Is foliar application of entomopathogenic nematodes (Rhabditida) an effective alternative to thiametoxam in controlling cereal leaf beetle (Oulema melanopus L.) on winter wheat?

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Abstract

The efficacy of entomopathogenic nematodes (high conc. 150.000 IJ m⁻²; low conc. 75.000 IJ m⁻²) in controlling Oulema melanopus on winter wheat was tested in a field experiment. A Slovenian strain of Steinernema carpocapsae (C101) was used with the commercial product NemasysC and the insecticide thiametoxam (60 g ha⁻¹). The preceding observation helped determine when the larvae of cereal leaf beetle started to hatch from eggs. Nematodes and insecticide were all applied on the same day when larvae were present and most eggs (≥90%) had hatched. Yield results of our research showed that the Slovenian strain of S. carpocapsae (C101) and commercial strain are both effective biological agents in foliar control of the CLB in the open field. A group analysis demonstrated that in 2010 (P<0.0001) and 2011 (P = 0.0359) the yield of wheat was affected by measures of pest control. In both years the highest yield was obtained in the thiametoxam treatment (2010: 7.3±0.1 t ha⁻¹; 2011: 8.1±0.4 t ha⁻¹) and lowest in the control (untreated) treatment (2010: 5.6±0.2 t ha⁻¹; 2011: 7.4±0.2 t ha⁻¹). In 2010 weaker efficacy among EPN treatment showed commercial strain NemasysC (low conc.: 6.0±0.1 t ha⁻¹; high conc.: 6.1±0.2 t ha⁻¹), while Slovenian strain gained better results (low conc.: 6.6±0.1 t ha⁻¹; high conc.: 6.8±0.2 t ha⁻¹). In 2011 among EPN treatments Slovenian strain (low conc.: 8.1±0.3 t ha⁻¹; high conc.: 8.1±0.2 t ha⁻¹) and commercial strain Nemasys C (high conc.: 8.2±0.1 t ha⁻¹) gained better yield results than commercial strain Nemasys C (low conc.: 7.4±0.2 t ha⁻¹). Concentration of the nematode suspension did not have any influence on the yield in both years (2010: P = 0.2315; 2011: P = 0.0937), which gives these biocontrol agents in integrated or organic agriculture better prospects from an economical point of usage, as the cost of plant protection is closely connected to the quantity of the applied EPNs.

Key words: Biological control, entomopathogenic nematodes, cereal leaf beetle, yield.

Introduction

Cereal leaf beetle (CLB), Oulema melanopus (L.) (Coleoptera: Chrysomelidae), is an important insect pest of wheat (Triticum aestivum L.), oat (Avena sativa L.), and barley (Hordeum vulgare L.) 21. The CLB is spread across Europe, the Middle East and Asia and in North America 10, 22. CLB has one primary generation per year 21. Adults overwinter in woodlots and windbreaks near cereal fields 3. Adults fly from overwintering sites to small grains after the first warm days of spring. Females lay eggs singly or in pairs on the leaf blades. Larvae skeletonise leaves by feeding on the outer leaf surface and parenchyma tissue and leaving the lower epidermis 5. Pupation occurs in the soil. Adults emerge after a few weeks, and feed on corn (Zea mays L.), and other grasses before dispersing to overwintering sites 3.

The economic impact of the CLB can be significant. Pest management recommendations prescribed insecticide application when infestations of one larva per flag leaf were encountered in winter wheat or two per flag leaf in oats and barley 21. Heyer 11 estimated that a single larva reduces assimilation by about 10%. A massive attack of larvae reduces total assimilation by up to 80% 9 causing losses of about one ton of grain per ha. Previous studies recommended an economic threshold of one larva per stem 16, but this infestation level often results in unacceptably high levels of defoliation 2. More recent studies have suggested a much lower economic threshold 12.

The application of entomopathogenic nematodes (EPNs) as biological control agents in protected environments is well documented 17. EPNs (Rhabditidae: Steinernematidae and Heterorhabditidae) are obligatory generalist insect parasites with a free-living stage, known as the infective juvenile (IJ), that seeks a suitable insect host in the soil. The application of EPNs in biological control was traditionally used to control soil pests until a few years ago 13. Research from the last two decades also indicates their potential against foliar pests, but only under special conditions 5, 19. Previous research has demonstrated that EPNs at high concentrations, together with favourable abiotic factors (high humidity, optimal temperature) can be effective biological control agents of chrysomelids 14, 18, 19, 26, 27.

Recently, it has been demonstrated that the EPNs including Steinernema feltiae strain B30, S. carpocapsae strain C101, and Heterorhabditis bacteriophora strain D54 have a potential to be used as biological control agents against the adults of the CLB under laboratory conditions 14, 18. Most efficient among the studied strains was S. carpocapsae C101, which caused 96% mortality rate of the beetles. We should not, however, neglect the important...
fact that laboratory results are not always comparable with the field experiments, since the efficiency EPNs outdoors depends on many other factors and interaction between them. In a related research the species *S. carpocapsae* was 100% efficient in suppressing larvae, pupae and adult specimens of Colorado potato beetles (*Leptinotarsa decemlineata* [Say]), while it reached only 31% efficiency when its activity was tested outdoors.

