

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

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

3

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

5

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24 **Author Contribution Statement**

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

27

28 **ABSTRACT**

29 **BACKGROUND**

30 The gypsy moth, *Lymantria dispar*, is one of the main pests of oak forests worldwide and it causes extensive defoliation
31 during its periodic outbreaks. In the Mediterranean area, the control of gypsy moth populations in cork oak forests is

32 based on the application of *Bacillus thuringiensis* serovar *kurstaki* (*Btk*) formulations. This research investigated the
33 effects of *Btk* applications done in two different population development phases on gypsy moth population dynamics.
34 With this aim, the temporal and spatial fluctuation patterns of *L. dispar* egg density were monitored in cork oak forests
35 treated with *Btk*-applications from 2004 to 2009 in Sardinia (Italy).

36

37 RESULTS

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

45

46 CONCLUSIONS

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

50

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

52

53 1. INTRODUCTION

54 The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) is one of the main forest defoliators worldwide and is
55 able to defoliate thousands of hectares in the same year during its outbreaks.¹⁻⁶ Although the gypsy moth feeds on more
56 than 300 host species,⁷ it is strongly associated with *Quercus* spp., such as cork oak (*Q. suber* L.), downy oak (*Q.*
57 *pubescens* Willd.), and holm oak (*Q. ilex* L.) in the Mediterranean area,^{3,5,8} and white oak (*Q. alba* L.) and northern red
58 oak (*Q. rubra* L.) in North America.⁹ Mediterranean cork oak forests represent one of the most interesting biodiversity
59 hotspots worldwide^{10,11} and, at the same time, an important cultural and economic resource for rural communities.¹² Cork
60 oak forests and woodlands cover approximately 2.5 million hectares across Europe and North Africa¹² and are mainly
61 exploited for cork extraction, with management systems that vary from semi-natural to pastoral. Agricultural and pastoral
62 activities in Mediterranean cork oak forests and woodlands are performed at different intensities, so that cork extraction
63 is often coupled with other activities, such as cropping, grazing, hunting and mushroom harvesting.¹³ Cork production
64 ranges from 150 to 5,000 kg of cork per hectare every 10 years, depending on the main land use.¹⁴ The time interval
65 between two consecutive cork extractions is generally 10-12 years, possibly with longer intervals when adverse climate
66 or pest infestation events occur in the year of debarking.³

67 *Lymantria dispar* infestations can harm the health status of trees,^{15,16} especially when an intensive defoliation occurs after
68 a prolonged drought period. Defoliation can cause a general decrease in plant growth, in terms of both height and
69 diameter,¹⁷ thus negatively affecting cork production in cork oak trees. Cambini¹⁸ estimated a reduction in cork production
70 up to approximately 60% and 40% in the years of complete or partial defoliation, respectively. Gypsy moth feeding
71 activity might also induce physiological imbalances in cork oak trees as a result of a delayed bud burst in the year
72 following the defoliation.¹⁹ Moreover, defoliation spoils the landscape at the beginning of summer, thus discouraging
73 tourism and leading to additional economic losses.

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

82 This population fluctuation pattern is common in many forest insect pests²³⁻²⁶ and can be potentially affected by several
83 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

84 species.^{22,32-34} Although these factors seem not to be effective enough in preventing periodic destructive infestations, they
85 could have a fundamental role in regulating the cyclical dynamics of Lepidoptera outbreaks²³ and the spatial
86 synchronization of separated populations.^{22,24,35} In particular, spatial synchrony (i.e. statistical congruence in the
87 oscillations of geographically separated populations) needs to be considered when analyzing the dynamics of forest
88 pests,³⁵⁻³⁷ as the population density that occurs in a large region can increase simultaneously, thus causing extensive
89 damage throughout a very large area.^{38,39} Spatial synchrony has been observed in different species,³⁸ including the gypsy
90 moth.^{24,35,40}

91 Given the negative impact of *L. dispar* on forest environments, several pest control programs have been carried out against
92 this insect in order to reduce the population density and damage during outbreaks^{41,42} or slow down its spread from infested
93 to non-infested territories.⁴³⁻⁴⁶ Among the control strategies developed against the gypsy moth,⁴⁷ the aerial application of
94 formulations based on *Bacillus thuringiensis* Berliner serovar *kurstaki* (*Btk*) exhibits few biological and practical
95 limitations and is currently the most used and effective method to suppress gypsy moth outbreaks. Several aspects should
96 be taken care of in order to enhance the effectiveness of *Btk* applications against *L. dispar*,⁴⁸ including the timing of
97 application relative to the phenology of 2nd instars,^{8,49} dose,^{41,50} droplet size and density of *Btk*-based insecticides.⁵¹ In
98 addition, an improper use of *Btk* applications against the gypsy moth could lead to unexpected outcomes in the long-term.
99 Recently, Reilly and Elderd⁵² developed a mathematical model on the long-term effects of different biological control
100 strategies against *L. dispar* populations, and hypothesized that *Btk* applications may reduce the density of the pest in the
101 short-term. On the other hand, microbiological applications could also alter the natural dynamics of gypsy moth by
102 dampening the outbreak peaks, thereby causing an unexpected outbreak occurrence over the long-term. In fact, pest
103 control strategies that prevent outbreaks could also impact on the density-dependent process that regulates population
104 cycles.⁵² However, no experimental study has investigated the indirect influence of different application timings on the
105 dynamics of the gypsy moth. In this perspective, we investigated the effects of *Btk* applications applied in different
106 population development phases on the spatial and temporal patterns of *L. dispar* population density in cork oak forests.

