

CYDALIMA PERSPECTALIS (WALKER, 1859) (LEPIDOPTERA,
CRAMBIDAE) AND THE THREATS FOR THE NATURE 2000
HABITAT 5110 IN LIGURIA (NW-ITALY)

VALTER RAINERI^{1*}, FRANCO BONECHI², DANIELA CARACCILO¹,
PAOLO CRESTA³, MAURO MARIOTTI⁴

¹ ARPAL, Ufficio Biodiversità. Via Bombrini 8, 16149 Genova, Italy.

² Comando Gruppo Carabinieri Forestali di Imperia, Via Argine sinistro, 78,
18100 Imperia, Italy

³ Ente Parco dell'Aveto, Via Marré 75 A, 16041 Borzonasca, Italy

⁴Università degli Studi di Genova, DISTAV, Corso Europa 26, 16132 Genova, Italy.

*Correspondence to: valter.raineri@arpal.gov.it

ABSTRACT

The authors report on the widespread occurrence of *Cydalima perspectalis* in spontaneous stands of Box (*Buxus sempervirens*) in Liguria corresponding to "Habitat 5110 - Stable xerothermophilous formations with *Buxus sempervirens* on rock slopes (*Berberidion* p.)" according to EU Habitats Directive. After a preliminary assessment, six Special Area of Conservation (SACs) are affected, where severe damage extends to some 370 hectares, corresponding to approximately 70% of the area. Finally, the authors suggest some guidelines for future surveys and control measures to limit the damage.

KEY WORDS

Cydalima perspectalis, *Buxus sempervirens*, Box Tree Moth, Habitat 5110, ALIEM project

INTRODUCTION

The Box tree moth, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae), is an alien invasive species accidentally introduced in Europe from China, out of its primary range which extends from India to the Russian Far East, Corea and Japan. It can produce from 1 to 5 generations per year, depending on the climate

(Wan et al., 2014). Other information on the biology and ecology of the species are reported by Wan et al. (2014) and Nacambo et al. (2014). Several species and varieties of Box, including cultivars and hybrids (Inoue *et al.*, 1982; Leuthard & Baur, 2013; Wan et al., 2014) [e.g. *Buxus sempervirens* L., *B. s.* var. *rotundifolia* Baill., *B. microphylla* Siebold & Zucc. and *B. sinica* (Rehder & E.H.Wilson) M. Cheng var. *insularis* (Nakai) M. Cheng] are the most important hostplants for its larvae. The preference would be for the *rotundifolia* variety (Wan, 2014), but further observations on field and experiments are needed. The first record of *C. perspectalis* from Europe dates back to 2007, when larvae were found defoliating a bush of *B. sempervirens* in Baden-Württemberg (Krüger, 2008; Korycinska, & Eyre, 2009). Since then the species underwent fast spreading into several other European countries. Tables 1a and 1b show the progress of species spread on the basis of the first records in each European countries and Italian regions.

Regarding Albania, where the Box tree moth was not recorded until now, one of the authors (Mauro Mariotti) on 8th June 2017, on the occasion of the mid conference tour of the 9th International Conference on Serpentine Ecology, observed the presence of *C. perspectalis* in the natural Box tree communities of Kuturman Massif near Librazhd (Prefecture of Elbasan).

Accidental release by the Italian delegation at the Sochi Olympic Games is considered responsible for diffusion of the moth in Imeretia Valley (Georgia) in 2012, as since that date the species rapidly spread in a relic forest near Sochi (Kintrishi State Nature Reserve and Mtirala National Park) and in the Caucasus Biosphere Area (Matsiakh, 2014; 2016), potentially threatening the existence of the local Box populations which, according to some authors, should be ascribed to an endemic species named *Buxus colchica* Pojark.

