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Influence of *Bacillus thuringiensis* application timing on population dynamics of gypsy moth in Mediterranean cork oak forests

Running title: Influence of *Btk* applications on gypsy moth population dynamics

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Author Contribution Statement

AL, PL and RM conceived and designed the research. AL and PL conducted the field observations. RM, AC and AL analyzed data. RM, AC, and AL wrote the manuscript. All authors read and approved the manuscript.

ABSTRACT

BACKGROUND

The gypsy moth, *Lymantria dispar*, is one of the main pests of oak forests worldwide and it causes extensive defoliation during its periodic outbreaks. In the Mediterranean area, the control of gypsy moth populations in cork oak forests is based on the application of *Bacillus thuringiensis* serovar *kurstaki* (*Btk*) formulations. This research investigated the effects of *Btk* applications done in two different population development phases on gypsy moth population dynamics. With this aim, the temporal and spatial fluctuation patterns of *L. dispar* egg density were monitored in cork oak forests treated with *Btk*-applications from 2004 to 2009 in Sardinia (Italy).

RESULTS

The applications done during progradation and culmination phases protected equally the oak canopies in the year of application, thus causing a similar decrease in pest population density in the following year. However, the medium-term effectiveness of *Btk* differed between the two application timings, because only applications in the culmination phase caused a gradual decrease in *L. dispar* infestations throughout the subsequent years. In contrast, when the application was done during the progradation phase, population density increased again after 2-3 years. Moreover, *Btk* applications performed in culmination reduced significantly the number of years in which the gypsy moth density was damaging compared to those done in progradation.

CONCLUSIONS

Our results indicate that *Btk* applications during the culmination phase were more effective than those in the progradation period, since application in the latter case did not suppress the population but only postponed the outbreak peak by 2-3 years.

Keywords: *Lymantria dispar*, aerial application, microbiological control, pest dynamics, cork oak

1. INTRODUCTION

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) is one of the main forest defoliators worldwide and is able to defoliate thousands of hectares in the same year during its outbreaks.[1–6] Although the gypsy moth feeds on more than 300 host species,[7] it is strongly associated with *Quercus* spp., such as cork oak (*Q. suber* L.), downy oak (*Q. pubescens* Willd.), and holm oak (*Q. ilex* L.) in the Mediterranean area,[3,5,8] and white oak (*Q. alba* L.) and northern red oak (*Q. rubra* L.) in North America.[9] Mediterranean cork oak forests represent one of the most interesting biodiversity hotspots worldwide[10,11] and, at the same time, an important cultural and economic resource for rural communities.[12] Cork oak forests and woodlands cover approximately 2.5 million hectares across Europe and North Africa[12] and are mainly exploited for cork extraction, with management systems that vary from semi-natural to pastoral. Agricultural and pastoral activities in Mediterranean cork oak forests and woodlands are performed at different intensities, so that cork extraction is often coupled with other activities, such as cropping, grazing, hunting and mushroom harvesting.[13] Cork production ranges from 150 to 5,000 kg of cork per hectare every 10 years, depending on the main land use.[14] The time interval between two consecutive cork extractions is generally 10-12 years, possibly with longer intervals when adverse climate or pest infestation events occur in the year of debarking.[3]

Lymantria dispar infestations can harm the health status of trees,[15,16] especially when an intensive defoliation occurs after a prolonged drought period. Defoliation can cause a general decrease in plant growth, in terms of both height and diameter,[17] thus negatively affecting cork production in cork oak trees. Cambini[18] estimated a reduction in cork production up to approximately 60% and 40% in the years of complete or partial defoliation, respectively. Gypsy moth feeding activity might also induce physiological imbalances in cork oak trees as a result of a delayed bud burst in the year following the defoliation.[19] Moreover, defoliation spoils the landscape at the beginning of summer, thus discouraging tourism and leading to additional economic losses.

The gypsy moth is characterized by cyclical fluctuations in population density,[1] which can be divided into different population development phases commonly named as latency, progradation (or release), culmination (or outbreak), and retrogradation.[1,20] During the latency phase, the population density remains at a harmless level for many years, after which it increases gradually (i.e. progradation) and, in 2-3 years, reaches a peak (i.e. culmination) that can last for two consecutive years. Subsequently, the population density naturally decreases (i.e. retrogradation) until returning to a latency period. The interval between two consecutive latency phases, also known as gradation,[8,21] is extremely variable, mainly depending on the forest type and management strategy,[4,6,8] and its periodicity tends to oscillate from 5 to 10 years.[2,4,22]

This population fluctuation pattern is common in many forest insect pests[23–26] and can be potentially affected by several factors,[27] including the natural enemy complex,[1,23,28,29] the quality of leaves[30,31] as well as the dispersal ability of the species.[22,32–34] Although these factors seem not to be effective enough in preventing periodic destructive infestations, they could have a fundamental role in regulating the cyclical dynamics of Lepidoptera outbreaks[23] and the spatial synchronization of separated populations.[22,24,35] In particular, spatial synchrony (i.e. statistical congruence in the oscillations of geographically separated populations) needs to be considered when analyzing the dynamics of forest pests,[35–37] as the population density that occurs in a large region can increase simultaneously, thus causing extensive damage throughout a very large area.[38,39] Spatial synchrony has been observed in different species,[38] including the gypsy moth.[24,35,40]

Given the negative impact of *L. dispar* on forest environments, several pest control programs have been carried out against this insect in order to reduce the population density and damage during outbreaks[41,42] or slow down its spread from infested to non-infested territories.[43–46] Among the control strategies developed against the gypsy moth,[47] the aerial application of formulations based on *Bacillus thuringiensis* Berliner serovar *kurstaki* (*Btk*) exhibits few biological and practical limitations and is currently the most used and effective method to suppress gypsy moth outbreaks. Several aspects should be taken care of in order to enhance the effectiveness of *Btk* applications against *L. dispar*,[48] including the timing of application relative to the phenology of 2nd instars,[8,49] dose,[41,50] droplet size and density of *Btk*-based insecticides.[51] In addition, an improper use of *Btk* applications against the gypsy moth could lead to unexpected outcomes in the long-term. Recently, Reilly and Elderd[52] developed a mathematical model on the long-term effects of different biological control strategies against *L. dispar* populations, and hypothesized that *Btk* applications may reduce the density of the pest in the short-term. On the other hand, microbiological applications could also alter the natural dynamics of gypsy moth by dampening the outbreak peaks, thereby causing an unexpected outbreak occurrence over the long-term. In fact, pest control strategies that prevent outbreaks could also impact on the density-dependent process that regulates population cycles.[52] However, no experimental study has investigated the indirect influence of different application timings on the dynamics of the gypsy moth. In this perspective, we investigated the effects of *Btk* applications applied in different population development phases on the spatial and temporal patterns of *L. dispar* population density in cork oak forests.

