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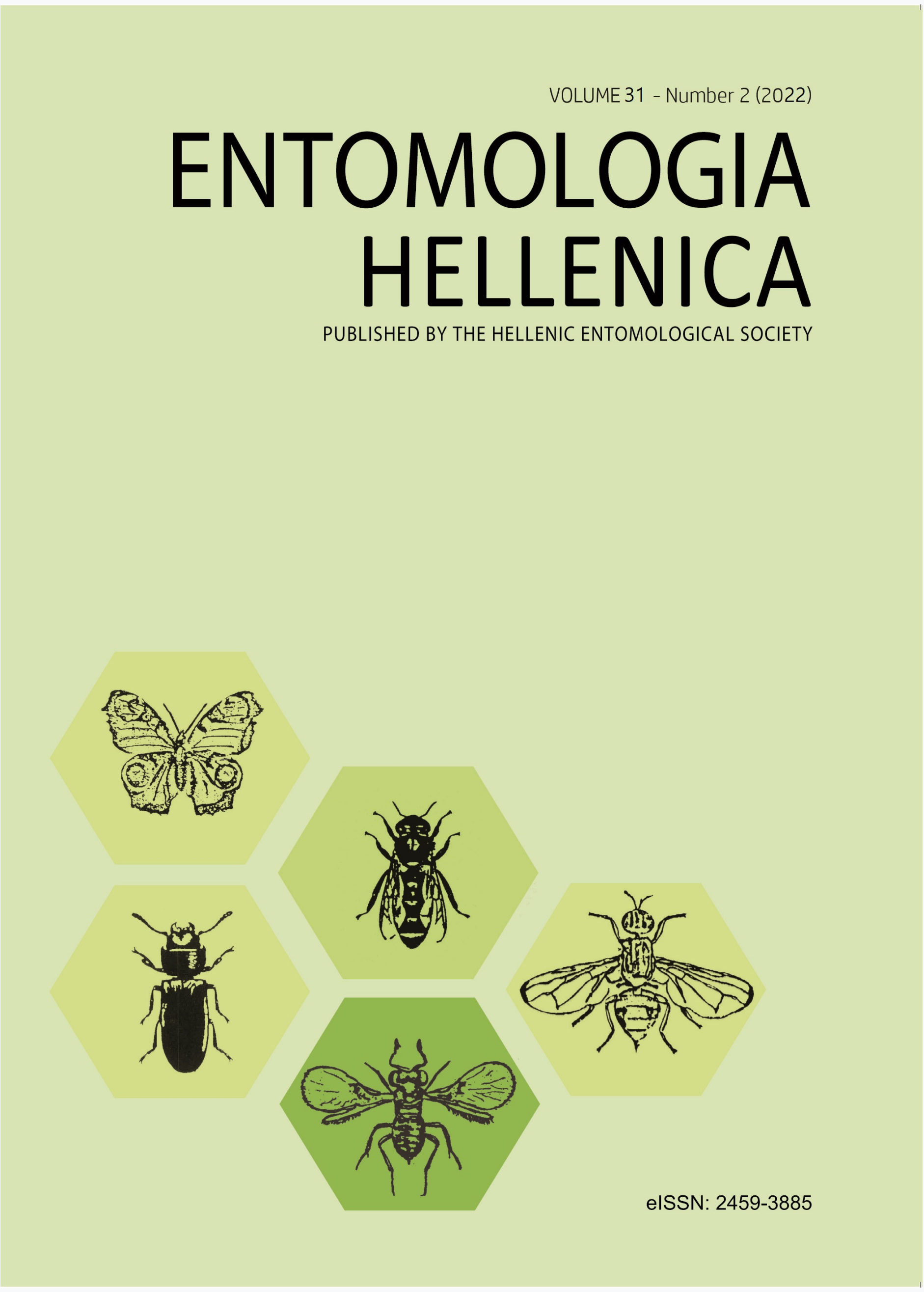

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Butterflies (Lepidoptera: Papilionoidea) of northwestern and western Crete (Greece) in early July, with the finding of new larval host plant for *Lampides boeticus*

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ABSTRACT

During the summer of 2021, the northwestern and western areas of the Greek island of Crete were surveyed to confirm certain observations of diurnal butterfly fauna of the island. Research was focused especially on the unsurveyed north-western parts of the island, as well as surveying the species that are extending their area of distribution (*Cacyreus marshalli* (Butler), *Lampides boeticus* (L.)). From 47 species reported from the island, 23 were observed. The surveys were all carried out during an especially hot and dry weather period (30-37 °C), between 30th of June and 6th of July. Additionally, a new larval host plant for *L. boeticus* was confirmed, as oviposition of females on *Campsis radicans* (Seem.) was observed on several occasions.

KEY WORDS: *Campsis radicans*; new localities; oviposition; northwestern peninsula.

Introduction

With an area of 8336 km^2, Crete is the fifth largest island in the Mediterranean Sea and the largest island of Greece (Sakellariou and Galanidou 2016). It is one of the most mountainous islands in Europe, having more than 50 mountain peaks exceeding 2000 m. The highest peak is located in Idi massif in the central part of the island and reaches an altitude of 2456 m (Agou et al. 2019; Kougioumoutzis et al. 2020). Due to the three mountain massifs, coupled with many lower semi-mountainous areas, Crete has a high fragmentation of land area, including numerous rivers and streams, further enriching its diverse geology (Agou et al. 2019).

Crete has a sub-humid Mediterranean climate with humid and relatively cold winters, and dry and warm summers (Morianou et al. 2021). The mean annual air temperature is around 20 °C, while frost temperatures are almost never registered at the lower parts (Bergmeier 1997). The highest mountain peaks, on the other hand, are covered with snow throughout the winter (Bretherton 1969). The coastal areas, where urban settlements and agriculture are concentrated, exhibit a typical Mediterranean climate, with transitions to semi-dessert (Agou et al. 2019; Kougioumoutzis et al. 2020). The coastal areas are the driest and the warmest, with 300-700 mm precipitation per year (Bergmeier 1997; Morianou et al. 2021), while the mountainous areas are the most humid, reaching 2000 mm of rainfall per year (Morianou et al. 2021). Most rainfall occurs from December to February, while the summer drought, which lasts up to 7 months, almost completely destroys the vegetation (Bergmeier 1997). The strong winds worsen the situation by desiccating

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certain flowering phrygana plants (Bergmeier and Matthäs 1996).

Crete is considered one of the richest hotspots in Europe in terms of endemic species of plants, which is mostly due to its unique geology, isolation and climate (Kougioumoutzis et al. 2020). The habitat types of Crete are also very diverse – from almost dessert-like cliffs, beaches, and lower hilly areas near the coast, where phrygana and macchia are the dominant plant communities (Troníček 1938), agricultural and urban areas with olive plantations, oleanders, cultivated plants and flowers, to the more humid mountain slopes, gorges, and river valleys. Phrygana, one of the most unique habitats on the island, is on the NW and W dominated by *Coridothymus capitatus* ((L.) Hoffmanns. & Link), *Sarcopoterium spinosum* ((L.) Spach) and *Ballota pseudodictamnus* ((L.) Benth.), typical for most of the island (Bergmeier 1997). Agricultural areas mostly consist of olive plantations (*Olea europaea* (L.)), which host a community dominated by grasses (*Hyparrhenia hirta* ((L.) Stapf), *Aristida caerulescens* (Desf.), etc.) (Bergmeier 1997) and individual flowers (*Picris altissima* (Desf.)) (Bergmeier 1997), whereas urban settlements mostly include different cultivated flowers like *Campsis radicans* (Seem.), *Pelargonium* sp., *Geranium* sp., trees like *Oleander* sp., different Palmaceae, etc. On the other hand, mountainous regions consist mainly of mixed and conifer forests in the gorges, while vast numbers of indigenous flowers reign the mountain slopes.

Crete also inhabits a relatively high number of butterfly species (47), some of which are endemic to the island (*Coenonympha thyrsis* (Freyer), *Hipparchia cretica* (Rebel), *Pseudochazara anthelea* ssp. *amalthea* (Frivaldsky), *Kretania psylorita* (Freyer) and *Zerynthia cretica* (Rebel)) (Nel and Nel 2003; Pamperis 2010). For these reasons, Crete butterfly fauna was widely studied. The first studies

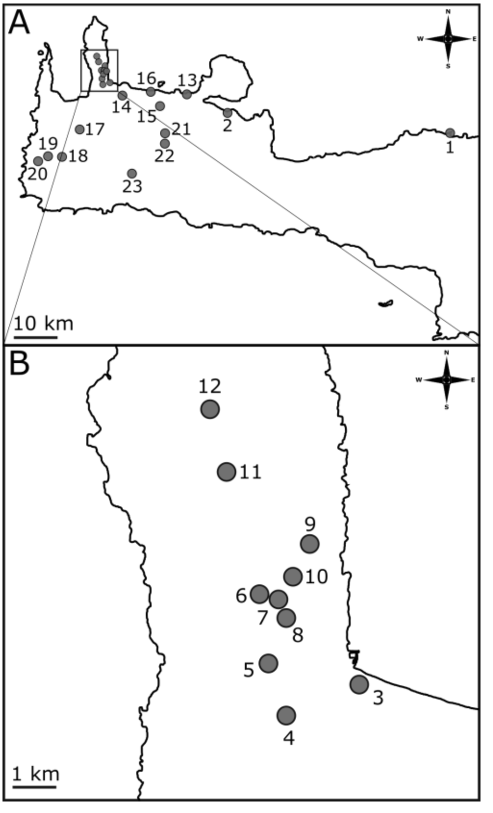

FIG. 1: Map of western Crete (1–2, 13–23) (A) and a close up of a mostly unsurveyed part of the biggest north western peninsula (3–12) (B) with marked surveyed localities.

concentrated mostly on the general overview of the species encountered, and also included a few species which were later unconfirmed or already disappeared from the island (e.g. *Cupido minimus* (Fuessly)) (Rebel 1916; Warnecke 1928; Troníček 1938; Bretherton 1969; Higgins 1973; Willemse 1975; Sala and Bollino 1997; Mérit and Mérit 1998; Nel and Nel 2003). The latest studies however, focus mainly on specific butterflies of the island, especially the species which emerged only recently (e.g. *Zizeeria karsandra* (Moore) and *Cacyreus marshalli* (Butler)) or are deemed endemic (e.g. *Z. cretica*) (Larsen 1986; Dennis 1996; Anastassiu et al. 2010; John et al. 2018; Rowlings and Cuvelier 2018). Since most of the island was already surveyed at one point or another, there are hardly any areas left unsearched, with the exception of certain remote or less interesting parts of the island (Pamperis 2010; www.butterfliesofcrete.com 2021). This article focuses mainly on the localities found on or near the biggest northwestern peninsula north of the town of Kolymvari and around the village of Rodopos, which are largely still unsurveyed. Some additional observations were also performed around the rivers Tavronitis and Keritis, as well as in some interesting localities previously surveyed by others (Willemse 1975; Mérit and Mérit 1998; Pamperis 2010) in the western and central northern (coastal) parts of the island (Fig. 1).

