NIKOLA GRUJIĆ^a - DRAGA GRAORA^a - BRANIMIR NJEŽIĆ^b - BORUT BOSANČIĆ^{bc} –
EUSTACHIO TARASCO^d

VIRULENCE OF ENTOMOPATHOGENIC NEMATODES TO THREE SOFT SCALE INSECT SPECIES

^aUniversity of Belgrade-Faculty of Agriculture, Department for Entomology and Agricultural Zoology, Nemanjina 6, 11080 Belgrade-Zemun, Serbia;

^bFaculty of Agriculture, University of Banja Luka, Bulevar vojvode Petra Bojovića 1A, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina;

^cGenetic Resources Institute, University of Banja Luka, Bulevar vojvode Petra Bojovića 1A, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina;

^dUniversity of Bari Aldo Moro, Department of Soil, Plant and Food Sciences, via Amendola 165/A, 70126 Bari, Italy;

Corresponding Author: Eustachio Tarasco, eustachio.tarasco@uniba.it

Grujić N., Graora D., Nježić B., Bosančić B., Tarasco E. - Virulence of entomopathogenic nematodes to three soft scale insect species.

Virulence of commercial strains of entomopathogenic nematode (EPN) *Steinernema carpocapsae* (Weiser), *Steinernema feltiae* Filipjev and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Steinernematidae, Heterorhabditidae), were tested on young females of *Pulvinaria hydrangeae* Steinweden, *Partenolecanium corni* (Bouché) and *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera, Coccidae) and a native strain of *H. bacteriophora* was tested on citricola scale, *C. pseudomagnoliarum*. The laboratory tests were carried out in small plastic Petri dishes with infective juveniles (IJ) applied on filter paper covering the bottom. Results showed that all EPN tested were virulent to all three soft scale insect species. Recorded mortality of insects at the highest concentration of approximately 50 IJ per cm² exceeded 64% in all combinations of nematode and scale species tested. Within the same nematode concentration, scales' mortality of 90% and above was recorded for *P. corni* with all nematode species, *P. hydrangeae* with *S. carpocapsae* (Nemastar) and *C. pseudomagnoliarum* with native *H. bacteriophora* strain. All scale species were good hosts for all nematode species. This is the first report of EPNs to parasitize soft scale insects and one of the few among Coccoidea. The level of parasitism recorded is promising for potential use in management of scale insects.

KEY WORDS: Coccidae; Steinernematidae; Heterorhabditidae; Biological control; citrus; EPN

INTRODUCTION

Scale insect species are phytophagous and a majority of them are polyphagous. They are feeding on sap of arborescent plants and disrupting physiological processes in plant. By the excretion of the excess of ingested fluid in form of honeydew, that allows sooty molds to grow, they further make various qualitative and quantitative damages to the crops. Coccidae are generally and usually controlled collaterally by use of chemical insecticides in conventionally grown orchards. Non-chemical control methods have been developed against scale insects, based on parasitoids and predators (ROSEN, 1973; OUVARD *et al.*, 2013) but they are with limitations.

Entomopathogenic nematodes (EPN) in families Steinernematidae and Heterorhabditidae are obligate parasites of insects. They have developed symbiotic relationship with bacteria in the family Enterobacteriaceae. The infective third stage juvenile (IJ) can survive outside the insect and can actively search for a host. IJs enter insect through any opening (mouth, anus, spiracles) or intersegmental membranes of insect cuticle. In the hemocoel of its host the IJ releases its symbiotic bacteria. The insect death occurs within 24-48 hours. EPNs are more frequently and successfully used for control of insects that

undergo at least one developmental stage in soil that has been extensively reviewed in literature (TOEPFER *et al.*, 2010; LACEY & GEORGIS 2012; LACEY *et al.*, 2015). Their success against foliar pests, where they are exposed to harmful abiotic factors, can be limited. However, in greenhouses, significant progress has been made against different aboveground pests including control of the Sweet Potato Whitefly *Bemisia tabaci* (Gennadius) (Hemiptera, Aleyrodidae) (CUTHBERTSON *et al.*, 2007), the Diamond Back Moth *Plutella xylostella* (L.) (Lepidoptera, Plutellidae) (SCHROER & EHLERS, 2005a; SCHROER *et al.*, 2005b), Tomato Leaf Miner *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) (BATALLA-CARRERA *et al.*, 2010) and Western Flower Trips *Frankliniella occidentalis* Pergande (Thysanoptera, Thripidae) (PREMA-CHANDRA *et al.*, 2003). Above ground pests in cryptic habitats are also targeted by EPN, like the Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera, Dryophthoridae) (LLÀCER *et al.*, 2009; TRIGGIANI & TARASCO, 2011) or the Codling Moth, *Cydia pomonella* (L.) (Lepidoptera, Tortricidae) (LACEY *et al.*, 2010; ODENDAAL *et al.*, 2016). Adding adjuvants or surfactants increases IJs persistence resulting in increase of their performances (GREWAL, 2002; SCHROER *et al.*, 2005a; SCHROER *et al.*, 2005b; DITO *et al.*, 2016). There are few

recent reports of pathogenicity of EPN species on some scale species of Pseudococcidae (ABD EL RAHMAN *et al.*, 2012, FUENMAYOR *et al.*, 2020) and Stictococcidae (NGO KANGA *et al.*, 2020) but data on susceptibility of Coccidae to EPN are lacking.

