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Destruction of a protected habitat by an invasive alien species: the case of *Cydalima perspectalis* (Walker, 1859) in the box tree formations of Liguria (North-West Italy) (Lepidoptera: Crambidae)

D. Badano, D. Caracciolo, M. Mariotti & V. Raineri

Abstract

The box tree moth, *Cydalima perspectalis* (Walker, 1859), an invasive alien species of Asian origin associated with box trees, gained immediate attention in Europe due to the damage caused to ornamental stands and its rapid spread. However, its effects on natural box tree formations remained poorly investigated, especially in Southern Europe. In Liguria (North-West Italy), this species attacked xerotermophilous box tree formations on rocky substrate, a protected habitat (5110) after the EU 92/43 Directive, seriously endangering its long-term survival. Since detection in 2013, this species defoliated almost the whole area covered by habitat 5110, until experiencing a population crash after depleting its only trophic resource. We present here the evolution of the invasion and the population dynamics of this invasive alien species in Liguria during the years 2016-2017.
KEY WORDS: Lepidoptera, Crambidae, invasive alien species, Natura 2000, special areas of conservation, *Buxus sempervirens*, Italy.

Distruzione di un habitat protetto da parte di una specie aliena invasiva: il caso di *Cydalima perspectalis* (Walker, 1859) nelle formazioni a bosso della Liguria (Italia nord-occidentale) (Lepidoptera Crambidae)

Riassunto

La piralide del bosso, *Cydalima perspectalis* (Walker, 1859), specie aliena invasiva di origine asiatica associata al bosso, ha immediatamente destato attenzione in Europa a causa del danno provocato alle piante ornamentali e alla sua rapida diffusione. Tuttavia, i suoi effetti sulle formazioni naturali a bosso sono rimasti scarsamente studiati, specialmente nell'Europa meridionale. In Liguria (Italia nord-occidentale), ha attaccato formazioni a bosso xerotermofile su substrato roccioso, un habitat protetto (5110) secondo la Direttiva EU 92/43, mettendo seriamente a repentaglio la sopravvivenza di quest'ultimo a lungo termine. A seguito del primo rilevamento, nel 2013, questa especie ha defogliato quasi l'intera area coperta dall'habitat 5110, fino a subire un crollo della popolazione a seguito dell'esaurimento della sua unica risorsa trofica. Presentiamo qui di seguito le dinamiche demografiche e l'andamento della popolazione di questa specie aliena invasiva in Liguria negli anni 2016-2017.
PAROLE CHIAVE: Lepidoptera, Crambidae, specie aliene invasive, Natura 2000, zone speciali di conservazione, *Buxus sempervirens*, Italy.

Destrucción de un hábitat protegido de parte de una especie invasora: el caso de *Cydalima perspectalis* (Walker, 1859), en las formaciones de boj de Liguria (Noroccidental de Italia) (Lepidoptera Crambidae)

Resumen

La mariposa del boj, *Cydalima perspectalis* (Walker, 1859), es una especie invasora de origen asiático asociada al boj, ha despertado enseguida la atención en Europa a causa del daño provocado a las plantas ornamentales y a su rápida difusión. Sin embargo, sus efectos sobre las formaciones naturales de boj, ha quedado escasamente estudiada, especialmente en Europa meridional. En Liguria (Italia Noroccidental), ha atacado formaciones de boj xerotermófilo sobre substrato rocoso, un hábitat protegido (5110), según la Directiva 92/43, poniendo en serio peligro la supervivencia de este último, a largo plazo. A continuación de la primera introducción, en el 2013, esta especie casi tiene defoliado el área entera cubierta por el hábitat 5110, hasta padecer un hundimiento de la población a causa del agotamiento de su único recurso trófico. Presentamos aquí el seguimiento de las dinámicas demográficas y el curso de la población de esta especie invasora en Liguria en los años 2016-2017.
PALABRAS CLAVE: Lepidoptera, Crambidae, especie invasora, Natura 2000, zona especial de conservación, *Buxus sempervirens*, Italia.

