

**EFFECTS OF BIOLOGICALLY-DERIVED PRODUCTS ON MOBILITY  
AND REPRODUCTION OF THE ROOT-LESION NEMATODE,  
*PRATYLENCHUS PENETRANS*, ON STRAWBERRY**

J. N. Pinkerton\* and M. L.C. Kitner

USDA-ARS, Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330 United States. \*Corresponding author: pinkertj@science.oregonstate.edu

---

**ABSTRACT**

Pinkerton, J. N., and M. L. C. Kitner. 2006. Effects of biologically-derived products on mobility and reproduction of the root-lesion nematode, *Pratylenchus penetrans*, on strawberry. *Nematropica* 36:181-196.

Seven biological products reported to have nematicidal activity were evaluated for suppression of *Pratylenchus penetrans* mobility *in vitro* and reproduction on 'Totem' strawberry plants. Mobility of nematodes was evaluated *in vitro* by exposing nematodes to a range of concentrations of products in aqueous solutions for 24, 48, and 72 h, followed by rinsing and incubating the nematodes for 24 h in deionized water. Nematodes exposed to deionized water or the organophosphate nematicide fenamiphos served as controls. During 72 h exposure in 70 µl/L a.i. solution of fenamiphos, >75% of nematodes were immobilized and 58% did not recover mobility in subsequent water incubation. Ninety percent of nematodes were immobilized during exposure to 1380 mg/L DiTera for 72 h, and 35% remained immobile after subsequent 24 h incubation in water. NatureCur, SLS-CA (Sodium lauryl sulfate-citric acid), and formulations of 3.5% thymol (Dominador and Promax) immobilized 45 to 70% of the nematodes, but mobility was restored by subsequent incubation in water. Sincocin, castor oil, and LCF (liquid compost factor) showed no activity. In greenhouse experiments, diluted products were applied as soil drenches at two or four week intervals, starting when strawberries were planted in soil infested with 1 *P. penetrans*/g soil, or four weeks after planting. Plant growth and nematode population densities were evaluated 16 and 20 weeks after planting in separate experiments. Applications that started at planting were more effective than those started four weeks after planting once nematodes had entered the roots. DiTera at 1380 mg/L and SLS-CA at 2857 µl/L applied at planting provided significant nematode suppression. However, SLS-CA stunted plant growth. No biological product was as effective as fenamiphos. Data suggest that multiple applications synchronized with host and nematode biology will be necessary to maintain efficacious concentrations of these biological products in agricultural soils.

*Keywords:* fenamiphos, nematicide, phytochemicals, plant-parasitic nematodes, organophosphate.

---

**RESUMEN**

Pinkerton, J. N., y M. L. C. Kitner. 2006. Efectos de productos biológicos sobre la movilidad y reproducción del nematodo lesionador, *Pratylenchus penetrans*, en fresa. *Nematropica* 36:181-196.

Se evaluó el efecto de siete productos biológicos en la movilidad *in vitro* y la reproducción en plantas de fresa 'Totem' de *Pratylenchus penetrans*. La movilidad se evaluó *in vitro* exponiendo los nematodos a un rango de concentraciones de los productos en solución acuosa durante 24, 48, y 72 h, seguido de lavado e incubación durante 24 h en agua desionizada. Los controles fueron agua desionizada y el nematicida organofosforado fenamifos. Durante 72 h de exposición en solución de 70 µl/L i.a. de fenamifos, >75% de los nematodos fueron inmovilizados y 58% no recuperaron la movilidad durante la subsiguiente incubación en agua. Se observó inmovilización de 90% de los nematodos expuestos a 1380 mg/L DiTera durante 72 h, y el 35% permaneció inmóvil después de la incubación en agua durante 24 h. NatureCur, SLS-CA (Sodium lauryl sulfate-citric acid), y las formulaciones de timol 3.5% (Dominador y Promax) inmovilizaron 45 a 70% de los nematodos, pero la movilidad se

recuperó con la incubación en agua. No se observó ninguna actividad con Sincocin, aceite de ricino, y LCF (liquid compost factor). En los experimentos de invernadero, se aplicaron diluciones de los productos al suelo en intervalos de dos o cuatro semanas, empezando al momento de la siembra en suelo infestado con 1 *P. penetrans*/g soil, o cuatro semanas después de la siembra. Se evaluó el crecimiento de la planta y la densidad de población del nematodo a las 16 y 20 semanas después de la siembra en experimentos separados. Las aplicaciones iniciadas al momento de la siembra fueron más efectivas que las iniciadas cuatro semanas después de la siembra una vez los nematodos ya habían penetrado las raíces. DiTera a 1380 mg/L y SLS-CA a 2857 µl/L aplicados en el momento de la siembra suministraron supresión significativa de nematodos a. Sin embargo, SLS-CA afectó el crecimiento de la planta. Ningún producto biológico fue tan efectivo como fenamifos. Los resultados sugieren que se requieren múltiples aplicaciones sincronizadas con la biología del hospedante y del nematodo para lograr mantener concentraciones eficaces de estos productos biológicos en suelos agrícolas.

*Palabras Clave:* fenamifos, fitoquímicos, nematicida, nematodos fitoparásitos, organofosfato.

---

## INTRODUCTION

The root-lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Shuurmans Stekhoven, is an economically important pest of agronomic and horticultural crops, including strawberry and raspberry (Brown *et al.*, 1993; Esnard and Zuckerman, 1998). This migratory endoparasite moves between the soil and the root cortex, where it feeds. Nematode activity kills the surrounding root tissue, and becomes visible as discrete necrotic lesions. When high *P. penetrans* population densities are present, lesions may coalesce and girdle the roots. Above-ground symptoms of infected plants are stunting, reduced runner or cane production, depressed fruit yields, and shortened life of plantings. In addition, *P. penetrans* has been implicated in disease complexes affecting strawberry and raspberry (Abu-Gharbieh *et al.*, 1962; Kurppa and Vrain, 1989; LaMondia, 1999; Szczygiel and Profic-Alwasiak, 1989; Vrain and Coopeman, 1987).

