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Improving methods in studying the *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) under laboratory and field conditions to better assess successes and failures of diverse control methods

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1. INTRODUCTION AND OBJECTIVES

Maize (*Zea mays* L.) is one of the most important crop plant for humans, in the human diet it has been estimated that around 50% of the world's consumed calories come from this plant. Its importance is also indicated by its cultivation area which is around 197 million ha worldwide in 2020 (in Europe: 9.2 million ha from that 1 million ha in Hungary) (FAO, 2021, KSH, 2021). One of the most important insect pests of this crop is the *Diabrotica virgifera* spp. *virgifera* (Coleoptera: Chrysomelidae) or Western Corn Rootworm both in North America and Europe (Krysan and Miller, 1986; Kiss et al. 2005). In Europe, this species considered as an invasive, alien species, because its successfully invaded the local maize fields in the 1990s (Edwards et al., 1999; Kiss et al., 2001). *Diabrotica v. virgifera* is an univoltine species with eggs overwintering in the soil of the maize fields and with three larval instars. The adults emerging from the soil end of June and beginning of July (Ball, 1957; Chiang, 1973). Despite that the adults can feed on pollen and clip the silk of the maize (Gyeraj et al., 2021), the main problem is that larval instars feeding and chewing the maize roots. The larvae injury on the roots cause reduced water and nutrient uptake, plant lodging and ultimately yield loss (Kahler et al., 1985). To manage damages by the beetle farmers can choose control methods including cultural practices.

The main pest management approaches include (1) synthetic insecticides such as granular or liquid soil insecticides as well as seed coatings against larvae, or foliar sprays against adults; (2) biological control agents (i.e. entomopathogenic nematodes and fungi, predatory mites) against the larvae; (3) cultural control of the larvae through crop rotation; or (4) GM maize through expressing insecticidal proteins or RNA interference (Levine and Oloumi-Sadeghi, 1991; Toepfer et al.,

2005; Whangbo and Hunter, 2008). However, all of the listed methods have its limitations. For example, resistance against insecticides, crop rotation and genetically modified maize plants has been detected. Moreover, even if resistance was not detected occasionally high variability of the efficacies of the soil insecticides, seed coatings and entomopathogenic nematodes was observed under field conditions (Meinke et al., 2021). Therefore, in my work first I tried to determine why the different control methods has variable efficacies to reduce pest populations and prevent root damage under field conditions. Secondly, because of this variability and the availability of some of the above listed chemical insecticides, I've examined a new alternative and environmentally friendly approach. Lastly, for better planning trials and assays with this pest's eggs, I have determined the shortest diapause length and the optimal incubation temperature which yields the largest number of larvae and the most synchronized egg hatching.

The specific objectives were the following:

- Investigate how the efficacy of the entomopathogenic nematodes and soil insecticides temporarily changes in the cropping season at reducing *Diabrotica v. virgifera* pest populations under field conditions
- Determine which and how the abiotic and biotic factors may be influencing the efficacies of the entomopathogenic nematodes and soil insecticides at reducing *Diabrotica v. virgifera* pest populations and at preventing root damage under field conditions.
- Assess alternative pest control options against *Diabrotica v. virgifera*, such as the botanical-derived azadirachtin as a granular soil insecticide, an establish LD and ED values under laboratory and greenhouse conditions.
- Determine the shortest diapause length and optimal incubation temperatures which still not compromises proper hatching rates and hatching synchrony of *Diabrotica v. virgifera* eggs, allowing a better planning of experimentation