In the present work susceptibility of *Pulvinaria hydrangeae* Steinweden- cottony hydrangea scale, *Partenolecanium corni* (Bouché) European fruit lecanium and *Coccus pseudomagnoliarum* (Kuwana), citricola scale (Hemiptera, Coccidae) to EPN was investigated. Many ornamental plants are hosts of these three species worldwide. *Coccus pseudomagnoliarum* is known as pest of citrus and *P. corni* of various continental fruit tree species. However, the latter two species can be serious pests in organic orchards where there is no use of conventional insecticides and where EPN can be their good alternative. Furthermore EPN have many advantages to be used against scales in urban areas on ornamental plants.

MATERIALS AND METHODS

SOFT SCALE SPECIES

Wild populations of *P. hydrangeae*, *P. corni* and *C. pseudomagnoliarum* taken from branches of *Hydrangea macrophylla*, *Prunus domestica* and *Celtis* sp., respectively, in Belgrade (Serbia) were used in the experiment. Young females of aforementioned species were gently detached from branches with small laboratory spatulas and used immediately.

ENTOMOPATHOGENIC NEMATODES

Three species of EPN were used in the experiments: *Heterorhabditis bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* Filipjev (Rhabditida: Heterorhabditidae and Steinernematidae). Commercial strains (Nematop®, Nemastar® and Nemaplus®, from company E-nema GmbH, Germany) of all mentioned EPN were used. Native population of *H. bacteriophora* (*H. bacteriophora* B&H) isolated from soil samples taken in Prnjavor, Bosnia and Herzegovina was tested additionally on *C. pseudomagnoliarum*. EPN populations were maintained in the last instars of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). Extracted IJs were stored in a refrigerator at 4 °C. Infective juveniles not older than 15 days were used in the experiments.

EXPERIMENTAL DESIGN

Pathogenicity bioassay was carried out in small plastic Petri dishes (35 mm in diameter) with filter paper covering the bottom surface. Four nematode concentrations of 50, 100, 200 and 500 IJs per Petri dish were used to test virulence of all EPN strains to *C. pseudomagnoliarum* and only the highest dose of 500 IJs was used against the other two soft scale insect species. Volume of 0.5 ml of suspension with nematodes in given concentrations has been transferred by pipetting on filter paper. In control treatment the same volume of water is applied. Subsequently, young soft scale females were transferred to Pe-

tri dishes and then sealed with parafilm. The results were checked after seven days and mortality of insects was recorded. Treatments with different nematode concentrations and control without nematodes were replicated five times with 10 insects per dish. All insects were dissected and only dead insects with presence of nematodes and/or clear symptoms of EPN infestation were considered killed by nematodes. All experiments were conducted in the laboratory at room temperature (20–23 °C).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) with appropriate post-hoc tests was performed on the data using SPSS 22 software (IBM, 2013). Measured data were presented by standard descriptive measures ($\bar{X} \pm S_{\bar{x}}$) i.e. mean and standard error of the mean. Analysis and comparisons were conducted by fitting general linear models in terms of practical applicative grouping between those groups which were of both scientific and practical agronomical interest. For improved precision data were log transformed prior to the analysis. Significant difference between compared groups was considered at $p < 0.05$. Beside p-value main effect size with its agronomical importance was also taken into consideration when comparing the studied groups and factors.

RESULTS AND DISCUSSION

Our results show that all EPNs caused high mortality of all three examined soft scale insect species. Mortality of *C. pseudomagnoliarum* females (Fig. 1) was increasing with the increase of concentration of IJs. The highest EPN virulence was observed with highest concentration (500 IJs per Petri dish). The lowest variability in virulence between concentrations was recorded for *S. carpocapsae* (Nemastar) where all concentrations above 100 IJ similarly induced mortality of insects between 60% and 65%. The highest two concentrations caused overall mortality of scales' females ranging from 52 to 96% with best results performed by native strain *H. bacteriophora* in all concentrations. The mortality of all scale species tested in control treatments were significantly lower than in ones exposed to nematodes.

Female individuals of *C. pseudomagnoliarum* were exposed to four strains (*S. feltiae*, *S. carpocapsae*, *H. bacteriophora* and *H. bacteriophora* B&H) of entomopathogenic nematodes at five different concentrations (0 – control, 50, 100, 200, 500). The analysis of the effect of the different species of nematodes on *C. pseudomagnoliarum* in different concentrations indicated that there was highly significant main effect of different species of nematodes ($p < 0.001$, $df=3$, $F=6.78$) hence different nematode species have in general different effect on mortality of the target insect species. There was also statistically highly significant main effect of different concentrations of nematodes ($p < 0.001$, $df=4$, $F=49.33$).

Concerning virulence, *H. bacteriophora* B&H caused superior effect on insect mortality in 500 IJ concentration in comparison to all other populations of EPN.

