Spirotetramat causes an arrest of nematode juvenile development

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Summary – Spirotetramat (Movento™, Bayer CropScience) (SPT), an effective insecticide, has also demonstrated potential activity as a nematicide. No significant effects on hatching rates of *Caenorhabditis elegans*, *Meloidogyne incognita* or *Heterodera glycines* were observed when eggs were soaked in a maximum concentration of 105 ppm of technical grade spirotetramat-enol (SPT-enol), the active form in plants. Synchronised first-stage juveniles of *C. elegans* soaked in SPT-enol concentrations as low as 30 ppm demonstrated arrested juvenile development with calculated EC₉₅ of 44–48 ppm. Single applications of formulated SPT (Movento 240SC) were applied to plant foliage at the labelled insecticidal rate of 87.6 g a.s. ha⁻¹ at 1-week intervals on soybean plants inoculated with *H. glycines* or tomato plants inoculated with *M. incognita* in glasshouse tests. SPT consistently inhibited nematode development to reproductive maturity when applied at 1–2 weeks after inoculation. Optimal SPT application timings coincide with early stages of root infection, when nematodes are still in vulnerable juvenile stages.

Keywords – *Caenorhabditis elegans*, chemical control, foliar application, *Heterodera glycines*, *Meloidogyne incognita*, Movento, root-knot nematode, soybean cyst nematode, systemic nematicide.

Plant-parasitic nematodes cause an estimated US\$ 125 billion in crop losses every year (Chitwood, 2003; Decraemer & Hunt, 2013). Different strategies have been employed to reduce nematode-related crop damage, including planting of resistant cultivars, rotating with non- or poor-host crops, and biological control (Cook & Evans, 1987; Kerry, 1987; Starr *et al.*, 2013; Viaene *et al.*, 2013). The cost-efficiency and durability of these management strategies is dependent on multiple factors including the crop host, species and population levels of the nematodes present, nature and availability of genetic resistance in the crop species, and a plethora of environmental factors. For example, the wide range of host plant species for the major species of root-knot nematodes (*Meloidogyne* spp.) makes nematode management using crop rotations a challenge, whereas the limited host range of the soybean cyst nematode (*Heterodera glycines*) allows greater management efficacy in rotations with non-host crops like corn (Viaene *et al.*, 2013). Host resistance to root-knot and cyst nematodes can provide effective management when genetic sources of resistance can be incorporated into commercial cultivars but the development of resistance-

breaking nematode populations remains a constant threat (Starr *et al.*, 2013).

Chemical control (fumigant and non-fumigant nematicides) can offer an effective means for management of plant-parasitic nematodes that is independent of the nematode species present. Chemical control of nematodes has been in practice since the late 19th century and is often used in combination with other nematode control methods in an integrated pest management strategy (Cook & Evans, 1987; Kerry, 1987; Starr *et al.*, 2013; Viaene *et al.*, 2013). However, the number of commercially available chemical nematicides has declined in recent years due to their inherent toxicity and potential to cause environmental damage. Since the late 1970s, bans and restrictions for a majority of nematicides have left an urgent need for new nematode management solutions (Hague & Gowen, 1987; Haydock *et al.*, 2013).

Spirotetramat (Movento™; Movento™ is a registered trademark of Bayer CropScience LP, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA) (SPT), a tetramic acid derivative, is currently labelled as an insecticide but has also been demonstrated to suppress nematode populations (McKenry *et al.*, 2009, 2010). SPT has a

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unique insecticidal mode of action (IRAC, Group 23) as a lipid biosynthesis inhibitor that reduces lipid content, inhibits ecdysis, and reduces fecundity and fertility (Nauen *et al.*, 2008). SPT also has very favourable toxicological, ecotoxicological and environmental profiles (Babczinski & Hellpointer, 2008; Klempner, 2008; Maus, 2008; Sur, 2008). Applied to plant foliage, SPT penetrates the leaf surface and is hydrolysed *in planta* to the active enol form. The physicochemical properties of SPT-enol allow it to enter both phloem and xylem transport systems of the plant, resulting in unique two-way systemicity that allows the active ingredient to travel from the point of application in the foliage down into the roots where many phytoparasitic nematode species feed (Fischer & Weiß, 2008; Vermeer & Baur, 2008).

