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The potential of entomopathogenic nematodes for the management of the mirid bugs *Lygus rugulipennis* (Poppuis), *Liocoris tripustulatus* (Fabricius) and *Macrolophus pygmaeus* (Rambur)

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ABSTRACT

Mirid bugs (Hemiptera: Miridae) represent a significant challenge for greenhouse cash crops like cucumber, tomato and eggplants, leading to huge economic losses. This study investigated the potential of the entomopathogenic nematode (EPN) *Steinernema carpocapsae* as biological control agent of the mirid bug species *Lygus rugulipennis*, *Liocoris tripustualtus*, and *Macrolophus pygmaeus* through aerial spray application under laboratory and greenhouse conditions (only for *L. rugulipennis* and *M. pygmaeus*). The laboratory trials showed a significant effect of *S. carpocapsae* on the average survival rate of the three mirid species. The highest efficacy was found for subadults of *L. rugulipennis* (50%), followed by *M. pygmaeus* (25%) and *L. tripustulatus* (15%). Microscopic dissections showed that EPNs can infect all studied mirid species and life stages. Under greenhouse conditions, a significant difference was observed between developmental stages, with no significant effect for adults, but an efficacy of 19% and 32% for nymphs of *L. rugulipennis* and *M. pygmaeus*, respectively. These results highlight the potential of EPNs in the control of problematic mirid bugs in greenhouse vegetable production, which could lead to a reduction in the use of synthetic pesticides and promote more sustainable agricultural practices.

1. Introduction

Plant-feeding bugs belonging to the mirid family (Hemiptera: Miridae) are ubiquitous in a large portion of crops, thus rendering it an important threat to agricultural production (Wheeler, 2000, 2001; Wheeler and Henry, 2008). In fruit production, they are significant pests on crops such as apple, pear, peach, and strawberry. Field crops such as wheat, sorghum and alfalfa are also heavily damaged. In vegetable production, various families including *Fabaceae*, *Cucurbitaceae*, *Apiaceae*, and *Solanaceae* are at risk. Feeding behaviour varies across the mirid species, most of them being phytophagous, while others being almost exclusively zoophagous or show a mixed type of feeding behaviour (Wheeler, 2001; Aukema et al., 2014). Phytophagous species often feed on nutrient and energy-rich plant parts such as young shoots, new leaves, inflorescences, nectar and pollen or (young) fruits.

In recent years, members of the mirid family have been the main focus of IPM of greenhouse vegetable production (Fischer, 2013; Ristord et al., 2019; Streito and Bout, 2019; Gard et al., 2022). Species of particular concern in Europe are *Lygus rugulipennis* (Poppius), *Liocoris tripustulatus* (Fabricius), *Nesidiocoris tenuis* (Reuter) and to a lesser extent *Macrolophus pygmaeus* (Rambur). *L. rugulipennis* feeds on over 400 different types of plants, leading to a significant impact on various crops such as strawberries, eggplants, and cucumbers (Wheeler, 2000; Holopainen and Varis, 1991; Łabanowska, 2007). *L. tripustulatus* is a common pest species in strawberry crops and certain vegetable crops including eggplants and cucumber (Wynde and Port, 2012; Messelink and Janssen, 2014; Jaccard and Fischer, 2016). *N. tenuis* and *M. pygmaeus* may be particularly problematic species in tomato production (Perdikis and Lykouressis, 2004; Pérez-Hedo and Urbaneja, 2016; Puentes et al., 2018; Sanchez et al., 2018).

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Few natural enemies are known and even fewer are currently commercially available, which considerably limits non-synthetic control options available to growers (Gard et al., 2022). As a consequence, vegetable producers are adopting various time-consuming and labour-intensive techniques that generally delay invasions (Ginez and Brun, 2017; Prisca et al., 2017; Gard et al., 2021) or physical methods of population control such as insect-proof nets (Gard et al., 2022; Clerc, 2019). As an alternative means, vegetable growers also rely on *M. pygmaeus* to restrict populations of other mirid bugs through competition (Jaworski et al., 2004). The use of biopesticides is also common, but registration and possible incompatibilities with beneficial species sometimes hamper this solution (Damalas and Koutroubas, 2018). Often only broad-spectrum multipurpose insecticides are effective against mirid bugs, which affect most beneficials (Fischer, 2013; Easterbrook, 1997; Fitzgerald, 2004). Other problems, such as the development of resistance or the preharvest intervals make this approach unattractive.

A recent avenue is the use of entomopathogenic nematodes (EPNs) from the families *Steinernematidae* and *Heterorhabditidae* to control mirid bugs. These obligate parasitic nematodes provide an alternative to synthetic pesticides for a multitude of economically important crop pests (Kaya and Gaugler, 1993; Mracek, 2002; Gulcu et al., 2017; Koppenhöfer et al., 2020). Once installed in the hemocoel of its host, the infective juveniles mutate and release mutualistic bacteria of the genera *Xenorhabdus* and *Photorhabdus* (Endo and Nickle, 1994). This association generally allows EPNs to kill their hosts within 24–48 h (Gaugler et al., 1997; Dowds and Peters, 2002). The highest efficacy is recorded against insects living in the soil environment or in cryptic habitats such as boreholes and galleries where infective juveniles are protected from environmental extremes (temperature, desiccation and UV) thus enhancing their development (Arthurs et al., 2004; Shapiro-Ilan et al., 2006, 2012).

