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Preliminary attempts to control overwintering populations of *Thaumetopoea pityocampa* (Den. et Schiff.) (Lepidoptera: Thaumetopoeidae) with *Steinernema feltiae* (Filipjev, 1934) (Nematoda: Steinernematidae)*

ABSTRACT

The Pine processionary caterpillar *Thaumetopoea pityocampa* (Den. et Schiff.) (Lepidoptera: Thaumetopoeidae) is a very dangerous lepidopterous pest spread in all mediterranean area. A preliminary survey was conducted with 3 different strains of Steinernematid entomopathogenic nematodes in the nests of pine processionary caterpillars in a *Pinus halepensis* Miller reforestation at "Pulicchie" (Gravina in Puglia, 338 m a.s.l.) of Apulia Region (southern Italy). A suspension of 300,000 IJs in 20 ml of gel (Idrosorb SR 2002 - Nigem®) was employed in each nest and mortality recorded every 10 days, for 30 days.

The results of this preliminary experiment pointed out:

- a real feasibility to reduce the overwintering larval populations injecting gel suspension of *Steinernema feltiae* IJs in their nests;
- the persistence in the nests of IJs for more than 20 days from treatment;
- the possibility of a limited number of nematodes to complete their life cycle in the larvae of *T. pityocampa*;
- very low effects on the parasite *Phryxe caudata* Rond. (Diptera: Tachinidae).

Key words: aqueous suspension, gel suspension, IJs injection, nests, *Pinus halepensis*, reforestation, *Phryxe caudata*.

INTRODUCTION

Pine processionary caterpillar, *Thaumetopoea pityocampa* (Den. et Schiff.) (Lepidoptera: Thaumetopoeidae) is the most endemic and important pest in southern Europe and north Africa pinewoods (ANDROIC, 1956). It causes not only important environmental damage and economic losses to *Pinus* spp., but also it represents a problem to human health because of the urticating hairs of the last instar larvae (DÉMOLIN *et al.* 1996).

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[1] The first author has planned the research and carried out the field tests. The second author has collaborated in gathering, breeding the nematodes and in the laboratory tests. Both authors have collaborated on processing the data and in layout of the paper.

*This research was supported by MURST "Advances in the integrated control of forest defoliating insects" and 60%.

In Italy, the insect shows a preference for both native *Pinus* species, like *Pinus nigra* Arnold (Austrian pine) and *P. halepensis* Miller (Aleppo pine), and introduced exotic species, like *P. radiata* D. Don (Monterey pine) and *P. canariensis* C. Smith (Canary pine) (BATTISTI *et al.*, 1998).

Numerous efforts have been made to slow down larval populations of *T. pityocampa* with ecologically acceptable methods like the picking up and destruction of winter nests, and treatments with *Bacillus thuringiensis* and nuclear polyhedrosis virus (TRIGGIANI and SIDOR, 1982; BATTISTI *et al.*, l.c.), but so far we have no notes on using *Steinernema* spp. or *Heterorhabditis* spp. nematodes in insect nests.

Moreover, even if these nematodes have been collected from numerous pinewood soil samples infested by *T. pityocampa*, no larvae of this lepidoptera have been found parasitized by *Steinernema* or *Heterorhabditis* in nature.

This paper is an effort to provide the framework for evaluating the possibility in controlling overwintering larval populations of *T. pityocampa* with *Steinernema feltiae* (Filipjev, 1934) and the impact of this nematode on the parasitoids associated with the lepidoptera larvae.

MATERIALS AND METHODS

LABORATORY BIOASSAYS

- Infectivity of *S. feltiae* Italian strains at low temperatures

Bioassays were performed in laboratory against *Galleria mellonella* L. (Lepidoptera: Galleriidae) to determine the infectivity at low temperatures of native *S. feltiae*, isolated from different biotopes in Apulia Region (southern Italy) to select the most suitable strain for field tests.

Their infectivity was determined by larval mortality rate assay.

