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Long-term monitoring and microbiological control programs against lepidopteran defoliators in Sardinian cork oak forests (Italy)

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ABSTRACT The gypsy moth, *Lymantria dispar (L.)*, and the tent caterpillar, *Malacosoma neustrium (L.)*, are the main cork oak, *Quercus suber L.,* pests in the Mediterranean area and cause complete defoliation in large forest districts. In order to control infestations, large scale aerial applications of insecticides based on *Bacillus thuringiensis* subsp. *kurstaki (Btk)* have been carried out in Sardinia (Italy) since 2001. This paper evaluated the frequency of outbreaks in forest districts with varying homogeneity of land use, forest areas annually exposed to defoliation and the effectiveness of control programs based on *Btk* insecticide applications.
The volume of areas annually exposed to defoliation depends on forest homogeneity, as infestations are more frequent in cork oak areas with a lower than 25% canopy cover rate. The microbiological control programme efficiently protected cork oaks from lepidopteran defoliators and caused an overall annual mean mortality of over 60%, with maximum rates of 89.9 and 98.0% for *L. dispar* and *M. neustrium,* respectively. To date, approximately 180,000 hectares of cork oak forests have been protected by spraying Btk-based insecticides.

KEYWORDS: *Bacillus thuringiensis kurstaki*, gypsy moth, tent caterpillar, *Quercus suber* L.

Introduction

Cork oak, *Quercus suber* L., is one of the most characteristic forest tree species in Sardinia (Italy), where it grows in pure or mixed oak woods, mainly together with holm oak, *Q. ilex* L., and downy oak, *Q. pubescens* Willd. Pure cork oak forests and woodlands cover approximately 140,000 hectares throughout the entire island. Cork oak trees perform multiple ecological roles in Mediterranean ecosystems, as they are a significant part of Europe's forest heritage and an important biodiversity hotspot, with a key conservation function (Mannu et al. 2018, Verdinelli et al. 2017). In addition, *Q. suber* forests are a fundamental rural economic resource due to cork production (Aronson et al. 2009). Cork production varies widely depending on principal land use and ranges from 150 to 5,000 kg of cork per hectare every 10 years (i.e. the time interval between two consecutive extractions) in woodlands and forests, respectively (Bullitta et al. 2011). In several areas, cork oak woodlands are also grazed intensively, mainly by sheep and beef cattle or, marginally, by other ungulates such as wild boar and horses, thereby providing an important source of food for livestock (Aronson et al. 2009). However, increasing use of cork oak woodlands as pastures in several countries, as a consequence of decreasing cork price trends (Acácio and Holmgren 2014), may amplify the impact of Mediterranean pests and pathogens on cork oak (Luciano and Prota 1986). In this context, the main cork oak forest health concerns are lepidopteran defoliators, which can lead to complete *Q. suber* tree defoliation across whole forest areas (Luciano et al. 1982, Luciano and Prota 1995). In particular, the most damaging species are the gypsy moth, *Lymantria dispar* (L.) *(Lepidoptera Erebidae)* and the tent caterpillar, *Malacosoma neustrium* (L.) *(Lepidoptera Lasiocampidae)*, univoltine species characterized by periodic or irregular population density fluctuations (Luciano et al. 2002).

The gypsy moth is a highly poliphagous species capable of developing on more than 300 shrub and tree species (Liebhold et al. 1995a), including grey birch, *Betula populifolia* Marsh., American beech, *Fagus grandifolia* Ehrh., red maple, *Acer rubrum* L., and eucalyptus, *Eucalyptus* spp. (Barbosa et al. 1986, Barbosa and Greenblatt 1979, Floris et al. 2018). In North America, where *L. dispar* was accidentally introduced at the end of the nineteenth century, this pest annually causes extensive defoliation on white oak, *Quercus alba* L., and northern red oak, *Q. rubra.* In Sardinia, gypsy moth outbreaks generally occur every 7-8 years depending on main land use (Luciano and Prota 1995).

The tent caterpillar is often considered a secondary cork oak pest as populations are generally less numerous than those of the gypsy moth (Luciano and Prota 1995, Tiberi et al. 2016). Nevertheless, *M. neustrium* can cause severe defoliation and outbreaks typically occur every 8 to 9 years in Sardinia (Luciano and Prota 1995). Although *M. neustrium* develops on different *Quercus* species, holm oak is its preferred host (Verdinelli and Sanna-Passino 2003).