However, research advances in the development of technologies that improve the longevity of EPNs, indicate an ability of these organisms to control foliar pests (Arthurs et al., 2004; Williams and Walters, 2000; Kim et al., 2015; Kagimu et al., 2017). Dipteran leafminers, *Liriomyza* spp., tobacco whiteflies (Hemiptera) and the South American tomato leafminer (Lepidoptera) are three examples of aerial pests that have been successfully controlled to date (Williams and Walters, 2000; Head et al., 2003; Batalla-Carrera et al., 2010; Garcia-del-Pino et al., 2018). For the management of mirid bugs, the use of EPNs is increasingly popular and hence there is the need to provide further information on their potential on pest control in vegetable production (Gard et al., 2022, 2021).

The aim of this study is to investigate the efficacy of the EPN *Steinernema carpocapsae* (Nematoda: Steinernematidae) in controlling the three mirid species *L. rugulipennis*, *L. tripustulatus*, and *M. pygmaeus*. Our initial investigation involved evaluating the ability of *S. carpocapsae* to infect these three species in a controlled laboratory setting, with the goal of determining the extent to which EPNs could penetrate the hosts and identifying any interspecific differences. To gain further insight into possible constraints on the potential application of *S. carpocapsae* to control the three mirid bugs, we further analysed EPNs on the survival rate at pupal and nymph stages of these pests. The same experiment has been replicated under greenhouse conditions to study the infestation potential in a realistic production scenario.

2. Material and methods

2.1. Insects

The species *M. pygmaeus* was supplied by Andermatt Biocontrol SA (Grossdietwil, Switzerland), while *L. rugulipennis* and *L. tripustulatus* originated from wild collected individuals in Western Switzerland in April and May 2021. All three species were reared following the same protocol as Fischer (Fischer, 2012) in climate chambers of the Plants and Pathogens Group, HEPIA, University of Applied Sciences of Western Switzerland. The conditions during the whole rearing period were the following: 23 ± 2 °C, 70 ± 10% Relative humidity and 16:8 L:D photoperiod. Adult bugs were isolated in "BugDorm" (MegaView Science Co., Taiwan) cages made of 680 μm fabric (60 ×60 ×60 cm). In terms of food and oviposition substrate, each of these cages contained sunflower seeds, cocoa beans, sprouted potatoes and eggs of *Ephestia kuehniella* (Zeller). Pods and tubers served as egg-laying material for the adults and were transferred to rectangular plastic containers covered with fine mesh netting until hatching. Emerged nymphs were kept in these containers until adulthood and then transferred to the cages. To confirm that individuals of the rearing were healthy (no latent pre-infection with EPN from natural sources) before the trial, individuals from the rearing were dissected on occasional basis and no nematodes were found.

2.2. Plant material

The plant material for both trials consisted of eggplants (Solanum melongena L. Shakira F1 Gautier Semences SAS, France), grown under greenhouse conditions in pots (Ø = 12 cm) with the commercial substrate (substrate 1, Klasmann-Deilmann GmbH, Geeste, Germany). Crop maintenance consisted of watering 2–3 times per week and fertilization with organic fertilizer "Biorga tomatoes" (Hauert, Switzerland) every 2 weeks. Greenhouse trials were carried out with eggplants in 3 litre pots with the same substrate and maintenance. Eggplants were placed in cages on 6th June 2021, with a drip irrigation system, to ensure water supply. Before each trial, all plants and cages were checked for any other pest species. Cellophane film was stretched over each pot to prevent the mirid bugs from entering the substrate.

2.3. Entomopathogenic nematodes (EPN)

Infective juveniles of the species *Steinernema carpocapsae* were obtained from the company Koppert Biological Systems Inc (Berkel en Rodenrijs, Netherlands). The product name is Capsanem® and contains 86% *S. carpocapsae* and 14% inert carrier. *S. carpocapsae* is an ambush-type nematode (Campbell and Gaugler, 1993). Such behavioural type will be most effective in cryptic and soil surface habitats (Lacey and Georgis, 2012). This species tolerates desiccation better than other species (Poinar and Simons, 1973; Kung et al., 1991; Koppenhöfer et al., 1995) and is the most commonly used to control foliar and other aerial pests (Arthurs et al., 2004). This is why we believe that this species is particularly suitable for investigating EPN pest control potential. Confirmation of the viability of the infective juveniles was done under the microscope before each experiment.

2.4. Experimental design

The laboratory experiment took place in climatic chambers and was replicated two times, each replication with 3 replicated blocks. The number of living mirid bugs was used to estimate the survival rate of the 3 mirid bug species (*M. pygmaeus*, *L. rugulipennis* and *L. tripustulatus*) in petri dishes when in contact with EPNs. To assess whether the treatments had different effects depending on the stage of the insects, individuals of each species were divided into 3 groups according to their life stages: young nymph with L2 and L3 instars, old nymph with L4 and L5 instars, and adults. Ten insects of the same life stage were placed in Petri dishes (Ø = 9 cm). Each dish was filled with an eggplant leaf and *E. kuehniella* eggs as supplementary food, reducing the risk of mirid cannibalism. The EPN treatment was dosed at 5 million infective juveniles per litre of autoclaved water, which was evenly sprayed on both sides of the eggplant leaves, up to the point of run-off. For the control eggplant leaves were sprayed with autoclaved water. Once the eggplant leaves with each different bug life stages were placed together in petri dishes, they were moved in an air-conditioned room at a temperature of 23 ± 2 °C, a relative humidity of 70 ± 10% and a photoperiod of 16 h.

The survival rate was assessed 72 h after the application of EPNs. After death, the bugs were dissected for microscopic confirmation of EPN infection. The set-up consisted of six randomised blocks on two different dates, i.e., a total of 6 replicates.

