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A STUDY OF INNOVATIVE OPTIONS FOR THE CONTROL OF WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA VIRGIFERA*) LARVAE AND IMAGOES

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1. INTRODUCTION AND OBJECTIVES

Supplying food in appropriate quantities and quality while minimising environmental damage is increasingly challenging due to a steadily growing world population and diminishing cultivation areas.

In addition to wheat (*Triticum aestivum*) and rice (*Oryza sativa*), maize (*Zea mays*) is one of the most important cereals of humankind with a worldwide sowing area of 140 to 160 million hectares. Maize is also the most significant cultivated plant in Hungary. In recent years, the cumulated cultivation area of maize and wheat has accounted for more than 50% of the arable lands of the country. Growing maize over large areas is partly due to the multiple uses the plant provides. It plays a major role in both food production (sweet corn, corn meal) and industrial uses (starch, oil, and alcohol production), and increasing quantities of maize are used for bioethanol production. In Hungary, 90% of the maize is grown for feeding (concentrated and mass feedstuffs) and industrial uses.

It is also worth mentioning that the growing technology of maize is easy and highly automated, and the associated plant protection practices are well established. As far as economic considerations are concerned, it is a highly profitable plant; therefore, farmers mostly prefer growing it in monocultures.

In addition to agrotechnical methods (soil cultivation, nutrient supply, weed control, sowing counts), the control of western corn rootworm

(*Diabrotica virgifera virgifera*)—one of the most significant current maize pests—is a growing challenge in maize production. The control of this particular pest has a significant impact on both the quantity and quality of the harvest.

In 2018, a massive withdrawal of active agents started in the European Union, and several insecticides effective against and providing secure control of this pest (e.g., neonicotinoids, organic phosphoric acid esters) have disappeared from the European markets as a result. Nowadays, the control of organisms causing damage in the soil and at the foliage level presents increasing challenges, including a major difficulty in cutting the numbers of western corn rootworm larvae, the damage of which appears 4 to 6 weeks after sowing.

During his PhD training, the author was involved in developing methods that offer an answer to the current challenges and fight the larvae and imagoes of western corn rootworm in an environmentally friendly manner—and all this in a way without causing environmental pollution and without risks to the health of the individuals working on the field or of useful organisms.

2. MATERIALS AND METHODS

2.1. CONTROL OF WESTERN CORN ROOTWORM LARVAE

The author conducted studies for several years (2020, 2021, 2022) at several sites with different larval density (Röjtökmuzsaj, Gyömöre, Perkáta, Tiszalök, Hajdúvid). One experimental material was an entomopathogenic nematode of the species *Heterorhabditis bacteriophara*, which belongs to the genus Heterorhabditis. Several recent publications reported that this entomopathogenic nematode can be successfully applied against western corn rootworm larvae. The author of this Dissertation aimed at determining whether the viability and larvicide effect of 2 billion nematodes applied using 200 L/ha of water, as suggested by the literature, was maintained when the amount of water was reduced to 100 L/ha and 50 L/ha. The biological agent applied using reduced quantities of water would significantly improve the practical application of this product and the performance of the sowing area.

The other experimental material was Azadirachtin, an active agent extracted from Neem tree seeds. The pesticide effect of this biological extract has already been confirmed against several foliage pests. The author of this Dissertation sought an answer to the question of whether the active agent Azadirachtin could be successfully applied as a soil insecticide against western corn rootworm larvae using seed

dressing technology. The above-mentioned seed treatment technology enables successful control at low per-hectare active agent concentrations and in a cost-effective manner.

Each year, the experiments were conducted in four replicates, using small experimental plots with a size of 3 m × 6 m, and in a randomised setup. Each experimental plot was sown with 24 linear meters (4 × 6 linear meters) of maize. Each year during the nematode experiments, 3 different per-hectare volumes of injection water (50 L/ha, 100 L/ha and 200 L/ha) were used. The active agent Azadirachtin was used in dressing dose concentrations between 10% (0.0043 mg/seed) and 300% (0.129 mg/seed). In each case, the author compared the efficacy of the experimental materials with a negative control (no treatment) and with Force 1.5G, a product in general use containing Tefluthrin as active ingredient (positive control).

The method of setting up the experiments was identical for each treatment and each year. The number of live 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. 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.