McKenry *et al.* (2009) first reported varying levels of suppression of plant-parasitic nematode species infecting citrus, grape and walnut by foliar applications of SPT. Subsequent field studies have further demonstrated promising effects of foliar applications of SPT on several species of nematodes in multiple cropping systems (Bayer CropScience, pers. comm.). Preliminary fundamental investigations suggest that SPT may affect nematodes in a similar manner to insects, with slowed/arrested development observed in *M. incognita* and *Caenorhabditis elegans*, inhibition of moulting observed in *H. schachtii* females, and absence of lipids under starved conditions observed in *C. elegans* (Bayer CropScience, pers. comm.). Although an effect on nematode juvenile development has been suggested, the relationship between SPT concentration and inhibition of juvenile development is not known, especially concentrations of the active SPT-enol generated *in planta*. Similarly, little is currently known about the effects of SPT on hatch or fecundity of females. Fully understanding the effects of SPT-enol on nematode biology could allow foliar SPT applications to be timed according to when nematodes are in the most vulnerable life stages for optimal nematode control. Understanding the relationship of SPT-enol concentration to nematode life stage development could promote more efficient usage of SPT to maximise nematode control with minimum cost and environmental impact.

In the present study, the first objective was to determine nematode life cycle stages most affected by SPT-enol. Hatching and development assays were initially conducted with the model nematode, *C. elegans*, because, unlike sedentary endoparasitic root-knot and soybean cyst nematodes, *C. elegans* has a short life cycle that can be completed in a Petri dish and is easily observable in

laboratory studies (Jones *et al.*, 2011). Where appropriate, similar tests were repeated with plant-parasitic nematodes, *M. incognita* and *H. glycines*, in order to show that results obtained with *C. elegans* were repeatable with economically important species of plant-parasitic nematodes. A dose response curve was also developed to model the relationship between SPT-enol concentration and observed nematode response. The second objective of this study was to determine the optimal time to apply SPT to plant foliage for maximum effects on either root-knot or soybean cyst nematodes infecting roots. The results combined could determine if timing foliar applications of SPT to coincide with vulnerable nematode life stages would result in increased efficacy.

Materials and methods

CAENORHABDITIS ELEGANS CULTURE

Caenorhabditis elegans wild-type strain, N2, stock cultures were maintained on NGM plates seeded with the OP50 strain of *Escherichia coli* as described previously (Brenner, 1974). Large quantities of gravid *C. elegans* hermaphrodites were grown on 15 cm peptone-enriched plates of medium seeded with the NA22 strain of *E. coli* (Schachat *et al.*, 1978; Lewis & Fleming, 1995). Eggs were harvested from these gravid adults by treatment with a hypochlorite solution (Recipe 5) for 4 min as previously described (Porta-de-la-Riva *et al.*, 2012).

HETERODERA GLYCINES AND MELOIDOGYNE INCOGNITA CULTURE

Heterodera glycines were propagated on roots of soybean plants (*Glycine max* cv. Hutcheson) grown in a glasshouse. Cysts were dislodged from soybean roots with a directed water stream and collected in a 250 µm sieve. Cysts were then crushed with a rubber stopper over a 25 µm sieve to collect eggs (Goellner *et al.*, 2001). *Meloidogyne incognita* were propagated on roots of tomatoes (*Solanum lycopersicum* cv. Rutgers) grown in a glasshouse. *Meloidogyne incognita* eggs were extracted from host roots by rinsing roots in 0.6% sodium hypochlorite for 30 s, similar to that previously described (Hussey & Barker, 1973), and collecting and rinsing the eggs on a 25 µm mesh sieve. Both *H. glycines* and *M. incognita* eggs were cleaned free of debris *via* the sugar flotation method for counting (Agrios, 2005).