2. MATERIALS AND METHODS

2.1. Study areas

The study was conducted in eight different areas located in six cork oak forest districts in the island of Sardinia (Italy) (Fig. 1), which is one of the most important cork oak growing areas across Europe.[12] Cork oak forests in Sardinia extend across different forest districts located mainly in the north-eastern part of the island. In Sardinia, *Q. suber* grows in sparse forests (tree coverage ranging from 5 to 25%) on 53,000 hectares and in dense forests (tree coverage greater than 25%)

on 85,000 hectares.[53] All forest districts are characterized by typical Mediterranean climatic conditions, with mild and rainy winters and hot and dry summers, and with soils mostly granitic and classified as Eutric Leptosols.[54]

In our observational study, data refer to areas sprayed with *Btk* from 2004 to 2009. Three areas were protected with a *Btk*-based insecticide during the progradation phase, whereas five areas were sprayed with the same product during the culmination phase (Table 1). All the forest areas included in the survey were larger than 1,000 hectares. Data from two unsprayed areas located in north Sardinia were used as control to compare natural and treatment-affected patterns (Table 1).

2.2. Estimation of population density

For this study, the population abundance of the gypsy moth in Sardinia was monitored by counting the number of egg masses as they are the optimal sampling stage for monitoring *L. dispar* populations due to the close relationship between egg mass density and the resulting defoliation.[55,56] In all sprayed and unsprayed areas, the estimation of population density was made from 2001 to 2014 on monitoring sites that are part of a network of permanent sites where *L. dispar* populations have been annually monitored since 1980.[4,57] The number of monitoring sites selected in each area was variable from 2 to 26 (Table 1). In each site, population abundance was estimated by counting the egg masses on 10 consecutive trees along the four cardinal directions starting from a common central reference point (40 trees/site/year), as proposed by Fraval et colleagues.[58]

2.3. *Bacillus thuringiensis* applications

In all the treated areas, the *Btk*-based formulation Foray 48B® (Valent BioSciences, Libertyville, IL, USA) was sprayed at the dose of 4 l ha^{-1} (50.8 Billion International Units ha^{-1}) by means of a helicopter equipped with a bar with 4 rotary atomizers.[59] Foray 48B® is a commercial formulation containing the *Btk* HD1 strain, which is considered the most effective strain to control gypsy moth and tent caterpillar, *Malacosoma neustrium* (L.) (Lepidoptera: Lasiocampidae) populations in Sardinia.[60] Control measures were performed against the 2nd-instars, as they are the most susceptible stage to *Btk* applications.[49] For this purpose, larval instar distribution was evaluated weekly by field observations in all the areas to be treated .

For each forest district, the gypsy moth population development phase in the year of *Btk* application was evaluated by analyzing historical data of egg mass density. In particular, *L. dispar* populations were considered in the progradation phase when their density increased following a latency period, whereas they were considered in the culmination phase when population density was higher than 100 egg masses (i.e. economic damage threshold above which defoliation is assumed to be complete) on at least 90% of the monitoring sites of the forest district. The boundaries of the areas to be treated, which included the infested monitoring sites, were digitally determined using a Geographical Information System (GIS).

The effectiveness of the *Btk* treatments on gypsy moth control was evaluated by estimating the reduction in larval population at 2-4 monitoring stations per treated area. The assessment was performed at each station comparing the number of live larvae before and 10 days after insecticide application. *Lymantria dispar* larvae were counted from 40 shoots (approximately 30 cm in length) randomly sampled from a linear transect of 10 trees (4 shoots/plant). The sampling sites were equally distributed within the treated area.

2.4. Data analyses

Statistical analyses were performed using R statistical software v. 3.10.[61] When necessary, a log(x+1) transformation was applied before the analyses to meet the assumptions of normality.

Differences in the reduction of gypsy moth larvae 10 days after the treatment (i.e. the effectiveness of *Btk*-based applications) between applications done in progradation and culmination phases were tested using a two-sided Wilcoxon signed-rank test (significance at 0.05).

In order to investigate differences in the average level of *L. dispar* infestation among unsprayed areas, areas sprayed in the progradation phase, and areas sprayed in the culmination phase, the number of egg masses over a complete gradation was analyzed by fitting a linear mixed model (LMM) using the lme4 package.[62] A linear mixed model approach was used, as strongly recommended by Pinheiro and Bates[63] for unbalanced samples. In the linear mixed model, fixed factors were treatment (sprayed in progradation, sprayed in culmination or unsprayed), year of observation within the gradation, and their interaction, whereas random factors were forest district and year of observation nested within each forest district.[64] Analysis of variance (ANOVA) was used to test the significance of each factor.

The effect of different *Btk* application timings on gypsy moth population dynamics was also evaluated in the sites sprayed in progradation and culmination phases, by evaluating both the number of years needed to return to the latency phase (i.e. the year when no egg masses were found) and the number of years with high infestations (i.e. population density higher than 100 egg masses per site) after the *Btk* applications. Differences between treatments were tested using Wilcoxon signed-rank test at the 0.05 level of significance.

The number of years in which the gypsy moth density occurred at damaging level over a complete gradation was analyzed for unsprayed, sprayed in culmination or sprayed in progradation forest sites, and differences were assessed with ANOVA test followed by Tukey post-hoc test at the 0.05 level of significance.

The annual spatial distribution of the abundance of *L. dispar* egg masses was mapped using geostatistical methods. Before the analysis, a log(x+1) transformation was applied to approximate data to a normal distribution.[65] Anisotropy was not considered and the semivariograms were fitted using ArcGIS software release 10.1.[66] The lag size was determined independently for each year, by computing the average distance between points and their nearest neighbors.[66] The average distance between points was different each year as a result of the variation in the number and location of the sites monitored annually. Gaussian, exponential and spherical models were tested between all the possible available methods.[4,67,68] The Akaike Information Criterion (AIC) was calculated for each model and used for selecting those best fitting the data. The following parameters were also calculated for the selected models: nugget (C0), range (A), sill (C0 + C, in which C is the variability attributable to the spatial dependence of the data).[69] The models fitted from the variograms were finally used for estimating the abundance of gypsy moth egg masses using ordinary kriging.

3. RESULTS

The average reduction of *L. dispar* larvae 10 days after the treatment was 64.8 and 82.7% for applications done in culmination and progradation phases, respectively (Fig. 2). No statistical difference in the reduction was found between applications carried out in progradation and culmination phases ($W = 32.5$, $p = 0.07$).

