Aerial Spraying of *Bacillus thuringiensis* var. *kurstaki* for the Control of *Thaumetopoea processionea* in Turkey Oak Woods

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Thaumetopoea processionea L. is an important oak defoliator whose outbreaks have become increasingly frequent in Europe and thus have received a great deal of attention from foresters. Field trials were carried out in central Italy (Tuscany) to test the efficacy of aerial spraying of *Bacillus thuringiensis* var. *kurstaki* [*Btk*] in early spring for the control of processionary infestations in *Quercus cerris* L. woods. The experimental sites were located in a hilly area of 1640 ha with a maximum altitude of 593 m a.s.l. and a yearly mean temperature of 10°C. Turkey oak woods were divided into three areas to be treated with 31.75 BIU ha^{-1} (2.5 l ha^{-1}) on 578 ha, 44.45 BIU ha^{-1} (3.5 l ha^{-1}) on 306 ha and 57.15 BIU ha^{-1} (4.5 l ha^{-1}) on 756 ha, respectively. Five days after treatment, larval mortality was less than 40% in the control plot, but over 60% in the treated areas. Thirteen days after treatment, larval mortality varied from 75.05% to 96.42% in the three treated areas. Surveys conducted 2 months after the treatment showed a strong decline in the number of oak processionary nests in all the treated plots. It was possible to control the pest effectively in turkey oak woods using *Btk* at 31.75 BIU ha^{-1} distributed at ultra-low volume at the time of bud opening when non-urticating larvae were present.

KEY WORDS: Forest protection; microbial insecticide; *Quercus cerris;* urticating larvae.

INTRODUCTION

The oak processionary moth *Thaumetopoea processionea* (L.) (Lepidoptera: Thaumetopoeidae) is a univoltine lepidopteran forest defoliator of deciduous oaks, widely distributed in central, western and southern Europe (1,16). The adults appear between July and September and the female lays the eggs on young oak twigs in uniplanar egg clusters destined to support overwintering. The larvae, equipped from the 3^{rd} instar with urticating hairs on the dorsum (18) and active in spring–summer, exhibit gregarious behavior throughout their life, constructing sack-shaped nests on the stem or axis of large branches inside which they pupate in a papery cocoon (27). This pest can completely defoliate oak stands but is also found on isolated trees in avenues and parks (14,24). Since the 1990s, outbreaks of this phytophage, with the associated medical problems, have become increasingly frequent throughout very large areas of oak forests in various European regions (38,42), including Holland, where a high incidence of this processionary had not been reported for a century (9,36,37). In addition to the outbreaks in areas where *T. processionea* is endemic, the recent first record of adult processionary moths in four sites in

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southern Sweden (21) confirms the tendency of this species to colonize new environments, particularly by northward expansion of its distribution area.

This defoliator has also become increasingly important in Italy due to heavy and extensive infestations both in Middle European oak environments with a high density of the pedunculate oak *Quercus robur* L. and in forests inhabited mainly by deciduous species of the submediterranean zone such as the turkey oak *Quercus cerris* L.; indeed, it has become a pest in forests where it was absent or occurred only occasionally (10,31). In central Italy, a *T. processionea* peak occurred in 1996 in the Santa Luce Forest in the Province of Pisa, Tuscany, strongly reducing activities and normal recreational uses of the forest (30). As a consequence, field experiments and laboratory trials were carried out to study the population dynamics of the oak processionary, to establish efficient monitoring methods and to identify low-environmental-impact strategies for the control of infestations.

