Control of western corn rootworm with entomopathogenic nematodes in maize monoculture

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Abstract. Western corn rootworm is one of the most dangerous pests of maize, and both the larvae and the imagoes thereof may cause significant damage to the plants. The options of controlling these pests have recently become highly limited, thus creating a great demand for new control methods complying with sustainable plant protection. These requirements are met by the natural enemies of these pests, such as entomopathogenic nematodes (e.g. Heterorhabditis bacteriophora, Gerritsen, 1994). The objective of this study was to determine whether the viability and larvicide effect of a single injection into the soil of 2 billion nematodes using various amounts of water (50, 100 or 200 L/hectare) was maintained even with the lower quantities. Our studies proved that the entomopathogenic nematodes retain their viability and larvicide effect when applied using 50 L/ha of water. The efficacy of the biological agent did not differ from that of Force 1.5G, a product containing Tefluthrin as active ingredient, which was used as positive control. *Keywords*: Western corn rootworm, *Diabrotica virgifera virgifera*, EPNs; biological control methods

1 Introduction

Maize (*Zea mays*) is one of the most important crops for humankind. Its world-wide sowing area covers approximately 140 to 160 million hectares [13]. The technology of growing maize is simple and highly automated, and the associated plant protection practices are well established. In addition to agrotechnical methods (soil cultivation, nutrient supply, weed control, and sowing counts), the control of western corn rootworm (*Diabrotica virgifera virgifera*) is an increasing challenge in maize production. The control of this particular pest has a significant impact on the quantity and quality of the harvest [11].

The gene centre of this harmful insect is in Mexico [8]. In Europe, it first appeared in 1992 in Belgrade [4]. It has a great invasive potential (50 km/year), and economic damage is observed within 5 to 7 years after the insect appears in an area [7]. Depending on the heat sum (268°C), the larvae hatch 4 to 6 weeks after maize sowing, and develop in the soil through three larval stages [9]. The primary pests are older larvae, which chew down the

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supporting roots, thereby causing a characteristic lodging of the plants referred to as goose-necking in the literature [2]. Especially in dry years, these roots are unable to regenerate, and a wind-storm or major rainfall may cause an entire stock to lodge, and the level of damage may be as high as 100% [6].

The number of authorised and effective insecticides against corn rootworm larvae is steadily declining. Therefore, current research efforts are focussed on biological solutions only killing the target species without presenting an environmental burden [12]. Such effective biological control methods include entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis*. Among the studied species, *Heterorhabditis bacteriophora* proved to be the most effective, causing 77% mortality among corn rootworm larvae [15]. The nematodes act as vectors that introduce bacteria (*Photorhabdus luminescens*) hosted by them into the pests, and the propagating bacteria kill the target species and liquefy their bodies, thereby providing food and opportunity for the nematodes to reproduce [19, 1].

Entomopathogenic nematodes seek out their prey during pheromone release [14, 5], and enter the target organism through the anal and oral orifices, as well as via the epidermal route, using their mouth stylet; the invaded corn rootworm larvae die within 2 to 3 days after such entry by the nematodes. In the body of the dead larvae, the nematodes continue to reproduce and develop, and (approximately 4000) new virulent nematode larvae (L3, juvenile infective) are released after 12 days to seek out new host organisms [15].

The results of Santos et al. (2011) [21] also provided evidence that nematodes of the *Steinernema* and *Heterorhabditis* genera show the best larvicide effect against Western corn rootworm larvae. Further experiments showed a 3% to 15% reduction in root damage on the Iowa scale and a 14% to 54% reduction on the scale for damage to the various root stages as a result of using *Heterorhabditis bacteriophora* [16].

