

Is *Bactra bactrana* (Kennel, 1901) a novel pest of sweet peppers?

E. Ruditakis^{1*}, S. Morin² and J. Baixeras^{3*}

¹Laboratory of Entomology, Hellenic Agricultural Organisation ‘Demeter’, Plant Protection Institute of Heraklion, Heraklion, Greece: ²The Robert H. Smith Faculty of Agriculture, Food and Environment, Department of Entomology, The Hebrew University of Jerusalem, Rehovot, Israel:

³Universitat de València, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Paterna, Spain

Abstract

This is the first report of *Bactra bactrana* (Kennel, 1901) (Lepidoptera: Tortricidae) attacking a major solanaceous crop, sweet pepper *Capsicum annuum* L. The infestation was detected in two greenhouses at the area of Tympaki (Southern Crete, Greece). The moth larvae caused typical symptoms of a fruit borer with numerous small holes on the surface of the peppers and extensive damage on the inside of the fruit as a result of the feeding activity. Unknown factors facilitated this major shift in host range since *B. bactrana* is typically a stem borer of sedges. In addition, the pest status of *B. bactrana* is currently under question, as in both cases the infestations by the moth were associated with significant yield losses. *B. bactrana* was moderately controlled with chemicals registered for Lepidoptera management in sweet pepper due to the boring nature of the infestation. Some comparative taxonomic notes are provided to facilitate accurate pest discrimination of related *Bactra* species. Finally, biological attributes of the species are summarized and are discussed from pest control and ecological perspectives. Because *Bactra* species have been used in augmentative releases for the control of sage, the implications of our findings on the release of biocontrol agents are placed in perspective.

Keywords: *Bactra bactrana*, *Capsicum annuum*, pest, Solanaceae, Greece, fruit borer

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Introduction

The host range of insect species is an important parameter in modern agriculture because significant decisions are based on this knowledge, such as the development or the release of a novel biological control agent in an area (Sheppard *et al.*, 2005; van Lenteren *et al.*, 2006). However, studies have shown extreme plasticity in the host range of insect species that can occur very slowly or in a relatively very short time

frame, with changes potentially observable in human life time in the latter. The factors facilitating the host shift and subsequently the pest status have been thoroughly reviewed by Price *et al.* (2011). Thus, native herbivores have been reported to shift to pest status, becoming threat to agro-ecological environments. Notable cases of pest outbreaks have been linked with climate change (Patterson *et al.*, 1999; Giorgi & Lionello, 2008; Jepsen *et al.*, 2008; Traill *et al.*, 2010; Kocsis & Hufnagel, 2011; Parkash *et al.*, 2013) or with changes in land use patterns. For example, the pollen beetle *Meligethes aeneus* (Fabricius) shifted to oilseed rape after the expansion of the crop cultivation in Northern Europe. The insect adapted successfully to the new environment and rapidly developed resistance to insecticides used for control, a combination that made pest control extremely difficult in many cases (Hokkanen, 2000; Slater *et al.*, 2011).

Here, we present a striking case of host shift for the moth *Bactra bactrana* (Kennel, 1901) (Tortricidae), a typical stem