In North America and Europe, *Bacillus thuringiensis* var. *kurstaki [Btk]* formulations have been used annually against lepidopteran defoliators in coniferous and broadleaved woods (4,39) because of their effectiveness, specificity (40) and the rapidity with which the spores are killed by UV radiation (20). At present, commercial formulations of *Btk* are widely used even though only standard employment protocols are available for some species (6). This is despite the fact that several studies have shown clearly that environmental factors (sunlight, temperature), the target species and insect stage, the preparation and handling of the product (concentration and distribution methods), and the plant where the pest feeds can influence the efficacy of different strains of the micro-organism (5,11,17). Unlike the situation for the similar *Thaumetopoea pityocampa* (Den. et Schif.) that colonizes coniferous trees of the genus *Pinus* (25,26), there are few literature data on methods to control *T. processionea* with microbiological insecticides (14,25), particularly in Mediterranean oak woods (29).

On the basis of previous investigations showing the high efficacy of FORAY 48B®for the control of defoliator lepidopterans in oak woods (19), trials were conducted in central Italy in 2004 to evaluate the effects of aerial spraying of different doses of *Btk*, serotype 3a,3b, on 1^{st} and 2^{nd} instar *T. processionea* larvae in *Q. cerris* woods a few days after the leaf buds started to open. The aim was to assess the possibility of controlling this pest by ensuring a highly efficient deposition of *Btk* spray droplets within the tree crown in order to kill the larvae that did not yet have urticating hairs.

MATERIALS AND METHODS

Study area The experimental sites were located in the Santa Luce Forest (Pisa), central Italy, a hilly area of 1640 ha, with a maximum altitude of 593 m a.s.l., a yearly mean temperature of 10°C and precipitation peaks in autumn and spring, with a marked reduction in rainfall during the hot, dry summer (minimum 39 mm for the month of July). The most representative stands are dominated by *Q. cerris* sometimes combined with *Quercus pubescens* Willd., *Fraxinus ornu* L., *Quercus ilex* L., *Acer opulifolium*, *Acer pseudoplatanus* L., *Acer campestre* L. and *Ulmus minor* Mill. Interspersion of the various vegetation types is particularly evident. In 1976, the turkey oak coppice woods began to be turned into high-trunk forest.

Monitoring the oak processionary population Since 1996, new oak processionary nests have been counted annually in July on ten randomly selected trees in each of 26 plots (area 1 ha) distributed throughout the study area. Nests were counted when all the oak processionary larvae reached maturity and feeding individuals were no longer observed on the trees. Care was taken to separate the new nests from those of the previous year by opening the nests to assess the contents when necessary.

In July 2003 and July 2004, the definitive nests on ten randomly selected trees were counted in another five plots situated in age-mixed coppices of turkey oak with various broadleaved species neighboring the areas to be treated (Universal Transverse Mercator [UTM] coordinates of the central point of each plot: C_1, 630412 N – 4815052 E; C_2, 630842 – 4812537; C_3, 631442 – 4812486; C_4, 630389 – 4812639; C_5, 630130 – 4809245).

Determination of the date of treatment Various authors have reported that the oak processionary larvae of each egg cluster hatch within a few hours and that, in large forest areas, there is good coincidence between larval hatching and oak leaf bud opening (3,7,28). The synchrony of the two events prompted us to try to obtain an effective reduction of the processionary population with a single treatment. The best date for treatment, *i.e.*, between the hatching of all the eggs and the development to the 2^{nd} instar of a large proportion of the larval population, was identified on the basis of intensive monitoring starting in the first week of April 2004: every 3 days, samples of branches, egg clusters and then larval colonies were collected in six plots in the Santa Luce forest.