FIG. 2: Different localities/habitats found in NW and W Crete: A) Elos (4.7.2021); B) Kefali (4.7.2021); C) Plokamiana (4.7.2021); D) Lakkoi (4.7.2021); E) Chania (3.7.2021); F) and G) Agia Irini gorge (6.7.2021); H) hills N of Rodopos; I) Astratigos with Kolymvari in the background; J) hills SW of Afrata.

Materials and Methods

The study was performed mostly in the northwestern and western parts of the island of Crete, including some localities from the central northern (coastal) part. The butterflies were determined via catch and release method and the species were determined using the Tolman and Lewington (2008) field guide. As most species of butterflies on Crete are easyli distinguishable, most identifications could take place without catching the butterfly. On the other hand, harder-to-identify species were either photographed, or caught in the net, identified and later released. Individuals which could not be 100 % correctly identified are not included in the list. The exact locality and details regarding coordinates (WGS 84 Web Mercator projection; EPSG:3857), altitude, habitat and date of each observation are given in Table 1. In the case of a broader locality, coordinates are given for the most appropriate observation spot. The localities are given by the order of observation date.

Results and Discussion

During the survey of the northwestern and western parts of the island of Crete, a total of 23 species were observed, out of the 47 (49 %) that have been reported for the island (Pamperis 2010). Among these, two (*Pontia edusa* (Fabricius) and *Pieris brassicae* (L.)) were observed only once, while five species were found at at least 10 different localities (Tab. 2). Among the most widespread butterflies were *Pieris rapae* (L.), *Papilio machaon* (L.), *Pararge aegeria* (L.), *Lasiommata megera* (L.) and *Lampides boeticus* (L.), which is in accordance with other authors (Rebel 1916; Higgins 1973; Mérit and Mérit 1998), especially when the lack of localities from any of the three mountain massifs is considered. Among the most numerous were *P. rapae* and *L. boeticus*, both of which could be found in most of the researched localities, almost always near agricultural or urban habitats. On the other hand, species such as *Hipparchia cretica*, otherwise regarded as one of the most common species on the island (Rebel 1916; Mérit and Mérit 1998; Pamperis 2010), was observed at only two occasions. While only two imagines were found in Elos (Fig. 2, A), hundreds of them were observed inside the Agia Irini gorge. Anyhow, the number of active butterflies was relatively low during the hottest parts of the day, similarly as observed by other authors at this time of year (Higgins 1973). Extremely hot and dry weather conditions sometimes known for Mediterranean likely also contributed to their generally lower numbers, as observed in a study by Herrando et al. (2019).

Low number of butterflies was also apparent on the largest, until now mostly unsurveyed, northwestern peninsula of the island. Here, in the central parts, notably at the localities north of Rodopos and near the Afrata-Astratigos road (Fig. 2A, J), where the habitat mostly consists of dried phrygana, only *Coenonympha thyrsis*, *L. megera* and *Polyommatus icarus* (Rottemburg) were observed in high numbers. These localities, besides being dried by immense heat, were also the windiest and highest parts of the peninsula. In this area, butterflies were found in small dried-up river gorges, where the wind and heat were less intensive, and at least some flowering plants were found. On the southern part of the peninsula, however, the butterflies were more numerous, especially in small villages, where flowering plants were still plentiful. Here, *L. boeticus*, *P. rapae* and *P. machaon* were the most abundant species. Especially abundant were the butterflies of *L. boeticus*, which were sometimes observed in dozens, mostly flying around the flowering plants inside small parks or larger gardens.

Despite the overall hot weather and relatively few species found, some interesting observations were nonetheless made. Most importantly, some species were reported at certain localities for the first time. New localities were thus confirmed for *Cacyreus marshalli* (3, 6), *Carcharodus alceae* (Esper) (3, 4), *Celastrina argiolus* (L.) (3, 8), *C. thyrsis* (11, 12), *L. boeticus* (1, 3, 4, 6, 8, 9, 10, 13, 18, 19, 23), *Gegenes pumilio* (Hoffmannsegg) (3, 4, 6), *Gonepteryx cleopatra* (L.) (3, 5, 8, 9), *Lycaena phlaeas* (L.) (4, 14, 18), *P. aegeria* (3, 6), *P. rapae* (1, 16), *P. icarus* (3, 7, 10, 11, 12, 16), *P. edusa* (3), *Vanessa cardui* (L.) (1), and *L. megera* (4, 6, 7, 8, 11, 12) (Rebel 1916; Warnecke 1928; Higgins 1973; Willemse 1975; Sala and Bollino 1997; Mérit and Mérit 1998; Pamperis 2010; www.butterfliesofcrete.com 2021).

The number of new localities for certain butterfly species is not surprising, since the biggest northwestern peninsula was very poorly explored until now, and most observations in this part of the island were made in the vicinity of Kolymvari or further east near Chania (Rebel 1916; Mérit and Mérit 1998; Pamperis 2010; www.butterfliesofcrete.com 2021).

TABLE 1: Localities visited during the 30.6.–6.7.2021 survey of Crete and their description.

No.	Locality	Coordinates	Description of the habitat	Altitude	Date
1	Bali	X: 35.411534, Y: 24.782511	Mostly anthropogenic: arid scrub, gardens, cultivated flowers, wild flowers and trees on rocky ground (oleander, *Heliotropium* sp.), close proximity to the sea	15–25 m	30.6.2021
2	Aptera (highway)	X: 35.466789, Y: 24.135580	Mostly anthropogenic: cultivated deciduous trees (oleander, olive), flowers by the road	80–90 m	30.6.2021
3	Kolymvari	X: 35.542742, Y: 23.780298	Mostly anthropogenic: arid scrub and grasslands, fields, gardens, parks, cultivated flowers, abandoned estates	5–40 m	30.6.–5.7.2021
4	Koumouli	X: 35.533797, Y: 23.759599	Mixed natural and anthropogenic: olive groves, fields, arid scrub, wild flowers	120–140 m	2.7.2021
5	Aspra nera (crossroad W of village)	X:35.545616, Y: 23.755192	Mostly anthropogenic: olive groves, arid scrub, wild flowers, abandoned buildings	200–220 m	2.7.2021
6	Rodopos	X: 35.561584, Y: 23.755217	Mostly anthropogenic: parks, gardens, cultivated flowers, arid scrub, olive groves	230–240 m	2.7.2021
7	Rodopos (hills SE above town)	X: 35.558834, Y: 23.757862	Mostly natural: arid scrub and grasslands, olive groves, macadam road with wild flowers	270–290 m	2.7.2021
8	Astratigos	X: 35.556435, Y: 23.762502	Mixed natural and anthropogenic: gardens, olive groves, cultivated flowers, rocky slopes, arid scrub	200–210 m	2.7.2021
9	Afrata	X: 35.570288, Y: 23.766473	Mostly anthropogenic: gardens, cultivated flowers, arid scrub, olive groves, fields	135–145 m	2.7.2021
10	Astratigos – Afrata (road between villages)	X: 35.559239, Y: 23.763268	Natural: phrygana – dense arid scrub on rocky ground	170–190 m	2.7.2021
11	Rodopos (beginning of a macadam road in the hills N of town)	X: 35.587692, Y: 23.749653	Mixed natural and anthropogenic: olive groves, phrygana – dense arid scrub on rocky ground, dry riverbed, rocky pastures, vineyards	420–440 m	2.7.2021
12	Oros Titiron (dry riverbed E below the hilltop, near the road, N of Rodopos)	X: 35.602380, Y: 23.743666	Natural: phrygana – dense arid scrub on rocky ground, dry riverbed, rocky pastures	500–520 m	2.7.2021
13	Chania	X: 35.518949, Y: 24.014405	Mostly anthropogenic: gardens, parks, cultivated flowers, cultivated trees, close proximity to the sea	1–10 m	3.7.2021

TABLE 1 (cont.)

14	Polemarchi (near Tavronitis riverbed)	X: 35.510011, Y: 23.817907	Mostly anthropogenic: gardens, cultivated flowers, arid scrub, olive groves, fields	45–55 m	3.7.2021
15	Kirtomados (road near Keritis river NW of village)	X: 35.488852, Y: 23.914757	Mixed anthropogenic and natural: olive groves, fields, arid scrub, riverbed, peach and orange groves	50–60 m	3.7.2021
16	Gerani – Platanias (Keritis river delta)	X: 35.517613, Y: 23.891034	Mixed anthropogenic and natural: regulated riverbed, scrub, cultivated and wild flowers, fields	5–10 m	3.7.2021
17	Topolia	X: 35.430428, Y: 23.686085	Mostly anthropogenic: gardens, cultivated trees and flowers, olive groves, arid scrub	230–260 m	4.7.2021
18	Elos	X: 35.359959, Y: 23.639097	Mixed natural and anthropogenic: gardens, parks, olive groves, cultivated flowers, scrub, phrygana – dense arid scrub on rocky ground, deciduous forest, small stream	520–580 m	4.7.2021
19	Kefali	X: 35.363863, Y: 23.597344	Mostly anthropogenic: gardens, olive groves, cultivated flowers, arid scrub, pastures	450-470 m	4.7.2021
20	Plokamiana (dry riverbed S of village)	X: 35.347799, Y: 23.584307	Mostly natural: dry riverbed, arid scrub, deciduous forest, overgrowing olive groves	190-210 m	4.7.2021
21	Botanical parks & gardens of Crete (S of Fournes)	X: 35.419093, Y: 23.939196	Mostly anthropogenic: botanical park with different (including tropical, subtropical and native) cultivated trees and flowers, dry stony riverbed, scrub	140–210 m	4.7.2021
22	Lakkoi	X: 35.394823, Y: 23.942221	Mostly anthropogenic: gardens, olive groves, cultivated trees and flowers, scrub	480–520 m	4.7.2021
23	Agia Eirini (Agia Irini gorge S of village)	X: 35.324126, Y: 23.842279	Natural: mixed forest, arid scrub, dry riverbed, stony cliffs	540–600 m	6.7.2021

And while certain findings are of no real interest (e.g., new localities for some of the most abundant species on the island), others are quite surprising. The most important observations were made for *L. boeticus*, which was found at 11 new localities, while previously not being observed on the northwestern coast at all (Rebel 1916; Mérit and Mérit 1998; Pamperis 2010), let alone from the biggest northwestern peninsula. Important observations were also made for the relatively new addition to the Cretan fauna, *C. marshalli* (John et al. 2018), and the relatively rare *G. pumilio*, which was so far reported only by a handful of authors (Troníček 1938; Pamperis 2010). It is especially surprising, that *C. marshalli* already consolidated on the island (www.butterfliesofcrete.com 2021), despite being unknown from the entire Greece not two decades ago (Anastassiu et al. 2010) and was firstly observed on Crete only a few years back (John et al. 2018). It seems, that certain plants commonly found in urban areas (*Geranium* sp., *Pelargonium* sp.), coupled with suitable weather conditions, are enabling fast colonisation of the species, similar as in the Canary Islands (Wiemers et al. 2013). Considering the fast colonisation of the urban areas there is also a justified concern, that the species might start using wild *Geranium* species as host plants as well, thus competing with the native species, such as *Aricia agestis* (Denis & Schiffermüller).

TABLE 2: Species of butterflies found in Crete during 30.6.–6.7.2021. The localities are indicated by numbers from 1 to 23 as in the list and description of localities.