107

108 2. MATERIALS AND METHODS

109 2.1. Study areas

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

114 on 85,000 hectares.⁵³ All forest districts are characterized by typical Mediterranean climatic conditions, with mild and
115 rainy winters and hot and dry summers, and with soils mostly granitic and classified as Eutric Leptosols.⁵⁴
116 In our observational study, data refer to areas sprayed with *Btk* from 2004 to 2009. Three areas were protected with a *Btk*-
117 based insecticide during the progradation phase, whereas five areas were sprayed with the same product during the
118 culmination phase (Table 1). All the forest areas included in the survey were larger than 1,000 hectares. Data from two
119 unsprayed areas located in north Sardinia were used as control to compare natural and treatment-affected patterns (Table
120 1).

121

122 **2.2. Estimation of population density**

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

131

132 **2.3. *Bacillus thuringiensis* applications**

133 In all the treated areas, the *Btk*-based formulation Foray 48B[®] (Valent BioSciences, Libertyville, IL, USA) was sprayed
134 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
135 atomizers.⁵⁹ Foray 48B[®] is a commercial formulation containing the *Btk* HD1 strain, which is considered the most
136 effective strain to control gypsy moth and tent caterpillar, *Malacosoma neustria* (L.) (Lepidoptera: Lasiocampidae)
137 populations in Sardinia.⁶⁰ Control measures were performed against the 2nd-instars, as they are the most susceptible stage
138 to *Btk* applications.⁴⁹ For this purpose, larval instar distribution was evaluated weekly by field observations in all the areas
139 to be treated .

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

145 treated, which included the infested monitoring sites, were digitally determined using a Geographical Information System
146 (GIS).

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

152

153 **2.4. Data analyses**

154 Statistical analyses were performed using R statistical software v. 3.10.⁶¹ When necessary, a $\log(x+1)$ transformation was
155 applied before the analyses to meet the assumptions of normality.

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

159 In order to investigate differences in the average level of *L. dispar* infestation among unsprayed areas, areas sprayed in
160 the progradation phase, and areas sprayed in the culmination phase, the number of egg masses over a complete gradation
161 was analyzed by fitting a linear mixed model (LMM) using the lme4 package.⁶² A linear mixed model approach was used,
162 as strongly recommended by Pinheiro and Bates⁶³ for unbalanced samples. In the linear mixed model, fixed factors were
163 treatment (sprayed in progradation, sprayed in culmination or unsprayed), year of observation within the gradation, and
164 their interaction, whereas random factors were forest district and year of observation nested within each forest district.⁶⁴
165 Analysis of variance (ANOVA) was used to test the significance of each factor.

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

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

174 The annual spatial distribution of the abundance of *L. dispar* egg masses was mapped using geostatistical methods. Before
175 the analysis, a $\log(x+1)$ transformation was applied to approximate data to a normal distribution.⁶⁵ Anisotropy was not

176 considered and the semivariograms were fitted using ArcGIS software release 10.1.⁶⁶ The lag size was determined
177 independently for each year, by computing the average distance between points and their nearest neighbors.⁶⁶ The average
178 distance between points was different each year as a result of the variation in the number and location of the sites
179 monitored annually. Gaussian, exponential and spherical models were tested between all the possible available
180 methods.^{4,67,68} The Akaike Information Criterion (AIC) was calculated for each model and used for selecting those best
181 fitting the data. The following parameters were also calculated for the selected models: nugget (C0), range (A), sill (C0
182 + C, in which C is the variability attributable to the spatial dependence of the data).⁶⁹ The models fitted from the
183 variograms were finally used for estimating the abundance of gypsy moth egg masses using ordinary kriging.

184

185 3. RESULTS

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

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

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

205 The average time to reach the latency phase after *Btk* application was significantly different between sites sprayed in both
206 culmination and progradation phases ($W = 39.5, p < 0.01$), with values significantly lower in sites sprayed during

207 culmination (mean \pm SEM: 1.56 ± 0.12 years) than in those sprayed in progradation (mean \pm SEM: 5.25 ± 0.43 years)
208 (Fig. 4). In addition, the average number of years in which the gypsy moth density occurred at damaging level after the
209 *Btk* applications was significantly higher in the sites sprayed in progradation (mean \pm SEM: 2.33 ± 0.31 years) than in
210 those sprayed in culmination (mean \pm SEM: 0.23 ± 0.08 years) ($W = 132.5$, $p < 0.05$) (Fig. 4).

