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Compatibility of entomopathogenic nematodes with fipronil

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Abstract

The survival and infectivity of infective juveniles (IJs) of three species of entomopathogenic nematodes, *Steinernema carpocapsae* Weiser, *S. arenarium* (Artyukhovsky) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), were determined after exposure to different concentrations (250, 500, 1000 and 2000 ppm) of fipronil, an insecticide acting on the GABA receptors to block the chloride channel. *Heterorhabditis bacteriophora* was very tolerant to all concentrations of fipronil, with the highest mortality of 17% being observed at 2000 ppm of fipronil after 72 h exposure. *Steinernema carpocapsae* showed a similar response, with the highest mortality of 11.25% of IJs being observed after 72 h exposure to 2000 ppm of fipronil. *Steinernema arenarium* was, however, more sensitive to fipronil, and at 2000 ppm mortality rates of 94.6% and 100% were observed after 24 and 72 h, respectively. Fipronil had negligible effects on the infectivity of the three nematode species tested. The IJs which survive exposure to all concentrations of fipronil tested can infect and reproduce in *Galleria* larvae. The moderate effects on entomopathogenic nematodes of a lower fipronil concentration (250 ppm) and the field rates (12–60 ppm) of fipronil used as insecticide, suggest that direct mixing of entomopathogenic nematodes and fipronil at field rates is a viable integrated pest management option.

Introduction

Entomopathogenic nematodes of the genera *Steinernema* (Rhabditida: Steinernematidae) and *Heterorhabditis* (Rhabditida: Heterorhabditidae) are important biological agents used to control a number of insect pests that can have significant economic consequences. With the implementation of integrated pest management (IPM) techniques, that use both chemical pesticides and biological control agents, the compatibility of entomopathogenic nematodes with chemical insecticides needs to be established. Previous studies have shown the consequences of direct exposure to solutions of insecticides on the behaviour and infectivity of selected entomopathogenic nematode species. Steinernematid and heterorhabditid nematodes have been shown to be relatively insensitive to a wide range of pesticides (Ishibashi & Takii, 1993; Zhang *et al.*, 1994; Alumai & Grewal, 2004; Bednarek *et al.*, 2004) although some organophosphate and carbamate compounds, which inhibit acetylcholinesterase in the nematode nervous system (Wright, 1981; Opperman & Chang, 1990), have been found to induce partial paralysis, reduce infectivity and inhibit development of *S. carpocapsae* (Prakasa Rao *et al.*, 1975; Fedorko *et al.*, 1977a,b; Hara & Kaya, 1982, 1983a,b; Kaya & Burlando, 1989; Rovesti & Deseö, 1990; Zimmerman & Cranshaw, 1990; Patel & Wright, 1996; Head *et al.*, 2000). However, some studies have shown that some pesticides can enhance the activity of infective juveniles (IJs) of entomopathogenic nematodes (Gaugler & Campbell, 1991; Ishibashi & Takii, 1993).

Fipronil belongs to the phenyl pyrazole class of insecticides (Buntain *et al.*, 1988), which act on the γ-aminobutyric acid (GABA) receptor as a non-competitive

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blocker of the GABA-gated chloride channel (Cole *et al.*, 1993). In insects and mammals, the behavioural effects of central GABA antagonists include hyperactivity, hyperexcitability, and convulsions, which are correlated with increased spontaneous nerve activity and the generation of prolonged, high frequency discharges following on from nerve stimulation, finally resulting in death. With the use of fipronil, there are three toxicants to consider, the parent compound, its sulphone, and its desulphinyl photoproduct (Hainzl & Casida, 1996). Fipronil sulphone is the major metabolite of fipronil in insects and vertebrates, and it is nine times more potent than fipronil (Hainzl *et al.*, 1998). Desulphinyl fipronil, although not a metabolite, is the principal photoproduct on plants and soils and is more toxic than fipronil (Hainzl & Casida, 1996). Due to the effect of fipronil being highly specific to the invertebrate GABA receptor (Hainzl & Casida, 1996), it has been used in terrestrial and aquatic environments for a wide range of economically significant insect pests (Colliot *et al.*, 1992; Tomlin, 1997). The mode of action of fipronil is similar to cyclodiene insecticides such as endosulfan, lindane and dieldrin. A distinct advantage of fipronil is that it is one of the most selective of the insecticidal blockers of the GABA-gated chloride channel with a favourable safety factor between insects and mammals (Cole *et al.*, 1993; Tomlin, 1997; Hainzl *et al.*, 1998).

