RESEARCH/INVESTIGACIÓN

EFFECT OF SPIROTETRAMAT (MOVENTO®) ON HATCH, PENETRATION, AND REPRODUCTION OF *ROTYLENCHULUS RENIFORMIS*

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ABSTRACT

Waisen, P., K.-H. Wang, and B. S. Sipes. 2019. Effect of spirotetramat (Movento®) on hatch, penetration, and reproduction of *Rotylenchulus reniformis*. Nematropica 49:194-199.

Spirotetramat is a lipid biosynthesis inhibitor active against hemipteran insect pests and plant-parasitic nematodes such as *Heterodera*, *Meloidogyne*, *Pratylenchu*s, and *Tylenchulus*. Reniform nematode (*Rotylenchulus reniformis*) has traits and behaviors that involve lipid biosynthesis; consequently, we wanted to know if spirotetramat could affect hatch, penetration, and reproduction of *R. reniformis*. To assess effects on hatch, *R. reniformis* eggs were exposed to Movento® solutions at 0, 50, 100, or 200 g a.i/ha and hatched juveniles counted up to 6 days later. Penetration on tomato (*Solanum lycopersicum*) roots and reproduction on pineapple (*Ananas comosus*) by *R. reniformis* as affected by spirotetramat at 0, 50, 100, or 200 g a.i/ha were also determined. Tomato and pineapple plants were treated with spirotetramat 14 or 28 days post-inoculation, respectively. Tomato roots were stained with acid fuchsin and checked for nematode penetration, or pineapple roots were shaken in sodium hypochlorite and eggs extracted. In insects, a secondary metabolite is the active moiety. In the hatching test, this secondary metabolite may not have been formed in the solution or may not have been mobile across the nematode eggshell, thus no effect was observed on nematode hatch. Penetration on tomato roots by *R. reniformis* was also not affected 14 days after spirotetramat application. However, in pineapple, reproduction of *R. reniformis* was suppressed by spirotetramat at 100 and 200 g a.i/ha compared to water control. Spirotetramat has the potential to control *R. reniformis* on crops.

Key words: Chemical control, Movento®, reniform nematode, *Rotylenchulus reniformis*, spirotetramat

RESUMEN

Waisen, P., K.-H. Wang, and B. S. Sipes. 2019. Efecto de spirotetramat (Movento®) sobre la escotilla, la penetración y la reproducción de *Rotylenchulus reniformis*. Nematropica 49:194-199.

Spirotetramates un inhibidor de la biosíntesis de lípidos activo contra plagas de insectos hemípteros y nematodos parásitos de plantas como *Heterodera*, *Meloidogyne*, *Pratylenchus* y *Tylenchulus*. El nematodo reniforme (*Rotylenchulus reniformis*) tiene rasgos y comportamientos que implican la biosíntesis de los lípidos, por lo que queríamos saber si el espirotetramato podría afectar la eclosión, la penetración y la

reproducción de *R. reniformis*. Para evaluar los efectos en la eclosión, los huevos de *R. reniformis* se expusieron a soluciones Movento® a 0, 50, 100 o 200 g a.i/ha y los juveniles eclosionados se contaron hasta 6 días después. Se determinó la penetración en las raíces del tomate (*Solanum lycopersicum*) y la reproducción en la piña (*Ananas comosus*) por *R. reniformis* según se vio afectada por el espirotetramat. Las plantas fueron tratadas 14 o 28 días después de la inoculación. Las raíces de tomate se tiñeron con fucsina ácida y se verificaron para detectar la penetración o se agitaron las raíces de piña en hipoclorito de sodio y se extrajeron los huevos. En insectos, un metabolito secundario es el resto activo que puede no haberse formado en la solución o en el móvil a través de la cáscara del huevo del nematodo, por lo que no se observó ningún efecto en la eclosión del nematodo. La penetración en las raíces de tomate por *R. reniformis* no se vio afectada 14 días después de la aplicación de spirotetramat. La reproducción se suprimió a 100 y 200 g a.i / ha en comparación con el tratamiento de control de agua. Spirotetramat tiene potencial para controlar *R. reniformis* en cultivos.