HATCHING ASSAYS WITH *CAENORHABDITIS ELEGANS*

The enol form of technical grade spirotetramat (SPT-enol) was synthesised and provided by Bayer Crop-Science (Monheim, Germany) to provide the active form for *in vitro* assays. The *C. elegans* hatching assays were conducted at room temperature in 96-well plates to determine the effect of SPT-enol on hatch rate, with a single well equaling one replication. Approximately 100 eggs in M9 buffer were pipetted into each well and then SPT-enol (dissolved in either 50% acetone-triton (5 ml Triton X-100 (1 l of acetone)⁻¹; Test 1) or 20% dimethyl sulfoxide (DMSO; Test 2)) was added to a final SPT-enol concentration of either 105, 90, 75, 60, 45, 30 or 3 ppm, in a total volume of 100 μ l. Buffer and solvent negative controls were included, as well as a positive nematicidal control of 0.1 ppm abamectin (ABM), for a total of ten treatments. Each treatment was replicated five times. The numbers of first-stage juveniles (J1) of *C. elegans* that hatched in each well after 3 days were counted and percentage hatch was calculated.

HATCHING ASSAYS WITH *HETERODERA GLYCINES* AND *MELOIDOGYNE INCOGNITA*

Hatching tests were conducted with *H. glycines* and *M. incognita* eggs at 28°C in bowls using the Baermann technique (Agrios, 2005). Approximately 20 000 eggs were placed on a double-layered Kimwipe™ on a mesh screen placed inside a plastic bowl. SPT-enol (dissolved in 20% DMSO) was used to prepare hatching solutions at concentrations of 90, 60 and 30 ppm. Water and solvent controls were also included for a total of five treatments. Hatching solutions were poured into respective Baermann bowls such that the surface of the liquid enveloped the eggs through the screen. Five replications were included per treatment. Hatched second-stage juveniles (J2) of *H. glycines* and *M. incognita* were collected from the bowls for 7 days and combined. Hatched J2 were then counted and percentage hatch was calculated.

STAGED DEVELOPMENTAL ASSAYS WITH *C. ELEGANS*

To determine the effects of SPT-enol on nematode development, synchronised *C. elegans* populations starting with hatched J1 were observed over the course of one life cycle. Populations of *C. elegans* were synchronised by plating eggs onto water agar plates with no food and collecting the hatched J1 in M9 buffer after 24–48 h (Lewis & Fleming, 1995). Approximately 40 J1 were pipetted into

each well of 24-well plates along with 800 μ l of test medium (10 ml of OP50 *E. coli* overnight culture, 5 mg of ampicillin, and 2 mg of nystatin in 100 ml of M9 buffer). SPT-enol was then added to a final concentration of either 105, 90, 75, 60, 45, 30 or 3 ppm, bringing the total volume to 1 ml in each well. Buffer and solvent controls were included, as well as positive nematicidal controls of 0.1 ppm ABM and 10 ppm mebendazole (MBZ), for a total of 11 treatments. ABM is a mixture of avermectin compounds derived from the soil bacterium, *Streptomyces avermitilis*, and works by targeting the nervous system. Nematodes become paralysed by low doses of ABM and, thus, ABM was used as a comparison for acute toxicity (Martin *et al.*, 2002). MBZ is an antihelmintic drug that inhibits the synthesis of microtubules in the intestine, blocking uptake of nutrients, causing a more gradual paralysis than ABM (Spence *et al.*, 1982). Therefore, MBZ was used as a comparison for chronic effects. Five replicates were included for each treatment. Developmental progress of the worms was observed over the course of 4 days and then the number of adult worms in each well was recorded.

DOSE-RESPONSE MODEL

The relationship between SPT-enol concentration and the number of *C. elegans* J1 that fully developed to adults in 4 days was modelled for each independent staged assay by fitting original data to a three-parameter log-logistic regression model (Equation 1) using the statistical software R version 3.0.3 (<http://www.r-project.org/>) with the analysis of dose-response curves (drc) package (Ritz & Streibig, 2005).

$$y = \left(\frac{d}{1 + \exp(b(\log x - \log e))} \right). \quad (1)$$

In the model, x is the SPT-enol concentration in ppm, while the parameters e , b and d represent the SPT-enol concentration in which the number of developed juveniles is reduced by half (EC_{50}), the slope around e , and the upper-response limit of the curve.