*Pratylenchus penetrans* is a major pest in red raspberry production in the Pacific Northwest, a region that accounts for greater than 95% of raspberry production in North America (NASS, 2006). McElroy (1992) developed a nematode manage-

ment program for raspberry production in this region. He recommended soil fumigation prior to planting raspberry in nematode infested fields, but *P. penetrans* densities can increase to damaging levels several years after planting in fumigated soil. Fenamiphos (Nemacur), an organophosphate nematicide/insecticide, is the only pesticide registered for managing nematodes in established plantings of raspberry and strawberry. Fenamiphos will not be manufactured after 2007, leaving the small fruit industries no options to manage nematode damage in established plantings. It is unlikely that organophosphate and carbamate nematicides will be developed or registered in the near future for use on minor crops, such as small fruits.

Phytochemicals have roles in management of plant-parasitic nematodes as components of host plant resistance, allelopathic cover crops, green manures, or nematicidal plant extracts (Chitwood, 2002). These chemicals may act directly as nematicides, by modifying nematode egg hatch, or indirectly by modifying plant resistance or populations of soil microflora that are antagonistic to plant-parasitic nematodes (Birch, 1993). Reviews of research on nematode-antagonistic phytochemicals noted that plants have a broad

spectrum of compounds with nematicidal activity (Akhtar and Mahmood, 1994; Chitwood, 1993 and 2002; Birch, 1993). Over 90% of the aqueous extracts from 153 Chinese herbal remedies representing 71 plant families were nematicidal or nematostatic to *Pratylenchus vulnus* or *Meloidogyne javanica* (Ferris and Zheng, 1999). These results and those compiled by Chitwood (2002) suggest that nematicidal phytochemicals extracted from plant tissue are common and may have potential as commercial nematicides. In addition, the registration of natural product-based pesticides generally requires less data and cost to register than synthetic chemical pesticides.

Nematicides derived from plants have several disadvantages. The composition and concentration of active phytochemical can vary with the plant source and the extraction process. Active compounds in biologically-derived products are rarely identified, including the products that we evaluated. Activity may not be broad-spectrum, so response of nematode genera to a phytochemical may differ (Chitwood, 2002; Ferris and Zheng, 1999). Nematicidal activity observed *in vitro* or in pot trials often require concentrations that may be cost-prohibitive for agricultural applications. The manufacturing processes must guarantee product formulations with consistent and stable active fractions, and the product must be economical for commercial agriculture. Claims of efficacy made and modes of action proposed by the manufactures often are not substantiated by experimental data. In spite of these limitations, biologically-derived compounds that have demonstrated utility for managing plant-parasitic nematodes are entering the marketplace.

Our research focused on biologically-derived products that are near or in commercial production. The objectives of our research were 1) to evaluate seven products, each at a range of concentrations, for

nematicidal or nematostatic activity on *P. penetrans in vitro*, 2) to use a strawberry-*P. penetrans* model to evaluate at planting prophylactic (AP) and a post-planting therapeutic (PP) applications on nematode population development and plant growth, and 3) to identify products as potential replacements for fenamiphos in red raspberry and strawberry production.

## MATERIALS AND METHODS

### *Biological Nematicides*

The biological products evaluated have active ingredients that are derived from plant extracts or fungal fermentation. These products were selected because they have been reported to have nematicidal/nematostatic activity or to ameliorate plant damage caused by plant-parasitic nematodes. The products evaluated and their plant source tissues, extraction methods and active components are listed in Table 1.

The biological compounds were each evaluated at two concentrations ranges; 1) a range of concentrations to determine dose response of nematodes and; 2) concentrations calculated based on the amount of product recommended by the manufacturer per hectare, assuming delivery with enough water to incorporate the product in the root zone. The later was based on standard practices that strawberry and raspberry growers use to apply fenamiphos in the Pacific Northwest. These calculations assume the product is applied as a 1.5 m band broadcast on the soil surface in the plant row, and incorporated into the soil with 6 mm of water, or applied in chemigation.

### *Nematode Preparation*

The *P. penetrans* population used in this research was collected from a peppermint (*Mentha piperita*) field in Benton County,

Table 1. Descriptions of the biologically-derived products evaluated for efficacy to manage *Pratylenchus penetrans*. Most products contain proprietary surfactants and adjuvants.

Product <sup>w</sup>	Source	Tissue	Extraction method	Active compounds
Castor oil	<i>Ricinus communis</i>	Seeds	Heat and pressure	Unknown
DiTera	<i>Myrothecium verrucaria</i>	Hyphae in fermentation	90% by weight heat treated solid and solutes	Unknown
Promax Dominator <sup>x</sup>	<i>Thymus vulgaris</i>	Fresh and partially dried leaves and flowers	Steam distillation	3.5% Thymol
LCF	<i>Ananas comosus</i> , <i>Carica papaya</i> , <i>Saccharum officinarum</i>	Pineapple and papaya fruit purees and molasses	Compost tea: Filtered liquid extract of fungal digestion	Unknown
NatureCur	<i>Juglans regia</i>	Ground wood	Proprietary	Unknown
Sincocin <sup>y</sup>	<i>Quercus falcata</i> , <i>Opuntia lindheimeri</i> , <i>Rhus aromatica</i> , <i>Rhizophora mangle</i>	Roots and bark	Tissues dried and ground. Extraction with alcohol/water solution	Unknown
SLS-CA <sup>z</sup>	<i>Cocos nucifera</i>	Coconut oil. Citric acid from fungal fermentation	Active ingredients dissolve in water	Sodium lauryl sulfate, citric acid

<sup>w</sup>Manufacturers: DiTera, Valent BioSciences Corporation, Libertyville, IL; Dominator and Promax, BioHumanetics, Inc., Chandler, AZ; NatureCur, Redox Chemical, Inc., Burley, CA; LCF, SLS-CA, and nematocidal castor oil, ABR LLC, Puunene, HI; Sincocin, Agriculture Sciences, Inc., Dallas, TX.

<sup>x</sup>Promax and Dominator are identical, except Dominator contains 5% alpha-amylase.

<sup>y</sup>Mixed with equal concentration of Titan, a proprietary soil wetting agent. A 0.5% solution of Nutri-Phite Sulfone, a 5-20-15 fertilizer, was applied as foliar spray with each application. Applied with Zap, 8-0-0 fertilizer with chelated micronutrients, in the second greenhouse trial.

