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LABORATORY TESTS ON THE BIOCONTROL OF CHESTNUT INSECT PESTS ON ETNA (SICILY, ITALY) BY MEANS OF ENTOMOPATHOGENIC NEMATODES

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Clausi M., Leone D., Vinciguerra M.T., Rappazzo G., Tarasco E. – Laboratory tests on the biocontrol of chestnut insect pests on Etna (Sicily, Italy) by means of entomopathogenic nematodes.

The virulence of seventeen strains of entomopathogenic nematodes, belonging to four species of *Steinernema* and two of *Heterorhabditis*, against the chestnut insect parasites *Curculio elephas, C. glandium, Pammene castanicola, Cydia splendana* and *C. fagiglandana*, was tested in laboratory. The bioassays were conducted on the insect larvae collected in three different years (2010-2012) in chestnut groves of Etna. Most of the strains tested resulted good biocontrol agents for all the treated insect pests. The strains *S. feltiae* ESA and *S. feltiae* EPP were the most effective strains autochthonous of Etna when all the species of insect pests are considered as a whole.

KEY WORDS: *Curculio, Pammene, Cydia, Steinernema, Heterorhabditis*.

INTRODUCTION

Among the insects there are numerous parasites that can cause damage to chestnuts, reducing their quality and commercial value. A research on the chestnut pests of Etna was considered useful because recently in Sicily there has been a revival of this crop, which has traditionally been used only for timber, and there are some attempts to convert coppice stands into orchards and to produce good quality nuts for the alimentary market. Moreover, because of the poor commercial use made of the nuts so far, there is no literature regarding the nut parasite situation in Sicily.

From studies conducted in Sicily (CLAUSI and VINCIGUERRA, 2005; VINCIGUERRA and CLAUSI, 2006; RAPPAZZO *et al*., 2006) chestnut pests in Etna Vulcan area belong to two species of Coleoptera Curculionidae, namely *Curculio elephas* Gyll. and *Curculio glandium* Marsh., and to three Lepidoptera Tortricidae, *Cydia splendana* (Hb.), *Cydia fagiglandana* (Zel) and *Pammene castanicola* Trematerra, 2009, the last one previously identified as *P. fasciana* (L.) and recently described as a new species (TREMATERRA and CLAUSI, 2009) and until now reported only from Etna. These species represent a real menace for chestnut and for this reason it would be very useful to adjust suitable tools of biological control against them. In the previous work (VINCIGUERRA and CLAUSI, 2006) some preliminary tests on the ability of different species and strains of EPN to infect these insect pests were conducted but a more punctual study was needed. The present investigation is based on several laboratory tests conducted on the biocontrol of late instar larvae of *C. elephas*, *C. glandium, C. splendana*, *C. fagiglandana* and *P. castanicola*, extracted from chestnuts and from acorns collected in seminatural mixed groves on Etna, by means of strains and species of entomopathogenic nematodes (EPNs) of the genera *Heterorhabditis* Poinar and *Steinernema* Travassos. The restriction on the use of many pesticides, particularly in protected areas as the natural Park of Etna, requires to resort to alternative products such as EPNs as biocontrol agents. Aim of this study was to determine which chestnut pests are sensitive or resistant to EPN + bacteria complexes and which species or strain of nematodes are the most suitable to such control strategies, also comparing commercial to autochthonous strains, most of which collected in the same sites where pests are present.

MATERIALS AND METHODS

COLLECTION OF INSECTS AND SELECTION OF EPN

The efficacy of 17 EPN strains (Table 1) was tested in laboratory; these strains belonged to the species *Steinernema feltiae* (Filipjev, 1934) Wouts, Mrácek, Gerdin & Bedding, 1982, *S. ichnusae* Tarasco, Mrácek, Nguyen, Triggiani, 2008, *S. kraussei* (Steiner, 1923) Travassos, 1927, *S. carpocapsae* (Weiser, 1955) Wouts, Mrácek, Gerdin & Bedding, 1982, *S. vulcanicum* Clausi, Longo, Rappazzo, Vinciguerra, 2011, *Heterorhabditis bacteriophora* Poinar, 1976 and *H. megidis* Poinar, Jackson & Klein, 1987; 13 of them were from Italy (12 from Sicily and 1 from Apulia) and 4 were commercial products. The commercial strains were *S. feltiae, H. megidis* and *H. bacteriophora,* furnished by IntrachemBio, and *S. feltiae* furnished by B.T. Biotecnologie; the native strains were 5 of *S. feltiae* (ESA, EPP, EMM1, EPC, ETA), 4 of *S. kraussei* (EBL, EPL, ESC3, EMM2), 1 of *S. vulcanicum* (ESC1), 1 of *S. ichnusae* (EMA), all collected in various sites of Etna, 1 of *H. bacteriophora* (BAL1) from the island of Pantelleria (Sicily) and 1 of *S. carpocapsae* (MR7) from Apulia (Tarasco *et al*., 2014). The native strains come from the EPN collections of the Department of Biological, Geological and Environmental Sciences, University of