Toepfer et al. conducted efficacy studies of nematodes against corn rootworm larvae in Hungary between 2004 and 2010 using various application technologies (e.g., surface spraying and injection into the soil). Whereas soil injection involved applying 230,000 nematodes per linear meter, surface spraying used 400,000 nematodes per square meter. The study concluded that these two methods of application reduced the level of root damage by at least 50%. The highest efficacy was observed when the nematodes were injected into the soil simultaneously with the sowing: the observed larvicide effect was up to 68%. In comparison with surface spraying, the advantages of injecting simultaneously with the sowing process include a lower water requirement and the absence of harmful UV exposure. In a subsequent experiment, nematodes were injected simultaneously with the sowing, at a rate of 2 billion/hectare and using a water quantity of 200L/ha; the observed efficacy in terms of root damage was 79% in this study [16]. Experiments conducted by Tóth et al. in 2019 [20] confirmed that the effect of nematodes applied simultaneously with the sowing shows a proportionately lesser decrease with the passing of time in comparison with soil insecticides comprising chemical active ingredients (cypermethrin, chlorpyrifos, tefluthrin) [20].

These studies concluded that nematodes applied at a rate of 2 billion per hectare show appropriate efficacy in the control of Western corn rootworm larvae [17]. In an experiment conducted in southern Hungary which compared biological (nematodes, fungi) and chemical (clothianidin, tefluthrin) insecticide products, it was found that chemical products reduced soil fauna to a greater extent, and killed numerous useful organisms other than the target species [3]. Further favourable effects of using entomopathogenic nematodes include the applicability in ecological farming, as well as a reduced risk of potentially developing resistance [10].

In the beginning of our research, it was hypothesised that the entomopathogenic nematode *Heterorhabditis bacteriophora* applied at a rate of 2 billion/ha retains viability and larvicide effect even if lower quantities (50 to 100 L/ha) of water are used.

2 Materials and methods

Experiments using a product called Dianem, which contains the entomopathogenic nematode *Heterorhabditis bacteriophora*, were conducted for 2 years (2021 and 2022) at various locations in Röjtökmuzsaj and Perkáta, Hungary.

Röjtökmuzsaj is located in the western part of Győr-Moson-Sopron County, and has a 60-year-old maize monoculture. This study site has a highly cohesive soil, which is rich in humus (3.25%), is slightly acidic (pH: 6.87), and has a good supply of potassium and a medium level supply of phosphorous.

The other study site in Perkáta is located in central Hungary, in the eastern part of Fejér County, and is considered one of the best maize growing areas of the country. It is characterised by favourable soil conditions, which refers to medium level cohesion, high humus content (3.5%), slightly alkaline pH (7.2), and good supply of both potassium and phosphorous. In each year, the experiments were conducted in four replicates, using small experimental plots with a size of 3×6 m, and in randomised experimental setup. Each experimental plot was sown with 24 linear meters (4×6 linear meters) of maize. In each year, the experiments were conducted using 3 different per-hectare injection volumes of water (50, 100 or 200 L/hectare), and were compared to a negative (untreated) control, and to the widely used product Force 1.5G containing tefluthrin as active ingredient (positive control). Since living organisms are used, special care had to be taken during the storage and transportation of the experimental materials. 500 g packages comprising 500 million nematodes were stored in the dark in refrigerators until use, and cooler bags with ice accumulators were used during transportation. Experimental materials could only be opened immediately before the treatments.

Before setting up the experiments, the first task was to check the viability of the nematodes. A microscope was used to examine the motility of the larvae, and the ratio of live and dead larvae was recorded to yield a viability percentage. Above 80%, the viability of the nematodes is considered excellent.

The setting up of the experiments was started after examining the viability of the nematodes. In each experimental plot treated with Dianem, a total of approximately 36 million nematodes had to be introduced, which was contained in 36 g of the experimental material. The treatments were carried out in four replicates; therefore, 12×36 g experimental materials were weighed out. The quantity of nematodes was constant across the experimental plots treated with them, the only variable being the volume of injected water. The first treatment involved dissolving 36 g of material in 90 mL of water, and the solutions thus prepared were evenly distributed among the 4 rows of an experimental plot using an electrical pipette. The second treatment involved dissolving 36 g of the appropriate experimental material in 180 mL of water, and the same values were 36 g of experimental material and 360 mL of water, respectively, in the third nematode treatment. In both years, DKC 1541 hybrid seeds were used (sowing depth: 8 cm, distance between the plants: 19 cm, row distance: 75 cm). Upon injecting the nematodes in a sowing bed, the seed bed was immediately closed in order to reduce the UV exposure of the nematodes. The sowing dates were 12 April in 2021 and 20 April in 2022.