***Btk* formulation and spraying** In Canada and the United States, most treatments are applied with airplanes. In Italy, as in other European countries like France (25), helicopters are preferred for the control of defoliator lepidopterans because of the small size and more or less irregular borders of the areas to be treated, their close association with agricultural areas, and the morphology of the environments (rarely uniform over large areas) (22). In the present trial, treatment was carried out with the commercial formulation FORAY 48B at 12.7 BIU l^{-1} (Valent Bio-Sciences Corp., Libertyville, IL, USA) on April 28, 2004, using an Aérospatiale SA 315 Lama helicopter with a total capacity of 500 l and equipped with four electrically operated rotary nozzles (Micronair) mounted on a 12-m-wide bar, which sprayed a 20-m band of forest at each passage. The helicopter flew under conditions of a partly cloudy sky and at a wind speed less than 16 km h^{-1}. Turkey oak woods were divided into three areas: (i) 578 ha to be treated with 31.75 BIU ha^{-1} (2.5 l^{-1}), (ii) 306 ha to be treated with 44.45 BIU ha^{-1} (3.5 l^{-1}), and (iii) 756 ha to be treated with 57.15 BIU ha^{-1} (4.5 l ha^{-1}), for a total of 1640 ha. Before each treatment, the atomisers were calibrated on the ground according to the speed at which the helicopter would be flying ($\sim$ 90 km h^{-1}) to regulate the quantity of product sprayed by each nozzle per unit time. The helicopter flights were recorded with a GPS system to ensure complete treatment coverage in each of the three areas.

Efficacy of the treatment In May 2004, the efficacy of the treatment was evaluated by recording the larval mortality in plots (1 ha in area) in each of the three treated areas: T_1 in the area treated with 31.75 BIU ha^{-1} (transitory high forest of mainly turkey oak with various broadleaved species, UTM coordinates of the central point of the plot 627765 N – 4813867 E); T_2 in the area treated with 44.45 BIU ha^{-1} (transitory high forest of mainly turkey oak with various broadleaved species, UTM coordinates of the central point of the plot 629662 N – 4815471 E); T_3 the area treated with 57.15 BIU ha^{-1} (aged mixed coppice of turkey oak with various broadleaved species, UTM coordinates of the central point of the plot 630803 N – 4813932 E). As a control, the same data were collected in plot C_1, with the same forest types and stand conditions as T_3, situated over 500 m from the borders of the treated areas to avoid any effects related to dispersion of the product.

The initial density was estimated in two samplings carried out on May 3 and May 11, 2004, in plots T_1, T_2, T_3 C_1: on the two sampling days, three trees were randomly selected in each plot and all the egg clusters and larval colonies on them were collected. The colonies were identified after the trees were pruned from an aerial platform and the branches were examined on the ground. In the laboratory, the egg clusters were desquamated and observed under a stereomicroscope to count the total number of eggs per cluster and the percentage of regularly hatched eggs (8). In this species, as in the congeneric pine processionary, the larvae from each egg cluster do not disperse but remain together and live gregariously (24). Therefore, the total number of dead larvae for each tree at the time of checking was taken as the sum of the larvae hatched but not found on the tree and the dead larvae hanging by silk threads on the branches. All live larvae were collected, counted, put in fine-mesh metal-screen cages (fed on *Btk*-free turkey oak leaves), transferred to the wild and maintained in the open under a canopy. They were checked regularly for subsequent mortality.

Fecundity (number of eggs/egg cluster) and percentage of eggs hatched/egg cluster were recorded to assess the efficacy of the *Btk* treatments at the different doses and also to obtain biological indicators of the processionary population dynamics. In previous studies carried out in the same areas, the mean number of eggs/female was correlated with the population trend of *T. processionea*: it oscillated between a maximum of 183.3 eggs/egg cluster in ovidepositions prior to the outbreak peak to a minimum of 104 eggs/egg cluster in the year of collapse (30). Therefore, the biological indicators were recorded to ensure that, in the case of a synchronous fluctuationacross the large area, any spatial heterogeneity of the cluster of population collapse would not result in biased data, *i.e.*, data referring to some plots with naturally declining populations (12).

Statistical analysis Egg cluster density, fecundity, percentage of egg hatching and larval mortality in the field were analyzed by one-way analysis of variance (ANOVA); the differences were compared using the Tukey HSD test. A level of $P<0.05$ was accepted as statistically significant. All percentage data were normalized using angular transformation. To evaluate the relative effectiveness of different *Btk*, the mortality of larvae collected in the field 5 (3 May) and 13 (11 May) days after treatment and reared on *Btk*-free oak leaves was analyzed by SPSS software 10.0 Function Analysis (*follow-up* Life Table) and Comparison of Survival Experience using the Wilcoxon (Gehan) statistic (35).

