THE EFFECTS OF DIFFERENT FUNGICIDES ON THE VIABILITY OF ENTOMOPATHOGENIC NEMATODES Steinernema feltiae (FILIPJEV), S. carpocapsae WEISER, AND Heterorhabditis downesi STOCK, GRIFFIN & BURNELL (NEMATODA: RHABDITIDA) UNDER LABORATORY CONDITIONS

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To increase our knowledge on the susceptibility of entomopathogenic nematodes (EPN) species to agrochemicals, the compatibility of the infective juveniles (IJ) of the entomopathogenic nematodes Steinernema feltiae, S. carpocapsae, and Heterorhabditis downesi with 15 chemical fungicides was investigated under laboratory conditions. The effect of direct IJ exposure to fungicides for 24 h was tested in a petri dish at 15, 20, and 25 ºC. The results showed that the compatibility of S. feltiae with azoxystrobin was high, and similar findings were obtained for S. carpocapsae (strain C67) and all of the tested fungicides, except for tebuconazole + spiroxamine + triadimenol, maneb, dinocap, and copper (II) hydroxide + metalaxil-M. Nematode H. downesi (strain 3173) suffered the highest mortality rate when infective juveniles were mixed with tebuconazole + spiroxamine + triadimenol. The integration of the aforementioned agents into a pest management program is also discussed.

Key words: Compatibility, fungicides, Heterorhabditis downesi, Steinernema carpocapsae, Steinernema feltiae, viability.

Rhabditid nematodes of the Steinernematidae and Heterorhabditidae family are lethal to a broad range of economically important insect pests (Journey and Ostlie, 2000). Entomopathogenic nematodes (EPN) are often applied to sites and ecosystems that routinely receive other inputs that may interact with nematodes, including chemical pesticides, fertilizers, and soil amendments (De Nardo and Grewal, 2003). To save time and money, it is often beneficial to determine if a pesticide can be tank-mixed or applied simultaneously with another pesticide. In addition, the compatibility of an agent with integrated pest management (IPM) and integrated production (IP) systems must be evaluated (Grewal, 2002).

EPN infective juveniles (IJ) can tolerate short-term exposure (2-24 h) to many chemical and biological insecticides, fungicides, herbicides, fertilizers, and growth regulators, which can be tank-mixed and applied together (Krishnayya and Grewal, 2002; De Nardo and Grewal, 2003). However, generalizations cannot be applied because the nematode’s susceptibility depends on several factors, including the species, strain, agrochemical formulation and application dose (Grewal, 2002).

Biological control agents are used to control a wide range of foliar insect pests (Trdan et al., 2007; Laznik et al., 2010a). When applied under conducive conditions, nematodes can be as effective as chemical insecticides (Trdan et al., 2007; Laznik et al., 2010a). Nematode-fungicide combinations in tank mixes could offer a cost-effective alternative to foliar integrated pest management (IPM) systems. However, before an ecologically integrated approach to pest management involving nematode-fungicide combinations in tank mixes can be developed for foliar application, the compatibility of nematodes with new and routinely used fungicides must be established.