2. MATERIALS AND METHODS

2.1 Assessing temporal effects of treatments

The study was carried out on 12 maize fields in southern Hungary between 2010 and 2018. All agricultural work was done by the farmers with their machineries. Maize fields were rotated (except one field). The target organism was *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) wild, diapausing population. Six to seven subsequent maize plants were artificially infected with ready-hatch-eggs two to three weeks after the plants were germinated per plot. The experimental design followed the PP 1/212 and PP 1/152 EPPO standards (Anonymous, 1999, 2007). We used four to five plots/treatments arranged in a systematic block design. All treatments were applied as in-furrow ones at sowing. The *Heterorhabditis bacteriophora* (Dianem™) entomopathogenic nematodes were sprayed with water into the furrow with commercial applicator. The concentration was 1.5 to 2 billon nematodes/ha. The chlorpyriphos (Kentaur™) granule insecticide was applied in-furrow with a seeder-mounted fine applicator. The concentration was 10 kg granules/ha. The cypermethrin (Belem™) micro-granule pyrethroid was applied in the same way as the chlorpyriphos. The rate was 12 kg granules/ha. The tefluthrin (Force™) fine granules were again applied in the same way as the other soil insecticides. The rate was 13 to 15 kg granules/ha. The negative control was the untreated, infected plots.

The *Diabrotica v. virgifera* adult emergence was measured with gauze cages covering the 6 to 7 six to seven subsequent maize plants. Adult beetles were counted on a weekly basis (sex differentiation on site and release). Later, adult emergence data were standardized to 100 eggs

per plant per week. The weekly cumulative emergence was calculated per time step for all experiments. The efficacy of each treatment was calculated as the *Diabrotica v. virgifera* emergence relative to the control (corrected efficacy % = 100 × (beetles in control plots − beetles in treated plots)/maximum (beetles in control or treated plots). Temporal dynamics of pooled sex, male and female adult emergence as well as their cumulative emergence were plotted over weeks using loess smoothed (local polynomial regression) lines. The cumulative adult emergence curves (= local polynomial regression lines) were used to estimate the date of start and end of adult emergence (Table 2). Differences between male and female emergence start, their 25, 50, and 75% emergence, and their peak emergence were analyzed for each treatment and controls using paired t-tests. Linear models were used to identify and compare control effects of treatments as well as to investigate the influence of treatments, sex, and their interaction on efficacies with additionally performed basic diagnostic plots for assumption of residual normality and homoscedasticity, as well as plots of Cook's distance for detecting influential data points. To assess the temporal effects of treatments on the reduction of *Diabrotica v. virgifera*, we assumed that temporal shifts in adult emergence patterns reflect potential shifts in larval control by the treatments. The cumulative emergence of the adults in cages of each treatment and experiment was standardized and plotted as a percentage of the corresponding total emergence.

2.2 Assessing the influence of abiotic and biotic factors influence on treatment efficacies

This series of experiments was carried out on 20 maize fields in southern Hungary between 2010 and 2020 on commercial maize fields. Maize fields were rotated (except field T, S, P, O, H with 2nd year maize) and one field (L) with 3rd year maize. The target organism was *Diabrotica virgifera* ssp. *virgifera* LeConte (Coleoptera: Chrysomelidae) wild, diapausing population. Six to seven subsequent maize plants were artificially infested with ready-hatch-eggs two to three weeks after the plants were germinated per plot. Another set of the plants was infested and marked for root damage assessment from the same plot. The experimental design followed the PP 1/212 and PP 1/152 EPPO standards (Anonymous, 1999, 2007). We used four to five plots/treatments arranged in a systematic block design. All treatments were applied into the furrow at sowing. The *Heterorhabditis bacteriophora* (Dianem™) entomopathogenic nematodes were sprayed with water into the furrow with commercial applicator. The rate was 1.5 to 2 billon nematodes/ha. The clothianidin (Poncho™) seed coating was used in a 0.006 clothianidin ml/seed. The cypermethrin (Belem™) micro-granule pyrethroid was applied in the same way as in the previous section (2.1). The treatment rate was 12 kg granules/ha. The tefluthrin (Force™) fine granules were again applied in the same way as the other soil insecticide. The rate was 13 to 15 kg granules/ha. The negative control was the untreated, infected plots.