<sup>z</sup>Inert ingredients are safflower oil, sugarcane molasses, and non-pasteurized blue cheese.

Oregon and was maintained in greenhouse pot cultures on peppermint. This population reproduced well on strawberry in previous research (Pinkerton and Finn, 2005) and on raspberry (Pinkerton, unpublished data).

Roots and rhizomes of peppermint plants were washed free of soil, cut into 4-8 cm sections, and placed in a funnel-test tube apparatus under intermittent mist (Ayoub, 1981). For the *in vitro* toxicity trials, nematodes were collected from tubes after 24 hours of misting. The suspension of nematodes was poured onto to a 43 µm sieve which was submerged in deionized water. Only active nematodes that moved

through the sieve within 4 hours were used in the trial. These nematodes were used immediately. Nematodes used in the greenhouse trials were extracted from roots for up to 4 days under intermittent mist. Nematodes were collected daily and stored in water at 4°C until adequate numbers were collected for the trial.

#### *In Vitro Toxicity Trials*

A suspension of active *P. penetrans* was adjusted to approximately 40 nematodes/ml by adding deionized water. Five ml of the nematode suspension was pipetted into 60 × 15 mm polystyrene Petri dishes, yielding 200 *P. penetrans* per dish. Stock solu-

tions of each product were prepared such that the desired final concentrations (Figs. 1 and 2) were achieved by combining 5 ml of nematode suspension and 10 ml of a stock solution in each dish. Products in liquid formulations were diluted with deionized water v/v, while the dry formulation of DiTera (Valent BioSciences Corporation, Libertyville, IL) was diluted w/v. Stock solutions were pipetted directly into the nematode suspension. Because the particu-

lates of DiTera and SLS-CA obscured the nematodes in the first experiment, in the second experiment stock solutions of these products were pipetted onto sieves (38 mm dia with 28  $\mu$ m mesh fabric) that were raised slightly off the bottom of the Petri plates. This system allowed soluble active ingredients to contact the nematodes, but trapped the particulates on the submerged sieves. Sieves were removed during evaluations of nematode movement. Petri dishes

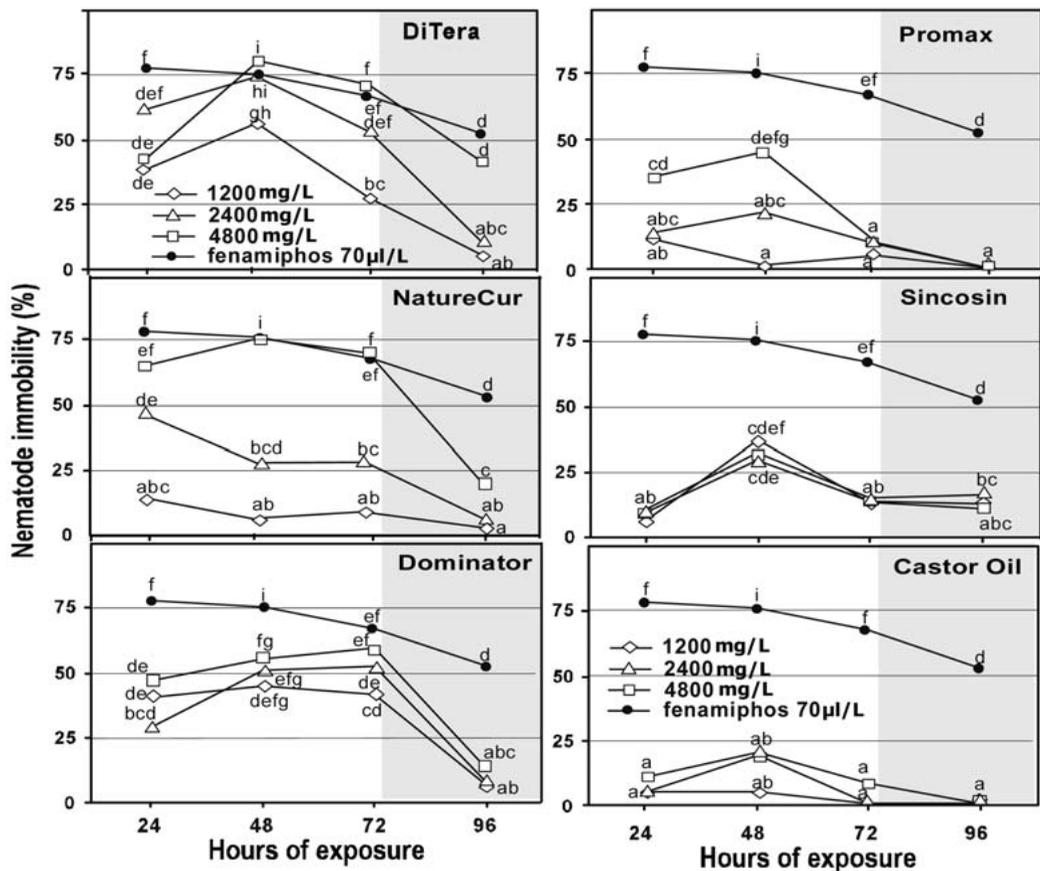


Fig. 1. Effect of biologically-derived products and fenamiphos on the mobility of *Pratylenchus penetrans* *in vitro*. Movement of nematodes was observed after 24, 48, and 72 hours exposure in solutions of each compound, followed by a rinse in deionized water. Responses of nematodes were observed after 24 hours incubation in water (gray area). Concentrations were mg/L for DiTera and µl/L for other products. Data were standardized by subtracting the percentage of immobile nematodes in the water control treatments at each time interval. For each product, percent immobility values at each time interval that differ ( $P < 0.05$ ) according to Fisher's Protected LSD are indicated by different letters above the symbols.