Palabras clave: Control químico, Movento®, nematodo reniforme, *Rotylenchulus reniformis*, spirotetramat

INTRODUCTION

Spirotetramat, commercially labeled as Movento® from Bayer Crop Science, is a group 23 chemical insecticide active against hemipteran insect pests and plant-parasitic nematodes such as *Heterodera*, *Meloidogyne*, *Pratylenchu*s, *Rotylenchulus,* and *Tylenchulus* (McKenry *et al*., 2009, 2010; Smiley *et al*., 2011; Sipes, 2014; Waisen and Sipes, 2014). Spirotetramat acts as a lipid biosynthesis inhibitor after ingestion by the pest (Nauen *et al*., 2006). The parent compound (spirotetramat) undergoes hydrolysis of side-chain ester bond to form a secondary metabolite or spirotetramat-enol (Australian Pesticides and Veterinary Medicines Authority, 2009). The spirotetramat-enol forms a weak acid in xylem and phloem sap and is translocated bidirectionally throughout the entire plant (Nauen *et al*., 2008; Safferling, 2008; Vermeer and Baur, 2008).

Reniform nematode (*Rotylenchulus reniformis*) shares traits and behaviors with other plant-parasitic nematodes and hemipteran insect pests. These shared traits include a sedentary intimate association with the host, as well as physiological processes such as embryogenesis and molting. Several of these physiological processes occur in *R. reniformis* (Krusberg, 1967; Sekora *et al*., 2009). Reniform nematode undergoes embryogenesis in an egg, hatches, undergoes several molts to reach an infective fourth-stage juvenile, and only the female penetrates the host root (Robinson *et al*., 1997). These physiological processes source energy from lipid metabolism, thus spirotetramat could interfere with the processes. The objective of this study was to determine the effect of spirotetramat on the hatch, penetration, and reproduction of *R. reniformis*.

MATERIALS AND METHODS

Nematode inoculum

Rotylenchulus reniformis was sourced from cultures maintained on cowpea (*Vigna unguiculata*) in a greenhouse at the University of Hawaii, Honolulu, HI. Nematode eggs were extracted from the roots by shaking them in 0.6% sodium hypochlorite (NaOCl; Hussey and Barker, 1973) followed by a density-dependent centrifugal sugar flotation method (Jenkins, 1964; Barker, 1985) to separate eggs from soil and debris. The collected supernatant was used to prepare a final concentration of 100 nematode eggs per ml of water for inoculation.

Hatching tests

To determine the effect of spirotetramat on egg hatch, an *in vitro* test was conducted. Spirotetramat treatment rates were 50, 100, and 200 g a.i/ha. A water control was included. The experiment was arranged in a completely randomized design (CRD) with 7 replications. To prepare respective treatment solutions, Movento® (240 g a.i/liter, Bayer Crop Science, Research Triangle Park, NC) was dispensed at 0.012, 0.024, or 0.048 µl into each 1-ml or 27-mm-diam. (8 mm high) watch glass (U.S Bureau of Plant Industry model) and made up to 900 µl with a tap water. By

pipetting up and down, the solutions were thoroughly mixed before addition of eggs. Then, the nematode suspension was pipetted 20 eggs/100 µl into each watch glass for a final volume of 1 ml. The experiment was incubated at room temperature in the dark, and number of hatched juveniles was recorded 2, 4, and 6 days after initiation of the test. Movento® solution was replaced at each assessment time. This test was conducted three times.

Penetration test

A greenhouse experiment was conducted to determine the effect of spirotetramat on tomato root penetration by *R. reniformis*. Spirotetramat treatment rates included 50, 100, and 200 g/ha, along with a water control. Each treatment was replicated six times with experimental units arranged in CRD, and the test was repeated twice. A field soil was collected, brought back to the laboratory, and autoclaved at 121°C and 103.4 × 10^3 Pa for 30 min. before use. A sterile Lapis Lustre® sand (CEMEX Lapis, Marina, CA) was mixed with the soil at a 1:1 (v:v) ratio. The sand-soil mix was added to twenty-four 1 L or 15-cm-diam. biodegradable pots (The HC Companies, Middlefield, OH). Tomato 'Patio F1 Hybrid' seeds were germinated in vermiculite (Greenhouse Megastore, Danville, IL), and 4-wk-old seedlings were transplanted individually per pot. Plants were watered daily and fertilized once with Miracle-Gro® (The Scotts Miracle-Gro Co., Marysville, OH) at 6 g/L of water. Seven days after transplanting, 1,000 eggs of *R. reniformis* were inoculated per plant at three equidistant locations around the base of each plant. After 14 days of inoculation, Movento® was mixed with Methylated Seed Oil® (MSO) surfactant (Loveland Products, Loveland, CO) at 0.25% (v:v) and applied at 50, 100, or 200 g a.i/ha delivered in 0.37, 0.74, or 1.47 µl per pot, respectively. The plants were foliar sprayed to runoff using a hand-held Delta Plant Care Pressure Sprayer® (Delta Industries, Allentown, PA). Irrigation was delayed for 3 days following spirotetramat application. The experiment was terminated 14 days post-treatment or 28 days post-inoculation. At the time of termination, tomato roots were gently removed from the pots, shaken to remove soil and debris, and a 1 g root subsample was randomly collected from each root system for assay. The root subsamples were stained following the NaOCl-acid fuchsin-glycerin method (Byrd *et al*., 1983) and number of nematodes penetrating recorded.