To increase our knowledge of the susceptibility of EPN species to agrochemicals (fungicides) and to explore the effect of their mechanism on the viability of these organisms, the aim of the present study was to select several commercial fungicides currently used in Slovenia for crop protection, evaluate their effects on the survival of IJ from native Slovenian strains of Steinernema feltiae (Filipjev) and Steinernema carpocapsae Weiser, a Hungarian strain of Heterorhabditis downesi Stock (Griffin & Burnell) and commercial ENTONEM® strains.
Fungicides
In the present study, 15 commercial fungicides registered against different fungal pathogens in Slovenia were evaluated. The tested fungicides were Fosetyl: ethyl hydrogen phosphonate (3.7 g L⁻¹; 0.25% Foltsyl; manufacturer: BASF SE Germany; distributed by BASF Slovenia, d.o.o.), Fluquinconazole: 3-(2,4-dichlorophenyl)-6-fluoro-2-[(1H-1,2,4-triazol-1-yl)quinazolin-4(3H)-one] + Pyrimethanil: N-(4,6-dimethylpyrimidin-2-yl) aniline (1.5 mL L⁻¹; 0.1% Clarinet; manufacturer: BASF SE Germany; BASF Slovenia, d.o.o.), Copper (II) hydroxide + Metalaxil-M: 0.15% Previcur 607 SL; manufacturer: Bayer CS; Bayer Cropscience d.o.o.), Dinocap: (a.i.)-1-(4-chlorophenox)-3,3,2-dioxaspiro[4.5]dican-2-ylmethyl(ethyl)(propyl)amine + Triadimenol: (1RS,2RS;1RS,2SR)-1-(4-chlorophenoxy)-3,3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol (0.4 mL L⁻¹; Falcon EC-460; manufacturer: Bayer CS; Bayer Cropscience d.o.o.), Folpet: N-(trichloromethylthio) phthalimide (150 mL L⁻¹; 0.15% Folpan 80 WDG; manufacturer: Makhteshim-Agan; Karsia, Dutovlje, d.o.o.), Sulfur (6 g L⁻¹; 0.2% Pepelin; manufacturer: BASF SE Germany; Cinkarna Celje, d.d., Slovenia), Metiram: zinc ammoniate ethylenebis(dithiocarbamate) - poly(ethylenethiuram disulfide) (1.2 g L⁻¹; 0.12% Polyym DF; manufacturer: BASF SE Germany; distributed by BASF Slovenia, d.o.o.), Propamocarb: propyl 3-(dimethylamino)propylcarbamate (2.5 mL L⁻¹; 0.15% Previcur 607 SL; manufacturer: Bayer CS; Bayer Cropscience d.o.o.), Copper (II) hydroxide + Metalaxil-M: methyl N-[(methylxycetyl)-N-(2,6-dimethyl)-D-alaninate (4 g L⁻¹; Ridomil Gold Plus 42.5 WP; manufacturer: Syngenta; Syngenta Agro, d.o.o.), Azoxystrobin: methyl (2E)-2-[(2-[(6-(2-cyanophenox)pyrimidin-4-ylxylo]) phenyl]-3-methoxyacrylate (1 mL L⁻¹; 0.075% Quadris; manufacturer: Syngenta; Syngenta Agro, d.o.o.), Dinocap: (RS)-2,6-dinitro-4-octylphenyl crotonates (0.4 mL L⁻¹; Sabithane; manufacturer: Dow Agrosciences; distributed by Karsia, Dutovlje, d.o.o.), Maneb + Propamocarb: (4 mL L⁻¹; Tattoo; manufacturer: Bayer CS; Bayer Cropscience d.o.o.) and Fenhexamid: 2’’,3’’,4’’-dichloro-4’’-hydroxy-1-methylcyclohexanecarboxanilide (2 mL L⁻¹; Teldor SC 500; manufacturer: Bayer CS; Bayer Cropscience d.o.o.).

Nematodes
All of the strains were reared using the last instar larvae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Bedding and Akhurst, 1975). *Galleria mellonella* production was executed in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) at 28 ± 2°C and 60% relative humidity (RH), and using a 12:12 h photoperiod (Woodring and Kaya, 1988; Parra, 1998). Four strains were included in the experiment. The commercial preparation Entanem (a.i. *Steinernema feltiae*) was obtained from Koppert B.V. (Berkel en Rodenrijs, The Netherlands). All of the other strains were isolated from the soil, *Steinernema feltiae* C76 (Laznik et al., 2009a) and *Steinernema carpocapsae* C67 (Laznik et al., 2008) were isolated in Slovenia, while *Heterorhabditis downesi* 3173 was isolated in Hungary (Tóth, 2006). Two strains (C67 and 3173) were tested for the first time in the present study, while strain C76 was proven to be effective in a laboratory assay against the third-stage larvae of the common cockchafer (Laznik et al., 2009b). Only IJ less than 2 wk old were used in the present study (Gutiérrez et al., 2008). The IJ were stored at 4 °C at a density of 3000 IJ mL⁻¹. The number of IJ in a previously prepared nematode suspension with an unknown concentration was determined by counting the number of nematodes in a droplet (5 μL × 5) and diluting (adding a tap water solution) or concentrating (reducing to an adequate volume with the assistance of centrifugation) the sample. In this manner, the selected concentrations of nematode suspensions were obtained (Laznik et al., 2010c). Prior to the compatibility experiment, nematode viability was determined, and only nematode stocks with > 95% survival rates were used (De Nardo and Grewal, 2003).

Compatibility test
All of the fungicides were tested at the highest recommended concentration. Stock solutions of the fungicides were prepared in water. To 30 mL of the fungicide at 120% of the recommended concentration, 6 mL of IJ at a density of 3000 IJ mL⁻¹ was added. The addition of the IJ solution brought the concentration of the fungicide down to the recommended rate. Each plastic Petri dish (40 × 10 mm; Kemomed d.o.o., Slovenia) contained 5 mL of the given solution. IJ were counted in Petri dish arenas at each step (before they were added to the fungicide and immediately after mixing). Five replicates were used for each treatment, and the experiment was repeated three times. Water was used as a control treatment. The Petri dishes were placed in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) without light at temperatures of 15, 20, and 25 °C and at 70% RH. The Petri dishes were placed in a chamber in blocks (each fungicide treatment represented one block). At all of the tested temperatures, the effect of evaporation
was negligible. The viability of IJ incubated in different chemicals was assessed after 24 h by removing 3 × 50-μL sub-samples from each of the replicated treatments. At least 100 nematodes were counted for each treatment and the control. Nematodes that did not move after prodding were considered dead.