Family/*species*	Locality	No. of localities
Papilionidae		
Iphiclides podalirius	3, 8, 9, 13, 17, 19, 21	7
Papilio machaon	2, 3, 5, 6, 7, 8, 9, 14, 17, 18, 19, 20, 21, 23	14
Pieridae		
Colias crocea	3, 7, 16, 18, 19, 23	6
Gonepteryx cleopatra	1, 2, 3, 5, 8, 9, 18, 19, 23	9
Pieris brassicae	23	1
Pieris rapae	1, 2, 3, 4, 5, 6, 7, 8, 13, 14, 15, 16, 17, 18, 19, 21, 23	17
Pontia edusa	3	1
Lycaenidae		
Aricia agestis	18, 19	2
Cacyreus marshalli	3, 6	2
Celastrina argiolus	3, 8, 19, 22	4
Lampides boeticus	1, 3, 4, 6, 8, 9, 10, 13, 15, 16, 17, 18, 19, 21, 23	15
Lycaena phlaeas	4, 14, 18	3
Polyommatus icarus	3, 7, 10, 11, 12, 16, 18, 23	8
Nymphalidae		
Coenonympha thyrsis	7, 10, 11, 12, 18, 23	6
Hipparchia cretica	18, 23	2
Lasiommata megera	4, 6, 7, 8, 11, 12, 18, 19, 21, 22, 23	11
Maniola jurtina	3, 18, 23	3
Pararge aegeria	3, 6, 13, 15, 17, 18, 19, 21, 22, 23	10
Polygonia egea	18, 19, 21, 22	4
Vanessa atalanta	7, 22	2
Vanessa cardui	1, 3, 4	3
Hesperiidae		
Gegenes pumilio	3, 4, 6, 15	4
Carcharodus alceae	3, 4, 18, 19, 20, 23	6

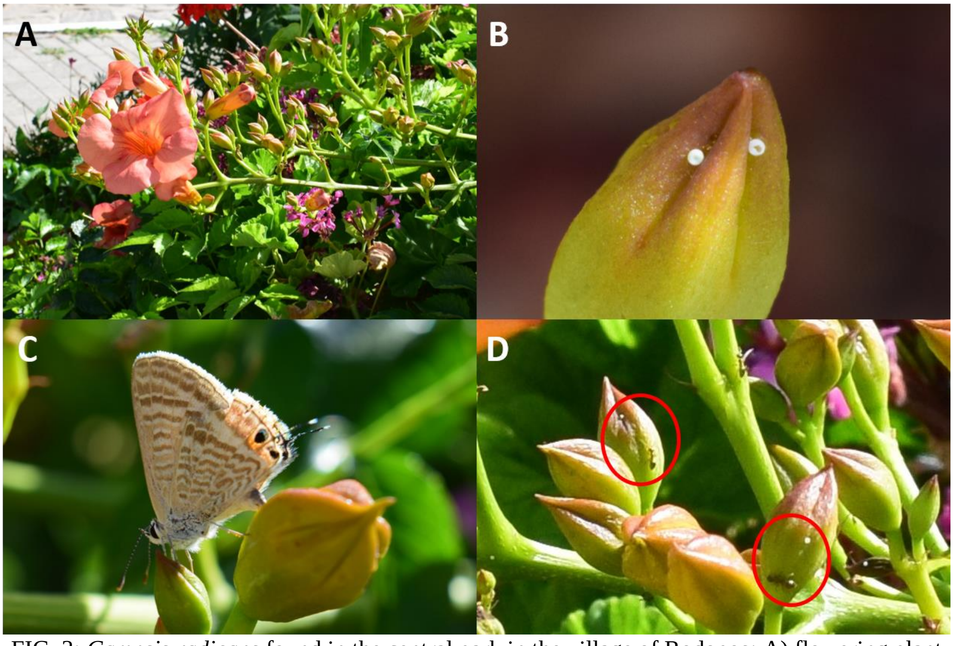

FIG. 3: *Campsis radicans* found in the central park in the village of Rodopos: A) flowering plant with buds; B) eggs of *Lampides boeticus* on the flower bud; C) oviposition of *L. boeticus* on the flower bud; D) encircled with red - ants tending the *L. boeticus* eggs on the flower buds.

Also important is the finding of a new larval host plant for the butterfly *L. boeticus*, one of the most widely distributed butterflies in the world, currently found across the Palaearctic region, parts of Africa, Madagascar, South-East Asia, Australia, Oceania and Hawaii (Lohman et al. 2008). It is otherwise a well-known polyphagous butterfly, feeding on many cultivated plants such as legumes (including *Vicia faba* (L.) and *Pisum sativum* (L.)) and sometimes considered as serious pest due to its high prevalence in peas in some countries (Larsen 1986; Lohman et al. 2008; www.plantwise.org 2021). It is also being connected with many other species of plants, especially cultivated flowers, like *Kennedia prostrata* (R.B.), *Virgilia oroboides* (P.J. Bergius), etc. (www.plantwise.org 2021). However, oviposition of females on the cultivated ornamental flower *Campsis radicans* (Fig. 3, A) was observed for the first time (Harding 1971; Lohman et al. 2008; www.plantwise.org 2021). *C. radicans*, a species native to North America, is a common cultivated liana/shrub, which was brought to Europe (Italy) in the 17th century and has quickly spread throughout the Mediterranean basin (Bergmeier 2011; Jeberean et al. 2016). Due to its high drought resistance and big yellow to red blossoms it is a very common plant in parks and gardens, especially in countries such as Greece (Bergmeier 2011; own observations), Croatia (Tafra et al. 2013) and parts of Slovenia (own observations). The oviposition of females on the flower buds and stems of the *C. radicans* was observed multiple times, mostly on the biggest northwestern peninsula in the villages of Rodopos (Fig. 3, C), Afrata and Astratigos. Here the butterflies were found in great numbers, especially in the village of Rodopos, where dozens of imagines were observed in a single spot. Sometimes, several eggs were observed on even just one flower bud/stem (Fig. 3, B). Also, ants were seen visiting the flower buds tending the newly laid eggs, which is common for myrmecophilous species such as *L. boeticus* (Obregón-Romero and Gil-T 2011; Obregón et al. 2015). Observation of oviposition on *C. radicans* is mostly important from the economical view, as *C. radicans* is also widely cultivated among florists (Bergmeier 2011; Tafra et al. 2013). Just like in the case of *C. marshalli*, where the butterfly is considered a non-negligible pest for the cultivated species of *Pelargonium* and *Geranium*, *L. boeticus* could cause a considerable damage on cultivations of *C. radicans*. Additionally, as *L. boeticus* was observed in many new localities along the coast, on a previously unknown host plant, the species might adapt to additional larval host plants and thus might amplify its economic damage or start competing with other species of butterflies in their native habitats.

Conclusions

During the surveys in the western and northwestern parts of Crete, including the so far mostly overlooked northwestern peninsula, 23 species of butterflies were observed, many on new localities. Among the recorded species were also two Cretan endemics, *Hipparchia cretica* and *Coenonympha thyrsis*. The relatively low number of butterflies/species can be attributed to the intense heatwave and summer drought, which were present at the time of the surveys. The most important new localities were found for the species *Gegenes pumilio*, *Lampides boeticus* and *Cacyreus marshalli*, the latter two of which were found to be expanding their areal and consequently consolidating on the island. While *C. marshalli* was found on two new localities, *L. boeticus* was observed on 11 previously unknown areas. Also, a new larval host plant – *Campsis radicans* – was found for the butterfly *L. boeticus*, where oviposition of the females was observed multiple times at different localities. As *C. marshalli* and *L. boeticus* are consolidating in urban areas of Crete, the fear exists, that in the foreseeable future they may begin to compete with other native species on the island as well.

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Butterflies (Lepidoptera: Papilionoidea) of northwestern and western Crete (Greece) in early July, with the finding of new larval host plant for *Lampides boeticus*

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ΠΕΡΙΛΗΨΗ

Κατά τη διάρκεια του καλοκαιριού 2021, οι βορειοδυτικές και δυτικές περιοχές του ελληνικού νησιού της Κρήτης ερευνήθηκαν για να επιβεβαιωθούν ορισμένες παρατηρήσεις για την πανίδα ημερόβιας πεταλούδας του νησιού. Η έρευνα επικεντρώθηκε ιδιαίτερα στα μη διερευνημένα βορειοδυτικά τμήματα του νησιού, καθώς και στα είδη που επεκτείνουν την περιοχή εξάπλωσής τους (*Cacyreus marshalli* (Butler), *Lampides boeticus* (L.)). Από 47 είδη που έχουν αναφερθεί για το νησί, παρατηρήθηκαν τα 23. Όλες οι έρευνες πραγματοποιήθηκαν κατά τη διάρκεια μιας ιδιαίτερα ζεστής και ξηρής περιόδου (30-37 °C), μεταξύ 30 Ιουνίου και 6 Ιουλίου. Επιπρόσθετα, επιβεβαιώθηκε ένα νέο φυτό ξενιστής προνυμφών για το *L. boeticus*, καθώς η ωοτοκία θηλυκών σε *Campsis radicans* (Seem.) παρατηρήθηκε σε αρκετές περιπτώσεις..

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Evaluation and economic analysis of ecofriendly biological approaches for the management of shoot and fruit borer (*Earias vittella* F.) of okra

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ABSTRACT

During the years 2020 and 2021, the effects of various biopesticides and the egg parasitoid *Trichogramma chilonis* on the okra shoot and fruit borer were investigated in open fields. All interventions outperformed over the untreated control. The entomopathogenic bacterium *Bacillus thuringiensis* was the most promising biopesticide tested, with the lowest shoot (5.49% and 6.87% in 2020 and 2021, respectively) and fruit damage (4.95% and 5.65% in 2020 and 2021, respectively), followed by the entomopathogenic fungi *Beauveria bassiana* (7.08%, 8.04% shoot damage and 6.78% and 6.73% fruit damage during 2020 and 2021, respectively). Interestingly, all biopesticides evaluated were shown to be safe for the polyphagous predators occurring in the okra habitat, such as the ladybird beetles *Menochilus sexmaculatus* and *Micraspis discolour* and the spiders *Marpissa* spp. and *Oxyopes lineatipes*. However, emamectin benzoate 5% SG was the most effective treatment in terms of minimizing okra shoot and fruit damage, resulting in a maximum percent reduction over control (PROC) (85.54 and 80.90 against shoot damage and 76.60 and 71.33 against fruit damage during 2020 and 2021, respectively). Each treatment's economics were also analysed. The experimental plots treated with emamectin benzoate had the highest cost:benefit ratio (1:11.16), while *B. thuringiensis* had the highest (1:7.06) among the biopesticide / parasitoid releasing plots.

KEY WORDS: *Earias vittella*; biopesticides; okra; pest management; fruit yield; economic return.

Introduction

Okra shoot and fruit borer (OSFB), *Earias vittella* (Fabricius, 1794) (Nolidae: Lepidoptera), is an oligophagous pest of okra (*Abelmoschus esculentus* (L.) Moench) that causes considerable damage in India. Both the vegetative and reproductive phases of okra are harmed by the OSFB larvae. During the vegetative stage, larvae bore into new shoots and feed on internal tissues, flower buds and fruits, during the reproductive stage. As a result, the infested shoots die early (Rai et al. 2014b). The infested fruits become malformed in shape, which drastically lowers their market value. In India, OSFB alone damages 21-51% of fruits, while in Bangladesh, 52.33-70.75% (Choudhury et al. 2021; Halder & Rai 2021).