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

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

221 The interpolation maps highlighted different spatial distribution patterns of *L. dispar* density when *Btk* applications were
222 made in progradation or culmination phase, as exemplified in Fig. 6. The gypsy moth population density was at a harmless
223 level (i.e. lower than 100 egg masses per site) in the majority of the monitoring sites during the progradation phase (2005-
224 2006) (Fig. 6a). In 2007, the population density in the area to be sprayed was higher than in the two preceding years. The
225 bio-insecticide applied in 2007 reduced the population density in the following year, when gypsy moth egg masses were
226 observed only in two of the seven monitoring sites inside the sprayed area. However, the reduction was restricted to the
227 area protected with the *Btk*-based insecticide, as a higher infestation level was observed in the surrounding untreated sites.
228 In 2009, gypsy moths spread out from the untreated to the treated area, causing an increase in the population at damaging
229 density throughout the forest district.

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

238

239 4. DISCUSSION AND CONCLUSION

240 The results of the present research show that the phase of the gradation in which the application is performed does not
241 influence the efficiency of *Btk*, as similar larval mortalities were observed in both treatments. A positive effect was also
242 obtained in the year following *Btk* application, as the *L. dispar* population density decreased in forests treated in both
243 progradation and culmination phases. However, the medium-term effectiveness of the *Btk*-insecticide differed
244 substantially depending on the application timing. Notably, when the bio-insecticide was sprayed in the culmination
245 phase, the population abundance decreased down to the latency phase faster than in the non-treated areas. On the other
246 hand, when *Btk* was applied in progradation, the gypsy moth density decreased only temporarily before increasing again
247 to reach the outbreak peak in two years. In other words, early applications during population growth shifted the abundance
248 peaks forward, thus increasing the duration of gradations. These results suggest that the long-term effects of *Btk*
249 application might be significantly influenced by the factors involved in the regulation of gypsy moth population cycles.
250 The population dynamics of forest Lepidoptera are the result of the interactions between the pest and a bioregulatory
251 complex represented by natural enemies (parasitoids and predators), diseases (pathogens and viruses), and nutritional
252 quality of the host foliage.²³ The effect of the natural enemies on the gypsy moth mortality varies depending on the phase
253 of the gradation. The population density of parasitoids naturally increases at high gypsy moth density, i.e. culmination
254 phase, and reaches the peak during the retrogradation phase,^{21,28,29,70-72} with *Parasetigena silvestris* Robineau-Desvoidy
255 and *Blepharipa pratensis* Meigen (Diptera: Tachinidae) being the most frequent oligophagous species. Although the
256 response of tachinids to the variation in gypsy moth abundance is delayed, it plays a key role in reducing the population
257 density to the latency phase.⁷³ Some species of generalist tachinid parasitoids, such as *Compsilura concinnata* (Meigen),
258 are more active at low pest population density and can actively concur to suppress incipient outbreaks.¹ Predators of *L.*
259 *dispar* are often generalist and could significantly affect the population dynamics of the defoliator in some forest types
260 and climatic conditions. In fact, egg predators can cause mortality up to 90%,⁷⁴ and larval predators, such as *Calosoma*
261 *sycophantha* (L.) (Coleoptera: Carabidae), can also notably increase their density during outbreaks.⁷⁵
262 In North America, low defoliator population densities are affected by predation by small mammals,^{76,77} whereas the
263 collapse of high population densities is attributable, especially during outbreaks, to specific entomopathogens, such as
264 *Entomophaga maimaiga*^{78,79} and the gypsy moth nuclear polyhedrosis virus (LdNPV).¹ Although *E. maimaiga* mortality
265 is largely density-independent, the fungus theoretically causes greater levels of mortality in hosts than the virus.⁸⁰
266 The quality of food can influence the biological parameters and population dynamics of the gypsy moth.^{27,81,82} Leaf quality
267 variations can occur in response to feeding activity of insects. For example, a higher level of phenolics, hydrolysable
268 tannins, dry matter content, and toughness were associated with a higher level of defoliation on red oak, *Quercus rubra*

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

275 To summarize, the population dynamics of the gypsy moth is regulated by several density-dependent mortality factors,
276 which reach the highest incidence in the culmination phase and beyond. Therefore, *Btk* applications during the
277 progradation phase, at low-medium gypsy moth population density, do not allow the biotic control factors to increase
278 enough to the extent of causing the collapse of the pest population density. In this case, the bio-insecticide spray protects
279 the vegetation for 1-2 years, and the *L. dispar* population density increases again to the culmination phase after few years.