*Author for correspondence

Phone: +30 2810 302309

Fax: +30 2810 245858

E-mail: eruditakis@gmail.com and

Phone: +34 963543636

Fax: +34 963543733

E-mail: joaquin.baixeras@uv.es

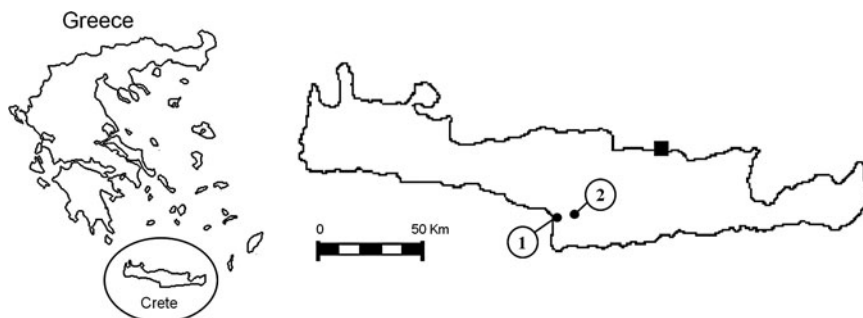


Fig. 1. Map of Crete showing the areas that the infestations by *B. bactrana* were detected: No 1, first pest report at Makrimaliana, No 2, second pest report at Anadasmos. Dark square is Heraklion, where the Institute is located. Side map: Crete in relation to mainland Greece.

borer of sedges with a widespread distribution, that is reported for the first time to infest pepper as a fruit borer. *B. bactrana* infestation was associated with economic losses in greenhouse crops of Crete, Greece. A comparative analysis for identification of the pest is provided and the main biological attributes are summarized.

First pest report

Location and crop

On 9 May 2014, reports of extensive damages on a greenhouse pepper crop in the area of Tympaki (Makrimaliana) were communicated to our laboratory (fig. 1). Initial observations indicated feeding activity of an unknown insect pest. An onsite inspection was immediately arranged with the local agronomists of the Tympaki Agricultural Cooperative. The damage levels were estimated based on fruit production of the particular day. Approximately 30% of the fruits were infested. A month after the initial report (10 June 2014), a second sweet pepper greenhouse was reported infested, with fruits bearing the same symptom pattern. The location was again in the area of Tympaki, however, at a different site (Anadasmos) approximately 7 km east of the initial report. The percent of infested fruit was estimated at 15% in this case.

Damage description

Infested fruits were bearing multiple holes on the surface, sometimes surrounded by intense green discoloration (fig. 2a, b). The opening of the hole was occasionally covered by silk fibres. Within the fruit, Lepidoptera larvae were found causing extensive damage on the internal surface followed by dark discoloration (fig. 2c). In some cases, secondary infestations were observed and macroscopic symptomatology denoted bacterial infestation. No symptoms or damage was observed on any other parts of the plant vegetation. Dense numbers of moths were found hiding under the debris on the soil surface. Moths were easily detected as they were flying in response to human activity during the thorough inspection process.

Specimen collection

Infested peppers as well as unidentified adult moths were collected for examination in the laboratory. Collected fruits were stored in an insect proof cage, under controlled conditions (16 h L: 8 h D, $25 \pm 1^\circ\text{C}$) to allow larval development.

Once development was completed, most larvae exited the fruit, moved under the tissue paper that was placed at the bottom of the container, and pupated within a silk cocoon. Pupae were collected, placed separately in a Petri dish and were monitored daily. Emerged adults matched the morphology and the wing colour pattern of the field collected adults.

Species identification

General dissection procedures followed standard methods for genitalia preparation for the optical microscope (Robinson, 1976). Tools for dissection and cleaning included spring microscissors, Dumont forceps (number 5) and fine model brushes (5/0) under a MZ9.5 Leica stereomicroscope in glass embryo dishes of 30 mm. Optical microscopic images were taken through a Leica Macroscope Z16 APO equipped with a digital camera DFC 500. Images were edited by Photoshop CS3 (Adobe).

Nomenclature of the taxa follows Gilligan *et al.* (2012). General and genitalia terminology follows Horak (1999) and Klots (1970), respectively. Setal map interpretation follows Hinton (1946). Five male adults, and two last instar larvae were examined. Based on the comparison with museum material and publications later discussed, the specimens were assigned to *B. bactrana*.