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Complete or partial defoliation prompts physiological imbalances in oak trees (Cambini 1975, Muzika and Liebhold 1999) and can cause significant cork growth reductions of 60% and 32% in years following total (100%) and partial (50%) defoliation, respectively (Cambini 1971). When intensive defoliation follows a prolonged drought, oak health status can be seriously compromised, leading to tree death and potentially increasing the spread of the oak decline (Davidson et al. 2006, Gottschalk et al. 1998). The latter seems to be caused by a combination of abiotic and biotic factors, including defoliator pests, and is one of the major Mediterranean cork oak forest concerns (Franceschini and Luciano 2009, Tiberi et al. 2016). In addition to this, defoliated trees in early summer may have a negative effect on tourism in the worst infested forest areas.

Given the negative impact of these pests, a network of georeferenced monitoring sites covering the main oak woods has been developed in Sardinia since 1980 (Cocco et al. 2010, Franceschini and Luciano 2009). In order to protect cork oak trees and limit the negative impact of defoliator outbreaks, aerial spraying of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) based insecticide formulations have been carried out annually in Sardinia's worst affected cork oak forests. The control programme started in 2001 following a long experimental period during which various *Btk* strains, commercial formulations and aerial distribution techniques were tested (Cerboneschi and Ruiu 2002, Lentini and Luciano 1995, Luciano and Lentini 2012).

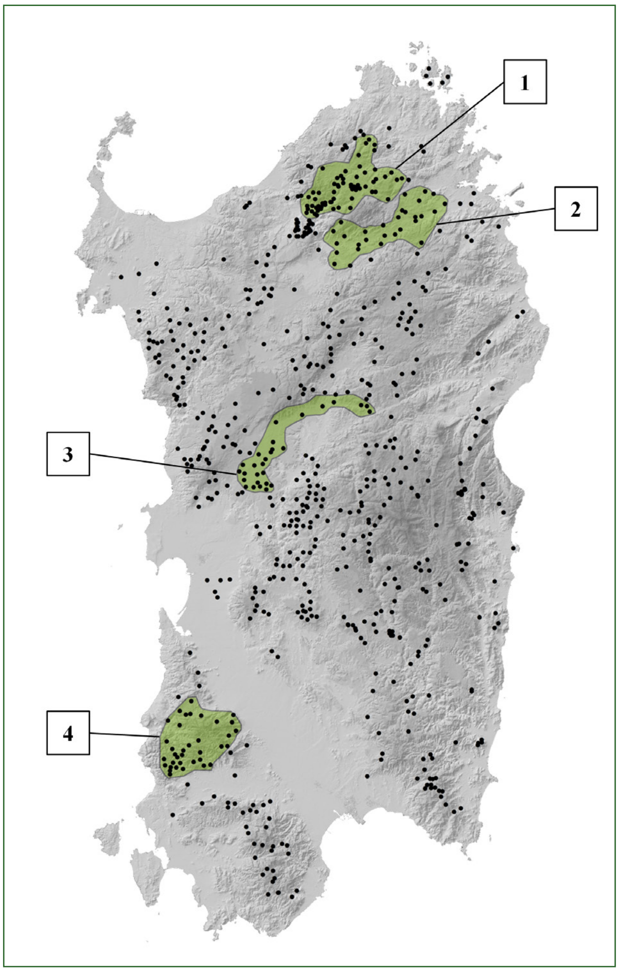
The aim of this work is to summarise the main results obtained in more than 30 years of monitoring, experimentation and control of *L. dispar* and *M. neustrium* in Sardinia's cork oak forests.

Materials and methods

Pest monitoring network

The monitoring network, originally made up of 107 sites distributed almost exclusively across Sardinia's main cork oak districts, was expanded to all pure and mixed *Quercus* forests of this region and today consists of 687 monitoring sites (Fig. 1). Gypsy moth population abundance on each site was estimated annually from 1980 to 2018 by counting egg mass numbers during winter. Egg masses are considered the optimal stage for sampling and monitoring purposes, mainly because of the significant relationship between their density and the resulting defoliation (Liebhold et al. 1995b, Mannu et al. 2017).

Figure 1 - Map of monitoring sites in Sardinia (Italy) and location of the cork oak forest districts of (1) North Gallura, (2) South Gallura, (3) Oristanese, and (4) Iglesiente.



Gypsy moth monitoring was carried out using a sampling method developed in Moroccan cork oak forests (Fraval et al. 1978), which took into consideration the counts in each monitoring site of all the egg masses from 40 trees selected on the main cardinal directions from a common starting point (i.e. 10 trees for each cardinal direction).