280 The results presented here suggest that the hypothesis of Reilly and Elderd,⁵² concerning the potential defoliation due to
281 the diffusion of pests from untreated to treated patches, more likely occurred in Mediterranean environments in which
282 aerial applications were made during progradation. The spread through extinction-colonization dynamics also needs to
283 be considered as an important factor directly influencing the temporal pattern of gypsy moth populations and the treatment
284 effectiveness. In fact, the geostatistical analysis showed that the gypsy moth spread from untreated to treated forest areas
285 in the years following the application only when the *Btk*-insecticide was applied in the progradation phase. In contrast, in
286 the years following the application done in the culmination phase, the gypsy moth population density did not increase
287 within the area treated with *Btk*-insecticides nor in its surroundings. These results suggest that the ability of the gypsy
288 moth to spread from more- to less-infested areas may be strongly related to the population density at a forest district
289 scale,^{35,45} also because of the higher influence of some biological factors affecting the gypsy moth mortality, such as
290 parasitoid and predator density at this spatial scale. In particular, their role increases from the progradation phase on,^{5,23}
291 thus allowing to naturally limit defoliator density in the entire forest district after its culmination phase.^{28,70,84}

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

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

307

308 5. ACKNOWLEDGEMENTS

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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

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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*

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* $p < 0.05$; NS = not significant

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505 **Figure captions**

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

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

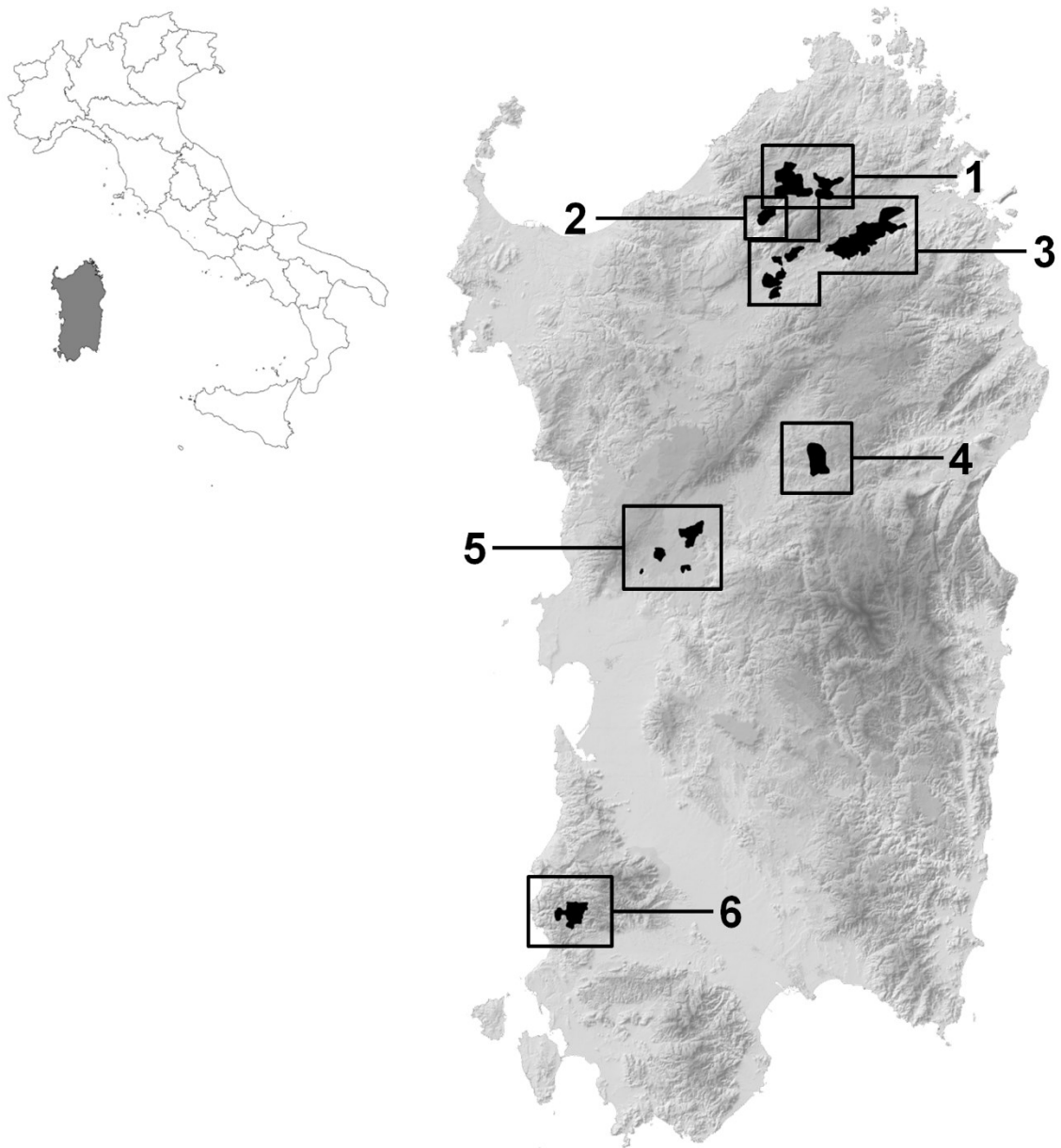
512 **Figure 3** Average temporal pattern of the gypsy moth population density in areas where *Btk*-based insecticides were
513 sprayed in progradation (A) or culmination (B) phases, and in areas left unsprayed (C). The arrows indicate the time of
514 spraying during the gradation (i.e. the time interval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

