UNIVERSITY OF BANJA LUKA

FACULTY OF AGRICULTURE

Branimir Nježić

Control of Plum Sawflies (*Hoplocampa flava* L. and *Hoplocampa minuta* Christ) with entomopathogenic nematodes

DOCTORAL DISSERTATION

Banja Luka, 2017

УНИВЕРЗИТЕТ У БАЊОЈ ЛУЦИ

ПОЉОПРИВРЕДНИ ФАКУЛТЕТ

Бранимир Њежић

Контрола осица шљиве (*Hoplocampa flava* L. и *Hoplocampa minuta* Christ) ентомопатогеним нематодама

ДОКТОРСКА ДИСЕРТАЦИЈА

Бања Лука, 2017.

Page with information about the promoter and dissertation

Promoter:
Prof. Dr. Ralf-Udo Ehlers, associate professor at Kiel University, Germany and guest professor at Ghent University, Belgium.

Key words:
Steinernema feltiae, S. kraussei, S. carpocapsae, Heterorhabditis bacteriophora, biological control.

Scientific field:
Agricultural science.

Scientific area:
Plant science.

Classification Code for a given scientific field according to the CERIF (Common European Research Information Format) codebook:
B250 Entomology, plant parasitology.

Summary in English:

Plum Sawflies *Hoplocampa flava* L. and *Hoplocampa minuta* Christ are the main pests of European plum. Currently control measures are exclusively based on application of synthetic pesticides in time of fetal fall and no other measures are available for commercial production. Therefore, the aim of this investigation was to evaluate the potential of entomopathogenic nematodes (EPN) as biocontrol agents of these pests.

First, a national survey on the presence of EPN was conducted on the territory of Bosnia and Herzegovina. In total, 221 soil samples were collected and the presence of EPN was evaluated. Eight samples were positive yielding in total of 12 isolates and four species. Molecular identification confirmed the presence of *Steinernema feltiae, S. kraussei, S. carpocapsae* and *Heterorhabditis bacteriophora*.

The susceptibility of different larval stages to commercial strains of *S. feltiae, S. carpocapsae* and *H. bacteriophora* was tested under laboratory conditions. The only susceptible stage of the pest (90-100%) were the larvae immediately after they exited infested fruits, but before making the cocoon. Cocooned larvae were not susceptible to the nematodes. This result was confirmed in the field experiment. Adult Plum Sawfly susceptibility was assessed in field trials in cages and open field trials. In the cages, EPN reached an efficacy of up to 100%, while in the open field trials efficacy was on lower levels, which can be explained by lower amounts of water used to deliver the nematodes on the soil surface compared to the trials in the cages.

To determinate optimal timing of EPN application against adult stages of Plum Sawflies, a temperature driven model for adult emergence was developed for conditions of Northern Bosnia and Herzegovina.

In conclusion, this research results indicate a promising potential of EPN as biocontrol agents of Plum Sawflies, but timing of application and amount of application water are the key factors influencing their efficacy.

Сажетак на српском језику:

Осице шљиве *Hoplocampa flava* L. и *Hoplocampa minuta* Christ су најзначајније штеочине шљиве. Мјере сузбијања које су тренутно доступне произвођачима су искључиво засноване на примјени синтетисаних инсектицида у вријеме отпадања латица. Стога је циљ ове дисертације био да се оцијени потенцијал ентомопатогених нематода (ЕПН) као биолошких агенаса у сузбијању ових штеточина.

Узорковање с циљем утврђивања просисутва ЕПН у Босни и Херцеговини по први пут је рађено кроз ову дисертацију. Узет је укупно 221 узорак, који је испитан на присуство ЕПН. Осам узорака који су били позитивни дали су укупно 12 изолата. Молекуларна идентификација је потврдила присуство четири врсте ЕПН *Steinernema feltiae, S. kraussei, S. carpocapsae* и *Heterorhabditis bacteriophora*.

Осјетивост различитиг стадијума ларви осица шљиве на комерцијалне препарате врста *S. feltiae, S. carpocapsae* and *H. bacteriophora* је испитана у лабораторијским условима. Једино су биле осјетљиве (90—100%) ларве које су напустиле оштећени плод, а прије прављења кокона. Нематоде нису имале фекеат на ларве унутар кокона. Ови резултати су потврђени у пољским условима. Ефикасност ЕПН у сузбијању имага осица шљиве испитана је у пољским огледима у тунелима прекривеним мрежама и на отвореном пољу. У тунелима, ЕПН су показале ефикасност до 100%, док је на отвореном пољу та ефикасноист била нижа, што се може објаснити да је у овим огледима кориштено значајно мање воде за апликацију нематода.

Да би се утврдило оптимално вријеме апликације ЕПН за сузбијање имага осица шљиве, развијен је модел почетка излијетања имага заснован на температури за услове сјеверне Босне и Херцеговине.

У закључку се може рећи да је ово истраживање показало да ЕПН имају потенцијал за сузбијање осица шљиве, али се за ефикасну примјену мора водити рачуна о прецизном времену апликације и количини воде са којом се нематоде аплицирају.

Aknowledgements

I am writing this chapter in a moment when all other parts in requirement of PhD are done except defense. Whole process was a long journey to unknown, but desired destination. I wanted to travel, but I was not sure where to go. When the destination was clarified I had to make a plan. I enjoy making plans. Just after the start of the journey I was taking the road that was not on the list. I liked it. But then I faced troubles with bad weather and blind roads. I lost confidence. However, next year passion found me again. Of course this time I was careful with my expectations. Finally, I came to this destination and it looks quite normal. I found that it is beside how far I go and where end up more important is how and with whom I walk.

Foremost, I would like to express my sincere gratitude to my promoter Prof. Ralf-Udo Ehlers for giving me a chance to work with him. He guided me through this journey, made me proud when I succeeded to reach aims, but also pushed me forward when I was disappointed with troubles on the road. Privilege to work with him was for me the greatest motivation.

I am profoundly grateful to Prof. Gordana Đurić, for pushing me in nematology when I was still bachelor student. It would never be my choice and I couldn't imagine myself fishing nematodes in that time. However, nematology had influenced last twelve years of my life in a amazing way.

My first contact with the nematodes was in the laboratory of Jan van Bezooijen. In that time he believed more than me that I will continue with nematodes. He supplied me with first materials for nematology lab. He had a wish to come and design it together with me, but unfortunately it didn't happen. Even Jan is not anymore with us, friendship with him still lasts through his son Marien.

My profound gratitude goes to Prof. Maurice Moens who was promoter of my master thesis for his passionate attitude to nematology research that had parasitic effect on me. He knitted the net of nematologists in which I get.

Many thanks to Prof. Nikola Mićić; for his encouragement and all time support. Thank to his advice I did PhD in home country.

I would like to express my special appreciation to members of dissertation Committee Prof. Snježana Hrnčić, Prof. Marek Tomalak and Prof. Stanislav Trdan for constructive comments on dissertation.

I would like to thank my former roommate Borut Bosančić, for having passion for discussions about my work.

My special thanks go to students without whom field work would not be possible. Petar and his humor were present almost in all expedition. Ćosa, Branko, Stana, Perica, Miloš, Dragan, Marko, Drago and Radoš, you did grate job and I enjoyed time with you.

My thanks go to farmers who offered me their orchards for field studies: Thomas Urlich, Boban Trivić, Mile Kuljić, Besim Salkić, Farmaland, Dušan Aleksić, Josip Rainović, Jiri Mikulić, Alen Cvitešić and Mario Blagec. Field work in Croatia would not be possible without Zdenko Freze and Margita Budiselić-Kutle.

The research was supports by Company e-nema who provided me with insect proof nets and Ministry of Science and Technology Government of Republic of Srpska who financial supported EPN survey.

I would like to thank my parents, sister Branka and her family for never ending support and patience for my developmental stages. They always believed in me.

Finally I wish to thank my family, who are my biggest inspiration, my wife Aleksandra for never complaining about work related to PhD, my son Aleksa who was always asking when it will get over with and my daughter Ana whose pets were sawflies larvae.

Table of Contents

Chapter 1
Introduction

Through history human beings always had the aim to have higher yields in agricultural production. To achieve this goal the greatest contribution gave breeding of new varieties with higher yields, improving production systems and increasing fertilization. Intensification of production systems created favorable conditions for many pests. Those pests belong to different groups of organisms like insects, nematodes, mites, fungi, bacteria, viruses, phytoplasmas and weeds. Research results and development of new technologies, especially in the fields of mineral plant nutrition and pesticides resulted in the "Green revolution". This revolution brought better global food security but very soon raised problems in production, human health and environmental issues. Introduction of pesticides during the twentieth century provided easy-to-use tools to eliminate pests and together with plant breeding and innovative plant nutrition contributed to achieve higher yields, particularly in developed countries.

Pesticides enable farmers to control pests but also to switch production to more intensive systems, which need more fertilization to increase yields. However, in these production systems plants are more sensitive to pests and more pesticide application is required. As a result of these innovations in agriculture, consumption of pesticides raised several times within the last five decades. Very soon the high consumption of pesticides brought along several problems. Development of pesticide resistance, risk to human health and the environment are major forces that push production systems more towards sustainable use systems.

Relying on control of pests based only on pesticides, farmers have faced problems with resistance against several groups of pesticides (Panini *et al.,* 2016). In some cases these problems left farmers without solutions for certain pests and diseases. Sometimes, resistance against different groups of pesticides made things worse. Newly developed pesticides should be more environmentally friendly and more selective. These challenges require from pesticide industry more investment and time to bring new compounds to the market.

Reports on risks to human health of farmers and consumers of food caused by pesticides and their residues confirmed the connection between serious diseases and pesticides. Moreover, contamination of water resources for human consumption, negative effects on non-target species and on the general environment increased public attention.

All those issues gradually resulted in phasing out of the most harmful active substances and mitigation of pesticide use. The main force in this process is public awareness which resulted in supermarket chains developing their own specific requirements regarding pesticide residues in food products. Moreover, recently, the European Union developed legislation that requires food safety and environmental friendly agricultural production (Directive 2009/128/EC). Change in production systems is necessary to achieve these demands. For example, in many cases cultivars that are widely accepted (like in apple production) are highly susceptible to pests and they should be replaced by varieties that have higher levels of resistance to particular pests and diseases.

A production system that is now mandatory at European level is Integrated Pest Management (IPM). It puts weight to not only control with more acceptable pesticides but also on monitoring, forecasting and prevention. Biological control, the use of natural enemies to control pests, is given priority over the use of chemical compounds in IPM. Biological control also is the only tool organic farming has available and this sector has a tremendous potential for growth.

Biological control is performed in four ways: classical biological control, conservation biological control, inoculation biological control and inundation biological control (Eilenberg *et al.,* 2001). Classical biological control is control of introduced pests, diseases or weeds by introduction of their natural enemies from the country of origin. Conservation of natural enemies means actions taken to protect and maintain biological control agents. The aim is to maintain the existing biocontrol potential by adapted use of production tools. Inoculation biological control refers to introduction of biological control agents that are expected to proliferate and distribute in the area by themselves. Inundative biological control is the periodic release of large numbers of living organisms to control pests and diseases. Host plant resistance, autosterilization, genetic manipulation of species, mechanical. phisical and cultural controls, non-conventional insecticides (insect growth regulators, etc.), and transgenic plants are covered by some definitions of biological control. Nowadays, biological control mostly refers to inundative biological control when biological control agents are used as a biopesticide. Biopesticides that are used as biological control agents are viruses, bacteria, protozoa, fungi, nematodes, insects, mites, snails and vertebrates.

Biopesticides have several advantages over synthetic pesticides. They are often safer for application personnel, food consumer and the environment. Biopesticides have a much narrower target spectrum compared with pesticides that affect many non-target organisms as well. They are very often effective in small quantities and decompose more quickly. In inundative biological control, that nowdays is the most common, effect depends on the effect of relised population, but not its offspring. They have an immediate effect.

Successful commercialization of several species of entomopathogenic nematodes (EPN) in the families Steinernematidae and Heterorhabditidae have occurred during the past three decades. Beside successful mass production their advantages as biological control agents are their extraordinary host range, at least proven under laboratory conditions and fast killing of hosts. They can actively seek or ambush hosts, can be applied with conventional application equipment, are safe for food consumers, vertebrates and non-targets and ease to register compared to synthetic chemicals (Lacey and Georgis, 2012). Their success as biological control agents is also due to their mutualistic relations with their symbiotic bacteria, *Xenorhabdus* and *Photorhabdus*. About 90 % of all insects spend at least one stage of their life cycle in the soil, presenting an excellent target for EPN. However, disadvantages of EPN that limit their higher market share are still higher production costs compared with pesticides, limited shelf life, refrigerated storage, and environmental limitation like requirements for adequate moisture, temperature, and sensitivity to UV light (Lacey and Georgis, 2012).

The Black Plum Sawfly (*Hoplocampa minuta* Christ.) and the Yellow Plum Sawfly (*H. flava* L.) (Hymenoptera, Symphyta, Tenthredinidae) are widespread key pests of plum fruits. They can cause significant damage, sometimes up to 100 %, if no control measures are applied. Currently control measures are based exclusively on pesticide application and no other measures are available for commercial production. Shift to integrated production with decreased pesticide application and development of organic production is demanding alternative measures for farmers since no control measures are or will be available for these key pests of plums.

This thesis deals with the evaluation of the efficacy of EPNs against Plum Sawflies. Since Plum Sawflies spend most of their time in the soil they present potential good targets for EPNs. In literature there is no evidences for studies related to the control of Plum Sawflies

with EPN or other biological control agents. This thesis project studied the potential of EPN against Plum Sawflies under laboratory and field conditions. Related to this topic, parameters of the life cycle of Plum Sawflies were studies in order to improve timing of EPN application and a survey on the presence of EPN in Bosnia and Herzegovina was conducted, since it is a prerequisite for biological control agents registration to confirm their presence in the country. Moreover, results obtained from this study could be extrapolated to a closely related species, the Apple Sawfly (*Hoplocampa testudinea*) and Pear Sawfly (*Hoplocampa brewis*).

Chapter 2

Literature review

2.1. About nematodes

Nematodes are a highly diverse, abundant and ubiquitous animal Phylum. These unsegmented worms are highly consistent in their anatomy, but buccal morphology suggests diverse feeding groups. Life style ranges from bacterial and fungal feeding, through plant and animal parasitism, to carnivores and omnivores (Yeates, 1993). They occupy almost all terrestrial, freshwater and marine niches. In the 1914 edition of the Yearbook of the United States Department of Agriculture, N.A. Cobb wrote on the abundance of nematodes: "If all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, valleys, rivers, lakes and oceans represented by a thin film of nematodes. The location of towns would be decipherable, since for every massing of human beings there would be a corresponding massing of certain nematodes. Trees would still stand in ghostly rows representing our streets and highways. The location of the various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases even their species could be determined by an examination of their erstwhile nematode parasites." In 2001, the phylum Nematoda comprised of 26,646 described species, out of them 8,359 are parasitic in vertebrate hosts, 10,681 are free-living species, 4,105 are species parasitic in plants, and 3,501 are species parasitic in invertebrate hosts (Hugot *et al.,* 2001). The most studied animal on earth, the bacterial feeding nematode *Caenorhabditis elegans*, is a model organism in biology, particularly in genetics and a source of spin-off results to nematologists.

2.2. Nematodes associated with insects

Nematodes associated with insects have been described from more than 30 families (Stock and Hunt, 2005). However, biocontrol potential is concentrated in seven families: Mermithidae, Allantonematide, Neotylenchidae, Sphaerularidae, Rhabditidae, Steinernematidae and Heterorhabditidae. The potential of nematodes is not restricted only to biocontrol of insect pests. There are commercial products of slug parasitic nematode, *Phasmarhabditis hermaphrodita* (Wilson and Gaugler, 2000), while predatory and fungal feeding nematodes have been studied as biocontrol agents of plant-parasitic nematodes

and plant pathogens respectively (Choudhury and Sivakumar, 2000; Lootsma and Scholte, 1997).

2.3. Entomopathogenic nematodes

Among all members of the phylum Nematoda the most attention as biocontrol agents have received obligate insect pathogenic nematodes of two families, Steinernematidae and Heterorhabditidae, known as entomopathogenic nematodes (EPN). Although the first species of EPN has been described almost 100 years ago, their innundative application only started 30 years ago. Increased understanding of the nematode biology, host range, epizootiology, advances in production technology, formulation, storage and application resulted in an exponential increase of interest on the market (Arthurs *et al.,* 2004). Beside research advances, their rapid expansion was supported also by exemption or ease of registration based on reports of no effect on humans, mammals and plants or environment (Ehlers, 2005). Their successful biocontrol attributes are based on the unique partnership of host seeking nematodes and lethal insect-pathogenic bacterium carried inside the nematode´s intestine, presumed to have arisen through convergent evolution (Poinar, 1993). Half a century had to pass from description of the first species to their mass production due to the development of cheap broad-spectrum insecticides that shifted attention away from biocontrol agents. Since the 1980s, application of EPN has experienced growth due to the development of relatively cheap, large scale liquid culture production technology and pressure on pesticide use due to the development of resistance, surpassing residue levels in food and negative impacts on the environment.

2.3.1. Classification

The families Steinernematidae and Heterorhabditidae are based in two orders of Rhabditida (de Ley and Blaxter, 2002) (Tab. 2.1.). Increased numbers of described species and new molecular characterization techniques have expanded species identification from morphometrical data, cross fertilization to the restriction fragment length polymorphism (RFLP) profiles and sequencing of the internal transcribed spacer (ITS) and D expansion segments of the 28S ribosomal RNA (Hunt, 2007). Useful stages for morphological identification are dauer juveniles (DJ) and males while in Heterorhabditidae, although a group with less number of species, this approach is more

difficult. Sequencing techniques revealed different polyphyletic origin of the two families (Blaxter *et al.,* 1998) that had already been stated based on morphological characterization by Spiridonov and Belostotskaya (1983). Hunt (2007) reported 55 valid steinernematid and 11 heterorhabditid species. The family Steinernematidae contains two genera, *Steinernema* Travassos, 1927 and *Neosteinernema* Nguyen & Smart, 1994, while the family Heterorhabditidae contains one genus, *Heterorhabditis* Poinar, 1976 (Hunt, 2005).

Table 2.1. Placement of the families Steinernematidae and Heterorhabditidae within the phylum Nematoda (de Lay & Blaxter, 2002)

NEMATODA (phylum)
Enoplea (class)
Chromadorea Inglis (class)
Rhabditida Chitwood (order)
Tylenchina Thorne (suborder)
Steinernematidae Chitwood and Chitwood (family)
Rhabditina Chitwood (suborder)
Heterorhabditidae Poinar (family)

2.3.2. Life cycle

Steinernema and *Heterorhabditis* belong to two phylogenetically distinct families but have many features in common. The similarities, including the association with the symbiotic bacteria, is probably due to their convergent evolution (Poinar, 1993). The only stage that is infective is the third juvenile stage, called dauer juvenile (DJ). It is a developmentally arrested stage that is adapted to endure harsh environmental conditions. During this stage, bacteria are in a quiescence and DJs are acting as a vector. They actively seek for their insects hosts, and this strategy can be in a range of ambusher to cruiser. Once the suitable host is found, the nematodes penetrate it through mouth, anus, spiracles or through intersegmental membranes of the insect cuticle (Peters and Ehlers, 1994). Once the DJ entered the insect haemocoel, bacteria are released. Pathogenesis and successful epizootics depend on each of the three, the insect, nematode and bacteria. The insect might have defence mechanisms to specific nematode-bacteria complexes, whereas

virulence factors of both, the nematode and bacteria, act separately or together to overcome hosts defence systems (Griffin *et al.,* 2005). The death of the insect ensues usually within 24-48 hours. The DJ starts to feed on bacteria and develop to the fourth juvenile stage and adult.

Whereas *Heterorhabditis* are heterogonic, having both, hermaphrodic (automictic) and amphimictic (male and female) modes of reproduction, *Steinernema* is characterized only by cross fertilization (amphimixis) (Griffin *et al.*, 2005). Only one exception, *S. hermaphrodita*, exists (Griffin *et al*., 2001). Thus, *Heterorhabditis* can reproduce in a host when a single DJ has invaded it, whereas for *Steinernema* at least two individuals of different sexes are required. Most of the juveniles of hermaphroditic heterorhabditids hatch within the mother body, feed on its uterus and intestine, causing maternal death (Johnigk and Ehlers, 1999). This phenomenon is called *endotokia matricida* and is common for rhabditid nematodes. Feeding on parental tissue provides juveniles with enough food supply to develop to DJs. Adult stages that reproduce by amphimictic reproduction only develop from laid eggs. DJ of both families retain the cuticle of the second pre-dauer stage juvenile, but it is more loosen in steinernematids and in most cases lost during soil movement (Campbell and Gaugler, 1991).

Depending of food resource, two to three generations of the nematodes are completed within the insect cadaver. EPN finish their development in the DJ stage that emigrates from the cadaver and seeks for other suitable hosts. Several hundred thousands of DJs can emigrate from a single insect cadaver, depending on its size (Griffin *et al.,* 2005).

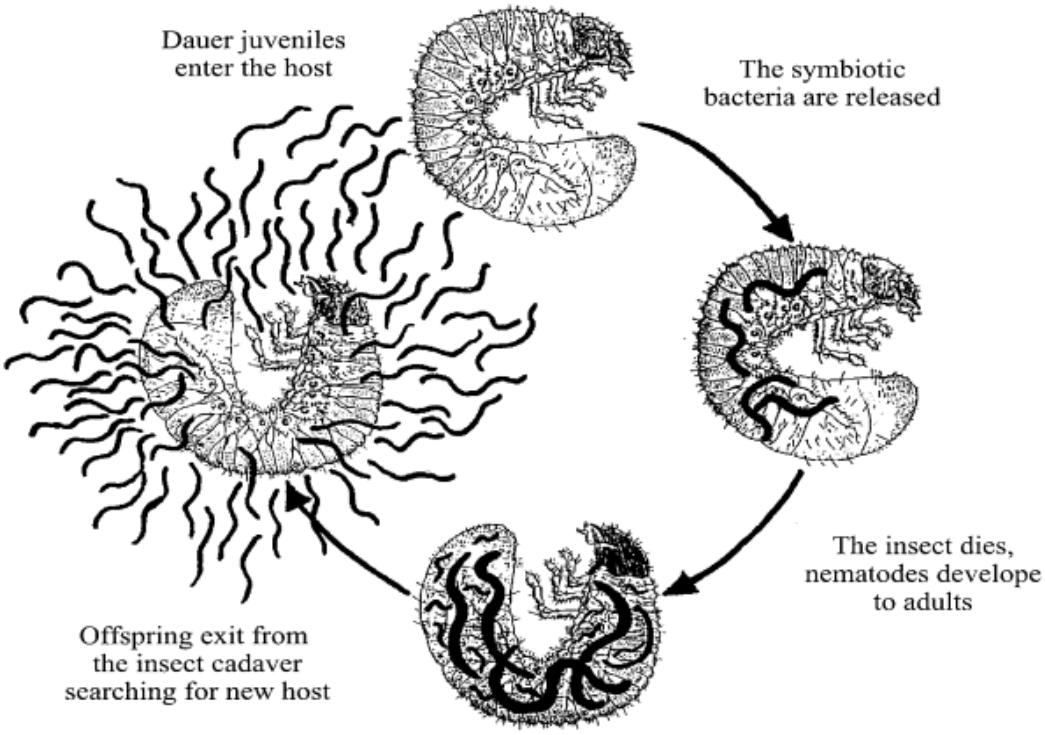

Figure 2.1. Life cycle of entomopathogenic nematodes (Ehlers 2001).

2.3.3. Bacterial association

The entomopathogenic nematode species belong to two families, while the bacterial symbionts of the genera *Xenorhabdus* and *Photorhabdus* are placed in a monophyletic clade of the family Enterobacteriaceae (Rainey *et al.,* 1995). *Xenorhabdus* is related with Steinernematidae while *Photorhabdus* is related with Heterorhabditidae. Specific feature of *Photorhabdus* is their ability to produce bioluminescence. The physical location of the bacterial cells carried within the DJs differs between the nematodes of the two families. While *Xenorhabdus* cells are situated in the anterior part of the DJ´s intestine in a bi-lobed vesicle in the anterior part of intestine (Ciche *et al.,* 2006), *Photorhabdus* are distributed throughout the intestine of the DJ. *Photorhabdus* are released through the mouth opening, while *Xenorhabdus* are defecated. Heterorhabditidae need their bacterial symbionts to kill the insect and for their reproduction, while Steinernematidae can reproduce on non-symbiotic bacteria as well, however, with much decreased yields. The

nematodes transmit only their specific bacterial species/strains, meaning that they are able to discriminate their symbiont from non-associated species (Akhurst, 1983). Once the nematode enters the host and the bacteria are released, the collaboration of the nematodes-bacteria complex starts to overcome the host immunity. The nematode inhibits the prophenoloxidase cascade, which is necessary for non-self-recognition and melanization, as well as the antimicrobial activity of the hemolymph (Ciche *et al.*, 2006). The bacterium influence insect immunity through suppression of the phenoloxidase activation, cytotoxicity to haemocytes, production of lipopolysaccharide (LPS) factors and proteases that kill haemocytes and by production of high molecular weight toxins (Ciche *et al.*, 2006). The relationship between the nematodes and their associated bacteria goes from complete dependence on each other to being able to independently kill an insect.

2.3.4. Distribution

Entomopatogenic nematodes have been reported from all continents, except Antarctica. The most sampled continent is Europe. Steinernematidae are more often recovered from samples with exceptions to surveys targeting sandy soils and warmer climatic conditions where Heterorhabitidae are dominat. *Steinernema carpocapsae, S. feltiae, Heterorhabditis bacteriophora* and *H. indica* are worldwide distributed (Hominick *et al.*, 1997). These species might be apparently efficient in their distribution by combination of wide host range, host movement, wind, water and human activities. The most common species in Europe are *S. affine* and *S. feltiae* (Sturhan and Liskova, 1999). Entomopathogenic nematodes have shown habitat preferences dependent on host distribution and vegetation type (Hominick, 2002). There have been no reports of the presence of EPN in Bosnia and Herzegovina due to lack of relevant surveys. The type isolate of *S. bicornutum* is from Vojvodina (Tallosi, Peters and Ehlers, 1995). Moreover, the presence of *S. feltiae* was confirmed in Vojvodina too. In Slovenia several species have been reported: *S. affine, S. feltiae, S. carpocapsae, S. kraussei* and *H. bacteriophora* (Laznik and Trdan, 2012). Divers climate and soil conditions, insects and vegetation diversity imply species richness of Bosnia and Herzegovina.

2.3.5. Host range

Range of insect's hosts of EPN species under laboratory conditions, inundatively realized, and naturally occurring populations can differ (Peters, 1996). Natural hosts of described species are often not obtained since nematodes are recovered from soil by trapping in larvae of the Great Wax Moth, *Galleria mellonella*. Even for *S. krausseri*, the very first steinernematid species described, only two insects species were reported to be infected with this nematode (Peters, 1996). However, *S. carpocapsae* has been recovered from insects belonging to four orders (Peters, 1996). The *S. carpocapsae* holotype population was described from *Cydia pomonella* (former *Carpocapsa pomonella*) but it is the only species recovered from this insect (Peters, 1996). The natural control potential of EPN is rather limited since they are found in more balanced relation with hosts and seldom cause epizootics. However, agricultural practice can increase mortality of pests supporting nematode populations and their persistence in soil. Successful establishment of EPN in a field is a prerequisite for biocontrol success and it depends on a high host density, a fact that was already obvious for pioneers of EPN application (Glaser and Farrell, 1935).