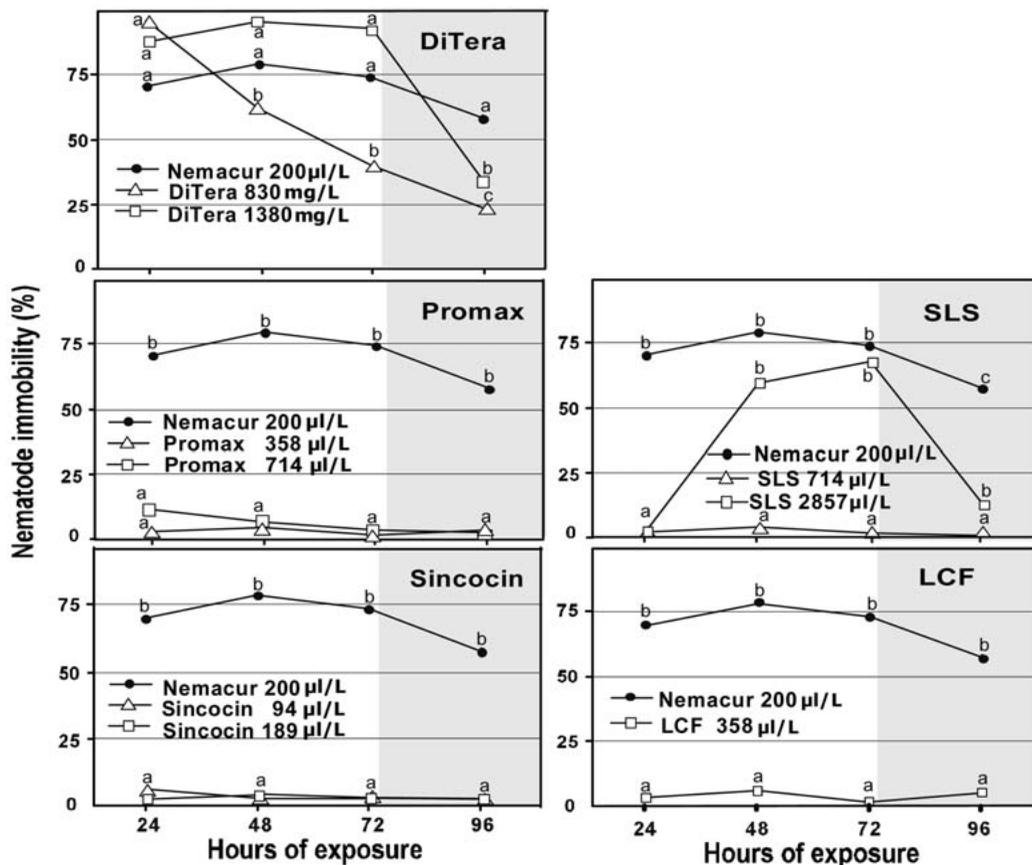


Fig. 2. Effect of field application concentrations of biologically-derived products and fenamiphos on the mobility of *Pratylenchus penetrans* *in vitro*. Movement of nematodes was observed after 24, 48, and 72 hours exposure in solutions of each compound followed by a rinse in deionized water. Responses of nematodes were observed after 24 hours incubation in water (gray area). Data were standardized by subtracting the percentage of immobile nematodes in the water control treatments at each time interval. For each product, percent immobility values at each time interval that differ ( $P < 0.05$ ) according to Fisher's Protected LSD are indicated by different letters above the symbols.

were covered between nematode observation intervals to prevent evaporation of the solutions. Plates were incubated in the dark at 20°C. In each experiment, four replicates were evaluated for each treatment and the trial was conducted twice.

In the first experiment, all products were evaluated at 1,200, 2,400, and 4,800 µl/L or mg/L to ascertain the dose response of the nematode. In the second experiment, the concentrations evaluated

were based on the amount of product per hectare recommended by the manufacturers and the amount of water required to deliver the product into the root zone, as discussed previously. Control plates containing deionized water or an aqueous solution of 70 µl a.i./L fenamiphos (200 µl/L of formulated Nematicur) were included in each trial.

For each evaluation, Petri plates were agitated to disperse the nematodes and

placed on a gridded plastic counting sheet. Nematodes in 50% of each plate were observed at 30× with a dissecting microscope. Nematodes that were moving actively at each time interval were recorded as mobile. Nematodes that were not moving were touched gently with a dental pulp file and their responses were recorded. Those that moved in response to touch also were considered mobile. Vigor of movement in response to touch was observed, but not quantified. Nematode mobility was evaluated after 24, 48, and 72 h of exposure to the compounds. After 72 h, the contents of each Petri plate were poured into a submerged 28 µm sieve and rinsed for 1 minute under a gentle stream of deionized water to remove the compounds. The contents of the sieve were rinsed into 50 ml centrifuge tubes, and centrifuged for 3 minutes at 400 G. Contents of the tubes were drawn down to ca. 15 ml and placed into clean Petri plates and mobility of the nematodes was evaluated.

#### *Greenhouse Trials*

Strawberry was chosen as a model system because of its compact size and susceptibility to *P. penetrans* (Pinkerton and Finn, 2005), which made it more amenable to greenhouse experiments than red raspberry. Tissue culture strawberry plants, cultivar 'Totem' were obtained from Sakuma Bros. Burlington, WA. Plants were grown in 10 cm pots in soil-less media for 1 month prior to the trials. At the start of the experiments, plant roots were washed free of potting media and roots were trimmed to 15 cm. Plants were sorted by size and those of similar size were selected for each replicate. A suspension of *P. penetrans* was adjusted to 200 nematodes/ml, and 20 ml of the suspension was mixed thoroughly into 2 liters of steam-pasteurized loam soil mixed 2:1 (v/v) with washed sand. One gallon pots were filled with 1.5 liters of

non-infested pasteurized soil mix, and strawberry plants were planted while filling the pot with nematode infested soil. Population density of *P. penetrans* was 1/g soil (4000 per pot).

Two greenhouse experiments were conducted. In the first experiment, the concentrations evaluated were those suggested initially by the manufacturer, while in the second trial concentrations were reduced to reflect those delivered in field applications at labeled rates (Tables 2 and 3). Both experiments evaluated two treatment series, one with the first application at the time of planting (AP), and the other with the first application 4 weeks after planting (PP). The first experiment was planted on 8 October 2003 and applications were made at planting (AP) and again at 4, 8 and 12 weeks, or (PP) at 4, 8, and 12 weeks after planting, except for DiTera, which was applied bi-weekly, as recommended by the manufacturer. Nematode population densities and plant biomass data were collected 16 weeks after planting. The second experiment was initiated on 10 August 2004. Applications were made AP and 4, 8, 12, and 16 weeks later, or PP at 4, 8, 12, and 16 weeks after planting. DiTera was applied biweekly in both series. Nematode and plant data were collected 20 weeks after planting.