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

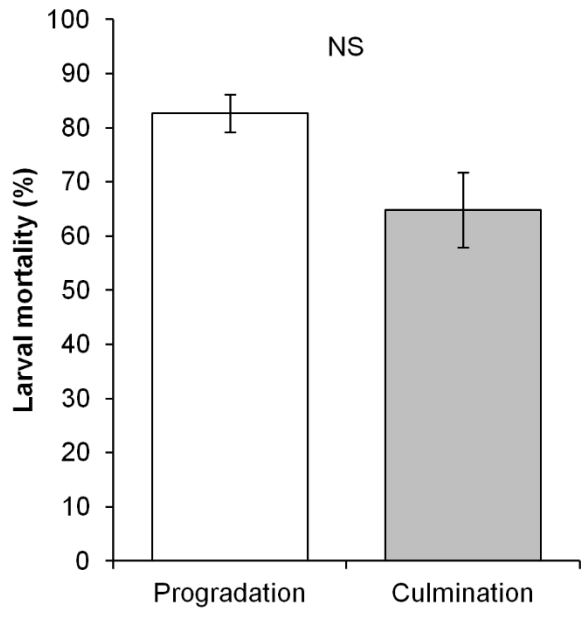
521 **Figure 5** Average (\pm SEM) number of years in which the gypsy moth density occurred at damaging level (i.e. population
522 density higher than 100 egg masses per site) over a complete gradation (i.e. the time interval between two consecutive
523 latency phases) in cork oak forest sites that were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

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

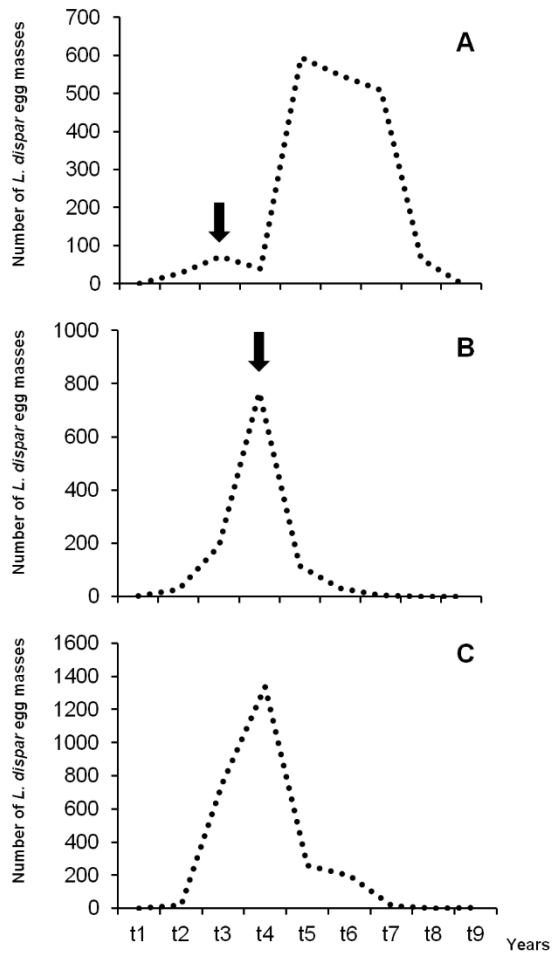
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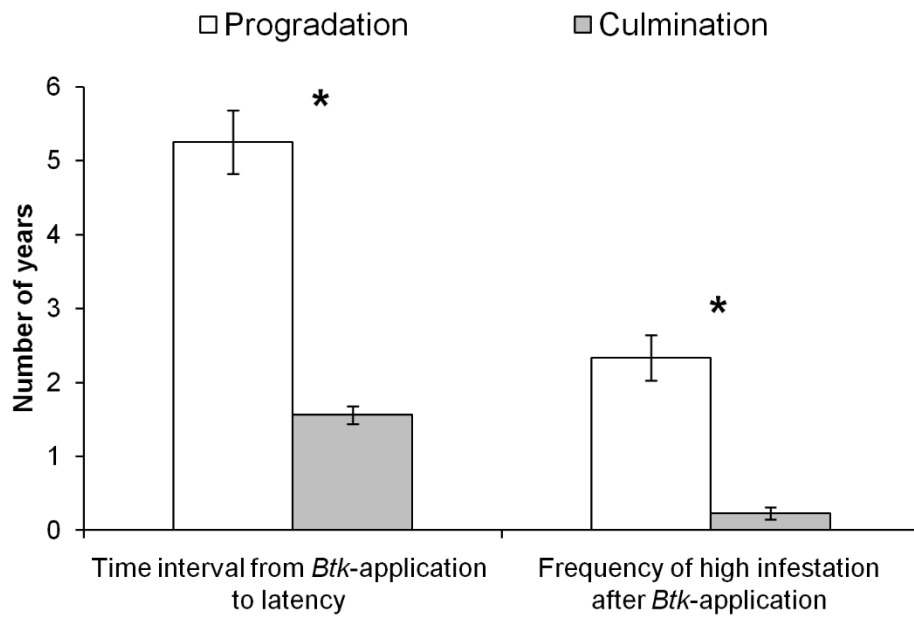