RESULTS

Monitoring the oak processionary population trends After the peak of the *T. processionea* outbreak in 1996–97 and the sudden decrease in 1998, only a few nests were observed in the following years in the Santa Luce Forest. In 2001, the oak processionary population began to increase again over a large area. Over the period 2001–2003, the mean number of trees with nests showed an almost threefold increase in the 26 permanent plots; consequently, in summer 2003, *T. processionea* nests were present on more than 60% of the sampled trees (Fig. 1). The monitoring program revealed a dangerous trend toward another strong outbreak of the *T. processionea* population, similar to what was happening in other European areas. This tendency to a marked increase of the defoliator population applied to the entire area, including neighboring stands.

TABLE 1. Larval mortality (% ± S.D.) observed in the field on May 3 and May 11. T_1, plot treated with 31.75 BIU ha^{-1}; T_2, plot treated with 44.45 BIU ha^{-1}; T_3, plot treated with 57.15 BIU ha^{-1}; C_1, untreated control plot

Sampling date	Plots			
	T_1	T_2	T_3	Control (C_1)
May 3	70.0(±22.2) a A	69.7(±9.1) a A	82.6(±2.8) b A	37.4(±0.1) c A
May 11	75.1(±7.8) a B	96.4(±4.1) b B	90.7(±3.9) c B	47.3(±17.1) d A

Within rows, different lower-case letters indicate significant differences; within columns, different capital letters indicate significant differences ($P<0.05$).

TABLE 2. Live larvae collected on May 3 and May 11 in treated and untreated plots and reared on *Btk*-free leaves until May 21.

Btk (BIU ha^{-1})	May 3		May 11	
	Larvae reared	% Mortality	Larvae reared	% Mortality
Untreated control (C_1)	340	0.00	396	2.30
31.75 (T_1)	622	98.38	810	100.00
44.45 (T_2)	1537	99.08	22	100.00
57.15 (T_3)	792	100.00	297	100.00

On May 3, 98 egg clusters (total of 15,759 eggs) were collected on the nine trees examined in the three plots inside the treated areas and four egg clusters (612 eggs) on the oaks in the control plot, C_1. In the May 11 sampling, the number of egg clusters in the three treated plots had dropped to 75 (8192 eggs), whereas 11 egg clusters (790 eggs) were collected in the control plot (C_1). On May 3, there was no significant difference in the mean number of egg clusters per tree between plot T_1, situated in the area treated with the lowest amount of *Btk*, and the control plot; likewise, there were no differences between plot T_1 and the two other plots in the areas of the oak wood treated with the higher doses (Fig. 2). In the May 11 sampling, there were no significant differences between the control plot and all treated plots.

No significant differences in mean fecundity were found in the May 3 and May 11

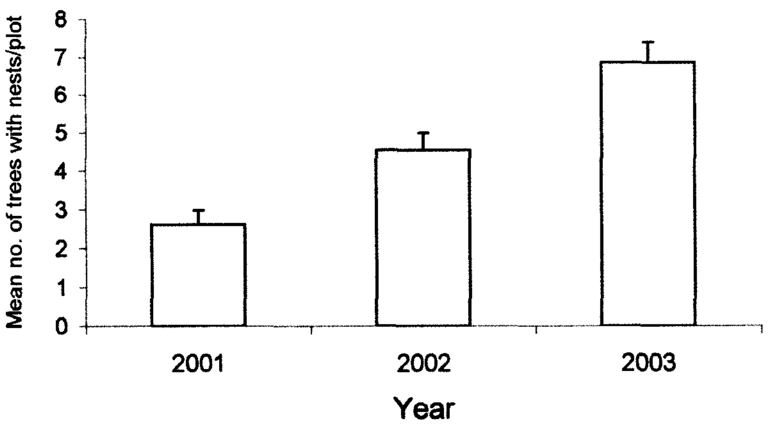

Fig. 1. Mean number of trees with nests/plot (ten randomly selected trees checked in each plot). Bars represent standard deviation.