Introduction

Invasive alien species pose one of the main threats to biodiversity because they can alter whole habitats, modifying their structure or targeting keystone species, with consequent deterioration of ecosystem services. Moreover, they can have a direct impact on native species through competition, predation and as vector of pathogens (NEW, 2016). The accidental introduction in Europe of the Asiatic box tree moth, *Cydalima perspectalis* (Walker, 1859), is indicative of how an invasive alien species can seriously endanger a protected habitat, in this case box tree formations on rocky substrates, preserved after the EU 92/43 Directive within the Natura 2000 habitat 5110 "Stable xerothermophilous formations with *Buxus sempervirens* on rock slopes (*Berberidion pp*.)". First reported from Germany and the Netherlands in 2007, the box tree moth explosively spread to most of Europe, reaching almost all central and southern European countries in less than 10 years (KRÜGER, 2008; VAN DER STRATEN & MUUS, 2010; NACAMBO *et al*., 2013, 2014; MATOŠEVIĆ *et al*., 2017). This species was found for the first time in Italy in 2010, quickly invading most Italian regions, including Liguria, where it was found in 2013 (BELLA, 2013; RAINERI *et al*., 2017). The larva of the box tree moth is monophagous, feeding on species of the genus *Buxus* (LEUTHARDT & BAUR, 2013). Although this moth has been reported to feed also on other plants, its ability to develop on hosts other than box trees is strongly debated and it has been dismissed (VAN DER STRATEN & MUUS, 2010; WAN *et al*., 2014; MATOŠEVIĆ *et al*., 2017). Larval development lasts from 14 to 30 days, according to the temperature (LEUTHARDT & BAUER, 2013). In its original range, this species is very adaptable, ranging in a wide variety of climates, from cold temperate to subtropical ones, probably explaining its quick adaptation and diffusion in Europe (NACAMBO *et al*., 2014). The number of generations per year varies according to the environmental conditions: from three to five in China, two or three in the introduced European range (NACAMBO *et al*., 2014; WAN *et al*., 2014; GÖTTING & HERZ, 2017; MATOŠEVIĆ *et al*., 2017). NACAMBO *et al*. (2014) demonstrated that *C. perspectalis* might colonize most of Europe by adapting its life cycle and the number of annual generations, according to mean temperature, potentially causing the maximum damage in the mild climates of Southern Europe, where this moth might reach the highest number of generations. The spread of box tree moth is not hampered by native predators or parasitoids, as this species is unpalatable due to sequestered alcaloids from the toxic box tree (LEUTHARDT *et al*., 2013). In fact, only a few parasitoids were reported from the introduced range (WAN *et al*., 2014; GÖTTING & HERZ, 2016). Moreover, few non chemical treatments appear effective against this species, especially *Bacillus thuringiensis* var. *kurstaki,* although there are some difficulties to use it within protected areas, besides being relatively expensive and not having a residual action. Some repellents also appear promising (GÖTTING *et al*., 2017; MOLNÁR *et al*., 2017). Sex pheromone traps appear to be a highly effective method to assess the number of generations per year, the spread of this moth and the timing of treatments (KAWAZU *et al*., 2007; KIM & PARK, 2013; SANTI *et al*., 2015). The box tree

moth gained immediate attention due to the damage caused on ornamental plants in urban settings (i.e. parks, garden, cemeteries), but the study of this pest in natural environments remained poorly investigated, mostly limited to Central European forests with box tree undergrowth (KENIS *et al*., 2013; JOHN & SCHUMACHER, 2016). RAINERI *et al*. (2017) and MARIOTTI *et al*. (2017) documented the presence of this moth in the box tree formations comprised in the habitat 5110 within Special Areas of Conservation (SAC), included in the Natura 2000 network of Liguria, also reporting for the first time the serious damage caused by this pest. Ligurian box tree formations, despite their relatively modest extension, are particularly noteworthy because they grow on ophiolitic bedrock, thus resembling the shrublands thriving under similar conditions in the Balkan Peninsula. Moreover, they are also remarkably ancient, in some cases existing for at least 10000 years (MARIOTTI, 1986, 1994, 2008; VAGGE, 1997).