Chemical pesticides are still the preferred choice of many Indian farmers due to their ease of use, quick action, and ease of application (Roy et al. 2017). However, the difficulties associated with chemical pesticide applications, such as resistance development of target pests, resurgence of sucking insects, secondary

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pest outbreaks, elimination of non-target organisms, pollution, and consumer health dangers, cannot be ruled out (Ahmad & Arif 2009; Halder et al. 2019; 2021).

Because of their target-specificity, self-perpetuity and safety, biological pest management methods employing macro- and micro-organisms is gaining appeal among growers and consumers. Plant-based insecticides, such as azadirachtin, which is generated mainly from the seeds of the neem tree (*Azadirachta indica* L.), are widely used to control various insect pests of various crops, particularly vegetables (Halder et al. 2010; Kodandaram et al. 2014). The soil bacterium *Bacillus thuringiensis* (Bt) produces delta-endotoxins, which are harmful to a variety of phytophagous insects and can be employed as biopesticides (Rai et al. 2014b). In the same way, *Beauveria bassiana* (Balsamo-Crivelli) and *Metarhizium anisopliae* (Metschnikoff) Sorokin are two significant entomopathogenic fungi (EPF) that are employed as mycoinsecticides against a variety of insect pests around the world (Butt et al. 2001; Zimmerman 2007a,b). In India, little research has been done on the ecofriendly management of OSFB. As a result, the current research was carried out to establish the individual efficacy of these bioagents under field conditions, as well as their cost:benefit ratio, to set up appropriate pest management modules.

Materials and Methods

Experimental site: The field experiments were conducted on a research farm of ICAR-Indian Institute Vegetable Research in Varanasi (82°52' E longitude and 25°12' N latitude), Uttar Pradesh, India. The experimental site is located in the Indo-Gangetic plains' alluvial zone, with silt loam soils that are low in organic carbon (0.43 percent) and available nitrogen (185 Kg/ha).

Seed sowing and crop management: During the rainy season (July - October) of 2020 and 2021, seeds of okra (cv. Kashi Pragati) were sown in a randomized block design with four replications and seven treatments, including an untreated control, in a plot size of 5 x 4 m with plant to plant spacing of 45 cm and row to row distance of 60 cm, during the first week of August. The prescribed fertilizer concentrations (N:P:K = 120:60:60) were utilized as a starting point. Standard agronomic practices were followed, except for plant protection measures. Hand weeding and irrigations were provided as needed.

Experimental design and treatments: The experiment adopted a randomized block design (RBD) with four replications, during the rainy season (July - October) in 2020 and 2021. Three entomopathogenic fungi as talc-based formulation viz., *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) (1×10^8 cfu/g) NBAIR strain, *Metarhizium anisopliae* (Metchnioff) Sorokin (Hypocreales: Clavicipitaceae) NBAIR strain (1×10^8 cfu/g) and *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) NBAIR strain were considered for the experiments. Azadirachtin 300 ppm were procured from the local market. Apart from these microbial insecticides, the egg parasitoid, *Trichogramma chilonis* (NBAIR strain) was also released in a separate plot (75 m^2) 500 m from the main plot. A chemical insecticide approved by the Central Insecticide Board and Registration Committee, Ministry of Agriculture & Farmers Welfare, Govt. of India, emamectin benzoate 5% SG was used as a chemical control treatment. The treatment details along with their concentrations were: T1: *Metarhizium anisopliae* (NBAIR strain) 1×10^8 spores/ g @ 5 g/L; T2: *Beauveria bassiana* (NBAIR strain) 1×10^8 spores/ g @ 5 g/L; T3: *Trichogramma chilonis* (NBAIR strain) @ 50,000 parasitoids/ha, 6 releases at weekly interval; T4: *Bacillus thuringiensis* (NBAIR strain) @ 2 ml/L; T5: Azadirachtin 300 ppm @ 5 ml/L; T6: emamectin benzoate 5% SG @ 0.3 g/L; T7: Untreated control. A high-volume knapsack sprayer equipped with a hollow cone nozzle was used for all applications. The spray volume was 600 L/ha during sunny and warm conditions with no or little wind. The first application was performed when the borer population reached the economic threshold level (5% of the fruit damage), followed by two sprays, fifteen days apart.

Shoot infestation: At weekly intervals, the total number of shoots, as well as the number of infested shoots, were visually inspected and recorded from five plants in each plot. The percentage of shoot infestation was calculated using the following formula:

% Shoot infestation =
100x infested shoots number/total shoots number

Fruit infestation and yield: Fruits were harvested at 4-5 days intervals, and the number of healthy and infested fruits was recorded. Healthy and infested fruits were weighed separately per plot and per treatment. During the fruiting season, harvests were made, and the percentage of fruit infestation was calculated using the following formula:

% Fruit infestation (by number) =
100x infested fruits number/total fruits number

The percentage reduction (PR) of the OSFB over the untreated control for each treatment was calculated using the following formula:

PR = 100x (Count in control–Count in treatment)/Count in control)

Observation on beneficial fauna: In addition, 10 days after each spray, the population of polyphagous ladybird beetles (grub, pupa and adults) i.e., *Menochilus sexmaculatus* (Fab.) (Syn: *Cheilomenes sexmaculata* (Fab.)) and *Micraspis discolor* (Fab.) (Coleoptera: Coccinellidae), jumping spiders (spiderling and adult) (*Marpissa* spp.) (Araneae: Salticidae) and true spiders like lynx (*Oxyopes lineatipes* (Koch)) (Araneae: Oxyopidae) was counted from 5 plants/plot and expressed as number of predators/plant.

Data analysis: All above mentioned data from the field experiment were collected, and the mean data from three replications was analyzed using the SAS program (Johnston 1993). For the parameters of shoot and fruit infestation, analysis of variance (ANOVA) was used to determine significant differences between the treatments. Using data from the mean population after spraying, the critical difference (CD) was estimated at a 5% level of significance. The incremental cost-benefit (ICBR) analysis for each treatment was carried out using the following formula:

Incremental Cost-benefit ratio =
Net return (₹ ha-1)/Cost of treatment (₹ ha-1)

Results

Shoot infestation

Table 1 shows the impact of several treatments on okra shoot and fruit borer (OSFB) infestation of shoots. The proportion of shoot infestation was greatly reduced in all treatments. The untreated control plot had the highest percentage of infested shoots (22.69), whereas the emamectin benzoate treated plots had the lowest (3.28%) during the first year. Among the biopesticides examined, *B. thuringiensis* treated plots had the lowest amount of shoot damage in both years (5.49% and 6.87% during 2020 and 2021, respectively).

During both years, the *B. bassiana* treated plots had the second lowest shoot damage (7.08% and 8.04%). The reduction percentage was calculated with respect to the control treatment, and all treatments showed a markedly decreased percentage of shoot infestation as compared to the control.

The highest reduction over the control (PROC) was detected for treatment 4 (*B. thuringiensis* treated plots), with corresponding values of 75.81 and 71.53 for the years 2020 and 2021, respectively, followed by the plots sprayed with *B. bassiana* (68.80 and 66.68 PROC). The lowest reduction was found in the azadirachtin-treated plots as compared to the control.

Fruit damage

In the case of *Earias* spp. fruit damage in okra, a similar pattern was observed. During the year 2020, plots treated with the entomopathogenic bacterium *B. thuringiensis* suffered only 4.95% fruit damage, resulting in a maximum protection percentage of 70.07 above the untreated control among the biopesticides evaluated. The white muscardine fungus *B. bassiana* was the next best biopesticide in 2020, with 6.78% fruit damage and 59.01 PROC. During the following year, the results were constant, with plots sprayed with *B. thuringiensis* showing the lowest fruit damage (5.65%), followed by *M. anisopliae* (6.73% fruit damage and 55.84 PROC), and *B. bassiana* (7.16% fruit damage and 53.02 PROC). However, emamectin benzoate 5% SG was the most effective treatment in terms of fruit damage, with 3.87% and 4.37% fruit damage in 2020 and 2021, respectively, and PROC values of 76.60 and 71.33.

Table 1. Bio-efficacy of different biocontrol agents against shoot damage (%) in okra

Treatment	2020[a]			2021[a]		
	Before spray	After spray	PROC*	Before spray	After spray	PROC[#]
T1	18.65	7.91[bc]	65.14	22.34	10.59[c]	56.11
T2	17.32	7.08[b]	68.80	21.06	8.04[b]	66.68
T3	20.36	11.97[d]	47.25	21.58	13.96[d]	42.15
T4	15.79	5.49[b]	75.81	20.19	6.87[b]	71.53
T5	18.05	12.64[d]	44.30	19.54	13.97[d]	42.11
T6	19.64	3.28[a]	85.54	20.36	4.61[a]	80.90
T7	19.43	22.69[e]	--	21.14	24.13[e]	--
SEm(±)	--	0.86	--	--	0.74	--
LSD (5%)	--	1.79	--	--	1.87	--

[#]PROC = Percent Reduction Over Control; Means followed by same letters in a column are not significantly different at 0.05P; [a]Mean of 15 observations over three sprays of different treatments at 15 days interval.

(T1: *Metarhizium anisopliae* (NBAIR strain) @ 5 g/L; T2: *Beauveria bassiana* (NBAIR strain) @ 5 g/L; T3: *Trichogramma chilonis* (NBAIR strain) @ 50,000 parasitoids/ha, 6 releases at weekly interval; T4: *Bacillus thuringiensis* (NBAIR strain) @ 2 ml/L; T5: Azadirachtin 300 ppm @ 5 ml/L; T6: emamectin benzoate 5% SG @ 0.3 g/L; T7: Untreated control).

SEM: Standard error of mean, LSD: Least Significant Difference

Table 2. Bio-efficacy of different biocontrol agents against fruit damage (%) in okra

Treatments	2020			2021		
	Before spray	**After spray**	**PROC***	**Before spray**	**After spray**	**PROC#**
T1	12.56	7.43[c]	55.08	13.59	7.16[bc]	53.02
T2	13.47	6.78[c]	59.01	15.67	6.73[c]	55.84
T3	11.69	8.29[cd]	49.88	17.58	7.44[bc]	51.18
T4	12.68	4.95[b]	70.07	15.43	5.65[b]	62.93
T5	13.57	9.81[d]	40.69	14.36	7.89[d]	48.23
T6	11.06	3.87[a]	76.60	13.58	4.37[a]	71.33
T7	12.34	16.54[e]	--	14.56	15.24[e]	--
SEm(±)	--	0.39	--	--	0.37	--
LSD (5%)	--	0.88	--	--	0.84	--

#PROC = Percent Reduction Over Control; Means followed by same letters in a column are not significantly different at 0.05P; [a]Mean of 15 observations over three sprays of different treatments at 15 days interval.
(T1: *Metarhizium anisopliae* (NBAIR strain) @ 5 g/L; T2: *Beauveria bassiana* (NBAIR strain) @ 5 g/L; T3: *Trichogramma chilonis* (NBAIR strain) @ 50,000 parasitoids/ha, 6 releases at weekly interval; T4: *Bacillus thuringiensis* (NBAIR strain) @ 2 ml/L; T5: Azadirachtin 300 ppm @ 5 ml/L; T6: emamectin benzoate 5% SG @ 0.3 g/L; T7: Untreated control)
SEM: Standard error of mean, LSD: Least Significant Difference

Economic Analysis

The incremental cost:benefit ratio (ICBR) was estimated on the basis of the crop protection expenses incurred and local market value of healthy fruits obtained against the treatment used in the present study (Table 3). It is important to note that the expenses incurred refer to those only related to pest management. It was revealed that the highest ICBR was 1:7.06 as estimated for the *B. thuringiensis* treated plots, followed by *T. chilonis* plots, whereas the lowest was estimated for azadirachtin 300 ppm (1:1.07).