For the greenhouse experiment, each eggplant was placed in a 580 μm canvas cage (60×60×90 cm) to avoid the dispersal of the bugs within the experimental units. Similar to the laboratory experiment, each species was divided into 3 groups according to stages (adults, young nymphs and old nymphs). The treatments also consisted of 2 modalities: a treatment consisting of EPN and a control with water. Ten insects per group, i.e. 10 young nymphs, 10 old nymphs, and 10 adults, were placed on an eggplant (n = 48). To ensure prey supply, *E. kuehniella* eggs were placed on each eggplant.

The EPN product was sprayed on the eggplant foliage at a dose of 5 million nematodes per litre and a volume of 600 litres/ha. The treatments were carried out in the evenings, between 8:30 and 9:30 pm, during optimal weather conditions (19–31 °C, 75–90% RH and low UV). The spray was applied up to the point of run-off, evenly on both sides of the leaves. The water controls were applied first and then the nematode treatments to ensure that the controls were not infected with EPNs. For the same reason, a plastic sheet was placed between the controls and treatments during spraying.

Survivor and death counts were taken 72 h after the treatments. Dead bugs were stored in a freezer, so that they could be dissected and microscopically confirmed as EPN infected.

The set-up was conducted in a block design with blocks consisting of 4 experimental units receiving the four modalities. Each block was repeated 12 times on two different dates.

2.5. Statistical analysis

The results obtained on the survival rate (number of dead individuals / number of released individuals) of bugs were processed using the "stats" package in R 4.2.1 (R Core Team, 2022). As there were no differences between the two temporal replications in both trials (ANOVA with "survival rate" dependent and "temporal replication" as independent variable), the replicates were pooled together to increase replication number and therefore statistical power. To test the difference in survival rate between stage and treatment, separate ANOVAs were performed for each species (Girden, 1992). Normality and homoscedasticity of residuals were tested by plotting theoretical against sampling quantiles and residuals against fitted values, respectively, for each ANOVA. To find significant differences between means of life stages and treatments we compared them pairwise using Tukey's HSD test from the "multcomp" package (Tukey, 1949).

3. Results

Visual qualitative inspection under the microscope showed that *S. carpocapsae* was able to penetrate all three mirid species studied in these trials (Fig. 1). In addition, the nematodes were observed in individuals of each life stage of the three species, thus confirming the potential of this species to penetrate all life stages from all three studied mirid species.

3.1. Efficacy of EPN in controlling mirid bugs under laboratory conditions

The results of the experiment showed a statistically significant main effect of the EPN treatment on the mean survival rate for all three mirid species (Table 1, Fig. 1) Across all species and life stages, EPN applications reduced survival rate of the mirid bugs by 25%.

The treatment effect was highest for *L. rugulipennis*, with a mean difference in survival rate of 30%, 50% and 42% for young nymphs, old nymphs and adults respectively (Fig. 2). Next, *M. pygmaeus* showed a difference in the mean survival rate compared to the control, of 23%, 18% and 35% for young nymphs, old nymphs and adults respectively. Finally, the smallest effect of EPNs was seen in *L. tripustulatus*, with an average reduced survival rate of 15% 12% and 12% for young nymphs, old nymphs, and adults respectively. The mortality in the control treatment was overall very low and therefore provided high confidence to the results.

Table 1
Summary of the ANOVA tests for the laboratory and greenhouse experiments for L. tripustulatus, L. rugulipennis and M. pygmaeus. EPN = entomopathogenic nematode treatment, life stage (young nymph, old, nymph, adult). Df = degrees of freedom, Sum Sq = sums of squares. Significant p-values are shown in bold.

Experiment	Species	Parameter	Df	Sum Sq	F value	P value
Laboratory	*L. rugulipennis*	EPN	1	14,803	107.87	**0.001**
		Life Stage	2	439	1.60	0.219
		EPN:life Stage	2	606	2.21	0.128
		Residuals	30	4117		
	L. tripustulatus	EPN	1	1469	15.84	**0.001**
		Life Stage	2	1089	5.87	**0.007**
		EPN:life Stage	2	22	0.12	0.888
		Residuals	30	2783		
	M. pygmaeus	EPN	1	4444	32.26	**0.001**
		Life Stage	2	150	0.54	0.586
		EPN:life Stage	2	72	0.26	0.771
		Residuals	30	4133		
Greenhouse	*L. rugulipennis*	EPN	1	3200	12.57	**0.001**
		Life Stage	2	40,269	79.10	**0.000**
		EPN:life Stage	2	658	1.29	0.281
		Residuals	66	16,800		
	M. pygmaeus	EPN	1	5000	18.47	**0.001**
		Life Stage	2	20,119	37.16	**0.001**
		EPN:life Stage	2	3658	6.76	**0.002**
		Residuals	66	17,867		

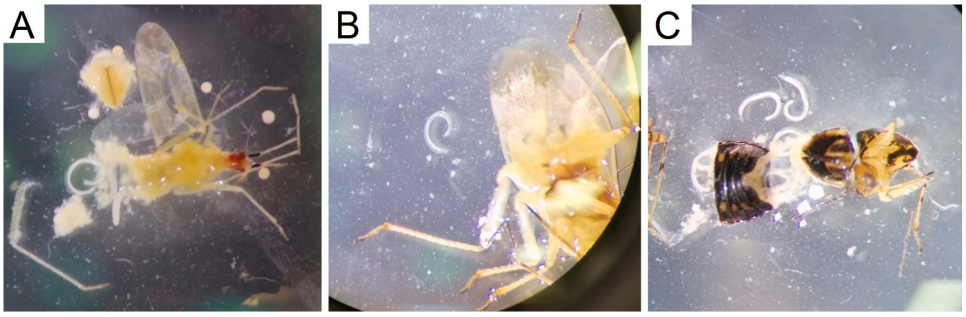

Fig. 1. Results from microscopic visual inspection of the EPN treated mirid bugs, showing fully grown individuals of S. carpocapsae, evidencing that the tested EPN is able to penetrate and infect all life stages of the three investigated species (A) Macrolophus pygmaeus (B), Lygus ruggulipennis and (C) Liocoris tripustulatus.