INFECTION ASSAYS WITH *HETERODERA GLYCINES*

To determine the optimal time to apply formulated SPT to plant foliage for maximum control of nematodes infecting roots, a single application of SPT was applied at 1-week intervals over the course of a single nematode life cycle. Formulated SPT (Movento SC 240) was applied with a spray bottle to leaves of 3-week-old soybean plants (*G. max* cv. Hutcheson) grown in pure sand in a

glasshouse at the labelled insecticidal rate of 87.6 g a.s. ha⁻¹ mixed in 38 l of water, a foliar application rate equivalent to 936 ppm of SPT a.s. that was not in the enol form. A methylated seed oil (MSO) concentration adjuvant, consisting of methylated oils and a nonionic surfactant (NIS) blend (Bayer CropScience), was mixed into the spray bottle at a rate of 0.25% (v/v). Plants were taken outside the glasshouse and sprayed to leaf drip. Plants were allowed to dry before being moved back into the glasshouse. Each assay consisted of seven treatments, with five plant replicates per treatment positioned in a randomised complete block design. Treatments consisted of a non-treated control, an adjuvant control applied 1 week after nematode inoculation, and Movento applied one time at either 1 week before inoculation, at the time of inoculation, or 1, 2 or 3 weeks after inoculation with 10 000 *H. glycines* eggs. Cysts were collected from each treated soybean plant at 4 weeks after inoculation. After counting, cysts were gently crushed to release and count the eggs. Egg collections had relatively little soil debris, so cleaning *via* sugar flotation was not necessary for counting. Roots were also weighed at cyst harvest and cyst (g root)⁻¹, eggs (cyst)⁻¹ and egg (g root)⁻¹ were calculated.

INFECTION ASSAYS WITH *MELOIDOGYNE INCOGNITA*

Formulated SPT (Movento SC 240) was applied to leaves of 5- to 6-week-old tomato plants (*S. lycopersicum* cv. Rutgers) grown in pure sand in a glasshouse at the labelled insecticidal rate of 87.6 g a.s. ha⁻¹. Single applications of SPT were made at weekly intervals as previously described for soybean plants. Each assay consisted of seven treatments, with five plant replicates per treatment arranged in a randomised complete block design in a glasshouse. Treatments consisted of a non-treated control, an adjuvant control applied 1 week after inoculation, and SPT applied once at either 1 week before inoculation with *M. incognita*, at the time of inoculation, or 1, 2 or 3 weeks after inoculation. Each plant was inoculated with 10 000 *M. incognita* eggs. Roots of each plant for the first assay were rinsed with water at 4 weeks after inoculation, weighed, and then stained with Phloxine B (0.03 g l⁻¹) for counting *M. incognita* egg masses (Fenner, 1962; Holbrook *et al.*, 1983). Few egg masses were present on control roots after 4 weeks so only galls were counted. The next two assays with *M. incognita* were taken down at 8 weeks after inoculation to allow more time for egg production. Stained egg masses were counted and then eggs were extracted from the roots for counting

using 0.6% sodium hypochlorite as described above. After counting, egg masses (g root)⁻¹, eggs (egg mass)⁻¹ and eggs (g root)⁻¹ were calculated.

STATISTICAL ANALYSIS

Data were log-transformed prior to analysis to satisfy assumptions of normality and homogeneity of variance (log(*y*) for *C. elegans* percentage hatch, both *H. glycines* infection assays and the first *M. incognita* infection assay with gall counts, log(*y* + 1) for counts of developed *C. elegans* and the second and third *M. incognita* infection assays with egg mass and egg counts, and log(*y* + 10) for *H. glycines* and *M. incognita* percentage hatch). A two-way ANOVA was performed for duplicate experiments using the general linear model (GLM) procedure in SAS version 9.4 (SAS Institute) to check for experiment-by-treatment interactions. If such interaction effects were not significant (*P* < 0.05) then data from two repeat experiments were combined for subsequent analysis. If interaction effects were significant (*P* < 0.05) then further analysis was conducted on individual experiments. When treatment means were significant, means were separated using the Scheffé test (*α* = 0.05).

Results and discussion

A delay in nematode juvenile development caused by SPT was observed in previous research (Bayer CropScience, pers. comm.). Cowpeas were sprayed with Movento at rates equivalent to 70.08 g a.s. ha⁻¹ and 109.5 g a.s. ha⁻¹ 2 days after inoculation with 2000 *M. incognita* J2. Infected roots were then stained and dissected at 1-week intervals following inoculation with nematodes. A significant increase in the proportion of third- (J3) and fourth-stage juveniles (J4) coinciding with a significant decrease in the proportion of females as compared to the non-treated controls was observed 20 days after inoculation, suggesting a delay in juvenile development. Studies (Bayer CropScience, pers. comm.) with mixed life stages of *C. elegans* suggested that SPT affected *C. elegans* development and movement at concentrations ≥ 30 ppm.