The weather data were recorded by hourly (David Instruments). One litre of mixed soil sample was taken from 5 to 30 cm from each experimental sites every year. These samples were sent to the Soil

Conservation Service, Szolnok, Hungary to determine the clay, silt loam sand, bulk density in May-June, CaCO3, humus and pH of the soil. Soil moisture was measured and calculated according to (Anonymous, 1999) on a monthly basis from April to August. Quality control assays with *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) were implemented as per Toepfer et al. (2008). The assessment pest populations and root damage were the followings: Four to five series of six to seven infested maize plants were assessed per treatment and per control per field. Plants were covered with gauze cages. The emerged adult beetles of *Diabrotica. v. virgifera* were counted on a weekly basis and re-moved from cages at each assessment Tóth et al. (2020). Weekly emergence data of adults were standardized to 100 eggs per plant. The efficacy of each treatment was calculated as the reduced emergence of beetles relative to the untreated control (corrected efficacy %=100 × (beetles in control plots − beetles in treated plots) / maximum (beetles in the control or treated plots). The roots of 24 to 30 maize plants per treatment were dug out from each field every year from early-July to beginning of August to assess the damage. Damage was rated using two scales: (i) the 1–6 Iowa scale for the general root damage (Hills and Peters, 1971), which is the most commonly used scale despite the fact that it may overestimate the importance of minor damage (such as feeding scars), and (ii) the 0.00–3.00 node injury scale for heavy root damage (Oleson et al., 2005), which is a linear and decimal scale that measures only totally destroyed roots or nodes. Corrected efficacy of root damages was calculated as 100 × (damage in control plots – damage in treated plots) / maximum (damage in control or treated plots).

We used ordinary least squares linear models to detect the overall effect of treatments on adult emergence and root damage. That followed by ANOVA tests with post-hoc Tukey test to detect differences between

the treatments. Then, Pearson's correlation coefficients (r coefficient) were calculated between the different factors and between control efficacies of treatments on the adult emergence as well as on general and heavy root damage. Factors appearing to have r coefficients of at least between -0.4 =< r coefficient >= +0.4 with p-value < 0.05 were chosen for developing regression models. This means factors with small r coefficients were dropped from further analysis. Simple or multiple linear regressions were performed for correlated factors to find relationships between factors and control efficacies. To avoid any misinterpretation of the model's non-linearity of the response-predictor pairs, correlation of the error terms, non-constant variance of error terms, outliers, high-leverage points and multicollinearity were investigated before model execution (Hastie et al., 2021).

2.3 Assessing the effects of azadiracthin botanical insecticide effect *Diabrotica v. virgifera*

The target organism was the non-diapause population of the *Diabrotica v. virgifera*. The population is originated from USDA ARS laboratories (Brookings, USA), and reared in-house since 2017.

Laboratory tests: The positive controls were the following: azadirachtin 1% (NeemAzal-T/S 10EC) with 9 different concentrations (1 µg a.i./ml – 10000 µg a.i./ml); imidacloprid 20% (Confidor 200 SL) with one concentration (2 µg a.i./ml); cypermethrin 0.8% (Belem 0.8 MG) with one concentration (100 µg a.i./ml); tefluthrin 1.5% (Force 1.5G) with one concentration (100 µg a.i./ml). The test agent was: azadirachtin 0.15% (Neem Azaal 0.15G) with 4 different concentrations (0.1 µg a.i./ml – 100 µg a.i./ml). The negative control was the wells treated with distilled

water. Three artificial diet-based bioassays were conducted under semi-sterile conditions. Each bioassay consisted 3 to 6 plastic 96-well plates. Each well contained 190 µl artificial diet (Frontier #F9800B diet + dried maize roots) and 20 µl top-treatment and 1 neonate larvae (sterilized the day before) and closed with an optically clear adhesive sheet. After that, the plates were placed in a dark, 24 C° incubator and after 3 and 5 days' mortality and sub-lethal effects were assessed.