For each isolate of *S. feltiae*, the third stage infective juveniles (IJs) of nematodes were suspended in deionized water; 1 ml of nematode suspension containing approximately 1,000 IJs was applied on a 9 cm diameter filter paper and placed in a 9 cm diameter Petri dish. In the control, deionized water only was applied on filter paper. Ten last instars of *G. mellonella* larvae were transferred in each of the dishes and exposed to the nematodes.

The assays were conducted at 8 and 10°C, the most frequent temperatures recorded during January and February 1999 in the nests in the same place where the field tests were performed the year after.

Each treatment was replicated three times. The insect mortality was recorded every 48 h for 10 days and the bioassay was repeated 3 times.

- Comparison between water and gel suspension of *S. feltiae*

Previous tests, performed in the field in order to estimate the applicability of injections of IJs in aqueous suspension in the Pine processionary nests, pointed out a severe loss for percolation through the inferior part of the nests. Therefore, further tests were performed in laboratory with a water-absorbent synthetic polymer to fill this gap.

This polymer has been developed by the chemical industry as soil conditioner in agriculture for dry biotopes and for the xenic culturing of plant nematodes (De Boodt, 1990; Rognon 1995; Reversat *et al.* 1999). In our tests Idrosorb SR 2002 (Nigem®) was used; it is an acrylic polymer of high molecular weight, formulated as small irregular pieces non-exceeding 3 mm, that jellified by absorbing water.

Ten g of Idrosorb in 1000 ml of water, after 24 h of water absorption, were spun in a blender for about 15 minutes to obtain a homogenized gel.

Cylindrical polyethylene containers of 12x12 cm with a 1 mm wide wire netting on the bottom, filled with sterilized contents of the insect nests (excrements, exuviae, pieces of silk web) were used as "artificial nests" in laboratory, to evaluate the loss of both suspensions. The nests with three stems at the bottom were placed on half of the 16 cm diam Petri dish to quantify the losses.

Thirty last instars of *G. mellonella* larvae were put in each "artificial nest" and after 24 h 10,000 IJs, suspended in 20 ml water, were injected with a 50 ml syringe in 10 nests; 20 ml of gel with the same concentration of nematodes were injected in the other ten nests and the upper side was closed with filter paper.

The artificial nests as control were treated with 20 ml of water or gel. The treatment was replicated three times.

ItS-GR1 and ItS-CL2 *S. feltiae* were tested in this bioassay at 10 ± 1°C and the insect mortality caused by nematodes was recorded after 10 days.

Field bioassays

The field tests were performed during January and February 2000 in a *Pinus halepensis* reforestation at "Pulicchie" (Gravina in Puglia, 338 m a.s.l.) where the plants are 4-5 m high with a great presence of nests; the nests collection during winter was not advisable to avoid an irregular growth of the *Pinus* and the reduction of parasites in the nests.

Two days before treatments 80 nests were selected and marked.

ItS-CL2, ItS-GR1 and one German strain of *S. feltiae* supplied by "e-nema" from Raisdorf were compared. The Italian ones were cultured in laboratory in the last instars of *G. mellonella* at 24 ± 1°C; IJs were harvested using modified

White traps and stored at 10°C for no more than a week before the treatment.

The IJs were added to the gel and left at room temperature for the whole night before the field tests.

A suspension of 300,000 IJs in 20 ml of gel was employed in each of 20 nests; 20 nests selected as control, were injected with 20 ml of gel suspension without nematodes.

A 50 ml syringe with a stainless steel cannula, 20 cm long and 3 mm diam, was used for injecting the suspensions. The cannula had the tip closed and 2 rows of 4 openings 1 mm diam, along the last 4 cm.

Every 10 days, during a month, 5 nests for each nematode strain and 5 as control were collected in the field and examined in laboratory. The larvae found dead in the nests were washed for 5 minutes under tap water to remove nematodes from the surface of their bodies, and then dissected in Ringer's solution under a stereoscopic microscope. The larvae with at least one IJ in the body were recorded as killed by nematodes.