Tab.1a. – Chronology of the spread of *C. perspectalis* in European countries

Year	Country	Source
2007	Germany	Krüger, 2008; Korycinska, & Eyre, 2009
	The Netherlands	Muus et al., 2009
	Switzerland	EPPO, 2008; Käppeli, 2008
2008	France	Feldtrauer et al., 2009
	Great Britain	Mitchell, 2009
2009	Austria	Rodeland, 2009
	Liechtenstein	Slamka, 2010
2010	Italy	FEI, 2010
2011	Belgium	Castells et al., 2011
	Czech Republic	Šumpich, 2011
	Hungary	Sáfián & Horváth, 2011
	Romania	Szekely et al., 2011
	Slovenia	Matjaž Jež in Sáfián & Horváth, 2011
	Turkey (European)	Hizal, 2012
2012	Croatia	Koren & Črne, 2012
	Georgia	Matsiakh, 2014
	Russia	CABI/EPPO, 2012; Matsiakh, 2014
	Slovakia	Pastoralis et al., 2013
2013	Denmark	Hobern, 2013
	Bulgaria	Beshkov et al., 2015
2014	Montenegro	Hrnčić & Radonjić, 2014
	Spain	Pérez-Otero et al., 2014
2015	Bosnia-Hercegovina	Ostojic et al., 2015
	Greece	Strachinis et al. 2015
	Serbia	Konjević et al., 2015; Stojanović et al., 2015
2017	Albania	Mariotti (unpublished data; see the text).

Tab.1b. – Chronology of the spread of *C. perspectalis* in Italian regions

Year	Italian region	Source
2010	Veneto	FEI, 2010
2012	Lombardia	Tantardini et al., 2012
2012	Piemonte	Regione Piemonte, 2014
2012	Emilia Romagna	FEI, 2012
2012	Toscana	CESPEVI, 2012
2013	Sicilia	Bella, 2013
2013	Friuli-Venezia Giulia	Governatori, 2013
2013	Lazio	Regione Lazio, 2014; FEI, 2014b
2013	Marche	FEI, 2013
2013	Liguria	Unpublished original data (see in the text)
2014	Abruzzo	Demetra, 2016; FEI, 2015
2014	Umbria	Demetra, 2016
2015	Trentino Alto Adige	Ferrari, 2015 (Province of Trento)
2015	Campania	Regione Campania, 2015
2016	Calabria	Demetra, 2016

In Italy it was firstly recorded in October 2010 from a garden nearby Vicenza (Veneto) (FEI, 2010), and thereafter in other regions (Tab. 1b).

As it happens for most invasive species during the first phases of their spreading, so far there are neither natural enemies capable of keeping the moth populations under control nor forms of resistance developed by infested plants. Regarding climate, the Box tree moth seems to be a “hard” resistant species, as in fact it tolerates also quite low temperatures, down to -16 C° according to Tuba *et al.* (2014). For this reason it has an enormous spread potential, especially in most southern European countries, in particular Italy. As in countries of origin *C. perspectalis* feeds also on *Euonymus japonicus* Thunb., *E. alatus* (Thunb.) Siebold, *Ilex purpurea* Hassk., *Pachysandra terminalis* Siebold & Zucc. and *Murraya paniculata* (L.) Jack, and in Sochi on *Ruscus colchicus* Yeo, *Ruscus aculeatus* L., *Eriobotrya japonica* (Thunb.) Lindl., *Acer campestre* L., *Fraxinus*

excelsior L. and *Rubus* spp., (Trokhov & Kaurova, 2015), there is a high risk that the species may shift on other spontaneous plants of our flora.

In Liguria the Box three moth was observed for the first time in summer 2013 in Hanbury Botanical Gardens (Ventimiglia, IM) by the Gardens's staff (unpublished data), probably arriving from France, but reputedly it was present in the surroundings of Genova before that time and it has been widespread in 2014 (Redazione IVG.it, 2014; FEI, 2014a). Records from this region, and from Italy as a whole, were at first exclusively from parks and gardens. Following observations carried out on 22nd July 2016 in Monte Carme di Stodomelli (SP), one of us (F.B.), working for the State Forestry Corp, informed the Regional Agency for Environmental Protection (ARPAL) about a severe attack suffered by wild *Buxus* plants via a concise report in which concern about ongoing defoliation was raised, and *C. perspectalis* put forward as the likely pest.



Fig. 1. Adults of *C. perspectalis* and some melanic specimens

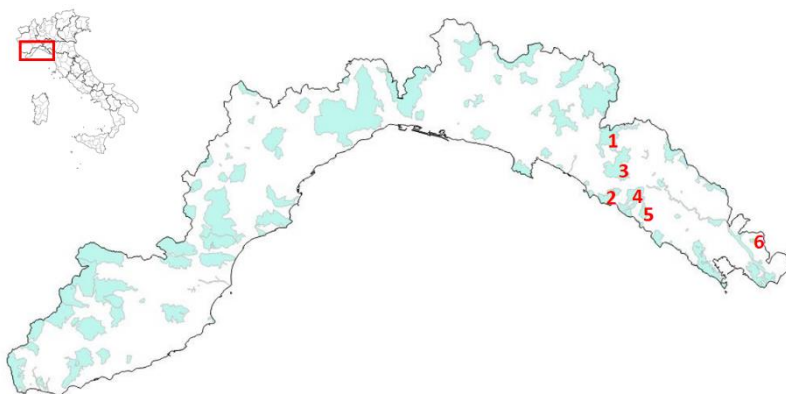


Fig. 2. Ligurian SACs and presence of the habitat 5110. The number is referred to the first column of Tab. 2 and Tab. 4.