2.2. CONTROL OF WESTERN CORN ROOTWORM IMAGOS

In addition to the control of the larvae of western corn rootworm, major emphasis should also be placed on the control of imagoes. This would not only allow for a significant mitigation of the damage caused by the fully developed insects, but would also enable cutting back oviposition, and thereby reducing the size of the larval population of the following year. The control of the imagoes of this pest would greatly benefit from an opportunity to minimise the per-hectare active agent cost and the applied amount of active agent. Between 2021 and 2023, the author carried out related experiments, and made use of the special palate of the pest: a taste material found in Cucurbitae (cucumber juice, 5 L/ha), which is highly preferred by these insects, was added to the insecticide most widely used against them (Acetamiprid), thereby reducing the amount of the chemical applied (to 50%, 25%, and 12.5%). These experiments were conducted in a laboratory setting in 2021 followed by field experiments in 2022 and 2023 using aerial application.

The laboratory experiment included four treatments carried out in four replicates, using 100 imagoes per cage. The four treatments included an untreated (negative) control, and the other three contained the active agents in an amount reduced to 50%, 25% and 12.5%, respectively, of that specified in the market authorisation, and 5 L/ha of cucumber juice, as attractant.

Following the methodology used in the laboratory experiments of 2021 and relying on the results thereof, field studies were conducted in 2022 and 2023. The experimental materials were sprayed via aerial application, in an amount of 50 L liquid per hectare, and providing appropriate isolation distance between the experimental plots corresponding to the treatments to take the flying intensity of the western corn rootworm imagoes into account. The spraying was carried out during the peak swarming period, the timing of which was determined by the author using plant examination (5×10 plant/area).

Data collected during the treatments against both the larvae and imagoes were continuously recorded. The author of this Dissertation used SPSS one-way ANOVA to assess treatment efficacy, and Tukey's Post Hoc Test to determine the significance levels between the treatments.

3. RESULTS

3.1. RESULTS OF THE TREATMENTS AGAINST WESTERN CORN ROOTWORM LARVAE

3.1.1. RESULTS OF THE NEMATODE TREATMENTS

A total of five experiments were set up with varying injection water doses at three locations (Röjtökmuzsaj, Gyömöre, Perkáta) in three years (2020, 2021, 2022) to assess the efficacy of entomopathogenic nematodes against the western corn rootworm. The experiment of 2020 at Röjtökmuzsaj is clearly separate from the other four experiments and should therefore be treated separately because the entomopathogenic nematodes did not cause significant changes in the larval counts or the degree of root chewing. The results of the viability tests conducted before the application of the experimental materials were devastating: 75% of the nematodes in the product were dead, and the remaining amount of the product was not sufficient for use four times more in order to compensate for the high mortality rates. The success of the treatments was also influenced by the fact that sowing was followed by a period of drought, which presented unfavourable ecological conditions for the remaining 25% of live nematodes.

In 2021, the experimental sites included one in Röjtökmuzsaj and one in Gyömöre. The results of the viability tests conducted before setting up the experiments of that year were excellent, showing 85% to 90%

nematode viability, which was clearly manifested in the subsequent experimental results.

The study site in Röjtökmuzsaj had a very high larval density that year, which was also reflected by an average m.Iowa score of 3.55 in the negative control plot. The statistical results of this experiment show that all treatments were successful in comparison with the negative control and that there was no statistically significant difference between the treatments involving different doses of injection water.

Despite being a 3-year-old monoculture, the Gyömöre study site showed lower larval density than the Röjtökmuzsaj study site, which was also reflected in an m.Iowa score of 2,83 in the negative control plot. In that year, the Gyömöre site also manifested significance between the negative control and the treatments in terms of root damage and as far as the injection water doses are concerned, the difference was again not significant.

Due to the lower larval density at the Gyömöre site, the author sought another highly infested area in addition to Röjtökmuzsaj in 2022 and found it in Perkáta in central Hungary. During the experiments of 2022, nematode viability was not a problem, which was clearly reflected in the results in comparison with those of the previous year. In this year of the studies, Röjtökmuzsaj had almost the same larval density as in 2021. There was a clear statistical difference between the negative control and the treatments administered in terms of both larval counts and root chewing scores. Like the treatments of the

previous year, the outcomes of the nematode treatments showed no differences. In the case of the Perkáta study site, the treatments significantly reduced larval counts (to less than 25% in comparison with the negative control), which was also reflected by the fact that the control plots showed a remarkably high number of fallen plants. The experimental materials again did well on the examination despite such a high m.Iowa score (3.93). Again, there was no statistical difference between the treatments with different injection water doses. Accordingly, wherever the viability of the nematodes in the biological product was appropriate, the treatments proved to have significant effects in comparison with the negative control at sites with different degrees of larval infestation. Furthermore, it was concluded that the efficacy of the biological agent was not inferior to that of Force 1.5 G, a widely used soil disinfecting product containing Tefluthrin as the active ingredient. The results of the experiments proved the initial hypothesis that the nematodes retain their larvicide effect at lower injection water doses.

