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Immediate and delayed mortality of the pine processionary moth treated by *Bacillus thuringiensis* var. *kurstaki* 3a 3b in the sub-Saharian pine reforestations

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Abstract: In the reforestations of *Pinus halepensis*, situated in the Algerian sub-Saharian region, 1 500 hectares were treated by an aerial application against the third and fourth larval stage of the pine processionary moth. The microbiological insecticide used contained *Bacillus thuringiensis* var. *kurstaki* 3a 3b. The immediate mortality was evaluated by sampling colonies of caterpillars 7 and 14 days after the application of the treatment, and the delayed mortality was determined firstly for caterpillars after 80 and 100 days in the field and secondly for pupae by collecting and rearing surviving caterpillars to *Btk* at the end of the larval cycle during the nomadic period. The mortality rate of the caterpillars increased between 7 and 100 days and averaged 93 % in the treated zone. The caterpillars exposed to the treatment were more contaminated by *Beauveria bassiana* and infested by the larval-pupal parasitoïds *Erigorgus femorator* Aub. (Hym, Ichneumonidae) and *Phryxe caudata* Rond. (Dip., Tachninidae). The sex ratio of surviving moths was 1.7 in favour of males which suggests that female caterpillars are more vulnerable to *Btk*.
Keywords: *Thaumetopoea pityocampa*, Parasitism, Insect control, *Pinus halepensis*

Aşağı Sahra'da çam ağaçlandırma sahalarında *Bacillus thuringiensis* var. *kurstaki* 3a 3b ile ilaçlanan çam kese böceklerinin anında ve geç ölümü

Özet: Cezayir'de Aşağı Sahra bölgesinde bulunan ağaçlandırılmış *Pinus halepensis* ormanında 1500 hektarlık alana çam kese böceğinin üçüncü ve dördüncü larva evresine karşı havadan ilaçlama uygulaması yapılmıştır. Kullanılan mikrobiyolojik insektisit, *Bacillus thuringiensis* var. *kurstaki* 3a 3b içeriyordu. İlaç uygulamasından sonra 7. ve 14. günde tırtıl kolonilerinden örnekler toplanarak anında ölüm değerlendirilmiştir. Geç ölüm ise, ilk olarak tırtıllar için 80 ve 100 gün sonra sahada belirlenmiş, ikinci olarak ise pupalar için Btk uygulamasına rağmen sağ kalan tırtılları göç döneminde larva döngüsünün sonunda toplayıp büyüterek belirlenmiştir. Tırtılların ölüm hızı, 7 ila 100 gün arasında artış gösterirken ilaçlama yapılan bölgede ortalama %93 olarak belirlenmiştir. İlaçlamaya maruz kalan tırtıllar *Beauveria bassiana* ile daha fazla kontamine olmuş ve *Erigorgus femorator* Aub. (Hym, Ichneumonidae) ve *Phryxe caudata* Rond. (Dipt. Tachninidae) tarafından istila edilmiş durumdaydı. Sağ kalan böceklerin cinsiyet oranı, erkeklerin lehine olacak şekilde 1,7 idi. Bu durum dişi tırtılların *Btk*'ya karşı daha duyarlı olduğunu düşündürmektedir.
Anahtar kelimeler: *Thaumetopoea pityocampa*, Parazitlik, Zararlı kontrolü, *Pinus halepensis*

1. Introduction

The pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775). (Lepidoptera: Notodontidae), is well known for its defoliation of different species of pines and cedars all over the Mediterranean Basin. The larvae are gregarious, inhabit silk nests and pupate below ground. Adults mostly emerge during summer, reproduce and die within a few days. In Algeria the success of the reforestations with *Pinus halepensis* Mill. in the "green barrier" located in the sub-Saharan region is limited by the permanent damage of this insect pest. To control the populations of this insect, and to preserve the progressive installation of its complex of natural enemies in these artificial forests, a biological insecticide using *Bacillus thuringiensis* var. *kurstaki* 3a 3b (*Btk*) have been used since 1980 (Zamoum and Demolin, 2004). Until now, these treatments are the only way to ensure a normal increase of the foliar biomass of the young pines (Zamoum *et al*., 2007).