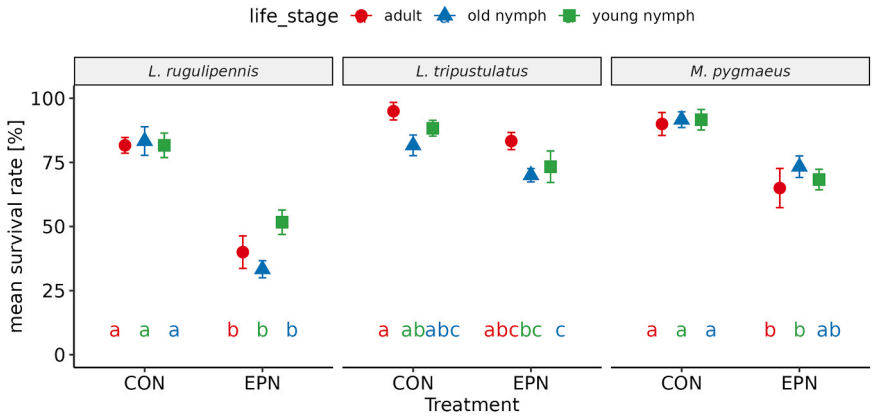

Fig. 2. Species (L. tripustulatus, L. rugulipennis and M. pygmaeus) and life stage (young nymph, old nymph, adult) dependent mean (+/- SE) survival rate for the laboratory experiment for control (CON) and entomopathogenic nematode (EPN) treatment (n = 6). Different letters show significant differences between groups according to Tukey post-hoc comparisons. For results of statistical models see Table 1.

3.2. Efficacy of EPN in controlling mirid bugs under greenhouse conditions

The average survival rate across both species and life stages was reduced by 15% through foliar application of EPN. However, the life stages of *M. pygmaeus* and *L. rugulipennis* were differently affected by the treatment with *S. carpocapsae* (Table 1, Fig. 3). In both species, we found no evidence in an increase of mortality of adult stages due to *S. carpocapsae*. However, twice as many young nymphs survived for *M. pygmaeus* (32%) than for *L. rugulipennis* (16%), while the survival rate of old nymphal stages was similar for both mirid species (19%).

4. Discussion

4.1. Proof of principle: species and life stage dependent efficacy of EPN

The present study highlights the potential of the EPN *S. carpocapsae* as an effective biological control agent against three common mirid pest species. Our laboratory results indicate a considerable reduction in the survival rate after exposure to *S. carpocapsae*, with a difference in efficacy of the EPN between the different species. Previous reports about the lack of efficacy may be related to differences in study design (Deneve and De, 2015). Reduced filter paper moisture influences the mobility of EPNs and thus their ability to infest their hosts (Shapiro-Ilan et al., 2006; Freckman and Caswell, 1985). Consequently, an environment with increased moisture is favorable for the control of mirid bugs using EPNs (Georgis, 1990). Furthermore, it is likely that the survival rate of mirid bugs treated with EPNs is dose dependent (Georgis, 1990; Lewis et al., 1996; Dlamini et al., 2020).

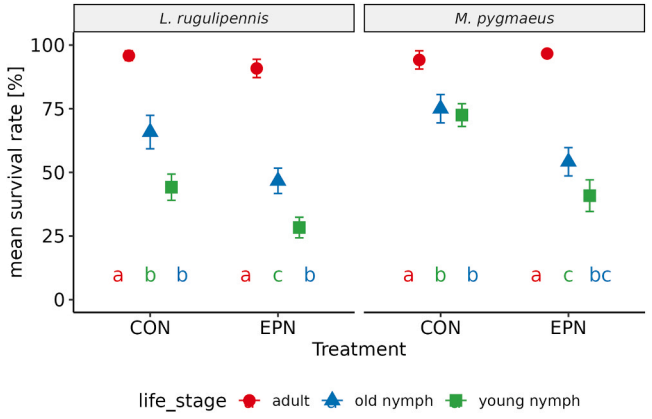

Fig. 3. Species (L. rugulipennis and M. pygmaeus) and life stage (young nymph, old nymph, adult) dependent mean (+/- SE) survival rate for the greenhouse experiment for control (CON) and entomopathogenic nematode (EPN) treatment (n = 12). Different letters show significant differences between groups according to Tukey post-hoc comparisons. For results of statistical models see Table 1.

As reported by previous studies, EPNs may vary in virulence between different host species, and also within the same host at different developmental stages (Fuxa et al., 1988; Ansari et al., 2008; Gorgadze et al., 2017). For example, Guevara et al (Guevara et al., 2020). found that the effect of *S. carpocapsae* on *Macrolophus basidiocornis* (Stål) is positively correlated with the host life stage. Deneve (Deneve and De, 2015) observed efficacy of *S. carpocapsae* on adults, but not on the fourth nymphal instars of *M. pygmaeus*. *S. carpocapsae* was classified by Garriga et al (Garriga et al., 2019). as slightly damaging to adults of *M. pygmaeus* and *Nesidiocoris tenuis* and harmless to the nymphal stages. These studies have not assessed the effect of the treatment in relation to the developmental stage of *L. rugulipennis* and *L. tripustulatus* and conclusions can differ depending on the species. In line with our results, for example, the effects of EPNs on *Collaria scenica* (Stål) (Miridae) showed no significant difference between the different developmental stage groups (Naranjo et al., 2011). A study on *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) showed that the mortality rate caused by EPNs was lower in the adults than in the nymphs (Gorgadze et al., 2017). The formation of groups of old and young nymphs is an important factor to consider in experimental design. Notably, among the studies cited earlier, only Naranjo et al. (2011) incorporated group formation in their design, and their results showed no significant difference in treatment effects across developmental stages.