Biological products were added to a 500 mg/L fertilizer solution (Peters Professional M-77, 20-10-20, Scott-Sierra Horticultural Products, Marysville, OH) and were applied as soil drenches with enough solution to fully saturate the soil without draining from the pot. Soil was allowed to dry for 3 to 5 days before each subsequent application. Then 250-300 ml of a solution was slowly added to soil surface of the pots. The amount of solution applied was determined at each application interval by recording the amount of fertilizer solution required to saturate soil in the control pots.

Sincocin was mixed with a proprietary soil wetting agent (Titan) and received a foliar fertilizer spray of 0.5% solution of Nutri-Phite Sulfone, a 5-20-15 fertilizer. SLS-CA was applied with an 8-0-0 fertilizer with chelated micronutrients (ZAP) in the second greenhouse experiment.

Plants were arranged on greenhouse benches in a randomized block design with six and eight replicates in the first and second experiments, respectively. The greenhouse was maintained at 18 to 22°C with 14 h of supplementary light (400  $\mu\text{mol}/\text{m}^2/\text{s}$ ) per day. Plants were fertilized bi-weekly. Between fertilizer applications, plants were sub-irrigated to restrict leaching of the biological compounds from the soil. Runners and flowers were removed from plants throughout the study.

Nematode and plant data were collected four weeks after the final treatments were applied. For each pot, the root system was removed and soil carefully shaken from the roots. The soil was screened through a 1 cm screen, mixed, and weighed. A 100 g sample of soil from each pot was processed in a Baermann funnel apparatus for 7 days to extract nematodes (Ayoub, 1981). Root systems were washed free of soil and placed in a mist chamber to extract nematodes (Ayoub, 1981). Nematodes were collected and counted after 7 and 14 days. Crowns and roots were oven-dried for 24 h at 70°C and then weighed.

#### *Statistical Analyses*

In the *in vitro* studies, the percentage of immobile nematodes in each treatment was standardized by subtracting the percentage of immobile nematodes in the deionized water control treatments at each time interval. Based on ANOVA of separate trials as blocks, homologous treatments from the two trials of each experiment were not different ( $P < 0.05$ ),

thus data from the trials were combined for further analysis. Percentage immobile nematode data were arcsine-transformed prior to analysis. Model variance components were estimated for *in vitro* and greenhouse studies using ANOVA procedures and means were separated by Fisher's protected LSD (Statgraphics Plus version 3, Manugistics, Inc., Rockville, MD, USA).

## RESULTS

### *In Vitro Toxicity Study*

In both experiments, movement of the control nematodes incubated in deionized water declined slightly over the 96 h trials. Mean percentage of immobile nematodes in controls was 2% after 24 h and reached 5.8% after 72 and 96 h. The percentage of immobile nematodes was adjusted by these values for all other treatments. Similarly, nematode movement in fenamiphos solutions was consistent within all trials. All nematodes exposed to fenamiphos were quiescent, with straight bodies at each observation period. Some nematodes responded to touch with slow, often single, movement. After being touched, between 70 and 80% of nematodes were judged immobile after 24, 48, and 72 h in fenamiphos solution. Following rinsing and incubation in water for 24 h, 50-60% of nematodes remained immobile. In contrast to nematodes exposed to fenamiphos or DiTera, nematodes exposed to other compounds responded to touch with active serpentine motion.

In the first experiment, dose responses were observed for several products (Fig. 1). Nematode immobility was similar among DiTera at 2400 and 4800 mg/L and 70  $\mu\text{l}/\text{L}$  fenamiphos during the first 48 h of exposure. As with fenamiphos, greater than 90% of the nematodes exposed to the 2 higher concentrations of DiTera were

quiescent and required touch to elicit movement. The effects of DiTera at 2400 mg/L on nematode movement declined after 48 h exposure. Nematode mobility was reduced, but to a lesser degree by exposure to 1200 mg/L DiTera. Less than 10% of nematodes exposed to 1,200 and 2,400 mg/L DiTera were immobile after rinsing and incubating nematodes in water, while 35% exposed to 4,800 mg/L remained immobile. The particulate fraction of DiTera in the dishes made it difficult to see quiescent nematodes, such that they may have been underestimated.

There was a strong concentration effect of NatureCur on nematode movement (Fig. 1). Mobility of nematodes exposed to 4,800  $\mu\text{L}$  of NatureCur was not different ( $P < 0.05$ ) from those exposed to fenamiphos at 48 and 72 h. Nearly 50% of nematodes exposed to 2400  $\mu\text{L}$  were immobile after 24 h exposure, but this effect declined to 25% after 48 h exposure. NatureCur at 1,200  $\mu\text{L}$  was not effective, with less than 13% of nematodes immobilized. The effect of NatureCur at all concentrations was removed after rinsing the nematodes in water.

Dominator at 2,400 and 4,800  $\mu\text{L}$  immobilized greater than 50% of the nematodes after 48 and 72 h exposure, and it was as effective as fenamiphos at 72 h (Fig. 1). However, the effect of the compound was removed after rinsing and incubation in water, with most nematodes regaining mobility. Promax has the same active ingredient (3.5% Thymol) as Dominator, but it had less effect on nematode movement than Dominator. Sincocin and castor oil had minimal effect on nematodes at all concentrations, with 25% immobile nematodes during exposure, and <15% after rinsing (Fig. 1). SLS-CA was not evaluated in the first trial because particulates in the suspension obscured the nematodes, and mobility could not be observed.