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Table 1 – EPNs tested in the experiments

Strain/species	Locality/Company
S. feltiae ESA	S.Alfio (CT, Sicily)
S. feltiae EMM1	Mt. Monaco (CT, Sicily)
S. feltiae EPP	Piano Porcheria (CT, Sicily)
S. feltiae EPC	Pietracannone (CT, Sicily)
S. feltiae ETA	Tarderia (CT, Sicily)
S. feltiae	IntrachemBio
S. feltiae	B.T. Biotecnologie
S. ichnusae EMA	Triciala (CT, Sicily)
S. carpocapsae MR7	Manfredonia (FG, Apulia)
S. vulcanicum ESC1	Salto del cane (CT, Sicily)
S. kraussei ESC3	Salto del cane (CT, Sicily)
S. kraussei EBL	Piano Balilla (CT, Sicily)
S. kraussei EPL	Piano Lepre (CT, Sicily)
S. kraussei EMM2	Mt. Monaco (CT, Sicily)
H. bacteriophora BAL1	Pantelleria (TP, Sicily)
H. bacteriophora	IntrachemBio
H. megidis	IntrachemBio

Catania, and from DiSSPA, University of Bari "A. Moro". The experimental bioassays were performed in laboratory against the chestnut pests *Curculio elephas*, *C. glandium*, *P. castanicola*, *C. splendana* and *C. fagiglandana*. The experiments were performed in a span of 3 years, 2010-2012, and following the trend of infestations of 5 chestnut groves of Etna in the three years. All insect pests used for testing were obtained from chestnuts collected on Etna; the larvae were extracted putting the chestnuts in a stove at a temperature of 40° C and collecting the larvae which came out from the nuts. Before the experiments, all larvae were previously checked to make sure there were no infections of any kind. In all bioassays a dose of 750 IJS x ml and 10 insect larvae in a 5x5 Petri dish for each EPN were used. The Petri dishes were kept at the temperature of 10° C, more or less corresponding to the mean soil temperature in Etna chestnut groves in Autumn. Each treatment was replicated three times. The insect larvae that died showing the typical characteristics caused by the infestation (colour, odour, consistence) were transferred in White traps where the nematodes completed their life cycle and abandoned the insect body.

Laboratory bioassays

2010. Efficacy of different EPNs strains against curculionid larvae.

In this bioassay all the already cited EPN strains were used but only to test the susceptibility of the larvae of each *Curculio* species.

2011. Efficacy of different EPNs strains against the main chestnut insect pests.

In 2011 only three species of chestnut pests were collected: *C. elephas*, *C. glandium* and *P. castanicola*. No commercial strain was used; three the selected native strains, *S. feltiae* EPP, *S. kraussei* EMM2 and *H. bacteriophora* BAL1, used against the chestnut pests. In this experiment the larvae of *Curculio* were tested without distinguishing the species. The selected EPNs were tested also on larvae of *Galleria mellonella* (L.) as control.

2012. Efficacy of selected EPNs against all chestnut pests

In 2012 all five species of chestnut pests were collected. No commercial strain was used; five the selected autochthonous strains, *S. feltiae* ESA, EPP, EMM1, *S. kraussei* EMM2 and *H. bacteriophora* BAL1, used against the 5 chestnut pests. Also in this case the larvae of both *Curculio* species and those of both *Cydia* species have been used without distinguishing the species of the same genus. The selected EPNs were tested also on larvae of *G. mellonella* as control.

Data analysis

The mortality data of larvae were assessed using analysis of variance (ANOVA); Tukey's (HSD) test was used to compare means. Before conducting ANOVA, all percentages were transformed using the arcsine square root transformation. All data were processed utilizing Statistic 9.0. A p value of 0.05 was used in all analyses.