The aim of the present work is to increment the previous observations of RAINERI *et al*. (2017) and MARIOTTI *et al*. (2017) on the population dynamics and behaviour of *C. perspectalis* in the box tree formations on the arid ultramaphic slopes of Liguria.

Materials and methods

Surveys were carried out in all Ligurian SACs with presence of habitat 5110, aiming to monitor the presence of the box tree moth and the state of the infestation in comparison with the previous visits made from the end of 2016 (RAINERI *et al*., 2017). We followed the same procedures of RAINERI *et al*. (2017), carrying out visual inspections of the phytomass, to check the damage to plant crowns, and estimating average damage both to individual plants and to the whole formation. The estimate of infestation intensity was based on the observed density of larvae, while the abundance of adults was assessed by collecting them with a hand net. Damage levels were represented after a three-grade scale: 1, minimum; 2, medium; 3, serious, following RAINERI *et al*. (2017). Particular attention was also dedicated at spotting eggs, larvae and pupae on the shrubs, following the so-called "walking census method" (JERVIS & KIDD, 1996).

Results and discussion

The life cycle of box tree moth in Liguria

Our observations confirm that the 1st instar larva is the overwintering stage of the Ligurian populations of box tree moth. At the dropping of temperatures in autumn, the 1st instar larva spins a loose cocoon of seta on the lower surface of a box tree leaf, for protection against adverse climatic conditions (Fig. 1). In Liguria, we observed overwintering larvae from the end of October/beginning of November. The larvae then remain in diapause for the whole winter, until resuming activity and feeding at the beginning of spring, usually at the end of March or April, according to altitude and exposition. After a month of continuous feeding, the larvae pupate, spinning a cocoon among leaves. The pupation of the overwintering generation occurs in May and the first adults emerge in June. At this point, probably due to the highly favourable environmental conditions, the population of the moth demographically explodes, reaching hundreds of thousands of specimens and causing severe damage to the plants. At the same time, there is a complex and complete overlapping of stages, hampering the recognition of the different generations (Figs. 2-3). Surveys made in August in the SAC IT1342806 Monte Verruga-Monte Zenone-Roccagrande-Monte Pu brought evidence of the simultaneous presence of larvae of all instars, from newly hatched specimens to mature 6th instar larvae, besides pupae and adults (Figs. 2-3). Therefore, while adults were feeding or mating, thousands of larvae continuously fed on box trees. In Liguria, we observed adults almost continuously from June to the beginning of October according to the climatic conditions, without a clear period of absence marking the end of well distinct and recognizable generations. Nevertheless, it is usually possible to distinguish a last generation of

adults flying in autumn (end of September-beginning of October), which lays the eggs from which the overwintering larvae hatch.

Destruction of habitat 5110 and population crash

Once the moth reaches and colonizes a box tree formation, the plants are subjected to a constant pressure by the larvae, which voraciously feed on their leaves, from spring to autumn. *C. perspectalis* disperses widely and it is very effective to locate its host plant. RAINERI *et al*. (2017) and MARIOTTI *et al*. (2017) reported the presence of *C. perspectalis* for all Ligurian box tree formations included in SACs, which were equally effected by the presence of this pest. Surveys carried out during the end of the year 2016, showed that in all Ligurian SACs the entire surface of box tree formations was infested by *C. perspectalis* and the damage was estimated as serious in all examined habitats (RAINERI *et al*., 2017; MARIOTTI *et al*., 2017) (Tab. 1). In preparation for a potential habitat destruction, seeds of *B. sempervirens* were collected in 2017 in the SAC IT1342806 Monte Verruga-Monte Zenone-Roccagrande-Monte Pu and then preserved in the seed bank of the University of Genoa. As expected, during the year 2017, the situation worsened considerably. In spring, the population density and the reproductive rate of box tree moth did not slow down, and the larvae consumed all the available foliage (Figs. 4, 5). We observed this trend in all surveyed sites, under a whole range of different conditions (e.g. surface, coverage, altitude, exposition and humidity). All box tree scrublands were equally and intensively affected, both those growing on arid, rocky slopes, and those in more humid settings, such as along brooks and rivulets. Moreover, we also registered high infestation rates and total defoliation in nearby box tree stands growing in understory, both within mixed broadleaves and pine wood forests. During the peak of the infestation, the soil under the box trees was also entirely covered in frass (Fig. 7).