Significancy of the issue derives from the Ligurian spontaneous formations of *Buxus* being essentially comprised within Natura 2000 Habitat 5110, “Stable xerothermophilous formations with *Buxus sempervirens* on rock slopes (*Berberidion p.*)”, which is protected after the EU 92/43 Directive, under the Annex I (Mariotti, 2008).

According to the Natura 2000 European Database (http://ec.europa.eu/environment/nature/natura2000/data/index_en.htm), in the European Union Habitat 5110 is known for 310 Natura 2000 sites (SCIs, SPAs and SACs) distributed across 9 countries: France (146 sites), Spain (109), Italy (33), Belgium (12), Germany (3), Greece (3), Portugal (2), Luxembourg (1), and the UK (1). Among 33 Italian sites, 11 are in Umbria, 6 in Liguria, 6 in Marche, 4 in Abruzzi, 2 in Tuscany, 2 in Latium, 1 in the autonomous province of Trento and another in Basilicata (this last needs verification).

In Tab. 2 the six Ligurian Natura 2000 sites (Fig. 2) including Habitat 5110 are listed along with their extension. Under the decree of 7th April 2017 of the Ministro dell’Ambiente e della Tutela del Territorio e del Mare, published 28th April 2017, all the sites have been designated

Special Areas of Conservation (SACs) (DM, 2017). They all pertain to the Mediterranean biogeographical region and have been subjected to conservation measures (Regione Liguria, 2017).

In some countries (e.g. France) natural formations of Box tree have a large extension while in others they represent fragmented isolated stands of ancient origin with great scientific importance and ecological value (Di Domenico *et al.*, 2012).

Tab.2. – Ligurian SACs with the presence of Habitat 5110.

N.	SAC code and Name	Surface (ha)	Surface habitat 5110 (ha)	%
1	IT1331909 Monte Zatta - Passo Bocco - Passo Chiapparino - Monte Bossea	3,034.00	95.78	3.16
2	IT1333307 Punta Baffe - Punta Moneglia - Val Petronio	1,308.00	13.08	1.00
3	IT1342806 Monte Verruga - Monte Zenone - Roccagrande - Monte Pu	3,757.00	391.47	10.42
4	IT1343412 Deiva - Bracco - Pietra di Vasca - Mola	2,031.00	3.89	0.19
5	IT1343415 Guaitarola	581.00	1.16	0.20
6	IT1344422 Brina e Nuda di Ponzano	239.00	23.90	10.00
Total		10,950.00	529.28	4.83

In Italian environments the Box tree enters either as a typical or differential species of several forest and semi-forest associations (Biondi *et al.*, 2006), namely *Buxo-Quercetum pubescentis* Br.-Bl. (1931) 1932 [within sub-alliance *Buxo-Quercenion pubescentis* Zólyomi & Jakucs ex Jakucs 1960]; *Cephalanthero longifoliae-Quercetum ilicis* Biondi & Venanzoni ex Biondi, Gigante, Pignatelli & Venanzoni 2002 *buxetosum sempervirentis* Biondi, Casavecchia & Gigante 2003; *Cytiso sessilifolii-Quercetum pubescentis* Blasi, Avena & Scoppola 1982 *buxetosum sempervirentis* Pirone, Corbetta, Ciaschetti, Frattaroli & Burri 2002; *Chamaecytiso spinescentis-Juniperetum oxycedri* Pirone & Cutini 2002

buxetosum sempervirentis Pirone & Cutini 2002. Limited to some Italian regions, the Box habitat extends onto calcareous soils.

