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Field Persistence of Some Entomopathogenic Nematodes

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Review article

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Abstract

Inoculation of some entomopathogenic nematodes (EPN) in the field was carried out to evaluate their ability to persist in the soil. The experiment was carried out in a chestnut grove on the slopes of Mount Etna and some native species strains were introduced: *Steinernema feltiae*, *S. kraussei*, *Heterorhabditis bacteriophora*, *H. megidis*. Checks were carried out to ascertain the presence of nematodes over time, for *S. megidis* the number of larvae present in the soil after 39 days from the introduction was calculated and the distribution of *S. feltiae* in the various layers of the land was followed. Persistence times varied according to the species: *S. feltiae* was found for several days and *H. bacteriophora* for a shorter time, in any case 14 weeks were never exceeded. The nematodes are able to penetrate deeply into the soil and this allows them to face adverse environmental conditions (mainly excessive drought) and to have more chances to meet the host insect.

Keywords: Entomopathogenic Nematodes; Persistence; Field Tests

Introduction

Entomopathogenic nematodes (EPN) are used in agriculture for biological control against crop pest insects. One of the advantages of using nematodes is their reduced environmental impact compared to that of traditional chemical pesticides. In order for this advantage to be considered decisive in increasing the use of biological control, it must be accompanied by real effectiveness and low cost. These aspects are related to the ability of nematodes inseminated into the soil to remain there long enough and with sufficient population density to avoid the necessity of repeating the treatment several times to obtain the desired results. Moreover, repeated treatments, in addition to being economically unattractive, could lead to intraspecific competition that is more damaging than profitable. The problem of persistence is therefore a crucial problem. The main causes that can hinder the persistence of nematodes introduced into the soil are to be found in dehydration and exposure to UV at the time of treatment Gaugler, et al. [1] and, subsequently, especially in microenvironmental factors, in the presence of antagonists and competitors and in the absence of suitable guests.

In recent decades, numerous studies have been conducted on the persistence of different species of entomopathogenic nematodes in the soil. These have shown that the introduced population is drastically reduced already in the first hours after the treatment, continues to decline in the following days and, in most cases, is reduced to less than 1% after 2-6 weeks [2]. At these low levels the population, if it finds a suitable host to reproduce in, can last even for quite long periods. Generally, *Steinernema* spp. appears to persist longer than *Heterorhabditis* spp. under laboratory and field conditions [3,4]. Several authors Forschler and Gardner [5]; Geden, et al. [6]; Warshaw [7]; Buhler and Gibb [8] have reported

a rather short persistence, from one to eight weeks, of populations belonging to different species of *Steinernema* and *Heterorhabditis*. In other cases it was possible to demonstrate a much longer persistence. The limit case is that of *S. glaseri*, introduced on a large scale in New Jersey in the period 1939 to 1942 and still present fifty years later, albeit in limited and limited areas [9].

In our study, in order to establish the persistence ability of some native EPN species, these were released into the soil of a chestnut grove; the soil was then tested periodically to ascertain their presence. The tests on persistence were carried out using different sampling techniques.

Any EPN strain was introduced after verifying with absolute certainty, by means of a careful and repeated screening, the absence of any native EPN species in the inseminated site.

Materials and Methods

The experiment started on 07/25/2016. We proceeded to enter 500,000 IJ larvae of each EPN species into the soil through a watering can. The soil was wet before and after the nematodes was introduced. The native species used in the experiments had previously been determined morphologically and molecularly Tarasco, et al. [10] and they were reproduced in the laboratory according to the Bedding and Akhurst [11] technique using *Galleria mellonella* as target insect.

Research Site

The experiments were carried out in a chestnut uncultivated on the slopes of Mount Etna, the site is represented by an isolated wood, surrounded by lava flows (dagala), in the territory of Fornazzo (Catania-Italy) at 800 m of altitude (Figure 1).

Figure 1: Uncultivated Chestnut Wood (Fornazzo, Etna)

Techniques Used

- Non-quantitative assessment: collection of approximately 2 kg of soil from each parcel treated using a gardening shovel. Check of the presence of nematodes using the Bedding and Akhurst technique [11]. In some cases the sampling was carried out by separating the surface layer (0-7 cm) from the underlying layer (7-15 cm) to test the possible different vertical distribution of the nematodes. In this case the percentage of infested larvae in each layer was calculated.

- Quantitative assessment: to determine the number of nematodes still present in the soil, the technique of Koppenhöfer, et al. [12] was used. This consists in extracting 150 cc of soil with a corer up to a depth of 15 cm, placing 10 *G. mellonella* larvae per 150 cc of soil, replacing the larvae every 3 days, counting the infected ones and applying the relation $Y = 10^{-0.25 + 2.08\log(x)}$ which gives the number of infectious nematodes present in the 150 cc (Y) knowing the number of infected larvae (X) .The sampling was carried out with a core drill of 10 mm diameter up to the depth of 15 cm. 9 carrots, each of 150 cc of soil, were taken using two different sampling methods:

- On a continuous surface of 10 cm^2 (sampling 1).
- Random (sampling 2).