Table 1.– Infestation rate of *Cydalima perspectalis* and damage estimation in Ligurian SACs with box tree formations in the years 2016 and 2017.

SAC code and name	Estimated area habitat 5110 (ha)	% infested area as of 2016	Damage grade as of 2016	% infested area as of 2017	Damage grade as of 2017	Presence of box tree sprouts	Presence of secondary fungal pathogens
IT1331909 Monte Zatta - Passo Bocco - Passo Chiapparino - Monte Bossea	95.78	70 30	3 - serious 2 - medium	100	3 - serious	•	
IT1333307 Punta Baffe - Punta Moneglia - Val Petronio	13.08	-	-	100	3 - serious		
IT1342806 Monte Verruga - Monte Zenone - Roccagrande - Monte Pu	391.47	70 20 10	3 - serious 2 - medium 1-minumum	100	3 - serious	•	
IT1343412 Deiva - Bracco - Pietra di Vasca - Mola	3.89	70 30	3 - serious 2 - medium	100	3 - serious	•	•
IT1343415 Guaitarola	1.16	70 30	3 - serious 2 - medium	100	3 - serious	•	
IT1344422 Brina e Nuda di Ponzano	23.90	70 30	3 - serious 2 - medium	100	3 - serious	•	•

During a survey made during August 2017 in the SAC IT1342806 Monte Verruga-Monte Zenone-Roccagrande-Monte Pu, i.e. the most extensive Ligurian box tree formation, we observed a very high density of larvae belonging to different stages, besides pupae and adults (see above) and the shrubs were already completely defoliated (Figs. 2-3). The surviving larvae were feeding on box tree barks, since no other resources were available. At the end of summer 2017, the moth population crashed, and we did not spot larvae or adults, while a year before they both were extremely abundant. Inspections in

all other formations in Liguria showed the same trend: the box trees plants were completely defoliated and largely decorticated, while the pest was apparently absent, well below detection level (Tab. 1). These observations are clearly indicative of a sudden and deep population crash resulting from the complete depletion of the only available trophic resource. We also did not detect any sign indicative of the occurrence of predators or parasitoids that can explain such a sudden and drastic drop in abundance. Finally, it is clear that *C. perspectali*s is not able to shift to other plants, even when its host, the box tree, is completely depleted. However, when the population density reached its peak and the box tree leaves did not offer any shelter, the last instar larvae pupated into nearby trees and shrubs. Therefore, these larvae spun the cocoon on other species, such as *Fraxinus ornus*, *Pinus nigra* and *Juniperus communis*, entirely wrapping and rolling up their leaves and causing a possible collateral damage to these plants (Fig. 6).

During the autumn of 2017, all the box tree formations were completely defoliated with several specimens massively decorticated, seriously questioning the possibility of habitat recovery (Tab. 1). In almost all surveyed sites, some box trees produced young sprouts after the disappearance of the moth, both at their base and at their apex, according to the damage caused by the larvae (Figs. 8, 9). However, the viability of the plants, and eventually the preservation of the habitat, still need to be assessed (Tab. 1). During careful inspections made in autumn 2017, we noted the presence of a handful of overwintering larvae and other clues of the presence of the pest, such as grass, filaments of seta and minor damages to the sprouts, suggesting that *C. perspectalis* is actually still present in the surveyed sites, but at an extremely low population density. These remaining specimens still pose a threat to the sprouting plants as they could potentially compromise the survival of the remaining box trees. In two sites, we also documented the outbreak of fungal pathogens following the box tree moth infestations, affecting young sprouts and causing a complete desiccation of the remaining foliage (Tab. 1). In the SAC IT1333307 Punta Baffe-Punta Moneglia-Val Petronio, an area previously characterized by an extensive coverage of habitat 5110, the survey did not detect any sprout or other sign of the survival of the plants after the invasion, suggesting that the whole habitat is already lost (Tab. 1) (Fig. 5).

