2.879	0.097
DR	1	683.3	13.13	0.000

a) Movements within and between habitats; b) Movements within a single habitat.

principal component accounted for 98% of the total variance, the second principal component accounted for about 1% of the total variance. The variables width of head and length of pronotum were correlated to each other, while the length of hind femur and hind tibia were correlated to each other (Figure S1). The PAM cluster analysis yielded seven instar categories.

After the initial sampling dates, corresponding to the earlier developmental stages, all individuals progressively shift to the melanic color (Figure S2).

#### 4. Discussion

This study provides the first insights into movement rates and behavior, offering valuable information for estimating the dispersal of *B. vicetinus* across both natural and agricultural habitats. Movement changed depending on developmental stage and habitat type, revealing high movement and dispersal abilities for a flightless insect.

We observed a general decrease of resight rates over time characteristic for capture- mark-resight experiments probably caused by an area-dilution effect or mortality factors often related to these types of experiments (Narisu & Schell 1999, Hein et al. 2003). Drops in resight rates could be due also to the peculiar behavior of this species, able to reach top of the elements as forest trees (Cavaletto et al. 2019), making detection difficult. Notably, despite thorough examination of the vegetation in both experiments, no individuals were found at ground level or in the grass.

Movement rates observed after releasing individuals in two adjacent patches suggest that habitat didn't influence patterns of movement of *B. vicetinus*. There were no significant differences between all parameters analyzed in the two

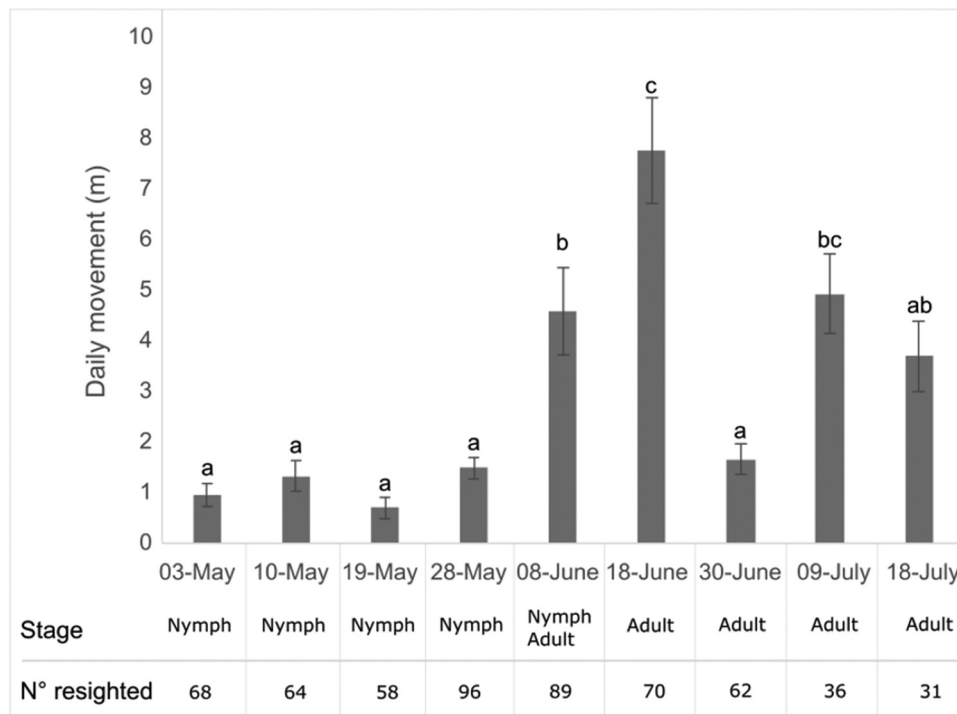


Figure 6. Observed movements (mean ± SE) in the vineyard of marked individuals of *Barbitistes vicetinus* during the nine release dates. Different letters indicate significant differences according to Tukey's multiple comparison test ( $p < 0.05$ ).