Estimates of *M. neustrium* population density were based on various sampling methods throughout the entire monitoring period. From the beginning of the monitoring programme (1980) to 2004, *M. neustrium* populations were not specifically monitored and infestations were observed only when they fell within areas infested by *L. dispar*. Starting from 2005, *M. neustrium* population density was estimated through a sequential sampling plan that considered egg mass counts from 5 branch tips on 28 trees, for a total of 140 branch tips per site (Verdinelli et al. 2005). However, this sampling method was applied in specific forest areas only from 2005 to 2012, as it was much too time consuming. In addition, sampling preferred shoots for oviposition, i.e. in the apical canopy, was difficult in trees higher than 6 m, making application unreliable.

Partly for this reason, from 2013 the boundaries of the cork oak forests to be protected from tent caterpillar infestations were based on defoliation estimates from the previous year.

Bacillus thuringiensis application and spraying efficiency evaluation

Btk-based insecticide application was carried out exclusively in years and areas of severe infestations. However, in 2008, applications did not take place due to the lack of registered product and government authorisation. The boundaries of the areas to be treated were defined by narrowing the network of monitoring sites to forest areas in which high pest density indicated potential defoliation. Boundaries were delimited manually on a paper map or digitally using a Geographic Information System (GIS) including monitoring sites with the highest egg mass numbers. A site was considered infested when estimated *L. dispar* and *M. neustrium* population densities were higher than 100 and 35 egg masses per sampling site, respectively, the economic damage thresholds above which defoliation is assumed to be complete (Luciano et al. 2002, Verdinelli et al. 2005).

Different *Btk* strains and formulations were tested in a number of experimental trials (Lentini and Luciano 1995, Luciano and Lentini 2012, Ruiu et al. 2012, 2013), highlighting that *Btk*-based formulations providing the highest larval density reductions were Foray® 48B and Foray® 76B (Valent BioSciences Corporation, Libertyville, Illinois, USA). Both commercial products - flowable concentrate formulations based on a mixture of spores and parasporal crystals of *Btk* strain ABTS-351 - were used in the control programme at doses of approximately 50 Billion International Units (BIU) per hectare. Foray® 48B, containing 12.7 BIU/L, was applied until 2017 in 4 L/ha doses whereas Foray® 76B, with a potency of 20 BIU/L" was used in 2018 in 2.5 L/ha doses. Both formulations were sprayed undiluted in ultra-low volumes using a helicopter equipped with a 12-meter bar and four electronic Micronair rotary atomizers (model AU) adjusted to sprinkle 160 micron-sized drops. The helicopter released the bio-insecticidal formulation at a speed of about 90 km/h and treated a 20 m-wide strip. Until 2008, the helicopter navigation devices used were rudimentary and product distribution homogeneity thus mainly relied on pilot skill. Since 2009, helicopters have been supplied with a GPS navigation system specifically designed for aerial applications (Guia Silver, AG-NAV Inc., Barrie, Canada) allowing uniform swathe coverage with a further improvement being added in 2017, when the spray system was equipped with a flow control system ensuring steady distribution rates at variable helicopter speed.

Spray applications were performed in optimal or sub-optimal weather conditions, within the following ranges: 19-28°C temperatures, 30-60% relative humidity, wind speeds under 1-2 m/s. *Btk* applications were timed to coincide with maximum population density of lepidopteran defoliator II-III larval instars. However, due to delayed *Btk*-insecticide formulation delivery, applications in 2015 targeted IV-V larval instars for the most part.

Treatment efficacy was evaluated in the field by estimating larval population reductions at 2-4 sites per treated area. At each site, larvae were counted before and 7-10 days after *Btk* applications on 40 shoots (30 cm long) randomly sampled from a linear transect of 10 trees (4 shoots per tree) (Luciano and Lentini 2012). In 2015, *M. neustrium* mortality caused by *Btk* treatment was evaluated under laboratory conditions, by breeding a sample of 100 II-III instar larvae randomly collected from each of four sites. The larvae were collected immediately after applications and fed fresh cork oak leaves from the same locations. Mortality data was recorded daily for 10 days after treatment. Moreover, the degree of defoliation was evaluated during the summer in all areas subjected to *Btk*-applications via visual tree crown inspection. Defoliation was expressed as a percentage of leaf surface eaten by defoliators compared to total leaf biomass (Connola et al. 1966).

Table 1 - Forest and dehesas areas, and index of homogeneity (HI) of land use in four pure cork oak forest districts of Sardinia (Italy).