The common predators observed in okra plants were identified as the predatory polyphagous ladybird beetles i.e., *Menochilus sexmaculatus* (Fab.) (Syn: *Cheilomenes sexmaculata* (Fab,)) and *Micraspis discolor* (Fab.) (Coleoptera: Coccinellidae). Also present were the jumping spiders *Marpissa* spp. (Araneae: Salticidae) and true spiders like lynx (*Oxyopes lineatipes* (Koch)) (Araneae: Oxyopidae).

The effect of these bioagents and botanicals on associated natural enemies (NEs) was investigated (Fig. 1). In all the biopesticide treated plots, *T. chilonis* released and untreated control, all stages of these predators were observed. The highest number of predators was recorded at the untreated control plots (3.94 lady bird beetles and 4.18 spiders per plant) followed by the *T. chilonis* plot (3.97 lady bird beetles and 4.07 spiders plant-1). The plots sprayed with emamectin benzoate 5% SG had the lowest spiders (2.87 per plant) and predatory coccinellid beetles (2.49 per plant). The other biopesticides viz., *B.*

bassiana, *M. anisopliae*, *B. thuringiensis* and azadirachtin treated plots had higher natural enemies' populations and these did not differ significantly to each other.

Table 3. Economic analysis of different treatments against the okra shoot and fruit borer

Treatments	Yield of healthy fruits (q ha^{-1})	Increase in yield over control (q ha^{-1})	Increase in yield over control (%)	Cost of increase yield (₹ ha^{-1})	Cost of treatment (₹ ha^{-1})	Net profit (₹)	Incremental Cost: Benefit ratio
T1	107.4^{c}	19.6	22.32	23520	4500	19020	1:4.23
T2	113.9^{d}	26.1	29.73	31320	4500	26820	1:5.96
T3	99.7^{b}	11.9	13.55	14280	2000	12280	1:6.14
T4	116.8^{d}	29.0	33.03	34800	4320	30480	1:7.06
T5	97.1^{b}	9.3	10.59	11160	5400	5760	1:1.07
T6	131.6^{e}	43.8	49.89	52560	4320	48240	1:11.16
T7	87.8^{a}	--	--	--	4500	--	--
LSD (5%)	3.4	--	--	--	--	--	--

Spray volume = 600 L ha^{-1}; Average cost of okra was ₹ 1200 q^{-1}; Means followed by same letters in a column are not significantly different at 0.05P.

Yield related parameters

The effects of different treatments significantly influenced the individual yields of okra (Table 3). Total yield was calculated by hectare of land, and the maximum yield (131 q ha^{-1}) was found in the emamectin benzoate 5% SG treated plots followed by *B. thuringiensis* (116.8 q ha^{-1}) and *B. bassiana* (113.9 q ha^{-1}) treated plots. The minimum healthy fruit yield (87.8 q ha^{-1}) was found in the untreated control treatment.

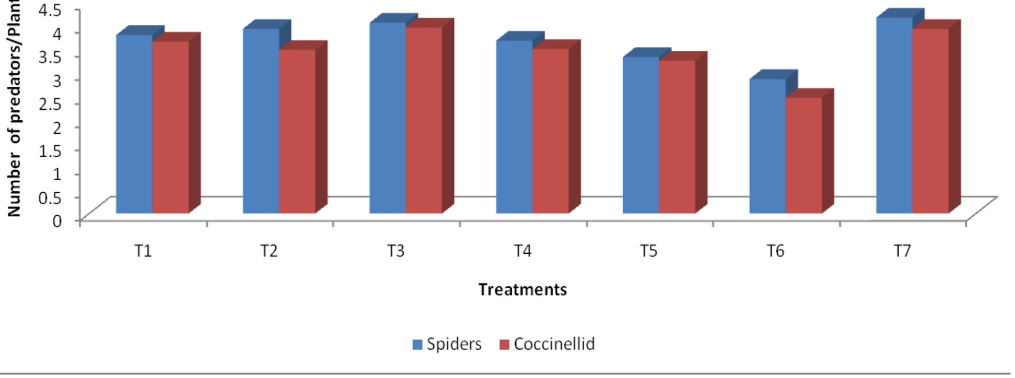

FIG. 1. Effect of different treatments on population of polyphagous spiders and lady bird beetles in okra

Discussion

The entomopathogenic bacterium *Bacillus thuringiensis* treated plots suffered lowest shoot and fruit damages by the okra shoot and fruit borer during both years amongst the biopesticide treated plots. The bacterium after entering the insect gut causes septicemia and eventual death of the insect host. Insects show different kinds of responses to the toxins, depending on the crystal proteins (delta-endotoxins), receptor sites, production of other toxins (exotoxins), and requirements of spores (Halder & Seni 2021). So, *B. thuringiensis* is an effective competitive alternative, compared to conventional insecticides in terms of efficacy and costs of production (Ruiu et al. 2013). Another entomopathogen, *Beauveria bassiana*, is used for the management of a wide range of insect pests. *B. bassiana* 1.15% WP @ 3000 g/ha and 2500 g/ha were highly effective in controlling pod borer populations in chickpea (Bajya et al. 2015). Pest control effectiveness of *B. bassiana* was documented against the maize stem borer, *Chilo partellus* from Kenya (Maniania 1993). Similarly, the black muscardine fungus *Metarhizium anisopliae* is also reported to be effective against a large number of crop pests (Revathi et al. 2011; Halder et al. 2016; Fite et al. 2020). Gaikwad (2013) concluded that *B. thuringiensis* var. *kurstaki* proved to be the toxic to the larvae of *Earias vittella* infesting okra whereas *M. anisopliae* and *B. bassiana* were moderately toxic. In contrast, the chemical insecticide, used in the present study, emamectin benzoate, is a novel group of insecticides, effective against lepidopteran and sucking pests (Rai et al. 2014b; http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides). The mode of action of this new molecule is unique in the panorama of insecticides as it is grouped together with avermectins and milbemycins (IRAC, 2022). It inhibits muscle contraction, causing a continuous flow of chlorine ions in the GABA and H-Glutamate receptor sites (Fanigliulo & Sacchetti 2008; Banik & Halder 2013). This unique mode of action could be the reason for superior activity of this molecule against okra shoot and fruit borer.

It is evident that all the biopesticide treated and *T. chilonis* released okra plots had higher numbers of predatory fauna indicating their target-specificity and safety towards associated non-target organisms. Biosafety of these bioagents and neem-based insecticides have been documented by several workers (Gracy et al. 2011; Rai et al. 2014b; Roy et al. 2017; Zimmerman 2017b).

Among the biopesticide treated plots the highest cost benefit ratio was registered from the *B. thuringiensis* treated okra plots. Relatively higher fruit yield and low cost of plant protection inputs were the reason for its higher ICB ratio. Treatment 3 i.e., inoculative release of *T. chilonis* plots had the second highest ICB ratio (1:6.14). This was due to lowest cost of plant protection inputs amongst the treatments although it had a lower healthy fruit yield (99.7 q per ha). However, the emamectin benzoate treated plots had the maximum ICB ratio (1:11.06) due to its maximum fruit yield (131.6 q per ha) and accompanied with lower input cost.

Conclusion

The present study revealed that among the different biopesticide treatments, *Bacillus thuringhensis* was the best in controlling shoot and fruit borer in okra. Other biopesticides viz., *Beauveria bassiana* and *Metarhizium anisopliae* were also proved effective against OSFB and may be considered as control tools in okra cultivation. However, amongst all examined treatments, emamectin benzoate showed the highest efficacy against OSFB. These individual components should be taken into consideration while formulating ecofriendly pest management module for managing OSFB.

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Αξιολόγηση και οικονομική ανάλυση οικολογικών βιολογικών προσεγγίσεων για την αντιμετώπιση του λεπιδοπτέρου *Earias vittella* F. στη μπάμιας

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ΠΕΡΙΛΗΨΗ

Κατά τη διάρκεια των ετών 2020 και 2021, διερευνήθηκαν οι επιδράσεις διαφόρων βιοπαρασιτοκτόνων και του ωοπαρασιτοειδούς *Trichogramma chilonis* ενάντια στο λεπιδόπτερο *Earias vittella* F. σε ανοιχτή καλλιέργεια. Όλες οι επεμβάσεις είχαν καλύτερη απόδοση σε σχέση με τον μάρτυρα. Το εντομοπαθογόνο βακτήριο *Bacillus thuringiensis* ήταν ο πιο πολλά υποσχόμενος βιολογικός παράγοντας που δοκιμάστηκε, με τη χαμηλότερη προσβολή βλαστών (5,49% και 6,87% το 2020 και το 2021, αντίστοιχα) και καρπών (4,95% και 5,65% το 2020 και 2021 αντίστοιχα), ακολουθούμενο από τον εντομοπαθογόνο μύκητα *Beauveria bassiana* (με 7,08% και 8,04% ζημιά στους βλαστούς και 6,78% και 6,73% ζημιά στους καρπούς κατά το 2020 και το 2021, αντίστοιχα). Είναι ενδιαφέρον ότι όλοι οι βιολογικοί παράγοντες που αξιολογήθηκαν αποδείχθηκαν ασφαλής για τα πολυφάγα αρπακτικά που απαντώνται στον βιότοπο της μπάμιας, όπως οι πασχαλίτσες *Menochilus sexmaculatus* και *Micraspis discolour* και οι αράχνες *Marpissa* spp. και *Oxyopes lineatipes*. Ωστόσο, το εντομοκτόνο emamectin benzoate 5% SG ήταν το πιο αποτελεσματικό όσον αφορά την ελαχιστοποίηση της ζημιάς στους βλαστούς και τους καρπούς της μπάμιας, με αποτέλεσμα τη μέγιστη ποσοστιαία μείωση έναντι του μάρτυρα (PROC) (με 85,54 και 80,90 έναντι της ζημιάς των βλαστών και 76,60 και 71,33 έναντι της ζημιάς των καρπών το 2020 και 2021, αντίστοιχα). Τα οικονομικά στοιχεία κάθε επέμβασης αναλύθηκαν επίσης. Τα πειραματικά αγροτεμάχια στα οποία εφαρμόστηκε το emamectin benzoate είχαν την υψηλότερη αναλογία κόστος/όφελος (1:11,16), ενώ ο *B. thuringiensis* την υψηλότερη (1:7,06) μεταξύ των επεμβάσεων με τους βιολογικους παράγοντες.

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Ecobiology of *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae, Danainae) throughout the autumn – early winter breeding period on Rodos Island, Greece

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ABSTRACT

The present study is focused on the biology and ecology of *Danaus chrysippus* on Rodos Island, Greece, contributing on the knowledge of its life cycle in nature throughout the autumn – early winter breeding period, which is described and illustrated here for the first time for the country. Distribution range, habitat requirements and larval hostplant preferences, which are closely related with specific microclimatic conditions and the availability of milkweed resources in the region, are discussed.