Conclusions

European box tree populations are undergoing a process of fragmentation, especially in Southern countries, therefore a highly invasive phytophagous species, such as *C. perspectalis,* can be devastating also representing a serious menace to the survival of *Buxus sempervirens* in the wild (DI DOMENICO *et al*., 2012). JOHN & SCHUMACHER (2013) documented the invasion of the box tree moth in Nature Reserve of Grenzach-Whylen, which harboured the largest German box tree formation. In this locality, *C. perspectalis* completely defoliated the box trees in a few years and the plants were not able to recover after such a heavy attack. The death of the box trees triggered a change in the ecological succession due to modification in ground cover, causing an irreversible alteration of the forest ecosystem (JOHN & SCHUMACHER, 2013; KENIS *et al*., 2013). While the environmental conditions of Ligurian sites with 5110 habitats are very different from those of a German forest, the results of the spreading of the box tree moth are perfectly comparable, with the alien species reaching a peak in abundance until complete defoliation of its host and resulting disappearance. Although some box trees may produce young sprouts after the attack, their survival is highly questionable due to the low grow rate of this shrub and the constant danger of re-invasion of the moth from nearby infested sites. In the SAC IT1333307 Punta Baffe-Punta Moneglia-Val Petronio, the once verdant thick formation of box trees, now reduced to an expanse of dead twigs, rises serious doubts about the survival of this unique habitat also in the other surveyed areas, which were apparently invaded later. Despite the invasion dynamics by *C. perspectalis* are relatively well known, the interactions of this alien species in natural ecosystems and especially with insect communities still need to be explored and should not be underestimated. Indeed, *C. perspectalis* may prove to be a highly successful competitor against indigenous insects associated with box tree, such as the geometrid moth *Peribatodes buxicolaria* (Mabille, 1873), an endemism of Southern France (Montagne d'Alaric, Aude) which develops on

Buxus sempervirens (HOFMANN, 1893; SEITZ, 1912; ROBINEAU *et al*., 2007). The potential impact of the box tree moth on natural box tree formations and associated communities is probably worse than initially assessed.

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D. B.
Università degli Studi di Genova
DISTAV
Corso Europa, 26
I-16132 Genova
ITALIA / *ITALY*
E-mail: davide.badano@gmail.com
https://orcid.org/0000-0001-9715-3107

D. C.
ARPAL
Ufficio Biodiversità
Via Bombrini, 8
I-16149 Genova
ITALIA / *ITALY*
E-mail: daniela.caracciolo@arpal.gov.it

M. M.
Università degli Studi di Genova
DISTAV
Corso Europa, 26
I-16132 Genova
ITALIA / *ITALY*
E-mail: m.mariotti@unige.it
https://orcid.org/0000-0002-9595-4629

*V. R.
ARPAL
Ufficio Biodiversità
Via Bombrini, 8
I-16149 Genova
ITALIA / *ITALY*
E-mail: valter.raineri@arpal.gov.it

*Autor para la correspondencia / *Corresponding author*

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Figs 1-3.– *Cydalima perspectalis* (Walker), larvae. **1.** Overwintering first instar larva inside cocoon; **2.** Different instars feeding on box tree bark; **3.** Young and old instars feeding defoliating box tree. (Photo credits: 1, D. Badano; 2-3 ARPAL, Li. Bi. Oss. Archive).

Figs 4-9.– Damage and traces left by *Cydalima perspectalis* (Walker). **4.** Serious damage to box tree formation at IT1344422 Brina e Nuda di Ponzano; **5.** Serious damage to box tree formation at IT1333307 Punta Baffe - Punta Moneglia - Val Petronio; **6.** Collateral damage to *Fraxinus ornus* caused by larvae of *C. perspectalis* while spinning cocoon; **7.** Frass of *C. perspectalis*; **8.** Box tree sprouts at the base of the plant after defoliation; **9.** Apical box tree sprouts (Photo credits: 4-5, D. Badano; 6-9 ARPAL, Li. Bi. Oss. Archive).