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Title of the mission : Curative and preventive use of entomophatogenic nematodes against the maize pest *Diabrotica v. virgifera* (Western Corn Rootworm)

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Introduction

The Western Corn Rootworm (WCR) *Diabrotica virgifera virgifera* Le Conte is a Coleoptera belonging to the family of Chrysomelids and was introduced from North America into Europe in the late 80`s. This introduction took place in Serbia and from there the beetle spread over Eastern Europe. The larvae of the Western Corn Rootworm (WCR) live in the soil and feed on the roots of the maize plants, where they can cause severe damage.

One of the most promising biological control agents are entomopathogenic nematodes (EPN), which have been proved in laboratory trials to kill WCR effectively. For the use of those nematodes, their establishment and their persistence in the soil are crucial factors to allow a successful control against WCR. Furthermore the influence of different soil types on the effectiveness of the EPN should be examined.

Finally, for the application of the EPN's it is important to optimise the amount of water which is applied together with the EPN's. All experiments were carried out in the south of Hungary near Hodmezovasarhely.

Main objectives

1. Determination of the establishment and the persistence of EPN's in the soil. This was done for three different nematode species (*Heterorhabditis megidis, Heterorhabditis bacteriophora, Steinernema feltiae)* and three different application methods.
2. Examination of the influence of the sand content in the soil on the ability of the nematodes to reach WCR larvae was examined.
3. Calculation of the amount of water which reaches the stem base and how much water is needed to fill the leaf base.

Methodology

1.Establishment and Persistence

Three fields of different soils were used, one in Szatymaz with light soil, one in Foiskola with medium soil and one in Hodmezovasarhely with heavy soil. Maize was sown and three different EPN species (*Heterorhabditis megidis, Heterorhabditis bacteriophora, Steinernema feltiae)* were used. The EPN's were applied with three different methods in April during sowing of maize.
In April EPN's were applied as fluid directly during sowing, as powder during sowing and as flat spray shortly after sowing.

Twenty-eight soil samples were randomly taken 1 hour and 48 hours after application and one *Galleria mellonela* (Waxmoth) was put into every soil sample. Sometimes *Tenebrio molito*r larvae had to be used instead of *Galleria*, because there were not always enough *Galleria* larvae available. The soil samples were checked two weeks for dead larvae and dead larvae were put into a nematode trap. In the nematode trap larvae were checked for visible nematodes under the skin, propagation of the nematodes and typical change in colour of the larvae. So the data were consisting of the mean mortality of the larvae, and the percentage of EPN Infection (colour, propagation, visible adult nematodes) of the larvae.

2. Influence of the soil type on the infectivity of EPN's

Single maize plants from the variety magister were grown in pots with soil which contents different amounts of sand (13.7%, 30%, 60%). Dense clay loam was used from the fields of Hodmezovasarhely with **13.7% sand**, 44.6% loam and 41.7 % clay. This soil was used for

one set of pots and it was mixed with different amounts of sand for two other sets . One set of soil was added up once to a sand content of **30%** and one set of soil to a sand content of **60%.** The maize plants were infected with 8 second and third instar WCR larvae per pot and one week later the EPN's were added in a distance of 15 cm to the maize plant. The used EPN species were *H. megidis, H. bacteriophora* and *S. feltiae.* Every of the three sets consisted of 108 pots (27 replicates per EPN species and control).Sleeve cages were put over of the pots to catch the emerging beetles.

3. Calculation for needed amount of water

Maize plants in the 2., 3., 4. and 5. leaf stage were used. First the amount of water which is needed to fill the leaf base was measured. Second, the time was measured which was needed to fill the leaf base with water by spraying from the height of 30 cm and 440 ml/ minute.

Third the amount of water which reaches the stem base after the plant had been sprayed for 1.5 s with 440ml/ min was measured. Because the time in which the fluid is sprayed on a single plant is very short in practical experience, the amount of water which reaches the stem base is calculated for one second only. This experiment was accessorily carried out with water to which the compound adhaesit had been added. Adhaesit reduces the tension of water and the question should be answered if this compound can increase the proportion of water which reaches the stem base.

Results

1.Establishment and Persistence

1.1 Differences in the persistence for the three used nematode species

Heterorhabditis megidis: This species showed strong variation in its establishment between applications and sites. In Szaytmaz (light soil) it reached EPN infection rates of 70% while in Hodmezovasarhely (heavy soil) only an EPN Infection rate of 17% was found. *H. megidis* persisted over three months in two of the three fields, but only with a low percentage of EPN infections (less than 10%). (example from Szatymaz see Fig1.)

Heterorhabditis bacteriophora: This species could establish well in two fields with an EPN infection rate of 40%. In the field in Hodmezovasarhely (heavy soil) the EPN Infection rate was significantly lower (12%). *H. bacteriophora* persisted in all three fields over the whole period and it was the EPN specie with the highest EPN Infection rate in Foiskola (medium soil) (25%). (example from Szatymaz see Fig1.)