District	ID	Pure cork oak forest[a] (ha)	Dehesas[b] (ha)	Total (ha)	HI[c]
North Gallura	1	4,239.03	1,975.18	6,214.21	2.15
South Gallura	2	5,710.65	5,911.20	11,621.85	0.97
Oristanese	3	3,265.42	3,688.37	6,953.79	0.89
Iglesiente	4	1,628.70	180.50	1,809.20	9.02

a) Continuous cork oak forested areas of at least 5'000 m² with a canopy cover higher than 25%.
b) Areas of at least 5'000 m² with cork oak trees and canopy cover from 5 to 25%.
c) Surface of pure cork oak forests divided by surface of dehesas.

Data analyses

Areas with the highest risk of defoliation caused by gypsy moth and tent caterpillar were estimated separately for each year by interpolating egg mass density in the network of monitoring sites. The risk of defoliation due to *L. dispar* was evaluated from 2001 to 2018 at both regional - pooling data from all Sardinia's cork oak forests - and district scale, including separate data from four homogenous cork oak forest areas located in north (North Gallura and South Gallura), central (Oristanese) and south (Iglesiente) Sardinia (Fig. 1). Annual *M. neustrium* defoliation risk was evaluated exclusively for the Oristanese district in the 2005-2012 period. Spatial analyses were conducted with ArcGIS 10.1 (ESRI 2012) using Inverse Distance Weighted (IDW) interpolation methods, as the technique best suited to avoiding errors due to irregular site distribution (McCoy and Johnston 2002).

In order to evaluate the relationship between canopy cover rate and *L. dispar* outbreak frequency in each of the four forest districts, pure cork oak forest (i.e. continuous cork oak forested areas measuring at least 5,000 m^2 with over 25% canopy cover) and dehesas (i.e. continuous areas measuring at least 5,000 m^2 with 5-25% cork oak trees and canopy cover) surface areas were determined from the Land Use Map of Sardinia (Progetti RDM 2003). An index of land use homogeneity (HI) was then calculated by dividing the number of hectares of pure cork oak forests by the number of hectares of dehesas. Index values higher and lower than 1 indicate higher and lower pure cork oak forest frequency, respectively. Outbreak frequency was calculated as the number of years in which over 80% of pure cork oak forests were at the highest risk of defoliation (i.e. interpolated areas with more than 100 gypsy moth egg masses) (Mannu et al. 2017). Chi square tests (å>0.05) were performed to test differences in outbreak frequency between districts. For each district, HI was related to outbreak frequency during the 1980-2018 period and regression analysis was carried out by fitting log-linear models.

Results

Sardinian cork oak areas at risk of defoliation due to *L. dispar* infestations varied from year to year during the entire monitoring period (Fig. 2) with virtually regular peaks of potentially defoliated cork oak forests every 8-10 years. The highest total area at risk of defoliation were 69,117 and 69,047 ha in 1989 and 1990, respectively. By contrast, cork oak areas at risk of defoliation showed values of 31.9, 94.7 and 149.5 ha in 2006, 1985 and 1986, respectively, and 0 in 2012, 2013 and 2015.

Temporal patterns in the most severely infested areas differed from one cork oak forest district to another (Fig. 3). The North Gallura and Iglesiente districts showed cyclical infestation peaks punctuated by periods when no risk of defoliation occurred (Fig. 3). Iglesiente district's outbreak frequency was lower than those of North Gallura ($\chi^2 = 6.09$, P = 0.05), South Gallura ($\chi^2 = 17.16$, P = 0.01) and Oristanese ($\chi2 = 9.59$, P = 0.01). North Gallura district showed a significantly lower outbreak frequency than South Gallura ($\chi^2 = 8.87$, P = 0.01) and Oristanese ($\chi^2 = 6.53$, P = 0.04) whereas there was no significant difference between Oristanese and South Gallura ($\chi^2 = 0.0$, P = 0.92). Outbreak frequency was significantly related to HI by a log-linear model ($y=-5.045*\ln(HI)+11.541$; $R^2=0.90$; F=18.17, P=0.05).

Tent caterpillar monitoring in Oristanese district highlighted a high defoliation risk area in 2007-2009 peaking at approximately 1,600 ha in 2008, one year before the gypsy moth culmination phase (Fig. 4).

Btk-based insecticides were applied for 13 years, protecting a total of 180,600 ha of cork oak forests against gypsy moth and tent caterpillar infestations

Figure 2 - Annual area of pure cork oak forests exposed to defoliation by *Lymantria dispar* in Sardinia (Italy) from 1980 to 2018.