The application strategy significantly affected the average level of *L. dispar* infestation ($F_{2,633} = 36.34$, $p < 0.01$) (Fig. 3). At the beginning of the population development phase, the average density of gypsy moth egg masses was similar regardless of the application timing, with average values of 0.8, 0.7, and 3.1 egg masses per site in untreated, progradation-treated, and culmination-treated sites, respectively.

The population density increased after the first two years in all the surveyed areas, reaching approximately 100 egg masses per site in the third year of gradation. The application of the *Btk*-based insecticide during the progradation phase (third year) caused a decrease in the population density in the fourth year (38.7 egg masses per site) (Fig. 3a). However, the gipsy moth population increased again over the following years, reaching, on average, 595.2, 547.7 and 509.0 egg masses per site in the fifth, sixth and seventh year, respectively (Fig. 3a). In the forest districts where no insecticide was applied in the third year of gradation, the progradation proceeded and more than 700 egg masses per site were observed in the fourth year, indicating the onset of the gypsy moth culmination phase (Fig. 3b, 3c). The *Btk*-based insecticide sprayed in the culmination phase triggered a steep decrease in the population abundance to 115.1 and 30.7 egg masses per site in the two years following the application (Fig. 3b). The gradation in the untreated areas followed a similar pattern to that of the sites sprayed in the culmination phase (Fig. 3c), but the moth density in the years following the culmination phase was higher in untreated areas than in culmination-treated areas and exceeded the economic damage threshold in the fifth and sixth year of gradation (258.7 and 198.4 egg masses per site, respectively) in untreated areas.

The average time to reach the latency phase after *Btk* application was significantly different between sites sprayed in both culmination and progradation phases ($W = 39.5$, $p < 0.01$), with values significantly lower in sites sprayed during culmination (mean ± SEM: 1.56 ± 0.12 years) than in those sprayed in progradation (mean ± SEM: 5.25 ± 0.43 years) (Fig. 4). In addition, the average number of years in which the gypsy moth density occurred at damaging level after the *Btk* applications was significantly higher in the sites sprayed in progradation (mean ± SEM: 2.33 ± 0.31 years) than in those sprayed in culmination (mean ± SEM: 0.23 ± 0.08 years) ($W = 132.5$, $p < 0.05$) (Fig. 4).

The average number of years in which the gypsy moth density occurred at damaging level from the beginning of population development phase to the return into latency phase differed significantly among forest sites not-sprayed or sprayed in culmination and progradation phases ($F_{2,85} = 13.93$, $p < 0.01$). The number of years with damaging *L. dispar* populations was the lowest in sites sprayed in culmination (mean ± SEM: 1.49 ± 0.11), whereas no significant differences occurred between unsprayed (mean ± SEM: 2.42 ± 0.22) and sprayed in progradation sites (mean ± SEM: 2.83 ± 0.39) (Fig. 5).

Different models provided a best fit of data for the spatial distribution of gypsy moth egg masses throughout Sardinia (Table 2). In particular, 42.9% of the years (i.e. 6 out of 14) were best represented by the spherical model. The range varied from 2.33 to 31.89 kilometers (mean ± SEM: 19.16 ± 2.53 kilometers). Spatial analyses allowed to produce maps indicating the temporal pattern of the outbreaks within and surrounding the areas in which *Btk* applications occurred.

The interpolation maps highlighted different spatial distribution patterns of *L. dispar* density when *Btk* applications were made in progradation or culmination phase, as exemplified in Fig. 6. The gypsy moth population density was at a harmless level (i.e. lower than 100 egg masses per site) in the majority of the monitoring sites during the progradation phase (2005-2006) (Fig. 6a). In 2007, the population density in the area to be sprayed was higher than in the two preceding years. The bio-insecticide applied in 2007 reduced the population density in the following year, when gypsy moth egg masses were observed only in two of the seven monitoring sites inside the sprayed area. However, the reduction was restricted to the area protected with the *Btk*-based insecticide, as a higher infestation level was observed in the surrounding untreated sites. In 2009, gypsy moths spread out from the untreated to the treated area, causing an increase in the population at damaging density throughout the forest district.

In a different area, the *Btk*-based insecticide was applied in the culmination phase in 2009, when all the monitoring sites exhibited an above-threshold population density (Fig. 6b). In the two years preceding the culmination phase, the number of egg masses per site showed an increasing trend, even though the outbreak threshold was exceeded only in a few sites throughout the cork oak forest district, thus indicating that the culmination phase had not been reached yet. The insecticide application in 2009 reduced the gipsy moth population density in all treated sites in 2010, whereas damaging infestation levels persisted in the surrounding untreated areas. Despite the presence of some latent foci in 2010, the gypsy moth population density further decreased in 2011 in culmination-treated and untreated cork oak areas and, at a wider scale, throughout the forest district in the subsequent years.

4. DISCUSSION AND CONCLUSION

The results of the present research show that the phase of the gradation in which the application is performed does not influence the efficiency of *Btk*, as similar larval mortalities were observed in both treatments. A positive effect was also obtained in the year following *Btk* application, as the *L. dispar* population density decreased in forests treated in both progradation and culmination phases. However, the medium-term effectiveness of the *Btk*-insecticide differed substantially depending on the application timing. Notably, when the bio-insecticide was sprayed in the culmination phase, the population abundance decreased down to the latency phase faster than in the non-treated areas. On the other hand, when *Btk* was applied in progradation, the gypsy moth density decreased only temporarily before increasing again to reach the outbreak peak in two years. In other words, early applications during population growth shifted the abundance peaks forward, thus increasing the duration of gradations. These results suggest that the long-term effects of *Btk* application might be significantly influenced by the factors involved in the regulation of gypsy moth population cycles.

The population dynamics of forest Lepidoptera are the result of the interactions between the pest and a bioregulatory complex represented by natural enemies (parasitoids and predators), diseases (pathogens and viruses), and nutritional quality of the host foliage.[23] The effect of the natural enemies on the gypsy moth mortality varies depending on the phase of the gradation. The population density of parasitoids naturally increases at high gypsy moth density, i.e. culmination phase, and reaches the peak during the retrogradation phase,[21,28,29,70–72] with *Parasetigena silvestris* Robineau-Desvoidy and *Blepharipa pratensis* Meigen (Diptera: Tachinidae) being the most frequent oligophagous species. Although the response of tachinids to the variation in gypsy moth abundance is delayed, it plays a key role in reducing the population density to the latency phase.[73] Some species of generalist tachinid parasitoids, such as *Compsilura concinnata* (Meigen), are more active at low pest population density and can actively concur to suppress incipient outbreaks.[1] Predators of *L. dispar* are often generalist and could significantly affect the population dynamics of the defoliator in some forest types and climatic conditions. In fact, egg predators can cause mortality up to 90%,[74] and larval predators, such as *Calosoma sycophantha* (L.) (Coleoptera: Carabidae), can also notably increase their density during outbreaks.[75]