The species *B. bactrana*

Introduction to the genus *Bactra* and distributional notes

Bactra Stephens 1829 is a worldwide distributed genus of Olethreutinae including about a hundred species (Gilligan *et al.*, 2012). It is a rather homogeneous monophyletic group of moths adapted to grass habitats. Their larvae are internal feeders mostly on Cyperaceae (sedges), but have been recorded also on other representatives of the order Poales as Juncaceae and Poaceae. Horak (2006) updated the diagnosis of the group and discussed the main relations with other genera of Olethreutini. The elongate wings with a cryptic grass habitat adapted wing pattern is a key characteristic and allows superficial identification of the adults, but, the species level taxonomy is far from obvious. The best taxonomical references on the genus are still the works by Diakonoff (1956, 1962, 1963, 1964). Aarvik (2013) included nine species of *Bactra* in Europe including Canary Islands and Azores. *B. bactrana* is distributed across Western and Southern Europe. Other *Bactra* species generally have broad distributions, although few are

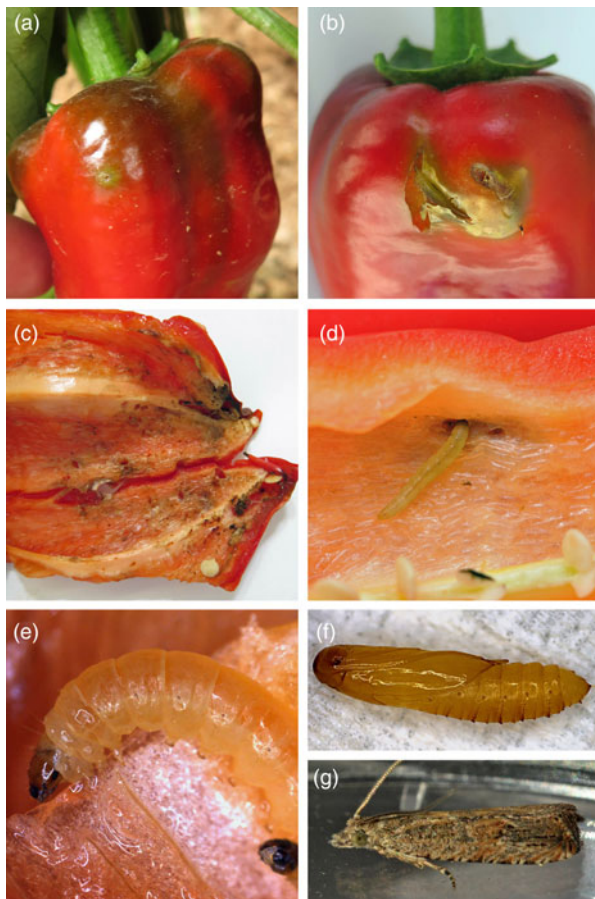


Fig. 2. Damages and general aspect of immatures and adult. (a) and (b) external surface of sweet peppers infested by *B. bactrana*. Cracks on the dry epidermis of the fruit can also be occasionally detected as the supporting tissue underneath is damaged by the galleries created by the mining activity of the larvae; (c) and (d) internal surface of the fruit showing galleries and tissue deterioration; (e) details of larva; (f) pupa; (g) adult.

considered geographically restricted taxa. *Bactra lancealana* (Hübner, 1799), *Bactra furfurana* (Haworth, 1811) and *Bactra robustana* (Christoph, 1872) are the most widely distributed representatives. Complementary to *Bactra lacteana* Caradja, 1916, which is mostly distributed in central and northern countries, *Bactra venosana* (Zeller, 1847) shows similar distributions to that of *B. bactrana*. *Bactra suedana* Bengtsson (1990) has a Nordic distribution (Bengtsson, 1990). Finally, *Bactra legitima* Meyrick, 1911 is an African species present in the Canary Islands and *Bactra minima* Meyrick, 1909 a South Asiatic representative that penetrates in the Canary Islands and Azores (Karsholt & Vieira, 2005; Aarvik, 2008). Most of the records of *B. bactrana* are referred to its junior synonym *Bactra gramini-vora* Meyrick. Diakonoff (1962) complained about the lack of reliable records for this species, which is systematically confused with *B. lancealana*. Out of continental Europe, *B. bactrana* records include Central Asia (India, Pakistan) and Africa (north and tropical, including Madagascar and Canary Islands). This suggests a broad distribution through the temperate regions of the Old World.