The aim of this study was to evaluate the immediate and delayed efficiency of a recent formulation of *Btk* spread during the third and fourth larval stage of the pine processionary moth. The delayed mortality by *Btk* was estimated firstly during the larval stage in the field and secondly for pupae by collecting and rearing surviving

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caterpillars to *Btk* at the end of the larval cycle during the nomadic period were the caterpillars migrate from the host tree to select a habitat underground to pupate. Based on the results obtained we propose an integrate strategy to control this insect pest all over the "green barrier".

2. Material and Methods

2.1. Study site and treatments

The study was carried out at Djelfa in the reforestation of Moudjebara, situated at an altitude of 1 100 m and characterized by a semi-arid bio-climate. The *P. halepensis* plantations have been realized since 1970 on 13 000 ha and the level of the infestation of pine processionary moth was high and permanent.

Btk was sprayed in winter (20-24 December) over 1 500 ha using an aircraft - type Gruman 600 with 8 micronairs AU 5000. *Btk*, serotype 3a 3b, commercialized under the name Foray 48B was used. The dose used was 3 L/ha with 1 litre of water as support; representing a concentration of 39 x 109 International Biological Units/L on the basis of a density of 1.15. At the day of the aerial application (J), 15.3% of the caterpillars were at the third larval instar and 72.7% at the fourth larval instar.

2.2. Treatment efficiency estimation

In addition to an untreated control plot which is located about 500 m far from the treated zone where four plots are delimited. For each plots, the mortality was evaluated by a random sampling of 30 infested trees per plot. The trees were chosen according to a perpendicular transect to the flight of the aircraft. For each tree in the treated and untreated plots, all the egg batches and nests were collected. The number of surviving and dead caterpillars was recorded. For the egg batches, the total number of eggs laid and the number of hatched eggs was recorded to estimate the initial population of caterpillars for each plot. Larval survival to *Btk* was estimated by the difference between averages number of the caterpillars hatched and those recorded into the nests. To evaluate the immediate efficiency of *Btk*, two sampling were carried out at 7 and 14 days after the date of the treatment. In a first step, delayed mortality was determined at 80 and at 100 days after the date of the treatment, to evaluate the survival rate of the caterpillars before the end of the winter season and the migration of the caterpillars from the host tree to the ground for pupation.

2.3. Trapping and rearing of surviving caterpillars

In a second step, the delayed mortality to the *Btk* was also estimated for the pupal stage. At the end of the larval stage and beginning of the nomadic period, 10 nests were randomly sampled in each of the treated and untreated plots. The nests were grafted in the same ecological conditions on two trees in each of the plot. The caterpillars at the end of the larval cycle leave their winter nests to pupation under the soil. To collect these caterpillars, two traps were placed on the trunks and visited daily.

The captured caterpillars were kept separately in a glass tube (8 x 1 cm) and all the treated (N = 535) and untreated (N = 771) caterpillars were placed under semi-natural conditions at Djelfa. We noted mortality of pupae and caterpillars and the emergences of all the larval-pupal parasitoïds and the pine processionary adults. The mycosis mortality was identified by the Professor E. Tarasco (Laboratory of Pathology - University of Bari, Italy).

2.4. Statistical analysis

The data related to the larval survival during the four samples in the treated and untreated plots were analyzed using the Newman-Keuls test to compare all pairs of means following one-way analysis of variance (ANOVA). Means are presented ± 1 S.E. Survival in plots was also expressed as a percentage of the total number average of eggs in the egg batches collected in each plot and period of sampling nests. Incidence of the different species of natural enemies was expressed as a percentage of the total number of caterpillars collected during the end of the larval cycle.

3. Results and discussion

3.1. Immediate and deferred mortality rate of caterpillars

Incidence to *Btk* vary significantly among plots notably for -the two first sampling (Table 1) and among the date of collect at J + 7, 14, 80 and 100 (ANOVA: $F_{(4,335)} = 76.02$, $P < 0.001$ - $F_{(4,339)} = 33.35$, $P < 0.001$ - $F_{(4,191)} = 160.54$, $P < 0.001$ - $F_{(4,224)} = 131.18$, $P < 0.001$) respectively. The average mortality rate observed for the four treated plots was 93 % (Figure 1). This is relatively low compared to a previous mortality assessment performed after an autumn treatment against larvae at the first, second and third larval stages (Zamoum et al., 1997).