The method of setting up the experiments was identical for each treatment and in each year. The number of larvae present in the root zone was recorded, and these were used to determine average larval counts per plant. The degree of chewing back on the roots that had been dug out was determined using the Modified Iowa Scale. In each year, recording the larval count was carried out based on forecasts prepared using yellow capture sheets equipped with sex pheromone traps, which were applied at different locations of the site. Work was started as soon as the first imagoes appeared because this confirmed that the given developmental stage of the soil-dwelling larvae was about to finish (L3; pupa). From a single

experimental plot, 5 plants were randomly selected and dug out together with an earth ball of the size of 20×20 cm. Upon digging out, live larvae were counted in the soil removed from the maize roots and in the pit that was dug out. Larvae in stage L3, which is the free pupa stage, and imagoes coming out of the soil were counted together. In each case, the roots that had been dug out from each experimental plot were collected in labelled bags and transferred to the Gyömöre Station, where root chewing was assessed using the Modified Iowa Scale after soaking. Larval counts and root chewing data from each experimental plot were recorded continuously, and were subsequently analysed using mathematical and statistical methods (one-way ANOVA; Tukey's post hoc test).

3 Results and discussion

3.1 Experiments at röjtökmuzsaj in 2021

In 2021 in Röjtökmuzsaj, larval counts from the experimental plots treated with different concentrations of Dianem (50, 100, 200) were compared to the results of experimental plots treated with Force 1.5G as positive control, and with those of a negative control.

According to the results, the lowest larval counts (2.40±1.79 larvae/plant) were detected in the experimental plots treated with Force 1.5 G. This was followed by the experimental plots treated with Dianem 50 (2.6±2.39 larvae/plant), Dianem 100 (2.50±2.07 larvae/plant) and Dianem 200 (2.50±2.12 larvae/plant). The highest average larval count per plant was observed in the untreated control plot (4.95±2.69 larvae/plant) (Figure 1). The high standard deviation values suggest significant differences between the plants in terms of larval counts.

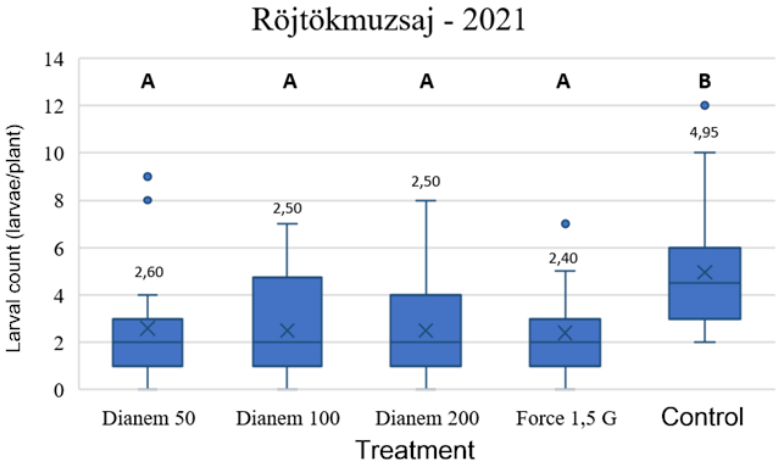

Fig. 1. Average larval count per plant upon treatment with Dianem, Röjtökmuzsaj 2021.