Biology – host range

Larvae of *Bactra* are stem borers. According to the food plant database compiled by Brown *et al.* (2008), there are records of food plants for 19 species of *Bactra*. Food plants include mostly Cyperaceae of the genera *Cyperus* (18 records), *Schoenoplectus* (two), *Carex* (four), *Bolboschoenus* (two), *Eriophorum* (three), *Scirpus* (five), *Cladium* (one) and *Kyllinga* (five). Few records make reference to Poaceae (*Cynodon*, two records) and Juncaceae (*Juncus*, nine records). Trematerra & Ciampolini (1989) updated all current biological knowledge, including food plants and life cycle, with special reference to the European species. According to these authors, *B. bactrana* is a bivoltine species overwintering as larvae in the stem of the plant.

Diagnosis of *B. bactrana* infestation

Identification of *B. bactrana* adults and its allies

Figures 2g and 3a show the general appearance of the adult of *B. bactrana*. Wing pattern of *Bactra* are cryptically variable and of difficult interpretation. No specific identification should be taken based on wing pattern. The shape and design of the forewing is characteristic of the genus. The forewing is elongate, with pointed apex. General colour is light to dark brown with greyish suffusion. As in most Olethreutinae, forewing upperside transversal elements appear degenerated. Forewing pattern includes typical strigulae variably developed on the costa. Scales on discal cell and from discal cell to apex tend to be variably darker giving a longitudinally striated general aspect to the forewing. Male genitalia examination (fig. 3b) is important for identification and the only reliable morphological way for identification of *Bactra* species. In all *Bactra*, the tegumen is much more developed than the vinculum. The tegumen may be rather globose, incurved, with the uncus-socii strongly connected. Preparing the tegumen separately (fig. 3c) increases the diagnostic power of this character. The vinculum is strongly connected to the juxta and phallus. The uncus usually is bent and edged by rod like spines. The sacculus is globular with an internal sac opened obliquely. There are typically 7–8 spines in the spc1 of *B. bactrana*. Additional short spines (or bristles) may appear at the posterior edge of the sacculus (Ms series; medio-saccular series of Diakonoff, 1962); their diagnostic value is more reduced. The cucullus is elongate in *B. bactrana* with the costa slightly sinuous, moderately pointed in the apex; the ventral edge is curved, somewhat quadrangular at the distal third, covered by hairs and bristles to the edge of the valva.

Larval description of *B. bactrana* and notes

In spite of a relatively well-known biology in some species of *Bactra*, little detailed information on the larval identification is available. The first data on the larvae of *B. bactrana* may have been collected by Fletcher (1932) but the author is unclear about the true identity of the larvae examined. Swatschek (1958), based on *B. lancealana*, and McKay (1959), based presumably on *B. verutana*, discussed the characteristics of the genus. Figure 4 includes a setal map of the *B. bactrana* larvae examined. Typical olethreutine character is the presence of a L2 closer to L1 than to L3 on a trisetose group in T1. However, SD1 is closer to SD2 than to XD2 where McKay (1959) finds them equidistant. On A7 the group SV is trisetose

