Biological control of the western corn rootworm (*Diabrotica virgifera virgifera*) by entomoparasitic nematodes

*Biologische Bekämpfung des Westlichen Maiswurzelbohrers (*Diabrotica virgifera virgifera*) mit entomoparasitischen Nematoden*

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1. Introduction

Entomoparasitic nematodes of the species *Heterorhabditis bacteriophora* are known to be highly virulent against larval stages of the western corn rootworm (WCR) under controlled laboratory conditions (Töpfer, 2005). However, a practical and sustainable plant protection strategy has to be efficient also under various field conditions. In addition the application of nematodes has to be easy to practice in order to be accepted by the farmers. Therefore only those application methods were used in our experiments that can be carried out in one step together with sowing of maize grains. To show the reliability of such control measures several field experiments were designed between 2009 and 2011 in the framework of the German Diabrotica research program (http://diabrotica.jki.bund.de/).

2. Material and Methods

During this project several field experiments were carried out in Deutsch Jahrndorf - 4 of them are reported here. The location is situated in the most Eastern part of Austria (17°-6′-4″ N – 48°-0′-3″ E) and exhibits a long history of WCR occurrence and monoculture of irrigated maize that leads to high natural pest populations. The region shows pannonian climate which is characterized by high summer temperatures and low precipitation. Preliminary experiments had shown a clustered distribution of WCR eggs in the field. To ensure even numbers of WCR larvae experimental plants were infested artificially with WCR eggs at various rates. These eggs had been derived from adults, caught in the former summer season and held under laboratory conditions with nutrition and soil for egg deposition. To ensure natural winter conditions, the oviposition substrate containing the eggs was buried in autumn at the experimental field site. After hibernation, the substrate was brought back to the lab and the eggs counted in subsamples to estimate their number per g of substrate. The egg containing soil was then applied in a furrow 20 cm apart from 6 maize plants in the center of each plot, at rates from 0 to 3000 eggs per plant.

The insect parasitic nematodes belonging to the species *Heterorhabditis bacteriophora*, were produced by e-nema GmbH, Schwentinental, Germany, and were applied as granules, as liquid formulations or as seed dressings in application rates ranging from 1.3×10^9 to 2.7×10^9 infective juveniles per ha.

Each plot had a size of 200 m². The experiments included a Clothianidin-treated control [25 g a.i./50 000 grains or in 50 g a.i./ha in granules], an untreated control and were carried out in five replications each. Efficacy of the treatment was evaluated by recording the emergence of adults in weekly intervals in cages put over the infested plants at the beginning of adult hatch and by rating the damage of maize roots according the "node injury scale" (Oleson *et. al.*, 2005) in August. Additionally the persistence of applied nematodes was evaluated at different times after the application. For this purpose *Tenebrio molitor* - larvae were buried in mixed soil samples for each plot and used as living baits. After storage for one week at 25 °C, infested larvae were submersed in water for validation. The statistical analysis of the experiments included ANOVA and was carried out with the help of SPSS.

3. Results and Discussion

In the experiments described herein the maize plants were infested artificially with various numbers of WCR eggs per plant. Fig. 1 shows the number of hatched beetles in untreated control groups as a function of numbers of infested eggs. The infestation in the experiment Hutweide 2009 gave extraordinary good results (datapoints marked with X in Fig. 1) – yet it is very likely that additional natural infestation may have played a major role in this case. The maximum number of beetles developing on a plant after artificial infestation appears as the upper limitation of the datapoints in Fig. 1. Up to 100 hatched beetles per plant could be achieved by the herein described artificial infestation under good conditions. The optimum number of WCR eggs per plant for infestation seems to be 300-400. It was not possible to achieve more hatching adults than 100 beetles per plant by infesting the maize plants with high numbers of 1000 or 3000 WCR eggs. It is very likely that this was the result of behavior of larvae which avoid such overcrowded roots or of density dependent mortality caused e.g. by predators like the staphylinid beetle *Platystethus sinosus* which feeds frequently on WCR eggs (Friedl S., pers. Communication, 2009).

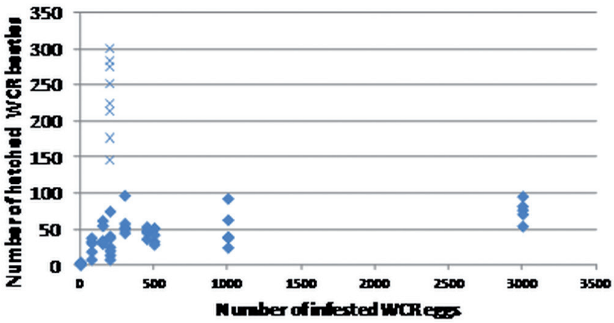

Fig.1 Number of hatched WCR beetles per plant in several untreated control groups after artificial infestation with various numbers of eggs. These pooled results are derived from various experiments in Deutsch Jahrndorf between 2009 and 2011. Datapoints marked with "X" refer to an experiment (field site Hutweide 2009) with artificial and natural infestation.