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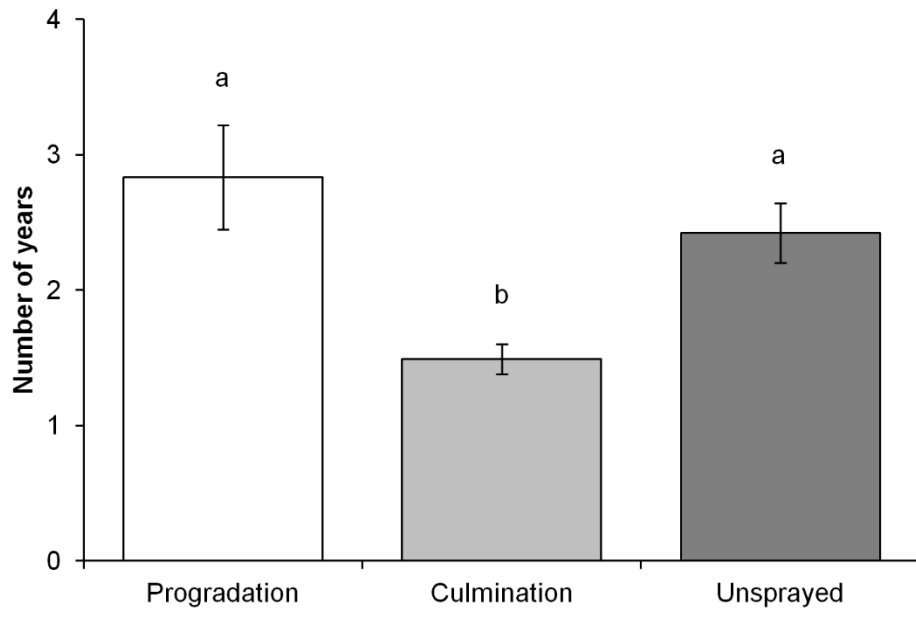
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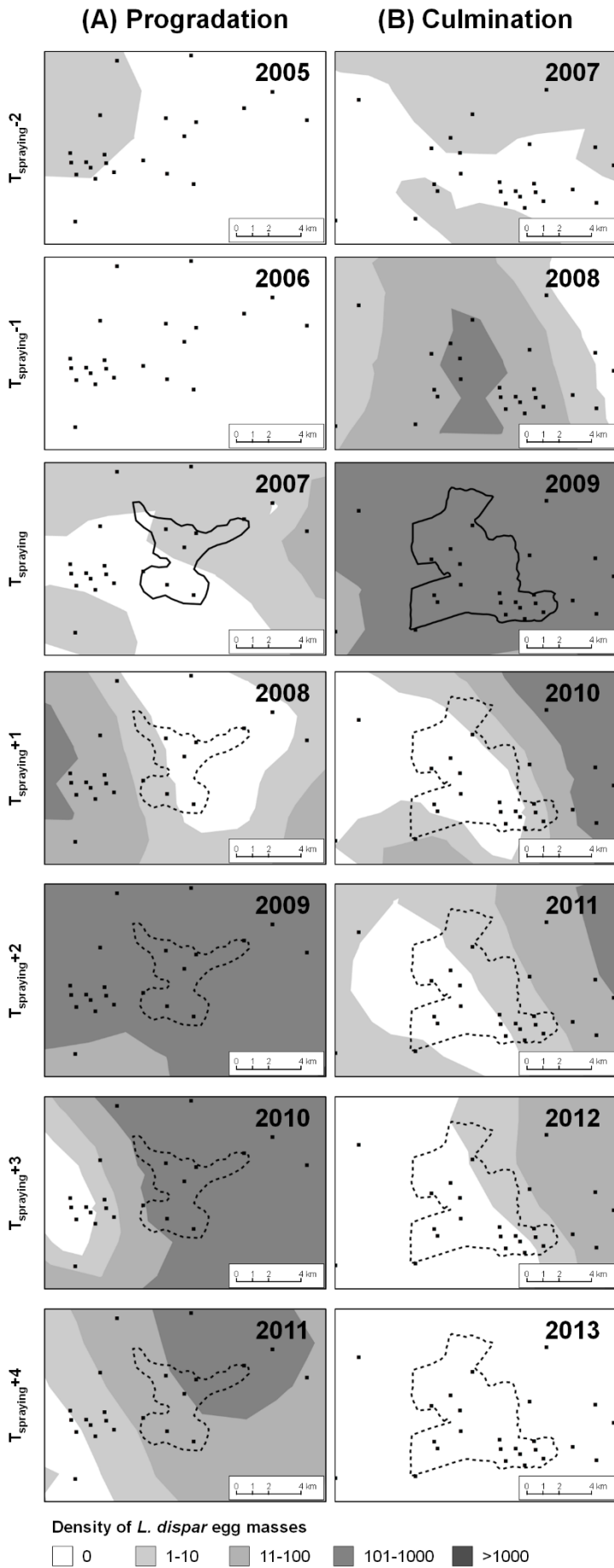
Figure 3