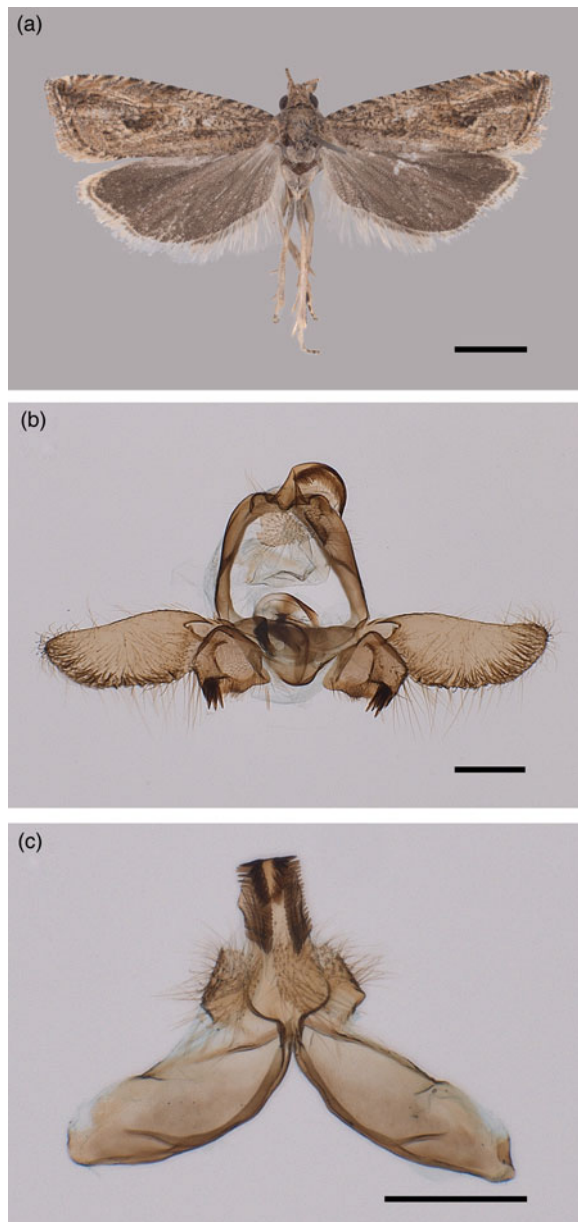


Fig. 3. Adult identification. (a) *B. bactrana* (from Greece); (b) male genitalia; (c) tegumen. Scale bars *a* = 2 mm; *b* and *c* = 200 µm.

in *B. bactrana* and *B. lancealana*, but bisetose in *B. verutana*. A8 and A9 coincident in the three species with a common pinacula for D1 (anterior) and SD1 (posterior).

The economic importance of *B. bactrana* globally

Zhang (1994) compiled records of eight economically important species of *Bactra* in Asia (*B. bactrana*, *Bactra commensalis*, *B. minima*, *Bactra phaeopis* and *B. venosana*), Europe (*B. furfurana* and *B. lancealana*) and North America (*B. verutana*). However, the consideration of economic importance requires consideration in relation to a geographical perspective. Some species of sedges (e.g., *Cyperus rotundus*) are among the

most invasive species of plants, and information available on their negative effects is considerable (Riemens *et al.*, 2008). Their distribution has been facilitated by human activity and there is no doubt that some species of *Bactra* have followed their distribution. Their narrow range of food plant hosts has made them adequate candidates for weed control. A good example is provided by the introduction into the Hawaiian Islands (from the Philippines) of *B. venosana*. The presence of this nutgrass natural enemy in Hawaii was confirmed by Swezey (1926) and was the subject of interesting research (Poinar, 1964a, b). The series of papers by Frick and collaborators (Frick & Garcia, 1975; Frick, 1978; Frick & Wilson, 1980; Frick, 1982) are also informative. However, *Cyperus* species (especially *Cyperus esculentus*) are commercially beneficial plants in some areas of the world, either as food (Sánchez-Zapata *et al.*, 2012) or as biofuel (Zhang *et al.*, 1996). Consequently, *Bactra* species may be considered as pests wherever nut-grass is considered as a crop (Tsung-Kai, 1978).

Current status and pest management

In this study, we found that *B. bactrana* can cause significant damages to commercial scale greenhouse crops and can therefore be considered as a potential future pest of sweet peppers. There are very few reports on *B. bactrana* as a pest and therefore the potency of the species and its damage capacity are basically unknown. To date, there are no additional reports of infestations by *B. bactrana* in the Tympaki region of Crete. However it is not until the end of the next cropping season that we will have a clear perspective on the level of expansion of the infestations. Unfortunately, valid predictions on infestation expansion at this stage are currently impossible due to absence of necessary information. Basic studies on the biology of *B. bactrana* may be required in order to estimate the risks from this novel pest of sweet peppers.