In North America, low defoliator population densities are affected by predation by small mammals,[76,77] whereas the collapse of high population densities is attributable, especially during outbreaks, to specific entomopathogens, such as *Entomophaga maimaiga*[78,79] and the gypsy moth nuclear polyhedrosis virus (LdNPV).[1] Although *E. maimaiga* mortality is largely density-independent, the fungus theoretically causes greater levels of mortality in hosts than the virus.[80]

The quality of food can influence the biological parameters and population dynamics of the gypsy moth.[27,81,82] Leaf quality variations can occur in response to feeding activity of insects. For example, a higher level of phenolics, hydrolysable tannins, dry matter content, and toughness were associated with a higher level of defoliation on red oak, *Quercus rubra*

L.[30] Oak trees might respond differently to various levels of defoliation, with a consequent variation in the chemical composition of leaves in which the gypsy moth develops. Consistent with this hypothesis is the fact that consecutive defoliations by *L. dispar* larvae over time significantly decreased the nutritional value of the host foliage, with a consequent reduction in pupal weight.[83] A higher decrease in food quality is more likely to occur at high population density (i.e. during the culmination phase), as larvae rapidly destroy the new leaves and move to one-year-old leaves, which grew following a defoliation and thus had a low nutritive value.[5,83]

To summarize, the population dynamics of the gypsy moth is regulated by several density-dependent mortality factors, which reach the highest incidence in the culmination phase and beyond. Therefore, *Btk* applications during the progradation phase, at low-medium gipsy moth population density, do not allow the biotic control factors to increase enough to the extent of causing the collapse of the pest population density. In this case, the bio-insecticide spray protects the vegetation for 1-2 years, and the *L. dispar* population density increases again to the culmination phase after few years. The results presented here suggest that the hypothesis of Reilly and Elderd,[52] concerning the potential defoliation due to the diffusion of pests from untreated to treated patches, more likely occurred in Mediterranean environments in which aerial applications were made during progradation. The spread through extinction-colonization dynamics also needs to be considered as an important factor directly influencing the temporal pattern of gypsy moth populations and the treatment effectiveness. In fact, the geostatistical analysis showed that the gypsy moth spread from untreated to treated forest areas in the years following the application only when the *Btk*-insecticide was applied in the progradation phase. In contrast, in the years following the application done in the culmination phase, the gypsy moth population density did not increase within the area treated with *Btk*-insecticides nor in its surroundings. These results suggest that the ability of the gypsy moth to spread from more- to less-infested areas may be strongly related to the population density at a forest district scale,[35,45] also because of the higher influence of some biological factors affecting the gypsy moth mortality, such as parasitoid and predator density at this spatial scale. In particular, their role increases from the progradation phase on,[5,23] thus allowing to naturally limit defoliator density in the entire forest district after its culmination phase.[28,70,84]

The spatial synchrony processes detected in gypsy moth populations in North America[2,35,40] also support our results and explain the patterns of gypsy moth populations sprayed at different timings in the Mediterranean area. In fact, the population density in the areas treated in the progradation phase increased to similar levels throughout the forest district from the second year following the application on. Differently, *Btk* applications in the culmination phase reduced the defoliator density to levels similar to surrounding forest areas. These results underline the importance of considering the population dynamics through time and space at a district level in order to achieve an effective gypsy moth control, because the factors affecting the forest pests (e.g. mortality rate) might have spatially synchronous dynamics as well.[35]

Our findings provide novel information for supporting decision-making in control programs against the gypsy moth in Mediterranean cork oak forests. Although *Btk*-based insecticides have always been effective in controlling *L. dispar* during outbreaks, an accurate choice of the timing and scale of the applications is necessary. Firstly, aerial application should be carried out throughout the forest districts rather than in smaller patches, in order to reduce and/or prevent the possibility of re-infestations from unsprayed surrounding areas to the treated areas. Secondly, culmination is the most suitable phase for the application of *Btk* to control the gypsy moth, since the growth of biotic mortality factors is not altered. Therefore, the bio-insecticide spray protects the foliage during outbreaks and the natural enemy complex reduces the defoliator density to latency.

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Table 1 Summary description of the monitoring periods, years of insecticide applications, and surface of the sprayed area in the surveyed districts

Cork oak district	Time period	Year of spraying	Spraying timing	Sampling sites (no.)	Sprayed area (ha)
West Gallura	1986-1995	-	No treatment	3	-
North Gallura	1987-1996	-	No treatment	16	-
Iglesiente	2001-2010	2004	Culmination	6	2,484.2
West Gallura	2005-2014	2009	Culmination	26	1,008.3
North Gallura	2005-2014	2009	Culmination	10	4,184.2
South Gallura	2005-2014	2009	Culmination	11	8,608.5
Oristanese	2005-2014	2009	Culmination	4	2,403.5
South Gallura	2002-2009	2005	Progradation	2	1,917.0
Nuorese	2004-2013	2007	Progradation	3	6,664.4
North Gallura	2004-2013	2007	Progradation	7	1,861.9

Table 2 Parameters of the semivariogram models best fitting the data for the spatial distribution of gypsy moth egg masses in Sardinia (Italy) from 2001 to 2014 and cross-validation coefficients (r^2)

Year	Model	Lag size	Nugget	Sill	Range (km)	r^2
2001	Exponential	2549.50	2.202	6.700	31.89	0.49*
2002	Spherical	2657.57	0.384	3.308	23.45	0.51*
2003	Gaussian	2687.79	0.961	2.399	16.16	0.38*
2004	Exponential	2410.21	0.093	1.989	16.13	0.43*
2005	Exponential	2448.05	1.083	3.424	29.38	0.32^{NS}
2006	Exponential	2641.77	0.704	1.910	31.70	0.22^{NS}
2007	Exponential	2642.97	0.328	4.644	17.76	0.23^{NS}
2008	Gaussian	2642.97	2.014	5.011	8.24	0.39^{NS}
2009	Spherical	2425.64	1.342	6.787	22.79	0.66*
2010	Gaussian	2650.17	0.701	6.072	7.83	0.59*
2011	Spherical	2424.91	1.381	3.152	16.31	0.39*
2012	Exponential	2365.89	0.223	0.926	28.39	0.40*
2013	Exponential	2363.89	0.091	0.149	28.37	0.10^{NS}
2014	Exponential	2344.43	0	0.286	2.33	0.30*

* $p < 0.05$; NS = not significant

Figure captions

Figure 1 Distribution of the surveyed cork oak forest districts in Sardinia (1 = North Gallura; 2 = West Gallura; 3 = South Gallura; 4 = Nuorese; 5 = Oristanese; 6 = Iglesiente). Black area indicate cork oak areas subjected to *Btk*-treatments against *L. dispar* infestations

Figure 2 Mean (± SEM) gypsy moth larval mortality observed in the field 10 days after *Btk* applications in forest sites sprayed in progradation or culmination phases. NS = not significant difference (p = 0.07) between means by two-sided Wilcoxon signed-rank test.