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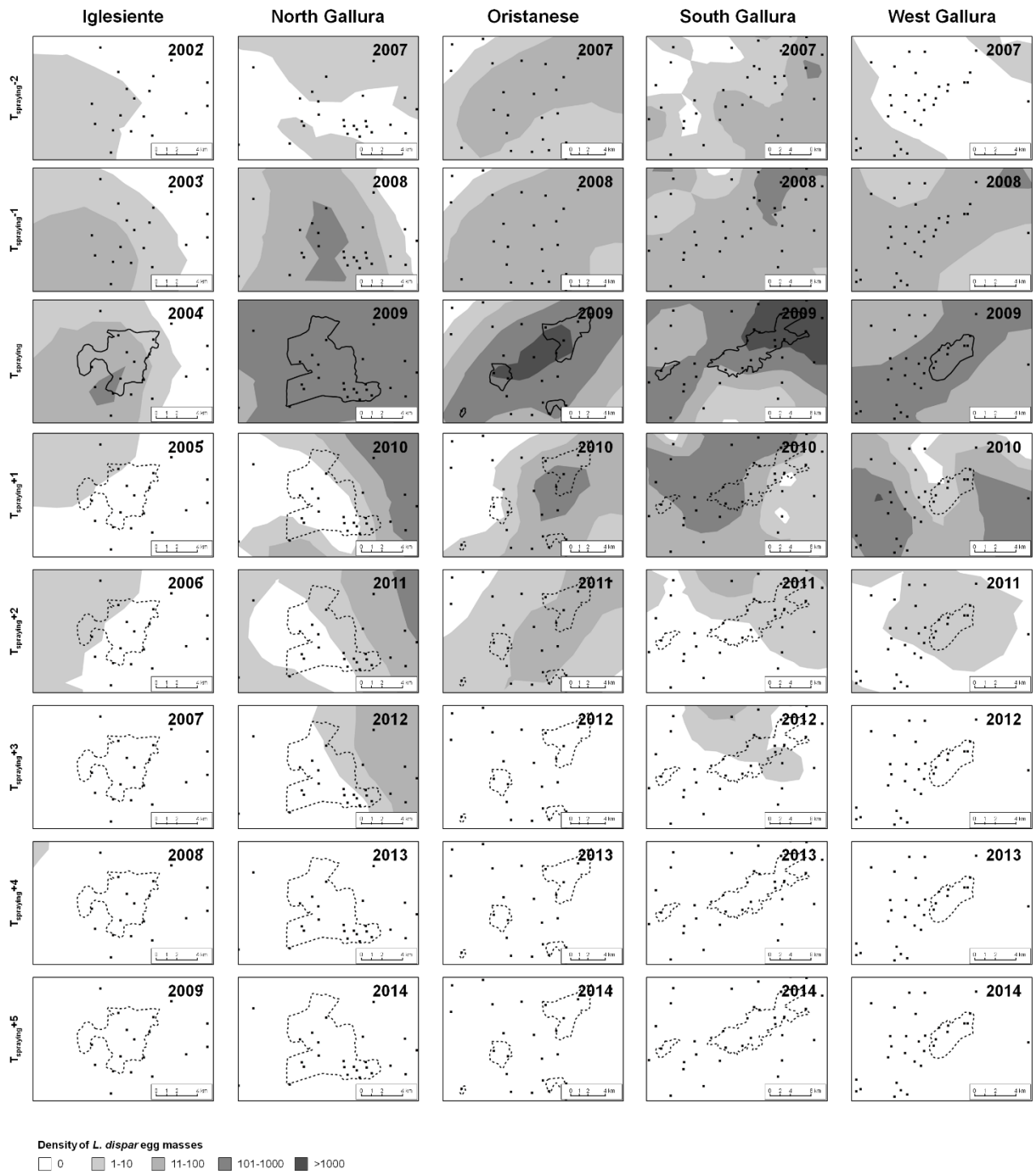
Figure 6