3.1.2. RESULTS OF THE SEED DRESSING TREATMENTS

During the Azadirachtin studies, the author wished to assess whether this botanical insecticide has appropriate long-term effect and efficacy when applied to the surface of the seeds using a seed dressing technology.

Research into this direction was conducted for three years and at several sites under different larval densities.

In 2020, the experiments in Gyömöre confirmed that this active agent, when used in a seed dressing technology, was able to fight back corn rootworm larvae and mitigate the resulting root damage. The study site in Gyömöre was under a lower larval pressure as shown by the m.Iowa score of the control plot (3.23). At these study sites, treatments with more than 50% dose concentration showed good efficacy against the larvae: identical to that of the positive control, i.e., Force 1.5G, a product containing Tefluthrin as the active ingredient.

In 2021, experiments were set up in Röjtökmuzsaj, Gyömöre and Hajdúvid. Among the study sites Röjtökmuzsaj and Hajdúvid had the largest larval populations as manifested in the corresponding m.Iowa scores; Röjtökmuzsaj: 3.55, Hajdúvid: 4.42, Gyömöre: 2.83. During the reproductive period, weekly field surveys were conducted, and especially at the sites located in the Great Plain region of Hungary, the experimental plots sown with seeds dressed using 50% to 100% dose concentrations had plants that were developing in a healthy way but showed mild stem tilting (although to a much lesser extent than in the untreated control plot), which could not be observed in the areas treated with the highest two doses (125% and 150%). This observation was also confirmed by the statistics, given that the best results were achieved by the seeds treated with the highest dose concentrations—125% and 150%—, which superseded even the results of the positive control plot. This gave rise to the conclusion that even higher dressing

doses may be necessary in areas with very large corn rootworm populations. In summary, the efficacy of the treatments in 2021 was equal to, or—in some cases—statistically superior to, that of the Tefluthrin-containing soil disinfecting product at all three study sites, as confirmed by the m.Iowa scores for root chewing.

Relying on the experience gained in 2021, study sites with high larval density (Röjtökmuzsaj, Perkáta, Tiszalök) were selected for 2022 to test seed dressing dose concentrations of 100%, 200%, and 300%. The entire country suffered extreme droughts in 2022, especially the Great Plain region but also the Western Transdanubian. During the field surveys, plants were observed to sprout late and unevenly, especially at the 300% dose. The Perkáta site received some precipitation later, and the developmental delays displayed by the plants disappeared by the middle of the reproductive period; however, this was not the case for the Tiszalök and Röjtökmuzsaj sites, which was also confirmed by the statistical evaluation of the experimental results, wherein the outcome of the 300% treatment was significantly inferior to that of the 100% and 200% dressing dose concentrations at both sites. This phenomenon resulted from the formulation used: it contained 95% vegetable oils and was applied to the surface of the seeds at high concentrations. The cumulative effect of the high oil levels and the dry period made it difficult for the seeds to absorb water, and this caused uneven sprouting. The still undeveloped, young maize plants suffered greater damage by the corn rootworm larvae in higher developmental stages (L2, L3). Despite these extreme cases, the outcome of the

treatment with the 300% dressing dose concentration was still significantly superior to that of the untreated control plot. The initial hypothesis that seed-dressing with appropriate Azadirachtin levels exhibits long-term efficacy and thus provides secure control of western corn rootworm larvae was again confirmed.

3.2. RESULTS OF THE TREATMENTS AGAINST WESTERN CORN ROOTWORM IMAGOS

Studies on corn rootworm imagoes were carried out for three years, as described above. The following hypothesis was successfully confirmed: the most widely used insecticide (Acetamiprid) can be efficiently applied after reducing its level by 90% in comparison with that specified in its market authorisation by adding an attractant (cucumber juice).