Figure 3 Average temporal pattern of the gypsy moth population density in areas where *Btk*-based insecticides were sprayed in progradation (A) or culmination (B) phases, and in areas left unsprayed (C). The arrows indicate the time of spraying during the gradation (i.e. the time interval between two consecutive latency phases). Note the different scale on the y-axis.

Figure 4 Average (± SEM) time of gypsy moth populations returning to latency phase (on the left) and frequency of high infestations (i.e. population density higher than 100 egg masses per monitoring site) (on the right) in the years following the applications of *Bacillus thuringiensis kurstaki* in forest sites sprayed in progradation or culmination phases. The asterisks above the bars indicate significant differences between the application timings following Wilcoxon Signed-Rank test at $p < 0.05$

Figure 5 Average (± SEM) number of years in which the gypsy moth density occurred at damaging level (i.e. population density higher than 100 egg masses per site) over a complete gradation (i.e. the time interval between two consecutive latency phases)in cork oak forest sites that were sprayed in progradation phase, sprayed in culmination phase, or unsprayed. Different letters above the bars indicate significant differences at $p < 0.05$ level following analysis of variance and Tukey post-hoc tests

Figure 6 Spatial interpolation of the annual gypsy moth population density in two areas of north-eastern Sardinia (Italy). The maps are sorted by time and indicate the temporal patterns of the number of *Lymantria dispar* egg masses per unit of area in the two years preceding and in the five years following the *Btk*-based insecticide applications ($T_{spraying}$). The sprayings were carried out in 2007 and 2009 in the progradation (A) and in the culmination (B) phases, respectively. Black lines indicate the boundaries of each sprayed area and black squares represent the monitoring sites