In the second experiment, lower concentrations of compounds than tested in the first trial were evaluated (Fig. 2). NatureCur was not included in the second trial because the concentrations tested previously covered the range of concentrations recommended by the manufacturer for field applications. Promax was the only thymol product tested in the second experiment. Because castor oil showed no nematicidal activity in the first *in vitro* and greenhouse experiments, it was dropped from further trials. Lower concentrations and the use of sieves, which trapped the particulates, made the evaluation of DiTera easier, and made SLS-CA possible in the second trial. DiTera at 1380 mg/L immobilized nematodes significantly better than fenamiphos over the 72 h exposure, but only 35% of the nematodes remained immobile after rinsing and 24 h of incubation in water (Fig. 2). At 830 mg/L, DiTera was as effective in immobilizing nematodes as fenamiphos during the first 48 h exposure, but this effect diminished after 48 h exposure. The effect of SLS-CA at 2857  $\mu\text{L}$  on nematode mobility increased with the length of exposure, and was similar to the effect of fenamiphos at 48 and 72 h exposure (Fig. 2). However, rinsing and incubating nematodes for 24 h in water restored nematode mobility. SLS-CA at 714  $\mu\text{L}$ , Promax at 358 and 714  $\mu\text{L}$ , Sincocin at 94 and 189  $\mu\text{L}$ , and LCF at 358  $\mu\text{L}$  had no significant effects on nematode mobility (Fig. 2).

#### Greenhouse Study

In the first greenhouse experiment with concentrations suggested by the manufacturers, all products, except LCF, applied AP reduced ( $P < 0.05$ ) the total number of nematodes recovered compared with the infested control (Table 2). Fenamiphos was the most effective product, with only 5 nematodes per pot and 1.1 nematodes/g root

Table 2. Effects of biologically-derived products and fenamiphos on the reproduction of *Pratylenchus penetrans* and on the growth of 'Totem' strawberry plants.<sup>w</sup>

At plant treatment <sup>s</sup>	Concentration ( $\mu\text{l/L}$ or $\text{mg/L}$ )	<i>P. penetrans</i> total	<i>P. penetrans</i> / $\text{g}^{-1}$ root	Root weight (g)	Crown weight (g)
Non-infested control	0	0 a <sup>v</sup>	0.0 a	6.7 bc	15.9 cd
Infested control	0	763 f	97.4 cd	7.3 cd	15.7 cd
Fenamiphos	70	5 a	1.1 a	7.7 c	19.4 e
SLS-CA	5000	102 ab	23.3 ab	4.4 a	12.3 ab
Dominator	1000	259 bc	30.6 ab	8.3 d	23.9 f
DiTera	500	361 cd	42.6 ab	7.8 cd	20.3 e
Promax	1000	413 cd	71.7 bc	7.2 bcd	16.4 d
LCF	750	625 ef	110.7 cd	5.8 b	13.6 bc
Castor Oil	5000	380 cd	129.2 de	3.5 a	10.0 a
Sincocin	4000	477 de	158.7 e	3.2 a	12.3 ab
Post-plant treatment <sup>t</sup>					
Non-infested control	0	10 a	0.3 a	8.7 bc	21.0 c
Infested control	0	1708 cd	221.7 bcd	8.6 bc	19.4 bc
Fenamiphos	70	96 a	4.9 a	13.8 d	21.5 cd
SLS-CA	5000	1185 bcd	303.8 de	5.1 a	14.2 a
Dominator	1000	857 b	93.1 ab	10.4 c	24.0 d
DiTera	500	1799 c	230.8 cd	7.3 ab	19.3 bc
Promax	1000	1119 bc	97.7 ab	10.7 c	20.2 bc
LCF	750	1037 bc	98.2 abc	9.8 bc	21.1 c
Castor Oil	5000	2572 e	397.6 e	7.6 ab	12.6 a
Sincocin	4000	1185 bcd	145.2 bc	9.0 bc	17.6 b

<sup>w</sup>Strawberry plants were planted in 4 liter pots containing loam:sand (2:1 v/v) soil infested with 4000 *P. penetrans* per pot and grown in a greenhouse for 16 weeks after the first treatment was applied.

<sup>s</sup>Biological products, except DiTera, were applied immediately at planting and 4 and 8 and 12 weeks after planting. DiTera was applied at planting and 2, 4, 6, and 8, 10, and 12 weeks after planting. Fenamiphos was applied only at planting.

<sup>v</sup>Values represent means of eight replications. Values within columns followed by the same letter are not significantly different according to Fisher's LSD tests ( $P < 0.05$ ).

<sup>t</sup>Biological products, except DiTera, were applied 4 and 8 and 12 weeks after planting. DiTera was applied 4, 6, 8, 10, and 12 weeks after planting. Fenamiphos was applied only 4 weeks after planting.

recovered after 16 weeks. DiTera (42.6 nematodes/g), Dominator (30.6/g), and SLS-CA (23.3/g) also reduced ( $P < 0.05$ ) nematodes recovered from roots compared with the infested control (97.4/g). When the nemati-

cides were applied PP, the biological products were less effective. Only plants treated with Dominator at 1000  $\mu\text{l/L}$  and fenamiphos had fewer ( $P < 0.05$ ) total nematodes recovered than the infested control plants.

The number of nematodes recovered from roots of plants treated with the biological products ranged from 93.1 to 397.6 nematodes/g, similar in number ( $P < 0.05$ ) to those recovered from roots of infested control plants (221.7/g). As with AP treatments, fenamiphos provided significantly ( $P < 0.05$ ) better nematode control than the biological products, with recovery of 96 total nematodes and 4.9 nematodes/g root.

Nematodes did not affect plant growth in the first experiment, i.e., root and crown weights were not different ( $P < 0.05$ ) between nematode infested and non-infested control plants (Table 2). When products were applied at planting, root weights were greater in the Dominator treatment compared to the non-infested control. Roots were stunted in the castor oil, SLS-CA, and Sincocin treatments compared to infested and non-infested plants (Table 2). Applications of fenamiphos, DiTera, and Dominator appeared to stimulate crown growth compared with the non-infested plants. Plants treated with castor oil, SLS-CA, and Sincocin had smaller crowns than plants of other treatments. Plants treated with Sincocin had some leaf burn following the first application of the foliar fertilizer, a phenomena that was not observed on plants receiving the PP applications of Sincocin. When applications were made starting 4 weeks after planting, root and crown growth was less than controls ( $P < 0.05$ ) in only a few treatments (Table 2). Compared to non-infested control plants, root weights were greater for plants treated with fenamiphos and less for those treated with SLS-CA ( $P < 0.05$ ). Crown weights were greater for plants treated with Dominator and less for those treated with castor oil and SLS-CA compared with controls.