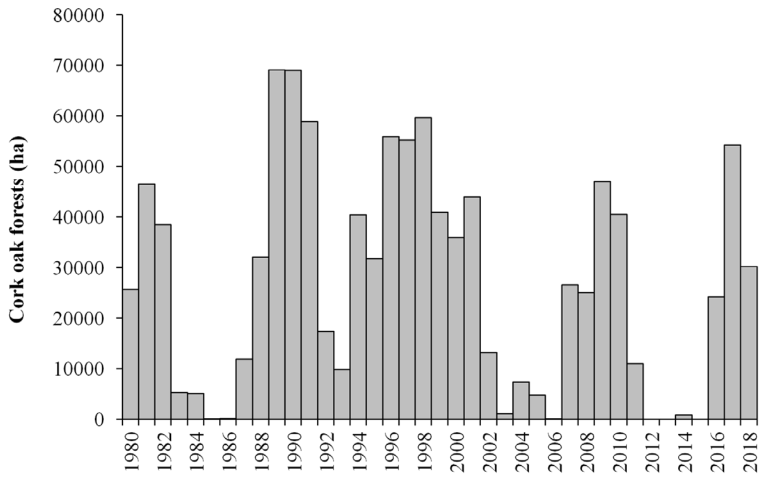


Figure 3 - Percentage of the pure cork oak forest areas at risk of defoliation by *Lymantria dispar* from 1980 to 2018 in four forest districts of Sardinia (Italy): (a) North Gallura, (b) South Gallura, (c) Iglesiente, and (d) Oristanese.

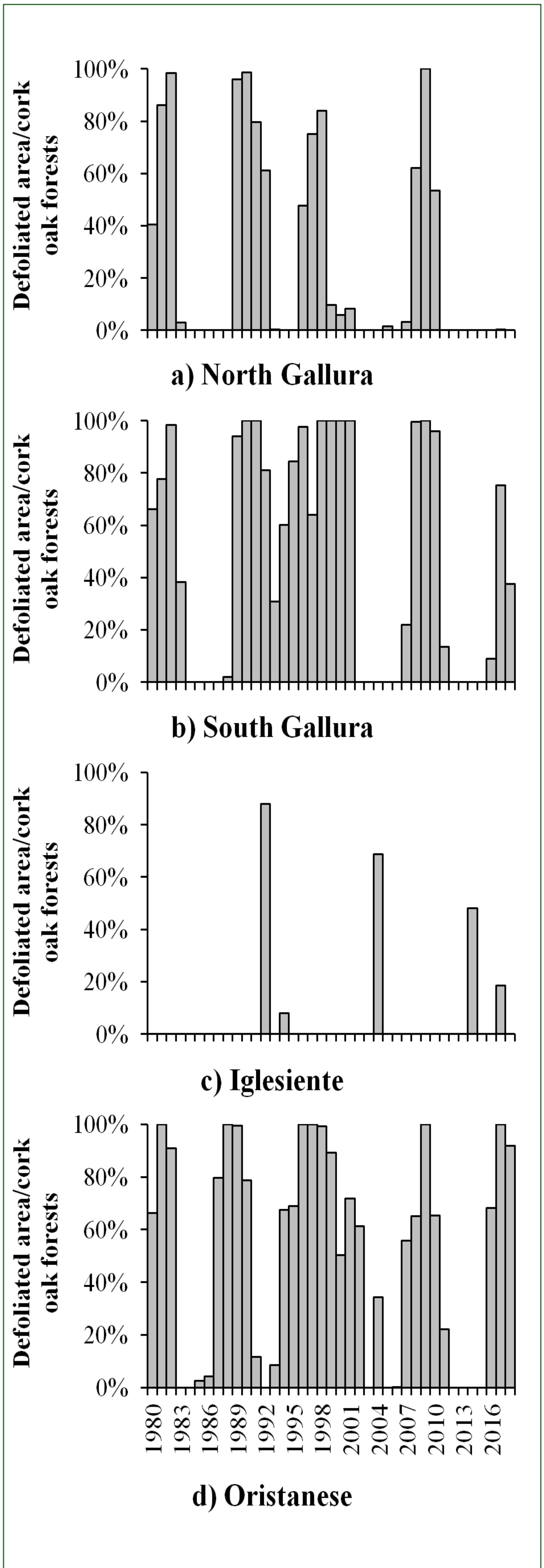
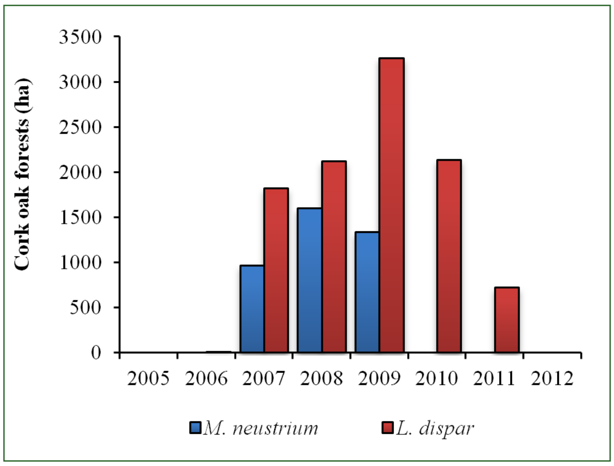