In Italy, Liguria is the only region to show particular formations of *Buxus* settled on ophiolitic bedrock which are reminiscence of those exclusively occurring in some localities between northern Greece and Albania. Most of these formations, from east Liguria, can refer to syntaxon *Euphorbio ligusticae* - *Genistetum desoleanae* Nowak 1987 corr. Vagge 1997 *buxetosum sempervirentis* Mariotti 1994, in the alliance *Alysson bertolonii* Pignatti 1977 (*Rosmarinetalia officinalis* Br.-Bl. ex Molinier 1934). Recently (Authors' Team, 2015) the association with *Genista desoleana* (and thereafter its sub-association with *Buxus*) has been included in *Genistion lobelii* Molinier 1934 (*Ononidetalia striatae* Br.-Bl. 1950; *Festuco valesiaca* - *Brometea erecti* Br.-Bl. & Tüxen ex Br.-Bl. 1949). Regarding its physiognomy and structure, the vegetation appears as “pseudomaquis” or “supra-mediterranean shrubs”, and it is naturalistically valuable for its restricted occurrence on ultramafic grounds and the presence of almost endemic plants associated to ophiolites, including several endemic species or with restricted range of distribution. Moreover, the Box tree enters with significant levels of coverage into shrub- and tree-layers of other forest associations, such as relatively xerophilous ones dominated by Holm oak, Cork oak or Pubescent oak, but also into more meso-hygrophilous ones, with Alders and Hornbeams in marshland or riparial areas.

Pollen analysis of peatland sediments in Pian del Lago (near Bocco di Bargone) set the presence of spontaneous Box trees to over 10,000 years ago (Mariotti, 1986, 1994, 2008; Parola, 2008, 2013; Vagge, 1997). In Liguria, the Box tree is also characteristic of some forest formations dominated by Maritime pine, described by Biondi and Vagge (2015) as a distinct association named *Buxo sempervirentis* - *Pinetum pinastri* Biondi and Vagge 2015.

The aim of this preliminary work is to report on the presence and distribution of *Cydalima perspectalis* in the spontaneous Box tree

communities, with special reference to the Ligurian Natura 2000 sites, and to provide a first evaluation of its impact.

MATERIALS AND METHODS

Following the alert raised by the State Forestry Corp (CFS) for Carme di Stodomelli, ARPAL, governing the Ligurian Biodiversity Observatory (Li. Bi. Oss.), run some surveys with the aim to monitor the development of the infestation both in the site and above all in SAC “IT1342806 Monte Verruga - Monte Zenone - Roccagrande - Monte Pu”, where Habitat 5110 has a major role in shaping the local environment, even as concerns its extension. During summer/autumn 2016 a number of visits have therefore been carried out (Tab. 3).

Tab. 3 – *Sites and dates of inspections*

Data	SAC's Code	Actors
3 August 2016	IT1342806	ARPAL
9 August 2016	IT1342806	ARPAL, CFS, UNIGE-DISTAV
11 August 2016	IT1344422	CFS
14 August 2016	IT1343415	CFS
14 August 2016	IT1343412	CFS
23 August 2016	IT1331909	Parco dell'Aveto
31 August 2016	IT1342806	ARPAL
13 September 2016	IT1331909	Parco dell'Aveto
11 October 2016	IT1344422	CFS
26 October 2016	IT1342806	ARPAL

Due to the lack of a standard protocol to monitor plant damage, visual inspections were carried out on plant crowns, during surveys, with particular attention to youngest leaves and twigs, recording the various developmental stages of the pest and estimating both average

damage on individual plants and land cover by the vegetation formation. Attack intensity was estimated as damaged phytomass (dry or lacking) for each plant and also as the amount of *C. perspectalis* larvae present on the plants, based on direct observations of massive infestations (e.g. a large density of specimens, at different larval instars, on the same plant; caterpillars using silk threads to increase their spread, etc.). Damage levels were then represented after a value scale subdivided into three grades: 1, minimum; 2, medium; 3, serious.

Visual inspections following also the so-called “walking census method” and attention was dedicated also to spotting eggs and other juvenile instars of the pest on Box plants (Jervis & Kidd, 1996).



Fig. 3. Larva of *C. perspectalis* and its damage on leaves and young twigs of Buxus tree

RESULTS

Infestation by *C. perspectalis* spans in Liguria from one extreme to the other that is from the French to the Tuscanian borders, and affects both gardens and spontaneous Box tree formations. Our surveys show that the most part of Habitat 5110 has suffered strong defoliation, between 70% and 80% of its extension.

In Tab. 4 we report the estimated area percentage of Habitat 5110 which shows clear signs of infestation in all considered SACs.

