Project title: Alternative methods of protection of maize from Western Corn Rootworm (*Diabrotica virgifera virgifera*)

Acronym: Diacont

Project number: 101111

Project goals

The project's aim was to test the effectiveness of four alternative biological methods to protect maize from the western corn rootworm (*Diabrotica virgifera*). Dianem®, a product already approved as a pesticide for use against the corn rootworm, contains insect pathogenic nematodes of the species *Heterorhabditis bacteriophora*. These nematodes infest the larvae of the corn rootworm and subsequently kill them. However, the effectiveness of this product varies from year to year. Therefore, the factors affecting the effectiveness of the product were examined. With the insect pathogenic fungus *Metarhizium brunneum* (GranMetTM), the aim was to test the efficacy against the larvae of the corn rootworm in field trials. The insect-pathogenic fungus *Lecanicillium lecanii*, infecting the eggs of the western corn rootworm, was tested under practical conditions on a Maize-field in Styria. The soil bacterium *Stenotrophomonas rhizophila* stimulates root growth of damaged corn roots and increases the stability after corn infestation has already occurred. Field trials were carried out to test the effectiveness of the soil bacterium.

Dianem®; insect-pathogenic nematodes of the species *Heterorhabditis bacteriophora*

Both good persistence and virulence were confirmed by experiments with the substitute host mealworm 2 months after application of Dianem in the field with mortality rates of 50 to 92 %.

In 2016 and 2017, beetle densities in untreated areas averaged 67 - 87 individuals per m², depending on the location. By using Dianem®, this value could be reduced by 10 - 70 %, while the insecticide Belem only reduced it by 4 - 26 %. The yield after use of Dianem® was on average 21.4% respectively 10% higher than that in the Belem variant and in untreated control. By adhering to crop rotation, infestation and lodging damage could be reduced despite the annual increase in beetle pressure: From 3 years on, no lodging was observed in the first maize year. One year of intermediate crop already reduced lodging by 57% compared to maize on maize. The importance of the choice of maize variety was also statistically proven.

Effectiveness in combination trials

Despite low mortality rates by insect-pathogenic nematodes and statistically proven highest mortality values by the fungus *Metarhizium* spp., the comparison of beetle hatching rates of the same variants showed the lowest numbers of hatched beetles in the variants Dianem and Metarhizium or the combination of both products. The harvest values of the Dianem variants were slightly above those of the untreated control and Belem without statistical significance.

***Metarhizium brunneum* (GranMet™)**

Based on the results of the efficacy and long-term study carried out in planting season 2018 and since 2012 in Styria, Austria, practical recommendations can be derived for consultants and practitioners. It has been shown that (1) the beetle catches *of Diabrotica v. virgifera* continue to be increasing in the Bad Radkersburg area. The catch rate in 2018 corresponds to a threefold increase in the number of adults compared to 2017 and an increase of almost 500 percent compared to 2016. Despite the high *Diabrotica* larval density, no damage to the maize roots could be detected. Also, no direct damage to the maize plant and its maize ear could be assessed. The alarm threshold of the larvae or beetles can thus be set much higher than has previously been derived from literature data. A threshold value at the height of 50-70 beetles per m2 (i.e. approx. 10 beetles per plant; depending on the sowing strength of the maize seed per hectare) can be confirmed as a realistic alarm number. (2) the active substances whether used alone or in combination with other active substance led to a significantly lower number of beetles. Each control measure led to a significant reduction in beetle abundance. (3) the desired abundance of 5.000 *Metarhizium brunneum* CFU g^{-1} TG soil and more could already be achieved after the first application of the fungal barley product GranMet™ in the soil, alone and in combination with the other biological agents. The *Metarhizium* density in the soil should be evaluated in the future as an indirect measure for the evaluation of the effectiveness of the biological active substance. (4) as an immediate measure, crop rotation should be continued to be used and continued to regulate the western corn rootworm population. The high efficiency of the measure enables a reduction of the beetles in the entire infested area (reduction of more than 2/3 of the beetles per year and crop rotation). (5) according to the recommendation of the EPPO experts (EPPO guideline 2017), as well as the experts of the Austrian Chamber of Agriculture, the use of the insecticidal fungus *Metarhizium brunneum* BIPESCO 5 can be recommended in addition to all listed countermeasures. (i.e. emergency approval). (6) direct control of beetles with biological agents is also conceivable. Therefore, alternative products based on the insecticidal fungus *Metarhizium* for beetle treatment could be tested.

Lecanicillium lecanii

The insect-pathogenic fungus *Lecanicillium lecanii* was isolated from infected eggs at the Austrian Agency for Health and Food Security. With regard to a new control strategy against the corn rootworm, the temperature characteristic of the fungus was investigated under laboratory conditions. This should help to estimate the persistence of the fungus in the field. It could be shown that sporulation and germination of *L. lecanii* begins very rapidly at high temperatures. Furthermore, the fungus is able to form colonies and germ tubes even at low temperatures around 0 ° C. Therefore, we assumed that the fungus is well suited for outdoor use. In the following year, the efficacy of *L. lecanii* was investigated on corn rootworm eggs within a field trial. The trial was conducted on an area in southern Styria heavily infested by the corn rootworm. In order to increase the likelihood of an infection, barley litter and cat litter infected with *L. lecanii* were plowed into lower soil layers. This way the fungus came in contact with the eggs of the corn rootworm which were deposited in the course of the summer. The results of the experiment have shown that *L. lecanii* was not able to establish sufficiently in the soil. For this reason, the fungus had no effect on the number of beetles. In order to investigate possible causes of the lack of persistence of *L. lecanii* in the soil, in the last year of the project an overwintering experiment was carried out. The aim of this trial was to test whether or not soil type, carrier material and temperature had an impact on the persistence of the fungus in the soil. The experiment has shown that the persistence of the fungus depends primarily on the water holding capacity of the soil. As carrier material, barley is much better suited than vermiculite. In addition, increasing temperatures and the availability of host insects promote the proliferation of the fungus in the soil.

Stenotrophomonas rhizophila

Root-associated microorganisms are known to enhance plant's strength and tolerance towards phytopathogens and abiotic stress. Plants impaired by drought or heat shows elevated susceptibility to pathogens and reduced ability to recover from damages. Within the project the potential of the stress protecting bacterium *Stenotrophomonas rhizophila* to stimulate root growth and to increase stress tolerance was tested on maize with the aim to prevent corn yield losses due to injuries of the root system caused by larvae of the Western corn root worm.

The effect of *S. rhizophila* was studied under field conditions in four consecutive years. To enable the strain to establish a protective layer around the on maize roots already with the development of the seedling, various seed treatment techniques were applied. Successful colonization of the root surface was verified microscopically and, in addition, by a highly specific detection method developed during the project. Results of the analyses of the root-associated microbial communities by 16S amplicon sequencing indicated a successful integration of the inoculated *S. rhizophila* strain within the microbiome without causing disturbances.

The results of the field trials demonstrated the potential of *S. rhizophila* to enhance corn yield. This effect was not only obvious in relation to damages cause by corn rootworms, but was also apparent at field sites with low or without disease incidence. Particularly under long-lasting drought conditions, the capacity of the stress protecting bacterium was notably evident. For the combined application of *S. rhizophila*, the entomopathogenic nematodes and fungi no synergistic effects were observed.