Species Entered

Steinernema feltiae strain ESA, 1 parcel of 1 m^2 (Figure 2): non-quantitative verification and by layers; *S. kraussei* strain EPL, 2 parcels of 1 m^2 (Figure 2): non-quantitative assessment;
Heterorhabditis megidis strain PR4, 1 parcel of 2 m^2 (Figure 3): non-quantitative and quantitative assessment (sampling 1 and 2).
H. bacteriophora strain CTSA10, parcel of 1 m^2 (Figure 2): non-quantitative and quantitative assessment (sampling 1).

Figure 2: plot 1 m^2

Figure 3: plot 2 m2

Results

Non-quantitative assessment (Table 1)

S. feltiae: found until 10/21/2016. Persistence time ascertained 98 days.

S. kraussei: placed in two areas of 1 square meter each; one of the areas had been cleaned from the litter. In the uncleaned area the nematodes were never found. 10/21/2016 was the last date of finding nematodes in the area without litter. Persistence time: 88 days

H. megidis: found until 10/21/2016 with an established persistence time of 88 days.

H. bacteriophora: found until 08/10/2016. Established persistence 75 days.

Sampling date	Temperatures and soil conditions	EPN Species				
		***S. feltiae* ESA**	***S. kraussei* EPL Area without chestnut litter**	***S. kraussei* EPL Area with chestnut litter**	***H. megidis***	***H. bacteriophora***
31/07/2016	Ext. Temp.: 27 °C Soil Temp.: 24 °C Very dry soil	Present	present	absent	present	present
06/08/2016	Ext. Temp.: 24 °C Soil Temp.: 21 °C Slightly damp soil surface	Present	present	absent	absent	present
03/09/2016	Ext. Temp.: 30 °C Soil Temp.: 25 °C Dry soil	Present	present	absent	present	present
10/09/2016	Ext. Temp.: 24 °C Soil Temp.: 22 °C Slightly damp soil surface	Present	present	absent	present	present
19/09/2016	Ext. Temp.: 17 °C Soil Temp.: 15 °C Slightly damp soil surface	Present	present	absent	absent	present
08/10/2016	Ext. Temp.: 17 °C Soil Temp.: 15 °C Damp soil	Present	absent	absent	present	present
21/10/2016	Ext. Temp.: 20 °C Soil Temp.: 16 °C Damp soil	Present	present	absent	present	absent
31/10/2016	Ext. Temp.: 19 °C Soil Temp.: 16 °C Damp soil	Present	absent	absent	absent	absent
10/11/2016	Ext. Temp.: 22 °C Soil Temp.: 18 °C Damp soil	Absent	absent	absent	absent	absent
27/11/2016	Ext. Temp.: 20 °C Soil Temp.: 16 °C Damp soil	Absent	absent	absent	absent	absent

Table 1: Presence/absence of species introduced at various sampling dates and environmental conditions

Quantitative assessment (Table 2), carried out on 03/09/2016:

EPN	Num. of infected larvae	N. EPN/m^2
H. megidis Sampling 1	5	120
H. megidis Sampling 2	6	137
H. bacteriphora Sampling 1	0	0

Table 2: Number of EPN per m2 calculated using the Koppenhöfer, et al. technique.

Assessment by layers (Table 3)

DATE	Num. of infected larvae (0-7 cm)	Num. of infected larvae (7-15 cm)
31/07/2016	16 i.l. / 20 (80%)	15 i.l. / 20 (75%)
06/08/2016	13 i.l. / 20 (65%)	0 i.l. / 20 (0%)
03/09/2016	15 i.l. / 20 (75%)	13 i.l. / 20 (65%)

Table 3: Percentage of *G. mellonella* larvae infected in the two soil layers. (i.l.= infected larvae).

Discussion and Conclusion

The persistence times of the species placed did not deviate too much from one another. *S. feltiae* showed to be the most resistant species, 14 weeks, while *H. bacteriophora* was not found again after about 12 weeks.

The absence of nematodes in some samples does not necessarily indicate the disappearance of the species but can be linked to the aggregate and non-uniform distribution of nematodes in soil. Only the absence in numerous samples can be indicative. The failure to find *S. kraussei* from the first check sampling probably shows that the presence of the litter hinders the penetration of nematodes into the soil and therefore it is advisable to clean the area to be treated before entering them.

There seems to be no correlation with atmospheric conditions: the only date, in which there was a noticeable drop in temperature, on September 19, we found nematodes almost everywhere. The quantitative assessment showed that the degree of persistence is very low even after 5 ½ weeks. Apparently, the sampling technique does not determine significant changes in the outcome of the number of nematodes.

The study of the distribution by layers showed that the nematodes tend to penetrate deeply into the soil, especially if the ground surface is dry, arranging themselves uniformly in both the analysed layers. This enables these animals to escape drying and increases their likelihood of encountering host larvae.

Taking into account, as previously mentioned, that the nematodes are strongly reduced in number immediately after having been introduced into the ground, that their persistence is often limited in time and that the possible stabilization of the population is linked to the presence of potential host larvae. It appears very important to evaluate case by case which is the right moment to enter the biological control agent nematodes in the soil to be treated to obtain a successful result.

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