In the second greenhouse experiment, concentrations were based on labeled rates and the volume of water required to incor-

porate product into the root zone (Table 3). All products applied AP reduced ( $P < 0.05$ ) the total number of nematodes recovered compared with the infested control (1285 nematodes), except Promax (Table 3). As in the first trial, fenamiphos had the lowest nematode densities after 20 weeks, 13 nematodes per pot and 0.6 nematodes/g root. Of the biological products, DiTera at 1380 mg/L had the lowest total nematode densities, 307 nematodes/pot and 29.2/g root, which were not different ( $P < 0.05$ ) from the non-infested control. Total nematode densities in SLS-CA and SLS-CA + LCF treatments were similar ( $P < 0.05$ ) to the non-infested control treatment. DiTera 830 mg/L (47.6/g), Sincocin (48.8/g), NatureCur (49.1/g), SLS-CA (49.1/g), and SLS-CA + LCF (44.3/g) also significantly reduced ( $P < 0.05$ ) nematodes recovered from roots compared to the infested control (83.0/g). When the products were applied PP, all products reduced ( $P < 0.05$ ) the total number of nematodes recovered compared to the infested control, except Promax (Table 3). Nematode densities recovered from fenamiphos treated plants were similar ( $P < 0.05$ ) to non-infested controls. Nematodes recovered from roots of DiTera 1380 mg/L (59.7/g), DiTera 830 mg/L (102.9/g), NatureCur (97.8/g), and Sincocin (99.5/g) were less ( $P < 0.05$ ) than recovered from infested control plants (159.2/g). SLS-CA, LCF and Promax did not reduce nematode densities in roots compared with the infested control.

In the second experiment, nematode infestation did not effect root growth, but did reduce crown weight in AP trial (Table 3). When the products were applied AP, applications of fenamiphos stimulated root and crown growth, while the other compounds did not effect root growth. Fenamiphos, DiTera 1380 mg/L, and Promax-Zap stimulated crown growth. Crown

Table 3. Effects of field application concentrations of biologically-derived products and fenamiphos on the reproduction of *Pratylenchus penetrans* and on the growth of 'Totem' strawberry plants.<sup>w</sup>

At plant treatment <sup>s</sup>	Concentration (µl/L or mg/L)	<i>P. penetrans</i> total	<i>P. penetrans</i> g <sup>-1</sup> root	Root weight (g)	Crown weight (g)
Non-infested control	0	2 a <sup>y</sup>	0.2 a	12.3 bc	10.6 cd
Infested control	0	1285 e	83.0 d	9.9 ab	8.6 ab
Fenamiphos	70	13 a	0.6 a	21.7 d	29.5 g
DiTera	830	606 bc	47.6 bc	11.7 bc	12.3 de
DiTera	1380	307 ab	29.2 ab	13.4 c	14.3 f
Sincocin	200	618 bc	48.8 bc	9.4 ab	10.4 bc
NatureCur	4800	658 bc	49.1 bc	9.7 ab	9.9 abc
Promax + Zap	714 + 358	1140 ef	63.2 cd	11.8 bc	13.2 ef
SLS-CA	2587	313 ab	49.1 bc	6.7 a	8.3 a
LCF	358	815 cd	67.2 cd	9.7 ab	10.3 bc
SLS-CA + LCF	2857 + 358	225 a	44.3 bc	6.6 a	8.4 a
Post-plant treatment <sup>t</sup>					
Non-infested control	0	0 a	0.0 a	9.6 ab	12.2 bcd
Infested control	0	1876 f	159.2 e	9.9 ab	12.9 cde
Fenamiphos	70	50 a	5.7 ab	10.4 b	12.4 cd
DiTera	830	1271 cde	102.9 cd	10.8 b	12.6 cde
DiTera	1380	827 b	59.7 bc	11.2 b	14.1 e
Sincocin	200	1234 cde	99.5 cd	10.6 b	11.7 abc
NatureCur	4800	1078 bcde	97.8 cd	9.7 ab	11.4 abc
Promax + Zap	714 + 358	1473 ef	131.3 de	11.2 b	13.8 de
SLS-CA	2587	957 bc	111.1 cde	7.6 a	10.1 a
LCF	358	1375 de	125.5 de	9.3 ab	11.5 abc
SLS-CA + LCF	2857 + 358	1067 bcd	117.7 de	7.7 a	10.5 ab

<sup>w</sup> Strawberry plants were planted in 4 liter pots containing loam:sand (2:1 v/v) soil infested with 4000 *P. penetrans* per pot and grown in a greenhouse for 16 weeks after the first treatment was applied.

<sup>s</sup> Biological products, except DiTera, were applied immediately at planting and 4, 8, 12, and 16 weeks after planting. DiTera was applied at planting and each 2 weeks through week 16. Fenamiphos was applied only at planting.

<sup>y</sup> Values represent means of six replications. Values within columns followed by the same letter are not significantly different according to Fisher's LSD tests ( $P < 0.05$ ).

<sup>t</sup> Biological products, except DiTera, were applied 4, 8, 12, and 16 weeks after planting. DiTera was applied at 4, 6, 8, 10, 12, 14, and 16 weeks after planting. Fenamiphos was applied only at planting.

and root weights were lowest with SLS-CA treated plants.

There were no differences in root weight and few differences in crown weight

between control plants and plants treated with any of the products starting four weeks after planting. DiTera 1380 mg/L appeared to slightly stimulate crown

growth and, as in AP treatments, crown and root weights were lowest with SLS-CA treated plants.

## DISCUSSION

The present research was limited to evaluating the nematicidal and narcotic activities of several products. However, these products may modify soil microbial communities to become antagonistic to plant-parasitic nematodes (Fernández *et al.*, 2001), which is unlikely to be expressed in pasteurized soil in the present research. Other mechanisms proposed are that biological-derived products can function as plant growth regulators, increase availability of soil nutrients, or induce nematode resistance (Birch, 1993), which are difficult to document and beyond the scope and experimental design of this research.