KEY WORDS: Lepidoptera; Danainae; life cycle; *Cynanchum;* Greece; Aegean; Rodos.

Introduction

Danaus chrysippus (Linnaeus 1758) is a wide-ranging migrant species belonging to Danainae, a subfamily of Nymphalidae. It is a polyvoltine, polyphagous species, the larvae of which feed on plants containing cardenolides, especially species of the families Asclepiadaceae, Apocynaceae and Moraceae. The species prefers bushy, rocky and coastal places, usually near gardens and cultivated areas (Pisciotta et al. 2008). According to its status in the most recent IUCN Red List, it is classified as of Least Concern (LC) (Westrip 2021). It is widespread in the North African coastal regions (western Morocco, northern Algeria and Tunisia), from where it has colonized the coastal areas of the Canary Islands, southern Spain (where the first European population was found in 1980), southern France, Corsica, Sardinia, Sicily, Italy, Malta, Serbia, Montenegro, Croatia, Albania, Turkey, Cyprus and Greece (Perković 2006; Baytas 2007; Pisciotta et al. 2008; Pamperis 2009; John et al. 2019). In Greece, it has been reported mainly from the western coastal regions (Ionian Islands, Epirus, Sterea Hellas, Peloponnese, Attiki), while regarding the Aegean Sea region, it has been reported from Chios, Samos, Iraklia and Kriti Islands (Pamperis 2009; Gavalas 2013). On the islands of the Dodecanese complex, it has so far been reported from Astipalea, Kalymnos, Kos, Nisiros, Tilos, Rodos and Simi (Albrecht & Kissling 2013; Cuvelier & Mølgaard 2014; Galanos 2016; Mølgaard 2002; Nemec 2016; Pamperis 2009). Moreover, new sightings have also been reported to date both from continental Greece – coastal areas in Macedonia are also included – as well as from other Greek islands (Pamperis 2022).

In Rodos, specifically, *D. chrysippus* was first discovered and collected by Ghigi in August 1926 (Olivier 1993). Since then,

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however, it should be noted that no thorough fieldwork has been done so far on the species' life cycle throughout the year on the island. Thus, following recent studies of the butterfly fauna in the Dodecanese Islands conducted by Galanos (2014, 2016, 2017, 2020), further investigations were carried out to define the number of species' generations throughout the year, as well as to obtain more accurate data by monitoring its seasonal stages and to evaluate the bionomic information. In addition, notes on the butterfly's hostplant range, larval polymorphism and duration of immature stages depending on weather conditions, were also examined, and are herein illustrated.

Materials and Methods

Photographs of living butterflies and oviposition on the larval hostplant were taken in situ. Early stages of *D. chrysippus* were measured recording larval body length, as well as the pupal length and width (at its broadest point). The moulted female larval head capsules of each instar, from 1 to 5, were collected and their widths were measured comparatively under laboratory conditions to determine accurately the number of moults and consequently of species' instars that have occurred. Moreover, we were able to track the different larval stages by measuring larval frass particles in accordance with Bean (1959) and Southwood and Henderson (2000). All measurements were taken using a digital calliper (Total TMT321501). No adult specimen was collected due to conservation considerations, considering that it was not necessary since the species could not be confused with any other. Taxonomy and nomenclature follow Wiemers et al. (2018) for the butterfly fauna, Hassler (2004-2022) and Kleinsteuber et al. (2016) for the flora. All photographs were captured by Christos Galanos.

Results and Discussion

During 2020 and 2021, a series of field surveys was carried out at selected locations, which had previously been confirmed as breeding habitats of *D. chrysippus*, to in-depth study the species' metamorphosis from the early life stages, i.e. egg, larva and pupa, to the adult, with the aim of ascertaining the number of generations over the year. In particular, butterflies of *D. chrysippus* begin to arrive along the coastal areas of the island, where they remain for breeding, subject to suitable climatic and habitat conditions, i.e. absence of winds, storms and freezing temperatures, exposure to dappled sunlight, prevalence of high humidity and availability of water and food (Pelton et al. 2016). More specifically, it was found that migrants gradually appear on the island from early September to October, although butterflies have occasionally been recorded flying earlier, i.e. in May (Rebel, 1936), 1st of June (Mølgaard, 2002), as well as in August 1926 and 2012 by Ghigi (Olivier 1993) and the author, respectively.

Life cycle

Three broods are produced over autumn and early winter, specifically from September to early January, each one succeeding in the completion of its life cycle. In particular, the developmental interval from egg to adult of the first, second and third autumn – early winter generations lasts for 26, 28 – 30 and 40 days, respectively (Fig. 1).

In order to accurately study the life cycle of *D. chrysippus*, mature hostplants of *Cynanchum acutum* ssp. *acutum* (Apocynaceae) (Fig. 2A), where females deposited their eggs, were examined in situ during the ovum, larval and pupal stage. Additionally, shoots of plants, where females deposited eggs, were transferred to the laboratory to allow further study. Fresh shoots with leaves were supplied on the morning of each day until pupation.

The female individuals were observed to lay eggs singly on the upper and the underside of the leaves, as well as on the stems of *C. acutum* plants (Fig. 2B). During oviposition, the forewings were seen to flutter continuously. It is worth noting that the presence of *D. chrysippus* was confirmed in every location where populations of *C. acutum* were recorded through the entire island, whereas the opposite did not occur. Thus, the specific plant species is here documented for the first time as its larval hostplant in Rodos, on which the butterfly has successfully developed through its full biological cycle.

C. acutum is an infrequent native perennial climbing shrub, which can be found along the coastline and prefers moist soils, rivers with patches of cane and reed beds. Such plants produce toxic cardenolides, which the larvae have adapted to remain unaffected and retain these toxins in their own bodies, which consequently pass on to the pupa and adults, protecting them against vertebrate predation. As a result, the majority of predators (birds, mammals, etc.) avoid consumption on account of their toxicity (Gil 2006; Malcom & Brower 1989).

Based on field and laboratory investigations it is clearly shown that the species' larvae develop through five instars, with developmental time dependent on temperature and availability of food sources. In accordance with the findings of comparative studies (Alam et al. 2019; Cockrell et al. 1993; Golestaneh et al. 2009; Smith et al. 1988; Talavera & Vila 2017; Zalucki 1982) and taking into account species' monitoring data collected by the author since 2012, the following may be stated: the first generation takes place in September and the duration from egg to adult lasts for about 26 days at an average maximum monthly temperature of 29°C ±2°C. The second generation takes place in October with an average maximum monthly temperature of 26°C ± 2°C and the development time from egg to adult lasts for 28 - 30 days. The third generation takes place in November and lasts up to December with average maximum monthly temperatures of 21°C ± 2°C and 18°C ± 2°C, respectively. The development time from egg to adult extends to about 40 days. Females were detected flying and laying eggs throughout December (6th, 12th and 22nd).

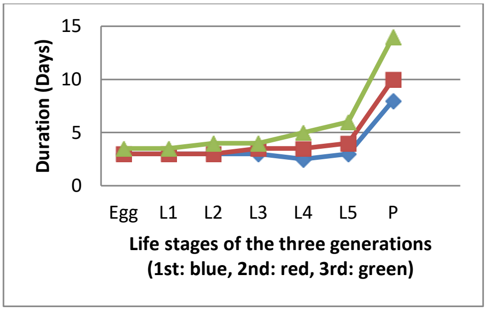


FIG. 1. Duration of early life stages of *D. chrysippus* over the three generations (L1, L2, L3, L4, L5 =1st, 2nd, 3rd, 4th and 5th instar, P=Pupa).

It is worth mentioning that during autumn (October, November, and early December), adults, eggs and larvae of all instars were also present at the same habitat, showing that there is an overlap of migrants gradually arriving in the region with the first-generation natives (male and female adults) (Fig. 2C). Final ovipositing was recorded on the 2nd of January, along with very few larvae of the first and the second instars, which fed on the last fresh leaves of the larval hostplant. At the end of January, with an average higher monthly temperature at 14°C ± 2°C, no traces of immatures were found, while the whole population of *C. acutum* plants had already withered, consequently larvae obviously died due to lack of food. It is pointed out that diapause was not confirmed at any biological stage. As perceived in the field, the seasonal absence of the larval hostplant lasts until April when new plants begin to sprout. However, it is noted that an extensive plant-to-plant research of the total population was carried out, from April to August, but neither adults nor traces of the species' immature stages were found.

Considering all the above, we conclude that the most important factor that affects species' overwintering population dynamic is the absence of its principal hostplant, *C. acutum*, for a limited time.

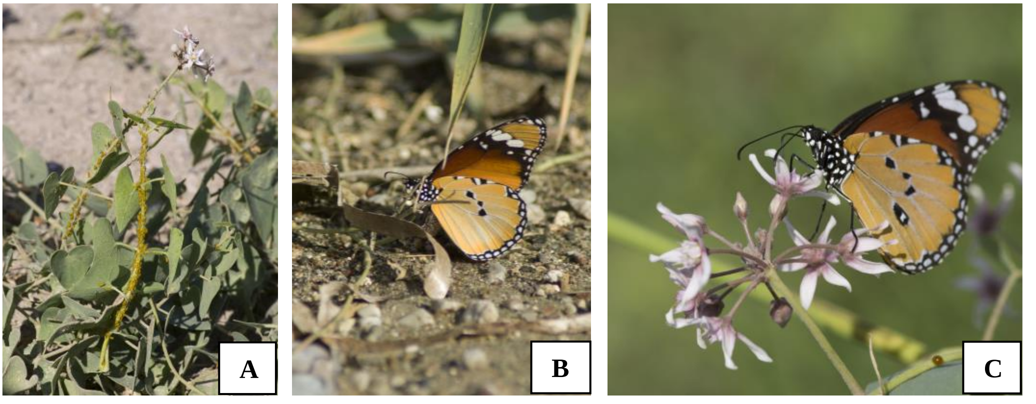


FIG. 2. (A) *C. acutum*, the larval hostplant of *D. chrysippus* in Rodos. Rodos, 12 Oct 2020; (B) Female of *D. chrysippus*, ovipositing on the leaves of its larval hostplant, *C. acutum*. Rodos, 19 Dec and 13 Nov 2020, respectively; (C) Male adult recently emerged, showing excreted meconium. Rodos, 25 Oct 2020. (Photos by Christos Galanos).

Immature stages

Egg: The eggs are white in colour when laid, turning creamy grey on the last day before hatching with an average size of 0.88 mm in diameter and 1.53 mm in height, barrel-shaped with a rounded top, flattened at base, longitudinally ribbed with ridges between the ribs (Fig. 3A, B).

Larva: The larvae passed through five instars, molted four times and the length of their fully grown bodies was measured (Fig. 4). Their frass pellets (Fig. 3L) and exoskeleton heads were collected and measured (Fig. 3M). Polymorphism in larval color was observed in the same studied areas, however it is not being considered as common (Fig. 6A, B, C).