Treatments were analyzed through unifactorial Generalized Linear Model (GLM) and multiple comparisons were applied using Tukey HSD post hoc comparison of data of equal variances and Games Howell post hoc comparison for unequal variances. Logistic regression analyses were applied to assess the dose response of each treatment including lethal dose leading to 50% or 90% mortality ($LD_{50/90}$).

Greenhouse tests: The positive controls were the following; cypermethrin 0.8% (Belem 0.8 MG) with one concentration (7.2 mg a.i./meter furrow); tefluthrin 1.5% (Force 1.5G) with one concentration (15 mg a.i./meter furrow). thiamethoxam 30% (Cruiser 350FS) with one concentration (6.25 mg a.i./5 seeds). The test agent was: azadirachtin 0.15% (Neem Azaal 0.15G) with 5 different concentrations (2 mg a.i./meter furrow – 280 µg mg a.i./meter furrow). The negative control were the pots infected with eggs. The blank control were the pots with no infestation. Each treatment was applied into the soil of three to four systematically arranged blocks (= replicates) of five pots. This totalled 15 to 20 data points (= sample size) per treatment per experiment. In detail, each pot (plastic garden pot, 15 cm inner diameter × 10 cm height, 2 l) was first filled with 1 l sterilized soil. Two maize seeds were added, one removed later. Thereafter, 200 ml water were applied to each pot. Treatments were applied either as granules along a 2 cm wide strip across

the 10 cm diameter of the pots, or as seed coating. Later, maize pots were infested with 50 -100 viable ready-to-hatch eggs per plant. At this point in time, the majority of plants was at 3 leaf stage (height 15 to 20 cm). After 40 – 50 days the experiments have terminated, and larvae numbers, fresh weight of the plants and root damage was assessed (for two damage scales see section 2.2).

The statistical analyses stated with the Levene's test to determine equality of the variances. Influences of treatments on assessed factors were analyzed through GLM analyses or through independent samples Kruskal–Wallis H test. Tukey HSD post hoc multiple comparison tests were applied following GLM in case of equal variances, and Games Howell test in case of unequal variances. Logistic regression analyses were applied to assess the dose response of each treatment including the effective dose leading to 50% suppression of the larval populations or root damage prevention (ED_{50}) The mean corrected efficacy of each treatment was calculated relative to the untreated control, this is corrected efficacy % = 100 × (larvae or damage in control plots – larvae or damage in treated plots)/maximum (larvae or damage in control or treated plots).

2.4 Understanding the hatching patterns of the *Diabrotica v. virgifera* eggs

We tested two different populations of *Diabrotica v. virgifera*. One was wild, diapausing, CSE population in Europe, collected in 2017 and 2018 in Csongrád-Csanád and Békés Counties. Adults were reared under standardized laboratory conditions at 23–25 °C and 40–60% r.h. The obtained eggs were held for pre-diapause development at around 24 °C during 2–3 weeks and then washed, cleaned and stored at 6–8 °C for diapause in dark conditions until use. The eggs were periodically checked

for quality control. The other population was the laboratory, non-diapausing, ND *Diabrotica v. virgifera*. These eggs were obtained from USDA-ARS (Brookings, USA) and reared since 2017 in the same conditions as the other population. Eggs were collected on a weekly basis, but no chilling phase was applied.

Egg batches from the wild population of *Diabrotica v. virgifera* were overwintered at 6–8 °C for eight diapause durations: no diapause, 0.5–1.5, 2–4, 5–7, 8–10 (i.e., natural diapause length), 11–13, 14–16, and >17 months of diapause. Eggs from the ND population were not exposed to cool temperature periods and used directly. Before usage, eggs were washed and cleaned. Eggs from each batch were pipetted in water onto a 5-cm-diameter filter paper in each of 12 Petri-dishes. Four Petri-dishes of eggs from each batch were incubated on the moist filter paper (no free water visible) in the dark at each of three temperatures (16 ± 0.5 °C, 20 ± 0.5 °C, and 24 ± 0.5 °C) and 50–60% r.h.