A one-way ANOVA was conducted to statistically evaluate the differences between the control and treated plots, and was completed with Tukey's post hoc test. According to the results of the analysis, at least two of the treatments used in the experimental parcels studied in Röjtökmuzsaj in 2021 are significantly different from each other in terms of larval counts at a significance level of 5% ($p=0.001$; $F=4.847$). The results of Tukey's post hoc test suggest that all treatments (Dianem 50, 100 and 200, and Force 1.5G) significantly differ from the larval count results of the negative control plots; however, the other studied Dianem treatments and Force 1.5G treatment do not show significant differences. The per-plant average larval count of the untreated control plot is significantly different from that of the plots treated with Dianem 50 ($p_{Tukey}=0.011$), Dianem 100 ($p_{Tukey}=0.007$), Dianem 200

(p_{Tukey}=0.007) and a Force 1.5G (p_{Tukey}=0.004). These results clearly indicate that both the Dianem treatments and Force 1.5G treatment were able to significantly reduce larval counts. The difference between the treatments using varying volumes of water is not significant (p>0.05).

As suggested by the degrees of root chewing, the most severe root damage (m.Iowa score) was observed in the untreated negative control plot (m.Iowa: 3.55±1.07). This score slightly exceeds the m.Iowa score of 3.5 considered as the limit value for economic damage. The degree of root chewing in the experimental plots did not reach said economic threshold. It can be observed that among the treatments, the experimental plot treated with Dianem 200 showed the highest m.Iowa score (2.40±1.08), followed by Dianem 100 (2.18±0.96) and Dianem 50 (1.93±0.65) (Figure 2).

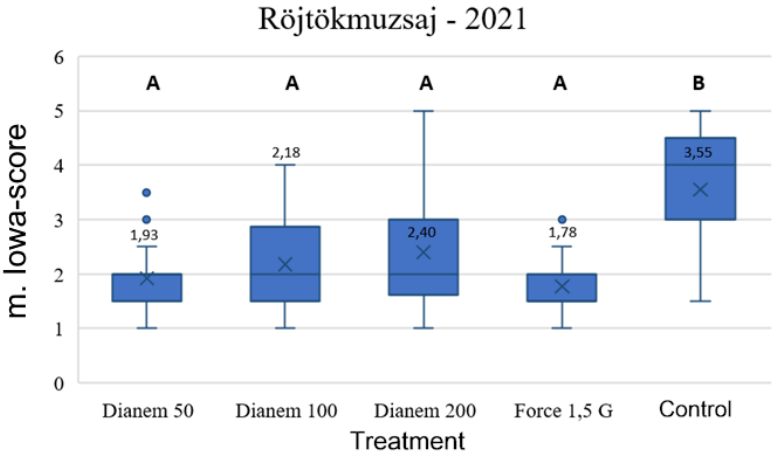

Fig. 2. Average degree of root chewing per plant upon treatment with Dianem, Röjtökmuzsaj 2021.

In order to assess the differences between the treatments, a one-way ANOVA analysis was performed, and this suggests that there exist at least two experimental plots that are significantly different from each other in terms of average m.Iowa scores (p=0.001; F=4.847). The results of Tukey's post hoc test demonstrate that all treatments are significantly different from the results of the control plots. The control plot is significantly different from the plots treated with Dianem 50 (p_{Tukey}=0.000), Dianem 100 (p_{Tukey}=0.000), Dianem 200 (p_{Tukey}=0.001) and a Force 1.5G (p_{Tukey}=0.000). Between the treated plots, no significance was observed.

3.2 Experiments at perkáta in 2022

In Perkáta, the highest per-plant larval count was observed in the control plot (3.20±2.46 larvae/plant), followed by the plots treated with Dianem 50 (0.95±0.95 larvae/plant), Dianem 200 (0.75±0.79 larvae/plant), Force 1.5G (0.76±0.66 larvae/plant), and Dianem 100 (0.60±0.82 larvae/plant) (Figure 3). The results suggest that the treated plots were characterised by significantly lower larval counts than the negative control.

The one-way ANOVA analysis indicates that there exist at least two experimental plots in Perkáta, which show significance (p=0.000; F=14.020). In view of the results of Tukey's post hoc test, the negative control plot is characterised by a significantly higher larval count than the plots treated with Dianem 50 (p_{Tukey}=0.000), Dianem 100 (p_{Tukey}=0.000), Dianem 200 (p_{Tukey}=0.000) and Force 1.5G (p_{Tukey}=0.000); however, no significant differences could be detected between the treatments. Treatment efficacies were not statistically different.