In hatching tests, SPT-enol had no apparent effect (Fig. 1) on the hatching rate of *C. elegans*, *H. glycines* or *M. incognita* eggs soaked in SPT-enol concentrations as high as 105 (*C. elegans*) or 90 ppm (*H. glycines* and *M. incognita*). *Heterodera glycines* did appear to be sensitive to the DMSO solvent, as indicated by the

Fig. 1. Percentage hatch of A: *Caenorhabditis elegans*; B: *Heterodera glycines*; and C: *Meloidogyne incognita* from eggs immersed in a range of spirotetramat-enol (SPT-enol) concentrations. No significant reductions in percentage hatch caused by SPT-enol were observed for any nematode species by two-way ANOVA ($P < 0.05$), although *H. glycines* did appear to be sensitive to the DMSO solvent. Differing letters represent a statistical difference (Scheffé test, $P < 0.05$). Data were transformed ($\log(y)$ for *C. elegans* and $\log(y + 10)$ for *H. glycines* and *M. incognita*) prior to statistical analysis; however, non-transformed data are presented in the graphs for ease of interpretation. A: Data are from a single representative hatching test with *C. elegans*; B: Data represent an average across two *H. glycines* hatching tests; C: Data represent an average across two *M. incognita* hatching tests.

significant reduction in hatch rate in the DMSO control treatment and all SPT-enol treatments compared to the water control. However, no significant differences in *H. glycines* hatching rates between the solvent control and the SPT-enol treatments were observed. From these data, we determined that SPT-enol either had no effect on hatch or that the eggshells were simply impervious to the chemical. Either way, for practical purposes, direct application of SPT-enol does not seem to have a direct effect on hatch of multiple nematode species. Plant nematode tests were only conducted with first generation eggs collected from stock plants that had never been treated with SPT. No direct evidence has been generated to test if SPT has an effect on the hatch rate or viability of second generation eggs produced by females feeding on plants treated with SPT. Evaluation of female nematode fecundity and hatch after plant host treatment with SPT and observation of reproductive rates over at least two nematode generations could provide evidence about the effects of SPT on viable egg formation and viability of a second generation of nematodes.

Results of nematode development assays starting with staged *C. elegans* J1 were consistent with those from previous research with mixed life stages, indicating a reduc-

tion in development of juveniles to adulthood (Fig. 2). No acute (lethal) effects were observed in worms soaked in SPT-enol at any concentration tested compared with abamectin (ABM) treatment. However, *C. elegans* soaked in SPT-enol remained small, suggesting an inhibition of juvenile development. Juveniles of *C. elegans* in SPT-enol treatments that had not fully developed to adults after one life cycle (3–4 days) continued to be observed for up to 10 days (the equivalent of at least three life cycles) but complete development was never observed, indicating an arrest, as opposed to a delay, in development. Significant reductions in the number of worms that developed to adults were observed in SPT-enol concentrations ≥ 30 ppm (Fig. 2). While it seems that SPT-enol causes an arrest of juvenile development, it is still unknown whether these effects are reversible.

The number of juveniles that failed to develop in SPT-enol treatments was dependent on SPT-enol concentration. Dose-response models generated for each independent assay with *C. elegans* indicated the EC_{95} to be 44 and 48 ppm (Fig. 3). Nematode juveniles appear to be somewhat less sensitive to SPT-enol than insects, as leaf-dip bioassays with SPT revealed the LC_{95} for aphid, *Aphis gossypii*, nymphs to be 21 ppm (Nauen *et al.*, 2008). Adult

Fig. 2. Number of *Caenorhabditis elegans* first-stage juveniles that developed to adults in a range of spirotetramat-enol (SPT-enol) concentrations. A significant reduction in the number of worms that developed after 4 days was observed in SPT-enol concentrations ≥ 30 ppm. Differing letters represent a statistical difference (Scheffé test, $P < 0.05$). Data were transformed ($\log(y + 1)$) prior to statistical analysis; however, non-transformed data are presented in the graphs for ease of interpretation. Data are from a single representative staged assay (Experiment 1) with *C. elegans*.