2.3.6. Mass production

For biocontrol application EPN can be produced *in vivo* or *in vitro* (Ehlers and Shapiro-Ilan, 2005). *In vivo* production in Wax Moth larvae is cheap but labour-intensive and suitable only for providing material for trials or small scale applications. Production *in vitro* can be on solid or in liquid media. Solid media production is suitable for medium scale applications having a main advantage in small investments in biotechnology equipment. Production in liquid media in biofermenters has high installation costs and needs advanced knowledge of nematode biology, but on the other hand, big markets can only be supplied with larger amounts of product at reasonable price when produced in liquid culture using fermenters. Factors that can contribute to further development of EPN production technology in order to become more competitive to pesticides are strengthening of process stability and downstream-processing, increasing product shelf-life, improvement of transport logistic and marketing. The most widely commercially available EPN species are *S. carpocapsae, S. feltiae and H. bacteriophora*, but also *S. kushidai*, *S. riobrave*, *S. scapterisci*, *H. indica* and *H. megidis* have been or are used commercially.

2.3.7. Application techniques

Application technology for field distribution of EPNs depends on cropping system and target pests. Virtually they can be applied by any agronomic or horticultural ground equipment keeping in mind agitation, nozzle type, pressure and temperature. Nematode density is about 1.05 g/cm^3 and they will settle in a tank without agitation. Nozzles should be at least of 500 μm diameter and sieves should be removed. Some species of EPN can stand pressure of up to 20 bars, but generally it should not exceed 10 bars. Temperature of water in tank should never exceed 30 °C, bearing in mind that pumping will heat water and sunshine can further heat the tank mix. EPNs are usually applied to soil at a rate of 2.5 x10^9 DJ/ha or higher (Shapiro-Ilan *et al.,* 2012). Soil biotic agents can have positive, neutral or negative effect on EPN applied to soil (Kaya, 2002). Interaction with other entomopathogens can be synergistic, like with *Bacillus thuringiensis* (Koppenhöfer and Kaya, 1997) and *Metarhizium anisopliae* (Ansari *et. al.,* 2004), but also antagonistic like with *Beauveria bassiana* (Brinkman and Gardner, 2000).

Since nematodes are aqueous animals they will be more efficient in moist soils. However, optimal soil moisture is species and soil type dependent (Koppenhöfer *et. al.,* 1995). Similar to interaction with other biocontrol agents, interaction with pesticides depend on nematode species, specific chemical, dosage, time of application, however, in most cases no inhibition of EPN activity was recorded (Koppenhöfer and Grewal, 2005).

2.3.8. Safety and regulation

A comprehensive overview on safety and regulation of EPN was published by Ehlers (2005). Entomopatogenic nematodes are exceptionally safe for users and the environment. Since their first release as biocontrol agents in 1935 to control *Popillia japonica* there is no report of any negative effect on the environment. They can hardly have long-lasting, negative impacts on non-target organisms since they do not persist for long time on the soil surface and usually disappear when host insects are absent. Risk in application of exotic species can hardly cause extinction of local insect populations but rather result in temporary reductions (Bathon, 1996). EPN do not have negative impacts on bacterivorous, fungivorous and omnivorous nematodes. However, they reduce

abundance and number of genera of plant-parasitic nematodes (Somasekhar, 2002). There are no reports of negative effects of associated bacteria on humans or mammals.

In most cases in approval procedure, EPN are covered within the group of macro-organisms together with beneficial arthropods and thus in many cases are exempted from registration. The European and Mediterranean Plant Protection Organization (EPPO) published a document (List of biological control agents widely used in the EPPO region - PM 6/3) in which they propose that national plant health authorities should only list macroorganism species and dispense them from authorization, or simplify the notification procedures as proposed in EPPO Standards PM 6/1 (First import of exotic biological control agents for research under contained conditions) and PM 6/2 (Import and release of non-indigenous biological control agents) (EPPO, 2016). The document contains a list of seven species of nematodes: *H. bacteriophora, H. megidis, S. carpocapsae, S. feltiae, S. glaseri, S. kraussei* and *P. hermaprodita*.

2.3.9. Examples of successful applications of EPN

Field application of EPN against insect pests has been extensively reviewed in literature (Arthurs *et al.,* 2004; Grewal *et al.,* 2005; Lacey and Georgis, 2012; Lacey *et al.,* 2015). Entomopathogenic nematodes showed high levels of efficacy against numerous tested insect pests. Most of the pests are soil dwelling organisms and present potentially good targets since EPN are from soil environment as well. Good efficacy was demonstrated against soil dwelling pests such as the Large Pine Weevil, *Hylobius abietis* L. (Williams *et al.,* 2013), Oriental Fruit Moth, *Grapholita molesta*, (Riga *et al.,* 2006), Small Hive Beetle, *Aethina tumida* (Shapiro-Ilan *et al.,* 2010), Western Corn Rootworm, *Diabrotica virgifera virgifera* (Toepfer *et al.,* 2008). Some successful examples against pests that are in cryptic habitats, like tree borers: the Mediterranean Flat-headed Root Borer *Capnodis tenebrionis* (Garcia del Pino and Morton, 2005) and the Peachtree Borer *Synanthedon exitiosa* (Shapiro-Ilan, 2009) are reported in the literature. In greenhouses, significant progress has been made against different pests including control of the Sweet Potato Whitefly *Bemisia tabaci*, (Cuthbertson *et al.,* 2007), the Diamond Back Moth *Plutella xylostella* (Schroer and Ehlers, 2005), Tomato Leaf Miner *Tuta absoluta* (Batalla-Carera *et al.* 2010), Western Flower Trips *Frankliniela occidentalis* (Premachandra *et al.,* 2003) and different fungus gnats (Diptera: Sciaridae) (Tomalak *et al.,* 2005). Above ground

pests in cryptic habitats are also targeted by EPN, like the Red Palm Weevil, *Rhynchophorus ferrugineus* (Llàcer *et al.,* 2009) or the Codling Moth, *Cydia pomonella* (Lacey *et al.,* 2010).

2.4. Plum Sawflies

2.4.1. Life cycle and distribution

In Southeast Europe two species of Plum Sawflies *Hoplocampa minuta* L. (Black Plum Sawfly) and *Hoplocampa flava* H. (Yellow Plum Sawfly) are the main pests of European plums (*Prunus domestica* L.). They belong to the order Hymenoptera and the family Tenthredinidae. Their only known hosts are European plums and Myrobalan plums (*Prunus cerasifer*a). Moreover, in Nordic countries, *Hoplocampa fulviconius* is dominant (Bovien and Stapel, 1940). Closely related species are *Hoplocampa testudinea* and *Hoplocama brevis,* which are important pests of apple and pear, respectively. The two species of plum sawflies are univoltin insects spending most of the time in the soil as diapausing larvae and prepupae. They have a common life cycle. Adults emerge in time of flowering of early plum cultivars. The females lay eggs into the calyxes of blossoms and larvae hatch when fruits begin to develop. During the course of feeding the larvae leave damaged fruit and burrow into intact one. As a result, one larva can destroy 3 to 6 fruitlets. Damaged fruitlets fall off. The insects overwinter as prepupae within a cocoon in the soil at depth of 5-20 cm. As in poikilothermic animals, developmental stages of plum sawflies are in relation to enviromental temperature. Graf *et al*. (1996a) in laboratory experiment found that the developmental threshold for post-diapause development of Apple Sawly was 4.0, 4.1 and 5.0 °C for coocons buried in vermiculite, potting compost and exposed cooccons, respectively. They found that post-diapausing development requires a thermal constant of 209±24 °C temperature sum at days with temperature above 4.5 °C. These data might be important for forcasting nematode application against adult stages.

2.4.2. Importance and management of Plum Sawflies

Plum sawflies are serious pests in plum orchards in Europe. Damage in organic production and non-treated orchards can be in the range of 36-96% (Bovien and Stapel,

1940; Caruso and Cera, 2004; Oroian, 2009; Andreev and Kutinkova, 2010; Rozpara, 2010). Plum Sawflies management is based on broad spectrum insecticides of the group of pyrethroides and neonicotinoides in countries where these products are still registered. Application of insecticides is in time of petal fall, but some growers apply already at the beginning of the blossom. The only biocontrol agent that was extensively studied against Apple Sawfly was the parasitoid wasp *Lathrolestes ensator*, but with limited results (Zijp and Blommers, 2002). Plant extract of *Quassia amara* can significantly reduce numbers of infested fruit by Apple Sawfly (Neupane, 2012).

2.5. Plum production

Plums are a diverse group of species of stone fruits that belong to the genus *Prunus*. Among more than a dozen described species only two are of worldwide commercial significance, the hexaploid European plum (*Prunus domestica*) and the diploid Japanese plum (*P. salicina* and hybrids). In Europe, the European plum is the most widely cultivated species. Plums are mostly consumed fresh, but large volumes of plums are used through Eastern and Central Europe for distillation and production of fruit brandy called „šljivovica-sliwovitz“, both comercially and homemade. The name derived from the Slavic word for plum „šljiva". Cultivars with high sugar content are also dried without removal of stone and called prunes. Although plums are on second place after apples by area among pome und stone fruits, they are on fourth place in total production (Table 2.2.) due to lower yield. World leading country is China producing more than half of the world plum production (Table 3.). However, two countries of South East Europe are on second and third place, Romania and Serbia, while Bosnia and Herzegovina depending on the year is in the top 10 or 20 countries. In Bosnia and Herzegovina plum production has intensified after a decline at the end of 20th century. The main reason for decline was sensitivity of the main cultivar Požegača to plum pox virus (PPV). In the last two decades new cultivars, tolerant to PPV, were introduced and high density plantation was implemented (Mićić, *et al.,* 2015).

Table 2.2. Pome and stone fruit crops, worldwide production 2014 (FAOSTAT).

Crop	Harvested area (ha)	Production Metric t x 1000	Yield Metric t/ha
Apple	5,5051,851	84,630	16.7
Plums	2,521,100	11,280	4.5
Pear	1,574,446	25,799	16.4
Peach	1,494,837	22,795	15.2
Apricot	508,974	3,365	6.6
Sweet cherry	440,228	2,245	5.1
Sour cherry	207,323	1,362	6.6

Table 2.3. Countries producing more than 100,000 t plums per year (FAOSTAT data 2017).

	Country	Production Metric t			Average Metric t
		2012	2013	2014	
1.	China	5,942,918	6,092,277	6,241,635	6,092,277
2.	Romania	424,068	512,459	495,287	477,271
3.	Serbia	297,446	568,840	401,452	422,579
4.	Iran	297,700	314,500	328,944	313,715
5.	Chile	315,172	313,994	296,439	308,535
6.	Turkey	297,026	305,393	265,490	289,303
7.	USA	266,000	193,800	231,800	230,533
8.	India	215,000	220,000	225,000	220,000
9.	Spain	210,700	172,400	232,765	205,288
10.	Italy	172,247	210,398	214,880	199,175
11.	France	198,970	170,960	194,000	187,977
12.	Argentina	161,942	166,345	171,232	166,506
13.	Ukraine	147,200	183,550	163,180	164,643
14.	Russian Federation	130,000	142,000	140,000	137,333
15.	B&H	111,005	226,898	74,075	137,326
16.	Algeria	105,490	128,786	107,191	113,822
17.	Uzbekistan	93,000	100,000	120,000	104,333
18.	Poland	102,498	102,402	106,057	103,652
	World	10,714,641	11,435,270	11,282,527	11,144,146

2.6. Thesis objective

2.6.1. Background

Until now, there is no biological control aproach that can be used by farmers as an alternative for broad spectrum insecticides in Plum Aawflies management. EPNs efficacy was evaluated through several studies on closely related species, Apple and Pear sawfly (Vicent and Belair, 1992; Zijp and Blommers, 1993; Curto *et al.,* 2007). However, only the larvae were targeted by foliar and ground application with limited success.

Biology of Plum Sawflies was mostly studied with the aim of scheduling pesticide applications, which is mainly in time of petal fall (Tamošiunas *et al.,* 2014). Insect development, as poikilothermic animals, is temperature dependent based on temperature and time, thus development of certain stages can be predicted. A model for prediction of adult emergence and hatching was developed for the Apple Sawfly (Graf *et al.,* 1996a; Graf *et al.,* 2002).

Surveys on the presence of EPN were done in many parts of the world with different aims, for screening for new species or strains with wider host range, better desiccation tolerance and temperature adaptation, extended longevity, or requirements for presence of indigenous species prior registration. These efforts contributed to broaden our knowledge of this group of animals. In the meantime, classical morphology-based identification, due to recent advances in molecular biology, is complemented with molecular tools. Even molecular based techniques are easier and allow less trained personnel, they should be used in combination with classical taxonomy tools.

2.6.2. The aim of the research

The aim of this research was to evaluate the biocontrol potential of entomopathogenic nematodes (EPN) against the key pests of plums, the Yellow Plum Sawfly (*Hoplocampa flava* L.) and the Black Plum Sawfly (*Hoplocampa minuta* Christ).

In order to reach this aim specific objectives were:

1. To screen Bosnia and Herzegovina for presence of EPN to eneble their registration as biocontrol agents.
2. To select the most suitable developmental stage of Plum Sawflies suitable for control with EPN and the most effective EPN species.
3. To develope temeperature driven model of the pest development forcasting to determinate time of EPN application.

2.5.3. Working hypothesis

The working hypothesis is:

- There are natural populations of EPN in B&H.
- Larval and adult stages of Plum Sawflies are susceptible to EPN.
- Precise application of EPN can be determined by a temperature-dependent forecasting model of the completition of Plum Sawflies stage that preceeds the target stage.

Chapter 3

Distribution of entomopathogenic nematodes in Bosnia and Herzegovina

3.1. Introduction

Among the numerous beneficial organisms that can be considered as biocontrol agents are nematodes. Many nematodes are associated with insects but entomopathogenic nematodes from families Steinernematidae and Heterorhabditidae are receiving the most attention as control agents of insect pests. These two nematode families belonging to the order Rhabditida are not closely related since they evolved insect associated parasitism independently (Blaxter *et al.,* 1998). They are obligate and lethal pathogens of insects in nature (Koppenhöfer, 2007) that kill their hosts in association with their symbiotic bacteria. Steinernematidae are associated with bacteria from the genus *Xenorhabdus* and Heterorhabditidae with *Photorhabdus*. The third stage infective juvenile, called dauer, the only free living stage, enters a host through natural openings (Griffin *et al*., 2005) or occasionally through the cuticle (Bedding and Molyneux, 1982) and release the bacteria into the haemocoel. The nematodes and bacteria collaborate to counteract insect immunity that results in insect's death usually within two days (Ciche *et al.,* 2006). After insect's death, bacteria proliferate and become the major source of food for the nematodes. The nematodes complete several life cycles, depending on the food supply, and leave the cadaver as dauer stages in search for other hosts (Griffin *et al.,* 2005).

The first entomopathogenic nematode was described by Steiner as *Aplectana kraussei* (now *Steinernema kraussei*) in 1923. Due to the successful use of chemicals for insect control further research of this subject was neglected until the 80-ties of the last century when mass production of EPN in solid and liquid cultures started to increase. With a growing market demand for nematodes as biocontrol agents the increase of research in this subject also augmented. Collection of indigenous EPN may provide isolates that are more suitable for inundative release and some countries restrict the import of exotic strains or species, although no major risks related to the use of EPN have been identified (Ehlers, 2003). The presence of EPN has been reported from all continents except Antarctica (Hunt, 2007). By the begining of 1990s only 9 steinernematide and 2 heterorhabditid species had been described (Hunt, 2007). Development of molecular techniques facilitated rapid increase of new species. Puža *et al.* (2016) reported 92 valid steinernematid and 18 heterorhabditid species.

Although many surveys have been conducted in Europe, the knowledge of EPN geographical distribution in the region of former Yugoslavia remains obscure. There are no reports on the presence of EPN from Bosnia and Herzegovina. The only neighboring country where EPN were reported is Serbia, from where *S. bicornutum* was described (Tallosi *et al.,* 1995) and there is a report on the presence of four steinernematids and one heterorhabditid in Slovenia (Laznik and Trdan, 2012).

3.2. Materials and methods

3.2.1. Sample collection

Soil samples were colected in autumn 2012 and 2014 from all territory of Bosnia and Herzegovina. In total, 221 soil samples were collected and the presence of EPN was evaluated. The sampling sites were within 100 m along passable roads and were selected based on accessibility and habitat. If different vegetation prevailed in close vicinity additional samples were taken. The distance between sampling sites was at least 10 km. Each soil sample was composed of approximately 1000 ml of soil collected randomly in 5 sub samples taken from corners and the center of an imagined one square meter at a depth of 15-20 cm. All subsamples were mixed together and placed in a polyethylene bag to avoid dehydration and transported to the laboratory. At each site, vegetation, GPS coordinates and elevation were recorded. The sampling was performed once per site.

3.2.2. Isolation of nematodes

The soil samples were processed after their arrival to the laboratory. Entire soil samples were thoroughly mixed and 250 ml subsamples were placed in a 400 ml plastic container. Ten last instar larvae of Greater Wax Moth *Galleria mellonella* (L.) were placed on the soil and the containers were inverted (Bedding and Akhurst, 1975). The containers were held at room temperature (20-25°C) for 15 days. Dead larvae were collected at 3-days intervals and placed on White traps (White, 1929). Recovered nematodes were propagated *in vivo* in *G. mellonella* and stored at 6°C. At regular intervals populations were passed through *G. mellonella* at 6 months intervals.

3.2.3. Identification of nematodes

To confirm identification of isolated nematodes, molecular identification was performed. DNA was extracted from 20 dauers, which were handpicked and transferred in 10 µl of distilled water on a glass slide. Each nematode was cut with a sterile scalpel into 2 or 3 pieces under a dissecting microscope. All pieces were transferred to one 1.5 ml Eppendorf tube and total DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany) following the protocol supplied by the manufacturer. Extracted DNA was stored at -20°C. Nucleic acids were not quantified prior to PCR amplification.

3.2.4. Amplification of internal transcribed spacer regions

The universal primers TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) described in Joyce *et al.* (1994) were used in the PCR reaction for amplification of the ITS region. PCR amplification was conducted in 50 µl reactions using the standard Taq DNA polymerase mixture (Sigma-Aldrich). The amplification profile was carried out using Applied Biosystems 2720 thermocycler, which was preheated to 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, and then 72°C for 8 min.

3.2.4.5. Electrophoresis

After DNA amplification, 5 µl of PCR product was mixed with 2 µl of 5x loading dye (Qiagen) and loaded on 1% agarose gel for checking the quality of isolated DNA. Ethidium bromide (2 µl) was added to 80 ml of gel for visualizing. The electrophoresis was performed at 100 mA and 100V for 45 min. Presence and size of amplified products was determined by comparison of 1kb DNA molecular ladder (Fermentas) on a transilluminator.

3.2.4.5. Sequencing and sequence analysis

Amplified DNA was sent to Macrogen (The Netherlands) for sequencing. Obtained sequences were visualized by Chromas software and edited manually by using Bio-edit software. The forward and reverse sequences were compiled into one contig sequence per sample. Obtained DNA sequences were compared with sequences from GenBank by

means of a Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI).

3.2.4.6. Phylogenetic analysis

Obtained sequences were aligned together with homologous sequences retrieved from GenBank using the default parameters of muscle alignment tools of SeaView (Gouy *et al*., 2010). A maximum likelihood analysis was performed using Mr Bayes 3.2.6. (Miller *et al*., 2010). *Oscheius* sp. obtained from this study and *Caenorhabditis elegans* were selected as outgoups.

3.3. Results

Entomopathogenic nematodes were isolated from 8 (3.6 %) out of 221 soil samples from different regions of Bosnia and Herzegovina (Fig 3.1. and Tab. 3.1.). Coloration of *G. mellonella* cadaver revealed presence of 11 steinernematides and 1 heterorhabditid isolate. One sample yielded 3 isolates and two samples two isolates.

Isolates were identified based on sequencing of the ITS region. PCR products showed a length of ±850 bp in the agarose gel (Fig. 3.1.).

Fig. 3.1. Map of Bosnia and Herzegovina showing distribution of EPN-positive samples.

Table 3.1. Data on sampling sites yielding EPN.

Species	Isolates	Location		Elevation (m)	Vegetation
		N	E		
S. kraussei	E-1	43°50'52"	18°28'03"	666	grassland
S. kraussei	230	44°04'51"	18°31'22"	995	pasture
S. carpocapsae	242.1, 242.2, 242.3	44°43'07"	18°17'54"	335	plum orchard
S. feltiae	226	43°54'21"	18°40'13"	1261	pasture
S. feltiae	202	44°38'15"	17°16'15"	401	shrubs
S. feltiae	02, 02.1	44°43'54"	17°08'45"	298	meadow
S. feltiae	E-17.1, E-17.2	44°39'24"	16°01'24"	297	river bank
H. bacteriophora	E-32	44°49'05"	17°38'27"	157	alfa-alfa (next to the river)

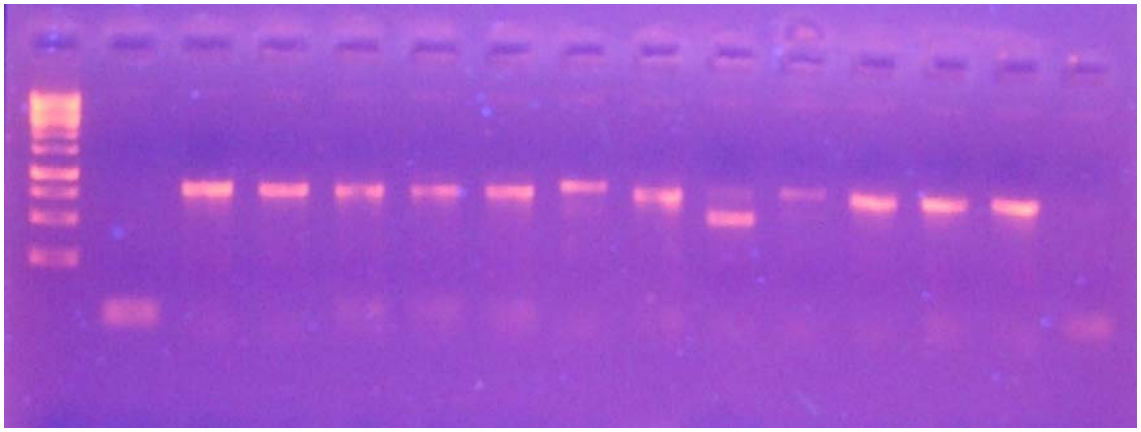

Figure 3.2. PCR products obtained following amplification of EPN isolates.

BLAST search revealed that 12 amplicons matched with sequences of different species of entomopathogenic nematodes from GenBank. Eleven sequences matched with species of the genus *Steinernema* and one with the genus *Heterorhabditis*. Three isolates showed similarity of 98-100% with *S. carpocapsae*, two with *S. kraussei* and six with *S. feltiae.* The heterorhabditid isolate revealed 100% similarity with *H. bacteriophora.*

The most common and widely distributed entomopathogenic nematode was *S. feltiae*. It was found on high elevation point (1261 m), but also in hilly areas in Western and Northern central parts of the country (elevation 300-400 m). Two samples were from grasslands, one from shrubs and one from river banks.

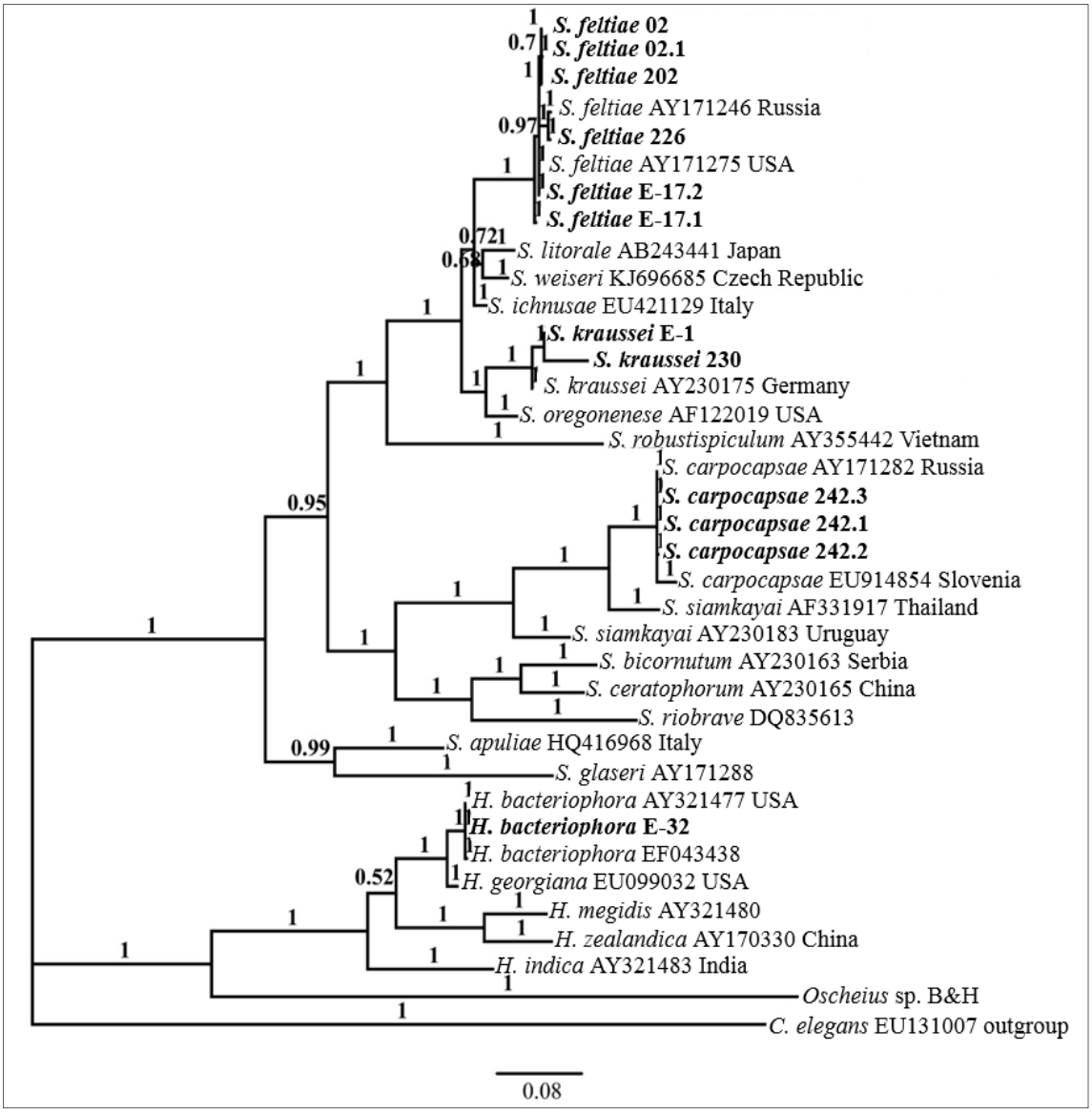

Figure 3.3. Phylogenetic relations of isolates from Bosnia ad Herzegovina (in bold) based on ITS-rDNA sequences as inferred from Maximum Likelihood (ML) analyses.

3.4. Disscusion

The present study represents the first survey on the distribution of entomopathogenic nematodes in Bosnia and Herzegovina. It reveales the presence of four species, three steinernematids and one heterorhabditid.

Eight samples were positive, with a prevalence of 3.6 %, yielding in total 12 isolates. From one sample three isolates (44°43'7"N, 18°17'54"E) and from two samples two isolates (44°39'24"N, 16°1'24"E and 44°43'54"N, 17°08'45") were obtained. Prevalence might vary widely (2-40%) between surveys (Hominick, 2002). More targeted surveys, sites with insect aggregations (Mracek and Becvar, 2000) or selected soil and vegetation types (Griffin et al., 1999), yielded much more isolates. In this survey natural habitats were preferrably targeted. Except for a very limited survey by Talloši *et al.* (1995) that yielded in the description of *S. bicornutum*, there was no surveys on the presence of EPN in neighbouring countries of Bosnia and Herzegovina. Surveys on the presence of EPN in neighbouring countries of former Yugoslavia were done in Italy (Tarasco *et al.,* 2014), Hungary (Toth, 2006) Bulgaria (Gradinarov *et al.,* 2010), Greece (Menti et al., 1997) and also Slovenia (Laznik and Trdan, 2012), with prevalence of 6,6%, 30.8%, 20.7%, 4,6% and 5.4%, respectively.