Reproduction test

A greenhouse experiment was conducted to determine the effect of spirotetramat on reproduction of *R. reniformis* on pineapple (*Ananas comosus*). Spirotetramat treatments included 50, 100, and 200 g/ha with a water control. Pineapple 'MG3' crowns were cured by sun drying for 7 days and planted singly in 4-L 20-cm-diam. biodegradable pots filled with the sterile sand-soil mix (1:1; v:v) prepared in the same way as described in penetration test. Four months later, the pineapple plants were inoculated with 4,000 eggs of *R. reniformis*. Movento® was mixed with MSO surfactant at 0.25% (v:v) in an application volume to 2 L. One month after inoculation, spirotetramat was foliar sprayed to runoff by delivering 0.66, 1.31, or 2.62 µl of Movento® per pot. Irrigation was delayed for 9 days after spirotetramat application. The experiment was terminated 3 months post-treatment or 4 months post-inoculation. At the time of termination, the potted pineapple plants were emptied into sampling bags, roots separated from soil, and a subsample of 250 cm^3 rhizosphere soil collected from each pot. Roots were shaken in 0.6% NaOCl (Hussey and Barker, 1973) using a Wrist Action® Shaker (Burrell Scientific LLC, Pittsburgh, PA) followed by density-dependent centrifugal sugar flotation method and nematode eggs extracted (Jenkins, 1964; Barker, 1985). Soil population of *R. reniformis* was also extracted from the 250 cm^3 soil subsample by elutriation (Byrd *et al*., 1972) followed by centrifugation. This trial was repeated once.

Statistical analysis

The number of *R. reniformis* hatched juveniles, nematodes penetrating tomato roots, root weights, eggs extracted from pineapple roots, and nematodes recovered from rhizosphere soil were checked for normality in Proc Univariate test in SAS version 9.4 (SAS Institute Inc., Cary, NC). The data were transformed by $\log_{10}$ (x+1) when necessary. All the data were subjected to one-way analysis of variance in SAS. When treatment was significant ($P \leq 0.05$), means were separated using

Waller-Duncan k-ratio (k=100) t-test, and only true means were presented.

RESULTS

Hatching tests

Spirotetramat did not suppress the hatch of *R. reniformis* eggs in any of the three tests conducted (Table 1). The number of hatched juveniles was comparable across all the spirotetramat treatments including the water control.

Penetration tests

Tomato root penetrations by *R. reniformis,* as affected by spirotetramat, were not statistically significant compared to the water control treatment (Table 1). Although tomato root growth tended to numerically decrease with increasing rates of spirotetramat, root biomasses were not significantly different among the treatments (Table 1). In fact, higher root penetrations by *R. reniformis* per gram of root or per root system were supported at higher spirotetramat rates, which could be indicative of phytotoxicity (Table 1).

Reproduction test

Spirotetramat suppressed reproduction of *R. reniformis* (eggs per gram of root) at 100 and 200 g a.i/ha on pineapple ($P \leq 0.05$). Spirotetramat also reduced the rhizosphere soil population of the nematode at 200 g a.i/ha and significantly increased corresponding root and shoot biomasses compared to water control (Table 2) ($P \leq 0.05$).