Fig. 3. Log-logistic curves modelling the relationship between spirotetramat-enol (SPT-enol) concentration and development of *Caenorhabditis elegans* first-stage juveniles to adults. Curves were generated from original data of two independent assays with staged *C. elegans*. Filled circles represent data from Experiment 1; open circles represent data from Experiment 2. The EC_{95} is estimated to be 48 ppm for Experiment 1 and 44 ppm for Experiment 2.

aphid fecundity and fertility were also more sensitive, with activity observed at SPT concentrations as low as 1 ppm. Whiteflies, *Bemisia tabaci*, were also highly sensitive, with the LD_{50} for nymphs below 1 ppm (Nauen *et al.*, 2008). Adult whitefly fecundity and fertility were also affected, with 90% reductions in fecundity observed

at 200 ppm and 60% reductions observed at 40 ppm of SPT. However, at 40 ppm, almost all whitefly failed to hatch and even at 8 ppm, 80% failed to hatch (Nauen *et al.*, 2008). Even though EC_{95} values for reduction in nematode development are not as low as those for sucking insects, they are still well below the labelled insecticidal rate of 936 ppm (87.6 g a.s. ha⁻¹) of SPT. Efficacy against nematodes infecting plant roots has been observed when the insecticidal rate was applied to plant foliage, suggesting that sufficient amounts of SPT are getting converted to the active enol form and translocated to the roots where nematodes feed (McKenry *et al.*, 2009, 2010; Bayer CropScience, pers. comm.).

SPT-enol is known (Bayer CropScience, pers. comm.) to be a lipid biosynthesis inhibitor (LBI). Nematodes have stored lipids that they use as a source of energy (Perry *et al.*, 2013). As nematodes deplete their energy reserves and are unable to restore them by synthesising new fatty acids, a lack of energy needed for development could explain the developmental arrest caused by SPT-enol. In a field setting, this lack of development would likely manifest as a gradual decrease in the population due to a reduction in reproduction rate. Effects on nematode development are similar to effects observed in insects. SPT is known to be particularly effective against juvenile stages of a variety of sucking insect pests, with symptoms on aphids including incomplete ecdysis and subsequent death (Nauen *et al.*, 2008). Since incomplete ecdysis has been observed in insects and, like insects, nematodes are also invertebrates that moult their exoskeletons during development, the

results of these and related investigations suggest that SPT-enol may inhibit moulting and prevent life stage progression in nematodes. The outermost layer of the nematode cuticle, the epicuticle, is a trilaminar layer composed of proteins and lipids. This is the first layer of the cuticle to be laid down during moulting (Decraemer & Hunt, 2013). Potentially a shortage of lipids needed to construct the new cuticle during moulting is responsible for an inhibition of nematode moulting and the observed arrest of juvenile development.

Consistent with results from *C. elegans* staged assays with SPT-enol, development to reproductive maturity was also consistently inhibited in the plant-parasitic nematode species *H. glycines* and *M. incognita* when SPT was applied to host plant foliage at the labelled insecticidal rate of Movento at either 1 or 2 weeks after root inoculation with nematodes (Figs 4, 5). Development of *H. glycines* cysts as well as production of eggs per g root were significantly reduced compared to non-treated controls when SPT was applied to soybean plants at the time of inoculation or 1 or 2 weeks after inoculation (Fig. 4A, B). Production of *M. incognita* egg masses and eggs per g root were also significantly reduced compared to non-treated controls when SPT was applied to tomato plants either 1

or 2 weeks after inoculation (Fig. 5A, B), while reductions were inconsistent across assays for other application timings.