Egg overwintering survival was assessed through counting healthy and dead eggs under a stereomicroscope. During egg incubation, hatching of larvae was assessed every 2nd or 3rd day. Hatched larvae were removed from the dishes with clean forceps. Egg overwintering survival and hatching rates, as well as the beginning, peak, termination, and duration of egg hatching were calculated per Petri-dish, and then averaged among the four corresponding Petri dishes of the same treatment.

Raw data were standardized to 100 eggs per dish to visualize temporal hatching dynamics. Mean values were used to plot start, peak, duration, and termination of egg hatching per diapause category. Synchrony of hatching was, next to a visual assessment, also characterized through calculating the standard deviation (SD) and coefficient of variation of each of those steps of hatching. Basic

diagnostic plots were used to examine assumptions of the normal distributions of the residuals and homoscedasticity. Generalized additive models (GAMs) were applied to analyze the effect of diapause length (eight categories) and post-diapause incubation temperature (16, 20, and 24 °C) on the beginning, peak, termination, and duration of egg hatching as well as on hatching rates. General linear hypothesis testing (GLHT) followed by post-hoc Tukey honestly significant difference tests were applied to detect variability in egg hatching patterns between the eight diapause length categories, three incubation temperatures, and two population types.

3. RESULTS

3.1 Missing temporal effects in the treatment efficacies

Our results showed that the cypermethrin, tefluthrin and the *H. bacteriophora* were able to control the pest populations according to the total adult beetle captures with gauze cages. Altogether, the treatment efficacies were around 33 to 42% with relatively high standard deviations. Variability in chlorpyriphos's efficacy indicated that it can only controls female beetles sufficiently.

We detected that tefluthrin's efficacy slightly increases over time to control females, thus they can better control late than early female larvae. Conversely, there was some minor slight indications that chlorpyriphos can better control early than late females. However, these changes are small, they are not reflected in the 25, 50, 75% and peak cumulative adult emergence compared to control. Cypermethrin and *H. bacteriophora* continuously reduced *Diabrotica v. virgifera* regardless of sex.

3.2 Limited influence of the abiotic and biotic factors on treatment efficacies

Generally, we detected that the applied control methods, the seed coated clothianidin, the granule cypermethrin and tefluthrin and the water-sprayed *H. bacteriophora* can reduce pest populations and the prevent general and heavy root damage with a relatively high variability. Results showed that 20% - 30% of the cases treatments were unable to reduce pest populations and in 5% - 20% unable to protect the roots from the heavy root damage.

We investigated 6 biotic and 20 abiotic factors influence on the soil insecticide efficacies to reduce pest population and prevent root damage. We found that there are very few factors which influences treatment efficacies. For example, clothianidin was slightly less effective at controlling *Diabrotica v. virgifera* with increasing $CaCO_3$ and humus content of the soil, and at preventing root damage at high soil bulk density. Late maize sowing and late treatment in April or May as well as high soil moisture in July improved the prevention of the heavy root damage by this chemical. Cypermethrin was more effective to prevent heavy root damage, when an increased amount of clay of the soil was there. Tefluthrin was less effective controlling *Diabrotica v. virgifera* with increasing soil moisture in June, but slightly more effective with increased amount of cumulative rainfall in July. It's efficacy to prevent heavy root damage was less effective with increased sand content of the soil.

We were not able to establish any relationships between the *H. bacteriophora*'s efficacies on controlling pest population and protect maize root damage and between the investigated 12 biotic and 20 biotic factors. However, there were some promising results in the point of view of the field application of nematodes. For example, all mortality and virulence indicators showed lack of influence on their efficacies, meaning the quality of the nematodes was satisfying during the field application. A wide range of water amount (133 L to 558 L) can be used to apply nematodes without compromising their efficacies, thus lower water amount can be used meaning that the farmers needs to carry less water quantities to the field.