**Statistical analyses**

Prior to analysis, all of the data were corrected for the mortality rate of the control group using Abbott’s correction (Abbott, 1925). Mortality data were analyzed using multifactor ANOVAs in Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc.), and different fungicides were applied as independent variables. Mean separation was performed using Tukey’s procedure with α = 0.05.

**RESULTS AND DISCUSSION**

Data analyses on the pooled results are presented in Table 1. Among the studied fungicides, the (P ≤ 0.05) highest rate of IJ mortality for strain C76 and Entonem was obtained with maneb (from -89.4% to -100%) and tebuconazole, spiroxamine + triadimenol (-100%) (Table 2). Compared to the control treatment, significant differences in the IJ mortality rate of strain C76 were observed at 15 °C with all of the studied fungicides, except azoxystrobin (-13.9%), which was not significantly different from the control group after 24 h. Compared to the control group, significant differences in the IJ mortality rate of strain C76 were observed at 20 °C and 25 °C with maneb, tebuconazole, spiroxamine + triadimenol (-100%) (Table 2). Among the studied fungicides, the highest (P ≤ 0.05) highest mortality rate in strain C67 and commercial product Entonem, S. carpocapsae strain C67, and Heterorhabditis downesi strain 3173. In a similar study, maneb did not affect the IJ mortality rate of Heterorhabditis bacteriophora (Rovesti et al., 1988), and S. feltiae was tolerant to four tested fungicidally active ingredients, including fenhexamid (N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide), kresoxim-methyl (methyl (E)-methoxyimino(o-tolyloxy)-o-tolylacetate), and nuarimol [(RS)-2-chloro-4’-fluoro-α-(pyrimidin-5-yl) benzhydral alcohol] (Radova, 2010). The results of the present study and those of previous investigations (Rovesti et al., 1988; Krishnayya and Grewal, 2002; De Nardo and Grewal, 2003), in which the compatibility of plant protection products with EPN was evaluated, revealed that compatibility is species-specific. The present findings on the compatibility of nematodes and fungicides also confirm the results of two similar preceding studies (Krishnayya and Grewal, 2002; De Nardo and Grewal, 2003), which demonstrated that azoxystrobin did not influence the mortality rate of S. feltiae. Similar results were also obtained in the present study. Namely, after 24 h, significant differences among strain C76 and the control treatment were not observed at all of the tested temperatures.

In our experiment, when the nematodes were mixed with fungicide, temperature had an important influence on IJ mortality rates. At higher temperatures, the mortality

![Table 1. ANOVA results for the corrected mortality rates of infective juveniles of entomopathogenic nematodes (EPN).](Image)

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>153.23</td>
<td>15</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>EPN strain</td>
<td>251.33</td>
<td>3</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Temperature</td>
<td>70.27</td>
<td>2</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Replication in time</td>
<td>1.85</td>
<td>4</td>
<td>0.0703</td>
</tr>
<tr>
<td>Replication in space</td>
<td>0.28</td>
<td>2</td>
<td>0.7590</td>
</tr>
<tr>
<td>EPN strain × treatment</td>
<td>13.48</td>
<td>45</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>EPN strain × temperature</td>
<td>122.40</td>
<td>6</td>
<td>&lt; 0.0001*</td>
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<tr>
<td>Treatment × temperature</td>
<td>10.39</td>
<td>30</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>EPN strain × temperature × treatment</td>
<td>7.11</td>
<td>90</td>
<td>&lt; 0.0001*</td>
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</table>

*Source of variation significant at α = 0.05.
Change in nematode survival after exposure to chemicals for various durations at different temperatures (%)