Abb. 1 *Anzahl geschlüpfter WCR-Käfer je Pflanze in mehreren unbehandelten Kontrollgruppen nach künstlicher Freisetzung unterschiedlicher Mengen von Eiern. Die Ergebnisse wurden aus unterschiedlichen Versuchen in Deutsch Jahrndorf zwischen 2009 und 2011 abgeleitet. Die mit einem „X" gekennzeichneten Datenpunkte beziehen sich auf einen Versuch (Standort Hutweide 2009) mit künstlichem und natürlichem Befall.*

The evaluation of efficacy was carried out by counting hatched beetles and by rating of damaged maize roots. Whereas exact results were achieved only by adult counts (time consuming), the root ratings were less time consuming, were not so exactly and were carried out mainly to facilitate comparison with experimental work of other authors. The results of these 4 experiments may be seen from Fig. 2, Fig. 3, Fig. 4 and Fig. 5:

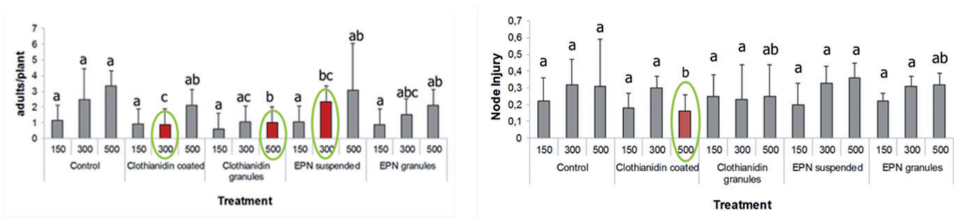

Fig. 2 The effect of the application of entomoparasitic nematodes (EPN, 1.3 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) after artificial infestation with various numbers (150, 300, 500) of WCR eggs per plant. The experiment was carried out in the field sites KARLHOF (sports ground) and FISCHWASSER in 2010.

***Abb. 2** Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 1,3 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach künstlicher Freisetzung unterschiedlicher Anzahlen (150, 300, 500) von WCR-Eiern je Pflanze. Die Versuche wurden an den Standorten KARLHOF (Sportplatz) und FISCHWASSER im Jahr 2010 durchgeführt.*

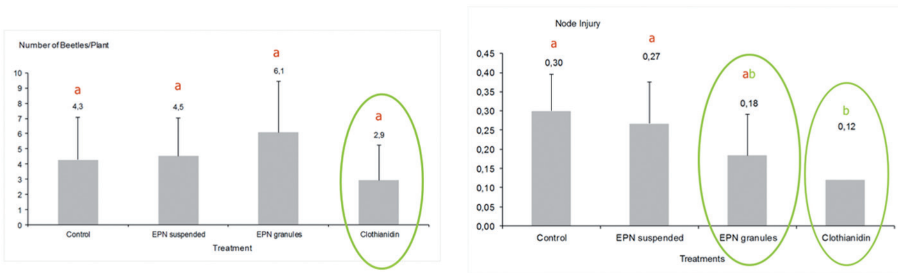

Fig. 3 The effect of the application of entomoparasitic nematodes (EPN, 1.3 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) after artificial infestation with 300 WCR eggs per plant. The experiment was carried out in the field site KARLHOF (Sportplatz) in 2010.

***Abb. 3** Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 1,3 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach künstlicher Freisetzung von 300 WCR-Eiern je Pflanze. Der Versuch wurde am Standort KARLHOF (Sportplatz) im Jahr 2010 durchgeführt.*

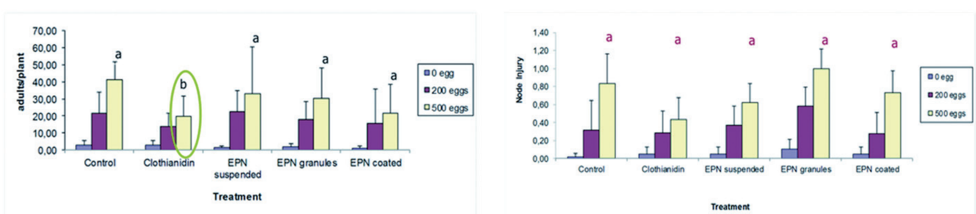

Fig. 4 The effect of the application of entomoparasitic nematodes (EPN, 2 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) after artificial infestation with various numbers (0, 200, 500) of WCR eggs per plant. The experiments were carried out in the field site FISCHWASSER in 2011.