S. feltiae is common in most parts of the world (Hominick, 2002), and its natural hosts range is among the insect orders Coleoptera, Lepidoptera and Diptera (Peters, 1996). It can be found in different habitats but it prefers sandy soils (Campos-Herrera *et al.,* 2007; Tarasco *et al.,* 2014).

S. kraussei was recovered from two samples from grassland and a scrub habitat in a mountain region. In other studies this nematode was also associated with forests and high altitude where average temperature is below 9°C (Campos-Herrera *et al.,* 2007; Tarasco *et al.,* 2014).

S. carpocapsae was isolated from one orchard with extensive plum production. This species was described from the Czech Republic by Weiser (1955) and it is indigenous for Europe. However, it is not frequent in samples from Central and Northern Europe (Hominick, 2002), presumably preferring more temperate regions. Although entomopathogenic nematodes are often associated with more sandy soils, this species can be present in soils with 30-32% clay content (Campos-Herrera *et al.,* 2007).

The only heterorhabitids isolate belongs to the species *H. bacteriophora.* The sample was taken from the edge of alfa-alfa field next to a small river. Its locality is from the Northeast and at the lovest elevation (157m) compared to other localities positive for EPN. This

nematode species has a cosmopolitan distribution but prefers warmer conditions (Hominick, 2002). Mostly it is found at lower elevations on sandy or sandy loam soils near the sea or rivers (Rosa *et al.,* 2000), but is also associated with other type of soils and vegetations (Tarasco *et al.,* 2014).

Although *S. bicornutum* is present in Serbia (type locality) and distributed in Central Europe (Hominick, 2002), it was not recovered from samples within this study. Moreover, although *S. affine* is after *S. feltiae* the most common steinernematid species in Europe (Hominick, 2002) its presence was not confirmed in this survey.

Entomopathogenic nematodes were recovered mostly from the Central part of Bosnia and Herzegovina from mountains and hills. Habitat and elevation of isolates were in consent with preferences of identifided species reported from various surveys, revealing preference of species for habitats. Entomopathogenic nematodes were not recovered from samples from Northern, Southern and Eastern parts of the country. Certain localities, although possibly attractive for EPN, were not sampled due to pollution with mines from the last civil war. Despite numerous samples were taken from forest habitats, EPN were not isolated. Only a limited number of samples were taken from agricultural fields, but from some of them EPN were isolated, indicating suitable environmental conditions for EPN. Specific climatic and eadphic characters of the positive localities may indicate conditions best suited for certain EPN species and their application in biological control.

Chapter 4

Efficacy of entomopathogenic nematodes against Plum Sawflies (*Hoplocampa minuta* L. and *Hoplocampa flava* H.) under laboratory and field conditions

4.1. Introduction

Plum Sawflies, black (*Hoplocampa minuta* L.) and yellow (*Hoplocampa flava* H.) are univoltin host specific, primary pests of European plums (*Prunus domestica* L.). Adults emerge in time of start of plum blossoming. Larvae attack young fruits which, when damaged, fall to the ground. Management tactics for Plum Sawflies is based on broad spectrum insecticides. Application of the pesticides can have harmful effects on bees and other beneficial insects and mites. The only biocontrol agent that was extensively studied against Apple Sawfly is the parasitoid wasp *Lathrolestes ensator*, but with limited results (Zijp and Blommers, 2002). Plant extract of *Quassia amara* can significantly reduce infestations of fruits caused by the Apple Sawfly (Neupane, 2012). Synthetic chemical insecticides cannot be used to control sawflies in organic production.

Incorporating effective biological control agents in management of Plum Sawflies might lead to reduction of pesticides use. The use of entomopathogenic nematodes (EPNs) could offer an interesting alternative to chemical control of sawflies (Vicent and Belair, 1992). Beside research advances, their rapid expansion was supported also by exemption or ease of registration based on reports of no effect on humans, mammals and plants or the environment (Ehlers, 2005). Their biocontrol success is based on a unique partnership of the host seeking nematode and a lethal insect-pathogenic bacterium carried in the nematode´s intestine, presumed to have arisen through convergent evolution (Poinar, 1993). The infective third juvenile stages (infective juvenile or dauer juvenile DJ) survive outside the insect and enter the insect host through any opening (mouth, anus, spiracles). The death of the insect is due to nematode activity together with the Gram-negative symbiotic bacteria, which are carried within the gut of the DJs and are released after host invasion.

Entomopathogenic nematodes are highly effective against numerous tested insect pests. Most of the target pests are soil dwelling organisms since entomopathogenic nematodes live in soil environment too. Good efficacy was demonstrated against soil dwelling pests such as the Large Pine Weevil, *Hylobius abietis* L. (Williams *et al.,* 2013), Oriental Fruit Moth, *Grapholita molesta*, (Riga *et al.,* 2006), Small Hive Beetle, *Aethina tumida* (Shapiro-Ilan *et al.,* 2010), Western Corn Rootworm, *Diabrotica virgifera virgifera* (Toepfer *et al.,* 2008). Some successful examples are against pests that are in cryptic

habitats like tree borers, like the Mediterranean Flat-headed Rootborer *Capnodis tenebrionis* (Garcia del Pino and Morton, 2005) and Peachtree Borer *Synanthedon exitiosa* (Shapiro-Ilan, 2009).

In the present study the potential of three entomopathogenic nematode species, *Steinernema feltiae, Steinernema carpocapsae* and *Heterorhabditis bacteriophora* was tested under laboratory and field conditions for biological control of different life stages of Plum Sawflies.

4.2. Material and methods

4.2.1. Laboratory assay

4.2.1.1. Source of sawfly larvae and cocoons

Plum Sawflies cannot be cultured on artificial media. Therefore, last instar larvae were sampled from a naturally infested plum orchard in Banja Luka, Bosnia and Herzegovina. Insect-proof nets were placed below plum tries at the beginning of May, before first fruitlets infested by sawflies started to fall to the ground. Infested fruits were collected daily and placed in a bucket with sterilized silver sand to allow larvae to exit fruits (Fig. 4.1.). Larvae at this age are fifth instar larvae and for nematode susceptibility assays larvae were used within 24 hours. The remaining larvae were left in moist silver sand to let them produce cocoons.

Figure 4.1. Fruits infested with Plum Sawflies larvae, collected for laboratory assay from naturally infested orchard.

4.2.1.2. EPN

The three EPN strains, *S. feltiae, S. carpocapsae* and *H. bacteriophora*, commercial strains of the company e-nema GmbH (Raisdorf, Germany) were used for this study. Infective juveniles were produced at 24°C on last instar larvae of the Great Wax Moth *Galleria mellonella* L. according to Woodring and Kaya (1988). Nematode strains did not exceed three times passages through the insect before used in the experiments. They were used within two weeks after the harvest. They were stored at 5°C, and left at 20°C one day before application.

4.2.1.3. Larval susceptibility in Petri dishes assay

Four ages of fifth instar larvae were tested, larvae immediately after they exited infested fruit before making the cocoon and cocooned larvae 10, 20 and 40 days after starting

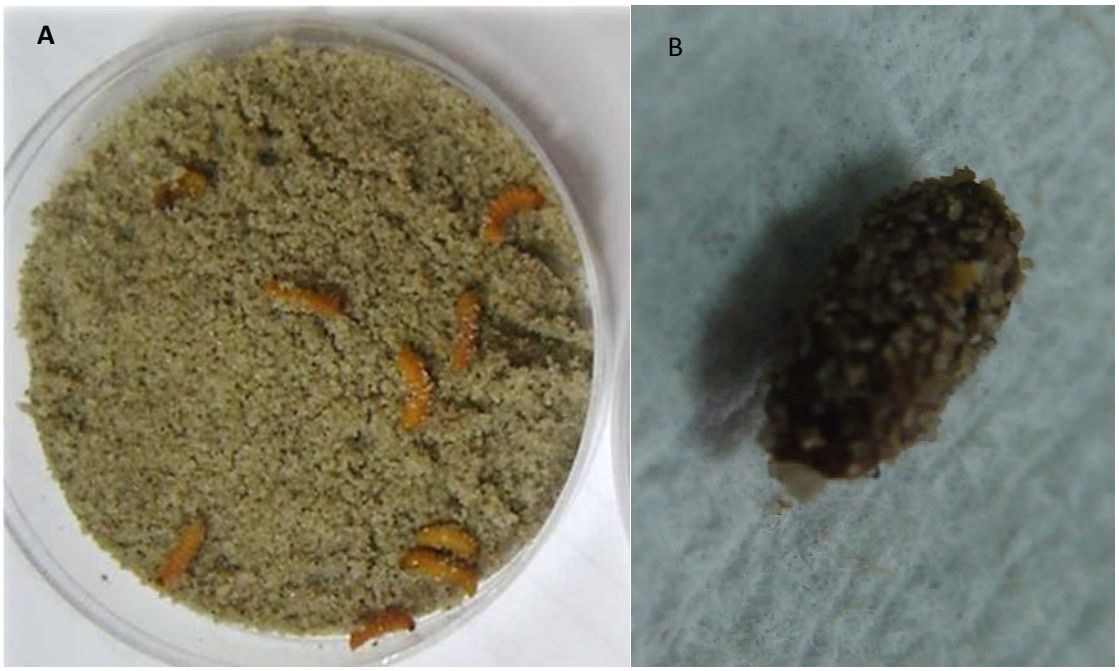

Figure 4.2. Petri dish with Plum Sawflies larvae seven days after treatment with EPN (A) and cocooned Plum Sawflies (B).

of constructions of cocoons. Additional assays were done with mechanically opened 50 days old cocoons. The assay was conducted in 5.5 cm Petri dishes. The Petri dishes were filed with 10 g of 180-500 µm sterilized silver sand moistened to 10 % by weight with distillated water. In each dish, 10 larvae or 10 cocoons were placed. Three dosages of nematodes were used, 500, 1000 and 2000 DJ per dish delivered within 300 µl of water, which dosage correspond to 21, 42 and 84 DJ per cm^{-2}. The untreated control was treated with water only. Petri dishes were sealed with Parafilm to avoid evaporation and maintained in dark at 20°C. Larval mortality was determined after 7 days (Fig. 4.2.). The larvae were dissected to confirm mortality caused by the nematodes. Each treatment contained 5 replicates. Using identical experimental parameters, the experiment was repeated once.

4.2.1.4. Larval susceptibility in foliar application assay

Branches from plum trees, each with at least 30 fruits infested with Plum Sawflies, were brought to the laboratory to test foliar application of entomopathogenic nematodes. The fruit size was up to 1 cm.The nematodes were applied on branches at concentration of 2,500 DJs ml-1 with back pack sprayer. The suspension was added until it started to drip. The branches were placed in jars with water to avoid their wilting and kept at room temperature (Fig. 4.3.). After 48 hours fruits were opened and larval moratlity was assesed.

Figure 4.3. Plum branches with fruits infested with Plum Sawflies larvae. The branches were treated with 2.500 DJs ml^{-1} of commercial products of *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora*. Larval mortality was assessed 48 hours following EPN application.

4.2.2. Larval susceptibility in open field trials

The orchard

A 10 years old orchard of 1 ha size planted with 3 varieties, Čačanska lepotica, Čačanska rodna and Stanley, situated 25 km East from Banja Luka, Bosnia and Herzegovina, was

selected for a field test to evaluate EPN efficacy against Plum Sawflies. The trees had been planted at a distance of 4 x 2 m.

EPN

The three nematode species supplied by e-nema GmbH as commercial products and tested earlier in the laboratory assay were also used for the open field trial. The nematodes were stored at 5°C prior to application and used in the original commercial formulation. They were applied at a rate of 0.5 x 10^6 m^{-2} with a sprinkling can before anticipated larval drop in the row space on 26th of April 2014. Application was done in a row space of two meter width under the canopy of three trees at 6 p.m.. The nematodes were suspended in water and applied in a rate of 0.75 l m^{-2}. Additionally, 1.5 l m^{-2} water was applied immediately following nematode application. Control plot recieved the same amount of water without EPN. Application was repeated the next year on the same experimental plots (21st of April 2015). The trial was not repeated. Weather conditions, precipitation and average temperature for both years are presented in Fig.4.4.

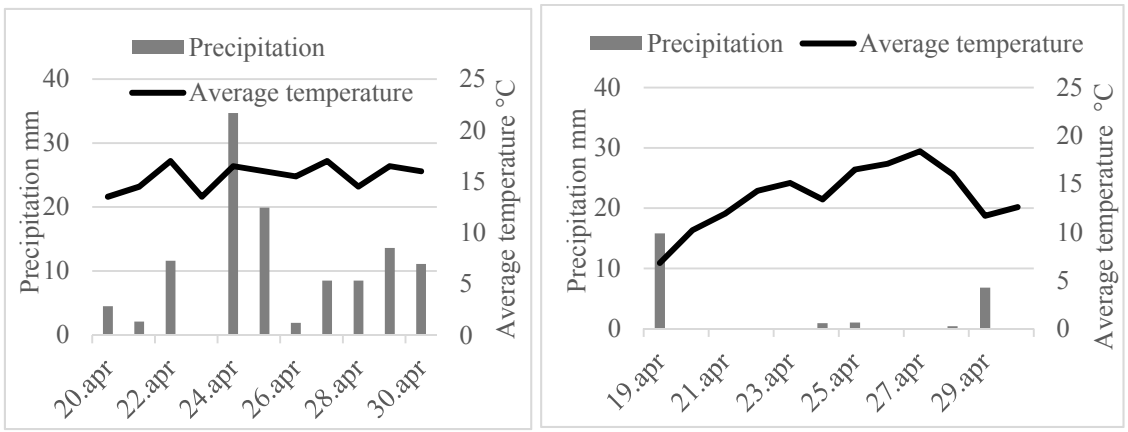

Figure 4.4. Precipitation and average temperature from the meteorological station Banja Luka, in 22 km distance from Crni Vrh, where field trials on larval susceptibility was conducted in 2014 (left) when EPN where applied on the 26th of April, and in 2015 (right) when EPN were applied on the 30th of April.

Assessment of efficacy

To assess efficacy, number of emerging adults was recorded in the following years (2015 and 2016). Before adult emergence was anticipated, each treatment area below the trees

(each 3 trees) was covered by a 6 m long, 2 m wide and 1 m high tunnel of insect-proof net of 1 mm mesh size (Fig. 4.5.). White sticky traps made of white tarpaulin of 22 x 13 cm size were coated with sticky plates and placed under the net to catch adults. At the end of the flight period, the number of adults on the white sticky traps were counted.

Figure 4.5. Insect-proof net placed before anticipated adult emergence (before start of plum blossom) and white sticky trap placed under it. After the end of the flight period (after blossom) number of caught adults on the trap was assessed.

4.2.3. Adults susceptibility in covered plot trials

Insect-proof cages

The trial in 2013 was conducted in a plum orchard planted in 2006 with a distance of 4 x 2 m in Popovac, municipality Čelinac, Bosnia and Herzegovina. The orchard had not been treated with pesticides during the previous 3 years. Significant damage caused by Plum Sawflies was observed during 2012. To isolate treatments from the rest of the orchard in order to avoid migration of flies, cages were constructed. The cage construction was temporary made of iron sticks placed over 12 plum trees to support an insect-proof net. It was divided by net into 4 compartments. Totally it covered a row space of an area of 2 m width and 24 m length. The net was thrips-resistant with a 150 x 490 μm mesh size or

mosquito net 1 x 1 mm and fixed to the ground to prevent adult Plum Sawflies to move outside or inside of the compartments.

Figure 4.6. Insect-proof cages for assessing efficacy of EPN against adult Plum Sawflies. The cages were constructed before anticipated start of adult emergence (just before first white balloons) A: Vijačani, B: Nevesinje, C: Popovac and D: Srebrenik.

In 2014, six cages were constructed at Popovac, Crni Vrh, Vijačani, Nevesinje and two at Srebrenik, all in Bosnia and Herzeovina. In 2015, five cages were constructed at Popovac, Crni Vrh, Srebrenik, Gradiška and Nevesinje.

Nematode application

Commercial products of three nematode species also used before were received from e-nema GmbH, Germany. The nematodes were stored at 5°C prior to application and used in the commercial formulation. They were applied at a rate of 0.5 x 10^6 m^{-2} with a

sprinkling can (Fig. 4.7.) before anticipated adult emergence from the ground. Anticipation was done by monitoring presence of first white baloons on plum trees. White sticky traps were hanged to confirm start of adult emergence. The nematode products were applied with 0.75 l m^{-2} of water. In addition, 1.5 l m^{-2} water was applied immediately after nematode release. Control plots recieved the same amount of water without EPN.

Figure 4.7. EPN application against adult Plum Sawflies inside cages in row space at a rate of 500,000 DJ m^{-2}. The amount of water with suspended nematodes was 0.75 l m^{-2} following 1.5 l m^{-2} water post application.

Assessing nematode efficacy

To assess the nematode efficacy, the number of infested fruits in treatments and control was counted in the same year application. The net was therefore placed under the trees (Fig. 4.8.), at the end of April before start of fruit drops, to catch infested fruits that were falling from the trees. Fruits with damage of Plum Sawflies larvae were counted.

Figure 4.8. Assessing nematode efficacy. A: The net was placed under trees canopy before start of first fruit drop (end of April) to collect fruits. B: Damaged fruits collected from different treatments.

4.2.4. Open field trials

Year 2015

A 20 years old plum orchard of 3 ha in Požega, Croatia with the cultivar Čačanska Rodna was divided into 6 plots each of 0.5 ha, representing 3 treatment plots and 3 control plots. The orchard was in first year of transition from conventional to organic production. *S. feltiae,* the commercial product of the company E-Nema GmbH (Raisdorf, Germany), was applied with a tractor blast sprayer at the rate of 0.5 million nematodes m^{-2} in the rows with 0.2 l m^{-2} water. Additionally, 0.2 l m^{-2} water was applied within 30 min following nematode application. Sawflies damage was assessed by flower inspection for the presence of deposited eggs. In each plot, 100 flowers per 10 trees were inspected at the stage of petal fall.

Year 2016

In 2016, in three plum orchards cultivars Čačanska Rodna, Čačanska Lepotica and Stanley in Vojnić, Croatia and one plum orchard cultivars Čačanska Rodna, Čačanska Lepotica Čačanska Najbolja and Stanley in Babići, Kozara, B&H the same nematode product as in 2015 for open field trial was applied on large scale. In Croatia the orchards were 7 to 8 years old and in organic production systems. The size of the treated area of the three orchards in Croatia was 3.2 ha, 3.6 ha and 2 ha and the size of the non-treated areas that were used as control were 1 ha, 1 ha and 2 ha, respectively. Damage by Plum Sawflies in previous year exceeded 80%. A twelve year old plum orchard in B&H in conventional management system was chosen, where pesticides had been regularly applied, but in 2015 it still suffered damage by Plum Sawflies of 10% due to late pesticides application. The size of the nematode-treated area in this orchard was 1 ha and the non-treated control 1 ha. The nematodes were applied with tractor blast sprayers at full doses of 0.5 and half dose of 0.25 million DJs m^{-2} with 0.2 l of water m^{-2} (Fig. 4.9.). The orchard floor after nematode application with blast sprayer is presented on Fig 4.10. The full dose was applied in the 1st orchard in Croatia and the orchard in B&H, while half the dose was applied in the 2nd and 3rd orchards in Croatia. An additional water application of 0.2 l m^{-2} followed nematode application within 30 min in the orchards in Croatia, while the nematode application in B&H was during rainfall (Fig. 4.11.). Application was done before presence of first white baloons on plum trees; in the orchard with full dose in Croatia on the 16th of March, in first orchard with half dose on the 17th of March, in second orchard with half dose on the 18th of March, and in the orchard in B&H on the 21st of March.

Figure 4.9. Nematode application in 2016 in Croatia, Vojnić against adult Plum Sawflies. A: Application by tractor blast sprayer at a rate of 500,000 DJs m^{-2} with 0.2 l m^{-2} following additional 0.2 l m^{-2} of water within 30 min. B: Application by tractor mounted sprayer at a rate of 250,000 DJs m^{-2} with 0.2 l m^{-2} following additional 0.2 l m^{-2} of water within 30 min. C: Application of tractor blast sprayer at a rate of 250,000 DJs m^{-2} with 0.2 l m^{-2} following additional 0.2 l m^{-2} of water with tractor mounted sprayer.

Figure 4.10. Density of water drops with EPN after application in Vojnić with 0.2 l m^{-2} showing surface coverage of suspension. A: Ground surface with Petri-dish. B: Petri-dish with water drops.

Figure 4.11. Nematode application in Babići, Kozara, B&H. Application was during rainfall (A) before first adults of Plum Sawflies were captured on white sticky trap (B).

At the stage of petal fall, 12-13 trees were inspected for eggs presence (Fig. 4.12.), except for the second orchard, where 6 trees per treatment were checked. About 500 flowers per tree were inspected.

Figure 4.12. Flowers with deposited eggs of Plum Sawflies. A: The egg deposited in the calix (blue arrow), B: The egg deposited in the receptaculum, C: Three eggs deposited on one flower.

4.2.5. Biometrical analysis

Data were compared by fitting General Linear Models with subsequent post-hoc tests in cases of observed statistically significant difference. The observed difference was considered statistically significant in cases of $p<0.05$.

4.3. Results

4.3.1. Larval susceptibility in Petri dishes assay

All three nematode species caused mortality of larvae exposed to DJ before forming a cocoon (Fig. 4.13.). Mortality of the larvae at a concentration of 2000 nematodes per 10 larvae was 100% for all nematode species. When 1000 DJs were applied, the mortality reached 99% with *S. carpocapsae* and 98.9% and 98.0% with *H. bacteriophora* and *S. feltiae*, respectively. Five hundred DJs caused the highest mortality again with *S. carpocapsae* (96%), while *H. bacteriophora* and *S. feltiae* caused 95% and 92% mortality, respectively. The overall mortality in the control was 12±7.5%. These larvae in the control died because they were not able to make the cocoon. Less than one third of the larvae managed to produce a cocoon, however, within the cocoon the nematodes were also able to infect the insects. Statistical analysis revealed that there was no significant difference of studied factors of dose and species (p=0.5). However,

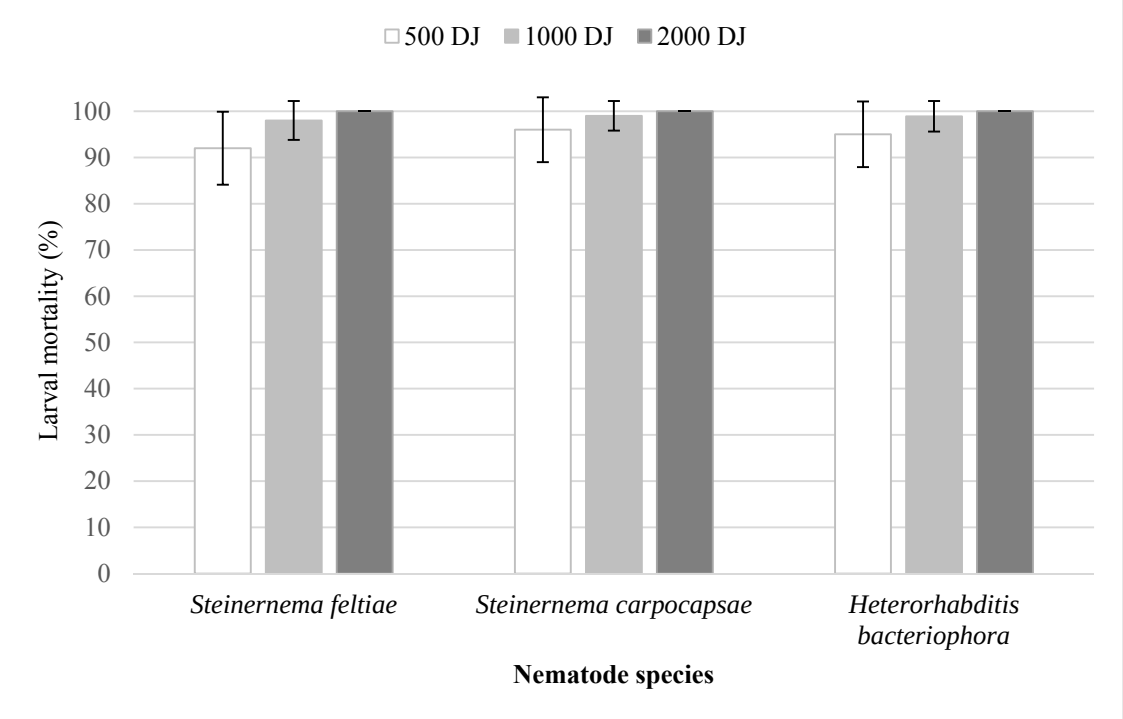

Figure 4.13. Percentage of last instar infected larvae of Plum Sawflies exposed to *Steinernema feltiae, S. carpocapsae* and *Heterorhabditis bacteriophora* under laboratory conditions before making cocoons. Ten larvae were exposed to 500, 1000 and 2000 DJs in 5.5 cm Petri dishes filed with sterile silver sand. Mean of mortality in % ± SD.

there was a statistically significant influence of dose (p=0.049), i.e. higher dose of 1000 nematodes resulted in higher mortality in all studied nematode species compared to 500, while there was no statistically significant impact of the various studied nematode species (p=0.382).

In contrast, to larvae without cocoon, cocooned larvae of 10, 20 and 40 days after starting to produce cocoons were not susceptible to the nematodes. When 50 days old cocoons were mechanically opened and larvae exposed to the nematodes 98% to 100% mortality was observed.

4.3.2. Larval susceptibility in foliar application trial

EPN could not cause mortality of Plum Sawflies larvae within fruits 48 hours after treatments with DJs. Mortality of larvae caused by EPN was not observed.

4.3.3. Efficacy of nematodes in the field trials

The experiments under field conditions revealed that the nematodes could control Plum Sawflies larvae. The control effect after one year was highest with *H. bacteriophora* 84.6%, while the lowest was obtained with *S. feltiae* 38.5 %. After a repeated application in the following year, the highest efficacy was in the plot treated with *H. bacteriophora* (94.1%), while on the two other treated plots an efficacy of 88.2 % was recorded (Fig. 4.14.).

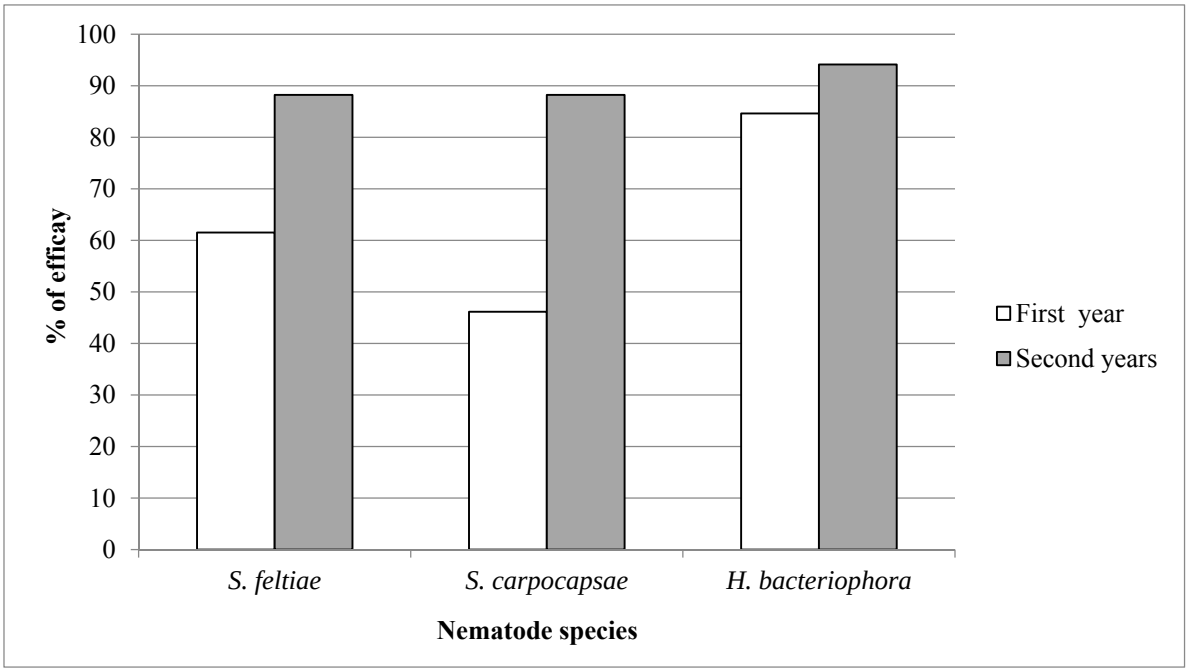

Figure 4.14. Reduction in number of adults of Plum Sawflies caught on white sticky plates on plots treated with *S. feltiae*, *S. carpocapsae* and *H.bacteriophora* at 500,000 DJs m^{-2} compared to untreated control in the assay with larval susceptibility under field conditions conducted in 2014 and 2015.

