*Entomologica,* Bari, 36 (2002): 157-163

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# Biocontrol of *Rhytidoderes plicatus* Oliv. (Coleoptera, Curculionidae) in potted savoy cabbages with entompathogenic nematodes[2]

## ABSTRACT

Laboratory experiments have been performed to control *Rhytidoderes plicatus* Oliv. (Coleoptera, Curculionidae) larvae on potted savoy cabbages with entomopathogenic nematodes.

Previous tests on last instar grubs of the weevil, in Petri dishes with a filter paper dampened with about 2,000 IJs in 1 ml of $H_2O$, showed the capacity of *S. carpocapsae* and *H. bacteriophora* to kill 100% of the insects after 24 h and a slower activity of *S. feltiae.*

The results of the second experiment, spraying 30,000 IJs in 50 ml of $H_2O$ on the soil of each potted savoy cabbage, put in evidence that *H. bacteriophora* was able to control 97% of the grubs after 5 days and 100% after 10 days while *S. carpocapsae* needed 5 days to kill 73% and 15 days to control 93% of the larvae. A slower activity was performed by *S. feltiae* that controlled only 30% of the weevil grubs after two weeks.

Key words: *Steinernema feltiae*, *S. carpocapsae, Heterorhabditis bacteriophora*, savoy cabbage.

## INTRODUCTION

*Rhytidoderes plicatus* is one of the most destructive pest of cultivated and wild *Brassicaceae* plants in the Southern Europe, North Africa, Asia Minor, Madera and Canary islands (HOFFMAN, 1963).

The adults emerge from the soil at the end of July until September and reach the leaves of the plants to feed on. The females are very prolific in laying more than thousand eggs on the soil near the host plants during the dark. The eggs take two, three weeks to hatch and the larvae can seriously damage or completely destroy the host plants, feeding on the young roots and reaching and boring into the taproot. Last instar larvae are present from October through May and pupate in the soils. The number of grubs for each plants is extremely variable, even 200-300 larvae per plant of cauliflower

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

[2] This research was supported by University of Bari funds (ex 60%).

Manoscritto accettato il 20 dicembre 2002

were observed on 1971 in the Apulia Region (Southern Italy) during a severe infestation of the weevil *R. plicatus* (MONACO, 1971). The weevil takes one year to complete one generation. The control is based on chemical pesticides against the adults while they are ineffective against the grubs, since they spend their entire larval stage in the soil around the roots, so alternative strategies have to be studied.

Entomopathogenic nematodes (EPNs) of the genus *Steinernema* and *Heterorhabditidis* are obligate parasites of the insects. They have symbiotic association with bacteria of the genera *Xenorhabdus* and *Photorhabdus* present in the intestine of the IJs (Infective Juveniles) (BOEMARE *et al.,* 1993). They penetrate into the insect body through natural openings (anus, spiracles, mouth) or areas of thin cuticle, perforate the tracheae or the gut to reach the body cavity of the host and release the associated bacteria. The victims are killed in a few days and the nematodes feed on the destroyed tissues and reproduce.

The results of numerous efforts to control different species of weevil larvae with nematodes have pointed out various degree of success (BEDDING & MILLER, 1981; BERRY *et al.*, 1997).

In particular, some experiments performed in Italy have pointed out the real feasibility to apply *S. carpocapsae* and *Heterorhabditis* sp. (DESEÖ, 1987; BOSELLI *et al.,* 1991, 1997; CURTO *et al.*, 1992; TACCONI *et al.*, 1999) against larvae of sugar-beet weevil *Temnorhinus* (=*Conorrhynchus*) *mendicus* Gyll., but no data are available on the use of EPNs in controlling *R. plicatus*.

Since numerous species and strains of *Steinernema* and *Heterorhabditis* have been collecting in Southern Italy by the authors and successfully applied against few insect pests (TRIGGIANI, 1983; IPPOLITO & TRIGGIANI, 1995; TARASCO & TRIGGIANI, 1997; TRIGGIANI & TARASCO, 2000, 2002) we have tested the virulence of three Italian nematode species toward 5$^{th}$ instar larvae of *R. plicatus*.

## MATERIALS AND METHODS

Ten samples of 1 kg soil were collected during January 2003, from a savoy cabbage irrigated cultivation on sandy loam soil of about 70 ha heavily infested by the insect, near Castellaneta town (Ionic Coast of Apulia Region), and searched for the presence of entomopathogenic nematodes using the technique of Bedding & Akhurst (1975). Afterwards 100 savoy cabbage plants were selected and pulled out; most of them supported 10-15 grubs on the roots and were significantly smaller than the undamaged ones. Sixty of them

were deprived of the larvae around the roots and transplanted in plastic pots (20x10x10 cm). Each pot was filled up with the soil and protected in a transparent plastic bag to save the original humidity (about 70%). In the same time more than 2,000 larvae of *R. plicatus* were collected, assembled as groups of 50 in the laboratory, set in plastic containers (40x20x20 cm) with soil and let fed on pieces of savoy cabbage roots for 15 days.

After that period only the most active last instar larvae were selected and used for the experiments.