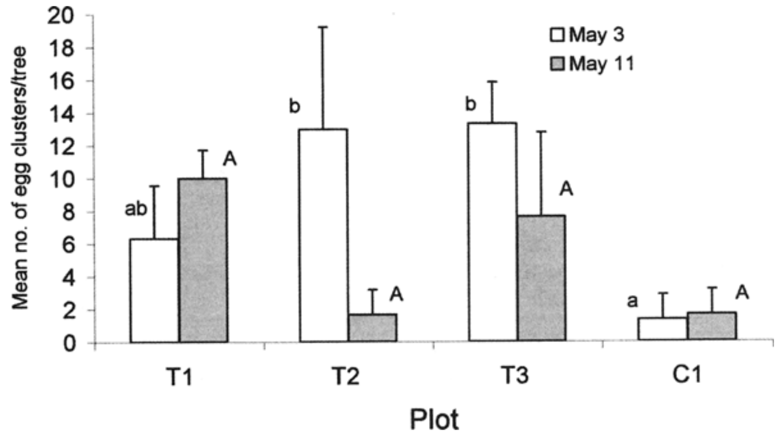

Fig. 2. Mean number of egg clusters per tree in the treated plots and the control plot. T_1, plot treated with 31.75 BIU ha^{-1}; T_2, plot treated with 44.45 BIU ha^{-1}; T_3, plot treated with 57.15 BIU ha^{-1}; C_1, untreated control plot. Bars represent standard deviation. Columns labeled with the same letter do not differ significantly ($P<0.05$) on May 3 (lower case letters) and May 11 (capital letters).

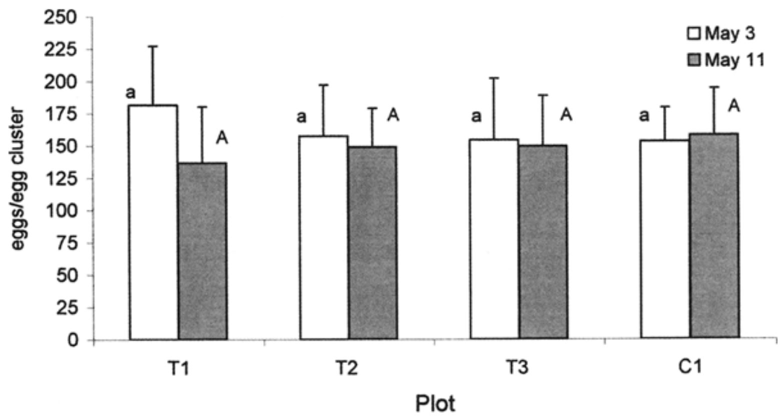

Fig. 3. Mean number of eggs per egg cluster in the treated plots and the control plot. T_1, plot treated with 31.75 BIU ha^{-1}; T_2, plot treated with 44.45 BIU ha^{-1}; T_3, plot treated with 57.15 BIU ha^{-1}; C_1, untreated control plot. Bars represent standard deviation. There are no significant differences among the mean values ($P<0.05$).

samplings either among the treated plots or between the treated plots and the control plot (Fig. 3). Examination of the number of hatched eggs per tree revealed that there were no significant differences among the plots in the areas treated with different doses of *Btk* nor between these environments and the control plots (Fig. 4).