The variation can be explained by various strategies and mechanisms that hosts put against EPNs attacks (Grewal et al., 1994). Target species may adopt different behaviors to eliminate the EPNs. Earwigs, for example, have been observed to adopt a higher grooming behavior when exposed to *S. carpocapsae* (Hodson et al., 2011). In contrast, personal observations (supported by Deneve (Deneve and De, 2015)) suggest that *M. pygmaeus* expressed the same grooming behaviour between *S. carpocapsae* treatment and control. Insect morphology such as natural orifices (mouth, anus and spiracles) may also facilitate the success of infestation by EPN species (Ishibashi and Kondo, 1990). Aphid nymphs with small natural orifices were less infected with EPNs than adults with larger orifices (Mràček and Růžička, 1990). Similarly, mortality rates were significantly higher in large earwigs than in small ones (Hodson et al., 2011). Another important obstacle to the successful establishment of EPN infection is the host's immune response, which can include mechanisms such as encapsulation and melanization of EPNs (Castillo et al., 2011). The strength and type of immune response elicited by the host, may vary depending on the specific EPN strain involved, the host

species and the developmental stage (Li et al., 2007; Hillyer, 2016).

Mortality in control individuals is rarely zero, and we have observed limited mortality in certain life stage, which can be explained by three main factors. The first is the natural mortality that can be expected. The experimental individuals have undergone considerable stress (complex environment in comparison to rearing conditions, eventual sub-optimal diet, variation in temperature and light) and although only young individuals were chosen for the trial, some stochastic mortality occurs even in the absence of an experimental treatment. The second reason is not true mortality but a potential experimental artefact. For the field trial, we may not have been able to find all the individuals due to the increased complexity of the environment at the end of trial. The third and final reason we mention here, although there may be others, is the potential moulting of immature individuals during the experiments, which again is not a true mortality, but could be interpreted as such. During the course of the experiment, individuals had the ability to undergo moulting and transition from one developmental stage to another. Consequently, the observed counts for young nymphs may have been underestimated compared to the counts for older stages. However, we would like to emphasize that none of these potential reasons for mortality are confounded by the treatments and therefore constitute a problem for the interpretation of the results.

4.2. Transfer laboratory results to the greenhouse

The semi-field experiment provided a more realistic environment for studying the efficacy of EPNs as biocontrol agents, by more accurately mimicking the conditions encountered by plants, pests, and EPNs in actual farming situations. Our results show that *S. carpocasae* had a significant effect on the survival rate of *M. pygmaeus* and *L. rugulipennis*, but only for their nymphal stages. A lower survival rate for nymphal stages of *M. pygmaeus* exposed to EPNs has also been reported in greenhouse experiments (Gard et al., 2022). This is probably caused by the flight ability of the adults which allows them to move away from the treated area more easily (Rezaei et al., 2015). It has been shown that evasion is a key response in aerial insects attacked by EPNs (Drees et al., 1992; Gambino et al., 1992). In the experimental setup used in the study, *L. rugulipennis* tended to take refuge against the walls of the cages, which were inevitably covered with EPN, while *M. pygmaeus* showed less tendency to exhibit this behavior. However, in a real-world situation where insects are more mobile and have access to crop shelters, direct contact with EPNs may be reduced (Kaya and Gaugler, 1993; Hajek and Goettel, 2007; Sanchez et al., 2012), which could result in lower efficacy of EPNs on *L. rugulipennis* compared to *M. pygmaeus*. This could also have contributed to the reduced efficacy observed in the greenhouse (15%) relative to the laboratory (30%).

4.3. Conclusions and perspectives

The findings of this study indicate that *S. carpocapsae* is a potential candidate for biological control of the three mirid bug species *L. rugulipennis*, *M. pygmaeus*, and *L. tripustulatus*, which are known to cause significant economic losses for greenhouse vegetable growers. However, the success of its use is complex and depends on many factors, difficult to control in on-farm conditions. Moreover, with relatively low efficacy values, especially for *L. tripustulatus* in the laboratory and for adult stages of the other mirid species in the greenhouse, it is likely that this approach alone is insufficient for mirid control. This would be in line with the conclusions from other studies that a successful control relies on a combination of methods. Further studies, particularly in collaboration with crop producers, could thus investigate the combination of methods to provide insights on how to improve biocontrol of mirid bugs. In combination with crop damage assessment or the relation between the costs and profitability of EPN in pest management programs the study could provide practical advice for vegetable growers.

CRediT authorship contribution statement

Emile Steenman, Gaëtan Jaccard, Ernest Ireneusz Hennig: Conceived the study. **Emile Steenman, Gaëtan Jaccard**: Collected the data. **Emile Steenman, Louis Sutter**: Analysed the data and led the writing of the manuscript. All authors contributed to the writing of the manuscript and gave final approval for its publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Ansari, M.A., Shah, F.A., Butt, T.M., 2008. Combined use of entomopathogenic nematodes and Metarhizium anisopliae as a new approach for black vine weevil, Otiorhynchus sulcatus, control. Entomol. Exp. Appl. 129, 340–347. https://doi.org/10.1111/j.1570-7458.2008.00783.x.

Arthurs, S., Heinz, K.M., Prasifka, J.R., 2004. An analysis of using entomopathogenic nematodes against above-ground pests. Bull. Entomol. Res. 94, 297–306. https://doi.org/10.1079/ber2003309.

Aukema, B., Chérot, F., Viskens, G., Bruers, J., 2014. Atlas des Miridae de Belgique (Insecta: Heteroptera), 3rd ed..,. Peeters,, Heren, Bruxelles.