Optimal application timings of 1 or 2 weeks after inoculation coincide with early stages of root infection when nematodes are in the juvenile stages. At the time of inoculation, most nematodes would probably still be inside the eggs, although some would hatch immediately to J2 that are able to penetrate plant roots. Juveniles of endoparasitic plant nematodes like *H. glycines* and *M. incognita* would probably be in the parasitic J2-J3 stages within roots at 1 week after inoculation. By 2 weeks after inoculation, most *H. glycines* and *M. incognita* within host roots would be in the J3-J4 stages (Davis & Tylka, 2000), although root-knot nematode feeding is paused during these stages and resumes upon completion of the fourth moult (Karssen *et al.*, 2013). Assays with *C. elegans* revealed significantly lower numbers of juveniles that fully developed to adults in SPT-enol concentrations ≥ 30 ppm, indicating an arrest of juvenile development. In the present studies with plant-parasitic nematodes, similar results were observed. When SPT was applied at times (1-2 weeks post infection) coinciding with early juvenile development, significant reductions in adult

Fig. 4. Reproduction of *Heterodera glycines* in roots of soybean plants after single foliar treatment with SPT (Movento) at different time intervals. Development of A: *H. glycines* cysts per g root; and B: Eggs per g root were maximally reduced at 4 weeks after inoculation when SPT was applied at the labelled insecticidal rate of 87.6 g a.s. ha⁻¹ to host plant foliage at 1 or 2 weeks after inoculation, although significant reductions were also observed when SPT was applied at inoculation; C: Significant reductions in eggs per cyst were only observed when SPT was applied 2 weeks after inoculation. Differing letters represent a statistical difference (Scheffé test, $P < 0.05$). Data were transformed prior to statistical analysis ($\log(y)$); however, non-transformed data are presented in the graphs for ease of interpretation. The data presented represent an average across two independent assays.

Fig. 5. Reproduction of *Meloidogyne incognita* in roots of tomato plants after single foliar treatment with SPT (MovenTo) at different time intervals. A: Production of egg masses per g root was significantly reduced at 8 weeks after inoculation when SPT was applied at the labelled insecticidal rate of 87.6 g a.s. ha⁻¹ to host plant foliage at 1 or 2 weeks after inoculation; B: Total egg numbers per g root were also significantly reduced when SPT was applied 1 or 2 weeks after inoculation, as well as when SPT was applied 3 weeks after inoculation; C: No significant reductions in eggs per egg mass were observed for any application timing of SPT. Different letters represent a statistical difference (Scheffé test, $P < 0.05$). Data were transformed prior to statistical analysis ($\log(y + 1)$); however, non-transformed data are presented in the graphs for ease of interpretation. The data presented are from one representative assay with *M. incognita* since two-way ANOVA indicated significant ($P < 0.05$) experiment by treatment interactions.

females (cysts) were observed in *H. glycines*. When SPT was applied at 3 weeks after inoculation many nematodes were likely to be developing to reproductive maturity; this was further confirmed since no significant cyst reductions were observed with SPT treatment at 3 weeks after inoculation. A slightly slower developmental rate of *M. incognita* to reproductive maturity (adult female) may explain the significant decrease in egg production with SPT treatment at 3 weeks after inoculation (although this specific observation was inconsistent among repeat experiments).

SPT applications made 1 week prior to inoculation and at the time of inoculation did not provide consistent statistically significant reductions in *H. glycines* cyst or *M. incognita* egg mass counts. Results are consistent with previous research that indicated curative application timings were more effective than preventative application timings (Bayer CropScience, pers. comm.) The EC₉₅ for SPT-enol effects on development in *C. elegans* is approximately 44–48 ppm. The lack of efficacy for preventative application timings suggests that SPT-enol concentrations in plant roots had dropped below the effective concentration by the time nematodes began to feed just a few days later,

indicating a short residual effect of less than 1 week *in planta*. SPT is known to be particularly effective against a variety of sucking insect pests, so ingesting the SPT-enol form at effective concentrations from host plants is likely to be key to activity against target nematodes and insects. Symptoms in aphids feeding from SPT-treated plants included incomplete moulting and subsequent death (Nauen *et al.*, 2008). In a field setting, repeat applications of SPT may be needed to combat a potential short residual of effective SPT-enol concentrations in plants, although correct timing of a single application as demonstrated here can provide significant reduction in nematode development to reproductive maturity.