3.3 Azadirachtin-based soil insecticide can become an alternative solution

Bioassays conducted in the laboratory revealed that after 3 days of the experiments the LD_{50} was 22.3 µg azadirachtin/ml which corresponds to 0.45 µg/neonate larvae. After 5 days this LD_{50} was 19.3 µg azadirachtin/ml which means 0.39 µg/first to second instar larvae. Due to their high and rapid kill, no sub-lethal effect was observed. Bioassays showed that, the newly tested azadirachtin-based product reached earlier the 100% mortality after 5 days on neonate larvae compared to a commercially available, liquid formulation product. Experiments in the greenhouse showed that the proposed standard dose of the granular formulated 38 g azadirachtin/hectare applied at sowing into the furrow were not able to control *Diabrotica v. virgifera* or prevent root damage. However, 10x of the standard dose can suppress pest populations as well as protect roots. This was better than the efficacy of the cypermethrin-based granules and comparable to tefluthrin-based granules or the thiamethoxam seed coatings. In this case, the ED_{50} was 92 g azadirachtin/ha to control *Diabrotica v. virgifera*, for preventing general root damage it was 220 g/ha and for preventing the heavy root damage it was 52 g/ha.

3.4 Diapause can be reduced at optimal incubation temperature

Generally, at 20-24 C° and 2 – 10 months of diapause hatching rates are comparable for the diapausing, wild population. At 16 C° incubation temperatures the hatching rates are lowered. Generally, at 20-24 C° and with 5 – 13 months of diapause the hatching start, peak, end

and duration are not different for the diapausing, wild population. At 16 C° incubation temperatures the hatching patterns are scattered.

As the result of the experiments, a data matrix assembled to provide detailed information for the experimenters to estimate egg hatching patterns for their experiments.

4. CONCLUSIONS AND OUTLOOK

In conclusion, all tested products, regardless of chemical or biological were able to control the *Diabrotica v. virgifera* populations and prevent root damage under variable field conditions and in most case across fields and years. There were no or only few and slight indications that the used treatments had any temporal effects in their efficacies during the cropping season. Farmers can be assured that these treatments have long-lasting effects under field conditions.

There were only few indications that the investigated abiotic and biotic factors can cause the variability of the treatment efficacies often observed in the control of *Diabrotica v. virgifera*. This indicates that environmental factors individually are not effects treatments efficacies in a major way, thus they universal usage is recommended. The generally good handling and application of the entomopathogenic nematodes and the fact the even 100 L/ha water is enough to apply them in the field are suggests that farmers can easily adapt this control method in their own fields. Our results suggest that there may be complex multi-interactions between the factors which are acting on each other or together and thus their added effect act on negatively on the treatment's efficacies. These kinds of fine interactions we were not able to detect, and may be worth studying further. As for the variability of nematode efficacy, it is possible that microbial communities of the soil and the belowground fauna play a larger role in influencing efficacies of nematodes than expected. This warrants further investigation.

We demonstrated that azadirachtin based granular products could replace phased-out soil insecticides, if delivered in sufficient rates.

Increased active ingredient containing granules and large field trials are needed before this solution could be a part of farmer's plant protection toolkit. Ultimately, farmers may be able to continue they traditional ways of controlling this serious pest under field conditions, i.e. through soil application of plant protection product granules during sowing of maize.

Our methodological research on the pest egg hatching in laboratory colonies revealed that researchers do not need to wait 8 – 10 months of natural egg diapause length before they could to use the eggs for experimentation. Eggs which underwent 2 – 10 months of diapause, and after that had been incubated at 20 – 24 °C can be used without compromising egg survival, hatching rates and hatching patterns. This significantly increases the frequency possible experiments. Diapause for <2 or >10 months negatively affected several egg hatching parameters, as did lower incubation temperatures (16 °C), and are therefore not recommend.