Batalla-Carrera, L., Morton, A., García-del-Pino, F., 2010. Efficacy of entomopathogenic nematodes against the tomato leafminer Tuta absoluta in laboratory and greenhouse conditions. BioControl 55, 523–530. https://doi.org/10.1007/s10526-010-9284-z.

Campbell, J.F., Gaugler, R., 1993. Nictation Behaviour and Its Ecological Implications in the Host Search Strategies of Entomopathogenic Nematodes (Heterorhabditidae and Steinernematidae). Behaviour 126, 155–169.

Castillo, J.C., Reynolds, S.E., Eleftherianos, I., 2011. Insect immune responses to nematode parasites. Trends Parasitol. 27, 537–547. https://doi.org/10.1016/j.pt.2011.09.001.

H. Clerc, Essai protection contre les punaises – IMPULsE, Invenio, Bordeaux, 2019.

Damalas, C.A., Koutroubas, S.D., 2018. Current status and recent developments in biopesticide use. Agric 8. https://doi.org/10.3390/agriculture8010013.

T. Deneve, P. De Clercq, Neveneffecten van entomopathogene nematoden op de roofwants Macrolophus pygmaeus, Universiteitsbibliotheek Gent, 2015. ⟨https://lib.ugent.be/catalog/rug01:002217127⟩.

Dlamini, B.E., Dlamini, N., Masarirambi, M.T., Nxumalo Kwanele, A., 2020. Control of the tomato leaf miner, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) larvae in laboratory using entomopathogenic nematodes from subtropical environment. J. Nematol. 52 https://doi.org/10.21307/jofnem-2020-013.

Dowds, B.C.A., Peters, A., 2002. Virulence mechanisms. Entomopathog. Nematol. 79–98. https://doi.org/10.1079/9780851995670.0079.

Drees, B.M., Miller, R.W., Vinson, S.B., Georgis, R., 1992. Susceptibility and behavioral response of red imported fire ant (Hymenoptera: Formicidae) to selected entomogenous nematodes (Rhabditida: Steinernematidae & Heterorhabditidae). J. Econ. Entomol. 85, 365–370. https://doi.org/10.1093/jee/85.2.365.

Easterbrook, M.A., 1997. The phenology of Lygus rugulipennis, the European tarnished plant bug, on late-season strawberries, and control with insecticides. Ann. Appl. Biol. 131, 1–10. https://doi.org/10.1111/j.1744-7348.1997.tb05392.x.

Endo, B.Y., Nickle, W.R., 1994. Ultrastructure of the buccal cavity region and oesophagus of the insect parasitic nematode, heterorhabditis bacteriophora. Nematologica 40, 379–398. https://doi.org/10.1163/003525994×00274.

S. Fischer, Méthode d'élevage de Lygus rugulipennis (Heteroptera, Miridae), Wädenswil, 2012.

Fischer, S., 2013. Le problèmes de punaises sous abris. Der Gemüsebau / Le. Maraîcher 27.

Fitzgerald, J., 2004. Laboratory bioassays and field evaluation of insecticides for the control of Anthonomus rubi, Lygus rugulipennis and Chaetosiphon fragaefolii, and effects on beneficial species, in UK strawberry production. Crop Prot. 23, 801–809. https://doi.org/10.1016/j.cropro.2003.12.005.

Freckman, D.W., Caswell, E.P., 1985. The ecology of nematodes in agroecosystems. Annu. Rev. Phytopathol. 23, 275–296. https://doi.org/10.1146/annurev.py.23.090185.001423.

Fuxa, J.R., Richter, A.R., Acudelo-Silva, F., 1988. Effect of Host Age and Nematode Strain on Susceptibility of Spodoptera frugiperda to Steinernema feltiae. J. Nematol. 20, 91–95. ⟨http://www.ncbi.nlm.nih.gov/pubmed/19290189%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2618776⟩.

Gambino, P., Pierluisi, G.J., Poinar, G.O., 1992. Field test of the nematode Steinernema feltiae (Nematoda: Steinernematidae) against yellowjacket colonies (Hym.: Vespidae). Entomophaga 37, 107–114. https://doi.org/10.1007/BF02372979.