The nests, after larvae removal, were vigorously rinsed out in 200 ml of water in polyethylene bags for 10 minutes 3 ml of this liquid was therefore examined and the nematodes still alive and their developmental stage recorded.

A HI 91610C (Hanna Instruments) thermohygrometer was used to report temperature and relative humidity in the field during the experiments and a "long-stem thermometer" (Spectrum technologies inc.) to check temperatures inside the nests.

A general linear model procedure and Tukey's multiple range test (SAS Institute, 1985) was performed to analyse the virulence data. The comparison was made at 0.1 level of significance.

RESULTS

Laboratory bioassays results

No mortality was observed in any of the control treatments. All the isolates of *S. feltiae* killed *G. mellonella* at low temperatures even if with different percentages of mortality. The killing activity of the nematodes was slowed down owing to low temperatures and only IS-CL2 and IS-GR1 performed the best results at 8° and at 10°C killing all the larvae in 10 days (figs 1-2)

The aqueous suspensions of these nematode strains tested in the "artificial nests" suffered a loss of 50-60% due to percolation during the first half an hour after the injection, while the gel suspension did not percolate.

Nematodes in gel suspension showed better results than in aqueous

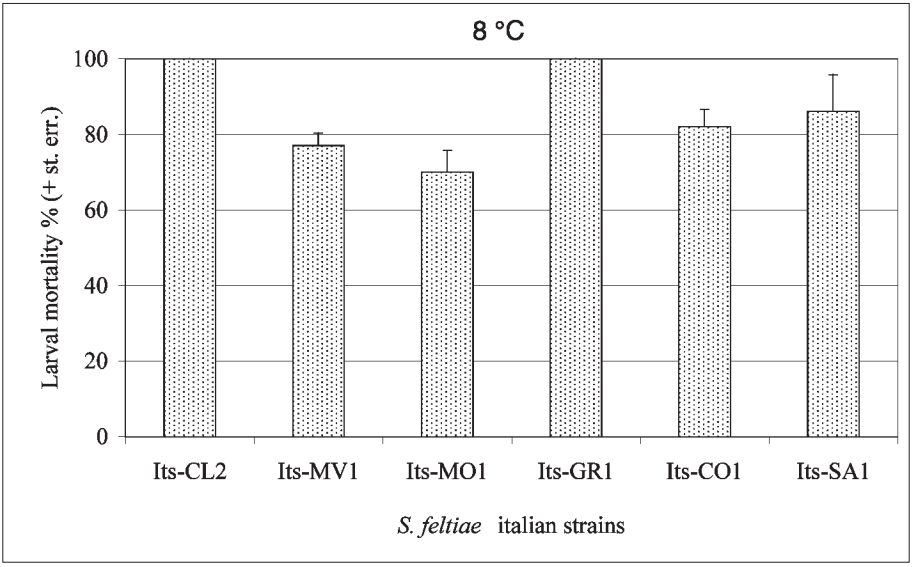

Fig. 1 - Infectivity comparison among 6 native *S. feltiae* strains: percentage mortality of *G. mellonella* larvae following 10 days of exposure to IJs at 8°C.