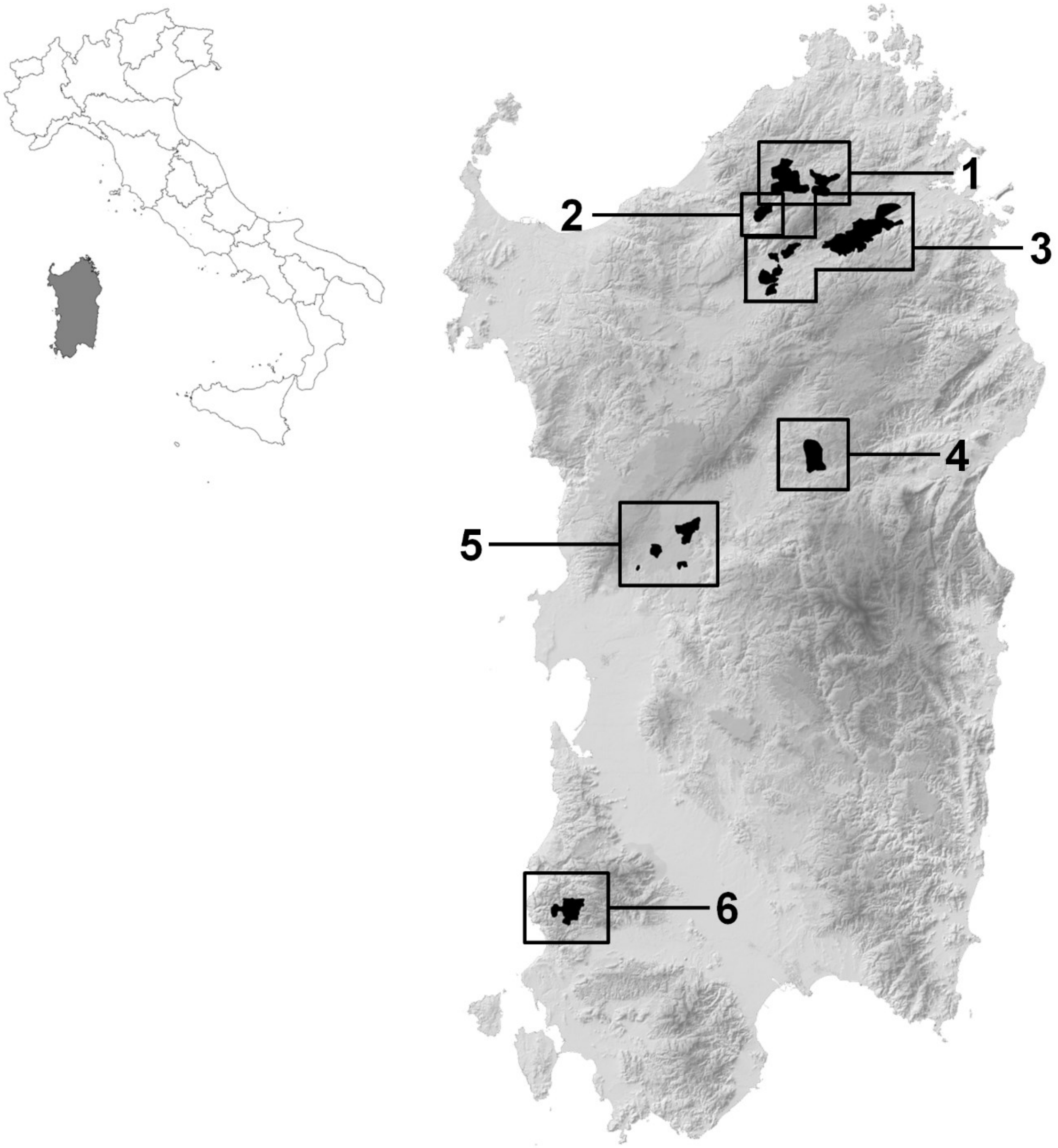


Figure 1

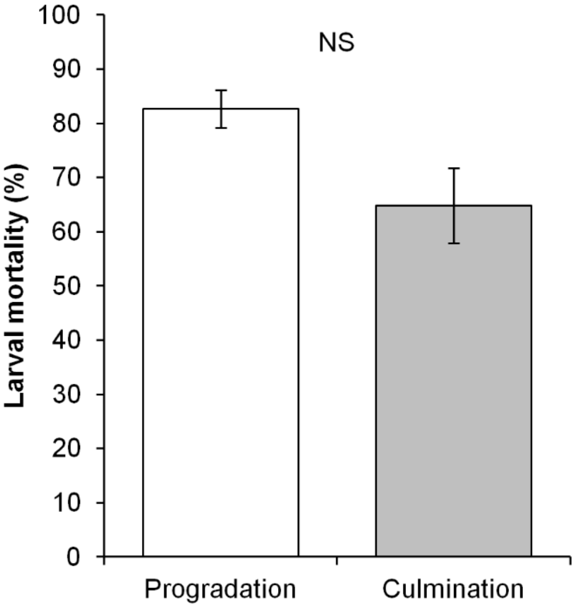
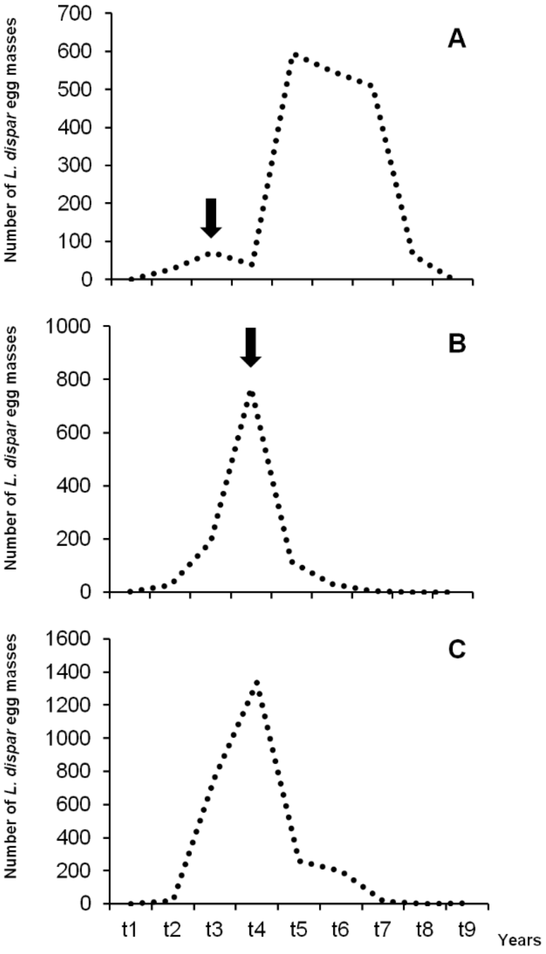
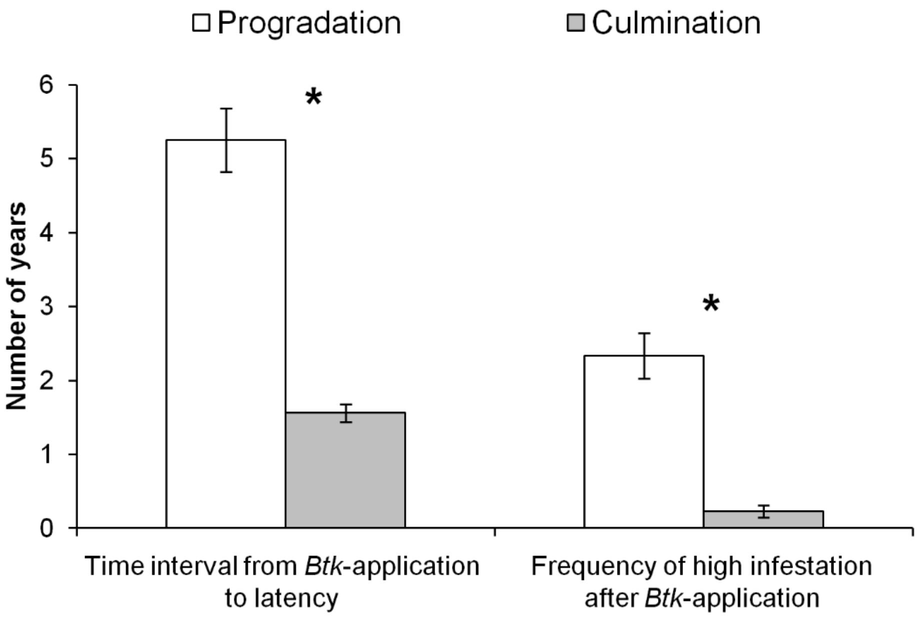

100
90
80
70
60
50
40
30
20
10
0
Larval mortality (%)
NS
Progradation
Culmination

Figure 2



A
B
C
Number of *L. dispar* egg masses
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
0
200
400
600
800
1000
1200
1400
1600
t1
t2
t3
t4
t5
t6
t7
t8
t9
Years

Figure 3



□ Progradation
▨ Culmination
Number of years
0
1
2
3
4
5
6
*
*
Time interval from *Btk*-application to latency
Frequency of high infestation after *Btk*-application