549 **Supplementary material captions**

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

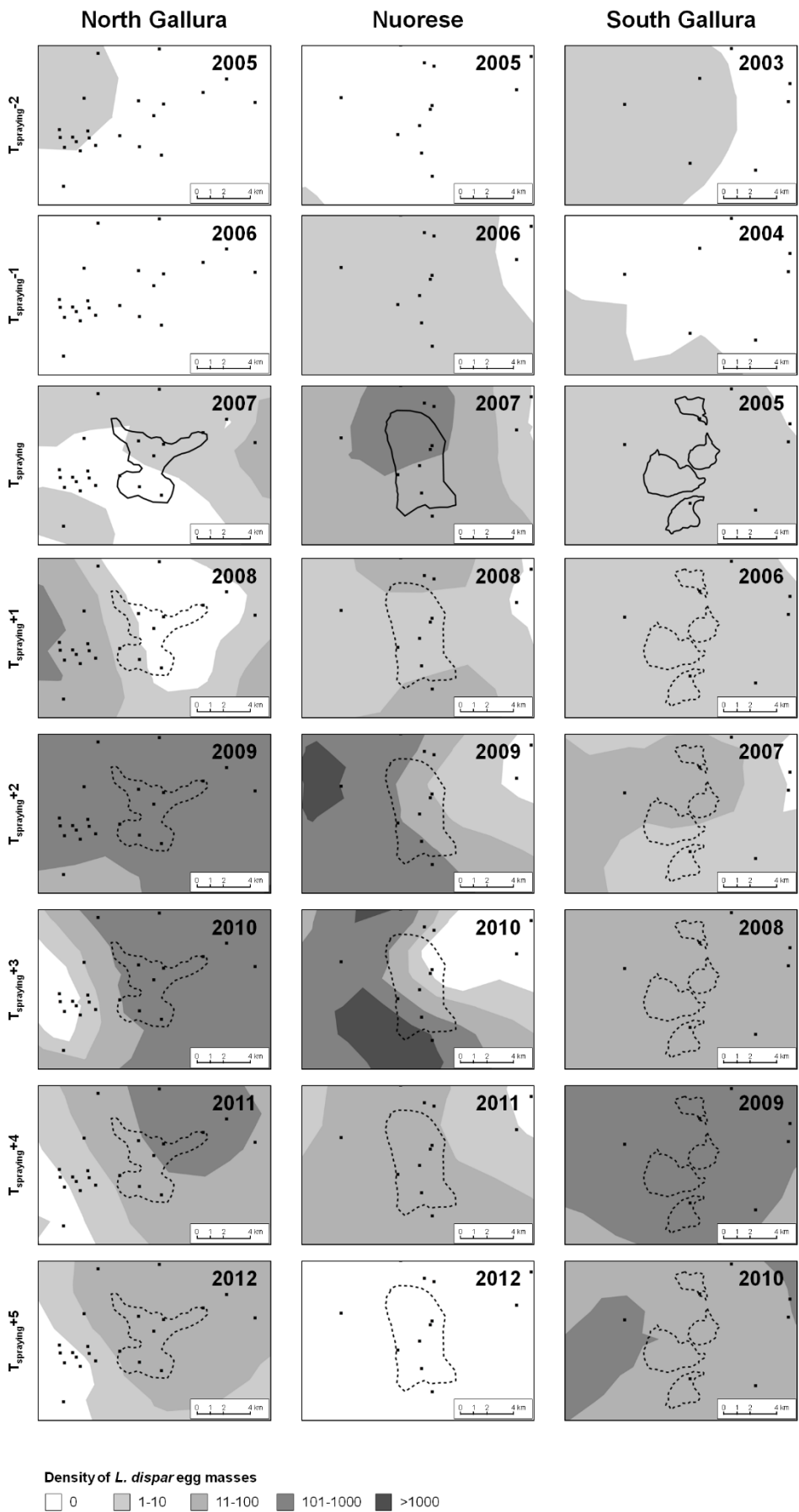
555 **S2** Spatial interpolation of the annual gypsy moth population density in different areas of Sardinia (Italy) sprayed with
556 *Bacillus thuringiensis kurstaki* (*Btk*) in culmination phase. The maps are sorted by time and indicate the temporal patterns
557 of the number of *Lymantria dispar* egg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

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Figure S1



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Figure S2