F. Garcia-del-Pino, A. Morton, D. Shapiro-Ilan, Entomopathogenic Nematodes as Biological Control Agents of Tomato Pests, Elsevier Inc., 2018. ⟨https://doi.org/10.1016/B978–0-12–802441-6.00012–7⟩.
Gard, B., Pierre, P., Clerc, H., Streito, J., Bout, A., Chaillout, S., Camoin, L., Tosello, L., Ginez, A., Delamarre, C., Lambion, J., 2021. Gestion des punaises phytophages en cultures maraîchères: le projet IMPULsE à l'heure du bilan. INFOS CTIFL 19–28.
Gard, B., Bout, A., Chaillout, S., Camoin, L., Canal, X., Al, E., 2022. IMPULsE - développement et Intégration de Méthodes innovantes pour la maîtrise des PUnaises en cultures LEgumières. Innov. Agron. 85, 199–212.
Garriga, A., Morton, A., García-López, D., 2019. Compatibility of entomopathogenic nematodes with natural enemies for horticultural pest control. Biol. Control. 138, 104050 https://doi.org/10.1016/j.biocontrol.2019.104050.
Gaugler, R., Lewis, E., Stuart, R.J., 1997. Ecology in the service of biological control: The case of entomopathogenic nematodes. Oecologia 109, 483–489. https://doi.org/10.1007/s004420050108.
Georgis, R., 1990. Formulation and Application Technology. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathog. Nematodes Biol. Control. CRC Press, Boca Raton, pp. 1–365. https://doi.org/10.1201/9781351071741.
A. Ginez, V. Brun, Stratégie de protection contre la punaise Nesidiocoris tenuis Projet IMPULsE, APREL, St Rémy de Provence, 2017.
E.R. Girden, ANOVA: Repeated measures, Sage, 1992.
Gorgadze, O., Bakhtadze, G., Kereselidze, M., Lortkhipanidze, M., 2017. The efficacy of entomopathogenic agents against halyomorpha halys (hemiptera: Pentatomidae). Int. J. Curr. Res. 9, 62177–62180.
Grewal, P.S., Selvan, S., Gaugler, R., 1994. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. J. Therm. Biol. 19, 245–253. https://doi.org/10.1016/0306-4565(94)90047-7.
Guevara, E.J., Porcel, M., Calixto, A.M., Bueno, V.D.P., Moino, A., 2020. Interactions Between the Nematode Heterorhabditis amazonensis JPM4 and the Predator Macrolophus basicornis: Two Natural Enemies of Tuta absoluta Native to South America. Neotrop. Entomol. 49, 108–115. https://doi.org/10.1007/s13744-019-00734-0.
Gulcu, B., Cimen, H., Raja, R.K., Hazir, S., 2017. Entomopathogenic nematodes and their mutualistic bacteria: Their ecology and application as microbial control agents. Biopestic. Int. 13, 79–112.
Hajek, A.E., Goettel, M.S., 2007. Guidelines for evaluating effects of entomopathogens on non-target organisms. In: F. Man. Tech. Invertebr. Pathol. Springer, Dordrecht, pp. 816–833. https://doi.org/10.1007/978-1-4020-5933-9_40.
Head, J., Palmer, L.F., Walters, K.F.A., 2003. The compatibility of control agents used for the control of the South American leafminer, Liriomyza huidobrensis. Biocontrol Sci. Technol. 13, 77–86. https://doi.org/10.1080/0958315021000054403.
Hillyer, J.F., 2016. Insect immunology and hematopoiesis. Dev. Comp. Immunol. 58, 102–118. https://doi.org/10.1016/j.dci.2015.12.006.
Hodson, A.K., Friedman, M.L., Wu, L.N., Lewis, E.E., 2011. European earwig (Forficula auricularia) as a novel host for the entomopathogenic nematode Steinernema carpocapsae. J. Invertebr. Pathol. 107, 60–64. https://doi.org/10.1016/j.jip.2011.02.004.
Holopainen, J.K., Varis, A. -L., 1991. Host plants of the European tarnished plant bug Lygus rugulipennis Poppius (Het., Miridae). J. Appl. Entomol. 111, 484–498. https://doi.org/10.1111/j.1439-0418.1991.tb00351.x.
Ishibashi, N., Kondo, E., 1990. Behavior of Infective Juveniles. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathog. Nematodes Biol. Control. CRC Press, Boca Raton, pp. 139–150. https://doi.org/10.1201/9781351071741.
Jaccard, G., Fischer, S., 2016. Etude et gestion de la punaise "Liocoris tripustulatus", ravageur émergent des fraises. Rev. Suisse Vitic. Arboric. Hortic. 48, 220–228.
Jaworski, C.C., Chailleux, A., Bearez, P., Desneux, N., 2004. Apparent competition between major pests reduces pest population densities on tomato crop, but not yield loss (2015). J. Pest Sci. 88, 793–803. https://doi.org/10.1007/s10340-015-0698-3.
Kagimu, N., Ferreira, T., Malan, A.P., 2017. The Attributes of Survival in the Formulation of Entomopathogenic Nematodes Utilised as Insect Biocontrol Agents. Afr. Entomol. 25, 275–291. https://doi.org/10.4001/003.025.0275.
Kaya, H.K., Gaugler, R., 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 181–206.
Kim, J., Jaffuel, G., Turlings, T.C.J., 2015. Enhanced alginate capsule properties as a formulation of entomopathogenic nematodes. BioControl 60, 527–535. https://doi.org/10.1007/s10526-014-9638-z.
Koppenhöfer, A.M., Kaya, H.K., Taormino, S.P., 1995. Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. J. Invertebr. Pathol. 65, 193–199. https://doi.org/10.1006/jipa.1995.1028.
Koppenhöfer, A.M., Shapiro-Ilan, D.I., Hiltpold, I., 2020. Entomopathogenic nematodes in sustainable food production. Front. Sustain. Food Syst. 4, 1–14. https://doi.org/10.3389/fsufs.2020.00125.
Kung, S.P., Gaugler, R., Kaya, H.K., 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. J. Invertebr. Pathol. 57, 242–249. https://doi.org/10.1016/0022-2011(91)90123-8.
Łabanowska, B.H.Ł., 2007. Strawberry fruit damaged by the tarnished plant bug (Lygus rugulipennis L.). J. Fruit. Ornam. Plant Res. 15, 147–156.