Table 1. Larval survival to *Btk* treatement with comparison between untreated and treated plots, for each date of sampling. * Different letters indicate significant differences amonf plots comparison of larval survival means (± S.E.) (Newman and Keuls test, $P < 0.05$)

Plots / date of collect	J + 7	J + 14	J + 80	J + 100
Untreated	$86.8 \pm 3.6^{a*}$	$45,4 \pm 2.3^{a}$	$85,6 \pm 3.9^{a}$	$75,3 \pm 3.0^{a}$
I	25.7 ± 2.4^{d}	$14,1 \pm 2.1^{c}$	$7,5 \pm 2.3^{b}$	$5,3 \pm 2.8^{b}$
II	$40,3 \pm 2.6^{c}$	$7,4 \pm 2.0^{c}$	$13,0 \pm 2.4^{b}$	$10,4 \pm 2.1^{b}$
III	$40,1 \pm 2.2^{c}$	$26,6 \pm 2.2^{b}$	$11,0 \pm 1.6^{b}$	$5,7 \pm 1.4^{b}$
IV	$59,2 \pm 2.2^{b}$	$27,7 \pm 2.4^{b}$	$11,2 \pm 2.6^{b}$	$6,3 \pm 2.6^{b}$

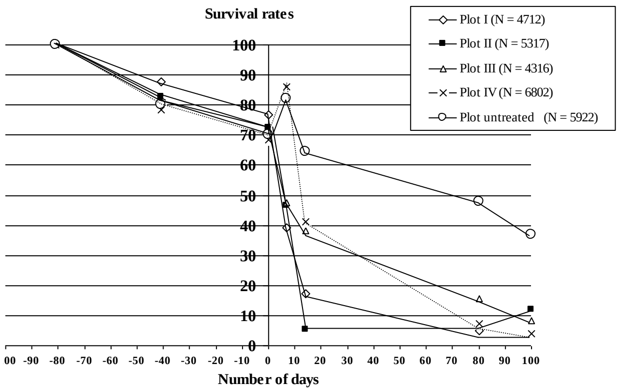

Figure 1. Evolution of larval survival rates in the treated and untreated plots, calculated from the average number of eggs collected and those hatched, the number of larvae recorded at the day of treatment *Btk* (0) and after 7, 14, 80 and 100 days

The immediate mortality rate evaluated at 7 and at 14 days after the treatment exceeded 80 % in plots I and II but it was only 62 and 59% in plots III and IV. Several causes can explain this weak *Btk* performance: firstly, four days after the treatment, rainfalls were registered in Djelfa. Consequently the insecticide might have been washed off in plots III and IV which were the last plots to be treated. Furthermore, the low temperatures during this winter season slowed down the feeding process of the caterpillars and consequently the ingestion of *Btk*.

Treated caterpillars were more vulnerable to the winter conditions than caterpillars from the untreated plot. Winter mortality has been observed by different authors in all the distribution area of this insect pest (Demolin, 1974; Geri, 1980; Battisti, 1988; Zamoum, 1998; Pimentel et al. 2010). In fact at J + 80 and J + 100 days we noted a clear increase in average mortality rates, which reached more than 90% compared to the mortality rates obtained in the untreated plot. This represents an average mortality rate of 19% compared to those obtained at J + 14 days in the four plots treated.

The phenological structure of the caterpillars surviving population at J + 100 days showed a clear abundance of the fourth larval instar in the treated plots compared to the untreated plot where a majority of the caterpillars are at the fifth larval instar (Table 2). This low development is certainly due to the *Btk* treatment; a similar situation was observed with *Euproctis chryssorrhoea* (Lep. Lymantriidae) by Ruelle et al. (1977).

Table 2. Comparison between larval phenology rates observed at the date of treatment *Btk* (J) and that observed during the different sampling periods from J + 7 to J + 100, in the untreated (UP) and treated plots (P I to P IV). C(1): Number of collected colonies; N(2): Number of caterpillars; L(3): Survival larval phenology at third, fourth and fifth larval instars