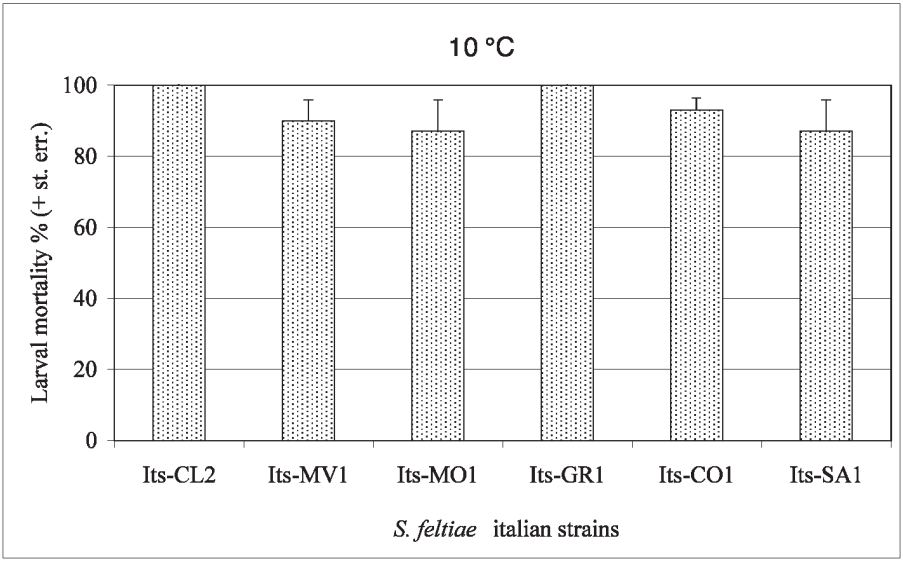

Fig. 2 - Infectivity comparison among 6 native *S. feltiae* strains: percentage mortality of *G. mellonella* larvae following 10 days of exposure to IJs at 10°C.

suspension: IS-CL2 in water caused the 55% of mortality, and in gel 79%, of the *Galleria* larvae after 10 days; aqueous suspension of IS-GR1 killed 68% of *G. mellonella* larvae after 10 days, while gel suspension the 86%.

Field bioassays results

On January 13th, at the moment of the treatment, temperature was 8°C in the field; the average lowered in the next 16 days and afterwards increased in a variable way (fig. 3). The relative humidity is reported in figure 4. Temperature in the nests was normally 1-2°C higher than outside.

The data on the mortality of *T. pityocampa* larvae 10 days after the treatment were not statistically different being very low for all the nematode strains, however it is interesting to highlight that a substantial amount of gel was still rich in water with about the 80% of the IJs still alive.

For all *S. feltiae* strains tested the data showed the ability of IJs to reduce the overwintering larval populations of *T. pityocampa* at 20 days post-treatment (tab. 1, fig. 5). In fact 16.64% and 15.78% mortality was recorded respectively for IS-GR1 and IS-GR2 and 5.48% for the German strain of *S. feltiae*. No mortality due to Nematodes was observed in control.

The last sampling after 30 days by the test, did not give statistically valuable data since most nests were empty and the larvae walking in procession on the soil.

Impact of nematode on parasitoids

Tab. 1 - Effect of different strains of *S. feltiae* on the mortality of overwintering larvae of *T. pityocampa* 20 days after treatment (± st. err.).

Nematode strains	**N. Larvae/nest**	**Dead larvae (%)**	**Larvae with nematodes (%)**	**Alive IJs in nests (%)**	**N. cadavers with adult nematodes**
IS- CL2	142.8 ± 24	20.48 ± 5	15.78 ± 5.3	13.8 ± 9.4	2.4 ± 2.4
IS- GR1	180.4 ± 21	17.88 ± 5.9	16.64 ± 5.4	1 ± 1	3.4 ± 3.4
S. feltiae Ger	213 ± 112	8.88 ± 5.8	5.48 ± 3.4	9 ± 6	1.4 ± 1.1
Control	216 ± 33.1	5.12 ± 0.7	--	--	--

Phryxe caudata Rondani (Diptera: Tachinidae) was the only parasite present in *T. pityocampa* nests during the tests. Its presence varied from 2% to 18% of the insects in the nests. Only two larvae of the *Phryxe* proved to be parasitized and killed by *Steinernema* in the body cavity of two Pine processionary larvae heavily infected by IJs.