Based on the records of the Tympaki Agricultural Cooperative, once the infestation was detected it was immediately controlled with insecticide. The first greenhouse received three applications of the insecticides indoxacarb and chlorantraniliprole. The second greenhouse received one application of spinosad, lufenuron and beta-cyfluthrin. All these insecticides are registered for Lepidoptera control in greenhouse sweet peppers. Pest control treatments were ongoing until acceptable damage levels were obtained. Efficacy of application has not been evaluated. However, based on preliminarily laboratory observation, the vitality of the larvae within the fruit was not affected after insecticide application. In all cases, larvae that were extracted from sprayed fruits responded normally to stimulus (disturbance with a fine paint brush) and completed development under laboratory conditions. This suggests that pest chemical control should focus mainly on the first instar larvae before entering the fruit and on the eggs laid on the plant surface. Beneficials preying on Lepidoptera eggs or egg parasitic hymenoptera may also play a key role in a revised integrated pest management (IPM) scheme. It has been demonstrated that under field conditions, natural parasitism by the egg parasitoid *Trichogrammatoidea bactrae* Nagaraja can successfully restrict the moth populations (Visalakshy & Jayanth, 1995). Control of the pest could also be achieved by targeting the adults as they come in contact with the treated plant foliage during oviposition. However, insecticides with adulticide activity should be identified and this may not be easy, since registered novel chemistries are in most