DISCUSSION

Spirotetramat is a systemic pesticide that targets inhibition of lipid biosynthesis in agricultural pests like plant-parasitic nematodes. Nematode fecundity and fertility can be altered using this treatment because it interferes with lipid biosynthesis. In *in vitro* tests, spirotetramat did not suppress *R. reniformis* hatch in aqueous solutions. When nematode eggs were exposed to the aqueous solutions of Movento®, the active secondary metabolite or spirotetramat-enol may not have been formed in the solution or the chemical may not have been mobile across the nematode eggshell. Therefore, spirotetramat may have been unable to interfere with lipid biosynthesis during embryogenesis or to prevent hatch. Our results support the fact that spirotetramat has to be hydrolyzed to secondary metabolite and the metabolite has to be ingested by the pest for any impact to occur (Nauen *et al*., 2008). Smiley *et al*. (2011) reported that spirotetramat was more effective on cereal cyst nematode (*Heterodera avenae*) before wheat (*Triticum* spp.) plants exhibited white females but not after the females were present. This means the females that were not exhibited on the plant were still feeding and, thus, spirotetramat had effect on them as opposed to those that were exhibited and non-feeding. Similarly, Fitzpatrick (2013) reported that chemigation with Movento® killed feeding instars of cranberry tipworm (*Dasineura oxycoccana*) but not the non-feeding pre-pupae or pupae of the pest.

Spirotetramat did not suppress root penetration by *R. reniformis*. In sucking insect pests, spirotetramat requires 2-10 days post-

Table 1. Hatch and penetration of *Rotylenchulus reniformis* and tomato root weight as affected by different rates of spirotetramat.

	Hatch (%)[w]			Penetration[x]		
Spirotetramat[y]	Test 1	Test 2	Test 3	Root	Root system	Dry root weight (g)[z]
0	100	47	67	1	7	13
50	83	40	60	0	0	12
100	100	53	65	3	30	11
200	87	57	73	2	18	9

[w]Values within a column are not different based on Waller-Duncan k-ratio (k=100) t-test.
[x]Values within a column are not different based on Waller-Duncan k-ratio (k=100) t-test.
[y]Rates in grams per hectare.
[z]Values within the column are not different based on Waller-Duncan k-ratio (k=100) t-test.

Table 2. Effect of spirotetramat on *Rotylenchulus reniformis* on pineapple.

	Nematode population[x]		Plant weight[y]	
Spirotetramat[z] (g)	Soil (#/250 cm^3)	Root (#/g)	Root (g)	Shoot (g)
0	171 A	41 a	8.8 B	501.5 b
50	85 AB	21 a	10.7 AB	608.2 ab
100	139 AB	3 a	11.7 AB	544.5 ab
200	165 B	2 b	12.6 A	673.3 a

[x]Values with the same letter within a column are not different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.
[y]Dry root and fresh shoot weights of pineapple.
[z]Rates of spirotetramat in grams per hectare.

treatment to kill the larvae when the vascular system of a plant is actively translocating the secondary metabolite of spirotetramat and an insect pest is actively feeding (Nauen *et al*., 2008). In this experiment, 14 days after treatment, no effect was observed on root penetration by *R. reniformis*. This suggests that *R. reniformis* were in the first generation and, therefore, had no prior exposure to spirotetramat-enol, thus no effect on penetration was observed. Similarly, embryogenesis in the egg to first-stage juvenile and subsequent molts to fourth-stage juvenile were not affected because they were non-feeding life stages. Therefore, tomato root penetration by *R. reniformis* was not suppressed among the spirotetramat treatments compared to water control.

Spirotetramat suppressed *R. reniformis* reproduction significantly on pineapple. One month after inoculation was sufficient for nematodes to penetrate the roots and a 3-month period after spirotetramat application was enough time for the spirotetramat-enol to be translocated throughout the plant parts including the roots. Due to the length of this study, the nematode underwent several generations exposing the nematode to the spirotetramat-enol; thus, spirotetramat-enol interfered with subsequent reproduction through interference of embryogenesis or molting processes. In previous studies, spirotetramat was promising to reduce population of *R. reniformis* on pineapple (Sipes, 2014; Waisen and Sipes, 2014). This study further supports the finding that spirotetramat has a potential to manage *R. reniformis* on crops.

Because spirotetramat had no effect on the hatch of *R. reniformis* nor any effect on the penetration of tomato by *R. reniformis*, the reduction of *R. reniformis* on pineapple must occur after infection of the host. Spirotetramat is not a contact nematicide but requires metabolization in the plant for the active moiety. Even with the requirement of metabolization, spirotetramat has the potential to manage *R. reniformis* on crops.

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