***Abb. 4** Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 2 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach künstlicher Freisetzung unterschiedlicher Anzahlen (0, 200, 500) von WCR-Eiern je Pflanze. Die Versuche wurden am Standort FISCHWASSER im Jahr 2011 durchgeführt.*

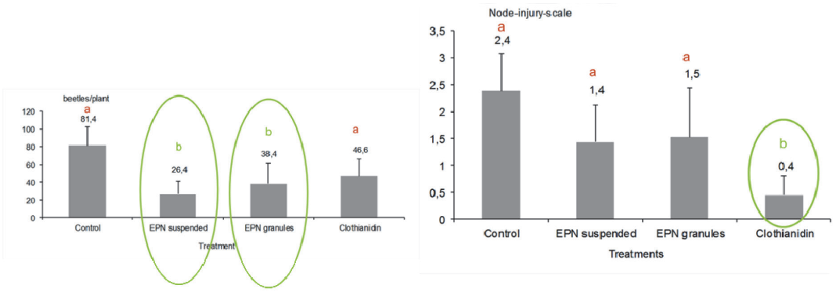

Fig. 5 The effect of the application of entomoparasitic nematodes (EPN, 2.7 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) under natural infestation and additional artificial infestation with 300 WCR eggs per plant. The experiment was carried out in the field site Hutweide in 2009.

***Abb. 5** Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 2,7 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach natürlichem und künstlichem Befall mit 300 WCR-Eiern je Pflanze. Der Versuch wurde am Standort Hutweide im Jahr 2009 durchgeführt.*

Clothianidin showed good efficacy against WCR larvae (according to adult hatch and root-rating). The application of EPNs during sowing leads to varying degrees of efficacy (according to adult hatch) ranging from poor to very good results. This variation was partially due to formulation type or application rate of the EPNs. Suspension of Nematodes in water showed high efficacy in some experiments (Fig. 5). So did granular formulations and seed coating (Fig. 4) in other cases. High concentrations of EPNs (2.7 x 10^9/ha) applied during sowing of the maize reduced the numbers of hatched adults significantly and reduced also root damages (Fig. 5). They showed equal efficacy in respect to hatched adults as Clothianidin treated seeds (Fig. 3). Low concentrated EPNs (1.3 x 10^9/ha), irrespective of their formulation were inappropriate for an efficient control (hatched beetles) in 2010. Further trials (Fig. 2, Fig. 4) with a moderate concentration (2 x 10^9/ha) lead to similar results.

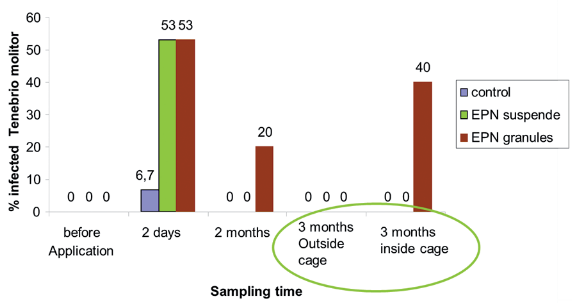

Fig. 6 The persistence of *Heterorhabditis bacteriophora* during the field experiment (Karlhof (sports ground), Fischwasser , 2010) was evaluated by exposition of Tenebrio molitor larvae in mixed soil samples from the field sites in the laboratory at 25 °C for one week.

***Abb. 6** Die Überlebensfähigkeit von* Heterorhabditis bacteriophora *im Freilandversuch (Karlhof (Sportplatz) und Fischwasser, 2010) wurde bewertet, indem Larven von* Tenebrio molitor *in Bodenmischproben von den Standorten im Labor für eine Woche bei 25 °C gehalten wurden.*

The persistence of EPN nematodes (Fig. 6) applied during sowing in April 2010 in the field sites Fischwasser and Karlhof, 2010 were sufficient to ensure parasitation of WCR larvae which lived approximately 6 weeks later. The entomoparasitic nematodes were able to multiply within the larvae and gave rise to another generation which could be detected 3 months after the initial release. This part of the project work has already been published separately (Pilz *et al.,* 2012).

4. Conclusions

Entomoparasitic nematodes in high concentrations of about 2.7 x 10^9 EPN/ha were able to reduce the emergence of adult corn rootworms in a significant way, regardless of the nematode formulation. Seed treatment with Clothanidin showed best results with respect to the prevention of root damage (according to node injury scale) in most experiments. A total eradication of WCR larvae was not possible - neither by EPNs nor by Clothianidin. None of the tested measures showed influence on the yield: this was most probably the result of irrigation used on the experimental sites, which enabled maize plants to compensate the loss of roots. Evaluation of the persistence of applied nematodes showed that they were able to survive as long as WCR-larvae were present in the soil despite their early application at sowing time.

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