4.3.3. Open field assays

Trials with cages

Application of all three nematode products/species to control adults before anticipated emergence from the soil in spring resulted in reduction in plum fruit infestation by plum sawflies larvae. However, these results were variable, indicating that some other factors could influence the nematode efficacy (Tab. 4.1).

Table 4.1. Efficacy of *Steinernema feltiae, S. carpocapsae* and *Heterorhabditis bacteriophora* against adult Plum Sawflies in trials with covered cages. Trials were conducted in 2013, 2014 and 2015. DJs were applied prior to anticipated adult emergence at a rate of 0.5 x 10^6 m^{-2} in 0.7 l m^{-2} water following additionally 1.5 l m^{-2}. Efficacy was assessed by counting infested fruits in treatments and untreated control.

Locality	Date of application	Efficacy (%)			Rainfall in mm within 7 days prior application
		S. f.	***S. c.***	***H. b.***	
Popovac	13/04/2013	**97.4**	**94.6**	**89.9**	28
Srebrenik	14/03/2014	**78.6**	**32.9**	**81.6**	5.2
Srebrenik	14/03/2014	No damage in control			5.2
Vijačani	14/03/2014	No damage in control			28
Popovac	13/03/2014	Fruits damaged by *Monilia*			28
Crni Vrh	13/03/2014	Fruits damaged by frost			28
Nevesinje	29/03/2014	Fruits damaged by frost			3.5
Topola	01/04/2015	**100**	**100**	**92.0**	8.4*
Popovac	03/04/2015	**-52.6**	**30.3**	**32.1**	25.2
Crni Vrh	03/04/2015	Fruits damaged by frost			25.2
Srebrenik	31/03/2015	No damage in control			1.8
Nevesinje	26/04/2015	**16.4**	**23.5**	**18.7**	0.0

* During application it was raining

In 2013, in the treatment with *S. feltiae,* 97.4% reduction of fruits infestation was observed compared to the untreated control. *S. carpocapsae* and *H. bacteriophora* reduced damage by 94.6% and 89.9%, respectively. The nematodes were applied 2 days before the first adults were captured on white sticky plates. Nematode application preceded high rain precipitation and followed warm weather during blossom with daily maximum up to 30°C.

In 2014, at the locality Srebrenik, the highest efficacy was recorded with *H. bacteriophora* with 81.6% control, followed by *S. feltiae* with 78.6%. At one locality high humidity caused an infection with *Monilia* sp.of all fruits of the sensitive variety Stanley which lead to a destruction of the fruit immediately after fruit set. At two localities late spring frost burned all flowers. At two other localities there was no damage in the control plots and all treatments.

In 2015, at the locality Topola, application of *S. feltiae* and *S. carpocapsae* caused 100 % efficacy, while *H. bacteriophora* reduced the sawfly population by 92.0%. At the locality Nevesinje, a reduction of 16.4%, 23.5% and 18.7% of the infested fruits in treatments with *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* was recorded compared to control, respectively. At the locality Popovac in the compartment treated with *S. feltiae* there were 52.6% more infested fruits compared to the control, while *S. carpocapsae* and *H. bacteriophora* reduced the infestation by 30.3% and 32.1%, respectively.

Open field application

2015

There was no Plum Sawflies eggs observed on flowers in both, control and treatment plots.

2016

Graphical presentation of infestation levels in treated and non treated plots on Fig. 4.15., with precipitation and mean air temperature around time of application on Fig 4.16..

In the first orchard in Croatia where 500,000 nematodes m^{-2} had been applied, the percentage of flowers with layed eggs was 9.9±2.9%, while in non treated control it was 31.5±6.4%. Reduction of infestation in the treated plot was 67.8 %.

In the second orchard, where 250,000 nematodes m^{-2} had been applied, 7.4±2.3% of the flowers were with deposited eggs on flowers receptaculum and in control 12.7±1.8%. A reduction of infested flowers in the treated plot was 41.7%.

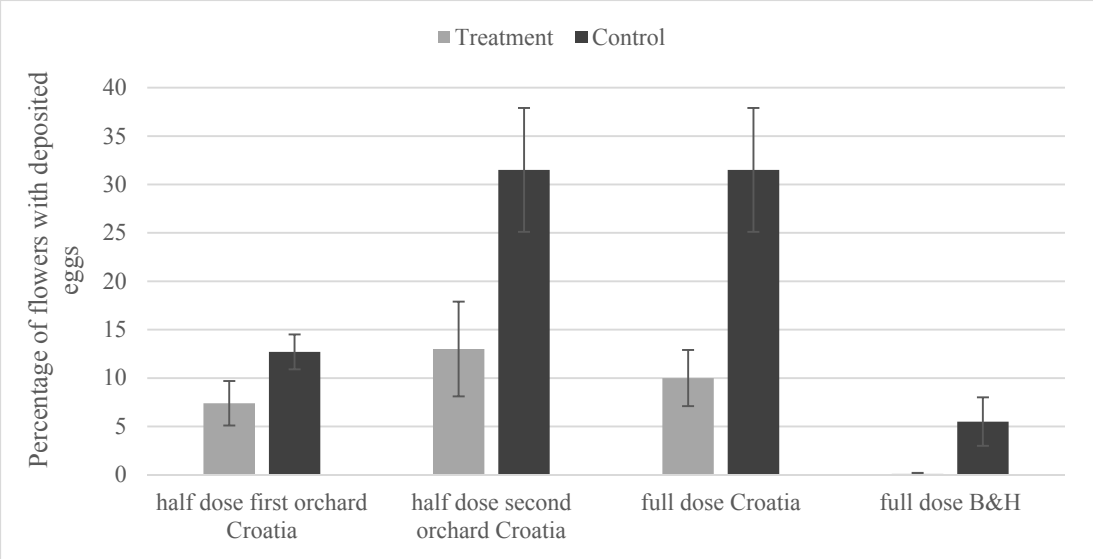

Figure 4.15. Percentage of flowers with deposited eggs in treatments and control. Field trials with application of *Steinernema feltiae* against adult Plum Sawflies were conducted in the orchards in Vojnić, Croatia and Babići, Kozara, B&H. Application in first orchard in Croatia with half dose (250,000 DJ m^{-2}) was on the 17th of March, in second orchard with half dose on the 18th of March, in orchard with full dose on the 16th of March, and in the orchard in B&H on the 21st of March during the rain.

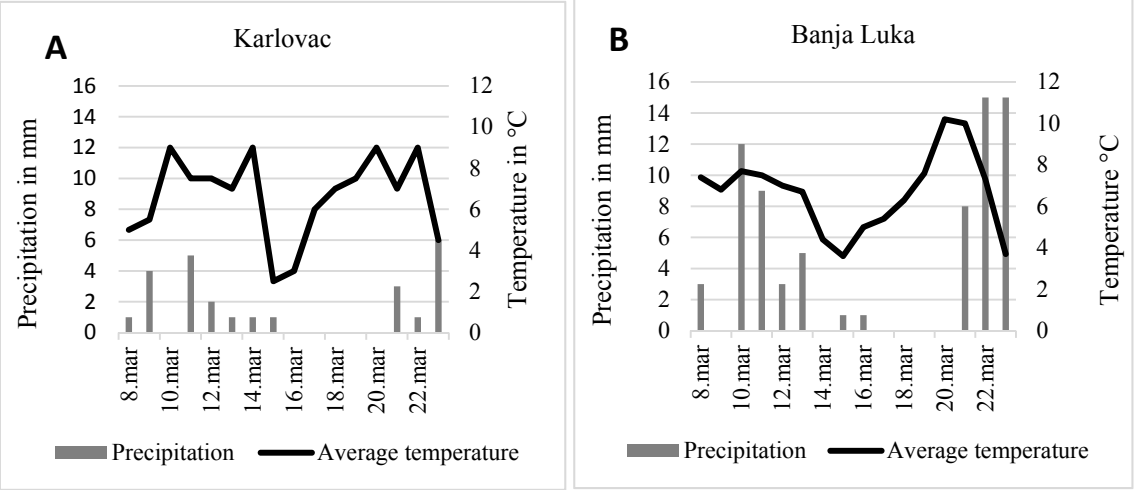

Figure 4.16. A: Precipitation and mean tempertaure for Karlovac, 15-20 km away from orcahrds in Vojnić Croatia, and B: Precipitation and mean tempertaure for Banja Luka, 30 km away from orchard in Babići, Kozara B&H.

In third orchard, where also half the dose of 250,000 nematodes m^{-2} had been applied, 13.0% of flowers were with deposited eggs in treatment and control plot was the same like in first orchard 31.5±6.4%. Therefore, reduction of fruit infestation in the treated plot was 41.2 %.

Difference in percentage of flowers with depsoited sawflies eggs in treatment and control in all three orchards in Croatia was statisticaly higly significant ($p<0.001$).

In the orchard in B&H, where EPN were applied at full dose, there were 0.1±0.1% flowers with deposited eggs recorded, while in the control 5.5±2.5% of the flowers were with eggs, meaning the control effect was 98.2%.

4.4. Discussion

Plum Sawflies spend most of their life cycle in cocoons buried in the soil. Our work demonstrates that at this stage they are not susceptible to EPN. However, at two short time intervals, when the larva enters the soil and when adults emerge after pupation leaving the cocoons, they are highly susceptible. At these stages successful control can be obtained with EPN.

There are only a few studies on the susceptibility of Plum Sawflies to EPN. Tomalak (2006) reported that mature larvae of the Black Plum Sawfly (*Hoplocampa minuta*) are susceptible to *Heterorhabditis megidis* and *S. feltiae,* while after construction of the insect pupal cocoon larvae within it were almost unaffected by the nematodes. It was confirmed in this study since no mortality of larvae was observed when cocoons were treated with EPNs. In laboratory assays Ulu *et al.* (2016) recorded the LD_{90} for *H. bacteriophora, H. marelatus, S, feltiae* and *S. carpocapsae* of 15, 19, 25 and 34 DJs, respectively, per last instar larva of the Yellow Plum Sawfly. Doses applied in our assay (21, 42 and 84 DJs cm^{-2}) are similar to the field application dose (50 DJs cm^{-2}) and larvae were highly susceptible, both, under laboratory and field conditions. Even with a treatment at the lowest number of DJs, larval mortality was more than 90 %. In our trials, a mixture of the two species of Plum Sawflies was recorded, but the Black Plum Sawflies was dominant.

Several studies stated susceptibility of the closely related Apple Sawfly(*H. testudinea*) and Pear Sawfly (*H. brevis*) to EPNs (Vincent and Belair, 1992; Zijp and Blommers, 1993; Curto *et al.*, 2007). *H. bacteriophora, S. feltiae* and *S. carpocapsae* caused 100% mortality after 72 h on directly exposed Apple Sawfly larvae (Vincent and Belair, 1992). In the same study *S. carpocapsae* application at a rate of 40 and 80 DJs cm^{-2} in the following year resulted in reduction of captured adults by 72% and 68%, respectively. Field trials in organic pear orchard carried out by Curto *et al.* (2007) showed that *S. feltiae* soil application reduced the adult Pear Sawfly population in the next year too. Moreover, foliar application in the same trial reduced the number of infested fruits on the same level like rotenone. Foliar application of *S. carpocapsae* after fruit set resulted in significant reduction of secondary damage of apples by Apple Sawfly (Vincent and Belair, 1992). Six foliar applications of *S. carpocapsae* during the adult flight resulted in significant reduction of primary damage in two years but not in the third (Belair *et al.*, 1998). Secondary damage was not reduced. In our study, foliar application of DJs did not result in Plum Sawflies larvae infestation by EPNs. The publication (Vincent and Belair, 1992) does not state time of application but in our trial application was in time when larvae were already inside infested fruits.

There are reports of two parasitoids of Apple Sawfly, *Lathrolestes ensator* and *Aptesis nigrocincta.* Low levels of parasitism with these parasitoids limits their potential for

strategy for Apple Sawfly control (Zijp and Blommers, 2002; Babendreier, 2002). In our study no parasitoids were observed. Beside parasitoids, the entomopathogenic fungus *Paecillomyces farinosus* was reported to cause 40 % mortality of apple sawflies larvae (Graf *et al.,*1996c). In our study cocoons that spent several months in the refrigerator were very often parasitized by fungi. Beside live organisms, several biopesticides of botanical origin were tested against Apple Sawfly. Extracts from plants, like *Quassia amara* and *Azadirachta indica*, provided efficacy of 10.3% to 60% and 0% to 43 %, respectively (Danelski *et al.,* 2014) and in another studies in treatments with *Q. amara* 3.8%, 3.2%, and 2.9% fruitlets were damaged while in the control 9.9 % were infested, but there was no difference in secondary damage (Neupane, 2012).

Organic fruit production is a growing market and new solutions for control of pest insects apart from pesticide applications should be adopted. Several approaches and control agents were tested against Plum Sawflies and closely related Apple and Pear Sawfly, but results were not consistent or low control effects were recorded. This study reveals that by precise timing of the application of EPN against emerging adults approximately two to one week before start of adult emergence and against larvae just before construction of their cocoon can be a successful strategy to control Plum Sawflies.

This is the first report of a successful adult Plum Sawflies control by EPN. There are not many reports of successful adult control of insects by EPN, while larval stages have been frequently reported to be susceptible. We observed a high efficacy of up to 100% with all three entomopathogenic nematode species *S. feltiae, S. carpocapsae* and *H. bacteriophora* in reducing damage on fruits by their application on the orchard floor against emerging adult Plum Sawflies. However, in some of our trials, the control effect was on lower level or was absent. Several factors could have influenced these results. Precise timing of nematode application, way of application and soil moisture are essential for successful Plum Sawflies control. EPN should be applied in row space just before first adult emergency. Application should be with enough water especially in orchards where tree row space is maintained with grass mulching. Priority should be given to *S. feltiae* since it is active at lower temperature which is necessary for application in time of plum blossom. Soil treatment against Plum Sawflies larvae in time before first larval drop to the ground and start of producing the cocoon showed high efficacy as well. However,

priority should be given to application against adults since duration of adult emergence is shorter compared to duration of descent of larvae into the soil, meaning that nematodes should be shorter time available in the soil.

The most important influencing factor for success of the EPN application is soil moisture Weather conditions when we consider soil moisture are more favorable for EPN activity in early spring in time of adult emergency than in time of larval drop from the trees as during the winter enough moisture has usually been provided.

Application against Plum Sawflies adults might not only have the potential to control of Plum Sawflies adults but also provide side effects by targeting overwintering stages of the Plum Fruit Moth (*Grapholita funebrana*). Whether these two pests can be controlled with one EPN application needs further investigation.

Result of this study reveals that EPNs can be effective in controlling adults and larval stages of Plum Sawflies. Proper timing of application is essential for success and therefore studies on the development of target stages of the Plum Sawflies are necessary. Foliar application and application against cocooned larvae did not provide satisfying control results. It can be expected that results obtained in control of Plum Sawflies can be extrapolated to the two closely related species, the Apple Sawfly and Pear Sawfly.

Chapter 5

Temperature-dependent prediction of emergence of the Plum Sawflies (*Hoplocampa flava* and *Hoplocampa minuta*)

5.1. Introduction

Ectotherms, among which are insects, relay on external sources of heat for their development. Because they require certain combinations of temperature and time their development is in the function of given temperature and time during which it acts. There are two values that define this process: the lower developmental threshold (LDT), at which development ceases and the sum of effective temperatures or degree days (DD) a sum of temperatures above the LDT necessary to complete the different developmental stages (Ludwig, 1928). Based on these two values, the development of certain life stages of an insect can be predicted. To define these values it is necessary to obtain data about the minimal thermal requirements for development, which is usually done through experiments. In case we lack these data, thermal requirements of closely related species can possibly be used to estimate those (Jarošik *et al.,* 2011).

Plum Sawflies – Black and Yellow (*Hoplocampa minuta* Christ, 1791 and *H. flava* Linnaeus, 1761) are the main pests of European plum (*Prunus domestica*), (Caruso and Cera, 2004; Oroian *et al.* 2009; Andreev and Kutinkova, 2010; Rozpara *et al.,* 2010; Tamošiunas *et al.,* 2014) but much more studied is the closely related species Apple Sawfly (*Hoplocampa testudinea*). They are univoltine species that belong to the order Hymenoptera, family Tenthredinidae. They hibernate in the soil as prepupa in a cocoon. Adults emerge in spring in time of blossom of early varieties and lay eggs in freshly opened flowers. These eggs hatch after petal fall. A larva of Plum Sawflies hatches from the egg when fruits start their development. They then penetrate fruits and feed on the seed and the flesh around of the seed. The fruits with the larva inside are recognized by entering hole. Damaged fruits drop to the ground. During the course of feeding one larva of the Plum Sawflies can destroy up to 6 fruits what makes these pests extremely destructive. Together with the last infested fruit, the larva of the Plum Sawflies falls to the ground and burrows into the soil and cocoons at a depth of 5 to 20 cm. Then it turns to prepupa and stays in diapause until the next spring, but a portion of the population can prolong diapause to two or even three years. Its life cycle is synchronized with the host tree phenology but they can differ in years (Tamošiunas, 2014).

Figure 5.1. Yellow Plum Sawly (*Hoplocampa minuta* Christ, 1791) on the left and Black Plum Sawfly (*H. flava* Linnaeus,1761) on the right.

Figure 5.2. Plum fruits infested with Plum Sawflies larvae.

Management of Plum Sawflies is based on synthetic insecticides application. Application of insecticides is in time of petal fall what can be harmful for beneficial insects and mites that are present in orchards at that time. Environmental friendly entomopathogenic nematodes have a potential as biological control agents against Plum Sawflies. Entomopathogenic nematodes are highly effective against soil dwelling stages of insects. Against Plum Sawflies they are applied into the soil before adult emergence, since the nematode invade the insect during adult emergence from the soil. For successful control, the correct timing of application is essential. The closer the application towards first adult emergence, the better is the activity of the nematode material and the higher is the chance for successful control. Precise forecasting models for adult emergence are crucial for successful control. There is a report by Tamošiunas *et al.* (2014) from Lithuania about condition that favour Plum Sawflies emergence at the beginning of May. They proposed a temperature sum model to predict first adult emergence and started calculating the degree days (DD) for the temperature sum accumulation on the 1st of April. However, in the Southern regions of Europe first adult catches in some years have already been recorded in March. Prediction of the start of the sawflies flight activity is based on DD (Graf *et al.,* 1996a; Zijp and Blommers, 1997; Tamošiunas *et al.,* 2014).

The aim of this study was to investigate when emergence of the adult stage of the Plum Sawflies can be expected based on LDT, starting day for DD accumulation and temperature sum based on conditions in Northern parts of Bosnia and Herzegovina.

5.2. Material and methods

Plum Sawfly flight was monitored during 2012-2016 in a 0.2 ha orchard situated in Banja Luka, with 45 plum trees of the varieties Čačanska rodna, Čačanska lepotica and Stanley grafted on Myrobalan Plum (*Prunus cerasifera* Ehrh.). Every year before the beginning of the flight, 5 hand-made white sticky traps where placed in the tree canopy at a height of 1.8 m. Sticky traps were made of white tarpaulin of 22 x 13 cm, to which on both sides transparent sticky traps for codling moth trapping were attached. The traps were checked daily for presence of adults.

Daily temperatures were recorded at 2 m above ground, 5 cm and 10 cm underground at an official meteorological station Banja Luka situated 4.5 km from the orchard.

5.3. Results

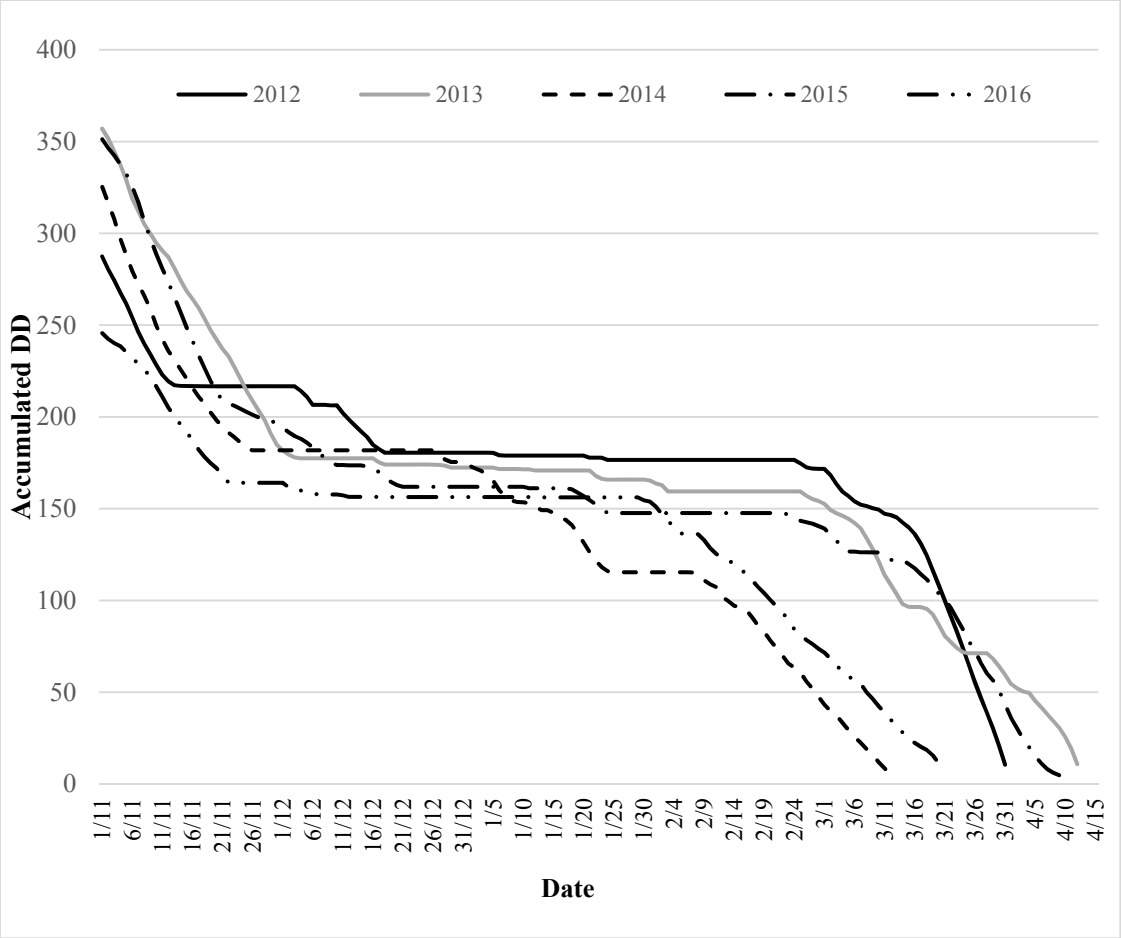

Figure 5.3. Accumulation of sum of the effective temperatures (DD) backward from the date of Plum Sawflies emergence over 5 years (2012-2016) for soil temperature at 10 cm and LDT 4.0°C.

The temperature sums, recorded at 5 cm under bare ground, that were accumulated from the 1st of January until the day of first adult captures in each of 5 experimental years were 169±10 and 145±10 when as LDT were taken 4.0°C and 4.5°C, respectively. When the 15th of January was taken as a starting day for accumulation of degree-days, average temperature sums and standard deviation were 163±12 and 132±13. At later starting days standard deviation increased even more, as for the 1st of February when accumulated DD were 151±23 and 134±21. The temperature sums measured at -10 cm showed a similar tendency of standard deviation and also dependence on the day of start of temperature accumulation (Fig. 5.3.). The lowest standard deviation of accumulated DD for soil was around the 1st of January while for air temperature around the 15th of January (Fig. 5.4.).

The date with lowest standard deviation can be taken as a starting point for accumulation. Difference in DD and days of observed adult emergence and average for 5 years are presented in Tab. 5.1. and Tab. 5.2.

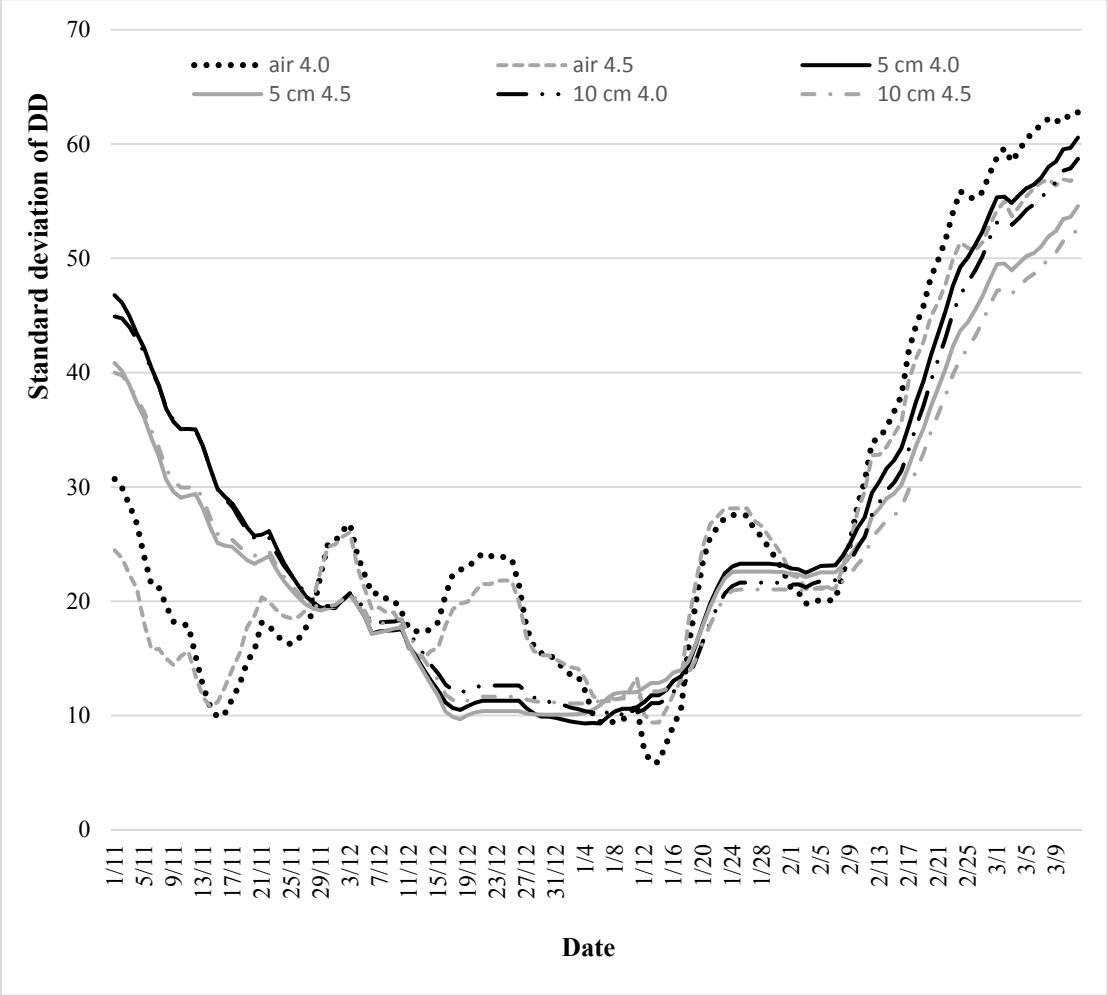

Figure 5.4. Standard deviation of mean of the accumulated temperature (DD) backward from the date of first adult emergence of Plum Sawflies over five years (2012-2016) for temperatures measured at 2 m above ground, 5 cm and 10 cm underground for two lower developmental threshold temperatures (LDT) of 4°C and 4.5°C.