Larval mortality In the May 3 sampling, 59 colonies were collected in the treated areas for a total of 2951 larvae, compared with the 11,695 larvae hatched from egg clusters collected in the same sampling on the same trees. In control plot C_1, four colonies for

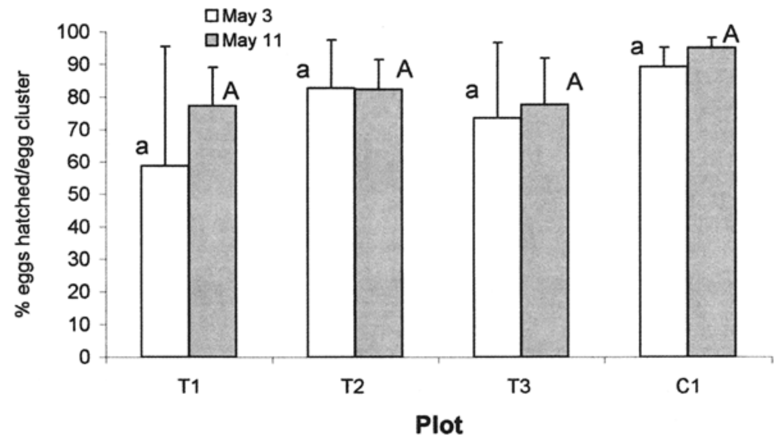

Fig. 4. Percentage of eggs hatched per egg cluster in the treated plots and the control plot. T_1, plot treated with 31.75 BIU ha^{-1}; T_2, plot treated with 44.45 BIU ha^{-1}; T_3, plot treated with 57.15 BIU ha^{-1}; C_1, untreated control plot. Bars represent standard deviation. There are no significant differences among the mean values ($P<0.05$).

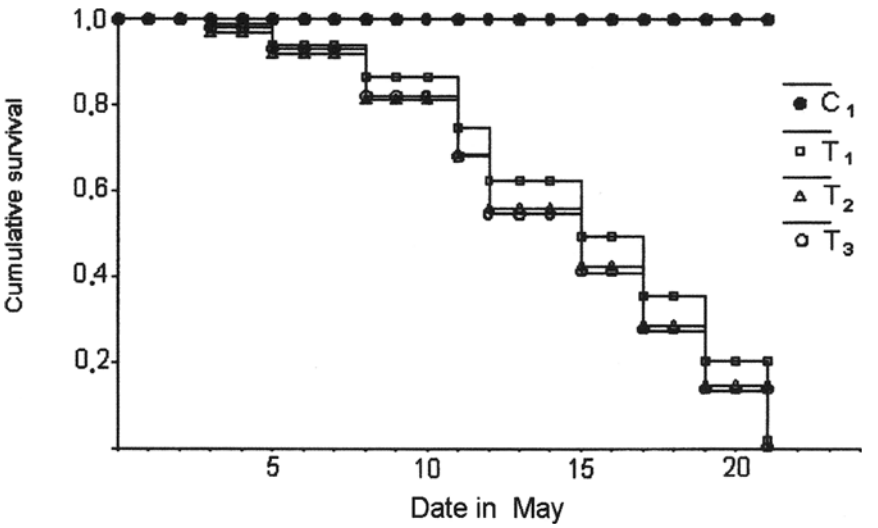

Fig. 5. Survival Function: larvae collected on May 3. T_1, plot treated with 31.75 BIU ha^{-1}; T_2, plot treated with 44.45 BIU ha^{-1}; T_3, plot treated with 57.15 BIU ha^{-1}; C_1, untreated control plot.

a total of 340 larvae were counted, compared with the 543 hatched larvae. Therefore, very high larval mortality was recorded in all the treated plots in the May 3 sampling: the minimum of 69.7% was in oak stands treated with 44.45 BIU ha^{-1}, compared with the significantly higher value of 82.6% in the area treated with 57.15 BIU ha^{-1}. Mortality was much lower (37.5%) in the control plot, C_1 ($P<0.05$). Smaller numbers of colonies and live larvae were collected in the treated areas in the May 11 sampling: 37 colonies *vs* 59