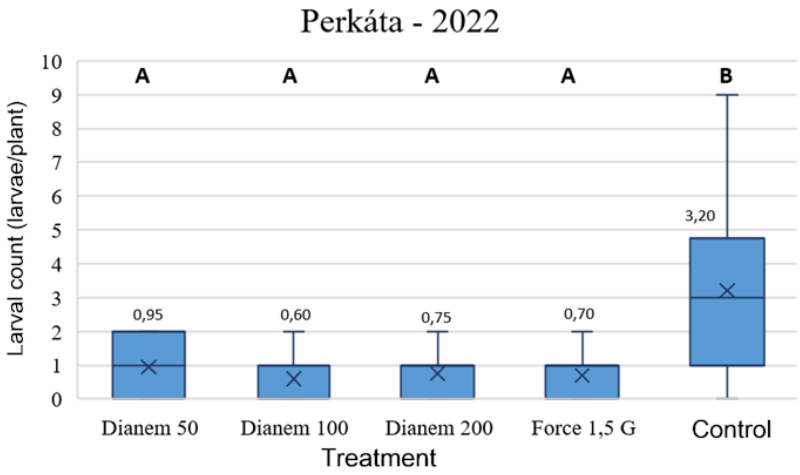

Fig. 3. Average larval count per plant upon treatment with Dianem, Perkáta 2022.

In Perkáta, the most notable degree of root chewing was observed in the negative control plots (3.93±1.23), and this value exceeds the economic damage threshold of 3.5. Among the treatments, the greatest root damage was detected in the experimental plot treated with Dianem 100 (2.03±0.57), followed by Dianem 200 (1.90±0.50), Dianem 50 (1.83±0.47) and Force 1.5G (1.80±0.57) (Figure 4). Entomopathogenic nematodes injected with different volumes of water, as well as Force 1.5G used as positive control strongly reduced root damage.

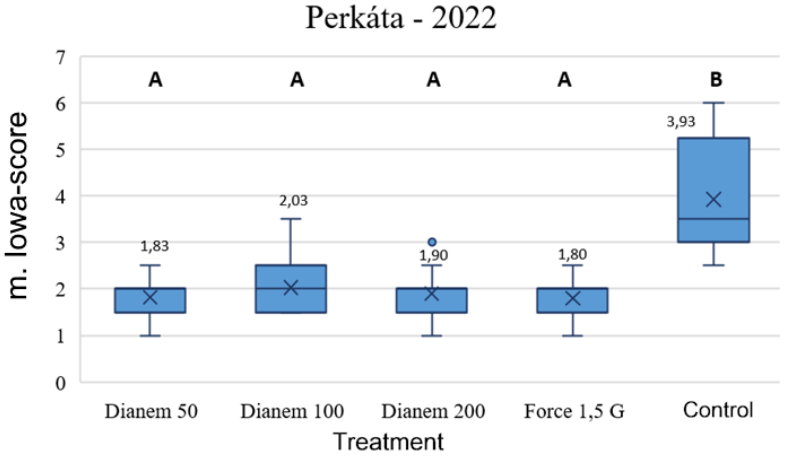

Fig. 4. Average degree of root chewing per plant upon treatment with Dianem, Perkáta 2022.

The evaluation of the differences between the treatments and the untreated control indicates that there exist at least two experiments that are significantly different from each other (p=0.000; F=31.834). In view of the results of Tukey's post hoc test, the negative control plot is characterised by a significantly higher m.Iowa score than any of the treatments with Dianem 50 (p_{Tukey}=0.000), Dianem 100 (p_{Tukey}=0.000), Dianem 200 (p_{Tukey}=0.000) and Force 1.5G (p_{Tukey}=0.000). Thus, the negative control is significantly different from all treated experimental plots (p>0.05). Both the Dianem treatments and Force 1.5G treatment were able to significantly reduce the degree of root chewing. At a significance level of 5%, no statistical difference was detected between the nematode-based treatments and the plots treated with Force 1.5G.