Figure 4- Annual area of pure cork oak forests in the Oristanese district (Sardinia, Italy) at risk of *Malacosoma neustrium* and *Lymantria dispar* defoliations from 2005 to 2012.



(Tab. 2). Control of *L. dispar* and *M. neustrium* populations was required in 13 and 7 years, respectively. Italicize "*L. dispar*" and "*M. neustrium*". *Btk*-insecticides were applied to control *L. dispar* and *M. neustrium* infestations on 121,400 and 25,400 ha, respectively, whereas 33,800 ha were treated to control mixed populations of the two defoliators (Fig. 5).

Btk applications were effective overall in protecting cork oak canopies (Tab. 2), even though in some areas and years significant defoliation was observed. In 2009, more than 22% (4,500 ha) of the total treated area (20,000 ha) defoliated as a result of a combination of a number of negative factors during the control programme. These factors were: the presence of an extremely high population of *L. dispar* before treatment (average density of 27 larvae per twig) in a 1,000 ha area, delayed spraying application on a population of IV-V instar larvae on 1,500 ha and adverse weather conditions (i.e. rain) immediately after *Btk* application on 1,000 ha. In other cases, delayed commercial product delivery (2015) and adverse weather conditions delayed *Btk* application to the extent that gypsy moth populations were mainly tolerant larval stages. Therefore, annual mean *L. dispar* larvae mortality was generally high although a wide range from 0% (2015) to 89.95% (2018) (Tab. 2) was visible. *M. neustrium* larvae showed higher susceptibility to *Btk* application than *L. dispar* with larval mortality ranging from 76.40% to 98.05%. In fact, defoliations in treated areas were entirely attributable to gypsy moth infestations.

Discussion

Population dynamics

The network of sites set up in Sardinia over 30 years ago for the purposes of monitoring *L. dispar* populations provided valuable information contributing to a better understanding of pest population dynamics and effective control programme development.

Annual forest areas exposed to gypsy moth outbreaks in all the main Sardinian cork oak forests indicated a strong positive relationship with the oak canopy cover rate, as fluctuations were irregular and more frequent in areas dominated by dehesas. By contrast, in pure cork oak areas with a > 25% canopy cover rate fluctuations were more periodic, occurring at approximately 8-10 year intervals. Cork oak districts characterized by higher HI (North Gallura and Iglesiente) (Tab. 1) showed cyclical infestation peaks separated by periods when no risk of defoliation occurred (Fig. 3). Conversely, an irregular temporal pattern was observed in forest districts with HI lower than 1 (Oristanese and South Gallura) (Fig. 3). Moreover, a higher number of outbreaks occurred in areas with a lower forest-dehesas ratio, as indicated by a significant relationship between outbreak frequency and HI. These findings accord with previous Sardinian studies which showed fluctuation patterns varying by forest ecosystem (Luciano et al. 2002, Luciano and Prota 1995). In particular, cork oak forests subjected to intense thinning and largely interspersed with pastureland, both natural or seeded for autumn-winter grass production, are prone to frequent defoliation risk, as high gypsy moth population density occurs every 5-6 years, causing total defoliations for up to three consecutive years (Luciano et al. 2002). By contrast, pure cork oak forests with well-preserved Mediterranean undergrowth subjected to traditional pastoralism, if at all, are subject to periodic defoliation occurring every 8-9 years. In regions with prevalent mixed-oak forests, defoliations due to gypsy moths recurring at over 10 year long irregular intervals have been observed (Luciano et al. 2002). Worldwide, gypsy moth populations tend to fluctuate on time frames ranging from 8 to 12 years, although in some areas of North America, Serbia and Hungary outbreaks take place on 4-5 year cycles depending on climatic condition and forest composition (Johnson et al. 2005, Marovic et al. 1998, McManus and Csóka 2007). The reason(s) for this, beyond different fluctuation patterns, have not yet been fully clarified, although the pest's natural enemy complex and oak forest complexity may play a part, as McManus and Csòka hypothesized (2007). In fact, more diverse ecosystems exhibit a more marked equilibrium and higher resilience to imbalance, meaning that they are able to delay population increase (Franceschini and Luciano 2009).