Studies of *Caenorhabditis elegans* neural connectivity indicate that muscles in nematodes are innervated by both γ-aminobutyric acid (GABA) and acetylcholine motor neurons (McIntire *et al.*, 1993). Patel & Wright (1996) determined the influence of neuroactive pesticides, that act on acetylcholinesterase, on the behaviour and infectivity of IJs of the entomopathogenic nematodes *Steinernema carpocapsae* and *S. feltiae*. However, there are a limited number of studies (Rovesti *et al.*, 1988; Rovesti & Deseö, 1990; Hussaini *et al.*, 2001) on the compatibility of entomopathogenic nematodes and some insecticides (lindane and endosulfan) acting at the GABA receptors to block the chloride channel, and no data are available on the effects of fipronil.

In the present study, the effect of fipronil is analysed on the behaviour, survival, infectivity and development of two species of entomopathogenic nematodes with a high level of mobility (*Heterorhabditis bacteriophora* and *Steinernema arenarium*), and one species with a low level mobility (*S. carpocapsae*).

Materials and methods

A commercially available fipronil (80% w/w a.i.) formulated as water-dispersible granules (WG) (Regente® from BASF Agro B.V. Zurich, Switzerland) was used. Stock solutions of fipronil were freshly prepared by means of agitation in distilled water, until an homogenate dispersible solution was observed.

Steinernema carpocapsae IJs were obtained from e-nema GmbH (Raisdorf, Germany), with *S. arenarium* (strain S2) being isolated in Salamanca (Spain) and *Heterorhabditis bacteriophora* (strain TF19) in Tenerife (Canary Islands, Spain). The nematodes were reared in last instar larvae of the wax moth, *Galleria mellonella* (Lepidoptera: Galleriidae) following the protocol of Woodring & Kaya (1998). Infective juveniles were stored in water or damp sponges at 5–8°C for a period no longer than two weeks before being used in the experiments. The nematodes were allowed to acclimate at room temperature for at least 12 h and checked for viability before use in the bioassays.

Survival of infective juveniles

Infective juveniles of each species were exposed to 250, 500, 1000 and 2000 ppm of fipronil in Eppendorf tubes (1.5 ml). For each concentration, ten IJs were transferred individually to each tube with 1 ml of fipronil solution. Infective juveniles incubated in tap water were used as controls. The tubes were incubated at 23 ± 2°C and the survival of IJs was recorded at exposure periods of 0, 24, 48 and 72 h. After each period, the ten IJs exposed to the chemical were removed from the tubes to watch glasses, washed three times in tap water, and their movement observed under a stereomicroscope. Immobile, presumably dead, IJs were maintained for 24 h in tap water and observed again after this period. Finally, nematode mortality (i.e. not responding to mechanical stimulation) was recorded. Each nematode species, concentration of fipronil and time of exposure was replicated eight times. Sublethal effects of fipronil on the IJs were determined by qualitative observations of the behaviour and active movements of the nematodes.

Infectivity of infective juveniles

Infective juveniles of each species were exposed to 250, 500, 1000 and 2000 ppm of fipronil in watch glasses. For each concentration, 200–300 IJs were transferred to each watch glass with 4 ml of fipronil solution and covered with a glass lid. Controls were maintained in watch glasses with 4 ml of tap water. After 24 and 48 h, the nematodes exposed to the chemical were observed. Ten of these exposed nematodes that showed mobility were washed three times with tap water, and transferred with 0.2 ml of water to an Eppendorf tube (1.5 ml) wrapped internally with filter-paper and with one late instar larva of *Galleria mellonella*. Watch glasses and Eppendorf tubes were incubated at 23 ± 2°C. Each nematode species, concentration of fipronil and time of exposure were replicated ten times. Larval mortality was determined daily for five days. Dead *Galleria* larvae were transferred to a Petri dish (5 cm diameter) with wet filter-paper, until the reproduction of the nematodes and the emergence of IJs.

Data analysis

Data, as percentage of IJs mortality were corrected for control mortality with Abbott's (1925) formula. This corrected mortality was transformed by arcsine and subjected to variance analysis (ANOVA, SPSS 11.0) to determine treatment effects in each nematode species. Treatment means were compared using the Scheffe multiple range test ($P < 0.05$).

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Results

Survival of infective juveniles

The effect of fipronil on the survival of infective juveniles varied according to exposure times and concentrations used on *Heterorhabditis bacteriophora* and *Steinernema arenarium*, but not on *Steinernema carpocapsae* (fig. 1). For all fipronil concentrations and exposure times, *S. arenarium* was significantly ($P < 0.05$) more sensitive than *H. bacteriophora* and *S. carpocapsae*, there being no observable differences ($P > 0.05$) in survival between the last two species.