Table 5.1. The difference between beginning of Plum Sawflies emergence temperature sum (DD) in studied years (2012-2016) and mean DD ($\bar{x}$DD) for five years presented as $\bar{x}$DD± DD and that difference presented in days ($\bar{x}$DD ± days) for s**oil** temperature measured at 10 cm for three starting days of accumulation (1st of January, 15th of January and 1st of February) and two thermal thresholds 4.0°C and 4.5°C.

	4.0°C						4.5°C					
	1st of January		15th of January		1st of February		1st of January		15th of January		1st of February	
	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	$\bar{x}$DD ± Days	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	$\bar{x}$DD ± days
2012	10	1	15	2	24	3	16	2	18	2	26	3
2013	2	0	8	0	13	2	2	1	6	1	11	1
2014	8	2	-12	-2	-33	-4	2	0	-12	-2	-31	-4
2015	-5	-1	-1	0	-2	0	-8	-1	-4	-1	-5	-1
2016	-15	-3	-10	-3	-2	0	-12	-2	-8	-1	-1	-1

Table 5.2. The difference between beginning of Plum Sawflies emergence temperature sum (DD) in studied years (2012-2016) and mean DD ($\bar{x}$DD) for five years presented as $\bar{x}$DD ± DD and that difference presented in days ($\bar{x}$DD ± days) for **air** temperature measured at 2 m for three starting days of accumulation (1st of January, 15th of January and 1st of February) and two thermal thresholds 4.0°C and 4.5°C.

	4.0°C						4.5°C					
	1st of January		15th of January		1st of February		1st of January		15th of January		1st of February	
	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	$\bar{x}$DD ± Days	$\bar{x}$DD ± DD	$\bar{x}$DD ± days	$\bar{x}$DD ± DD	x̄DD ± days
2012	0	0	+5	+1	+32	+4	+4	0	+18	+2	+22	+3
2013	-11	+1	+1	0	+14	+1	-10	-1	+6	+1	+15	+2
2014	+20	+8	-10	-2	-32	-4	+15	+7	-12	-2	-34	-4
2015	-16	-2	-5	0	-8	-1	-20	-2	-4	-1	-15	-2
2016	+7	+2	+8	+2	+3	+1	+10	+2	-8	-1	+12	+3

The day of first adult capture was during the period from the 12th of March until the 12th of April, average on day 89.0±12.7 (28th of March) since January 1st.

Postdiapausal development of sawflies occurs in the soil and thus it seems obvious to use the soil temperature to predict adult emergence. However, most weather stations in orchard only measure temperature at 2.0 m above ground. Therefore, temperature sums at 2.0 m were analysed for two LCD 4.0°C and 4.5°C. When the 1st of January as a starting day of DD accumulation was chosen, the average temperature sums were 200±14 and 177±15 for LCD 4.0°C and 4.5°C, respectively. The average DD when accumulation started on the 15th of January were 181±8 and 161±11, while on the 1st of February they were 152±21 and 138±24.

The purpose of the study was not to measure length of sawflies diapause, but it could be seen from the Figure 5.3. that in 2014 it lasted 30 days. In November and December 2013, soil temperature at 5 cm were below the LCD for 30 consecutive days. There were additional 12 and 14 days for the LCD of 4°C and 4.5°C, respectively, during the second part of January below the LCD. But between these two periods below LCD additionally 65 and 53 DD were accumulated for lower and higher LCD, meaning that diapause was already broken.

5.4. Discussison

The date of the first Plum Sawflies catch varied over the years. Since it occurred in a range of 32 days, a fixed date cannot be used as a source for prediction of start of sawflies emergence. Accumulation of degree days, (DD) is used as a useful tool to predict start of sawflies flight (Graf *et al.,* 1996a; Zijp and Blomers, 1997; Tamošiunas *et al.,* 2014; Sjöberg *et al.,* 2015). Empirically, the lowest standard deviation for accumulated DD was when accumulation started on the 1st of January for both soil temperature measurements at 5 cm and 10 cm (Fig 5.2.). There was no difference between the two thermal thresholds of 4.0°C and 4.5°C (Tab 5.1.). Standard deviation increased if the starting day was chosen later. Therefore it is proposed to start accumulation of temperature data on the 1st of January and consider the accumulated DD temperature sum of 169±10 and 145±10 for LCD of 4.0°C and 4.5°C, respectively, when soil temperature is measured at 5 cm for start of Plum Sawflies flight in the Northern part of Bosnia and Herzegovina. In a study

performed in Lithuania (Tamošiunas *et al.,* 2013) it was proposed to take the 1st of April as a staring day for accumulation. However, this study revealed that in conditions of Northern Bosnia and Herzegovina the average date of first adult flight advanced more than a month compared to Lithuanian conditions. Moreover, they calculated 84 DD for 4°C as LCD while in our study the smallest variation was assessed for a DD of 169. Different climatic conditions of Southern parts of Europe require different parameters of prediction of the start of the flight of Plum Sawflies compared to studies in Northern countries.

For farmers it is more suitable to use air temperature, since the majority of them do not have thermometers for soil temperature. The smallest variation of temperatures during the 5 year study was assessed when the starting day was the 15th of January. Farmers could use this date as starting point of accumulation of temperature and can expect that first adults will appear on 181±8 DD and 161±11 DD for LCD of 4.0°C and 4.5°C, respectively. The prophylactic manner of entomopathogenic nematodes application therefore requires application before first adult emergence. To avoid late application in practice, due to deviations from the mean temperature sum, it is suggested to subtract twice the standard deviation (2 x 8 and 2 x 11 DD) from the mean air temperature. Consequently application should be at 165 DD or 138 DD for a LCD 4.0°C or 4.5°C, respectively, measured from the 15th of January.

The length of diapause for Plum and Apple Sawflies seems quite different although they are closely related species. Graph *et al.* (1996b) concluded that diapause of Apple Sawfly came to the end at the begining of March for conditions of Switzerland, but when cocoons were exposed earlier to temperasture above developmental threshold they needed more DD to finish their development. This could explain the smaller variation of DD when calculation started on the 15th of March, since sawflies just finished diapause (Zijp and Blommers, 1997; Tamošiunas and Valiuškaite, 2013). However, our study revealed that the smallest variation in DD for Plum Sawflies was when the 1st of January was chosen as a starting day for temperature accumulation suggesting that the length of diapause is shorter in Plum Sawflies compared to Apple Sawfly. From this study it could be concluded that the diapause of studied population lasted less than 32 days but more than 21. In 2012, 21 days below 4.0°C were not enough to break diapause, but in 2014 after

32 days below the thermal threshold accumulation of DD started. Diapause in Plum Sawflies terminates already by the 1st of January for conditions of North Bosnia and Herzegovina, while for Apple Sawfly it is at March for conditions of Central and Northern Europe. The Apple Sawfly is an important pest only in North of the Alps (Graf *et al.*, 2002), since they need longer time for diapausal development, conditions which are often not achieved in Southern Europe. Apple Sawfly is present only in mountain regions of Southern Europe with a longer period of temperatures below LDT. Due to a shorter diapause length of Plum Sawflies and their ability to fulfil diapause requirements they are key pests of plums in South Europe as well.

Different climatic conditions of Southern Europe compared to Northern and Central Europe require different approaches for calculation of first adult emergence that is crucial for effective entomopathogenic nematode application against adult Plum Sawflies. Farmers can apply the nematodes on 165 DD when air temperature is measured for LDT and starting day of accumulation is the 15th of January. In case that farmers have data of soil temperature at 5 cm, accumulation of temperature should start on the 1st of January and application should be on 149 DD. Due to a shorter diapause length of Plum Sawflies they are important pests all around Europe, while a longer diapause of Apple Sawfly restricts them more to Central and Northern Europe.

Chapter 6
General Discussion

Plum Sawflies are key pests of plums. They are predominantly suppressed by synthetic pesticides. Recent bans of many registered pesticides for sawfly control and the introduction of integrated and organic approaches in production required by consumers and retailers, impose the search for new tactics for the control of these pests. Entomopathogenic nematodes (EPN), natural insect pathogens, have a high efficacy against several soil-dwelling insect pests. Improvements in production and application technologies for EPNs have brought them into the position of being much more competitive with pesticides. They are exceptionally safe for humans and the environment and do not require registration in most countries, why the market is exponentially growing for these nematodes and their bacterial biocontrol symbionts.

Larval susceptibility of Plum Sawflies under laboratory conditions was proven in the studies of Tomalak (2006) and Ulu *et al.* (2016) with mortality of over 90%. However, there are no reports on the susceptibility of other life stages, timing of application and field test results with recommendations for farmers. Moreover, the closely related species of the Apple Sawfly (*Hoplocampa testudinea*) and the Pear Sawfly (*Hoplocampa brevis*) could be controlled in the same or similar way based on the approach proven for Plum Sawfly control. With this background the present study was initiated to evaluate the potential of commercially available strains of EPN for Plum Sawfly control by: 1) laboratory and field tests of different EPN species targeting the most susceptible stage of Plum Sawflies; 2) optimizing timing of field application by monitoring temperature requirements of the target stage of Plum Sawflies and 3) a survey for EPN in Bosnia and Herzegovina to check for the presence of certain species, which is a prerequisite for registration of EPN as a biocontrol agent.

In this study, EPN showed potential to suppress populations of Plum Sawflies. The waste majority of reports targeted larval stage with EPN. In this study, beside larvae adults of Plum Sawflies were suppressed by EPN successfully. Soil stages are vulnerable but only during their migration through the soil. Larva inside cocoons are protected and EPN were not able to penetrate the cocoon. Only migratory stages in the soil were susceptible, larvae during migration through the soil before they enclose in the cocoon and adults during their emergence. The cocoon presents a mechanical barrier, which EPN are unable to penetrate. This structure presents a mechanical barier that protects the pest during its

diapausing stage when it is exposed to soil inhabiting natural enemies and environmental factors (Danks, 2004). Cocooned larvae or pupae would present the most desirable target stage since the complete population could be targeted at once. Since Plum Sawflies spend the majority of the year in the soil inside the cocoon, almost 10 months, it would leave plenty of time for EPN application. However, this stage is not accessible, but migratori stages are. EPN can infect Plum Sawflies in short time during larval migration through soil, but before it encloses itself inside cocoon and during adult emergence.

While under laboratory conditions mortality of larvae of Plum Sawflies caused by *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* did not differ significantly, under field conditions the lowest number of emerging adults after one and two years treatments was in the plot applied with *H. bacteriophora*, although all nematodes had an efficacy above 90% after two years of applications on the same plots. However, the highest efficacy against adults during their emergence from the soil was achieved with *S. feltiae*. Environmental factors such as temperature, moisture, aeration and soil type, but also biotic factors as target insect and soil biota can influence success of EPN application against certain pests (Lacey and Georgis, 2012).

Difference in efficacy of EPN against different life stages of Plum Sawflies might be influenced by both, environmental and biological factors. Since under laboratory conditions all nematodes showed similar efficacy against larvae of Plum Sawflies, one could assume that differences after field application do not lie in the host susceptibility, but in better performance of *H. bacteriophora* at environmental conditions during the trial.

Application of nematodes against the larval stage require their presence before the start of emergence of larvae from the infected fruitlets. This process starts at conditions of Northern Bosnia and Herzegovina at the end of April or beginning of May and lasts for approximately three weeks. During this period the nematode should be able to parasitise larvae that have emigrate from infested fruit, drop to the soil and migrate to deeper soil horizonts for cocooning and later pupation in spring. The larvae usually migrate to soil layers of 0-5 cm and seldomly down to 20 cm. Since the nematodes do not need to search for the target host, but rather can wait and invade it, foraging strategy of cruiser nematodes are not advantagous over ambushers. However, the nematodes should persist up to three

weeks and be able to infest larvae of Plum Sawflies. Soil moisture is the most critical abiotic factor that affects the nematodes (Stuart *et al.,* 2006). Therefore it would be crucial to keep the soil moist at the level close to field capacity, providing optimal conditions for EPN activity. However, targeting adult stage one month to 45 days earlier would provide much better enviromental condition since average temperatures are lower and soil moisture is higher due to accumulated water during the winter. Soil temperatures in time of blossoming is lower than in time of larval drop to the soil. In conditions of Banja Luka, Bosnia and Herzegovine during the study period 2012-2016 plum blossoming started from mid of March till mid of April, while the larvae start to drop at the end of April until the beginning of May. Moreover, adult emergence of Plum Sawflies happens over a shorter time compared to larval descent into the soil.

Efficacy of EPN against adult Plum Sawflies varied during the 3 years trials, but generally *S. feltiae* provided the highest efficacy against adult Plum Sawflies among treatments in cages. Temperature could be the reason why this nematode was the most succesful in suppression of damage produced by Plum Sawflies larvae. During the time of blossoming soil temperatures are at lower levels and cold active nematodes provide a better potential for control. In the study of Chen *et al.* (2003), *S. feltiae* was able to invide a host at 10°C, *S. carpocapsae* at 15°C and *H. bacteriophora* at 20°C. However, their efficacy increased with increase of temperature. Variation in efficacy among treatments could be due to weather conditions in different years. When the rainy weather preceeded, nematode application efficacy reached up to 100%.

In open field trials, *S. feltiae* efficacy differed depending on nematode concentration and weather conditions during nematode application. The highest efficacy was in the trial when application was during rain fall. Moreover, in this orchard floor in the rows, in time of application, was without grass. Application of 0.2 l m^{-2} water suspension of EPN over grass in open field trials resulted in efficacy much lower compared to trials in cages when the nematodes were applied in suspension of one liter m^{-2} following application of additional one liter m^{-2} post nematode application. Applied EPN should reach top layer of the soil. The soil is a more suitable environment for EPN survival compared to the grass surface and is better to parasitise emerging adults of Plum Sawflies. It seems that in case of application over grass, much more water is needed to assure that nematodes

reach the soil. Field application with the rate of 10,000 liters of water/ha is not practical for farmers. Application during rain revealed efficacy of almost 100%. However, rain periods did not preceed adult emergence for an acceptable time period in some years. Moreover, the slope of orchards would not allow application during rain at many sites. Keeping row space grass-free in time before blossoming could provide better conditions for nematode application as well. When EPN were applied during rain or sufficient amounts of water were provided for application to the bare ground, levels of control were comparable to insecticide applications. It might be possible to even use less nematodes under these conditions to achieve similar efficacy and thus contribute to a better economy of Plum Sawflies management.

In some trials application of EPN was even two weeks before first adult emergence. Mortality of EPN post application is highest during the first minutes and hours following application, reaching approximately 40-80% (Smits, 1996). Remaining population decrease was estimated at 5-10 % per day. Decreasing the time of nematode persistence in the soil can be obtained by more precise timing of the application. In the case of application of EPN against adult Plum Sawflies, ideal timing would be just before start of adult emergence. In poikilotermic animals, like insects, development of certain stages is temperature dependent. Emergence of adults of Plum Sawflies in conditions of Lithuania is at the beginning of May (Tamošuinas *et al.,* 2014). The model that anticipates this event is based on calculating of sum of temperatures from the 1st of April. However, in this study under conditions of Northern Bosnia and Herzegovina emergence of adults started in a range from the 12th of March until the 12th of April suggesting that a different model should be proposed. The lowest standard deviation of accumulated soil temperatures at 5 cm during the five years was when calculation started on the 1st of January, whereas for air temperature it was from the 15th of January. Calculated temperatures were 169±10 DD and 181±8 DD for soil and air temperature and thermal threshold 4°C. To avoid late application in practice it is suggested to subtract twice the standard deviation from the mean. Consequently, application should be at 149 DD for soil temperature at 5 cm calculated from the 1st of January, and 165 DD for air temperature from the 15th of January for a thermal threshold of 4°C.

During the study the presence of parasitoids was not observed in Plum Sawflies, but pathogenicity of entomopathogenic fungi to cocooned larvae was frequent. Probably, their populations are not able to cause epizootics, since in untreated orchards Plum Sawflies are highly devastating. There is about 700 described species of fungi determined as entomopathogenic, and 171 products were registered as mycoinsecticides or mycoacaricides by 2007 (Faria and Wright, 2007) giving space for another research area of their application against Plum Sawflies.

Based on this study EPN are able to control Plum Sawflies if application is done under certain conditions. At the moment, biological control of Plum Sawflies by EPN is still more expensive than application of synthetic insecticides. On the other hand, production costs of EPN are lowering continuously, bringing them into better position and more frequently used by farmers (Shapiro-Ilan *et al.,* 2012). Farmers in organic production do not have product that can successfully control these pests. Entomopathogenic nematodes could be incorporated in their control strategy. In strategy of management of Plum Sawflies, farmers have to consider that they are univoltine species and that portion of population stays in diapause for two years. This means that if in two consecutive years there was no observed damage or it was very limited, in next year population of sawflies will not make damage above economical threshold.

Considering common parts of the life cycle of Plum Sawflies and Apple and Pear Sawfly, management of the pests can be based on a similar approach (Happe *et al.,* 2016). Moreover, application of EPN in spring could affect overwintering populations of Plum Fruit Moth (*Grapholita funebrana*) and Codling Moth (*Cydia pomonella*).

Graph *et al.* (1996b) concluded that lenght of diapause of Apple Sawfly came to the end at the begining of March for conditions of Switzerland. When they were exposed earlier to temperatures above developmental threshold accumulated sum of efective temperture increased lineary but when cocoons were exposed earlier to above developmental threshold temperasture they needed more acumuated sum to finish their development. This could explain the smaller variation of DD when calculation started on the 15th of March, since sawflies have just completed their diapause (Zijp and Blommers, 1997; Tamošiunas and Valiuškaite, 2013). However, our study revealed that the smallest variation in DD for Plum Sawflies was when the 1st of January was chosen as a starting

day of temperature accumulation, suggesting that the length of diapause is shorter in Plum Sawflies compared to Apple Sawfly. From this study it could be concluded that the diapause of studied population lasted less then 32 days but more than 21. In 2012, 21 days below 4.0°C were not enough to brake diapause, but in 2014, after 32 days below thermal threshold, accumulation of DD started. Diapause in Plum Sawflies terminates already by the 1st of January for conditions of Northern Bosnia and Herzegovina, while for Apple Sawfly it is in March for conditions of Central and Northern Europe. The Apple Sawfly is an important pest only northof the Alps (Graf *et al.,* 2002) since they need longer time for diapause development, while Plum Sawflies are key pests in all Europe, due to shorter diapause length. The lengths of diapause for Plum Sawflies and Apple Sawfly seems quite different although they are closely related species, what could be the main reason for their distribution within Europe.

Results of the survey revealed presence of four species of EPN in Bosnia and Herzegovina. *S. feltiae* appears to be the most common species, followed by *S. kraussei* and *S. carpocapsae*. *H. bacteriophora* was the only representative of heterorhabditids. Besides geographic diversity in the country the nematodes were isolated from soils from central parts in the mountains or hills. The only species that was isolated from lower elevation was *H. bacteriophora.* The nematodes were isolated from their preferred habitats reported from previous reports. Two isolates of *S. kraussei* were found from mountain regions where annual temperature is below 9°C. *S. feltiae* was isolated from different altitude and vegetation types confirming its cosmopolitan adaptation. *S. carpocasae* was isolated from an orchard and from clay soil. Interestingly, nematodes were not isolated from samples collected from the northern flat areas of the country, and eastern and southern parts. A significant number of samples were taken from forests, but entomopathogenic nematodes were not obtained. Another type of survey, more targeted to sites with more insects prevalence could yield more isolates. In legislation of the country it is obligatory to confirm that a species is indigenous for the country before it can be introduced in the environment as a biological control agents. Therefore, results of this survey are the basis for registration of species of entomopathogenic nematodes as biocontrol agents in Bosnia and Herzegovina. Beside Plum Sawflies some other insect pests, such as Plum Fruit Moth (*Grapholita funebrana*) can be controlled by

entomopathogenic nematodes too as a part of integrated pest mangement or in organic production.

In conclusion...

The survey revealed presence of three steinernematids and one heterorhabditid species in Bosnia and Herzegovina. This is the first report of the presence of *S. feltiae*, *S. kraussei*, *S. carpocapsae* and *H. bacteriophora* for the country.

Adults and larval stages of Plum Sawflies can be effective controlled with EPNs. For adult control *S. feltiae* could be proposed and for larvae *H. bacteriophora.*

Efficacy of EPN depends on soil moisture, soil cover and amount of applied water. Optimizing these factors provides control level similar to sinthetic pesticides.

To determinate optimal timing of EPN application against adult stages of plum sawflies, a temperature driven model for adult emergence was developed for conditions of Northern Bosnia and Herzegovina.

Chapter 7

References

1. Akhurst, R.J. (1983). species: specificity of the association with bacteria of the genus *Xenorhabdus*. *Experimental Parasitology* 55, 258-263.

2. Andreev, R. and Kutinkova, H. (2010). Possibility of reducing chemical treatments aimed at control of plum insect pests. IX International Symposium on Plum and Prune Genetics, Breeding and Pomology. *ISHS Acta Horticulturae* 874, 215-220.

3. Ansari, M.A., Tirry, L. and Moens, M. (2004). Interaction between *Metarhizium anisopliae* CLO 53 and entomopathogenic nematodes for control of *Hoplia philantus*. *Biological Control* 31, 172-180.

4. Arthurs, S., Heinz, K.M. and Prasifka, J.R. (2004). An analysis of using entomopathogenic nematodes against above-ground pests. *Bulletin of Entomological Research* 94, 297-306.

5. Babendreier, D. (2000). Life history of *Aptesis nigrocincta* (Hymenoptera: Ichneumonidae), a cocoon parasitoid of the Apple Sawfly, *Hoplocampa testudinea* (Hymenoptera: Tenthrinidae). *Bulletin of Entomological Research* 90, 291-297.

6. Baumgartner, J., Graf, B. and Zahner, P. (1984). A stochastic population model to simulate the annual growth pattern of mature Golden Delicious apple tree. *Schweizerische Landwirtschaftliche Forschung* 23, 489-501.

7. Batalla-Carera, L., Morton, A. and Garcia del Pino, F. (2010). Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *BioControl* 55, 523-530.

8. Bathon, H. (1996). Impact of entomopathogenic nematodes on non-target hosts. *Biocontrol Science and Technology* 6, 421-434.

9. Bedding, R.A. and Molyneux A.S. (1982). Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica* 28, 354-359.

10. Belair, G., Vincent, C. and Chouinard, G. (1998). Foliar sprays with *Steinernema carpocapsae* against early-season apple pests. *Supplement to the Journal of Nematology* 30, 599-606.

11. Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T. and Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* 392, 71-75.

12. Bovien, P. and Stapel, C. (1940). Forsøg med bekæmpelse af blommenvespen (*Hoplocampa fulvicornis*). *Tidsskrift for Planteavl* 44, 699-730.

13. Brinkman, M.A. and Gardner, W.A. (2000). Possible antagonistic activity of two entomopathogens infecting workers of the red imported fire ant (Hymenoptera : Formicidae*). Journal of Entomological Science* 2, 205-207.

14. Bedding, R.A. and Akhurst R.J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21, 109-110.

15. Campbell, J.F. and Gaugler, R. (1993). Nictation behavior and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* 126, 155-169.

16. Campos–Herrera, R., Escuer, M., Labrador, S., Robertson, L., Barrios, L. and Gutierrez, C. (2007). Distribution of the entomopathogenic nematodes from La Rioja (Northern Spain). *Journal of Invertebrate Pathology* 95, 125-139.

17. Caruso, S. and Cera, M. C. (2004). Control strategies for Plum Sawflies (*Hoplocampa flava, Hoplocampa minuta*) in organic farming. *IOBC/WPRS Bull.*27 (5), 107-111.

18. Chen, S., LI, J., Han, X. and Moens, M.(2003). Effect of temperature on the pathogenicity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) to *Delia radicum*. *BioControl* 48, 713-724.

19. Choudhury, B.N. and Sivakumar, C.V. (2000). Biocontrol potential of *Mylonchulus minor* against some plant-parasitic nematodes. *Annals of Plant Protection Science* 8, 53-57.

20. Ciche, T.A. Darby, C., Ehlers, R.-U., Forst, S. and Goodrich-Blair, H. (2006). Dangerous liaisons: The symbiosis of entomopathogenic nematodes and bacteria. *Biological Control* 38, 22-46.

21. Cobb, N. (1914). Nematodes and their relationships. *Yearbook United States Department of Agriculture* pp. 457-490.

22. Cuthbertson, A.G.S., Walters, K.F.A, Northing, P. and Luo, W. (2007). Efficacy of the entomopathogenic nematode, *Steinernema feltiae*, against sweetpotato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) under laboratory and glasshouse conditions. *Bulletin of Entomological Research* 97, 9-14.

23. Curto, G., Boselli, M., Vergnani, S. and Reggiani, A. (2007). Effectiveness of entomopathogenic nematodes in the control of sawflies (*Hoplocampa brevis*) in pear orchards. *IOBC/wprs Bulletin* 30 (1), pp.13-17.

24. Danelski, W., Badowska-Cyubik, T., Rozpara, E. and Pniak, M. (2014). A study on the possibility of limiting damage to frit by the apple sawfly (*Hoplocampa testudinea* Klug) in organic apple orchards. *Journal of Research and Applications in Agricultural Engineering* 59, 27-30.

25. Danks, H.V. (2004). The roles of insect cocoon in cold conditions. *European Journal of Entomology* 101, 433-437.

26. De Lay, P. and Blaxter, M. (2002). Systematic position and phylogeny. In: Lee D.L. (ed.) *Biology of Nematodes*. London: Taylor and Francis, pp. 1-30.

27. Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 Establishing a framework for Community action to achieve the sustainable use of pesticides (O. J. L 309, 24.11.2009), 71-86.

28. Ehlers, R-U. (2001). Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology Biotechnology* 56, 623-633.

29. Ehlers, R.-U. (2003). Biocontrol nematodes. In: Hokkanen, H.M.T. and Hajek, A. (eds.). *Environmental Impact of Microbial Insecticides: Need and Methods for Risk Assessment*. Kluwer Scientific Publisher, Dordrecht, NL, pp.170-220.

30. Ehlers, R.-U. (2005). Forum on Safety and Regulations. In: Grewal, P.S., Ehlers, R.-U. and Shapiro-Ilan, D.I. (Eds.). *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 107-114.

31. Ehlers, R.-U. and Shapiro-Ilan, D. (2005). Mass production. In: Grewal, P.S., Ehlers, R.-U. and Shapiro-Ilan, D.I. (Eds.) *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 65-77.

32. Ehlers, R.-U. (2007). Entomopathogenic nematodes: from science to commercial use. In: Vincent, C., Goettel, M.S. and Lazarovits, G. (eds). *Biological Control: A Global Perspective*, pp. 136-151.

33. Eilenberg, J., Hajek, A. and Lomer, C. (2001). Suggestions for unifaying the terminology in biological control. *BioControl* 46, 387-400.

34. EPPO (2016). List of biological control agents widely used in the EPPO region - PM 6/3. Available at: http://archives.eppo.int/EPPOStandards/biocontrol_web/bio_list.htm.

35. FAOSTAT (2016). Food and Agricultural Organisation of the United Nations Statistics. *Available online at http://www.fao. org/documents/card/en (accessed 8 February, 2017).*

36. Faria, M. and Wraight, S. (2007). Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control* 43, 237-256.

37. Garcia del Pino, F. and Morton, A. (2005). Efficacy of entomopathogenic nematodes against neonate larvae of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae) in laboratory trials. *BioControl* 50, 307-316.

38. Glaser, R.W. and Farrell, C.C. (1935). Field experiments with Japanese beetle and its nematode parasite. *Journal of the New York Entomological Society* 43, 345-371.

39. Gradinarov, D., Petrova, E., Mutafchiev, Y. and Karadjova, O. (2012). Distribution of entomopathogenic nematodes of the genus *Heterorhabditis* (Rhabditida: Heterorhabditidae) in Bulgaria. *Nematologia Mediterranea* 40, 173-180.