RESULTS

Figure I, concerning the bioassays of 2010, shows the percentages of larvae of the two species of *Curculio* infected by each EPN strain. Both species were infected by almost all EPN strains used in the test. The highest mortality was obtained with both commercial strains of *Heterorhabditis: H. bacteriophora* caused 90% of mortality in *C. glandium* and 80% in *C. elephas. H. megidis* caused 60% of mortality in *C. glandium* and 100% in *C. elephas*. All the native strains showed to be less efficacious of the commercial strains of *Heterorhabditis*. Among the native strains the most virulent against *C. glandium* were *H. bacteriophora* BAL1, found in Pantelleria, and *S. kraussei* EMM2 and *S. feltiae* EMM1, both found in chestnut soil of Etna, at "Monte Monaco". On the contrary, the two industrial strains of *S. feltiae*, BT and Intrachem, and the native strain *S. feltiae* ETA did not show any virulence against the weevils. Both species of *Curculio* were also susceptible to *S. feltiae* EMM1 and, to a lesser extent, to *S. feltiae* EPC and to *S. vulcanicum* ESC1, both from Etna chestnut groves, and to *S. carpocapsae*, from Apulia.

Figure II, concerning the bioassays of 2011, shows that the three selected EPN strains had the same efficacy against the larvae of *P. castanicola* (about 60%), while against the weevils the highest mortality was obtained with *S. feltiae* EPP (60% of infected animals); *S. kraussei* EMM2, differently from the previous test, resulted inexplicably less virulent (only 20% of larvae infected) while *H. bacteriophora* BAL1 showed the same virulence on *C. elephas* (about 42%) as in the previous test. All the three strains, however, were very effective with the larvae of *G. mellonella*.

Figure III concerns the bioassays of 2012. In this test all the EPN strains used were very active against pests larvae. As regards the larvae of *Curculio*, the mortality ranged from 30 to 45 %; *S. feltiae* EMM1 was less effective than in 2010 while *S. feltiae* EPP was more effective and *S. feltiae* ESA maintained the same virulence degree. The least virulent strain towards almost all insects was *H. bacteriophora* BAL1. *Cydia* species were mainly infected by *S. feltiae* ESA and *S. feltiae* EMM1, with a mortality of about 80%. Against *P. castanicola* the most effective strains were *S. feltiae* ESA and *S. feltiae* EPP, with a mortality of about 70%. In many cases the mortality of the moths was higher than that of *G. mellonella*.

DISCUSSION AND CONCLUSION

The tests showed that the virulence of the same infectious strain of EPN is often different in respect of the target