<table>
<thead>
<tr>
<th>Steinerema feltiae strain C76</th>
<th>S. feltiae strain Entomem</th>
<th>S. carpocapsae strain C67</th>
<th>Heterorhabditis downesi strain 3173</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>15 ºC</td>
<td>20 ºC</td>
<td>25 ºC</td>
</tr>
<tr>
<td>Alillete flash</td>
<td>-59.9b</td>
<td>-20.0ab</td>
<td>-37.8bc</td>
</tr>
<tr>
<td>Bellis</td>
<td>-56.8b</td>
<td>-4.1a</td>
<td>+1.5ab</td>
</tr>
<tr>
<td>Clarinet</td>
<td>-68.2b</td>
<td>-2.4a</td>
<td>-3.6abc</td>
</tr>
<tr>
<td>Cuprablu-2</td>
<td>-65.1b</td>
<td>-8.4a</td>
<td>-2.1ab</td>
</tr>
<tr>
<td>Dithiane M-45IV</td>
<td>-98.2ed</td>
<td>-98.6c</td>
<td>-92.8de</td>
</tr>
<tr>
<td>Falcon EC-460</td>
<td>-100.0d</td>
<td>-100.0e</td>
<td>-100.0e</td>
</tr>
<tr>
<td>Folpaon 80 WDG</td>
<td>-61.4b</td>
<td>-32.0ab</td>
<td>-41.0bc</td>
</tr>
<tr>
<td>Pelder</td>
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<td>-8.4a</td>
<td>-4.4ab</td>
</tr>
<tr>
<td>Polyram D9</td>
<td>-65.7b</td>
<td>-17.4ab</td>
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<td>Pevivic 607 SLIII</td>
<td>-72.8bc</td>
<td>-7.6a</td>
<td>-7.3a</td>
</tr>
<tr>
<td>Rikidim Gold Plus</td>
<td>-77.2bcd</td>
<td>-68.0bc</td>
<td>-87.6de</td>
</tr>
</tbody>
</table>

*Values were significantly different (P ≤ 0.05) in Tukey’s multiple range tests. Small letters indicate that statistically significant differences were observed between the control treatment and treatment with the same EPN strain at the same temperature.

The effect of pesticides on IJ movement (behavior) is difficult to evaluate because nematodes (especially *S. carpocapsae*) tend to remain quiescent and stay in a J-shaped position after treatment, but they can respond to movement as indicated by the reduction, dispersion, and attraction of host attraction may be affected, as well as reproduction of plant diseases are similar and the proposed approach saves time and money spent in the control of pest organisms (Georgis, 1990). EPN combined with fungicide treatment is a cost-effective alternative to chemical pest control because the effects of simultaneous application of fungicide and IJ on fungal and insect agents of plant diseases are similar. Several studies have shown that when EPN are correctly applied, they are effective on several types of cucumber pests, including western flower thrips (*Frankliniella occidentalis* Pergande) (Trdan et al., 2007) and the greenhouse whitefly (*Trialeurodes vaporariorum* [Westwood]) (Laznik et al., 2011). Synchronous application of fungicide and EPN for the control of the aforementioned pests is justifiable, especially under greenhouse conditions, where fungal and insect pests often occur together (Trdan et al., 2007; Laznik et al., 2011). Similar conclusions can be drawn for metiram, copper (II) hydroxide, and maneb + propamocarb, which can be used to control potato blight (*Phytophthora infestans* [Mont.] de Bary), lettuce downy mildew (*Bremia lactucae* Regel), wheat leaf blotch (*Septoria tritici* Thüm.), and early tomato blight (*Alternaria solani* Sorauer) (Milus, 1994; Stepanović et al., 2009; Stevenson, 2009), along with several pest insects that often occur with the aforementioned fungi, such as the Colorado potato beetle (*Leptinotarsa decemlineata*)
Agency. We thank Sanja Ljubi for technical assistance and spatial planning of the Republic of Slovenia. A portion which was granted by the Slovenian Research Ministry of Agriculture, Food, and Forestry of the present work was performed within project V4-1067, an IPM program. Specifically at higher temperatures, the IJ mortality rates increased. Based on our research, EPN combined with fungicides must be supported by field experiments because the results of laboratory experiments cannot be wholly extrapolated to environmental conditions.

CONCLUSIONS

According to the results of previous investigations and the current study, maneb and tebuconazole, spiroxamine + triadimenol are not compatible with S. feltiae, S. carpocapsae and H. downesi. In our experiment, when H. downesi (cepa C67) and all the fungicides tested, except for azoxystrobin in the control of Erysiphe cichoracearum and Psedoperonospora cubensis on cucumber. Journal of Plant Protection Research 48:147-158.


Laznik, Ž., T. Tóth, T. Lakatos, M. Vidrih, and S. Trdan. 2010c. Control of the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) on potato under field conditions: a comparison of the efficacy of foliar application of two strains of *Steinernema feltiae* (Filipjev) and spraying with thiamethoxam. Journal of Plant Diseases and Protection 117(3):129-135.