During the treatments carried out in the laboratory setting, even the preliminary expectations were exceeded by the fact that practically 100% of the adult individuals were killed within a few (5 to 10) minutes after applying the experimental materials. In view of sustainability, the results of the laboratory tests can have great significance in practical plant protection given that the amount of the chemical active ingredient could be reduced to almost 10%. Relying on the results of the laboratory tests, field experiments over large plots were conducted for 2 years. The author of this Dissertation wished to

confirm that the attractant maintains its attracting efficacy under field conditions too. The treatments showed high efficacy under field conditions in both 2022 and 2023. The results of both years demonstrated clearly that the effect of reduced amounts of the active ingredient Acetamiprid appeared much faster when mixed with cucumber juice as an attractant than the effect of the amount of Acetamiprid specified in the market authorisation—i.e., the positive control—after spraying the materials from a helicopter. For the positive control, it took three days after the treatment to exhibit similar results as the treatments which included cucumber juice. This can be explained as follows: Acetamiprid is a member of the neonicotinoids' family of active ingredients and acts as a stomach poison. When used in a traditional manner, it first gets absorbed through the photosynthetically active plant surfaces, followed by a transfer into the circulatory system of the plant, and is finally accumulated in the above-ground parts upon acropetal flow. Western corn rootworm imagoes get into contact with the active agent via consuming plant parts and are killed by the active agent upon its absorption into their digestive systems. This technology makes use of the special palate of these insects to ensure that the western corn rootworm quickly gets a high concentration of the active agent. When using this attractant-based technology, waiting for the plant to first absorb the active agent to make it available for the pest to consume is not necessary. Insect counts drop quickly to below the specific per-plant economical threshold. The applied plant protection method can be fully integrated into agricultural practices and ensures a successful control of western

corn rootworm imagoes. Acetamiprid used in combination with cucumber juice as an attractant may not only reduce the associated environmental burden but may also significantly delay the potential appearance of resistant individuals.

4. NOVEL SCIENTIFIC RESULTS (THESIS)

(1,) The author concluded that entomopathogenic nematodes of the species *Heterorhabditis bacteriophora* applied to the sowing rows at a dose of 2 billion/ha using an injection water volume of 50 L/ha retained viability and larvicide efficacy, and thereby significantly reduced the number of western corn rootworm larvae and the degree of root chewing in comparison with the untreated control, both under low and high levels of larval infestation.

(2,) The experiments demonstrated that the efficacy of entomopathogenic nematodes of the species *Heterorhabditis bacteriophora* applied to the sowing rows directly after sowing at a dose of 2 billion/ha and using an injection water volume of 50 L/ha, was not inferior to that of a Tefluthrin-based soil disinfecting product (Force 1.5 G) against the western corn rootworm larvae.

(3,) The author of this Dissertation was the first to experiment with the active ingredient Azadirachtin in a seed dressing technology against soil-dwelling organisms. With regard to the active ingredient Azadirachtin, he concluded that the efficient seed dressing concentration is from 50% (0.022 mg/seed) to 100% (0.043 mg/seed) at low western corn rootworm larval density (average: 1.88 per plant; m. Iowa score: 3.03), and is from 125% (0.053 mg/seed) to 300% (0.129 mg/seed) at higher larval pressure (average: 3.84/plant; m. Iowa score: 4.08).

(4,) The author was the first to prove the remarkably long-term (4 to 6 weeks) efficacy of the active ingredient Azadirachtin against the western corn rootworm larvae in his experiments.

(5,) The experiments demonstrated that the efficacy of the active ingredient Azadirachtin applied at a dressing dose concentration of 50% (0.022 mg/seed) to 300% (0.129 mg/seed) in a seed dressing technology, was not different from that of a Tefluthrin-based soil disinfecting product (Force 1.5 G) against the western corn rootworm larvae.

(6,) The author proved that cucumber juice used as attractant exhibits sufficient attractive efficacy for western corn rootworm imagoes under laboratory conditions.

(7,) In field experiments it was proven that the original dose specified in the market authorisation of the active ingredient Acetamiprid against the western corn rootworm imagoes (30 g/ha) can be reduced by almost 90% in the presence of cucumber juice (5 L/ha) without reducing its efficacy against the pest.

5. LIST OF PUBLICATIONS

5.1. SCIENTIFIC ARTICLES IN HUNGARIAN JOURNALS

1. *VÖRÖS L., ÁBRAHÁM R., ENZSÖL E. (2020):* The effect of chemical and biological control on the western corn rootworm larvae (*Diabrotica virgifera virgifera* LeConte) in field trials. Acta Agronomica Óváriensis: 61(2): 53–72.

2. *VÖRÖS L. (2021):* Az amerikai kukoricabogár elleni védekezési eljárások áttekintése. Acta Agronomica Óváriensis: 62 (2): 141–162.