Plots / Sampling periods	C(1)	N(2)	L3(3)	L4(3)	L5(3)
UP / J	10	544	19,1	80,9	0,0
P I / J	10	429	15,8	2,3	0,0
P II / J	10	762	21,2	18,9	0,0
P III / J	10	378	17,5	25,4	0,0
P IV / J	10	543	10,3	39,4	0,0
			$F_{(2,12)}$ = 4.64, P = 0.03		
UP / J + 7	56	4863	17,3	82,7	0,0
P I / J + 7	72	1851	22,3	7,3	0,0
P II / J + 7	62	2499	17,4	29,0	0,0
P III / J + 7	51	2046	7,3	38,9	0,0
P IV / J + 7	99	5859	11,9	56,3	0,0
			$F_{(2,12)}$ = 8.40, P = 0.0005		
UP / J + 14	84	3815	0,1	52,2	0,0
P I / J + 14	58	818	1,6	14,7	0,0
P II / J + 14	44	288	0,3	7,3	0,0
P III / J + 14	62	1649	0,5	30,1	0,0
P IV / J + 14	101	2795	1,6	30,3	0,0
			$F_{(2,12)}$ = 11.7, P = 0.002		
UP / J + 80	37	2826	0,0	9,3	78,6
P I / J + 80	32	240	0,0	4,6	4,1
P II / J + 80	31	317	0,0	2,0	9,8
P III / J + 80	61	672	0,0	1,5	17,4
P IV / J + 80	44	491	0,0	1,7	5,5
			$F_{(2,12)}$ = 2.3, P = 0.14		
UP / J + 100	29	2184	0,0	0,0	86,7
P I / J + 100	32	169	0,0	5,9	0,2
P II / J + 100	62	645	0,0	11,6	0,4
P III / J + 100	62	352	0,0	9,4	0,3
P IV / J + 100	44	277	0,0	12,9	0,0
			$F_{(2,12)}$ = 0.8, P = 0.49		

3.2. Numerical change in the surviving caterpillars

The surviving caterpillars were much more vulnerable to their natural enemies, especially the entomycosis *Beauveria bassiana* (Bols-Criv. Vuillemin, 1912) and to the specific larval-pupal parasitoïds *Erigorgus femorator* (Aubert, 1960) (Hym. Ichneumonidae) and *Phryxe caudata* (Rondani, 1859) (Dip., Tachnidae) (Figure 2). This could result from a higher number of badly or unstructured nests in the treated plots. The caterpillars cannot escape easily to the attacks of the different species of parasitoïds, as already mentioned by Ruelle et al. (1977) in the case of *E. chryssorhoea.* The sex ratio was 1.7 in favour of male adults. This could be due to the fact that female caterpillars are more affected by natural enemies. The decline in female number could lead to a decrease of the pine processionary moth population for the next generation.

No prolonged diapause was recorded for caterpillars collected in the treated zone (Table 3). It would be necessary to perform additional experiments to study the physiological incidence of *Btk* on the breaking or induction of diapause.

3.3. Possibility of winter treatment

The efficiency of the microbiological preparations with *Btk* against the pine processionary caterpillars from the third and fourth larval stages (Demolin et al., 1993) opens a new opportunity to control the populations of this insect pest. The possibility to delimitate easily the infected zones during the winter season allows targeting precisely the infected zones during aerial applications, and thus avoid the large scale application in autumn on the young caterpillars at the first and second larval stages. Furthermore, in the case of the "Green barrier" reforestation the winter treatments have the advantage to preserve an important zoocenosis which is inactive at this season, and to have a reasonable control by treating when and where it is necessary, as suggested by Delorme (1991).

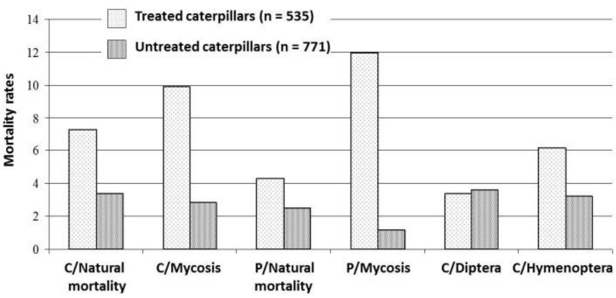

Figure 2. Comparison of mortality due by natural enemies between the caterpillars (C) and pupal stage (P) which survived to *Btk* treatment and those untreated

Table 3. Percentage of male and female adults emerging in year n and n+1 from caterpillars collected in the treated and untreated zones

	Treated plots (N = 305)		Untreated plot (N = 642)	
	Year n	Year n+1	Year n	Year n+1
Adult male (%)	35.9	0.0	38.3	3.4
Adult female (%)	21.1	0.0	38.5	3.1
Sex-ratio	1.7	—	0.9	1.1

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