Italian strains of *Steinernema feltiae* Filipjev, 1934 (ItS-G16), *S. carpocapsae* Weiser, 1955 (ItS-MR7), and *Heterorhabditis bacteriophora* Poinar, 1976 (ItS-CE1), were cultured separately in laboratory on larvae of *Galleria mellonella* L. (Lepidoptera, Galleriidae) at room temperature (≈20°C) (Dutky *et al.*, 1964). Their IJs, after harvesting, were suspended in tap water and stored in refrigerator at 10°C to be used for experimental purpose within two weeks.

The virulence of the nematode isolates was compared in Petri dishes and in pot tests in the laboratory at 20°C.

In the first experiment for each of the three isolates, 1 ml tap water suspension containing ≈2,000 IJs was applied on 9 cm diam filter paper in a 9 cm Petri dish. In the control, only tap water was applied to the filter paper. Ten last instar of *R. plicatus* larvae were transferred in each of the dishes and exposed to the nematodes. The test, with 3 replications for each isolate, was repeated three times. Larval mortality was assessed every 24 hr.

In the second experiment 10 last instar of the weevil grubs were deeply placed (about 15-18 cm from the soil surface) in each of the 60 pots around the plant roots and let settle for 7 days.

Afterwards, nematode suspensions counting 30,000 IJs in 50 ml of tap water were spread with a 100 ml syringe on the soil surface on the pots. Fifteen pots of each strain and fifteen as control were used. Five pots for each nematode species and five for the control were checked every 5 days for larval mortality.

All larvae used for the bioassays, after been washed with water, were dissected: the dead ones to determine whether death was caused by the presence of nematodes or another cause, live ones to find out whether they were infected with nematodes but not yet moribund.

Data on mortality of *R. plicatus* were analyzed by linear model procedure (ANOVA – analysis of variance) and significant differences among means were separated by HSD Tukey's test (Statistica, 1999). All comparisons were made at 0.05 level of significance.

## RESULTS

In our first experiment, *S. carpocapsae* and *H. bacteriophora* caused significantly higher mortality of the root weevil larvae, killing 100% of the larvae after 24 hr, than did *S. feltiae*. *S. feltiae*, in fact, controlled 57% after 48 hr and occurred 96 hr to obtain the complete mortality. No larvae died in the control Petri dishes (Fig. 1).

In the second experiment, *H. bacteriophora* killed 97% of the grubs after 5 days and 100% mortality was reached after 10 days. *S. carpocapsae* controlled 73% of the weevil larvae after 5 days and 80% after 10 days. Fifteen days were necessary to the nematode to kill 93% of the insects.

*Steinernema feltiae* controlled 26% of grubs after 5 days and 27% after 10 days. Only 30% of the tested larvae were found moribund or dead by this nematode after 2 weeks (Fig. 2). No larvae dead in the control.

## DISCUSSION AND CONCLUSIONS

The results of the experiments have demonstrated that the EPNs tested on Petri dishes are able to kill the late instar larvae of the weevil in short time, in fact only 48 h are necessary to *H. bacteriophora* and *S. carpocapsae* to control 100% of the grubs while *S. feltiae* needs a longer time.

The same nematode species sprayed on the soil surface of potted savoy cabbages have also confirmed the great ability of *H. bacteriophora* to reach and kill the insects deepened in the soil and in a short time, as well as *S. carpocapsae* very active against the grubs in the soil, even with a slower action. While IJs of *S. feltiae* have showed no good results toward *R. plicatus* in Petri dishes and in soil.

The Italian nematodes previously tested in Petri dishes, have confirmed their capability of approaching the grubs even in the soil where their action is limited by the soil structure and by the soil mixture: *H. bacteriophora* is considered an active-hunting, have a cruiser strategy making it more efficient against sedentary prey living deeper in the soil (KAYA & GAUGLER, 1993); *S. carpocapsae* features a shallowly-waiting-hunting "ambushing" characteristic, it sits and waits; *S. feltiae* presents a shallowly-present habit similar to *S. carpocapsae*, but is an intermediate active-hunting "cruising" characteristic as well as *H. bacteriophora*.

Even if our laboratory tests have proved that *R. plicatus* grubs constitute an excellent target for *H. bacteriophora*, able to reach and kill the weevil into the soil, we also need field test.