Figure 4

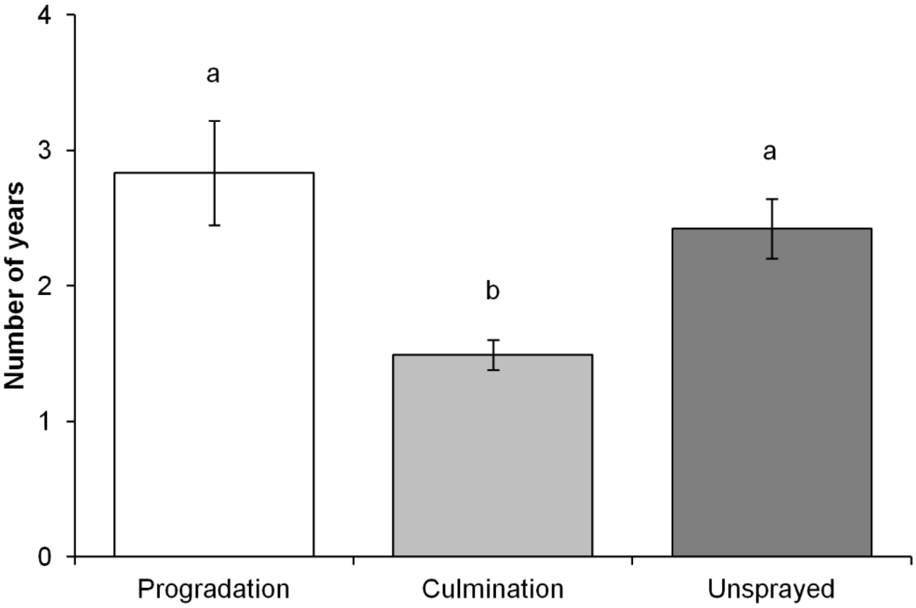

4
3
2
1
0
Number of years
a
b
a
Progradation
Culmination
Unsprayed

Figure 5

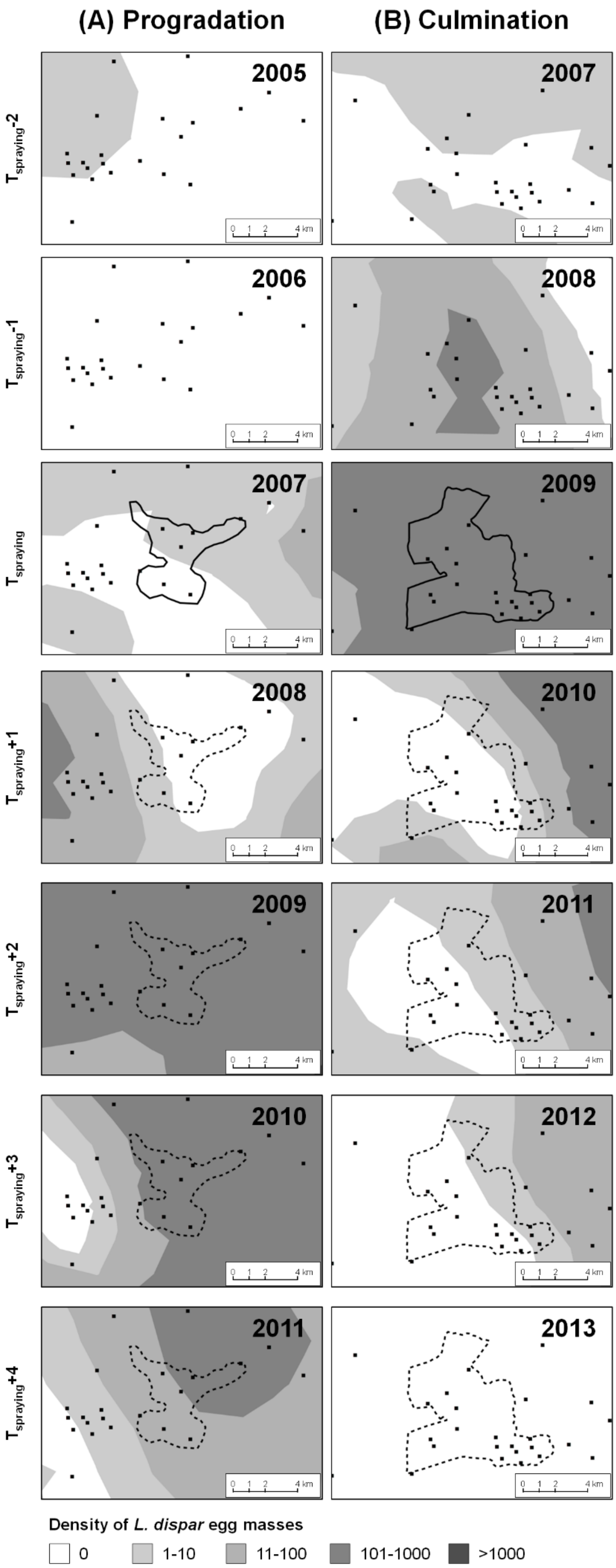

(A) Progradation
(B) Culmination
T$_{spraying}$-2
T$_{spraying}$-1
T$_{spraying}$
T$_{spraying}$+1
T$_{spraying}$+2
T$_{spraying}$+3
T$_{spraying}$+4
2005
2006
2007
2008
2009
2010
2011
2012
2013
0 1 2 4 km
Density of *L. dispar* egg masses
0
1-10
11-100
101-1000
>1000

Figure 6

Supplementary material captions

S1 Spatial interpolation of the annual gypsy moth population density in different areas of Sardinia (Italy) sprayed with *Bacillus thuringiensis kurstaki* (*Btk*) in progradation phase. The maps are sorted by time and indicate the temporal patterns of the number of *Lymantria dispar* egg masses per unit of area in the two years preceding and in the five years following the *Btk*-based insecticide applications ($T_{spraying}$). Black lines indicate the boundaries of each sprayed area and black squares represent the monitoring sites

S2 Spatial interpolation of the annual gypsy moth population density in different areas of Sardinia (Italy) sprayed with *Bacillus thuringiensis kurstaki* (*Btk*) in culmination phase. The maps are sorted by time and indicate the temporal patterns of the number of *Lymantria dispar* egg masses per unit of area in the two years preceding and in the five years following the *Btk*-based insecticide applications ($T_{spraying}$). Black lines indicate the boundaries of each sprayed area and black squares represent the monitoring sites