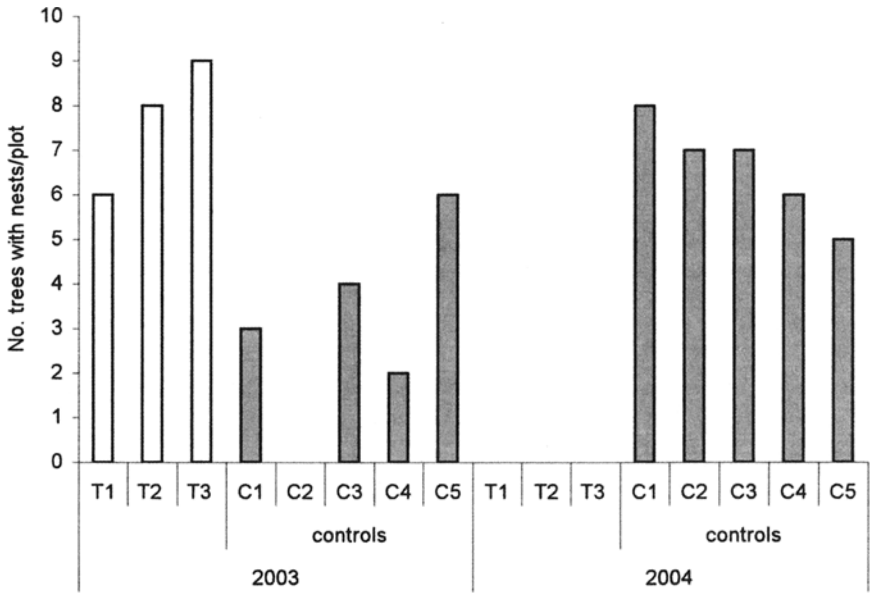

Fig. 6. Number of trees with nests in 2003 and 2004 in the three plots treated with increasing doses of *Btk* (T_1– T_3) and in five control plots (C_1– C_5). [Ten trees checked per plot]

colonies collected on May 3, and 1080 larvae *vs* 2951 larvae collected on May 3. Mortality ranged from 75.1% in the area treated with 31.75 BIU ha^{-1} to 96.4% in the area treated with 44.45 BIU ha^{-1}. Larval mortality was significantly different among the three plots treated with increasing doses of *Btk*, and also between the three treated areas and the control plot. The percentage of dead larvae in the control plot was not significantly different from that recorded in the same plot on May 3.

Survival Function Analysis of the *T. processionea* larvae collected on May 3 and reared on *Btk*-free turkey oak leaves showed no difference in the rapidity of action of different *Btk* dosages in killing larvae (T_1 *vs* T_2, prob = 0.1323; T_1 *vs* T_3, prob = 0.1315; T_2 *vs* T_3, prob = 0.9293) (Fig. 5). Also the Survival Function Analysis of larvae collected on May 11 did not reveal any significant difference, compared with the significant differences in the larval mortality registered in the sampling among the three different *Btk* dosages (T_1 *vs* T_2, prob = 0.4490; T_1 *vs* T_3, prob = 0.9325; T_2 *vs* T_3, prob = 0.4402).

Definitive nests Surveys conducted in July to identify definitive *T. processionea* nests showed a strong decline from 2003 to 2004 in the number of nests in the three treated plots (Fig. 6). In contrast, there was a strong increase in the number of trees with nests in the five control plots, particularly in plots C_1 (used as a control for the sampling of eggs and larvae), C_2 and C_4.

DISCUSSION

The values of the biological indicators used to interpret the *T. processionea* population trend (number of eggs/egg cluster and percentage of eggs hatched/egg cluster) did not reveal an incipient population collapse in any of the plots. In defoliator lepidopterans, sudden population collapses can occur locally because of the diffusion of epizootics, especially under conditions of stress due to overcrowding. However, these disease agents usually strike final instar larvae rather than the larval stages considered here (L1–L2) (41). Therefore, the data on population dynamics and the development stages considered in this study exclude the possibility that the results were biased by local natural declines of the defoliator.