5. NEW SCIENTIFIC RESULTS

- I have justified that soil insecticides as tefluthrin, cypermethrin and the clorpyriphos, seed coating as the clothianidin and biological control *H. bacteriophora* entomopathogenic nematodes are able to reduce *Diabrotica v. virgifera* populations and prevent the root damage under field conditions in different years and fields with a high variability in their efficacies.
- I have found that the temporal effects of the above listed treatments are not the cause of their variable efficacies against controlling *Diabrotica v. virgifera* populations.
- I have detected that the investigated abiotic and biotic factors only slightly influence of the efficacies of the above-mentioned treatments efficacies against *Diabrotica v. virgifera.* populations and preventing general and heavy root damage.
- The azadirachtin based soil insecticide granules are able to induce high mortality among *Diabrotica v. virgifera* larvae under laboratory conditions. Also, it is able to reduce *Diabrotica v. virgifera* larval populations and prevent root damage under greenhouse conditions with an elevated dosage compared to the recommended one.

- I have determined that the diapausing phase of the eggs of the wild populations of *Diabrotica v. virgifera* can be reduced until almost to 2 months compared to the natural diapause length of 8 to 10 months without compromising it's successful and synchronized hatching at 20 -24 C° incubation temperatures.

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7. PUBLICATIONS AND SCIENTIFIC ACTIVITIES

7.1 Peer-reviewed papers (In English)

TOTH, SZ., SZALAI, M., KISS, J., TOEPFER, S. (2020) Missing temporal effects of soil insecticides and entomopathogenic nematodes in reducing the maize pest *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Journal of Pest Science 93, 767-781. (DOI: 10.1007/s10340-019-01185-7) (IF 5.9)

TOEPFER, S., **TOTH, SZ.**, SZALAI, M. (2021) Can the botanical azadirachtin replace phased-out soil insecticides in suppressing the soil insect pest *Diabrotica virgifera virgifera*? CABI Agriculture and Bioscience 2:28. (DOI: 10.1007/s10340-019-01185-7)

TOTH, SZ., SZALAI, M., TOEPFER, S. (2022) On understanding and manipulating the hatching patterns of *Diabrotica virgifera virgifera* eggs to improve design of experiments. Entomologia Experimentalis et Applicata 170: 122–133. (DOI: 10.1111/eea.1312) (IF 2.14)

JABEUR, R., GUYON, G., **TOTH, SZ.**, ET AL. (2022) A novel binary pesticidal protein from *Chryseobacterium arthrosphaerae* controls *Diabrotica virgifera virgifera* via a different mode of action to existing commercial pesticidal proteins. Plos One (Accepted) (IF 3.75)

7.2 Peer-reviewed papers (In Hungarian)

VÖRÖS L., ÁBRAHÁM R., NAGY K., **TÓTH SZ.,** TOEPFER S. (2022) Megtartja-e a *Heterorhabditis bacteriophora* a kukoricabogár lárvára (*Diabrotica v. virgifera*) gyarkolort ölő hatását kisebb vízmennyiséggekkel történő kijuttatás esetén? (Can *Heterorhabditis*

bacteriophora nematodes still control western corn rootworm larvae when applied with low amounts of water?) Növényvédelem 83(5): 192-200.

7.3 Conference proocedings (In English)

TOEPFER, S., **TOTH, SZ.** (2020) Entomopathogenic nematode application against root-damaging *Diabrotica* larvae in maize: what, when, and how? Microbial and Nematode Control of Invertebrate Pests. IOBC-WPRS Bulletin. 150, 185-188.