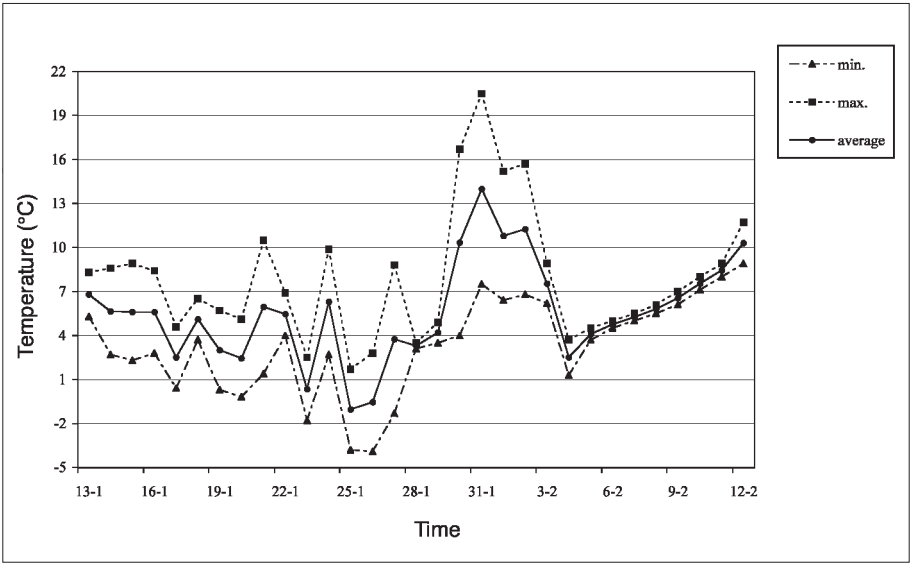

Fig. 3 - Temperature variation in the pinewood during field tests.

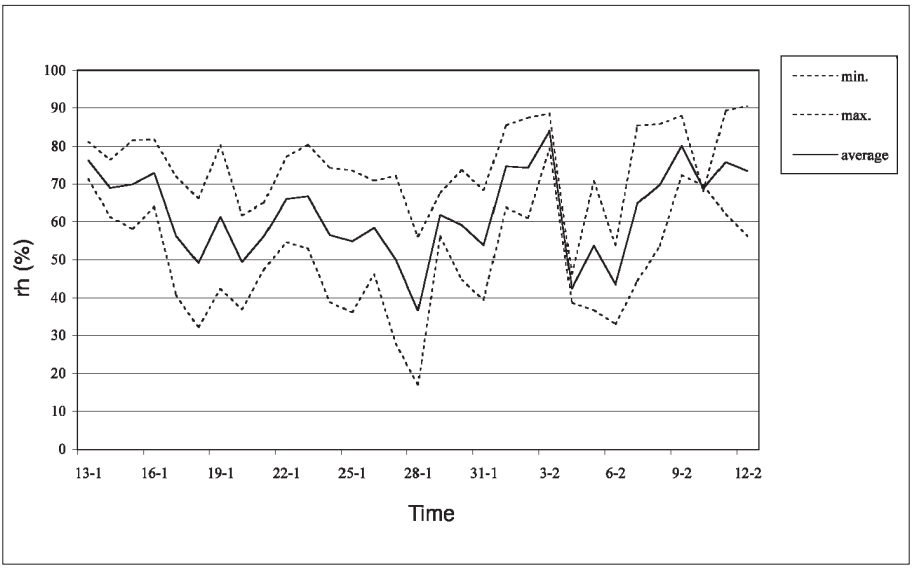

Fig. 4 - Relative humidity (rh) variation in the pinewood during field tests.