Heterorhabditis bacteriophora was very resistant to all concentrations of fipronil tested after 24 h (fig. 1). Infective juveniles were significantly ($P < 0.05$) more affected by the highest concentrations tested (1000 and 2000 ppm) only after 48 h of exposure. The highest mortality observed after 72 h of exposure was 17% at 2000 ppm of fipronil. No differences in mortality between concentrations were observed after 72 h, except for the lowest concentration (250 ppm) that at none of the exposure times caused lethal effects on this nematode species. *Steinernema carpocapsae* showed a similar response to fipronil exposure (fig. 1) as in *H. bacteriophora*. After 72 h of exposure, no differences in mortality were observed between concentrations. The highest mortality of IJs observed in this species after 72 h of exposure was 11.25% at 2000 ppm of fipronil.

Steinernema arenarium was significantly more sensitive to fipronil than the other two nematodes tested. At the high concentration (2000 ppm) and 24 h of exposure, a high mortality (94.6%) of IJs was observed. After 72 h exposure to fipronil (fig. 2), all concentrations, with the exception of 250 ppm (ANOVA: $F = 2.19$, $P > 0.05$), caused significantly higher mortalities in *S. arenarium* IJs than in the other nematode species tested (ANOVA: $F = 30.22$, $P < 0.01$ for 500 ppm; $F = 20.00$, $P < 0.01$ for 1000 ppm; $F = 530.87$, $P < 0.01$ for 2000 ppm; and a Scheffé multiple rank test carried out a posteriori). The high concentration of fipronil (2000 ppm) proved particularly toxic for this species at 72 h, with a 100% rate of mortality for those IJs exposed.

Sinusoidal movement in *S. carpocapsae* and in *H. bacteriophora* was not affected by fipronil exposure in any concentration. However, *S. arenarium* IJs exhibited slow movements or an inactive straight posture and a slow reaction to mechanical stimulation after 24 h exposure to fipronil.

Infectivity of infective juveniles

In all three nematode species, the IJs surviving the four concentrations of fipronil (250, 500, 1000 and 2000 ppm) for two of the exposure times (24 and 48 h), resulted in a 100% *Galleria mellonella* mortality in 48 h. In all *Galleria* larvae parasitized, reproduction of nematodes and emergence of new infective juveniles were observed.

Discussion

The present study shows that fipronil has moderate effects on entomopathogenic nematodes depending on the nematode species, together with the concentration of the chemical and times of exposure. No detrimental effects were observed at the lowest concentration tested (250 ppm) in *S. carpocapsae* and *H. bacteriophora*, and only a decline in movement, but no lethal effects, in *S. arenarium*. Although fipronil had some effect on *S. arenarium* motility, its effect on infectivity was negligible. This

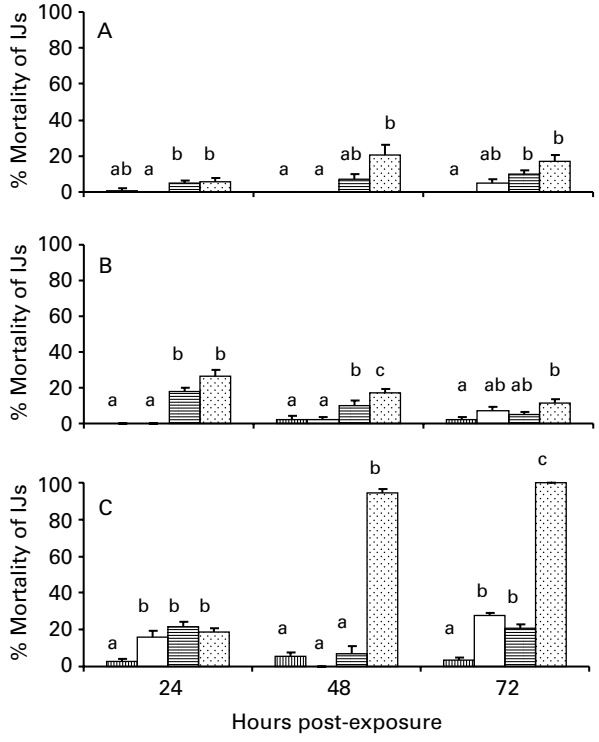

Fig 1. Mortality (mean ± SE) of infective juveniles (IJs) of (A) *Heterorhabditis bacteriophora*, (B) *Steinernema carpocapsae* and (C) *S. arenarium* exposed to different concentrations of fipronil (▥, 250 ppm; □, 500 ppm; ▤, 1000 ppm; ▨, 2000 ppm). Bars with the same letter are not significantly different ($P > 0.05$ Scheffe multiple range test).