Tab. 4. - Estimated damage caused by *C. perspectalis* during assessment in 2016.

n.	SAC code and name	Estimated area Habitat 5110 (ha)	% infested area	Damage grade
1	IT1331909 Monte Zatta - Passo Bocco - Passo Chiapparino - Monte Bossea	95.78	70	3- serious
			30	2- medium
			-	1- minimum
3	IT1342806 Monte Verruga - Monte Zenone - Roccagrande - Monte Pu	391.47	70	3- serious
			20	2- medium
			10	1- minimum
4	IT1343412 Deiva - Bracco - Pietra di Vasca - Mola	3.89	70	3- serious
			30	2- medium
			-	1- minimum
5	IT1343415 Guaitarola	1.16	70	3- serious
			30	2- medium
			-	1- minimum
6	IT1344422 Brina e Nuda di Ponzano	23.90	70	3- serious
			30	2- medium
			-	1- minimum
Total			516.20 ha	361.34 ha 3- serious
				115.71 ha 2- medium
				39.15 ha 1- minimum

In the Western Liguria, until now, the infestation seems limited to the gardens as wild Box tree plants are extremely rare. During the inspection carried out on 17th March 2017 in the wild box tree population at Fanghetto (Olivetta San Michele), near the SAC IT1315717 “M.Grammondo-Torrente Bevera”, one of the authors (MM) observed some dried branches and leaves, but no presence of *C. perspectalis*.

Particular attention was dedicated to the conservation status of Habitat 5110 in SAC IT1342806 ‘Monte Verruga - Monte Zenone - Roccagrande - Monte Pu’, where the local relevance of widespread Box tree communities is testified, inter alia, by big centuries-old arboreal individuals (Parola, 2008; 2013).

According to local people interviewed, in the considered SACs the pest infestation started at least two or three years ago. Its spreading developed after the different conditions prevailing at various elevations. In the range between 150 and 700 m a.s.l. in July and early August the Box plants had almost completely been defoliated (Fig. 4) and were suffering.

C. perspectalis is a multivoltine species with at least three generations, which is why plants face difficulty in springing new leaves between two lifecycles by the insect. The xeric conditions of the environment and stressing factors that may develop on ophiolitic substrates further hamper recovery by the Box trees. During inspections carried out on 3rd and 9th August, caterpillars were exceedingly abundant, and were at different instars. It was impressive to see younger larvae hanging down the plants along silk threads to reach the ground and look for plants left unexploited by already fully grown larvae. On 9th August, 2016 we noticed also the first moths on the wing; moreover, numerous pupae were over there (Fig. 5), and new adults are emerging continuously. On 31st August 2016, thousands of moths were flying across the village of Bargone (GE), and residents were seriously concerned that the invasion might turn out harmful to cultivations. On that date some melanic individuals were noticed (Fig. 1 and 6), which seems evidence of the last annual generation (Tuba *et al.* 2014).



Fig. 4. Significant damage caused by *C. perspectalis* to the Box tree, dominant species of the Habitat 5110, in the SAC IT1342806 (July 2016).



Fig. 5. A *C. perspectalis* pupa on Box tree.



Fig. 6. Melanic specimen of *C. perspectalis* on a wall at the Village of Bargone (August 2016).

On 31st August, at Pian del Lago (GE), at 700 m. a.s.l. near the marsh of Monte Roccagrande, *Buxus* plants were leaves-rich, but by a more accurate inspection we found some caterpillars at different stages; the flight of non-melanic adults was considerable. There was then a displacement in the age distribution of moth populations set at slightly different altitudes, corresponding to a few degrees of difference in temperature. Adults were massively resting on *Eupatorium cannabinum* L. (cf. Pinzari *et al.* 2015), known nectar plant for *Euplagia quadripunctaria* (Poda, 1761) too, a priority species under conservation according to Annex. II of Habitats Directive 92/43 EC, possibly ecologically displaced by the crambid. In fact, during our inspections *E. quadripunctaria* was never observed despite the fact in previous years numerous individuals had frequently been observed in the site.

During the visit on 26th October, 2016 around Bargone, at the lower altitude of ca. 150 m a.s.l., where during preceding months strong attacks were recorded, there was some regeneration by Box trees, corresponding to a few cm growth of internodes. At higher elevation, above 700 m, plants had foliage, but some of them were already suffering from attacks and showed first signs of defoliation. Caterpillars from very early instars could be noticed (Fig. 5); in order to overwinter they hide into loose silken shelters, which will be abandoned in spring to conclude their development. As a complement to our survey report for 3rd August 2016, we observed in different plants also symptoms apparently due to infection by the box blight, *Cylindrocladium buxicola* Henricot, a fungal species rapidly spreading in numerous countries (Gehesquière *et al.*, 2013) whose presence in Italy has been confirmed by Saracchi *et al.* (2008).