40. Graf, B., Höhn, H. and Höpli, H.U. (1996a). The apple sawfly, *Hoplocampa testudinea*: a temperature driven model for spring emergence of adults. *Entomologia Experimentalis et Applicata* 78, 301-307.

41. Graf, B., Höhn H. and Höpli, H.U. (1996b). Moddeling spring emergence of the apple sawfly *Hoplocampa testudinea* Klug (Hymenoptera, Tenthredinidae). *Acta horticulturae* 416 (416): 263-272.

42. Graf, B., Höhn, H. and Höpli, H.U. (1996c). Optimizing the risk assessment for the apple sawfly *Hoplocampa testudinea* Klug (Hymenoptera, Tenthredinidae). *Acta horticulturae* 422,113-122.

43. Graf, B., Höhn H. and Höpli, H.U. (2002).The apple sawfly, *Hoplocampa testudinea*: egg development and forecasting of egg hatch. *Entomologia Experimentalis et Applicata* 105, 55-60.

44. Grewal, P.S., Ehlers, R.-U. and Shapiro-Ilan, D.I. (2005). Nematodes as biocontrol agents. Wallington: CABI Publishing.

45. Griffin, C.T., Dix, I., Joyce, S.A., Burnell, A.M. and Downes, M.J. (1999). Isolation and characterisation of *Heterorhabditis* spp. (Nematoda: Heterorhabditidae) from Hungary, Estonia and Denmark. *Nematology* 1, 321-332.

46. Griffin, C.T., O'Callaghan, K. and Dix, I.A. (2001). Self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematodes? *Parasitology*. 122, 181-186.

47. Griffin, C.T., Boemare, N.E. and E.E. Lewis (2005). Biology and bahaviour. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (Eds.) *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 47-64.

48. Gouy M., Guindon S. and Gascuel O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27, 221-224.

49. Happe, S., Nježić, B. and Ehlers, R.-U. (2016). Control of apple sawfly (*Hoplocampa testudinea* Klug) and plum sawflies (*H. flava* L. and *H. minuata* Christ.) with entomopathogenic nematodes. *Proceedings of 17th International Conference on Organic Fruit-Growing*, Hohenheim 15-17 February 2016, 258-260.

50. Hominick, W.M., Briscoe, B.R., del Pino, F.G., Heng, J., Hunt, D.J., Kozodoi, E., Mracek, Z., Nguyen, K.B., Reid, A.P., Spiridonov, S., Stock, P., Sturhan, D., Waturu, C. and Yoshida, M. (1997). Biosystematics of entomopathogenic nematodes: current status, protocols, and definitions. *Jurnal of Helminthology* 71, 271-298.

51. Hominick, W.M. (2002). Biogeography. *In:* R. Gaugler (Ed.) *Entomopathogenic Nematology*. Wallingford, UK: CABI Publishing, pp.115-143.

52. Hugot, J.P., Baujard, P. and Morand, S. (2001). Biodiversity in helminths and nematodes as a field study: an overview. *Nematology* 3, 199-208.

53. Hunt, D.J. (2007). Overview of taxonomy and systematics. In: Nguyen, K.B. and Hunt, D.J. (eds). *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts 5*. Brill, pp. 27-57.

54. Jarošík, V., Honěk, A., Magarey, R.D. and Skuhrovec J. (2011). Developmental database for phenology models: Related insect and mite species have similar thermal requirements. *Journal of Economic Entomology* 104, 1870-1876.

55. Johnigk, S.-A. and Ehlers, R.-U. (1999). Endotokia matricida in hermaphrodites of *Heterorhabditis* spp. and the effect of the food supply. *Nematology* 1, 717-726.

56. Joyce, S.A., Reid, A., Driver, F. and Curran, J. (1994). Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. In: Burnell, A.M., Ehlers, R.U., Masson J.P. (Eds.) *Biotechnology: Genetics of Entomopathogenic Nematode Bacteria Complexes*. European Commission, Luxembourg, DG XII, pp. 178-187.

57. Kaya, H.K. (2002). Natural enemies and other antagonists. In: Gaugler, E. (ed.), *Entomopathogenic Nematology*. Wallington: CABI Publishing, pp.189-204.

58. Koppenhöfer, A.M., Kaya, H.K. and Taormino, S.P. (1995). Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. *Journal of Invertebrate Pathology* 65, 193-199.

59. Koppenhöfer, A.M. and Kaya, H. K. (1997). Additive and synergistic interaction between entomopathogenic nematodes and *Bacillus thuringiensis* for scarab grab control. *Biological Control* 8, 131-137.

60. Koppenhöfer, A.M. (2007). Nematodes. In: Lacey, L.A. and Kaya, H.K. (eds.*), Field manual of techniques in invertebrate pathology*. Springer Netherlands, pp. 249-264.

61. Lacey, L. A., Shapiro-Ilan, D. I.,and Glenn, G. M. (2010). Post-application of anti-desiccant agents improves efficacy of entomopathogenic nematodes in formulated host cadavers or aqueous suspension against diapausing codling moth larvae (Lepidoptera: Tortricidae). *Biocontrol Science and Technology* 20, 909-921.

62. Lacey, LA. and Georgis R. (2012). Entomopathogenic nematodes for control of insects pests above and below ground with comments on commercial production. *Journal of Nematology* 44, 218-225.

63. Lacey, L.A. and Georgis R. (2012). Entomopathogenic nematodes for control of insects pests above and below ground with comments on commercial production. *Journal of Nematology* 44, 218-225.

64. Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M. and Goettel, M.S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology* 131, 1-41.

65. Laznik, Ž. and Trdan, S. (2012). Entomopathogenic nematodes (Nematoda: Rhabditida) in Slovenia: from tabula rasa to implementation into crop production systems. In: Perveen, F. (ed.). *Insecticides – Advances in Integrated Pest Management.* Intech, Rijeka, Croatia, pp. 627-656.

66. Llacer, E., Martinez de Altube, M.M. and Jacas, J.A. (2009). Evaluation of the efficacy of *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil, *Rhynchophorus ferrugineus*, in *Phoenix canariensis*. *BioControl* 54, 559-565.

67. Lootsma, M. and Scholte, K. (1997). Effects of springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* on *Rizoctonia solani* stem infection of potato at temperatures of 10 and 15°C. *Plant Pathology* 46, 203-208.

68. Ludwig, D. (1928). The effects of temperature on the development of an insect (*Popilia japonica* Newman). *Physiological Zoology* 1, 358-389.

69. Menti, H., Wright, D. and Perry, R. (1997). Desiccation survival of populations of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* from Greece and the UK. *Journal of Helminthology* 71, 41-46.

70. Mićić, N., Đurić, G. and Životić, A. (2015). Yield potential of long bearing shoots of ten plum cultivars (*Prunus domestica* L.). *Agro-Knowledge Journal* 1, 15-19.

71. Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010) Creating the CIPRES Science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp. 1-8.

72. Mráček, Z. and S. Bečvář (2000). Insect aggregations and entomopathogenic nematode occurrence. *Nematology* 2, 297-301.

73. Neupane, D. (2012). Apple sawflly (*Hoplocampa testudinea*, Klug) situation, forecasting, minitoring and use of extract from *Quassia amara* for controling the apple sawfly in organic apple orchards in Sweden. *Master thesis at Alnarp* Univerity, Sweden, pp. 1-50.

74. Nguyen, K.B. and Hunt, D.J. (2007). Heterorhabditidae: species descriptions. In: Nguyen, K.B., Hunt, D.J. (Eds) *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts. Nematology Monographs and Perspectives, 5*. Brill, Leiden, The Netherlands, pp. 611-692.

75. Oroian, I., Oltean, I., Odagiu, A., Paulette, L., Iederan, C. and Brasovean, I. (2009). The control and monitoring of the orchard pests in Transylvania. *Research Journal of Agricultural Science,* 41, 277-283.

76. Panini, M., Manicardi, G.C., Moores, G.D. and Mazzoni, E. (2016). An overview of the main pathways of metabolic resistance in insects. *Invertebrate Survival Journal* 13, 326-335.

77. Peters, A. and Ehlers, R-U. (1994). Susceptibility of Leatherjackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. *Journal of Invertebrate Pathology* 63, 163-171.

78. Peters, A. (1996). The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. *Biocontrol Science and Technology* 6, 389-402.

79. Poinar, G.O. (1993). Origins and phylogenetic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema*. *Fundamental and Applied Nematology* 16, 333-338.

80. Premachandra, W.T.S.D, Borgemeister, C., Berndt , O., Ehlers, R.-U. and Poehling, H.-M. (2003). Combined releases of entomopathogenic nematodes and the predatory mite *Hypoaspis aculeifer* to control soil-dwelling stages of western flower thrips *Frankliniella occidentalis. BioControl* 48, 528-543.

81. Půža V., Mráček Z., Nermut, J. (2016). Novelties in pest control by entomopathogenic and mollusc-parasitic nematodes. In: Harsimran G. (ed.), *Integrated Pest Management (IPM): Environmentally Sound Pest Management.* InTech, pp. 71-102.

82. Rainey, F.A., Ehlers, R.-U. and Stackebrandt, E. (1995). Inability of the polyphasic approach to systematics to determine the relatedness of the genera *Xenorhabdus* and *Photorhabdus*. *International Journal of Systematic Bacteriology* 45, 339-381.

83. Riga, E., Lacey, L.A., Guerra, N. and Headrick, H.L. (2006). Control of the oriental fruit moth, *Grapholita molesta*, using entomopathogenic nematodes in laboratory and fruit bin assays. *Journal of Nematology* 38, 168-171.

84. Rosa, A.J.S., Boinifassi, E., Ameral, J., Lacey, L.A., Simoes, N. and Laumond, C. (2000). Natural occurrence of entomopathogenic nematodes (Rhabditida: *Steinernema*, *Heterorhabditis*) in the Azores. *Journal of Nematology* 32, 215-222.

85. Rozpara E., Badowska-Czubik T. and Kowalska J. (2010). Problems of the plum and cherry plants protection in ecological orchard. *Journal of Research and Applications in Agricultural Engineering,* 55, 73-75.

86. Schroer, S. and Ehlers, R.-U. (2005). Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*). *Biological Control* 33: 81-86.

87. Shapiro-Ilan, D.I. (2001a). Virulence of entomopathogenic nematodes to pecan weevil adults (Coleoptera: Curculionidae). *Journal of Entomological Science* 36, 325-328.

88. Shapiro-Ilan, D.I. (2001b). Virulence of entomopathogenic nematodes to pecan weevil larvae *Curculio caryae* (Coleoptera: Curculionidae) in the laboratory. *Journal of Economic Entomology* 94, 7-13.

89. Shapiro-Ilan, D.I., Cottrell, T.E., Mizell, R.F., Horton, D.L. and Davis, J. (2009). A novel approach to biological control with entomopathogenic nematodes: Prophylactic control of the peachtree borer, *Synanthedon exitiosa*. *Biological Control* 48, 259-263.

90. Shapiro-Ilan, D.I, Morales-Ramos, J.A., Rojas, M.G. and Tedders, W.L. (2010). Effects of a novel entomopathogenic nematode-infected host formulation on cadaver integrity, nematode yield, and suppression of *Diaprepes abbreviatus* and *Aethina tumida*. *Journal of Invertebrate Pathology* 103:103-108.

91. Shapiro-Ilan, D.I., Han, R. and Dolinksi, C. (2012). Entomopathogenic nematode production and application technology. *Journal of Nematology* 4, 206-217.

92. Sjöberg, P., Swiergiel, W., Neupane, D.,Lennartsson, E., Thierfelder, T., Tasin, M. and Rämert, B. (2015). Evaluation of temperature sum models and timing of *Quassia amara* (Simaroubaceae) wood-chip extract to control apple sawfly (*Hoplocampa testudinea* Klug) in Sweden. *Journal of Pest Science*; 88, 301-310.

93. Smits, P. (1996). Post-application persistence of entomopathogenic nematodes. *Biocontrol Science and Technology* 6, 379–387.

94. Somasekhar, N., Grewal, P.S., De Nardo, E.A.B. and Stinner, B.R. (2002). Non-target effects of entomopathogenic nematodes on the soil nematode community. *Journal of Applied Ecology* 39, 735-744.

95. Steiner, G. (1923). *Aplectana kraussei* n. sp., eine in der Blattwespe *Lyda* sp. parasitierende Nematodenform, nebst Bemerkungen über das Seitenorgan der parasitischen Nematoden. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankenheiten und Hygiene, Abteilung* 2, 14-18.

96. Stock, S.P. and Hunt, D.J. (2005). Morphology and systematics of nematodes used in biocontrol. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as Biocontrol Agents.* Wallington: CABI Publishing, pp. 3-43.

97. Stuart, R.J., Barbercheck, M.E., Grewal, P.S., Taylor, R.A.J. and Hoy, C.W. (2006). Population biology of entomopathogenic nematodes: Concepts, issues, and models. *Biological Control* 38, 80-102.

98. Tallosi, B., Peters, A. and Ehlers, R.-U. (1995). *Steinernema bicornutum* sp. n. (Rhabditida: Steinernematidae) from Vojvodina, Yugoslavia. *Russian Journal of Nematology* 3, 71-80.

99. Tamošiunas R, Valiuškaite A. (2013). The study on temperature sum model for predicting apple sawfly spring emergence and flight intensity in Lithuania. *Scientific works of the institute of horticulture, Lithuanian research center for agriculture and forestry*. 32: 23-36.

100. Tamošiunas, R, Valiuškaite, A., Survieliene, E. and Rasiukevičiute, N. (2014). Species ratio, spring emergence, population dynamics and damage of plum sawflies *Hoplocampa minuta* and *H. flava* in plum orchard. *Zembdirbyste-Agriculture* 101, 91-100.

101. Tarasco, E., Clausi, M., Rapazzo, G., Panzavolta, T., Curto, G., Sorino, R., Oreste, M., Longo, A., Leone, D., Tiberi, R., Vinciguerra, M.T. and Triggiani, O. (2014). Biodiversity of entomopathogenic nematodes in Italy. *Journal of Helminthology* 10, 1-8.

102. Toepfer, S., Peters, A., Ehlers, R.-U. and Kuhlmann, U. (2008). Comparative assessment of the efficacy of entomopathogenic nematode species at reducing western corn rootworm larvae and root damage in maize. *Journal of Applied Entomology* 132: 337-348.

103. Tomalak, M., Piggott, S., and Jagdale, G.B. (2005). Glasshouse application. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 191-214.

104. Tomalak, M. (2006).Potential of entomopathogenic nematodes for control of orchard and urban tree sawflies. *Progress in Plant Protection* 46, 249-255.

105. Tóth, T. (2006). Collection of entomopathogenic nematodes for the biological control of insect pests. *Journal of Fruit and Ornamental Plant Research* 14, 225-230.

106. Ulu, T.C., Sadic, B., Susurluk, I.A. and Aksit, T. (2015). Virulence of four entomopathogenic nematode species for plum sawfly, *Hoplocampa flava* L. (Hymenoptera: Tenthredinidae). *Invertebrate Survival Journal* 12: 274-277.

107. Vincent, C. and Belair, *G.* (1992): *Biocontrol* of the *apple sawfly*, *Hoplocampa testudinea*, with entomogenous nematodes. *Entomophaga* 37, 125-149.

108. Weiser, J. (1955). *Neoaplectana carpocapsae* n. sp. (Anguillata, Steinernematidae) novy cizopasnik housenek obalece jablecneho, *Carpocapsae pomonella* L.. *Vestnik Ceskoslovenske Spolecnosti Zoologicke* 19, 44-52.

109. White, G. F. (1929). A method for obtaining infective nematode larvae from cultures. *Science* 66, 302-303.

110. Williams, C.D., Dillon, A.B., Harvey, C.D., Hennessy, R., Mc Namara L. and Griffin C.T. (2013). Control of major pest of forestry, *Hylobius abietes*, with entomopathogenic nematodes and fungi using eradicant and prophylactic strategies. *Forest Ecology and Management* 305, 212-222.

111. Wilson, M.J. and Gaugler, R. (2000). Terrestial molusc pests. In: Lacey, L. and Kaya, H.K. (eds). *Field Manual of Techniques in Invertebrate Pathology*. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp. 787-804.

112. Woodring, J. L. and Kaya, H. K. (1998). Steinernematid and heterorhabditid nematodes: Hand book of biology and techniques. *South Cooperative Series Bulletin of the Arkansa Agricultural Experimental Station, Fayetteville* 331, 1-30.

113. Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. and Georgieva, S.S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology* 25, 315-331.

114. Zijp, J.P. and Blommers, L. (1993). *Lathrolestes ensator*, a parasitoid of the apple sawfly. *Proceedings of the Section Experimental and Applied Entomology*, N.E.V. Amsterdam 4, 237-242.

115. Zijp, J.P. and Blommers, L.H.M. (1997). Prediction of flight of apple sawfly, *Hoplocampa testudinea*, using temperature sums. *Entomologia Experimentalis et Applicata* 84, 71-75.

116. Zijp, J.P. and Blommers L. (2002). Apple sawfly *Hoplocampa testudinea* (Hym., Tenthredinidae) and its parasitoid *Lathrolestes ensator* in Dutch apple orchards (Hym., Ichneumonidae, Ctenopelmatinae). *Journal of Applied Entomology* 126, 265-274.

Chapter 8
List of tables and figures

LIST OF TABLES

LIST OF FIGURES

Figure 4.8. Assessing nematode efficacy. A: The net was placed under trees canopy before start of first fruit drop (end of April) to collect fruits. B: Damaged fruits collected from different treatments.

Figure 4.9. Nematode application in 2016 in Croatia, Vojnić. A: Application by tractor blast sprayer at a rate of 500,000 DJs m^{-2} with 0.2 l m^{-2} following additional 0.2 l m^{-2} of water within 30 min. B: Application by tractor mounted sprayer at a rate of 250,000 DJs m^{-2} with 0.2 l m^{-2} following additional 0.2 l m^{-2} of water within 30 min. C: Application of tractor blast sprayer at a rate of 250,000 DJs m^{-2} with 0.2 l m^{-2} following additional 0.2 l m^{-2} of water with tractor mounted sprayer.

Figure 4.10. Density of water drops with EPN after application in Vojnić with 0.2 l m^{-2} showing surface coverage of suspension. A: Ground surface with Petri-dish. B: Petri-dish with water drops.

Figure 4.11. Nematode application in Babići, Kozara, B&H. Application was during rainfall (A) before first adults of Plum Sawflies were captured on white sticky trap (B).

Figure 4.12. Flowers with deposited eggs of Plum Sawflies. A: The egg deposited in the calix (blue arrow), B: The egg deposited in the receptaculum, C: Three eggs deposited on one flower.

Figure 4.13. Percentage of last instar infected larvae of Plum Sawflies exposed to *Steinernema feltiae, S. carpocapsae* and *Heterorhabditis bacteriophora* under laboratory conditions before making cocoons. Ten larvae were exposed to 500, 1000 and 2000 DJs in 5.5 cm Petri dishes filed with sterile silver sand. Mean of mortality in % ± SD.

Figure 4.14. Number of adult Plum Sawflies caught on white sticky plates on plots treated with *S. feltiae, S. carpocapsae* and *H.bacteriophora* at 500,000 DJs m^{-2} in the assey with larvae susceptibility in the field conditions.

Figure 4.15. Percentage of flowers with deposited eggs in treatments and control. Field trials with application of *Steinernema feltiae* against adult Plum Sawflies were conducted in the orchards in Vojnić, Croatia and babići, Kozara, B&H. Application in first orchard in Croatia with half dose (250,000 DJ m^{-2}) was on the 17th of March, in second orchard with half dose on the 18th of March, in orchard with full dose on the 16th of March, and in the orchard in B&H on the 21st of March during the rain.

Chapter 9
Curriculum Vitae

Branimir Nježić was born on 4th of January 1979 in Banja Luka, Bosnia and Herzegovina. He graduated Gymnasium of Banja Luka and enrolled at the Faculty of Agriculture University of Banja Luka. During his study he was for two years student representative in the education committee of the faculty. He spent also three months in 2005 as a part of the TEMPUS project of student exchange program at the Laboratory for Nematology at Wageningen University, The Netherlands. He graduated 2006 with an average mark of 8.79 and received the golden plaque of the University of Banja Luka as the best student of his batch.

In 2006 he got a position at the Faculty of Agriculture University of Banja Luka as a teaching assistant. From 2008 to 2010 he attended the European Master of Science in Nematology, Erasmus Mundus Master Program supported by the European Commission. Coordinating university was Ghent University Belgium. Three more universities from EU were part of the consortium and four more were associated partners. He graduated with great distinction and the title of his master thesis was: Effects of *Tagetes patula* cv Single Gold on *Meloidogyne chitwoodi, Pratylenchus penetrans* and *Steinernema feltia.* He was student representative of the program in the education committee for two years.

He participated in several scientific projects and developed the nematology unit within the Faculty of Agriculture and the Laboratory for Certification of Horticultural Plants. This unit processes official samples for phytosanitary inspections for the presence of quarantine nematodes. It does research activities on different groups of plant-parasitic nematodes and entomopathogenic nematodes. He supervised several bachelor and master students to produce their thesis related to nematology. He is coauthor of 4 scientific papers and has attended over 20 scientific meetings where he presented his research.

He received a bursary grant from the European Society of Nematologists (ESN) to attend the IFNS meeting in 2014 in Cape Town, South Africa and gave an oral presentation related to his PhD thesis during the meeting.

He was representative of Bosnia and Herzegovina in EMA (Erasmus mundus students association) for two years 2010-2012. He is a member of the Society for Plant Protection of Bosnia and Herzegovina, the European Society of Nematologists and the IOBC (International Organization for Integrated and Biological Control). He is married and father of two children..

Образац -3

УНИВЕРЗИТЕТ У БАЊОЈ ЛУЦИ
ПОЉОПРИВРЕДНИ ФАКУЛТЕТ

ИЗВЈЕШТАЈ

о оцјени урађене докторске дисертације

I ПОДАЦИ О КОМИСИЈИ

1) Одлуком Наставно-научног вијећа Пољопривредног факултета број 10/3.1347-6-15/17 од 23.05.2017. године именована је комисија за писање извјештаја о оцјени урађене докторске дисертације и за одбрану докторске дисертације кандидата мр Бранимира Њежића под насловом "Контрола осица шљиве (*Hoplocampa flava* L. и *Hoplocampa minuta* Christ.) ентомопатогеним нематодама" у сљедећем саставу:
   - Проф. др Гордана Ђурић, редовни професор Универзитета у Бањој Луци, Пољоприввредни факултет, уже научне области: Хортикултура и Очување генетичких ресурса, предсједник,
   - Проф др Ралф-Удо Елерс, ванредни професор Универзитета у Килу, Пољопривредно-прехрамбени факултет, Њемачка, ужа научна област Заштита биљака, ментор,
   - Проф. др Сњежана Хрнчић, редовни професор Универзитета Црне Горе, Биотехнички факултет, ужа научна област Ентомологија, члан,
   - Проф. др Марек Томалак, редовни професор Националног истраживачког института, Институт за заштиту биља, Познан, Пољска, ужа научна област Ентомологија и нематологија, члан,
   - Проф. др Станислав Трдан, редовни професор Универзитета у Љубљани, Биотехнички факултет, Словенија, ужа научна област Заштита биљака, члан.

1) Навести датум и орган који је именовао комисију;
2) Навести састав комисије са назнаком имена и презимена сваког члана, научно-наставног звања, назива уже научне области за коју је изабран у звање и назива универзитета/факултета/института на којем је члан комисије запослен.

II ПОДАЦИ О КАНДИДАТУ

1. Бранимир, Милоша, Њежић.
2. Рођен 04.01.1979. године у Бањој Луци, Босна и Херцеговина.
3. Ерасмус Мундус програм: Европски магистериј из нематологије, магистар пољопривредних наука.
4. Универзитет у Генту, Белгија, наслов магистарске тезе: "Утицај *Tagetes patula* cv Single Gold на *Meloidogyne chitwoodi, Pratylenchus penetrans* и *Steinernema feltiae*".
Научна област: пољопривредне науке, одбрањена 30.06.2010. године.
5. Научна област пољопривредне науке
6. Докторска дисертација је пријављена 2015. године, складу са Законом о високом образовању, Члан 149. (Службени гласник Републике Српске 73/10; 104/11; 84/12;

108/13; 44/15; 90/16).

1) Име, име једног родитеља, презиме;
2) Датум рођења, општина, држава;
3) Назив универзитета и факултета и назив студијског програма академских студија II циклуса, односно послиједипломских магистарских студија и стечено стручно/научно звање;
4) Факултет, назив магистарске тезе, научна област и датум одбране магистарског рада;
5) Научна област из које је стечено научно звање магистра наука/академско звање мастера;
6) Година уписа на докторске студије и назив студијског програма.

III УВОДНИ ДИО ОЦЈЕНЕ ДОКТОРСКЕ ДИСЕРТАЦИЈЕ

1. Наслов докторске дисертације "Контрола осица шљиве (*Hoplocampa flava* L. и *Hoplocampa minuta* Christ.) ентомопатогеним нематодама",
2. Тема дисертације је прихваћена од стране Наставно-научног вијећа Пољопривредног факултета одлука бр 10/3.3951-1-12/15 од 23.11.2015. године и Сената Универзитета бр 02/04-3.4139-128/15 од 28.12.2015. године.
3. Садржај докторске дисертације по поглављима је сљедећи:
   1 Увод 1-5;
   2 Преглед литературе 6-20;
   3 Мониторинг на присуство ентомопатогених нематода у Босни и Херцеговини 21-31;
   4 Ефикасност ентомопатогених нематода у сузбијању осица шљиве (*Hoplocampa minuta* L. и *Hoplocampa flava* H.) у лабораторијским и пољским условима 32-56;
   5 Температурни модел појаве првих имага осица шљиве (*Hoplocampa flava* и *Hoplocampa minuta*) 57-67;
   6 Општа дискусија 68-75;
   7 Литература 76-88;
   Прилози у дисертацији су:
   Списак табела, слика и графика 89-93;
   Биографија кандидата 94-95.
4. Докторска дисертација је написана на укупно 103 странице, А4 формата од чега је 9 уводних страница: поднасловница, подаци о ментору, сажетак на енглеском и српском језику, захвала и садржај; затим 88 страница текста дисертације на енглеском језику са 7 табела, 24 графикона и слика, попис 115 коришћених литературних извора, као и 6 страница са листом табела, графикона и слика и биографијом канидата.

1) Наслов докторске дисертације;
2) Вријеме и орган који је прихватио тему докторске дисертације
3) Садржај докторске дисертације са страничењем;
4) Истаћи основне податке о докторској дисертацији: обим, број табела, слика, шема, графикона, број цитиране литературе и навести поглавља.

IV УВОД И ПРЕГЛЕД ЛИТЕРАТУРЕ

1. Осице шљиве *Hoplocampa flava* L. и *Hoplocampa minuta* Christ су најзначајније штеточине шљиве. Мјере сузбијања које су тренутно доступне произвођачима су искључиво засноване на примјени синтетисаних инсектицида у вријеме отпадања латица. Стога је циљ ове дисертације био да се оцијени потенцијал ентомопатогених нематода (ЕПН) као биолошких агенаса у сузбијању једних од најзначајнијих штеточина шљиве, жуте шљивине осице (*Hoplocampa flava* L.) и црне шљивине осице (*Hoplocampa minuta* Christ). Комерцијални препарати три врсте ЕПН *Steinernema feltiae*, *S. carpocapsae* и *Heterorhabditis bacteriophora* су тестирани за сузбијање различитих развојних стадијума осица шљиве. Огледи су извођени у

контролисаним лабораторијским условима, полуконтролисаним пољским условима и пољским условима. Кроз ова тестирања одабрана је врста нематода која је показала највећу ефикасност, те испитани најпогоднији услови њене примјене. Да би се побољшала ефикасност дјеловања ЕПН праћен је животни циклус штеточине, с посебним освртом на стадијум који је сузбијан. Пошто су се имага осица шљиве показали као најпогоднији стадијум за сузбијање, развијен је модел праћења појаве имага у зависности од темепературе. Земљишни узорци из цијеле Босне и Херцеговине су сакупљени и испитани на присуство аутохтоних популација ЕПН. Пошто локална легислатива, за регистрацију препарата за биолошку контролу, захтијева потврду присуства врсте на територије државе, потврда присуства врста ЕПН омогућиће регистрацију и примјену њихових препарата на територији БиХ.