Beside the strain *S. carpocapsae* C101, foliar control of CLB in the open was included in the experiment. The commercial product Nemaysys C, whose active ingredient is also *S. carpocapsae* and the systemic insecticide thiametoxam were also used. The aims of our research were (1) to establish the influence of the studied products when controlling CLB on winter wheat yield, and (2) to study the concentration effect of EPNs on control efficacy.

### Materials and Methods

**Experimental field:** We planted the Isengrain winter wheat variety in a plot measuring 44.4 m x 20.0 m on the Laboratory field of the Biotechnical Faculty in Ljubljana, Slovenia (46°04'N, 14°31'E, 299 m alt.) on 5 October 2009 and on 11 October 2010. In both years mineral fertilizer NPK (7-20-30) in a dose of 650 kg ha⁻¹ was used prior to the start of seeding winter wheat. Additional fertilization took place in both years three times (2010: 1 April, 26 April, 26 May; 2011: 23 March, 20 April, 23 May) in a dose of 200 kg of nitrogen (27% conc.). Winter wheat was seeded by mechanical seeder for enclosed seeding (D9 30 Super, manufacturer Amazone) at working speed of 6 km h⁻¹. Distance between two rows was 12 cm and seeding rate was 220 kg ha⁻¹. We divided the field into four blocks, and in each there were six treatments: control (unsprayed), *S. carpocapsae* C101 low conc. (ScC101L), *S. carpocapsae* C101 high conc. (ScC101H), Nemaysys C low conc. (NL), Nemaysys C high conc. (NH), and thiametoxam. The size of each treatment parcel was 37 m² (3.7 m x 10 m).

**Agri-technical measures:** The herbicide Hussar OD, (0.1 l ha⁻¹), a.i. iodosulfuron-methyl sodium; manufacturer Bayer CS, Germany; supplier Bayer d.o.o., Ljubljana, Slovenia) was used on 9 April 2010. On 24 March 2011 we used the herbicide Alister Grande (1 l ha⁻¹) (a.i. diflufenican and iodosulfuron-methyl sodium; manufacturer Bayer CS, Germany; supplier Bayer d.o.o., Ljubljana, Slovenia). The application of the insecticide Actara 25 WG (a.i. thiametoxam; manufacturer Syngenta; supplier Syngenta Agro d.o.o., Ljubljana, Slovenia) and entomopathogenic nematodes was performed on 7 June 2010 and 25 May 2011. The preceding observation helped determine when the larvae of CLB started to hatch from eggs. Treatments were all applied on the same day when larvae were present and most eggs (>90%) had hatched. We applied EPNs and insecticide Actara using the backpack sprayer Solo 425. The jet stream nozzle number 04110 with a pressure of 2 bars was used. Two concentrations of nematode suspension were chosen: low at 75,000 IJ m⁻² and high at 150,000 IJ m⁻². A dose of 60 g ha⁻¹ of thiametoxam was used. To gain better spreading of EPNs on the leaves of wheat we added wetting agent Break Thue S240 (a.i. 100% polyether-polymethylsiloxane-copolymer; manufacturer Goldschmidt GmbH, Germany; supplier BASF Slovenia d.o.o., Ljubljana).

Due to the risk of cereal infestation with fungi from genus *Fusarium* the experimental field was sprayed in each year two times with fungicide. On 26 April 2010 and 23 March 2011 we sprayed the winter wheat with the fungicides Sphere 553 SC (0.5 l ha⁻¹) (a.i. cyproconazole and trifloxystrobin; manufacturer Bayer CS, Germany; supplier Bayer d.o.o., Ljubljana, Slovenia) and Modulus 250 EC (0.4 l ha⁻¹) (a.i. trinexap-acetyl; manufacturer Syngenta; supplier Syngenta Agro d.o.o., Ljubljana, Slovenia). On 26 May 2010 we sprayed the plants with the fungicide Folicur EW 250 (1 l ha⁻¹) (a.i. tebuconazole; manufacturer Bayer CS, Germany; supplier Pinus TKI d.d., Slovenia). On 24 May 2011 we sprayed winter wheat with the fungicide Prosurao (1 l ha⁻¹) (a.i. prothioconazole and tebuconazole; manufacturer Bayer CS, Germany; supplier Bayer d.o.o., Ljubljana, Slovenia). We harvested the wheat on 19 July 2010 and 25 July 2011 with a plot combine Wintersteiger. Plant density at harvesting was from 400 to 500 of spikes per m². The yield of wheat was weighed and later calculated to the t ha⁻¹.