Fig. I - Mortality of *Coccus pseudomagnoliarum* females caused by 50, 100, 200 and 500 infective juveniles (IJ) of *Steinernema feltiae* (SF), *S. carpocapsae* (SC) and *Heterorhabditis bacteriophora* (HB) commercial (Nemaplus, Nemastar and Nematop) and *Heterorhabditis bacteriophora* native (B&H) strains, in experiment in Petri dishes. Error bars represent standard error of the mean. Bars with the same letter are not significantly different ($P < 0.05$).

Fig. II - Mortality of females of three soft scale insect species caused by 500 infective juvenile of *Steinernema feltiae* (SF, gray bars), *S. carpocapsae* (SC, dark gray bars) and *Heterorhabditis bacteriophora* (HB, black bars) commercial (Nemaplus, Nemastar and Nematop) and native (B&H) strains, in experiment in Petri dishes. Error bars represent standard error of the mean. Bars with the same letter are not significantly different ($P < 0.05$).

Concentration of 200 IJ in *H. bacteriophora* B&H was also significantly superior to all other tested nematode species with 200 IJ concentration, but not with their 500 IJ concentration.

In specific cases within tested populations of *H. bacteriophora* concentration of 500 IJ was significantly superior in causing insect mortality than all other tested concentrations.

In treatments with *S. carpocapsae* similar insect mortality at 100 IJ, 200 IJ and 500 IJ concentrations was achieved without statistically significant difference between those three concentrations. Lower concentrations and control in *S. carpocapsae* had significantly lower mortality without significant difference between those two.

In treatments with *S. feltiae*, the highest and statistically significant insect mortality was achieved at concentration of 500 IJs in comparison to other applied concentrations except for the 200 IJ which had similar effect to 500 IJ.

Different nematode species are compared at concentration 500 IJ to assess mortality of three target insect species. Analysis indicated highly significant interaction between studied factors ($p < 0.001$, $df = 6$, $F = 5.58$), i.e. EPN species and insect species. There was also highly significant main effect of both factors individually.

From all scale insect tested, the most susceptible to all EPNs was *P. corni* with recorded female mortality ranging from 90% caused by *S. feltiae* (Nemaplus), to 100 % done by *H. bacteriophora* (Nematop). *Pulvinaria hydrangeae* was the most susceptible to *S. carpocapsae* with 92 % recorded young females mortality (Fig. II).

Concerning susceptibility of *C. pseudomagnoliarum* there was statistically best effect of *H. bacteriophora* in comparison to other tested nematode species ($p = 0.034$ compared to *S. carpocapsae*, $p = 0.016$ compared to *S. feltiae*). In *P. corni* there was no statistically significant difference among studied nematode species ($p = 0.126$ for *H. bacteriophora* compared to *S. feltiae*, $p = 0.016$ for *H. bacteriophora* compared to *S. carpocapsae*, and $p = 0.537$ for *S. feltiae* compared to *S. carpocapsae*). In *P. hydrangeae* there was statistically best effect of *S. carpocapsae* compared to other tested EPN species ($p = 0.034$ compared to for *H. bacteriophora*, $p = 0.001$ compared to *S. feltiae*).

The results of the work show the high susceptibility of all three tested scale insect species to all three species of EPN presently used. To our knowledge that has not been previously presented in the literature. Development of nematodes was recorded in vast majority of dead insects. All scale species appear to be good hosts for all EPN species with new generation of IJ recorded for all of them.

The highest mortality of *C. pseudomagnoliarum* females was caused by *H. bacteriophora* B&H strain. The differences were significantly higher in most of the treatments and in all concentration above 100 IJ comparing to other EPN strains and species. The reason for that may be due to the loss of virulence of bacterial symbionts because of longer in vivo maintenance and in-fridge-storage of commercial strains than native *H. bacteriophora* B&H population. Also it could be due to difference in pathogenicity of populations within the same species of

EPN that could be further used as a good material for breeding for specific traits (MUKUKA *et al.*, 2010, SUMAYA *et al.*, 2018).

Important aspect of this study results is observed reproduction of EPN in parasitized scale insects. In dense scale colonies females are found close to each other and that could allow easy spreading to neighboring females and prolong persistence of virulent infective juveniles. However, in the field conditions, high temperatures lethal to the nematodes may hamper this effect.

EPN are soil organisms and shown to be the most efficient against soil dwelling pests (LACEY & GEORGIS, 2012). However, scale insects are aboveground pests and their control with EPN would require foliar application. Major obstacle in foliar application that limits nematode persistence is rapid juvenile desiccation (GLAZER *et al.*, 1992). These factors limit nematode persistence and ability to find and penetrate a host leading to dramatical decrease in their efficacy. Adding adjuvants and surfactants increase adhesion and dispersal of nematodes that contribute to nematode persistence in foliar application (SCHROER & EHLERS, 2005a; DE WAAL *et al.*, 2013).

These results for the first time promote use of EPN against Coccidae with special potential in managing soft scales in urban areas and organic orchards, due to their safety and eco-toxicological benefits (EHLERS, 2005). To achieve that goal, further experiments of testing effect of EPN in natural environment on plants are planned.

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