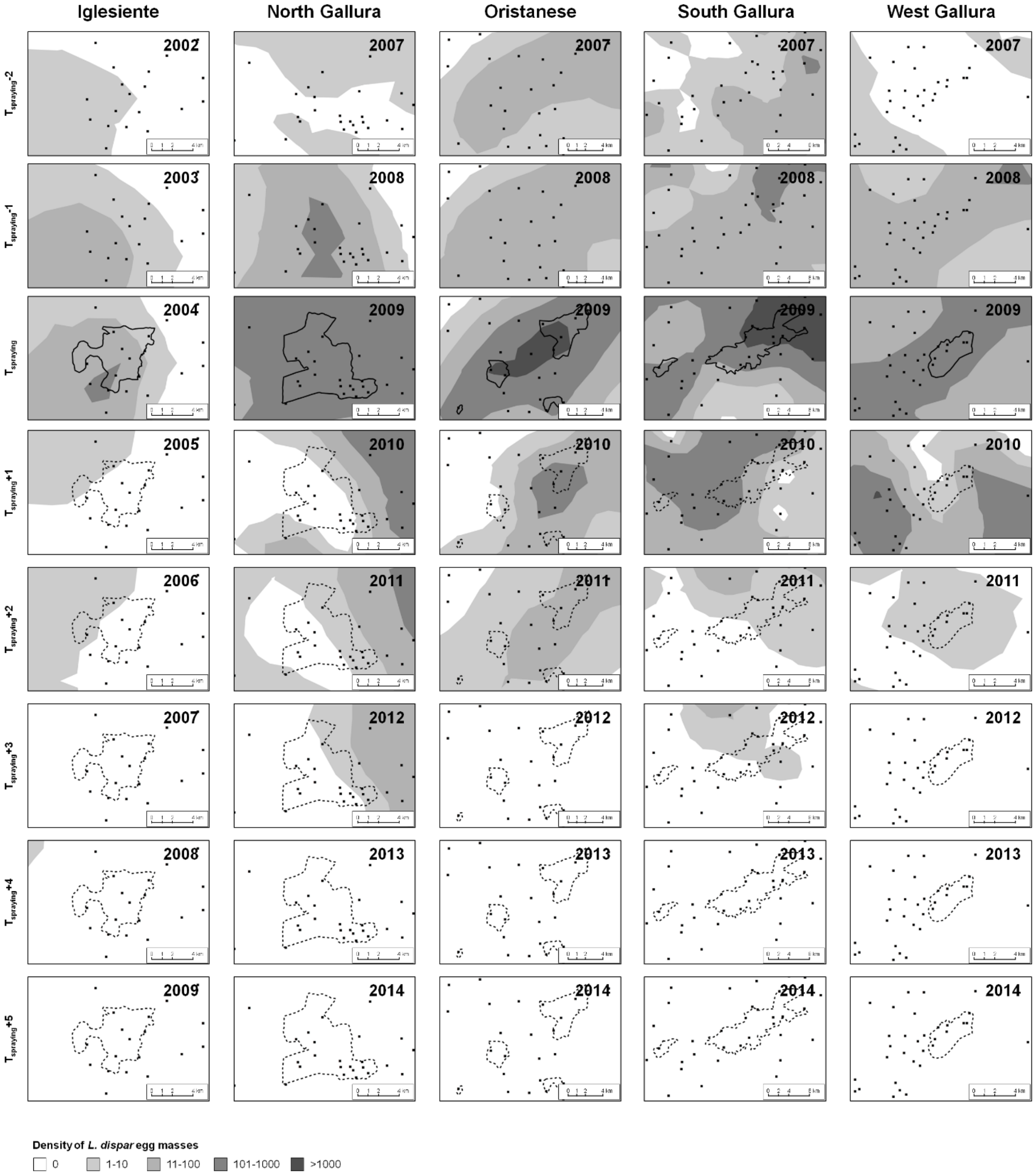

Iglesiente
North Gallura
Oristanese
South Gallura
West Gallura
$T_{spraying}-2$
$T_{spraying}-1$
$T_{spraying}$
$T_{spraying}+1$
$T_{spraying}+2$
$T_{spraying}+3$
$T_{spraying}+4$
$T_{spraying}+5$
2002
2007
2007
2007
2007
2003
2008
2008
2008
2008
2004
2009
2009
2009
2009
2005
2010
2010
2010
2010
2006
2011
2011
2011
2011
2007
2012
2012
2012
2012
2008
2013
2013
2013
2013
2009
2014
2014
2014
2014
Density of *L. dispar* egg masses
☐ 0
1-10
11-100
101-1000
>1000

Figure S1

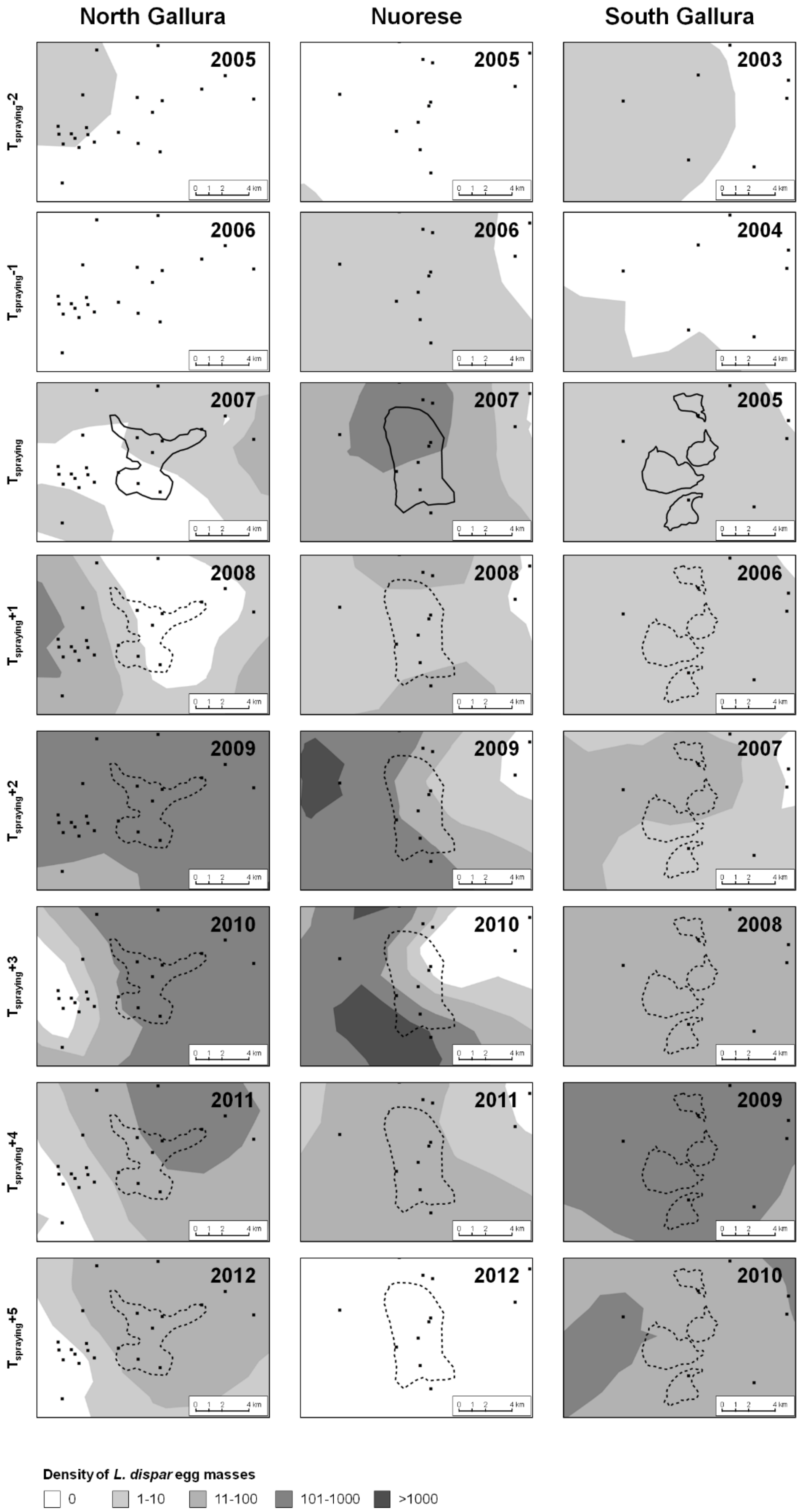

North Gallura
Nuorese
South Gallura
T_spraying-2
T_spraying-1
T_spraying
T_spraying+1
T_spraying+2
T_spraying+3
T_spraying+4
T_spraying+5
2005
2005
2003
2006
2006
2004
2007
2007
2005
2008
2008
2006
2009
2009
2007
2010
2010
2008
2011
2011
2009
2012
2012
2010
0 1 2 4 km
Density of L. dispar egg masses
0
1-10
11-100
101-1000
>1000

Figure S2