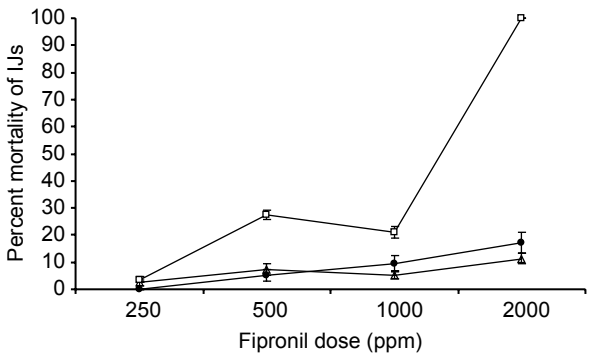

Fig. 2. Percent mortality (mean ± SE) of infective juveniles (IJs) of *Heterorhabditis bacteriophora* (•) *Steinernema carpocapsae* (△) and *S. arenarium* (□) after 72 h of exposure to fipronil.

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reduction in the movement of *S. arenarium* IJs without effect on infectivity was also observed by Rovesti & Deseö (1990) in *S. carpocapsae* with different neuroactive pesticides. However, Patel & Wright (1996) observed a reduction in the infectivity of *S. feltiae* and *S. carpocapsae* exposed to sublethal concentrations of oxamyl and fenamiphos. These authors reported that both pesticides probably impair the ability of IJs to locate the host, affecting locomotion and possibly sensory perception, and therefore reducing nematode pathogenicity. In our infectivity test, where IJs exposed to fipronil were in direct contact with *Galleria* larva, the ability of IJs to locate the host was not evaluated. In the highest concentration tested (2000 ppm), fipronil had moderately lethal effects (less than 25% mortality) in *S. carpocapsae* and *H. bacteriophora,* but a high mortality (95–100%) was observed in *S. arenarium*. These results are in agreement with the findings of other authors (Hara & Kaya, 1982; Heungens & Buysse, 1987; Rovesti *et al.*, 1988; Rovesti & Deseö, 1990) who reported that *S. carpocapsae* and *H. bacteriophora* can survive exposure to high concentrations of many neuroactive pesticides. Especially useful are the studies on the compatibility of entomopathogenic nematodes with endosulfan and lindane, which are similar to fipronil in having a high potency action on insect GABA receptors. Heungens & Buysse (1987) reported that lindane was very toxic and endosulfan was only slightly toxic for *Heterorhabditis* spp. Rovesti & Deseö (1990) observed negligible effects of endosulfan on the survival and infectivity of *S. carpocapasae* and *S. feltiae*, and Rovesti *et al.* (1988) showed that endosulfan and lindane had no effects on the survival and infectivity of *H. bacteriophora*. These and the present study agree insofar as the negligible effects of GABA antagonist pesticides on these entomopathogenic nematodes are concerned. Limited data are available on the susceptibility of 'long cruiser' nematodes (*glaseri* group), such as *S. arenarium*, to chemical pesticides. Palomo & Garcia del Pino (2000) reported that *S. arenarium* (strain S2) was more sensitive to oxamyl than *H. bacteriophora*, which supports the present results concerning the greater susceptibility of long nematodes such as *S. arenarium* to chemical pesticides like fipronil.

The negligible effects of a lower fipronil concentration (250 ppm) on entomopathogenic nematodes, and the moderate effects of a high concentration (2000 ppm) on *S. carpocapsae* and *H. bacteriophora* (25 and 20% respectively) contrast with the high activity of low concentrations of fipronil as an insecticide (Colliot *et al.*, 1992). Field rates for fipronil used as an insecticide (12–60 ppm) and the results of the present study, suggest that compatible combinations of entomopathogenic nematodes and fipronil may be suitable for co-application against certain insect pests.

The differing effects of fipronil on insects and entomopathogenic nematodes appear to be related to the high specificity of fipronil to GABA receptors. Little is known about the GABA receptor specificity of nematodes and other invertebrates. The understanding of the differences between nematode and insect GABA-gated chloride channels probably helps to explain the differing degrees of susceptibility to fipronil between nematodes and insects.

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