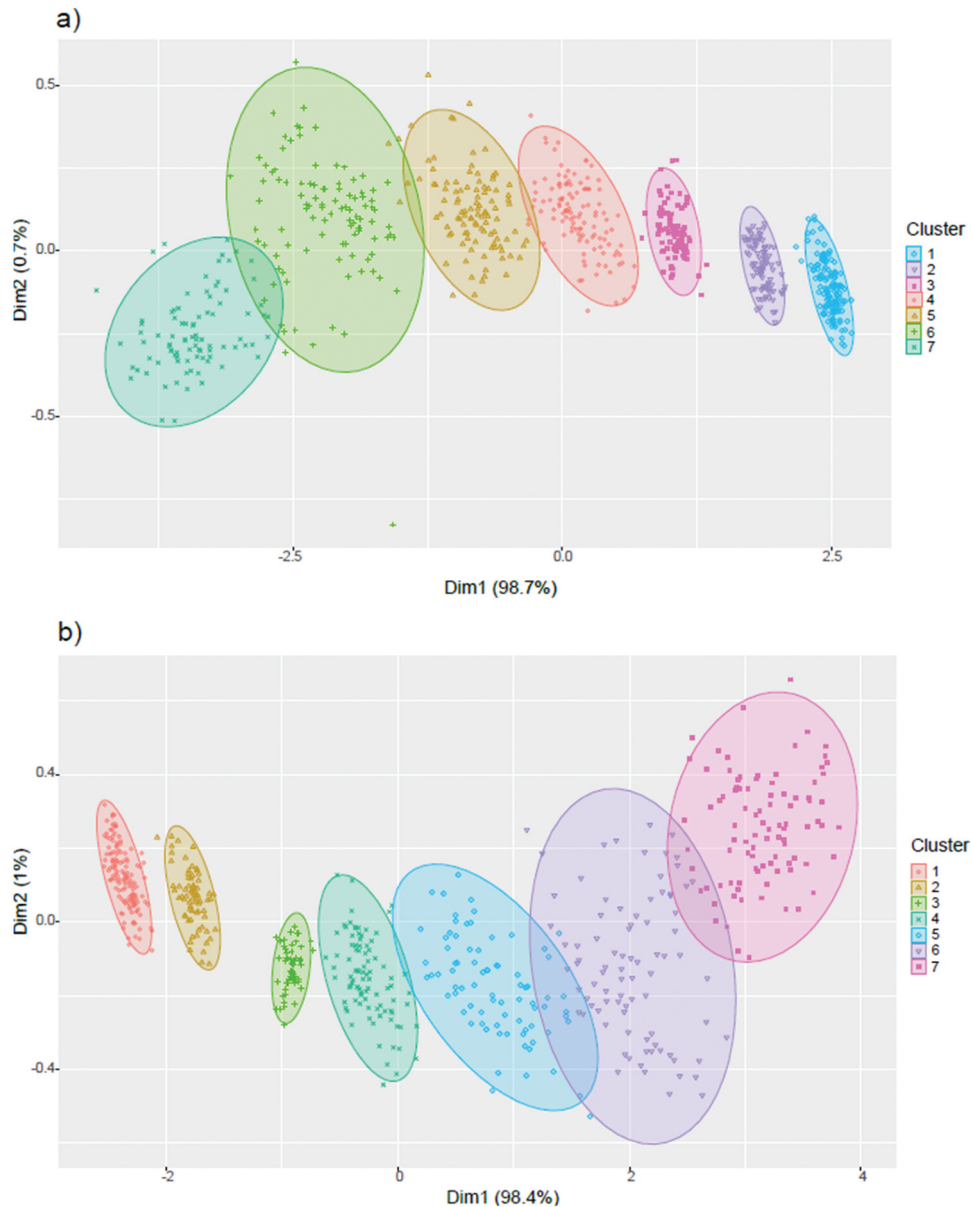


Figure 7. Partitioning around medoids (PAM) clustering of all morphometric data for: (a) females and (b) males. Ellipses enclose the seven groups.

habitats. Similar movement patterns suggest a comparable behavioral to persist within both habitats, which may offer suitable food sources for this bush-cricket. Even if our release experiment was conducted in one site, we think that the effect of habitat type could have only a minor effect on movement behavior of this species, contrary to what has been reported for other orthopteran species (Diekötter et al. 2007) that moved with different speed within different environments (Kindvall 1999). Typically, individuals tend to move through unprofitable habitats faster compared to suitable habitats, avoiding mortality risks caused by

starvation or predation (Kindvall 1999). Generally, vineyards are not suitable to support ensiferans (Ortis et al. 2021), instead *B. vicetinus*, due to its high polyphagia, can survive in different habitats without seeking new patches. Notably, one-third of the resighted individuals released in the hedgerow moved to vineyard while no individuals were detected inside the hedgerow after vineyard release, confirming that managed crops adjacent to forest edges represent suitable feeding habitats for *B. vicetinus* during outbreaks. This suggests that the species can persist in environments other than those suitable for reproduction, such as

Table II. Size and average silhouette width by PAM algorithm for morphometric data.