Figure 5- Annual area of pure cork oak forests subjected to *Bacillus thuringiensis kurstaki* (Btk) applications from 2001 to 2018 in Sardinia (Italy).

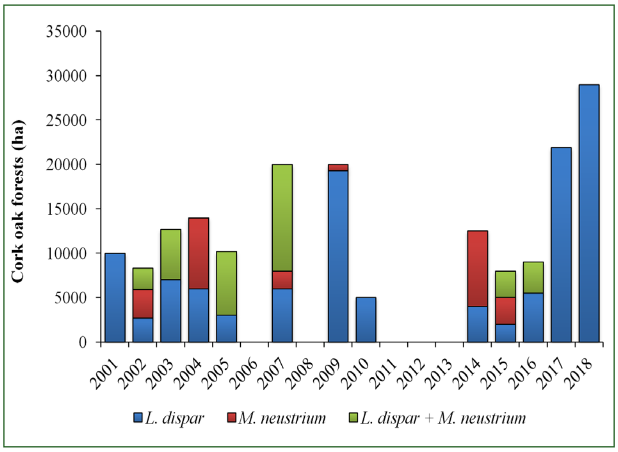


Table 2 - Sum of cork oak areas sprayed with *Bacillus thuringiensis kurstaki* (Btk) in Sardinia (Italy), and larval mortality (mean ± SD) observed for *Lymantria dispar* and *Malacosoma neustrium* from 2001 to 2018. The percentages of defoliated area on total sprayed area are reported in brackets.

Year	Sprayed area (ha)	Mortality (mean % ± SD)		Defoliated area (ha)	
		L. dispar	*M. neustrium*	*L. dispar*	*M. neustrium*
2001	10,000	84.98 ± 19.50	-	0*	-
2002	8,300	79.97 ± 8.94	98.05 ± 1.37	0*	0
2003	12,700	80.82 ± 23.71	91.32 ± 5.22	0*	0
2004	14,000	78.27 ± 18.69	96.43 ± 7.51	0*	0
2005	10,200	80.12 ± 16.53	80.40 ± 30.24	0*	0
2007	20,000	84.98 ± 8.46	97.97 ± 0.02	0*	0
2009	20,000	46.53 ± 33.20	NA**	4,500 (22.5)	0
2010	5,000	87.81 ± 5.76	-	0*	-
2014	12,500	63.48 ± 10.53	95.83 ± 10.21	1,400 (11.2)	0
2015	8,000	0.00 ± 0.00	76.40 ± 17.57	1,000 (12.5)	0
2016	9,000	67.85 ± 23.10	NA**	500 (5.6)	0
2017	21,900	74.77 ± 8.84	-	1,950 (8.9)	-
2018	29,000	89.95 ± 9.51	-	900 (3.1)	-

* *Observed defoliation on either isolated cork oak trees or limited areas (e.g., defoliated area < 1 ha)*
** *NA: not available*

M. neustrium population monitoring was effective in estimating population densities in forest areas with trees under 6 m in height, allowing shoots from apical canopies to be sampled. In areas with higher trees shoots could be sampled only from low and median canopies. As a result, pest density assessments were not reliable as total defoliation also occurred in forest areas in which egg mass numbers were below the damage threshold.

Although it was carried out for about 10 years in a single forest district, *M. neustrium* monitoring showed patterns similar to that of the gypsy moth but with peaks occurring one year earlier, in accordance with previous studies carried out in northern Sardinia (Delrio et al. 1991). In fact, the two species often jointly defoliate cork oak forests reaching peaks of 60,000 ha when outbreaks of both populations occur at the same time (Luciano and Prota 1985).

Control programme organization and technical requirements

Overall, the Sardinian microbiological control programme efficiently protected cork oaks from lepidopteran defoliators (Luciano and Lentini 2012). However control programme efficiency has improved over the years after overcoming both organisational and technical difficulties. In fact, control programmes were funded by the regional government year by year, thereby making intervention and programme implementation plans difficult. The main problem related to severe restrictions in aerial insecticide application under Italian law with Italian Legislative Decree 150/12 issued to implement the Directive 2009/128/EC (European Union 2009) prohibiting all aerial insecticide application either chemical or microbiological. Temporary specific permits can be issued by the regional government on Ministry of Health agreement only if no alternative application methods exist and for commercial products previously registered for aerial spray. For these reasons, no applications took place in 2008, due to the lack of a registered product and government permit. In other years (e.g. 2015), temporary delays in registering the commercial formulation led to later applications meaning that spraying was applied to a pest population made up mainly of IV-V instar larvae with greater resistance to *Btk*-based insecticides.