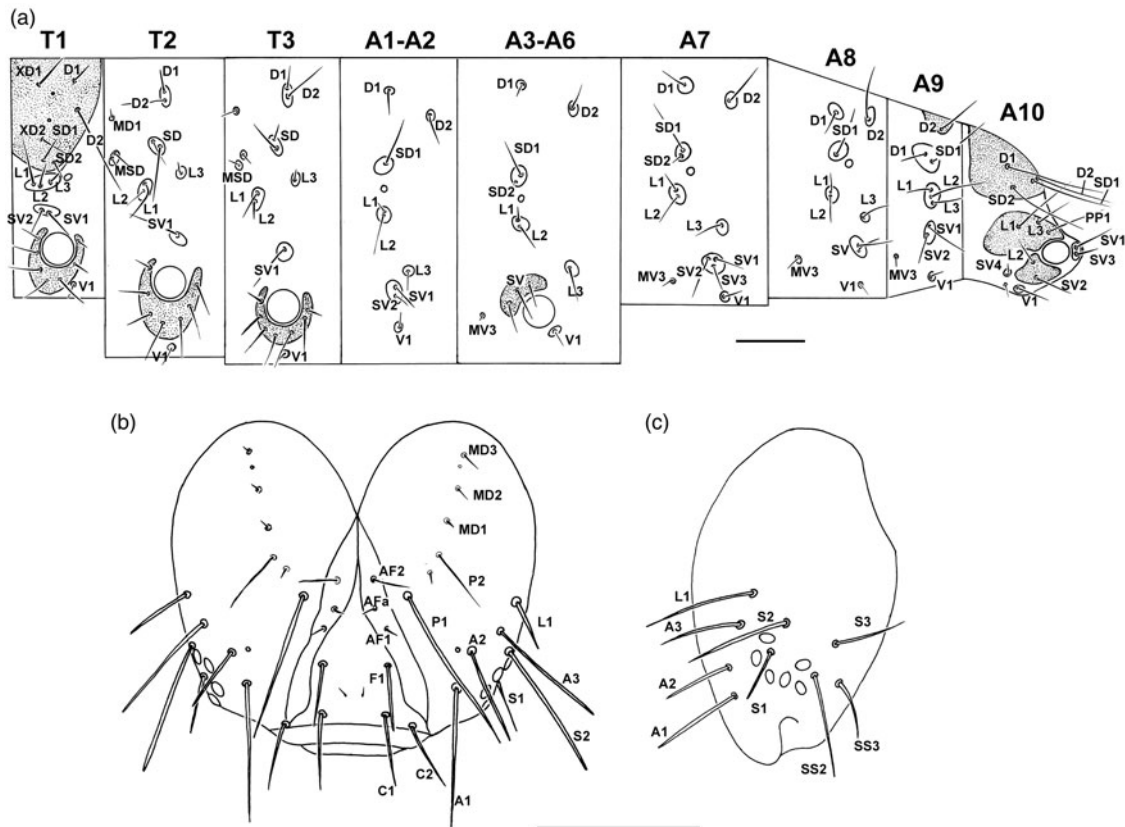


Fig. 4. Larval chaetotaxy. (a) Thorax and abdomen; (b) head frontal view; (c) head left side. Scale bars = 0.5 mm.

cases efficient but exclusive larvicides (Roditakis *et al.*, 2014). Monitoring and eventually mating disruption by pheromone traps is another possible future step (Booij & Voerman, 1984). Based on laboratory observations, pupation is expected to occur in the soil or under protected niches. Potentially, soil inhabiting beneficials (i.e., entomopathogenic nematodes) may be an important parameter in a successful pest control scheme.

Conclusion

This is the first report of *B. bactrana* developing as fruit borer on a solanaceous host, such as *Capsicum annuum*. Based on the observed damage levels in a commercial scale crop, it is suggested that *B. bactrana* can be considered as a potential pest for sweet pepper. However, the most striking finding in this study is the rare and notable shift in host range. This finding is even more important if one considers that other species of this genus, such as *Bactra verutana* Zeller, have been used in augmentative releases to facilitate biological weed control (Frick & Chandler, 1978; Prick *et al.*, 1983). The value of biological control has been demonstrated in numerous studies (van Lenteren & Woets, 1988; Cruttwell McFadyen, 1998; Thomas & Reid, 2007; Bale *et al.*, 2008; Naranjo *et al.*, 2015). However, there are some limitations and risks involved when releasing natural enemies (Collier & Van Steenwyk, 2004; Andersen *et al.*, 2005; Delfosse, 2005; Barratt *et al.*, 2010; Simberloff, 2012). Delfosse (2005) analyzed the risks of biological control and listed factors that are linked to uncertainties

with respect to hazards that can result from the release of natural enemies. One of the least investigated parameters is the adaptation of the biocontrol agent population to the new environment (Hufbauer & Roderick, 2005; Phillips *et al.*, 2008). Several cases of rapid adaptive evolution for biocontrol agents have been reported (Bean *et al.*, 2012; Szűcs *et al.*, 2012) and such changes in key life parameters have resulted in significant adverse effects on non-target organisms, that could not be predicted prior to the release of the organism (Louda *et al.*, 2003). For example, the weevil *Rhinocyllus conicus* Froeh exhibited both geographical and host range expansion significantly effecting native thistles and subsequently the density of native tephritid flies (Louda *et al.*, 1997). Recently, Lu *et al.* (2015) demonstrated that additional factors, such as the climate change, are forcing biocontrol agents to evolve. Global warming is driving shifts in phenology and geographical range of species, influencing the effect of biocontrol agents on non-targets (Simberloff, 2012).

Our study is a striking example of the unexpected risks involved in the release of insect biocontrol agents. It is not clear why and how this transition from a stem feeder to a fruit borer occurred. Several factors facilitating host plant shifts have been summarized by Price *et al.* (2011). Unfortunately, with the current level of knowledge, it is not possible to identify the causes of this phenomenon. Most of all, we are unaware of a report on the presence of *B. bactrana* in Crete prior to this report. A recent invasion to the island could be an explanation. However, based on the published data of the species

distribution, *B. bactrana* has been previously reported in areas where the cultivation of sweet pepper under glass is extensive for at least a decade (i.e., Southern Europe such as Spain and Italy). This suggests that a number of factors coexisted in the area of Tympaki that eventually enabled this rare host shift. It will be important to identify the factors that drive the host shift, thus potentially enabling prediction of potential damage prior to its occurrence or efficient screening of biocontrol agents for potential non-target effects.

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