The lack of reduction in galls per g root for *M. incognita*-infected tomatoes (Fig. 6) suggests that SPT does not prevent nematodes from invading roots, regardless of application timing. It is known that insects must ingest SPT for observable effects (Nauen *et al.*, 2008). The data presented here also suggest that nematodes must infect and feed from roots in order to be affected by SPT. Once *M. incognita* J2 invade roots, the symptoms of gall formation occur within 24–48 h (Perry *et al.*, 2013). Thus, gall formation is likely to occur before nematodes have

Fig. 6. Galls induced by *Meloidogyne incognita* in roots of tomato plants after single foliar treatment with SPT (Movento) at different time intervals. No significant reductions in root galling by *M. incognita* at 4 weeks after inoculation were observed for any application timing of SPT at the labelled insecticidal rate of 87.6 g a.s. ha⁻¹ to host plant foliage. Differing letters represent a statistical difference (Scheffé test, $P < 0.05$). Data were transformed prior to statistical analysis ($\log(y)$); however, non-transformed data is presented in the graphs for ease of interpretation. The data presented is from one representative assay with *M. incognita*.

had adequate time to feed and be exposed to SPT-enol for observed effects on development and gall formation. However, once the nematodes have been exposed to adequate levels of SPT through feeding activity, juvenile development could be inhibited and reduced development of mature females and egg production would then be observed, even on heavily galled roots. Consequently, using a galling index is not recommended for rating the efficacy of SPT applications. Experiments to expose (non-feeding) hatched J2 to SPT-enol in solution or soil application prior to root infection may be able to demonstrate whether nematode ingestion of SPT-enol is critical to its effects on subsequent nematode development.

The observed reductions in eggs per g root were possibly due to reduced total numbers of juveniles that developed to become gravid females. There was limited evidence for a reduction in fecundity with *H. glycines* when SPT was applied 2 weeks after inoculation, but no significant reduction in *H. glycines* fecundity was observed at 1 week or 3 weeks after SPT application. Numerical reductions in the number of eggs plus juveniles per cyst were also observed in *H. avenae* in wheat but reductions in fecundity were not statistically different from the non-treated controls (Smiley *et al.*, 2011). The lack of any significant reduction in eggs produced per *M. incognita* female in the study here at any SPT treatment time point suggests limited effects of SPT on nematode fecundity.

The present study showed that SPT consistently reduces *H. glycines* and *M. incognita* development to re-

productive maturity with a single application to foliage at 1-2 weeks after inoculation with nematodes. If these timings were to be translated into a field setting, SPT applications should be made early in the growing season, when the first generation of nematodes is just starting to infect and develop in roots. However, since SPT must be absorbed by plant foliage in order to be translocated to the roots where nematodes feed, applications should not be made before sufficient foliage is present to absorb the active ingredient. Further studies could be done in a field setting to balance the need for early applications targeting nematodes in juvenile stages and requirements of sufficient plant foliage to receive application. Combining use of SPT with other early season chemical or biological nematode control agents targeted at reducing the incidence and/or severity of nematode invasion of roots should also be investigated.

The labelled rate of SPT (for insect control) applied to plants in this study was equivalent to 936 ppm of active ingredient; however, assays with *C. elegans* have indicated that EC₉₅ of SPT-enol for inhibition of juvenile development is between 44 and 48 ppm, approximately one twentieth of the applied rate of SPT. It is unknown how much of the applied SPT is absorbed by plant foliage, converted into the active enol form, and translocated down to roots where nematodes feed. However, our data suggest that within 1 week after Movento application at the labelled insecticidal rate, SPT-enol levels drop below the effective concentration to inhibit nematode development in roots significantly. Further research is suggested to determine the percentage and titre of foliar-applied SPT that becomes available to nematodes in plant roots and how quickly the available SPT-enol can be metabolised by the plant. Such information would allow field performance data to be correlated with data obtained from laboratory assays. Application rates could then be adjusted accordingly to ensure adequate concentrations/residual of SPT-enol in roots (at least 44 ppm), without applying excess formulated product to plant foliage. Knowing how much SPT-enol initially reaches the roots and how long it remains effective could help inform decisions as to whether a second application is needed. Perhaps higher rates of SPT could be applied to increase the titre/residual of SPT-enol in the roots for longer-lasting effects or second applications could be made to target subsequent nematode generations. Fine-tuning application rates combined with effective application timing could allow more efficient use of SPT to reduce nematode damage to plants.

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