Lacey, L.A., Georgis, R., 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. J. Nematol. 44, 218–225.
Lewis, E.E., Ricci, M., Gaugler, R., 1996. Host recognition behaviour predicts host suitability in the entomopathogenic nematode Steinernema carpocapsae (Rhabditida: Steinernematidae). Parasitology 113, 573–579. https://doi.org/10.1017/s0031182000067627.
Li, X.Y., Cowles, R.S., Cowles, E.A., Gaugler, R., Cox-Foster, D.L., 2007. Relationship between the successful infection by entomopathogenic nematodes and the host immune response. Int. J. Parasitol. 37, 365–374. https://doi.org/10.1016/j.ijpara.2006.08.009.
Messelink, G.J., Janssen, A., 2014. Increased control of thrips and aphids in greenhouses with two species of generalist predatory bugs involved in intraguild predation. Biol. Control. 79, 1–7. https://doi.org/10.1016/j.biocontrol.2014.07.009.
Mracek, Z., 2002. Use of Entomoparasitic Nematodes (EPANs) in Biological Control. In: Upadhyay, R.K. (Ed.), Adv. Microb. Control Insect Pests, 1st ed..,. Springer, New York, pp. 235–264. https://doi.org/10.1007/978-1-4757-4437-8.
Mràček, Z., Růžička, Z., 1990. Infectivity and development of Steinernema sp. strain Hylobius (Nematoda, Steinernematidae) in aphids and aphidophagous coccinellids. J. Appl. Entomol. 110, 92–95. https://doi.org/10.1111/j.1439-0418.1990.tb00099.x.
Naranjo, N., Montero, D.A.V., Sáenz, A., 2011. Aponte, First record of infection by entomopathogenic nematodes of the grass bug Collaria scenica Stål (Hemiptera: Miridae). Entomotropica 26, 117–125.
Perdikis, D.C., Lykouressis, D.P., 2004. Macrolophus pygmaeus (Hemiptera: Miridae) population parameters and biological characteristics when feeding on eggplant and tomato without prey. J. Econ. Entomol. 97, 1291–1298. https://doi.org/10.1093/jee/97.4.1291.
Pérez-Hedo, M., Urbaneja, A., 2016. The Zoophytophagous Predator Nesidiocoris tenuis: A Successful But Controversial Biocontrol Agent in Tomato Crops. In: Horowitz, A.R., Ishaaya, I. (Eds.), Adv. Insect Control Resist. Manag. Springer International Publishing, pp. 121–138. https://doi.org/10.1007/978-3-319-31800-4.
Poinar, G., Simons, W., 1973. The ability of Neoaplectana carpocapsae (steinernematidae: Nematodea) to survive extended periods of desiccation. J. Invertebr. Pathol. 22, 228–230.
P. Prisca, B. Gard, Y. Trottin, Maîtrise des punaises en cultures légumières: Les méthodes innovantes du projet impulse, INFOS CTIFL, Bellegarde, 2017.
Puentes, A., Stephan, J.G., Björkman, C., 2018. A systematic review on the effects of plant-feeding by omnivorous arthropods: Time to catch-up with the mirid-tomato bias?. In: Front. Ecol. Evol, 6. https://doi.org/10.3389/fevo.2018.00218.
R Core Team, R: A language and environment for statistical computing, 2022. ⟨https://www.r-project.org/⟩.
Rezaei, N., Karimi, J., Hosseini, M., Goldani, M., Campos-Herrera, R., 2015. Pathogenicity of two species of entomopathogenic nematodes against the greenhouse whitefly, trialeurodes vaporariorum (Hemiptera: Aleyrodidae), in laboratory and greenhouse experiments. J. Nematol. 47, 60–66.
J. Ristord, H. Claes, G. Jaccard, M. Baladou, Rapport phytosanitaire 2019 cultures maraîchères des cantons de Vaud et Genève, Office Technique Maraicher, Morges, 2019.
Sanchez, J.A., La Spina, M., Perera, O.P., 2012. Analysis of the population structure of Macrolophus pygmaeus (Rambur) (Hemiptera: Miridae) in the Palaearctic region using microsatellite markers. Ecol. Evol. 2, 3145–3159. https://doi.org/10.1002/ece3.420.
Sanchez, J.A., López-Gallego, E., Pérez-Marcos, M., Perera-Fernández, L.G., Ramírez-Soria, M.J., 2018. How safe is it to rely on Macrolophus pygmaeus (Hemiptera: Miridae) as a biocontrol agent in tomato crops. ?, Front. Ecol. Evol. 6, 1–10. https://doi.org/10.3389/fevo.2018.00132.
Shapiro-Ilan, D.I., Gouge, D.H., Piggott, S.J., Fife, J.P., 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biol. Control. 38, 124–133. https://doi.org/10.1016/j.biocontrol.2005.09.005.
Shapiro-Ilan, D.I., Han, R., Dolinksi, C., 2012. Entomopathogenic nematode production and application technology. J. Nematol. 44, 206–217.
Streito, J.-C., Bout, A., 2019. Punaises phytophage: quelle diversité sur nos cultures ? Phytoma 53–56.
Tukey, J.W., 1949. Comparing Individual Means in the Analysis of Variance. Biometrics 5, 99–114.
Wheeler, A.G., 2000. Plant Bugs (Miridae) as Plant Pests. In: Schaefer, C.W., Panizzi, A.R. (Eds.), Heteroptera Econ. Importance. CRC Press, Boca Raton, pp. 37–83.
Wheeler, A.G., 2001. Biology of the Plant Bugs (Hemiptera: Miridae): Pests, Predators, Opportunists. Cornell University Press,, New York.
Wheeler, A.G., Henry, T.J., 2008. Plant Bugs (Hemiptera: Miridae). In: Capinera, J.L. (Ed.), Encycl. Entomol. Springer, Dordrecht, pp. 2909–2914. https://doi.org/10.1007/978-1-4020-6359-6_2983.
Williams, E.C., Walters, K.F.A., 2000. Foliar application of the entomopathogenic nematode Steinernema feltiae against leafminers on vegetables. Biocontrol Sci. Technol. 10, 61–70. https://doi.org/10.1080/09583150029396.
Wynde, F.J.H., Port, G.R., 2012. The Use of Olfactory and Visual Cues in Host Choice by the Capsid Bugs Lygus rugulipennis Poppius and Liocoris tripustulatus Fabricius. PLoS One 7, 1–8. https://doi.org/10.1371/journal.pone.0046448.