**Nematodes:** Nemaysys C was purchased (Becker&Underwood, UK) through the importer Metrob d.o.o. (Ljubčena, Slovenia). The nematode *Steineremena carpocapsae* isolate C101 ⁴⁶ was produced in a mechanically stirred, internal loop bioreactor with an 8000 ml capacity (BR021, Inel Ltd., Budapest, Hungary). After sterilization, the bioreactor containing the P2 culture media (23 g yeast extract, 12.5 g dried egg yolk, 5 g NaCl and 40 ml corn oil in 1000 ml water) ⁴⁶ was inoculated with 100 ml of overnight culture *Xenorhabdus nemataphila*, the bacterial symbiont of *S. carpocapsae*, isolated from infective juveniles of the C101 strain. The temperature was 24°C, and the aeration rate was 1.0 vvm. After two days, when the oxygen consumption of the bacterial culture decreased, the bioreactor was inoculated with five million infective juveniles of the C101 strain in 500 ml of culture media. The nematodes were harvested 14 days after, when the total number of nematodes was 50,000 individuals per ml, and the ratio of infective juveniles was 90%. The nematodes were centrifuged, washed twice with sterilized tap water and stored in sterile M9 solution (5 g NaCl, 3 g KH₂PO₄ and 6 g Na₂HPO₄ in 1000 ml water).

**Statistical analysis:** Differences in yield were analysed with the use of ANOVA. Prior to analysis, each variable was tested for homogeneity of variance, and the data found to be non-homogenous was transformed to log(Y) before ANOVA. Significant differences (P≤0.05) between mean values were identified using Student-Newman-Keuls’s multiple range test. All statistical analyses were done using Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc.). The data is presented as untransformed means ± SE.

**Results and Discussion**

A group analysis demonstrated that in 2010 (F = 19.08; df = 5, 15; P < 0.0001) and 2011 (F = 2.82; df = 5, 15; P = 0.0359) the yield of wheat was affected by measures of pest control. In both years the highest yield was obtained in the thiametoxam treatment (2010: 7.3±0.1 t ha⁻¹; 2011: 8.1±0.4 t ha⁻¹) and lowest in the control treatment (2010: 5.6±0.2 t ha⁻¹; 2011: 7.4±0.2 t ha⁻¹) (Fig. 1).

When compared treatments with EPNs we determined in 2010 statistically significant differences between both tested strains. Weaker activity showed commercial strain Nemasys C (low conc.: 6.0±0.1 t ha⁻¹, high conc.: 6.1±0.2 t ha⁻¹), while Slovenian strain gained better results (low conc.: 6.6±0.1 t ha⁻¹; high conc.: 6.8±0.2 t ha⁻¹). In 2011 among EPN treatments Slovenian strain (low conc.:...
treatment, which also coincide with our previous research in which we studied the effect of EPNs when applied foliar in controlling Colorado potato beetle.

Despite report of Welch and Briand that foliar application of EPNs is not recommended due to the faster drying of nematode suspension on leaves, which leads to weaker efficacy, we have established that when key limiting factors (i.e. temperature, moisture, UV radiation) are considered properly, a proper application can give us satisfying results in foliar usage of EPNs, also confirmed in other research. The problem of application of such kinds of biological products still lies in their poorer efficacy in comparison to chemical products and their high price. Some further results from studies on the activity of EPNs on other species of beetles have also shown that these agents could be an effective alternative to insecticides. A research has also shown that with proper application techniques and right timing as regards the insect developmental stage, we can reach almost the same results as with the use of insecticides.

Conclusions

Yield results gained in our research showed that the Slovenian strain of S. carpocapsae (C101) and commercial strain are both effective biological agents in foliar control of the CLB in the open. Highest yield in both years was obtained in the thiametoxam treatment and lowest in the control treatment. In 2010 weaker efficacy among EPN treatments showed commercial strain Nemasys C. In 2011 among EPN treatments Slovenian strain (low conc. and high conc.) and commercial strain Nemasys C (high conc.) gained better yield results than commercial strain Nemasys C (low conc.). Concentration of the nematode suspension did not have any influence on the yield in both tested years, which gives these biocontrol agents in integrated agriculture better prospects from an economical point of usage, as the cost of plant protection is closely connected to the quantity of the applied EPNs.

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Table 1. Cost differences between biological control and insecticide control of CLB in our experiment with price ratio of wheat/t between conventional and ecological production.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Treatment cost ratio/ha*</th>
<th>Price of wheat/t ratio*</th>
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<tbody>
<tr>
<td>EPN high conc. (150.000 IJm⁻²)</td>
<td>20</td>
<td>Conventional reduction</td>
</tr>
<tr>
<td>EPN low conc. (75.000 IJm⁻²)</td>
<td>10</td>
<td>Ecological production</td>
</tr>
<tr>
<td>Insecticide</td>
<td>1</td>
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*All data values are corresponding to the market prices of biological control agents, insecticides and wheat in 2011.
References