2. Црна осица шљиве (*Hoplocampa minuta* Christ.) и жута осица шљиве (*H. flava* L.) (Hymenoptera, Symphyta, Tenthredinidae) се једним именом називају осице шљиве. Оне представљају једне од најзначајнијих штеточина шљиве и широко су распрострањене. Могу да проузрокују веома значајне штете, које понекад износе и до 100%. Тренутно мјере сузбијања се заснивају искључиво на примјени синтетичких пестицида јер произвођачима нису доступне друге мјере сузбијања које су довољно ефикасне. Примјена инсектицида је у вријеме отпадања латица, мада у неким подручјима произвођачи врше третмане и на почетку цвјетања шљиве. Једини биолошки агенс који је детаљно проучаван за сузбијање блиске штетне врсте, јабукине осице, је паразитска осица *Lathrolestes ensator*. Резултати су показали да ефекат сузбијања није задовољавајући. Такође, сличан ниво контроле постигнут је и са примјеном екстраката биљке *Quassia amara*.

   Веома блиске врсте осицама шљиве су и штетне врсте јабукина осица *Hoplocampa testudinea* и крушкина осица *Hoplocama brevis*. Осице шљиве имају једну генерацију годишње и већину времена проведу у земљи као одрасле ларве у кокону. Њихов животни циклус је готово идентичан. Еклозија имага се догађа у вријеме цвјетања раних сорти шљиве. Женке полијежу јаја у чашичне листиће из којих се када плод почне развој пиле ларве и убушују у плод. Током развоја ларве оштећују сјемену кућицу што доводи до отпадање плода. Једана ларва може да оштети 3-6 плодова. Штеточине презимљавају као одрасле ларве у кокону у земљи на дубини 5-25 cm. Пошто су осице шљиве, као и сви инсекти, поикилотермне животиње њихов развој је у директној вези са вањском температуром.

   До сада није развијен метод за биолошко сузбијање ових штеточина који би био прихватљив произвођачима. Пошто већину времена осице шљиве проведу у земљи, оне представљају потенцијално погодне организме за сузбијање коришћењем ЕПН. Кроз преглед литературе, може се видјети да су ЕПН тестиране за сузбијање јабукине и крушкине осице у одређеним условима. Међутим, једини стадијум који је сузбијан је ларва. Бршени су и фолијарни третмани и третмани земљишта али са ограниченим резултатима.

   Ентомопатогене нематоде припадају типу Nematoda. Ова група животиња је веома разнолика, бројна и широко распрострањена. По типу исхране могу бити бактериоворне, фунгиворне, предаторске, омниворне, те паразити животиња и биљака. Међу овим групама највећу пажњу научника привлаче нематоде паразити животиња и биљака. Нематоде повезане са инсектима су описане у преко 30 фамилија, али је потенцијал за биолошко сузбијање штених инсеката ограничен на 7 фамилија. Ипак, највећу пажњу као средство за биолошко сузбијање привлаче нематоде из двије фамилије Steinernematidae и Heterorhabditidae, познате као ентомопатогене нематоде. Иако је прва врста ентомопатогених нематода описана прије готово 100 година, њихова масовна примјена за сузбијање штених инсеката почела је тек прије 30-так година. Нова сазнања о биологији нематода, кругу домаћина, развој технологије масовне производње, формулација, чувања и примјене

ЕПН, резултирали су експоненцијалним повећањем употребе ових биолошких агенаса. Поред научног и технолошког напретка, брзом расту употребе ЕПН допринијела је и легислатива која или не захтијева регистрацију препарата или је она доста једноставна. Овакав статус је заснован на подацима који кажу да нема негативног утицаја на људе, сисаре, животну средину и биљке. Успјешност у сузбијању штених инсеката заснована је на односу нематоде и бактерија, које нематоде носе у свом пробавном систему. Сматра се да је овај однос развијен кроз конвергентну еволуцију. Брзо повећање употребе ЕПН од 80-тих година прошлог вијека, у заштити биљака је засновано на смањењу цијене коштања препарата, повећању проблема са резистентношћу на инсектициде, налазима о повећаним концентарцијама остатака пестицида у храни те њиховом негативном ефекту на животну средину. ЕПН се могу произвести у *in vivo* или *in vitro* условима. *In vivo* је производња у ларвама восковог мољца. Овај вид производње је јефтин, али захтијева велику потребу за радном снагом. На овај начин се могу произвести мање количине за огледе. Производња *in vitro* може бити у чврстом или течном медијуму. Производња у чврстом медијуму је погодна за средње велике апликације, а основна предност је ниска цијена инвестиција у опрему. Производња у течним медијима има велике инсталационе трошкове за биоферменторе, а потребно је и високо технолошко знање о процесу гајења нематода у овим условима. Ипак, конкурентноист на великим тржиштима могуће је постићи само уз производњу великог обима као што су биоферментори. Примјена ЕПН зависи од саме културе те циљаног штетног организма. Теоретски ЕПН се могу аплицирати било којом опремо за примјену пестицида, водећи рачуна о мијешању, типу дизни, притиску и температури радног раствора. ЕПН су потпуно сигурне за раднике који рукују са препаратима, али и животну средину. Од прве примјене ЕПН за сузбијање неког штеног организма, а то је *Popillia japonica* из 1935. године, нема података о негативном утицају на животну средину. ЕПН готово да не могу имати никакав негативан дугорочни ефекат на нециљане организме пошто нису перзистентне на површини земље и у случају одсутва домаћина брзо угину. У већини слузчајева, ЕПН су у процесу регистрације третиране као и други макроорганизми, заједно са корисним зглавкарима, те су често изузете од регистрације. Примјена ЕПН за сузбијање штетних организама је у литератури детаљно обрађена. Оне су се показале ефикасне у сузбијању многих штетних инсеката. У већини случајева примјењују се у сузбијању земљишних стадијума инсеката пошто су и саме присутне у земљи у природним стаништима. Поред тога, постоје и примјери гдје су ЕПН ефикасне у сузбијању штеточина унутар биљног ткива, на надземним органима и у затвореним просторима.

Присуство ЕПН је потврђено на свим континентима изузев Антарктика. Континент на коме је узорковање вршено најчешће је Европа. Steinernematidae су чешће присуте у узорцима изузев у узорцима са пјесковитих земљишта.

3. У литератури не постоје подаци о биолошком сузбијању осица шљиве. Ова дисертација имала је за циљ да испита потенцијал ЕПН у сузбијању осица шљиве у лабораторијским и пољским условима. Да би се повећала ефикасност ЕПН праћени су параметри животног циклуса циљаних штеточина с циљем прецизније апликације. Узорковање на присуство аутохтоних популација ЕПН у БиХ је урађено јер је присуство врсте у природним популацијама предуслов регистрације препарата. Поред тога резултати добијени кроз ово истраживање, могу се екстраполирати под одређеним условима у циљу сузбијања осица јабуке и крушке.
4. Научни допринос дисертације огледа се у примјени новог приступа у сузбијању осица шљиве, коришћењем ентомопатогених нематода. Сузбијање имага ентомопатогеним нематодама није уобичајено, а ово истраживање је показало да такав приступ може бити изузетно ефикасан. Такође, кроз дисертацију је показано

да у природним стаништима у БиХ обитавају најмање 4 врсте ентомопатогених нематода, што представља први налаз ЕПН у БиХ.

Цитирана литература:

Akhurst, R.J. (1983). species: specificity of the association with bacteria of the genus *Xenorhabdus*. *Experimental Parasitology* 55, 258–263.

Andreev, R. and Kutinkova, H. (2010). Possibility of reducing chemical treatments aimed at control of plum insect pests. IX International Symposium on Plum and Prune Genetics, Breeding and Pomology. *ISHS Acta Horticulturae* 874, 215-220.

Ansari, M.A., Tirry, L. and Moens, M. (2004). Interaction between *Metarhizium anisopliae* CLO 53 and entomopathogenic nematodes for control of *Hoplia philantus*. *Biological Control* 31, 172-180.

Arthurs, S., Heinz, K.M. and Prasifka, J.R. (2004). An analysis of using entomopathogenic nematodes against above-ground pests. *Bulletin of Entomological Research* 94, 297-306.

Babendreier, D. (2000). Life history of *Aptesis nigrocincta* (Hymenoptera: Ichneumonidae), a cocoon parasitoid of the Apple Sawfly, *Hoplocampa testudinea* (Hymenoptera: Tenthrinidae). *Bulletin of Entomological Research* 90, 291-297.

Baumgartner, J., Graf, B. and Zahner, P. (1984). A stochastic population model to simulate the annual growth pattern of mature Golden Delicious apple tree. *Schweizerische Landwirtschaftliche Forschung* 23, 489-501.

Batalla-Carera, L. Morton, A. and Garcia del Pino, F. (2010). Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *BioControl* 55, 523-530.

Bathon, H. (1996). Impact of entomopathogenic nematodes on non-target hosts. *Biocontrol Science and Technology* 6, 421-434.

Bedding, R. A., and A. S. Molyneux. (1982). Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica* 28, 354–359.

Belair, G., Vincent, C. and Chouinard, G. (1998). Foliar sprays with *Steinernema carpocapsae* against early-season apple pests. *Supplement to the Journal of Nematology* 30, 599– 606.

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T. and Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* 392, 71–75.

Bovien, P. and Stapel, C. (1940). Forsøg med bekæmpelse af blommenvespen (*Hoplocampa fulvicornis*). *Tidsskrift for Planteavl* 44, 699–730.

Brinkman, M.A. and Gardner, W.A. (2000). Possible antagonistic activity of two entomopathogens infecting workers of the red imported fire ant (Hymenoptera : Formicidae*). Journal of Entomological Science* 2, 205-207.

Bedding, R.A. and Akhurst R.J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21, 109–110.

Campbell, J.F. and Gaugler, R. (1993). Nictation behavior and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* 126, 155-169.

Campos–Herrera, R., Escuer, M., Labrador, S., Robertson, L., Barrios, L., and Gutierrez, C. (2007). Distribution of the entomopathogenic nematodes from La Rioja (Northern Spain). *Journal of Invertebrate Pathology* 95, 125–139.

Caruso, S. and Cera, M. C. (2004). Control strategies for Plum Sawflies (*Hoplocampa flava, Hoplocampa minuta*) in organic farming. *IOBC/WPRS Bull.*27 (5), 107–111.

Chen, S., LI, J., Han, X. and Moens, M.(2003). Effect of temperature on the pathogenicity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) to *Delia radicum*. *BioControl* 48, 713-724.

Choudhury, B.N. and Sivakumar, C.V. (2000). Biocontrol potential of *Mylonchulus minor* against some plant-parasitic nematodes. *Annals of Plant Protection Science* 8, 53-57.

Ciche, T.A. Darby, C., Ehlers, R.-U., Forst, S. and Goodrich-Blair, H. (2006). Dangerous liaisons: The symbiosis of entomopathogenic nematodes and bacteria. *Biological Control* 38, 22-46.

Cobb, N. (1914). Nematodes and their relationships. *Yearbook United States Department of Agriculture* pp. *457–490.*

Cuthbertson, A.G.S., Walters, K.F.A, Northing, P. and Luo, W. (2007). Efficacy of the entomopathogenic nematode, *Steinernema feltiae*, against sweetpotato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) under laboratory and glasshouse conditions. *Bulletin of Entomological Research* 97, 9-14.

Curto, G., Boselli, M., Vergnani, S. and Reggiani, A.. (2007). Effectiveness of entomopathogenic nematodes in the control of sawflies (*Hoplocampa brevis*) in pear orchards. *IOBC/wprs Bulletin* 30 (1), pp.13-17.

Danelski, W., Badowska-Cyubik, T., Rozpara, E. and Pniak, M. (2014). A study on the possibility of limiting damage to frit by the apple sawfly (*Hoplocampa testudinea* Klug) in organic apple orchards. *Journal of Research and Applications in Agricultural Engineering* 59, 27-30.

Danks, H.V. (2004). The roles of insect cocoon in cold conditions. *European Journal of Entomology* 101, 433-437.

De Lay, P. and Blaxter, M. (2002). Systematic position and phylogeny. In: Lee D.L. (ed.) *Biology of Nematodes*. London: Taylor and Francis, pp. 1-30.

Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 Establishing a framework for Community action to achieve the sustainable use of pesticides (O. J. L 309, 24.11.2009), 71-86.

Ehlers, R-U. (2001). Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology Biotechnology* 56, 623-633.

Ehlers, R.-U. (2003). Biocontrol nematodes. In: Hokkanen, H.M.T. and Hajek, A. (eds.). *Environmental Impact of Microbial Insecticides: Need and Methods for Risk Assessment*. Kluwer Scientific Publisher, Dordrecht, NL, pp.170-220.

Ehlers, R.-U. (2005). Forum on Safety and Regulations. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.). *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 107-114.

Ehlers, R.-U. and Shapiro-Ilan, D. (2005). Mass production. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 65-77.

Ehlers, R.-U. (2007). Entomopathogenic nematodes: from science to commercial use. In: Vincent, C., Goettel, M.S. and Lazarovits, G. (eds). *Biological Control: A Global Perspective*, pp. 136-151.
EPPO (2016). List of biological control agents widely used in the EPPO region - PM 6/3. Available at: http://archives.eppo.int/EPPOStandards/biocontrol_web/bio_list.htm.
FAOSTAT (2016). Food and Agricultural Organisation of the United Nations Statistics. *Available online at http://www.fao.org/documents/card/en (accessed 8 February, 2017).*
Faria, M. and Wraight, S. (2007). Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control* 43, 237– 256.
Garcia del Pino, F. and Morton, A. (2005). Efficacy of entomopathogenic nematodes against neonate larvae of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae) in laboratory trials. *Biocontrol* 50, 307-316.
Glaser, R.W. and Farrell, C.C. (1935). Field experiments with Japanese beetle and its nematode parasite. *Journal of the New York Entomological Society* 43, 345-371.
Gradinarov, D., Petrova, E., Mutafchiev, Y. and Karadjova, O. (2012). Distribution of entomopathogenic nematodes of the genus *Heterorhabditis* (Rhabditida: Heterorhabditidae) in Bulgaria. *Nematologia Mediterranea* 40, 173-180.
Graf, B., Höhn, H. and Höpli, H.U. (1996a). The apple sawfly, *Hoplocampa testudinea*: a temperature driven model for spring emergence of adults. *Entomologia Experimentalis et Applicata* 78, 301–307.
Graf, B., Höhn H. and Höpli, H.U. (1996b). Moddeling spring emergence of the apple sawfly *Hoplocampa testudinea* Klug (Hymenoptera, Tenthredinidae). *Acta horticulturae* 416 (416): 263-272.
Graf, B., Höhn, H. and Höpli, H.U. (1996c). Optimizing the risk assessment for the apple sawfly *Hoplocampa testudinea* Klug (Hymenoptera, Tenthredinidae). *Acta horticulturae* 422,113-122.
Graf, B., Höhn H. & Höpli, H.U. (2002).The apple sawfly, *Hoplocampa testudinea*: egg development and forecasting of egg hatch. *Entomologia Experimentalis et Applicata* 105, 55–60.
Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (2005). Nematodes as biocontrol agents. Wallington: CABI Publishing.
Griffin, C.T., Dix, I., Joyce, S.A., Burnell, A.M. and Downes, M.J. (1999). Isolation and characterisation of *Heterorhabditis* spp. (Nematoda: Heterorhabditidae) from Hungary, Estonia and Denmark. *Nematology* 1, 321–332.
Griffin, C.T., O'Callaghan, K. and Dix, I.A. (2001). Self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematodes? *Parasitology*. 122, 181–186.
Griffin, C.T., Boemare, N.E., and E.E. Lewis (2005). Biology and bahaviour. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 47-64.
Gouy M., Guindon S. and Gascuel O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27, 221-224.
Happe, S., Nježić, B. and Ehlers, R.-U. (2016). Control of apple sawfly (*Hoplocampa testudinea* Klug) and plum sawflies (*H. flava* L. and *H. minuata* Christ.) with entomopathogenic nematodes. *Proceedings of 17th International Conference on Organic Fruit-Growing*, Hohenheim 15-17 February 2016, 258-260.
Hominick, W.M. (2002). Biogeography. *In:* R. Gaugler (ed.) *Entomopathogenic Nematology*. Wallingford, UK: CABI Publishing, pp.115–143.
Hominick, W.M., Briscoe, B.R., del Pino, F.G., Heng, J., Hunt, D.J., Kozodoi, E, Mracek, Z., Nguyen, K.B., Reid, A.P., Spiridonov, S., Stock, P., Sturhan, D., Waturu, C. and Yoshida, M. (1997). Biosystematics of entomopathogenic nematodes: current status, protocols, and definitions. *Jurnal of Helminthology* 71, 271-298.
Hugot, J.P., Baujard, P. and Morand, S. (2001). Biodiversity in helminths and nematodes as a field study: an overview. *Nematology* 3, 199-208.
Jarošik, V., Honěk, A., Magarey, R.D. and Skuhrovec J. (2011). Developmental database for phenology models: Related insect and mite species have similar thermal requirements. *Journal of Economic Entomology* 104, 1870-1876.
Lacey, L. and Georgis R. (2012). Entomopathogenic nematodes for control of insects pests above and below ground with comments on commercial production. *Journal of Nematology* 44, 218-225.
Johnigk, S.-A. and Ehlers, R.-U. (1999). Endotokia matricida in hermaphrodites of *Heterorhabditis* spp. and the effect of the food supply. *Nematology* 1, 717–726.
Joyce, S.A., Reid, A., Driver, F. and Curran, J. (1994). Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. In: Burnell, A.M., Ehlers, R.U., Masson J.P. (eds.) *Biotechnology: Genetics of Entomopathogenic Nematode Bacteria Complexes*. European Commission, Luxembourg, DG XII, pp. 178-187.
Kaya, H.K. (2002). Natural enemies and other antagonists. In: Gaugler, E. (ed.), *Entomopathogenic Nematology*. Wallington: CABI Publishing, pp.189-204.
Koppenhöfer, A.M., Kaya, H.K. and Taormino, S.P. (1995). Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. *Journal of Invertebrate Pathology* 65, 193–199.
Koppenhöfer, A.M. and Kaya, H. K. (1997). Additive and synergistic interaction between entomopathogenic nematodes and *Bacillus thuringiensis* for scarab grab control. *Biological Control* 8, 131-137.
Koppenhöfer, A.M. (2007). Nematodes. In: Lacey, L.A. and Kaya, H.K. (eds.), *Field manual of techniques in invertebrate pathology*. Springer Netherlands, pp. 249-264.
Hunt, D.J. (2007). Overview of taxonomy and systematics. In: Nguyen, K.B. and Hunt, D.J. (eds). *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts 5*. Brill, pp. 27-57.
Lacey, L. A., Shapiro-Ilan, D. I., and Glenn, G. M. (2010). Post-application of anti-desiccant agents improves efficacy of entomopathogenic nematodes in formulated host cadavers or aqueous suspension against diapausing codling moth larvae (Lepidoptera: Tortricidae). *Biocontrol Science and Technology* 20, 909-921.
Lacey, L. and Georgis R. (2012). Entomopathogenic nematodes for control of insects pests above and below ground with comments on commercial production. *Journal of Nematology* 44, 218-225.
Lacey, L., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M. and Goettel, M.S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology* 131, 1-41.
Laznik, Ž. and Trdan, S. (2012). Entomopathogenic nematodes (Nematoda: Rhabditida) in Slovenia: from tabula rasa to implementation into crop production systems. In: Perveen, F. (ed.). *Insecticides – Advances in Integrated Pest Management.*

Intech, Rijeka, Croatia, pp. 627-656.
Llacer, E., Martinez de Altube, M.M. and Jacas, J.A. (2009). Evaluation of the efficacy of *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil, *Rhynchophorus ferrugineus*, in *Phoenix canariensis*. *BioControl* 54, 559-565.
Lootsma, M. and Scholte, K. (1997). Effects of springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* on *Rizoctonia solani* stem infection of potato at temperatures of 10 and 15°C. *Plant Pathology* 46, 203-208.
Ludwig, D. (1928). The effects of temperature on the development of an insect (*Popilia japonica* Newman). *Physiological Zoology* 1, 358-389.
Menti, H., Wright, D. and Perry, R. (1997). Desiccation survival of populations of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* from Greece and the UK. *Journal of Helminthology* 71, 41-4.
Mićić, N., Đurić, G. and Životić, A. (2015). Yield potential of long bearing shoots of ten plum cultivars (*Prunus domestica* L.). *Agro-Knowledge Journal* 1, 15-19.
Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010) Creating the CIPRES Science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp. 1 - 8.
Mráček, Z. and Becvar, S. (2000). Insect aggregations and entomopathogenic nematode occurrence. *Nematology* 2, 297–301.
Neupane, D. (2012). Apple sawflly (*Hoplocampa testudinea*, Klug) situation, forecasting, minitoring and use of extract from *Quassia amara* for controling the apple sawfly in organic apple orchards in Sweden. *Master thesis at Alnarp* Univerity, Sweden, pp. 1-50.
Nguyen, K.B., Hunt D.J. (2007). Heterorhabditidae: species descriptions. In: Nguyen, K.B., Hunt, D.J. (Eds) *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts. Nematology Monographs and Perspectives,* 5. Brill, Leiden, The Netherlands, pp. 611 – 692.
Oroian I., Oltean I., Odagiu A., Paulette L., Iederan C. and Brasovean I. (2009). The control and monitoring of the orchard pests in Transylvania. *Research Journal of Agricultural Science,* 41, 277–283.
Panini, M., Manicardi, G.C., Moores, G.D. and Mazzoni, E. (2016). An overview of the main pathways of metabolic resistance in insects. *Invertebrate Survival Journal* 13, 326-335.
Peters, A. (1996). The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. *Biocontrol Science and Technology* 6, 389-402.
Peters, A. and Ehlers, R-U. (1994). Susceptibility of Leatherjackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. *Journal of Invertebrate Pathology* 63, 163-171.
Poinar, G.O. (1993). Origins and phylogenetic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema*. *Fundamental and Applied Nematology* 16, 333-338.
Premachandra, W.T.S.D, Borgemeister, C., Berndt , O., Ehlers, R.-U. and Poehling, H.-M. (2003). Combined releases of entomopathogenic nematodes and the predatory mite *Hypoaspis aculeifer* to control soil-dwelling stages of western flower thrips *Frankliniella occidentalis*. *BioControl* 48, 528-543.
Půža V., Mráček Z., Nermut, J. (2016). Novelties in pest control by entomopathogenic and mollusc-parasitic nematodes. In: Harsimran G. (ed.), *Integrated Pest Management (IPM): Environmentally Sound Pest Management.* InTech, pp. 71-102.
Rainey, F.A., Ehlers, R.-U. and Stackebrandt, E. (1995). Inability of the polyphasic approach to systematics to determine the relatedness of the genera *Xenorhabdus* and *Photorhabdus*. *International Journal of Systematic Bacteriology* 45, 339–381.
Riga, E., Lacey, L.A., Guerra, N. and Headrick, H.L. (2006). Control of the oriental fruit moth, *Grapholita molesta*, using entomopathogenic nematodes in laboratory and fruit bin assays. *Journal of Nematology* 38, 168-171.
Rosa, A.J.S., Boinifassi, E., Ameral, J., Lacey, L.A., Simoes, N. and Laumond, C. (2000). Natural occurrence of entomopathogenic nematodes (Rhabditida: *Steinernema*, *Heterorhabditis*) in the Azores. *Journal of Nematology* 32, 215-222.
Rozpara E., Badowska-Czubik T. and Kowalska J. (2010). Problems of the plum and cherry plants protection in ecological orchard. *Journal of Research and Applications in Agricultural Engineering,* 55, 73–75.
Schroer, S. and Ehlers, R.-U. (2005). Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*). *Biological Control* 33: 81-86.
Shapiro-Ilan, D.J. (2001a). Virulence of entomopathogenic nematodes to pecan weevil adults (Coleoptera: Curculionidae). *Journal of Entomological Science* 36, 325-328.
Shapiro-Ilan, D. I. (2001b). Virulence of entomopathogenic nematodes to pecan weevil larvae *Curculio caryae* (Coleoptera: Curculionidae) in the laboratory. *Journal of Economic Entomology* 94, 7–13.
Shapiro-Ilan, D.I., Cottrell, T.E., Mizell, R.F., Horton, D.L. and Davis, J. (2009). A novel approach to biological control with entomopathogenic nematodes: Prophylactic control of the peachtree borer, *Synanthedon exitiosa*. *Biological Control* 48, 259-263.
Shapiro-Ilan, D.I, Morales-Ramos, J.A., Rojas, M.G. and Tedders, W.L. (2010). Effects of a novel entomopathogenic nematode-infected host formulation on cadaver integrity, nematode yield, and suppression of *Diaprepes abbreviatus* and *Aethina tumida*. *Journal of Invertebrate Pathology* 103:103-108.
Shapiro-Ilan, D.I., Han, R. and Dolinksi, C. (2012). Entomopathogenic nematode production and application technology. *Journal of Nematology* 4, 206-217.
Sjöberg, P., Swiergiel, W., Neupane, D.,Lennartsson, E., Thierfelder, T., Tasin, M. and Rämert, B. (2015). Evaluation of temperature sum models and timing of *Quassia amara* (Simaroubaceae) wood-chip extract to control apple sawfly (*Hoplocampa testudinea* Klug) in Sweden. *Journal of Pest Science*; 88, 301–310.
Smits, P. (1996). Post-application persistence of entomopathogenic nematodes. *Biocontrol Science and Technology* 6, 379–387.
Somasekhar, N., Grewal, P.S., De Nardo, E.A.B. and Stinner, B.R. (2002). Non-target effects of entomopathogenic nematodes on the soil nematode community. *Journal of Applied Ecology* 39, 735-744.
Steiner, G. (1923). *Aplectana kraussei* n. sp., eine in der Blattwespe *Lyda* sp. parasitierende Nematodenform, nebst Bemerkungen über das Seitenorgan der parasitischen Nematoden. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankenheiten und Hygiene, Abteilung* 2, 14-18.