7.4 Peer-reviewed papers under publication (In English)

TOTH, SZ., TOEPFER, S., SZALAI, M., KISS, J. (2022) Limited influence of abiotic and biotic factors on the efficacy of soil insecticides and entomopathogenic nematodes when managing the maize pest *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae). Agronomy (Submitted)

TARIGAN, S.I., **TOTH, SZ.**, SZALAI, M., KISS, J., TUROCZI, GY., TOEPFER, S. (2022) Biological control properties of microbial plant biostimulants. A review. Biocontrol Science & Technology (Minor revision)

7.5 International conference talks

TOEPFER, S., **TOTH, SZ.**, KISS, J. AND KUHLMANN, U. (2018) Current status of biological control in Hungary: An EU member state and B&R target country. First International Congress of Biological Control, 14–16 May 2018, Beijing, China. In: Qiu, D. (ed.) Biological control for a healthy planet. China Agricultural Science and Technology Press, Beijing, China, p. 249

TOTH, SZ., SZALAI, M., KISS, J., TOEPFER, S. (2019) Factors influencing the efficacy of soil insecticides and entomopathogenic nematodes at reducing the maize pest *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae) under field conditions. IOBC IWGO conference. Engelberg. Switzerland. 14 to 17 October 2019. p 42 (oral presentation).

TOEPFER, S., **TOTH, SZ.** (2019). Entomopathogenic nematode application against root-damaging *Diabrotica* larvae in maize: what, when, and how? International Congress on Invertebrate Pathology and Microbial Control & 52nd Annual Meeting of the Society for Invertebrate Pathology & 17th Meeting of the IOBC-WPRS Working Group "Microbial and Nematode Control of Invertebrate Pests" Valencia Spain, 28 July - 1 August 2019 p.72

TOTH, SZ., SZALAI, M., KISS, J., TOEPFER S. (2022) Can entomopathogenic nematodes replace synthetic soil insecticides for suppressing populations of the maize pest *Diabrotica virgifera virgifera* in Europe? European Scientific Conference – Towards Pesticide Free Agriculture. Dijon, France, 2 & 3 of June 2022.

7.6 National conference talk

TOTH, SZ., SZALAI, M., VOROS, L., ABRAHAM, L.R., DOSHI, P., TOEPFER, S. (2021) Can azadirachtin-based soil biopesticides be used for sustainable management of the invasive *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae) I. Debreceni Alkalmazott Rovartani Konferencia (online). 2021. January 20.

7.7 International and national posters

TOTH, SZ., SZALAI, M., KISS, J., TOEPFER, S. (2019) Reasons behind inconsistency of soil insecticides and entomopathogenic nematodes at reducing the maize pest *Diabrotica v. virgifera* (Coleoptera:

Chrysomelidae) under field conditions. LXI. Georgikon Napok Nemzetközi Tudományos Konferencia (2019. okt. 3-4.)

TOTH, SZ., SZALAI, M., ZELLNER, M., KUNTH, P. GLAS, M., KISS, J., TOEPFER, S. (2019) Temporal effects of soil insecticides and entomopathogenic nematodes at reducing *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae) under field conditions. A *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae) lárvája elleni kémiai és biológiai védekezési lehetőségek hatékonyságának időbeli különbségei szabadföldön 65th Hungarian Plant Protection Days. 65. NÖVÉNYVÉDELMI TUDOMÁNYOS NAPOK. Budapest, Hungary, 19 to 20 to February 2019, p. 90-91.

TOTH, SZ., SZALAI, M., KISS, J., TOEPFER, S. (2019) Diapause and hatching patterns of *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae) to better plan experimentation with neonate larvae. IOBC IWGO conference. Engelberg. Switzerland. 14 to17 October 2019, p. 91.

TOEPFER, S., **TOTH, SZ.**, ZELLNER, M. (2019) How to use entomopathogenic nematodes against the root-damaging *Diabrotica* larvae in maize? IOBC IWGO conference. Engelberg. Switzerland. 14 to 17 October 2019 p. 82

TOTH, SZ., SZALAI, M., TOEPFER, S. (2021) How do abiotic and biotic factors affect *Heterorhabditis bacteriophora* at controlling *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae)? 2nd International Congress of Biological Control – ICBC2, Davos, Switzerland, 2021 April 26-30 (online)