Steinernema feltiae: This specie could well establish in Foiskola and Szatymaz , but in Hodmezovasarhely only a small percentage of EPN Infection were found (12%). *S .feltiae* persisted in all three fields and shows in Szatymaz (light soil) the highest EPN infection rate after three months of the three tested EPN species (39%).(example from Szatymaz see Fig1.)

H. bacteriophora and *S. feltiae* showed strong fluctuations in their rate of EPN infections between different fields and dates. Still it can be clearly seen that the persistence of *H. megidis* is less in comparison to the other two species (see Fig.1).

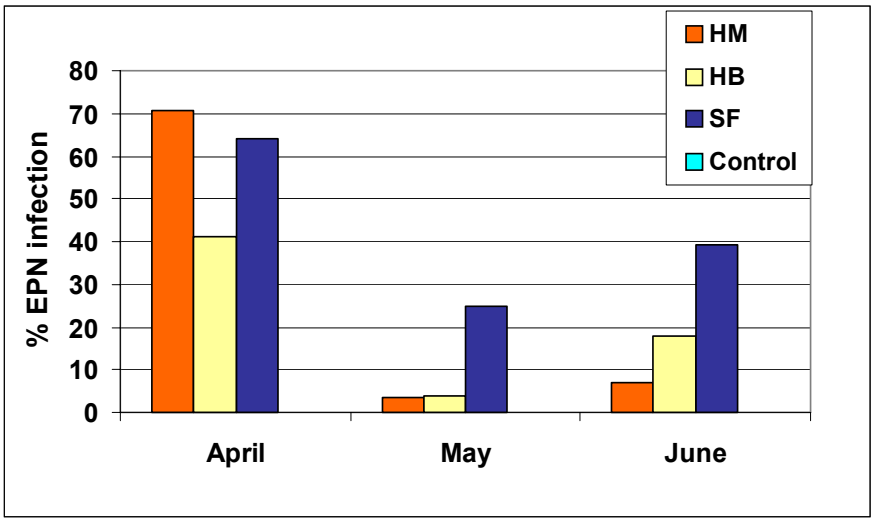

Fig1.: Persistence of different EPN species in maize fields in southern Hungary over time
HM= *Heterorhabditis megidis,* HB= *Heterorhabditis bacteriophora,* SF = *Steinernema feltiae*
Example from Szatymaz (light soil),

1.2 Differences in persistence for three different application methods

A: fluid row spray with sowing
The EPN's applied with this method persisted in all three fields. In two fields they showed the highest infection rates among the tested application methods after a three month period (18 and 25% infection).

B : powder row spray with sowing
The EPN persisted in all three fields but only low rates of EPN infection were found (around 10%).

C: fluid flat spray after sowing
The EPN infection rate already went down to 0% in two of three fields in May. The EPN persisted only in foiskola (medium soil).

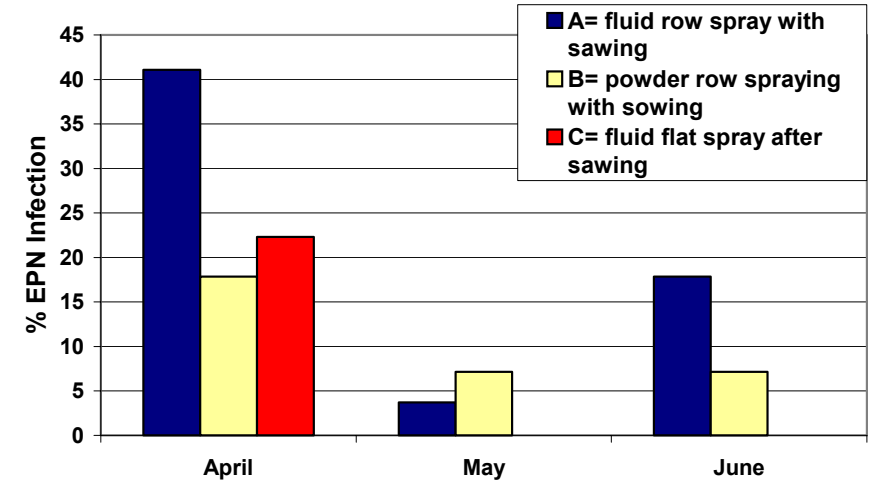

Fig.2: Persistence of *H. bacteriophora* applied with three different application methods,
Example from Szatymatz (light soil)

1.3 Differences between the three fields (soil types)

The Mean Mortality is relatively high in the soil samples of all three fields. Nevertheless there is a significant difference between the EPN infection rate in Hmvhely 12% and 40% in Foiskola and 39% in Szatymaz.