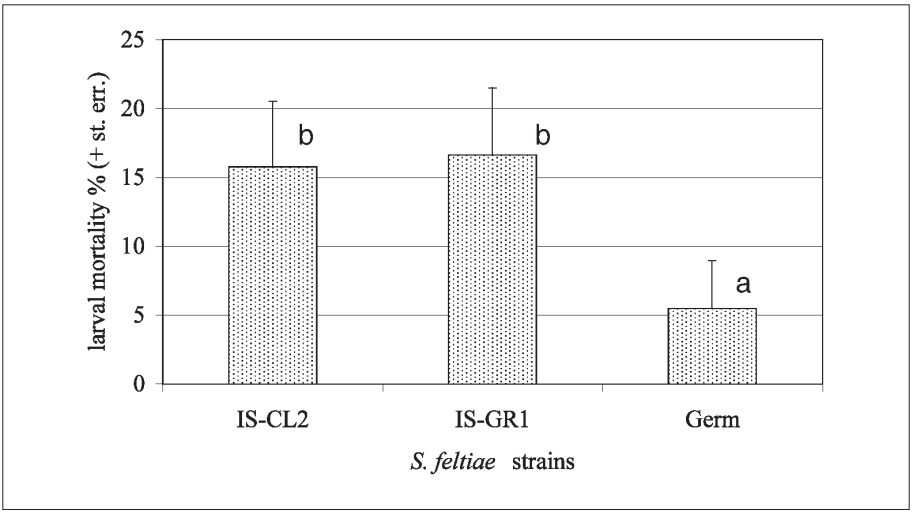

Fig. 5 - Mortality (%) of *T. pityocampa* larvae following 20 days of exposure to Italian and German *S. feltiae* isolates.

CONCLUSIONS

The results obtained regarding nematode efficacy against overwintering larvae of *T. pityocampa* have demonstrated a real feasibility to reduce the overwintering larval populations by injecting gel suspensions of *S. feltiae* IJs in the nests. The use of gel instead of water supports a longer survival of the nematode in the nest slowling the water evaporation and allows IJs waiting for the optimum temperature to enter the host. Very interesting is the capability of all the *S. feltiae* strains tested to reach the adult stage even if in a limited number of overwintering Pine processionary caterpillar, as reported in table 1.

The only chance for IJs to enter *T. pityocampa* larvae appears to be through mouth and anus. Infact, the larvae of the lepidoptera have morphological defenses or barrier to penetration as hard body surfaces and tightly closed spiracles; these spiracles have a vertical 10-12 µm wide opening while the *S. feltiae* IJs are about 30 µm wide. One more obstacle is the presence of numerous hair on the surface of the larvae as impediment to the IJs movement.

RIASSUNTO

PROVE PRELIMINARI PER IL CONTROLLO DI POPOLAZIONI SVERNANTI DI *THAUMETOPOEA PITYOCAMPA* (DEN. ET SCHIFF.) (LEPIDOPTERA: THAUMETOPOEIDAE) CON *STEINERNEMA FELTIAE* (FILIPJEV, 1934) (NEMATODA: STEINERNEMATIDAE)

Sono state effettuate preliminari prove di controllo con *Steinernema feltiae* in un rimboschimento di *Pinus halepensis* Miller gravemente infestato da *Thaumetopoea pityocampa* (Den. et Schiff.) (Lepidoptera: Thaumetopoeidae). Una sospensione di 300.000 IJs in Idrosorb SR 2002 (Nigem®) è stata iniettata nel gennaio 2000 in ciascun nido contenente larve svernanti del lepidottero. La mortalità è stata verificata ogni 10 giorni, per 30 giorni. I risultati hanno evidenziato:

- la capacità di *S. feltiae* di ridurre le popolazioni larvali svernanti di *Thaumetopoea pityocampa* tramite iniezioni di gel con IJs nei nidi;
- la persistenza di IJs nei nidi per più di 20 giorni dal trattamento;
- la abilità da parte dello Steinernematide di completare il ciclo nelle larve di *T. pityocampa*, anche se in un numero limitato;
- basso livello di parassitizzazione nei confronti delle larve di *Phryxe caudata* Rond. (Diptera: Tachinidae).

Parole chiave: sospensione in acqua, sospensione in gel, iniezioni con IJ, nidi, *Pinus alepensis*, rimboschimento, *Phryxe caudata*.

ACKNOWLEDGEMENTS

We thank Dott. Arne Peters to have supplied the German isolated of *Steinernema feltiae* and Prof. Parwinder Grewal for critically reviewing the manuscript.

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