High percentages of natural mortality of the young larvae were recorded in all the plots, including the controls. However, such high mortality values in the first larval stages have long been observed in various species, even in the phase of expansion of populations of these defoliators (33,34).

Treatment with a *Btk* suspension for the control of 1^{st} and 2^{nd} instar larvae of an oak processionary population was carried out in turkey oak stands at the end of April 2004, when the leaf buds were in the initial opening phase and before the defoliator reached peak density. This treatment resulted in effective control of the non-urticating *T. processionea* larvae, even in the area treated with 2.5 l ha^{-1} of FORAY 48B (31.75 BIU ha^{-1}). This was confirmed by surveys performed in summer, which showed only the sporadic presence of new nests in the large area adjacent to the treated oak woods.

In view of the high efficacy of the treatment as early as the May 3 sampling, it should be noted that Flemming and van Frankenhuyzen (15) hypothesized that the efficacy should be closely related to the degree of mortality caused by *B. thuringiensis* in a defoliator population (spruce budworm) in the first 48 h after an application.

The results of the Survival Analysis are particularly important since they demonstrated the lack of significant differences in the mortality of larvae collected in the plot treated with 2.5 l ha^{-1} with respect to the areas treated with the higher doses. This suggests the use of products with the lowest dose of *Btk* tested, which would limit the amount of product introduced into the environment and lower the cost of treatments per unit of area.

The high levels of mortality recorded in the trials with aerial-sprayed *Btk* were favored by the contemporary hatching of the *T. processionea* larvae in the study area. This allowed effective control with just a single early treatment. However, such synchronic hatching is rather infrequent in many of the defoliator lepidopterans harmful to Mediterranean oak woods, *e.g. Lymantria dispar*, whose graded hatching often requires a late intervention or adoption of the strategy of two successive interventions, with increased costs. The possibility of effective control of *T. processionea* populations in turkey oak woods with a single treatment of 31.75 BIU ha^{-1} at the time of bud opening requires accurate timing but could limit the effects on the species-rich lepidopteran fauna of these woods, since only a few of them are active at the beginning of spring (23). A preliminary study of this aspect showed that *Btk* treatment carried out at the beginning of spring for control of the oak processionary had only a limited effect on a few other lepidopteran species (32).

Other authors have reported on trials of the control of oak processionary moths with *Btk* aerial spraying. The recent paper by Martin and Bonneau (25) is a brief review focused almost exclusively on the pine processionary. The paper by Pascual *et al.* (29) refers to the use of soluble powders at the dose of 35 l of diluted product ha^{-1}, which resulted in high mortality of oak processionary larvae in colonies artificially transferred to areas that were then experimentally treated. The later study by Flemming (14) on small-scale control of the oak processionary also resulted in high levels of larval mortality.

During the present study, there was no host plant interference with *Btk*'s microbial pesticide action by substances in the *Q. cerris* leaves in the initial phase of leaf bud opening, which otherwise might have influenced its effectiveness on the insects. This contrasts with what was found for the gypsy moth in other broadleaved forests (2). This finding is particularly important for the control of the oak processionary since, as reported by Dissescu (13), suitable conditions for *T. processionea* outbreaks occur in the aged formations of *Q. cerris* that are widely distributed in Italy, where several coppice woods have been turned into high-trunk forests.

In the near future, it will be crucial to monitor the defoliator in the treated woods, as well as in surrounding areas where *T. processionea* populations persist, to record eventual migrations of adult moths from neighboring areas toward the zone in which the phytophage has been drastically reduced by the treatment. Analysis of possible modifications of *T. processionea* population cycles in these environments will be of particular interest in ongoing studies on the population ecology of this dangerous defoliator, especially with regard to the duration of the effects of the treatment.

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