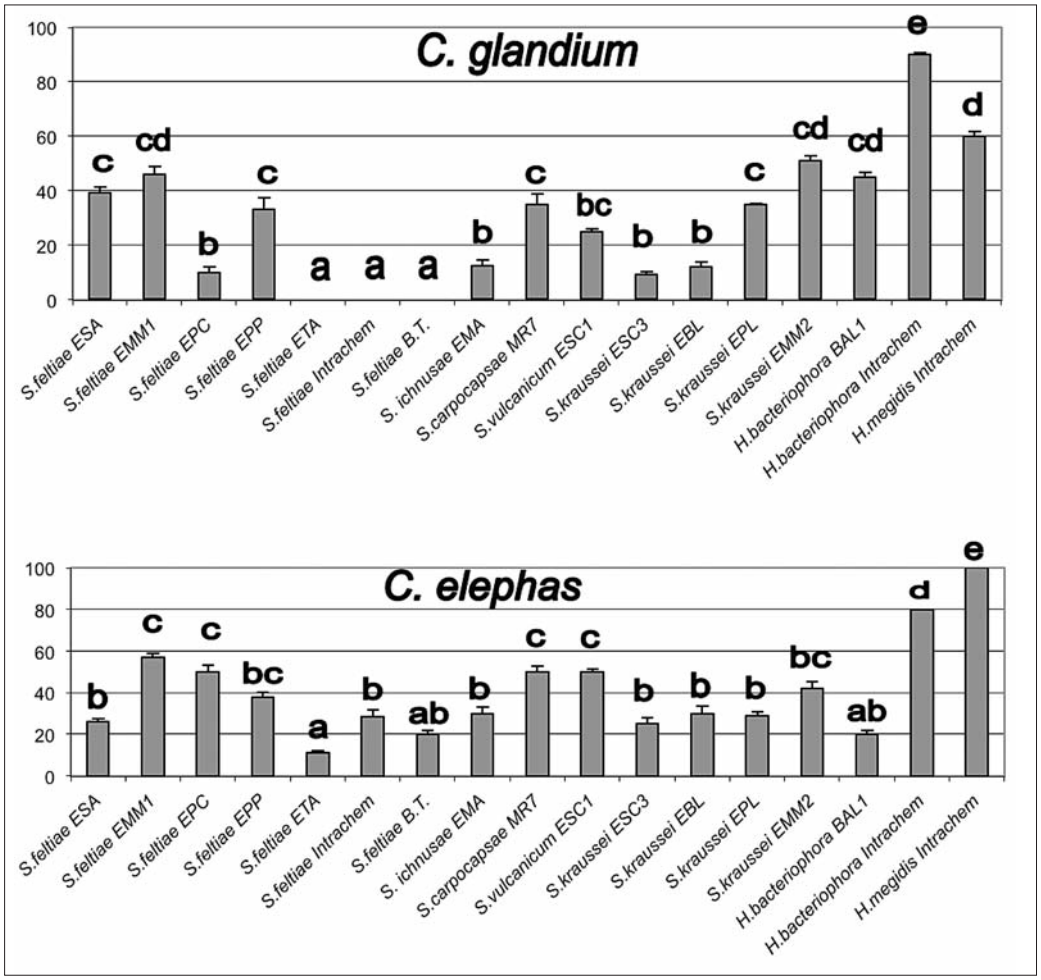

Fig. I – Mortality of *Curculio glandium* and *C. elephas* larvae 10 days after the treatment with 17 species and strains of EPNs. Columns marked with the same letter are not statistically different at $p < 0.05$, according to the Turkey's HSD test.

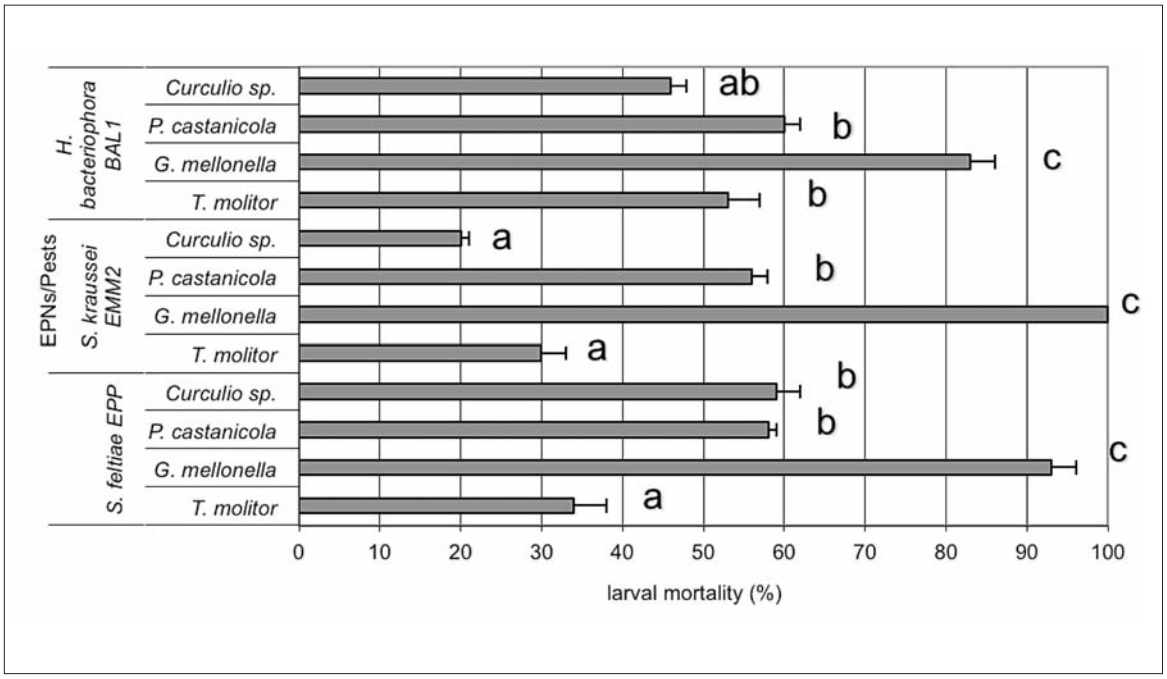

Fig. II – Mortality of chestnut pests' larvae 10 days after the treatment with 3 species and strains of EPNs. Columns marked with the same letter are not statistically different at $p < 0.05$, according to the Turkey's HSD test..