In major maize growing areas, and especially in places where monocultures are predominant, the control of Western corn rootworm presents substantial challenges. In recent years, the value of biological products protecting useful organisms is increasing due to the need for sustainable agricultural/plant protection practices, and such products may contain live organisms.

One such experimental material that was used in the present study is a live entomopathogenic nematode of the species *Heterorhabditis bacteriophora*, which belongs to the genus *Heterorhabditis*. Several researchers have conducted studies regarding the efficacy of entomopathogenic nematodes against corn rootworm larvae.

The experimental results of [21] demonstrated that nematodes of the *Steinernema* and *Heterorhabditis* genera show the highest efficacy against *Diabrotica* larvae, and our studies also confirmed that *Heterorhabditis bacteriophora* from the genus *Heterorhabditis* was indeed successful in controlling the larval form of the pest, and reduced the numbers thereof to below the economic threshold.

Toepfer et al. (2010b) [16] proved that *Heterorhabditis bacteriophora* reduced the degree of root chewing by 14% to 54%. The efficacy of such live organisms is greatly influenced by the method of introducing them into the soil. In experiments conducted between 2004 and 2007, Toepfer et al. (2010) [18] evaluated the efficacy of nematodes introduced via soil injection and via spraying onto the soil surface. This study found that both methods reduced the damage by the larvae. In addition, it was observed that the most successful control can be achieved by directly introducing the nematodes into the opened seed bed using 200 L/ha of water.

The authors believe that in addition to the excellent efficacy of nematodes, another important issue is the method of application, which should preserve the viability and larvicide effect of the live organisms while not diminishing the performance of the sowed surface area. This can be achieved by identifying the minimum volume of water required for spraying/injection. Experiments were conducted for several years and at several sites under different larval pressure using injection volumes of 50, 100 and 200 L/ha. On the basis of the results of these experiments, it was concluded that there were no significant differences between the larval counts or the degree of root chewing in those conducted using different quantities of water, and this suggests that the nematodes remained alive and retained their larvicide capacity even when lower amounts of water were used.

From a practical point of view, this technology is of paramount importance because the injection volumes reported in prior publications could be reduced to 25% in our experiments.

It was concluded that the efficacy of the product used by us was as good as that of Tefluthrin. Other advantages include the lack of presenting an environmental burden and adverse effects on useful organisms or on the health of the individuals carrying out the work. Said product may also be used in ecological farming.

References

1. A.R. Dillman, P.W. Sternberg, Entomopathogenic Nematodes. Curr. Biol., **22**, 11, 430–431 (2012) https://doi.org/10.1016/j.cub.2012.03.047
2. B.P. Spike, J.J. Tollefson, Yield response of corn subjected to western corn rootworm (Coleoptere, Chrysomelidae) infestation and lodging. J. Econ. Entomol., **84**, 1585–15 (1991) https://doi.org/10.1093/jee/84.5.1585
3. D. Badendreier, P. Jeanneret, C. Pilz, S. Toepfer, Non-target effects of insecticides, entomopathogenic fungi and nematodes applied against western corn rootworm larvae in maize. J. Appl. Entomol., **139**, 6, 457–467 (2015) https://doi.org/10.1111/jen.12229