3. *VÖRÖS L., ÁBRAHÁM R., NAGY K., TÓTH SZ., STEFAN T. (2022):* Megtartják-e a *Heterorhabditis bavteriophora* fonálférgek a kukoricabogár lárvára (*Diabrotica virgifera virgifera*) gyakorolt ölő hatást kisebb vízmennyiséggel történő kijuttatás esetén is? Növényvédelem: 83 (58): 192-200.

4. *VÖRÖS L., ÁBRAHÁM R. (2024)*: Botanikai inszekticiddel az amerikai kukoricabogár (*Diabrotica virgifera virgifera*) lárvái ellen. Acta Agronomica Óváriensis: 64 (2): 49-63.

5.2. TECHNICAL ARTICLES IN HUNGARIAN JOURNALS

1. *VÖRÖS L., ÁBRAHÁM R. (2022):* Rovarpatogén fonálférgek (*Heterorhabditis Bacteriophora*) alkalmazása az amerikai kukoricabogár (*Diabrotica virgifera virgifera*) lárvái ellen. Biokultúra: 32 (6): 21–23.

5.3. SCIENTIFIC ARTICLES IN INTERNATIONAL JOURNALS

1. *VÖRÖS L., LEDÓNÉ ÁBRAHÁM R. (2023):* Effect of azadirachtin applied as seed dressing on the larval density of and root injury caused by the western corn rootworm/*Diabrotica virgifera virgifera*/. Journal of Plant Diseases and Protection (1861-3829 1861-3837) 130: 757-767 DOI:10.1007/s41348-023-00763-3

5.4. INTERNATIONAL PATENT BULLETIN

1. *VÖRÖS L. (2022):* Azadirachtin for seed dressing of field crops. International research report. International Publication Number: WO 2022 /195308 A1

5.5. PRESENTATIONS AT SCIENTIFIC CONFERENCES

1. *VÖRÖS L., ÁBRAHÁM R., TÓTH SZ., NAGY K., TOEPFER S. (2021):* Fenntartható védekezési eljárások az amerikai kukoricabogár (*Diabrotica virgifera virgifera*) lárvája ellen. 38. Óvári Tudományos Nap. p. 127.

2. *TÓTH SZ., SZALAI M., VÖRÖS L., LEDÓNÉ ÁBRAHÁM R., DOSHI P., TOEPFER S. (2021):* Azadirachtin aktív hatóanyagot tartalmazó talajfertőtlenítőszerek képesek védelmet nyújtani a *Diabrotica v. virgifera* (Coleoptera: *Chrysomelidae*) lárvái ellen. Növényvédelmi Tudományos Napok. p.24.

3. *TÓTH SZ., SZALAI M., VÖRÖS L., LEDÓNÉ ÁBRAHÁM R., DOSHI P., TOEPFER S. (2021):* Azadirachtin aktív hatóanyagot tartalmazó talajfertőtlenítőszerek képesek védelmet nyújtani a *Diabrotica v. virgifera* (Coleoptera: *Chrysomelidae)* lárvái ellen. I. Debreceni Alkalmazott Rovartani Konferencia. p.38.

4. *VÖRÖS L., ÁBRAHÁM. R, NAGY K., TÓTH SZ., TOEPFER S. (2022):* Entomopatogén fonálférgek (*Heterorhabditis bacteriophora*) gyakorlati alkalmazása az amerikai kukoricabogár (*Diabrotiva virgifera virgifera*) lárvái ellen. 68.

Növényvédelmi Tudományos Napok: Magyar Növényvédelmi Társaság. p.19.

5. *VÖRÖS L., ÁBRAHÁM R. (2023):* Botanikai inszekticiddel az amerikai kukoricabogár (*Diabrotica virgifera virgifera*) lárvája ellen. 39. Óvári Tudományos Nap. p.88.

5.6. POSTERS AT SCIENTIFIC CONFERENCES

1. *VÖRÖS L., ÁBRAHÁM R., TÓTH SZ., NAGY K., SZALKA É., TOEPFER S. (2021):* A rovarpatogén *Heterorhabditis bacteriophora* Poinar, mint biológiai védekezési lehetőség az amerikai kukoricabogár lárvái ellen. Növényvédelmi Tudományos Napok. p.60.

2. *TÓTH SZ., SZALAY M., VÖRÖS L., ÁBRAHÁM R., DOSHI P.,* TOEPFER *S. (2021):* Azadirachtin based granular soil biopesticides can control larvae of *Diabrotica v. virgifera* (Coleoptera: *Chrysomelidae*): 67. Növényvédelmi Tudományos Napok. 9. 24.