1^{st} *Instar*: Immediately after hatching, the young larva consumes its eggshell. Body is cream to yellowish with lateral hairs and shiny black head with a pair of black horns. Larvae measured from 2.88 mm up to 3.16 mm in length (Fig. 3C, D) and were observed to consume the soft leaves of the hostplant. At this stage the larvae grow up to 4.30 mm. The exoskeleton head measured 0.67 mm in width.

2^{nd} *Instar*: At this stage the head capsule has a triangular white mark and a prominent white arch (Fig. 3E). Larvae grow up to 9.30 mm, while the exoskeleton head measured 1 mm in width.

3^{rd} *Instar*: An extra white arch becomes apparent at the rear periphery of the head capsule (Fig. 3F). At the same time, frass pellets became clearly bigger than in the previous instar and larvae grow up to 19.86 mm. Exoskeleton head measured 1.81 mm in width.

4^{th} *Instar*: Larvae were observed immediately after ecdysis to consume their discarded larval skins (Fig. 3G). At this stage frass pellets were found to be distinctly larger than in the previous instar, while larvae grow up to 25 mm. Exoskeleton head measured 2.02 mm in width.

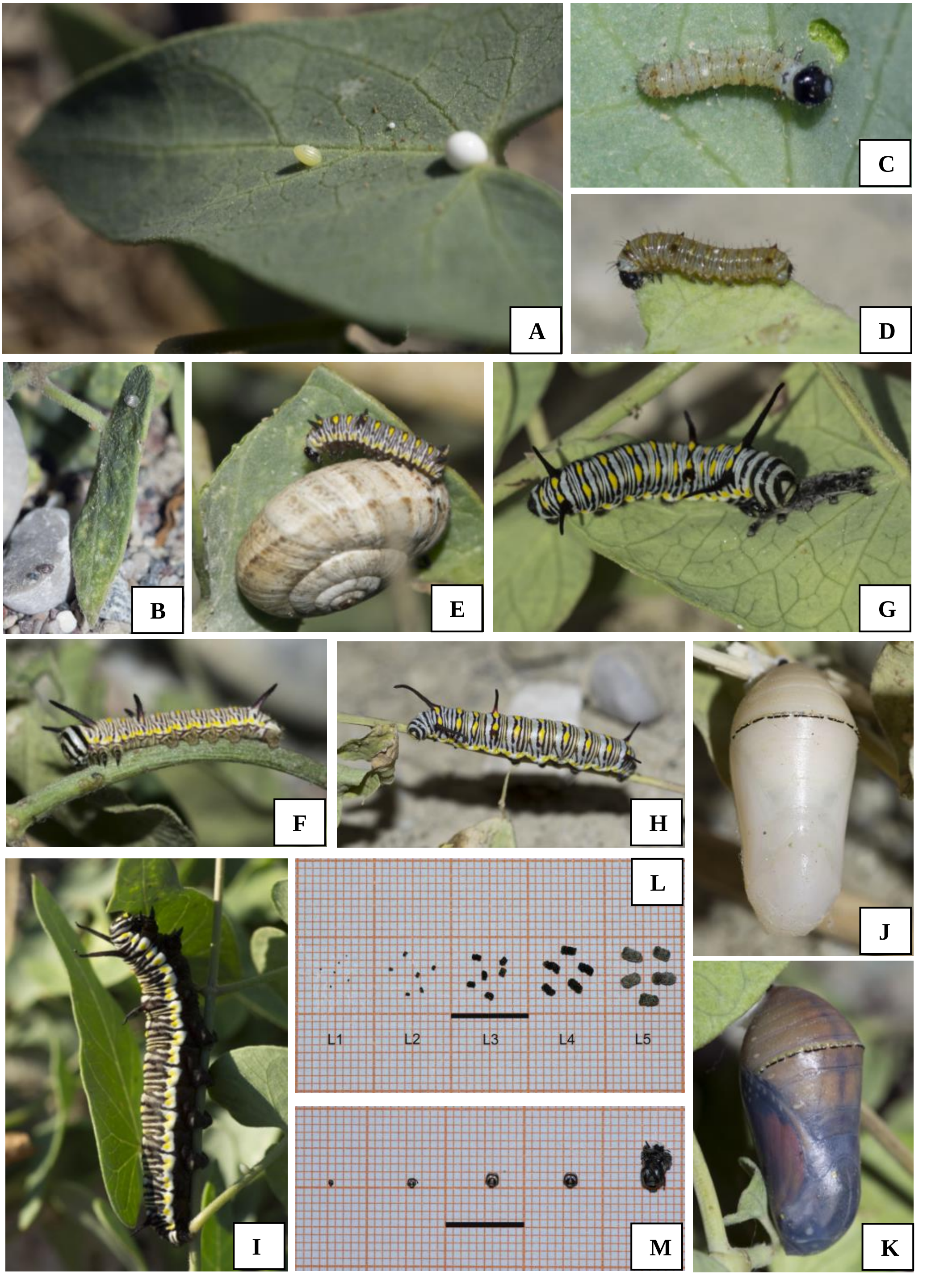
5th Instar: At this stage larvae grow up to 34.60 - 40 mm (Fig. 3H, I). Exoskeleton head measured 3.11 mm in width. Frass was collected and measured comparatively. The comparison revealed each instar to be noticeably distinct from the foregoing. It should be noted that the frass pellets were collected from a limited area where the larvae fed exclusively on the particular hostplant, *C. acutum*, both localised frass and larval specialisation being regarded as significant parameters in such an experiment (Southwood and Henderson 2000).

Pupa: At the pre-pupal stage the fully grown fifth-instar larvae become inactive, stop feeding and the last frass pellets are excreted. Subsequently, larvae become shorter and thicker before pupation and measured 21.10 mm in length. The pupa is translucent pale yellowish-green when first formed, eventually darkening while the wings and other parts of the body becoming visible through the pupal case. It measured 17.70 mm in length and 8.12 mm in width at its broadest point (Fig. 3J, K).

Conclusions

In conclusion, the present study aimed to assess the main reason for the species' temporary absence during winter, spring and summer, in particular, from late January to late August (Table 1). The results showed that the cessation of breeding is associated with the temporary absence of milkweed (*C. acutum*) from the end of January until April, which for the first time is documented here as the exclusive hostplant of the species on the island. No oviposition on other plant species of Apocynaceae or Convolvulaceae families, stated to be acceptable in other regions (Olivier 1993; Robinson et al. 2010) and which are present in Rodos, such as *Nerium oleander* L., *Ipomoea* sp. L. or *Gomphocarpus fruticosus* (L.) Ait. were observed. Despite the fact that suitable climatic conditions prevail in the area, specifically factors which favour the reproduction of the species throughout the year, i.e. protection from winds, absence of freezing temperatures, exposure to the right extent of sunlight, high humidity, as well as species' habitat requirements, such as the availability of water and nectar, the absence of the specific hostplant during the winter is decisive in terms of the lack of any form of life, up to arrival of the next wave of migration to the island at the end of the summer. Furthermore, this study contributes to the knowledge of the number of generations over the year, the identification of each larval instar of this species, the morphological traits and the development time of each life stage, as well as establishing life cycle duration depending on seasonal variations in ambient temperatures throughout the period of study.

FIG. 3. Metamorphosis of *D. chrysippus* during the early stages of its life cycle. A, B: *D. chrysippus* egg before and after hatching on fresh leaves of *C. acutum*; C, D: newly hatched 1st instar larva, dorsal and lateral view; E: 2nd instar larva moving on hostplant; F: 3rd instar larva, lateral view; G: newly moulted larva next to its exuvia after ecdysis from 3rd to 4th instar; H, I: fully grown 5th instar larva, lateral view; J, K: newly formed and fully grown pupa, dorsal and lateral view; L: comparison of collected frass pellets of fully grown larvae of the 1st, 2nd, 3rd, 4th and 5th instars. Scale bar: 1 cm; M: comparison of collected exoskeleton heads of all instars. Scale bar: 1 cm. Rodos 2020 - 2021. (Photos by Christos Galanos)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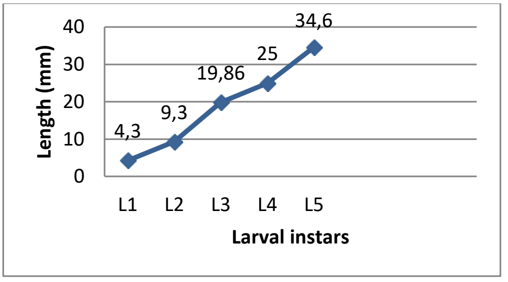

FIG. 4. Length of larval instars of *D. chrysippus*.

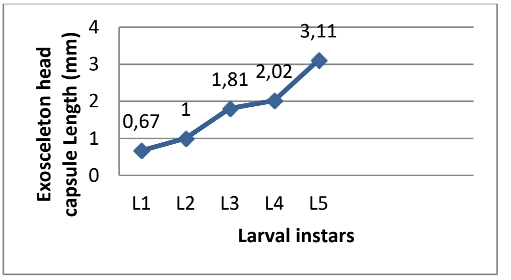

FIG. 5. Length of exoskeleton head capsules of different larval instars of *D. chrysippus*.

FIG. 6. Polymorphism in *D. chrysippus* larval color, in the same area of study. Rodos, 22-30 Oct 2020. (Photos by Christos Galanos).

Table 1: The life cycle of *D. chrysippus*, showing the annual emergence of adults, eggs, larvae and pupae in Rodos island. Each box corresponds to about 10 days.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Adult	■							■	■■■	■■■	■■■	■■■
Egg	■								■■	■■■	■■■	■■■
Larva	■								■■	■■■	■■■	■■■
Pupa									■	■ ■	■ ■	■

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Οικοβιολογικά χαρακτηριστικά της ημερόβιας πεταλούδας, *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae, Danainae) στη Ρόδο, Ελλάδα, καθ' όλη την περίοδο αναπαραγωγής του κατά τη διάρκεια του φθινοπώρου και, ειδικότερα, τον χειμώνα

ΧΡΗΣΤΟΣ ΓΑΛΑΝΟΣ

Ανεξάρτητος Ερευνητής επί της άγριας χλωρίδας και πανίδας λεπιδοπτέρων στο σύμπλεγμα των νήσων της Δωδεκανήσου, Πάροδος Φιλερήμου, 85101 Ιαλυσός, Ρόδος, Ελλάδα

ΠΕΡΙΛΗΨΗ

Η παρούσα έρευνα επικεντρώνεται στη βιολογία και την οικολογία της ημερόβιας πεταλούδας, *Danaus chrysippus*, στο νησί της Ρόδου, στην Ελλάδα, συνεισφέροντας στη γνώση του βιολογικού της κύκλου στη φύση, καθ' όλη την περίοδο αναπαραγωγής του είδους κατά τη διάρκεια του φθινοπώρου, και ιδιαίτερα κατά την περίοδο του χειμώνα, η οποία περιγράφεται και απεικονίζεται εδώ για πρώτη φορά για τη χώρα. Επίσης, το εύρος κατανομής, οι απαιτήσεις ενδιαιτήματος, οι οποίες σχετίζονται με τις μικροκλιματικές συνθήκες, που επικρατούν στις επιλεγμένες θέσεις αναπαραγωγής του είδους και η προτίμησή του προς το φυτικό είδος *Cynanchum acutum* ssp *acutum*, της οικογένειας Apocynaceae, ως μοναδικό ξενιστή των προνυμφών του στην περιοχή μελέτης για πρώτη φορά, συζητούνται.