Cluster	Size		Av. Sil. width	
	a)	b)	a)	b)
1	133	140	0.78	0.77
2	93	71	0.78	0.74
3	93	58	0.78	0.76
4	116	90	0.63	0.65
5	104	79	0.65	0.53
6	103	86	0.52	0.49
7	88	93	0.67	0.62

a) Females and b) Males.

forests. However, even if comparable movement rates were detected between two habitats, movements of individuals within vineyard were still higher (Figure 5(b)), suggesting the preference of this bush-cricket to live in shrubby and tree habitats rather than in the open habitat of an agricultural crop.

The observed movement rates were slightly higher in comparison to other bush-crickets, probably due to the high population density reached during the outbreak phase. The mean of the MDMs for *B. vicetinus* exceeded the means of daily movement distances observed for the flightless bush-cricket *Pholidoptera griseoptera* (De Geer) (Diekötter et al. 2005, 2007) and the movement rates of *Bicolorana bicolor* (Philippi) (Kindvall 1999), while were similar to movements of the winged species *Platycleis albopunctata* (Goeze) in suitable habitat (Hein et al. 2003). However, we found a considerable variation in the movement behavior of individuals, with only a minority covering long distances, while some individuals were relatively sedentary or moved to other habitats. This skewed distribution of movement distances has been reported for other orthopteran species (Uvarov 1977; With 1994) and could reflect the typical behavior of this outbreak species to congregate in certain areas forming groups of numerous individuals, increasing movement rates particularly during the mating season, when both males and females climb down vegetation crossing roads and forest ground to find partners and oviposition sites. Our experiments confirmed this behavior, revealing lower movement rates among nymph stages compared to adult stage, which exhibited a tendency to cover more distance per day, as reported also for other orthopteran species (With 1994; Leisnham & Jamieson 2002).

Considering the morphometric analysis, we found that *B. vicetinus* has 6 nymphal instars and the adult stage. These results differed from a study on morphometric analyses on *Barbitistes*

*constrictus*, which reported five nymphal instars and the adult stage (Holuša et al. 2006). Probably, in this latter experiment, the morphometric analyses were carried out on few individuals due to the scarce density of the bush-cricket, thus some unclear clusters were not attributed to an additional instar. Additionally, six instars were reported also from past studies on different species of the genus *Barbitistes* (Kaňuch et al. 2014). Past studies on tettigoniid species phylogenetically close to the genus *Barbitistes* (Mugleston et al. 2018) reported a range of five to seven instars. However, a specific research has consistently identified six instars in various tettigoniid species (Cejchan 1977). Therefore, it is more plausible that the six instars we observed are the more accurate number.

## 5. Conclusion

The higher mobility rates and inter-habitat movement found in this study are related to the abnormal densities reached by *B. vicetinus* during outbreaks, which allowed a wider dispersal of the species in the surrounding habitats, a typical behavior of outbreak species (Lorch et al. 2005). Indeed, before the occurrence of the first outbreaks, this species was considered rare and relegated to forest habitats, thus it seems plausible that its movement was limited. In support of this claim, a genetic study on *B. vicetinus* in the same area (Martinez-Sañudo et al. 2021) reported a high geographical structuring among populations, indicating a limited gene flow and a lack of dispersion over distances greater than a few kilometers.

In conclusion, although *B. vicetinus* is a species relegated to natural habitats, it can temporarily exploit vineyards as feeding sites during outbreaks. The higher dispersal rates coupled to the higher polyphagia and the outbreak propensity suggest a high risk for crops adjacent to the natural habitats of this species. As shown in the present study, this species exhibits the capability to colonize neighboring crops from adjacent habitats without demonstrating a propensity to leave them. Management of reservoirs habitats such as hedgerows and forest patches should be considered to avoid spillover of individuals, for example, maintaining a wide grass cover at the edges of crops which do not represent a suitable habitat for this species.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Supplementary material

Supplemental data for this article can be accessed online at <https://doi.org/10.1080/24750263.2025.2497398>

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