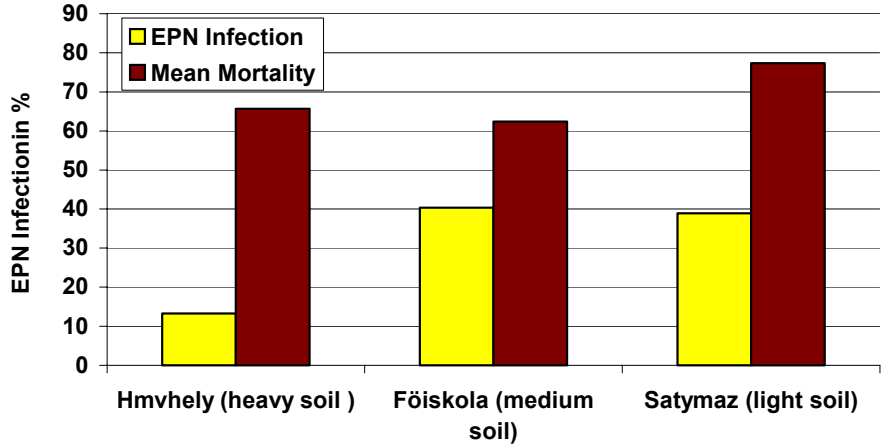

Fig.3: EPN Infection and Mean Mortality of all three nematode species together in different fields soils in maize fields in southern Hungary in April 2005

2. Influence of the soil type on the infectivity of EPN's

With this experiment we had problems, because only very few beetles emerged even from our control. These data can not be analysed and we are going to retry this experiment. We suppose that the reason for the suprising high mortality may lie in a low viability of the WCR larvae.

3. Calculation for needed amount of water

Tab.1: Calculation of water which would be needed to fill leaf base at maize plants using XR 8006 Nozzles and 1 bar = 440ml/min

	2. leaf stage	3. leaf stage	4. leaf stage	5. leaf stage
per plant in ml	6.65	8.12	16.43	26.65
per hectare in l	589.29	719.69	1454.89	2360.16

In practical experience farmers could often apply not more than 400 l per hectare. As expected only small amounts of water (second leaf stage 123 µl , third leaf stage 243 µl) could be calculated for reaching the stem base after a one second spraying period. This amounts of water may be increased by adding a additive which is called adhaesit, but the difference between sprays with and without adhaesit have been found not to be significant after being tested with an independent sample t-test. (see Fig. 4).

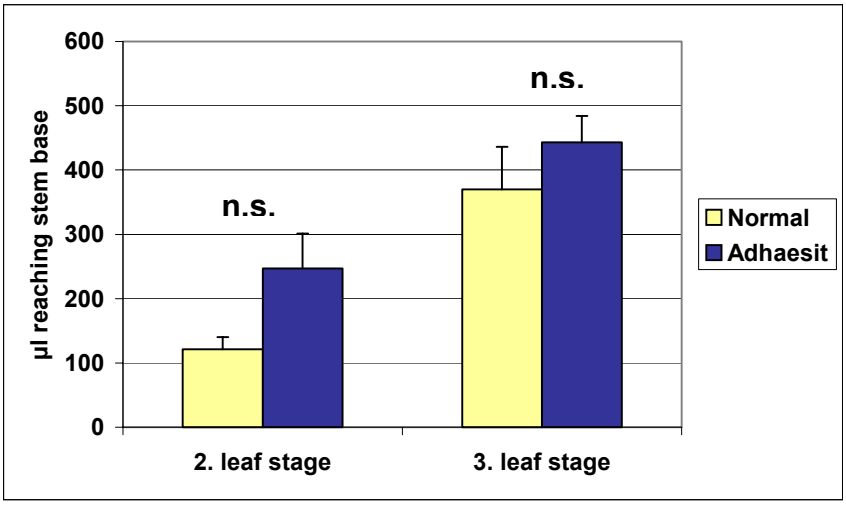

Fig.4: Water reaching stem base in µl, calculated for one s of spraying with 440ml/min at maize plants in two different leaf stages and one set with normal water and one set with water + adhaesit (0,1%)

Profit to the fellow

In the Plant Health Service in Hodmezovasarhely I could combine gaining of knowledge in the use of EPN with the control and monitoring of an important exotic pest.
I learned how to handle, how to apply and how to detect EPN's in soil samples. I based close to the mayor pest outbreak in Europe and could observe the pest itself and the symptoms of damage on the maize plants in the fields. As in the Plant Health Service of Hodmezovasarhely already ten years of research experience on WCR exist, I could profit from talking to local experts well as from having contact to visiting international collaborators.

Outlook

Due to the success of the STSM it was decided to conduct a diploma thesis as a frame up to this mission. So the research topics can be worked on in more detail. For the experiments of the persistence I will carry on taking soil samples in the following month, to see how the persistence of the EPN will evolve during a colder season period.
As already mentioned the experiment on the soil type has to be repeated.
Furthermore I would like to arrange one experiment to examine the different susceptibility to EPN attack of different larval stage of WCR.

Benedikt Kurtz

Hmvhely, 23.7.05

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