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First occurrence of the black field earwig, *Nala lividipes* (Dermaptera: Labiduridae) in Greece

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ABSTRACT

The cosmopolitan black field earwig, *Nala lividipes* (Dufour, 1820) (Dermaptera: Labiduridae) is observed for the first time in Greece. A photographic record was obtained by the first author in 2016, from Salamis Island followed by a photographic citizen-science observation from Rhodes in 2021. Despite frequent inspections and efforts to raise public awareness, no additional samples or observations have been recovered. The poor study of Dermaptera in Greece and the absence of early warning systems for alien insects, may have delayed the detection of this alien species in the country. However, given the agricultural significance of *N. lividipes*, a short description is given to assist further data collection and monitoring. Finally, the potential establishment of the earwig in Greece, along with the impacts on the native flora and fauna are discussed.

KEY WORDS: Agricultural pest, alien species, cosmopolitan, early warning systems.

Introduction

Globalization and the constant rise of international trade have led to an unprecedented rise of species translocations and a great distortion of their native ranges (Hulme 2009). Many of these alien species have been associated with socioeconomic, human health and environmental impacts (Simberloff et al. 2013; Schindler et al. 2015; Vilà and Hulme 2016), something that highlights the need to monitor their occurrence and dynamic shifts in distribution ranges.

The reported cases of introductions and translocation of earwigs (Dermaptera) are rather uncommon, supported by only seven alien earwig species being recorded in Europe (Zafeiriou et al. 2021). The small number of reported alien Dermaptera could be related to both their low species richness as well as their cryptic nature. Until now, only two alien earwigs have been recorded from Greece, namely *Euborellia femoralis* (Dohrn, 1863) and *Forficula lucasi* Dohrn, 1865 raising the Greek earwig fauna to 18 species (Haas 2015, 2018; Demetriou et al.

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2021; Fontana et al. 2021; Zafeiriou et al. 2021; Kalaentzis et al. 2022).

The black field earwig, *Nala lividipes* (Dufour, 1820) is a cosmopolitan species, presumably of Afrotropical origin, which has gradually spread across all biogeographic realms. The species is widely distributed along the Mediterranean basin with records from Algeria, France, Italy (including Sardinia and Sicily), Malta, Morocco, Portugal, Spain (including the Balearic and Canary Islands), Tunisia, and Turkey (Albouy and Caussanel 1990; Mifsud and Taglianti 2008; Rasplus and Roques 2010; Anlaş and Kočárek 2012; Pages 2012) (Fig. 1).

The alien insect-fauna of Greece has been recently updated, holding a total of 469 species (Demetriou et al. 2021) in anticipation of further additions. Citizen scientists have been supplementing our knowledge around the presence and distribution of alien species, providing photographic observations and specimens of new alien to the country insects such as the feather-legged fly *Trichopoda pictipennis* Bigot, 1876 and lantana plum moth *Lantanophaga pusillidactylus* (Walker, 1864) (Demetriou et al. 2020; Kazilas et al. 2020; Dios et al. 2021). Alientoma, an online database for the alien insects of Greece, promoting public participation in scientific research (Kalaentzis et al. 2021b, c), was utilised to track the presence of *N. lividipes* in the country. In this publication, the black field earwig *N. lividipes* is reported for Greece for the first time.

FIG. 1: Known distribution of *Nala lividipes* (Dufour, 1820) in the Mediterranean basin. Countries where the species has been previously reported are shaded red, while the new records from Greece are depicted with a dot. The map was created with QGIS, v.3.14.16. Inset: The adult, female individual observed on Salamis island, Greece.

Materials and Methods

A female individual was observed and photographed in Greece: Saronic Gulf, Salamis island, Paloukia [37.962ºN, 23.514ºE], vii/2016, alt. 25 m, observed by E. Koutsoukos, in a house garden (Fig. 2A). The observed female was identified as *N. lividipes* following the identification key of Anlaş and Kočárek (2012). The species identification was also confirmed by taxonomic expert (Dr. Petr Kočárek, pers. comm., 2021). A second observation of a female *N. lividipes* was recorded in Greece: Dodecanese, Rhodes island, [36.3848ºN, 28.1312ºE], 23/vii/2021, alt. 50 m, observed by Mr. Benoît Segerer in a hotel garden, uploaded to iNaturalist platform (iNaturalist 2021) (Fig. 2B).

Online educational material regarding the taxonomy, distribution, morphology, ecology and impacts of *N. lividipes* was uploaded to the Alientoma webpage and social media platform (Kalaentzis et al. 2021a), in order to investigate the presence of further photographic observations.

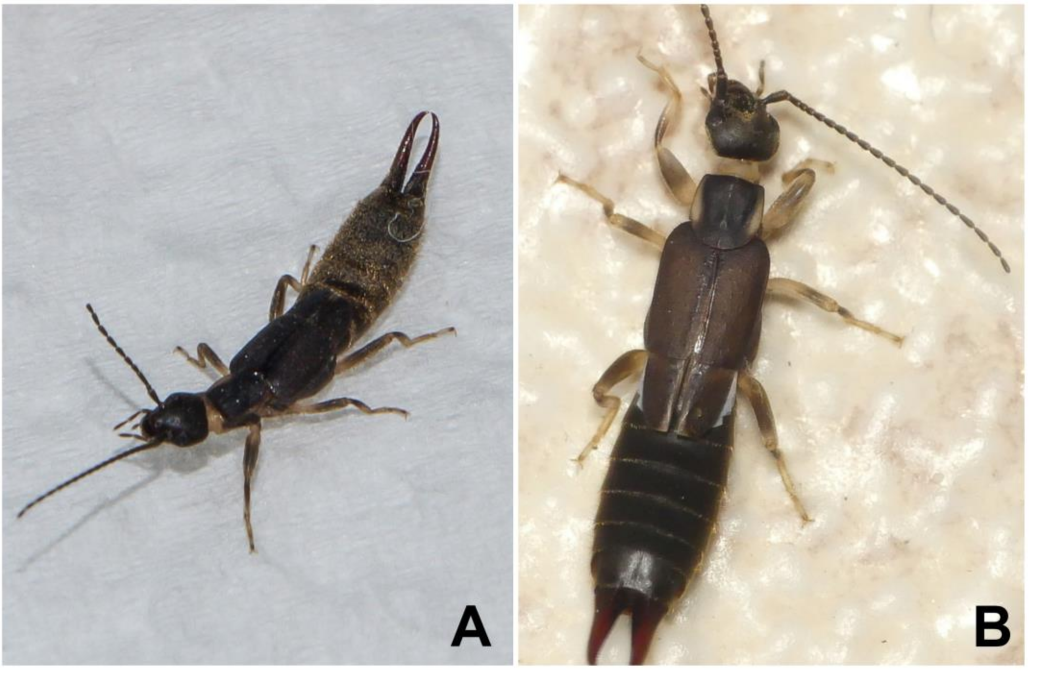

FIG. 2: Adult, female individual of *Nala lividipes* (Dufour, 1820) observed and photographed by Evangelos Koutsoukos on Salamis (A) and Mr. Benoît Segerer on Rhodes island (B).

Results and Discussion

These are the first reported occurrences of the black field earwig in Greece, which comprises the third alien species of the order Dermaptera in the country. Taking into consideration its broad distribution along the Mediterranean basin, its report in Greece comes as no surprise, although, the species is considered rather rare in Europe (Rasplus and Roques 2010). Despite of further inspections on the observation sites as well as the educational material uploaded to Alientoma, no additional material has been recovered. Consequentially, any speculations regarding the presence of established populations in the country seem to be rather uncertain. However, the long distance between the two observation sites may indicate that the species has long been present in the country, probably remaining unnoticed due to the insufficient study and public interest in Dermaptera on a national level.

A short diagnosis of the species is provided to assist further data collection by citizen-scientists and researchers: Head and thorax dark brown, antennae with >20 segments and wings fully developed. Tegmina and wing scales brown, opaque, rugose; tegmina with a ridge along the costal margin. Abdomen darker, black with sparse pale pubescence. Overall body length with forceps <15 mm (8.5-11 mm) (Fig. 2). Female forceps slender and almost straight; male forceps curved holding small teeth basally and a larger tooth towards their apex (Mifsud and Taglianti 2008; Anlaş and Kočárek 2012).

The black field earwig is a polyphagous insect feeding mostly on decaying organic matter or plant material (i.e. seeds, stems, roots), while it has also been reported as carnivorous (Albouy and Caussanel 1990; Simpson 1993). On one hand, it is often described as a crop pest, affecting beetroot, cotton, maize, sorghum, soybean, sunflower, and winter cereal cultivations, particularly when high population density is reached (Hargreaves 1970; Radford and Allsopp 1987; Simpson 1993). On the other hand, *N. lividipes* has also been utilised in classical biological control programs, preying on crop-damaging pests (i.e. aphids and moths) (Albouy and Caussanel 1990; Binns et al. 2020). For example, in Australia *N. lividipes* is acknowledged as the main predator of *Pieris rapae* (Linnaeus, 1758) (Kapuge et al. 1987), which is a serious, accidentally introduced pest of crucifers (CABI 2021). To our best knowledge, no damages have been reported by the black field earwig in Europe. However, given the socioeconomic effects associated with the species in question, it is important to monitor its distribution and feeding habits in the invaded territories, as well as to assess its diverse effects on agriculture and native fauna.

In conclusion, this article renders *N. lividipes* the third alien earwig reported from Greece, raising the number of its Dermaptera fauna to 19 species. This manuscript is intended to alert the scientific community and the public of the potential threats of a new alien insect in Greece, using citizen science towards the implementation of early warning systems for alien and invasive alien species.

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Πρώτες παρατηρήσεις του Δερμαπτέρου *Nala lividipes* στην Ελλάδα

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ΠΕΡΙΛΗΨΗ

Το κοσμοπολίτικο δερμάπτερο, *Nala lividipes* (Dufour, 1820) (Dermaptera: Labiduridae) παρατηρείται για πρώτη φορά στην Ελλάδα. Το είδος φωτογραφήθηκε από τον πρώτο συγγραφέα το 2016, από το νησί της Σαλαμίνας ακολουθούμενο από μια φωτογραφική παρατήρηση ενός πολίτη-επιστήμονα από τη Ρόδο, το 2021. Συχνές επιθεωρήσεις στον τόπο παρατήρησης και προσπάθειες ευαισθητοποίησης του κοινού, δεν έχουν μέχρι στιγμής παράσχει επιπλέον υλικό. Η ελλιπής μελέτη των δερμάπτερων στην Ελλάδα και η απουσία συστημάτων έγκαιρης προειδοποίησης για τα ξενικά έντομα, πιθανώς να καθυστέρησαν την παρατήρηση του ξενικού αυτού είδους στην χώρα. Παρόλα αυτά, δεδομένης της γεωργικής σημασίας του είδους, δίνεται μια σύντομη περιγραφή του προς υποβοήθηση της περαιτέρω συλλογής δεδομένων αλλά και της παρακολούθησης του. Τέλος, η πιθανή εδραίωση του δερμάπτερου στην Ελλάδα, καθώς και οι επιπτώσεις στην αυτόχθονη χλωρίδα και πανίδα, είναι υπό διερεύνηση.