It is important to put in evidence that the Italian *H. bacteriophora* and *S.*

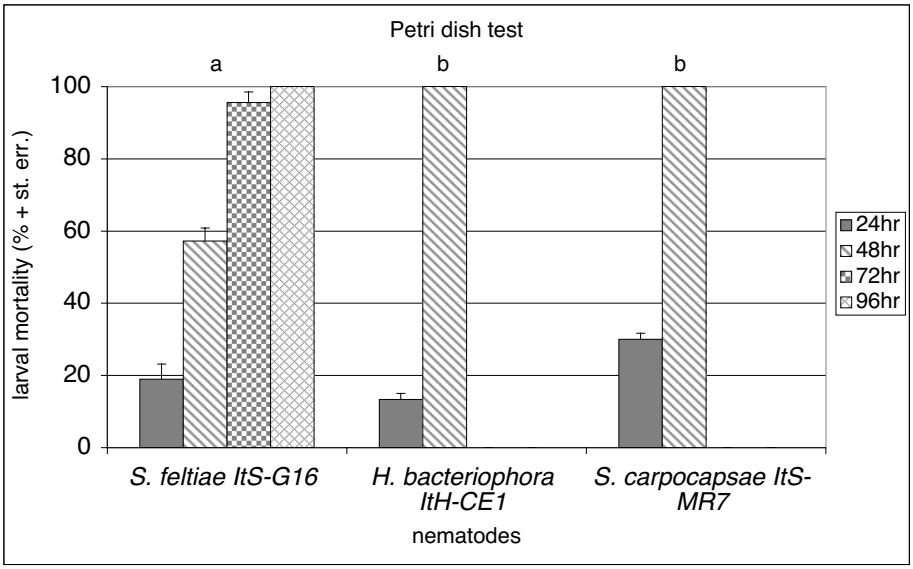


Fig. 1 - *R. plicatus* larvae (%) killed by different Italian nematode species in Petri dishes. Bars with the same letter are not significantly different ($p < 0.05$).

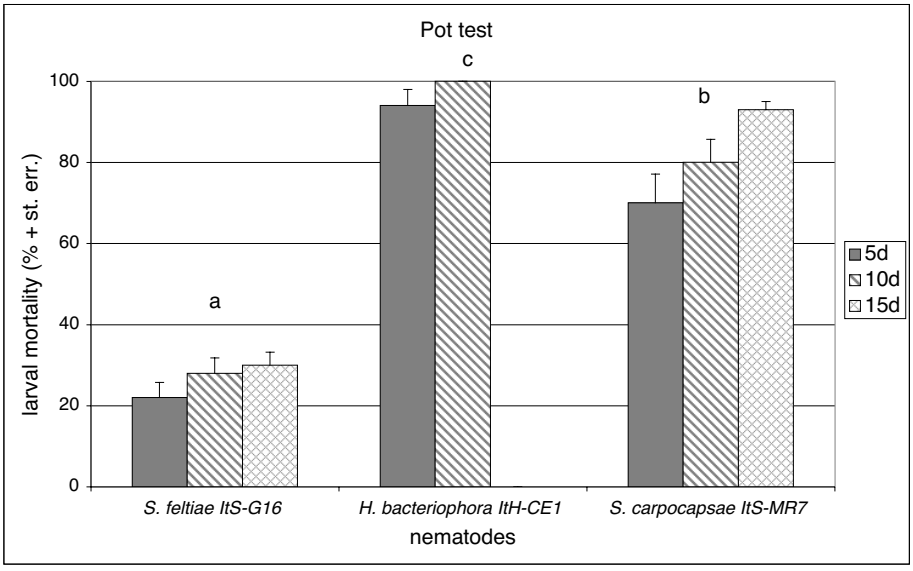


Fig. 2 - *R. plicatus* larvae (%) killed by different Italian nematode species in potted savoy cabbage plants. Bars with the same letter are not significantly different ($p < 0.05$).

*carpocapsae* can represent a valid tool in the reduction of the populations of *R. plicatus* in the soil where the use of the pesticides are not active against the larvae. The sandy loam soil on which cabbage is often cultivated in Apulia Region (Southern Italy) is the right place for the nematode applications due to the fact that it is kept wet during the entire period since there are irrigated artificially or by natural rainfall. The control with the EPNs will also permit to solve the request of the consumers asking for "natural foods" free of pesticides.

## RIASSUNTO

CONTROLLO BIOLOGICO DI *RHYTIDODERES PLICATUS* OLIV. (COLEOPTERA, CURCULIONIDAE) SU PIANTE DI CAVOLO VERZA IN VASO, CON NEMATODI ENTOMOPAGENI

Sono state effettuate prove di controllo su piante di cavolo attaccate alle radici da larve di *Rhytidoderes plicatus* Oliv. (Coleoptera, Curculionidae) utilizzando ceppi di *Steinernema feltiae*, *S. carpocapsae* e *Heterorhabditis bacteriophora* rinvenuti in Italia meridionale.

Test di laboratorio su larve mature del coleottero in scatole petri contenenti un disco di carta bibula inumidita con circa 2.000 stadi infettivi (IJs) dei nematodi in 1 ml di $H_2O$ avevano messo in evidenza che *S. carpocapsae* ed *H. bacteriophora* uccidevano il 100% delle larve del curculionide dopo 24 ore mentre allo *S. feltiae* ne occorrevano 96.

Nelle prove effettuate sulle piante di cavolo verza in vaso furono distribuiti 30.000 IJs in 50 ml di $H_2O$ sul terreno. *H. bacteriophora* controllò il 97% delle larve dopo 5 giorni e il 100% dopo 10, mentre *S. carpocapsae* ridusse le larve del 73% dopo 5 giorni e dell' 80% dopo 10 giorni. Dopo due settimane il 93% degli insetti era stato ucciso. *S. feltiae* evidenziò una azione molto più lenta arrivando a controllare il 30% delle larve dopo 2 settimane.

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