4. D. Čamprag, R. Sekulič, F. Bača, Western corn rootworm is a new maize pest in Yugoslavia. Agrofórum, **4**, 7, 41–42 (1994)
5. E.A. Hallem, A.R. Dillman, A.V. Hong, Y. Zhang, J.M. Yano, S.F. DeMarco, P.W. Sternberg, A sensory code for host seeking in parasitic nematodes. Curr. Biol., **21**, 5, 377–383 (2011) https://doi.org/10.1016/j.cub.2011.01.048
6. G. Vörös, Another enemy of corn: Western corn rootworm. Gyakorlati Agrofórum ("Practical Agroforum Booklets"), **6**, 35, 2002
7. J. Kiss, C.R. Edwards, European distribution of western com rootworm. Gyakorlati Agrofórum, **12**, 5, 2–3. (2001)
8. J.L. Krysan, Diapause in the nearctic species of the virgifera group of Diabrotica: evidence for tropical origin and temperate adaptations. Annals Entomol. Soc. America, **75**, 36–142 (1982) https://doi.org/10.1093/aesa/75.2.136
9. J.R. Fisher, G.R. Sutter, T.F. Branson, Influence of corn planting date on the survival and on some reproductive parameters of Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). Environ. Entomol., **20**, 185–189 (1991) https://doi.org/10.1093/ee/20.1.185
10. L. Vörös, An overview of the methods for controlling Western corn rootworm. Acta Agron. Óváriensis, **62**, 2, 141–162 (2021)
11. L. Vörös, R. Ábrahám, Effect of azadirachtin applied as seed coating on the larval density of and root injury caused by the Western Corn Rootworm /Diabrotica virgifera virgifera/. J. Plant Dis. Prot. **130**, 757-767 (2023) https://doi.org/10.1007/s41348-023-00763-3
12. R.K. Raja, K. Padmanaban, S. Sivaramakrishnan, Entomopathogenic nematodes: a best bio-control agent for insect pest: isolation and identification of entomopathogenic nematodes from agricultural land. Lambert Acad. Publishing. (2011).
13. S. Bekele, M.P. Boddupalli, H. Jonathan, B. Marianne, Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. Food Secur., **3**, 307-327 https://doi.org/10.1007/s12571-011-0140-5
14. S. Rasmann, T.G. Köllner, J. Degenhardt, I. Hiltpold, S. Toepfer, U. Kuhlmann, J. Gershenzon, J. Turlings, Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature, **434**, 7, 732–738 (2005) https://doi.org/10.1038/nature03451
15. S. Toepfer, C. Gueldenzoph, R.U. Ehlers, U. Kuhlmann, Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae) in Europe. Bull. Entomol. Res., **95**, 5, 473–482 (2005) https://doi.org/10.1079/ber2005379
16. S. Toepfer, I. Hatala-Zseller, R.U. Ehlers, A. Peters, The effect of application techniques on field-scale efficacy: Can the use of entomopathogenic nematodes reduce damage by western corn rootworm larvae? Agricul. Forest Entomol., **12**, 4 (2010b) https://doi.org/10.1111/j.1461-9563.2010.00487.x
17. S. Toepfer, P. Knuth, M. Glas, Sz. Tóth, M. Zellner, Field level dose-efficacy response of entomopathogenic nematodes at controlling at Diabrotica v. virgifera (Coleoptera: Chrysomelidae). Poster. 65. Növényvédelmi Tudományos Napok ("65th Plant Protection Science Days"), Budapest, 19 to 20 February 2019
18. S. Toepfer, R. Burger, R.U. Ehlers, A. Peters, U. Kuhlmann, Controlling western corn rootworm larvae with Entomopathogenic nematodes: effect of application techniques on plant-scale efficacy. J. Appl. Entomol., **134**, 5, 467–480 (2010) https://doi.org/10.1111/j.1439-0418.2009.01469.x
19. S.P. Stock, H. Goodrichblair, Entomopathogenic nematodes and their bacterial symbionts: The inside out of a mutualistic association. Symbiosis, **46**, 2, 65–75 (2008)

20. Sz. Tóth, M. Szalay, M. Zellner, P. Knuth, M. Glas, J. Kiss, S. Toepfer, Temporal differences in the field efficacy of different methods for the biological and chemical control of the larvae of Diabrotica v. virgifera (Coleoptera: Chrysomelidae). Poster. 65. Növényvédelmi Tudományos Napok ("65th Plant Protection Science Days"), Budapest, 19 to 20 February 2019
21. V. Santos, A.M. Junior, V. Andaló, C.C. Moreira, R. Alves de Olinda, Virulence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) for the control of Diabrotica speciosa germar (coleoptera: chrysomelidae). Ciencia e Agrotechnol., **35**, 6 (2011) https://doi.org/10.1590/s1413-70542011000600015