Stock, S.P. and Hunt, D.J. (2005). Morphology and systematics of nematodes used in biocontrol. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 3-43.
Stuart, R.J., Barbercheck, M.E., Grewal, P.S., Taylor, R.A.J. and Hoy, C.W. (2006). Population biology of entomopathogenic nematodes: Concepts, issues, and models. *Biological Control* 38, 80-102.
Tallosi, B., Peters, A. and Ehlers, R. U. (1995). *Steinernema bicornutum* sp. n. (Rhabditida: Steinernematidae) from Vojvodina, Yugoslavia. *Russian Journal of Nematology* 3, 71-80.
Tamošiunas R, Valiuškaite A. (2013). The study on temperature sum model for predicting apple sawfly spring emergence and flight intensity in Lithuania. *Scientific works of the institute of horticulture, Lithuanian research center for agriculture and forestry*. 32: 23-36.
Tamošiunas, R, Valiuškaite, A., Survieliene, E. and Rasiukevičiute, N. (2014). Species ratio, spring emergence, population dynamics and damage of plum sawflies *Hoplocampa minuta* and *H. flava* in plum orchard. *Zembdirbyste-Agriculture* 101,91-100.
Tarasco, E., Clausi, M., Rapazzo, G., Panzavolta, T., Curto, G., Sorino, R., Oreste, M., Longo, A., Leone, D., Tiberi, R., Vinciguerra, M.T. and Triggiani, O. (2014). Biodiversity of entomopathogenic nematodes in Italy. *Journal of Helminthology* 10, 1–8.
Toepfer, S. Peters, A., Ehlers, R.-U. and Kuhlmann, U. (2008). Comparative assessment of the efficacy of entomopathogenic nematode species at reducing western corn rootworm larvae and root damage in maize. *Journal of Applied Entomology* 132: 337-348.
Tomalak, M., Piggott, S., and Jagdale, G.B. (2005). Glasshouse application. In: Grewal, P.S. Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallington: CABI Publishing, pp. 191-214.
Tomalak, M. (2006).Potential of entomopathogenic nematodes for control of orchard and urban tree sawflies. *Progress in Plant Protection* 46, 249-255.
Tóth, T. (2006). Collection of entomopathogenic nematodes for the biological control of insect pests. *Journal of Fruit and Ornamental Plant Research* 14, 225- 230.
Ulu, T.C., Sadic, B., Susurluk, I.A. and Aksit, T. (2015). Virulence of four entomopathogenic nematode species for plum sawfly, *Hoplocampa flava* L. (Hymenoptera: Tenthredinidae). *Invertebrate Survival Journal* 12, 274-277.
Vincent, C. and Belair, G. (1992): *Biocontrol* of the *apple sawfly*, *Hoplocampa testudinea*, with entomogenous nematodes. *Entomophaga* 37, 125-149.
Weiser, J. (1955). *Neoaplectana carpocapsae* n. sp. (Anguillata, Steinernematidae) novy cizopasnik housenek obalece jablecneho, *Carpocapsae pomonella* L.. *Vestnik Ceskoslovenske Spolecnosti Zoologicke* 19, 44–52.
White, G. F. (1929). A method for obtaining infective nematode larvae from cultures. *Science* 66, 302–303.
Williams, C.D., Dillon, A.B., Harvey, C.D., Hennessy, R., Mc Namara L. and Griffin C.T. (2013). Control of major pest of forestry, *Hylobius abietes*, with entomopathogenic nematodes and fungi using eradicant and prophylactic strategies. *Forest Ecology and Management* 305, 212-222.
Wilson, M.J. and Gaugler, R. (2000). Terrestial molusc pests. In: Lacey, L. and Kaya, H.K. (eds). *Field Manual of Techniques in Invertebrate Pathology*. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp. 787-804.
Woodring, J. L. and Kaya, H. K. (1998). Steinernematid and heterorhabditid nematodes: Hand book of biology and techniques. *South Cooperative Series Bulletin of the Arkansa Agricultural Experimental Station, Fayetteville* 331, 1-30.
Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. and Georgieva, S.S. (1993). Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology* 25, 315–331.
Zijp, J.P. and Blommers, L. (1993). *Lathrolestes ensator*, a parasitoid of the apple sawfly. *Proceedings of the Section Experimental and Applied Entomology*, N.E.V. Amsterdam 4, 237–242.
Zijp, J.P. and Blommers, L.H.M. (1997). Prediction of flight of apple sawfly, *Hoplocampa testudinea*, using temperature sums. *Entomologia Experimentalis et Applicata* 84, 71-75.
Zijp, J.P. and Blommers L. (2002). Apple sawfly *Hoplocampa testudinea* (Hym., Tenthredinidae) and its parasitoid *Lathrolestes ensator* in Dutch apple orchards (Hym., Ichneumonidae, Ctenopelmatinae). *Journal of Applied Entomology* 126, 265-274.

1) Укратко истаћи разлог због којих су истраживања предузета и представити проблем, предмет, циљеве и хипотезе;
2) На основу прегледа литературе сажето приказати резултате претходних истраживања у вези проблема који је истраживан (водити рачуна да обухвата најновија и најзначајнија сазнања из те области код нас и у свијету);
3) Навести допринос тезе у рјешавању изучаваног предмета истраживања;
4) Навести очекиване научне и прагматичне доприносе дисертације.

V МАТЕРИЈАЛ И МЕТОД РАДА

1) Сви огледи и испитивања су добро организовани, а методе су правилно одабране и примјењене. Поглавље 3 се односи на преглед присуства ЕПН у БиХ и њихову идентификацију. Узорци су узети са цијеле територије Босне и Херцеговине. Укупно је узет 221 узорак и испитан на присуство ЕПН. Мјеста узорковања су била на удаљености до 100 m од проходних путева, а одабрана су на основу типа вегетације и приступачности. За сваки локалитет узети су подаци о типу вегетације, ГПС координатае и надморска висина локалитета. Цијели земљишни узорак је по

доношењу у лабораторију измијешан од чега је узето 250 ml узорка и стављено у пластичну посуду од 400 ml. Десет јединки посљедњег ларвалног стадијума восковог мољца (*Galleria mellonella* L.) је стављено на површину земље и посуда је поклопљена и окренута наопачке. Мртве ларве су сакупљане у интервалима од 3 дана и стављане на Вајтову клопку. Добијене нематоде су умножене *in vivo* на ларвама восковог мољца и чуване на 6°C. Да би се идентификовали добијени изолати извршена је молекуларна идентификација. ДНК је изолована из 20 индивидуа дауер стадијума. Умножена ДНК је послата у Макроген (Холандија) гдје је извршено секвенционирање. Добијене ДНК секвенце су упоређене са базом података у GenBank уз помоћ Basic Local Alignment Search Tool (BLAST) Националног центра за биотехнолошку информатику (the National Center for Biotechnology Information, NCBI). Добијене секвенце су филогенетски поређене са секвенцама из GenBank уз помоћ програма SeaView. и Mr Bayes 3.2.6.. *Oscheius* sp. добијен у процесу мониторинга и *Caenorhabditis elegans* из GenBank су кориштени као аутгруп.

Поглавље 4 се односи на оцјену ефикасности ЕПН у сузбијању осица шљиве. Испитивања и огледи су обављени у лабораторијским и пољским условима. Кориштени су комерцијални препарати три врсте ЕПН *Steinernema feltiae, S. carpocapsae* и *Heterorhabditis bacteriophora*, компаније Е-нема из Њемачке. За лабораторијски оглед кандидат је сакупио ларве осица шљиве из воћњака који се налази у Бањој Луци. Почетком маја, прије него су први плодови оштећени од осице шљиве пали на земљу, постављене су мреже за заштиту од инсеката су испод стабала шљиве. На мреже су падали плодови који су свакодневно сакупљани и стављени у канту са стерилним кварцни пијеском. Ларве које су напустиле плодове су сакупљане и кориштене у експериментима.Кориштене су ларве четри старости: оне које су тог дана изашле из плода, те ларве које су прије 10, 20 и 40 дана формирале кокон. 10 ларви или кокона је стављено у петријеву посуду пречника 5,5 cm у коју је стављено 10 g стерилног кварцни пијесак величине 180-500 µm овлажен са дестилованом водом до 10 % запреминске влаге. За фолијарну примјену гране са оштећеним плодвима донешене су у лабоарторију и третиране ЕПН. Након 3 дана плодови су отворени, те утврђен морталитет ларви. Тестирање осјетљивост ларви у пољским условима рађено је у 10 година старом воћњаку. Три препарата ЕПН кориштених у лабораторијским испитивањима кориштени су и у пољском огледу. Нематоде су аплициране 26.04.2014. године у дози 0.5 x 10^6 m^{-2} са кантом за залијевање, прије очекиваног силазка првих ларви у земљу. Апликација препарата је урађена у редном простору ширине два метра. Нематоде су аплициране са 0.75 l m^{-2} воде, уз додатних 1.5 l m^{-2} чисте воде након апликације средстава да би се утврдила ефикасност. Број имага са сваке третиране површине утврђен је у 2015. и 2016. години. Прије очекиваног излијетања имага постављене су изнад третиране површине мреже за заштиту од инсеката. Испод сваке мреже постављена је бијела љепљива плоча. На карју лета осице бијеле плоче су прегледане и утврђен је број јединки у сваком третману и у контроли. Оглед са утврђивањем ефикасности у сузбијању имага у полуконтролисаним пољским условима рађен је у 2013., 2014. и 2015. години. Да би се третирана површина изоловала од остатка воћњака стабла су прекривана мрежом за заштиту од инсеката. Мрежа је стављена преко привремене жељезне конструкције која је направљена преко 12 стабала. Мрежа је учвршћена а унутрашњост тунела подијељена је на 4 дијела. Мрежа је прекривала редни простор ширине 2 m а била је дугачка 24 m. Мрежа је анти трипс величине отвора 150 x 490 µm или за заштиту од комараца отвора 1 x 1 mm. Нематоде су аплициране у редном простору у дози од 0.5 x 10^6 m^{-2} са кантом за залијевање прије очекиване еклозије имага осице шљиве. Процјена очекивене еклозије првих имага одређивана је на основу појаве првих бијелих балона, а потврђена постављањем бијелих љепљивих

плоча. Да би се утврдила ефикаснот појединих врста нематода вршено је бројање оштећених плодова. У ту сврху мрежа је постављена испод стабала крајем априла, прије почетка отпадања плодова усљед оштећења од осица шљиве. За огледе на отвореном пољу одабрани су по могућности органски воћњаци са великим процентом оштећења у претходној години. Одабрани воћњаци су из Хрватске и БиХ. Нематоде су аплициране помоћу атомизера или тракторских прскалица у двије дзе. Пуна доза је износила 0.5 x 10^6 m^{-2} а примјењивана је и упола мања доза. Нематоде су аплициране са 0,2 литра воде m^{-2}. У воћњацима у Хрватској у року од 30-так минута аплицираниo је 0,2 l/m^2 док је за вријеме апликације у воћњаку у БиХ падала киша. У вријеме отпадања латица 12-13 стабала је прегледано. Преглед је подразумијевао утврђивање присуства положених јаја на 50 цвјетова по стаблу. Подаци су упоређени коришћењем општег линеарног модела са одговарајућим тестирањем у случају утврђивања статистичке разлике. Статистички значајна разлика је подразумијевана уколико је $p<0.05$.

Поглавље 5 обрађује еклозију имага осица шљиве засновану на доњем температурном прагу развоја, акумулираној температури у условима сјеверне Босне и Херцеговине. Праћење је вршено током 5 година 2012-2016. године у воћњаку површине 0,2 ha са 45 стабала шљиве смјештен у Бањој Луци. Сорте шљиве су чачанска родна, чачанска љепотица и стенлеј калемљени на џанарику (*Prunus cerasifera* Ehrh.). Сваке године прије почетка лета у воћњак је постављано 5 ручно израђених бијелих љепљивих плоча. Плоче су направљене од бијеле цераде димензија 22 x 13 cm. На обје стране постављене су безбојне љепљиве плоче. Овако направљене клопке објешене су у вањски дио крошње на висину од 1,5 m. Клопке су провјераване дневно на присуство имага осице шљиве. Мјерене су средње дневне температуре ваздуха на 2 m изнад земље, те температура земље на дубини 5 cm and 10 cm су вршена у метеролошкој станици Бања Лука. Метеоролошка станица је удаљена 4,5 km од воћњака.

2) 1. Примјењене методе су адекватне, прецизне и савремене. Резултати истраживања су представљени јасно.
2. Није било одступања од плана истраживања.
3. Истраживани параметри дају довољно информација за доношење научно заснованих закључака.
4. Статистичка обрада података је адекватно урађена.

1) Објаснити материјал који је обрађиван, критеријуме који су узети у обзир за избор материјала;
2) Дати кратак увид у примијењени метод истраживања при чему је важно оцијенити сљедеће:
1. Да ли су примијењене методе истраживања адекватне, довољно тачне и савремене, имајући у виду достигнућа на том пољу у свјетским нивоима;
2. Да ли је дошло до промјене у односу на план истраживања који је дат приликом пријаве докторске тезе, ако јесте зашто;
3. Да ли испитивани параметри дају довољно елемената или је требало испитивати још неке, за поуздано истраживање;
4. Да ли је статистичка обрада података адекватна.

VI РЕЗУЛТАТИ И НАУЧНИ ДОПРИНОС ИСТРАЖИВАЊА

1. Резултати мониторинга на присуство ЕПН показали су да су у БиХ присутне четири врсте ентомопатогених нематода. *S. feltiae* је врста која је нађена у највише узорака, након чега слиједе *S. kraussei* и S. *carpocapsae*. *H. bacteriophora* је једина врста из рода *Heterorhabditis*. И поред географске разноликости БиХ, већина позитивних узорака је из средишњег брдско-планинског региона. Једина врста која је нађена у равничарском предјелу је *H. bacteriophora*. Карактеристике вегетације локалитета са

којих су нематоде изоловане одговарају литературним наводима. Двије популације *S. kraussei* су нађене на локалитетима у планинском региону гдје је средња дневна температура испод 9°C. *S. feltiae* је нађена на локалитетима са различитих надморских висина и типова вегетације, што потврђује њено космополитско распрострањење. *S. carpocasae* је изолована са локалитета са глиновитим земљиштем на којем се екстанзивно гаје шљиве. Значајан број узорака узет је из шума, али у њима нису нађене ЕПН. Наредни мониторинг, који би укључивао локалитете са већом бројношћу инсеката, могао би имати већу шансу за проналазак популација ЕПН. У домаћој легислативи је обавезно да се потврди присуство врсте у природној средини прије него се да дозвола средству да се користи у биолошкој заштити. Због тога, резултати овог мониторинга представљају основу за регистрацију четири врсте ентомопатогених нематода за биолошку контролу.

Резултати оцјене ефикасности ЕПН у сузбијању осица шљиве. Кроз ово истраживање, се показало да ЕПН имају потенцијал за сузбијање осица шљиве. У свим истраживањаима циљани животни стадијум је била ларва. Кроз ово истраживање, поред ларви показано је и да се имага осица шљиве могу сузбијати са ЕПН. Земљишни стадијуми осица шљиве су осјетљиви на ЕПН, али искључиво покретни стадијуми. Ларве унутар кокона су заштићене, јер ЕПН не могу да продру кроз кокон. Само покретни стадијуми су осјетљиви на ЕПН, ларве током кретања кроз земљу, прије него формирају кокон и имага током еклозије. Кокон представља механичку баријеру која штити ларве од ЕПН и за нематоде представља непремостиву препреку. Поред ЕПН, кокон представља механичку баријеру и заштиту за ларву и од других природних непријатеља. Ларва у кокону или лутка, представљају потенцијално најпогоднији стадијум за третман са ЕПН, пошто би сви чланови популације могли да се сузбију у једном тренутку. У овом стадијуму осице шљиве проведу готово 10 мјесеци што би оставило више него довољно времена за апликацију ЕПН. Међутим, ово истраживање је показало да ови стадијуми нису погодни за сузбијање. ЕПН нематоде могу да инфицирају током кратког времена када се ларве или имага крећу кроз земљу.

Док се у лабораторијским условима мораталитет ларви осица шљиве није разликовао у зависности од врсте ЕПН, најмањи број имага је ухваћен у третманима са *H. bacteriophora*, иако су све три врсте имале ефикасност преко 90% након апликације ЕПН кроз двије узастопне године. На ефикасност ЕПН у пољу могу утицати услови животне средине као што су температура, влажност, прозрачност и механички састав земљишта, али и биотски фактори као што су бројност организма који се сузбија, те присуство осталих организама у земљишту.

Приликом апликације ЕПН за сузбијање ларви осица шљиве, потребно је обезбиједити да су нематоде присутне у земљишту прије него прве ларве падну на земљу и почну да праве коконе. У условима сјеверне БиХ овај процес почиње крајем априла или почетком маја и траје отприлике три седмице. Да би се постигла жељена ефикасност потребо је да су нематоде способне да паразитирају ларве осица сво ово вријеме. Ларве најчешће формирају кокон у површинском слоју земљишта на дубини 0-5 cm а веома ријетко и до дубине од 20 cm. Пошто нематоде не треба да трагају за ларвама осица, већ да "чекају", ЕПН које имају изражену карактеристику за активним тражењем домаћина нису у предности у односу на врсте које домаћина чекају на једном мјесту. Међутим, нематоде треба да буду способне да најмање три седмице задрже високу бројност и виталност у површинском слоју земљишта да би биле способне да изврше паразитирање ларви осица. Влажност земљишта је најзначајнији абиотски фактор који утиче на ефикасност ЕПН. Због тога би било неопходно држати влажност земљишта близу пољског водног капацитета, чиме би се обезбиједили оптимални услови за дјеловање нематода. Међутим, уколико се третирају имага, онда се период апликације помјера за неких мјесец до 45 дана, чиме

се обезбјеђују бољи еколошки услови за дјеловање нематода. Темепратура земљишта у вријеме еклозије имага је значајно нижа у односу на вријсме када ларвс падају на земљу. У условима сјеверне БиХ почетак цвјетања шљиве и еклозије имага осица шљиве у периоду 2012-2016. године се кретао од средине марта до средине априла. Поред тога еклозија имага се дешава кроз знатно краћи период времена у поређењу са силаском ларви у земљиште.

Ефикасност ЕПН у полуконтролисаним пољским огледима за сузбијање имага осица шљиве је варирала током три године. Ипак, *S. feltiae* се показала као врста која посједује најбољи потенцијал за сузбијање осица шљиве. Разлог за ово би могао бити и темепратура у вријеме апликације и очекиваног дјеловања нематода. Током цвјетања, температуре земљишта су нешто ниже и врсте које су активније на нижим температурама имају повољније услове. Варијација у ефикасности у појединим огледима у различитим годинама може бити због ралике у временским приликама. Када су апликацији нематода претходиле оборине ефикасност је ишла и до 100%.

У огледима на отвореном пољу ефикасност *S. feltiae* зависила је од концентрације нематода и временских услова прије и током апликације. Највећа ефикасност је постигнута у условима када су нематоде аплициране током кише. Поред тога у овом воћњаку редни простор је био без присутних корова. Апликација ЕПН са 0.2 l/m^2 плус 0.2 l/m^2 воде у року од 30 мин у редном простору на отвореном је дала много мању ефикасност у односу на апликацију нематода у 0,75 l/m^2 плус 1,5 l/m^2 воде непосредно након апликације нематода. Аплициране нематоде треба да доспију на површински слој земљишта, јер је земљиште много повољније станиште за ЕПН у односу на површину листа. У случају када се апликација врши по трави, потребно је значајно више воде да би нематоде доспјеле на површину земље. Апликација нематода са 10 000 литара воде по хектару не чини се изводљивом за произвођаче. Такође кишни период не претходи увијек еклозији имага. Чак и у случају кишног периода, нагиби воћњака често нс дозвољавају апликацију нематода током падавина. Механичка или хемијска обрада редног простора, чиме би се постигло да у вријеме апликације нематода нема травног покривача, дала би значајно повољње услове за апликацију нематода. Када је земљиште било влажно и када су ЕПН аплициране са довољном количином воде њихова ефикасност је достигла ефикасност инсектицида. Такође, обезбјеђењем оптималних услова апаликације могла би се смањивати и доза ЕПН, чиме би се утицало на смањење цијене коштања сузбијања осица шпљиве овим биолошким агенсом.

Резултати еклозије имага осица шљиве. Еклозија имага осицс шљиве у условима Литваније је почетком маја (Tamošiunas, *et al*., 2014). Модел који предвиђа појаву имага је заснован на суми ефективних температура од првог априла. Међутим, у овом истраживању у условима сјеверне БиХ почетак склозије имага се дешавао у периоду од 12.03. до 12.04. сугеришући да је за услове БиХ потребан другачији модел. Најмања вриједност стандардне девијације акумулираних вриједности температуре за земљиште на дубини од 5 cm од времена хватања првог имага током петогодишњег истраживања, била је када је акумулација почињала првог јануара, док је за температуру ваздуха то било 15.01. Акумулирана сума средњих дневних темература изнад прага развоја је износила 169±10 DD за земљиште и 181±8 DD за ваздух када је за температурни праг развоја одабрана вриједност од 4°C. Да би се избјегло кашњење у апликацији средстава у пракси се за тренутак апликације препоручује да се од средње вриједности одузме двострука вриједност стандардне девијације. С тога би у случају да се прати температура земљишта апликација требала бити када се акумулира 149 DD од 01.01., а за температуру ваздуха 165 DD када се акумулација почне од 15.01. У оба случаја температурни праг је 4°C.

Резултати овог истраживања су показали да се ЕПН могу успјешно користити за сузбијање осица шљиве у одређеним условима. Ипак, биолошко сузбијање осица

шљиве је још увијек скупље од сузбијања синтетисаним инсектицидима. У другу руку цијсна коштања производње ЕПН се стално смањују, чиме се стављају у бољу позицију за коришћење од стране произвођача. Ипак произвођачи немају друге могућности за сузбијање осица шљиве у органској производњи. Стога би ЕПН могле бити укључене у сузбијање осица шљиве. У процесу планирања сузбијања осица шљиве произвођачи треба да имају на уму да се ради о штеточинама које имају једну генерацију годишње. То значи да уколико се квалитена апликација изврши током двије године, у наредној години осице шљиве не би требало да могу причинити економске штете. Поред тога, ако се узме у обзир сличност у животном циклусу јабукине и крушкине осице, резултати овог истраживања се могу екстраполирати и за сузбијање ових штеточина, те примјенити сличан концепт сузбијања.

2. Добијени резултати су јасно приказани, те адекватно критички анализирани и интерпретирани. Сазнања до којих се дошло кроз истраживање су веома добро смјештена у контексту литературе која описује сличну проблематику. Кандидат је јасно образложио како његова достигнућа доприносе рјешавању одређене проблематике, као и значај самих достигнућа са научног и практичног аспекта.
3. Најзначајнији научни резултати научног истраживања су:
   I. Утврђено је присуство четири врсте ЕПН на територији БиХ: *S. feltiae*, *S. kraussei*, S. *carpocapsae* и *H. bacteriophora*.
   II. Стадијуми ларве и имага осице шљиве се могу сузбијати ентомопатогеним нематодама. При одређеним условима ЕПН се могу успјешно користити за сузбијање осица шљиве.
   III. Већа ефикасност ЕПН у сузбијању осица шљиве се може постићи тачнијом апликацијом, која је заснована на примјени модела за одређивања почетка лета имага осице.

   Кандидат је навео да се резултати његових истраживања могу интерполирати и за блиске штетне организме осице јабуке и крушке. Такође навео је да је могуће да се сузбијањем осица шљиве може можда истим третманом сузбити и шљивин савијач.

1) Укратко навести резултате до којих је кандидат дошао;
2) Оцијенити да ли су добијени резултати јасно приказани, правилно, логично и јасно тумачени, упоређујући са резултатима других аутора и да ли је кандидат при томе испољавао довољно критичности;
3) Посебно је важно истаћи до којих нових сазнања се дошло у истраживању, који је њихов теоријски и практични допринос, као и који нови истраживачки задаци се на основу њих могу утврдити или назирати.

VII ЗАКЉУЧАК И ПРИЈЕДЛОГ

1. Дисертација кандидата мр Бранимира Њежића обрађује примјену ентомопатогених нематода за сузбијање осица шљиве, као једених од најважнијих штеточина ове воћне врсте. Прегледом доступне литературе је утврђено да досада нема података о успјешном сузбијању осица шљиве са ЕПН или другим биолошким агенсима. Истраживање је обављено у лабораторијским, полуконтролисаним пољским и пољским условима с циљем испитивања могућности сузбијања појединих стадијума осица шљиве ентомопатогеним нематодама. Да би се поспјешила ефикасност примјене ЕПН проучавани су и параметри животног циклуса осица шљиве. Такође, мониторинг на присуство врста ЕПН на територији БиХ је био један од предмета истраживања, што је рађено с циљем остваривања могућности за регистрацију препарата на бази ЕПН. Поред тога, резултати добијени кроз ово истраживање могу се примјенити и за моделирање сузбијања сродних врста јабукине и крушкине осице.

Дисертација је јасно написана и добро документована. Текст је добро написан на јасан и концизан начин. Резултати су јасни и презентовани на јасан начин. Хипотеза је јасно аргументована. Закључци потврђују да је циљ истраживања успјешно остварен. Најважнији резултати истраживања су представљени на већем број интернационалних и националних научних скупова.

Дисертација представља висок ниво оригиналног научног рада. Она укључује различите аспекте рада. Отвара потпуно нове могућности за биолошку контролу осица шљиве ентомопатогеним нематодама, приказује нове параметре животног циклуса ових штеточина и распрострањеност врста ЕПН у БиХ.

2. На основу спроведеног увида, анализа и закључака, Комисија једногласно предлаже Наставно-научном вијећу Пољопривредног факултета у Бањој Луци да прихвати овај позитиван извјештај и прослиједи у даљу процедуру ка Сенату Универзитета у Бањој Луци , и да се кандидату одобри јавна одбрана.

1) Навести најзначајније чињенице што тези даје научну вриједност, ако исте постоје дати позитивну вриједност самој тези;
2) На основу укупне оцјене дисертације комисија предлаже:
- да се докторска дисертација прихвати, а кандидату одобри одбрана,
- да се докторска дисертација враћа кандидату на дораду (да се допуни или измијени) или,
- да се докторска дисертација одбија.

Датум и мјесто: Бања Лука – Кил – Подгорица – Познан – Љубљана 28.07.2017

ПОТПИСИ ЧЛАНОВА КОМИСИЈЕ

1. Проф. др Гордана Ђурић,
редовни професор Пољопривредног факултета Универзитета у Бањој Луци, уже научне области: Хортикултура и Очување генетичких ресурса, предсједник

.....

2. Проф др Ралф Удо Елерс,
ванредни професор на Пољопривредном и прехрамбеном факултету Универзитета у Килу, Њемачка,
ужа научна област: Заштита биља, ментор

.....

3. Проф. др Сњежана Хрнчић,
редовни професор, Биотехничког факултета Универзитета у Подгорици, Црна Гора
ужа научна област: Ентомологија, члан

.....

4. Проф. др Марек Томалак,
редовни професор, Институт за заштиту биља – Национални истраживачки институт, Познан, Пољска,
ужа научна област: Ентомологија и нематологија, члан

.....

5. Проф. др Станислав Трдан,
редовни професор Биотехничког факултета Универзитета у Љубљани, Словенија,
ужа научна област: заштита биљака, члан

.....

ИЗДВОЈЕНО МИШЉЕЊЕ: Члан комисије који не жели да потпише извјештај јер се не слаже са мишљењем већине чланова комисије, дужан је да унесе у извјештај образложење, односно разлог због којих не жели да потпише извјештај.

Изјава 1

Прилог 3.

ИЗЈАВА О АУТОРСТВУ

Изјављујем
да је докторска дисертација

Наслов рада Контрола осица шљиве (*Hoplocampa flava* L. и *Hoplocampa minuta* Christ) ентомопатогеним нематодама

Наслов рада на енглеском језику Control of Plum Sawflies (*Hoplocampa flava* L. and *Hoplocampa minuta* Christ) with entomopathogenic nematodes

- [x] резултат сопственог истраживачког рада,
- [x] да докторска дисертација, у цјелини или у дијеловима, није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- [x] да су резултати коректно наведени и
- [x] да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

У Бањој Луци 17.12.2017.

Потпис докторанта

_____

Изјава 2

Изјава којом се овлашћује Универзитет у Бањој Луци
да докторску дисертацију учини јавно доступном

Овлашћујем Универзитет у Бањој Луци да моју докторску дисертацију под насловом

Control of Plum Sawflies (*Hoplocampa flava* L. and *Hoplocampa minuta* Christ) with entomopathogenic nematodes

која је моје ауторско дјело, учини јавно доступном.

Докторску дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у дигитални репозиторијум Универзитета у Бањој Луци могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (*Creative Commons*) за коју сам се одлучио/ла.

1. Ауторство
2. Ауторство – некомерцијално
3. Ауторство – некомерцијално – без прераде
4. Ауторство – некомерцијално – дијелити под истим условима
5. Ауторство – без прераде
6. Ауторство – дијелити под истим условима

(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

У Бањој Луци 17.12.2017.

Потпис докторанта

Изјава 3

Изјава о идентичности штампане и електронске верзије докторске дисертације

Име и презиме аутора	Бранимир Њежић
Наслов рада	Control of Plum Sawflies (*Hoplocampa flava* L. and *Hoplocampa minuta* Christ) with entomopathogenic nematodes
Ментор	проф. др Ралф-Удо Елерс (Ralf-Udo Ehlers)

Изјављујем да је штампана верзија моје докторске дисертације идентична електронској верзији коју сам предао/ла за дигитални репозиторијум Универзитета у Бањој Луци.

У Бањој Луци 17.12.2017

Потпис докторанта