DISCUSSION AND CONCLUSIONS

Concerns for the spreading of *C. perspectalis* in Italy have so far been raised only regarding damage to plants in urban public space such as gardens, historical parks and cemeteries, following a vision focused on aesthetic values of the landscape and an economic-commercial side. Till now the vast natural extensions of wild Box tree had not been considered, particularly those referred to Habitat 5110, protected by the 92/43 EU Directive. Also if the results of our survey are only preliminary and relate to a single region, clear effects on natural biodiversity could be assessed. In Liguria the Box tree moth has rapidly spread in numerous gardens and cemeteries since 2013. Trials performed in the Hanbury Botanic Gardens (Ventimiglia) since summer 2013 with the use of pyrethroids and, subsequently *Bacillus thuringiensis* v. *kurstaki* or v. *aizawai*, led to no satisfying results; yet in 2017 no significant reduction of damage occurred, except for brief temporary periods. Same outcome was observed in several other sites (e.g. Finalborgo, Loano, Botanical Garden of Genoa University, etc.).

Different approaches should in any case be followed in Natura 2000 sites, where chemicals and pesticides are forbidden by law, including use of *Bacillus*. The only option would be to use species-specific pheromone traps or UV traps, though the latter are poorly selective. Interesting alternatives would consist of employing natural control agents which limit populations of the moth in its primary range. From the selection of natural enemies *Vespa velutina* and other species likely to turn out more detrimental than the Box moth itself should obviously be excluded, in that they would introduce additional disequilibria to natural habitats.

In 2016 (8th September) a meeting at the headquarters of Region Liguria was held, with the participation of the Parks Office, Regional Phytopathologic Observatory, Genoa University (DISTAV), ARPAL and managing Agencies of SACs affected by the problem, to tackle the issue. On that occasion, as a first step it was agreed to inform the Ministry of Environment asking for the registration of *C. perspectalis* in the Official Italian List of Invasive Alien Species. This request was sent on 13rd October 2016 by the Parks and Biodiversity Office of Region Liguria. This action was considered necessary because the Box tree moth is not listed at present in the “Executive Regulation (EU) 2016/1141 of the Commission of 2016 July 13rd” that provides a list of exotic invasive species of relevance for the Union in application of the 1143/2014 (EU) regulation by the European Parliament and Council. In fact, in 2012 this species was taken off from the European and Mediterranean Protection Plants (EPPO) alert list in which it had been inserted in 2007, because “starting from its registration no particular action of international relevance has been carried out by member Countries” (EPPO, 2012). *C. perspectalis* has been included among the target species of mounting concern within the European ALIEM Project: “Action pour Limiter les Risques de Diffusion des Espèces Introduites Envahissantes en Méditerranée”, dedicated to cope with the problem of different exotic invasive alien species (IAS) in a cross borders context within a

Cooperation Interreg Programme V-A Maritime Italy-France 2014-2020. ARPAL, that supports Regione Liguria in sites Natura 2000 management, and UNIGE (DISTAV) are involved in this project.

First step will be monitoring the spread of *Cydalima* at local level and investigate whether damages of boxwood are permanent or not.

Second step will be the increase of knowledge about species that characterize the habitat 5110, to understand possible impacts due to use different eradication systems (remember that we are operating into Nature 2000 site).

Third step will be to try several methods in small areas to test both results on population of *Cydalima* and impacts on surrounding environment.

Surely more investigations are required to verify if damages to wild Box tree stands are also due to other agents, as shown by the National French programme “Save Buxus 2014-2017”, in particular the Box blight *Cylindrocladium buxicola* and Volutella blight agent, *Pseudonectria buxi*, the second recorded for the first time in Italy on *Buxus microphylla* near Settime (Asti Province) at the beginning of 2016 (Saracchi *et al.*, 2008; Garibaldi *et al.*, 2016).

The resilience of natural Box tree formations will also have to be assessed, so for the capacity by *C. perspectalis* larvae to shift “*in situ*” to other host-plants such as the above mentioned *R. aculeatus*, *A. campestre*, *F. excelsior* and *Rubus* (Trokhov & Kaurova, 2015) in case Box tree would be missing. Particularly, new research should bring clarity on whether or not a niche overlap between the Box tree moth and *Euplagia quadripunctaria* is actually ongoing, so as to assess if the latter did actually suffer from trophic competition or its “disappearance” is only a matter of sampling artifact.

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