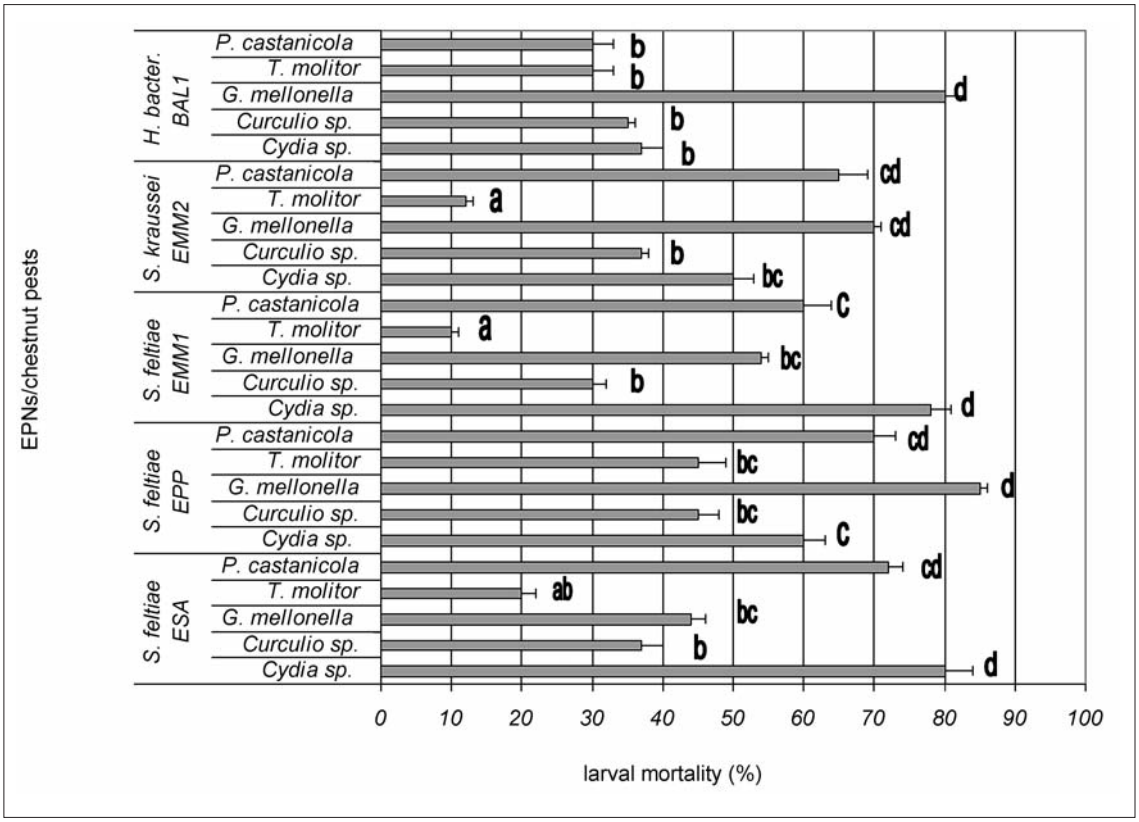

Fig. III – Mortality of chestnut pests' larvae infested 10 days after the treatment with 5 species and strains of EPNs. Columns marked with the same letter are not statistically different at $p < 0.05$, according to the Turkey's HSD test.

species and also that different strains of the same nematode species can have a different ability to infect the same insect species; moreover, even the virulence of a single strain against the same pest has resulted different in different tests. Notwithstanding all these variations, still these tests assessed the virulence of many EPN strains, most of which autochthonous of Etna, against all chestnut pests.

To obtain useful results in the biological control of such parasites, preliminary laboratory tests of the relative efficacy of the strains to be used are needed to select the most suitable ones, but the success of such a tool in the field, however, largely depends also on the correct methodology of application of the EPN suspension and on the ability of the nematodes to survive in the soil for the time needed to localize and infest the larvae. In the specific case of the chestnuts of Etna, it is important to evaluate the EPN ability to persist for long after insemination in the chestnut soil. The results of the bioassays show that the strains *S. feltiae* ESA and *S. feltiae* EPP were the most effective strains autochthonous of Etna when all the species of insect pests are considered as a whole. The commercial strains of *H. bacteriophora* showed to be more effective than *H. bacteriophora* BAL1, native of Pantelleria, but a comparison with strains of the same species autochthonous of Etna was not possible, since no species of *Heterorhabditis* has ever been found so far in chestnut soil. Some autochthonous strains of *Steinernema*, however, showed good applicative potentialities against all chestnut insect pests and probably they would be more able to persist in Etna soil than the species of *Heterorhabditis*, more susceptible to the low temperatures present at the high altitudes where chestnut are present, so much so that no species of this genus have ever been found during the thorough screening for autochthonous EPN strains and species made by the authors in Etna chestnut soil.

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