All Foray formulations were effective in protecting oak forests from gypsy moth defoliation. High efficacy was also associated with Foray 76B whose more concentrated formulation allowed doses to be virtually halved as compared to Foray 48B, with consequent lower product shipment and distribution costs. Moreover, improved application devices, such as the flow control system, contributed to further reducing application time frames and rationalising the application schedule, the key to successful pest control. The optimal time window is quite narrow as the pest target is II-III larval instars and unpredictable rainfall events may prevent insecticide sprays for several days. In line with this, defoliation after *Btk* application was observed mainly when *Btk* was sprayed late on a population made up of IV-V instar larvae (2009 and 2015) as well as when adverse climatic conditions occurred after spraying (2009) (Tab. 2). Nevertheless, overall only 10,250 ha (approximately 5.7% of the total treated area) were defoliated in the 2001-2018 period, demonstrating the effectiveness of the *Btk*-based control strategy in protecting cork oak foliage from lepidopteran defoliators.

Control programme effectiveness and environmental impact

The defoliator pest control programme covered almost 30,000 ha of protected cork oak forests in 2018. Although *Btk*'s high specificity allows non-target insect and arthropod fauna to be safeguarded, applying microbiological insecticides on such large areas may potentially affect lepidopteran species susceptible to spring applications (Cerboneschi and Ruiu 2002, Sample et al. 1996). However, a previous study carried out in Sardinia showed that *Btk* applications affected non-target lepidopteran species in a similar manner to total forest defoliation (Luciano and Lentini 1999) with competition for food causing high native lepidopteran fauna mortality rates. Therefore *Btk* sprays against high *L. dispar* population densities prevent oak defoliation and preserve plant health and cork production.

Another aspect of control programmes involving the use of microbiological insecticides to take into account is the potential insurgence of insect resistance and its management. From a long-term perspective, repeated treatments in the same area could lead to possible insect adaptation to *Btk* toxins, as with other crop pests (Griffitts and Aroian 2005). In forest ecosystems, resistance management might include treatment-free areas, in which *Btk*-susceptible populations are maintained and use of formulations ensuring different modes of action, e.g. based on different microbial strains or active ingredients. For this reason, although the most effective *Btk*-commercial formulations are based on the ABTS-351 strain, strain EG 2348 was recently successfully tested in preliminary studies (Ruiu et al. 2013).

The ABTS-351 strain was more effective against tent caterpillar than gypsy moth larvae. Since applications against *L. dispar* and *M. neustrium* took place under the same conditions, our results could be due to varying susceptibility of the two defoliators to the *Btk* formulation, as has already been observed for other lepidopteran species (Boulton et al. 2002, Van Frankenhuyzen and Fast 1989). This could be due to a range of causes, including the affinity of Cry toxins with *L. dispar* and *M. neustrium* midgut receptors and/or the presence and combination of different Cry toxins which can result in greater pore formation activity, depending on insect species (Pardo-López et al. 2013).

Long-term programmes for lepidopteran defoliators through *Btk* application could also affect the natural population dynamics of target pests. Theoretical studies indicated that spray applications lowered population peaks in the short term while levelling out pest population at high densities in the long term (Reilly and Elderd 2014). The results of the Sardinian control programme show that control sprays when gypsy moth populations are in the culmination phase lead to a fast transition to latency phase. By contrast, preliminary studies showed that modification of gypsy moth outbreak periodicity by spraying insecticides during the progradation phase is difficult to achieve (Mannu et al. 2020). In fact, spraying a *Btk*-based product over 3,500 ha of cork oak forests dramatically decreased population density and delayed the culmination phase by two years rather than driving the population to the latency phase (Lentini et al. 2012). Moreover, bioassays conducted on Sardinian *L. dispar* populations showed no significant debilitating effects on larvae and adult survivors of infection with *B. thuringiensis* (Cerboneschi 2002). According to Herms (2003), gypsy moth control programmes should focus on protecting oak forests during the pest culmination phase rather than on long-term pest population density reduction.

Conclusions

Microbiological control of forest pests should be seen as a temporary solution, although it does represent an effective way of protecting forests from defoliation. In fact, as treatments significantly reduce plant damage, they do not prevent repeat defoliator species outbreaks. A wider strategy for cork oak forest protection and conservation should include other management strategies aiming to restore the complexity of the forest ecosystem.

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