No biological compound was as effective as fenamiphos in immobilizing nematodes or restricting population increase on strawberry plants. During 72 h exposure *in vitro* to fenamiphos, 75% of the nematodes were immobile, which was similar to *in vitro* experiments with the endomigratory *Radopholus similis* (Marin *et al.*, 2000). The reaction of *P. penetrans* individuals was not different during exposure to fenamiphos and after rinsing with water. The quiescent nematodes that responded to touch did so with slow single movement. Extremely low nematode population densities recovered from soil and roots in fenamiphos treated pots suggest that nematodes were killed both in the soil and roots.

The biological products evaluated appeared to have transitory activity on nematodes under the conditions of our experiments. DiTera reversibly immobilized  $\geq 70\%$  of the nematodes during a 72 h exposure to concentrations  $>1380$  mg/L and were as effective as fenamiphos. After rinsing nematodes exposed to these products and

incubating in water, less than 35% were immobile. Ferris and Zheng (1999) reported a similar revival of *Pratylenchus vulnus* individuals following exposure to plant extracts. Marin *et al.* (2000) reported concentrations less than 15,000 mg/L of DiTera did not significantly immobilize *Radopholus similis* individuals compared to water controls, but at 75,000 mg/L DiTera was as effective as fenamiphos in immobilizing more than 70% of nematodes. However, they did not observe the nematodes after removal from DiTera solutions and incubation in water. DiTera appears to affect multiple aspects of nematode biology. DiTera at 1% and 10% was reported to adversely effect movement, sensory perception, feeding behavior, and hatch of *Globodera rostochiensis* (Twomey *et al.*, 2000; Twomey *et al.*, 2002). Both these studies demonstrate that DiTera has nematicidal activity at very high concentrations. Our data suggest that these effects are present at lower concentrations and are concentration dependent and reversible after the nematodes are no longer exposed to the compound.

Several plant-derived products tested in our research have demonstrated activity against several nematode genera. The essential oil thymol, 3.5% a.i. in Promax and Dominator, has been reported to act as a bio-fumigant with broad-spectrum activity against plant pathogenic bacteria (Ji *et al.*, 2005), fungi (Wilson *et al.*, 1997), and nematodes. Thymol inhibited the mobility and egg hatch of *Meloidogyne javanica* at 250 and 500  $\mu\text{L/L}$  *in vitro* (Oka *et al.*, 2000). Soler-Serratos *et al.* (1996) estimated the LC90 of thymol in soil for *Meloidogyne arenaria*, *Heterodera glycines*, and non-parasitic nematodes at 161, 145, and 225  $\mu\text{L/L}$ , respectively. Promax applied at 9.3 liters/ha on English boxwood suppressed populations of four genera of plant-parasitic nematodes 30 days after application, but efficacy decreased after 60

days (Pérez *et al.*, 2006). In our *in vitro* research, the two 3.5% thymol products inhibited mobility of *P. penetrans* for 72 h at the highest concentration, but efficacy was lost upon incubation in water. Volatile compounds, such as thymol, also may have lost some activity during the 72 h exposure period in our experiments. McKenry and Anwar (2003) reported water extracts from wood of *Juglans* spp. caused 100% mortality of *M. incognita* and multiple applications on grape vines infested with *M. incognita* over a two year period provided nematode control equal to fenamiphos. NatureCur, which is derived from wood of *Juglans*, inhibited movement of *P. penetrans* only during exposure to concentrations  $\geq 2400$   $\mu\text{L/L}$  in our *in vitro* trials. In our greenhouse trials, it showed some activity, but not on par with fenamiphos. SLS-CA followed a similar pattern, with *P. penetrans* reversibly immobilized at a high concentration *in vitro* and moderate efficacy in strawberry trials.

Nematode population densities in greenhouse studies were lower when bio-nematicide applications started at planting than when nematodes were allowed to infect plant roots for four weeks prior to the first application. Endoparasitic nematodes are more difficult to control once they enter the root. These data suggest that the biological products acted as prophylactics, in contrast to fenamiphos, and had little therapeutic activity on nematodes once inside the roots. Products that inhibit nematode movement, orientation, and feeding behavior could reduce penetration of nematodes into the roots and delay population increase. Among the bionematicides evaluated in greenhouse trials, DiTera at 1380 mg/L had the lowest *P. penetrans* population density, with root population densities not different from the fenamiphos treated plants. DiTera was applied at two week intervals compared

with four week intervals for the other products, so the activity may have been more persistent than the other products that showed activity *in vitro*. Plants treated with SLS-CA also had low total nematode populations, but stunted plant growth suggests that the concentration applied was phytotoxic. SLS-CA, a 12 carbon chain anionic surfactant used in soaps and detergents, may affect the integrity of nematode membranes. Maintaining high concentrations of SLS-CA in the rhizosphere may have adverse effects on plant growth offsetting any benefit from controlling nematodes.

Plants were not adversely affected by *P. penetrans* as indicated by no difference between root and crown biomass of infested and non-infested control plants in the greenhouse trials. Similar observations were made in previous research with *P. penetrans* on 'Totem' strawberry (Pinkerton and Finn, 2005). Young vigorous plants grown under luxurious conditions of water, fertilizer and environment compensated for nematode damage during these 16 to 20 week experiments. Although claims have been made that several biological products can stimulate plant growth, we observed no consistent pattern in our research. Fenamiphos, DiTera, and Dominator/Promax stimulated root or crown growth in one or more trials. Conversely, SLS-CA and nematicidal castor oil appeared to be phytotoxic at concentrations used in our trials.

Biologically-derived nematicides will not substitute directly for organophosphate and carbamate nematicides. Biological nematicides will require different strategies, such as multiple applications at critical times during flushes of root growth (Grau, 1996) or synchronized with egg hatch and when nematode population densities are high in the soil. We have demonstrated that nematodes once established in new strawberry roots were not affected

by drenching the soil with biological products at 4 week intervals. Population densities of *P. penetrans* were reported to be greatest on feeder roots of strawberry, with few extracted from soil (LaMondia, 2002). In our experiments, most nematodes also were extracted from the strawberry roots. In the case of *P. penetrans* on raspberry, nematodes are active in the soil and roots throughout the year, with ca. 40% of the population residing in the perennial roots (Vrain *et al.*, 1997). More frequent applications and/or higher concentrations may be required to manage nematode populations that move between the soil and roots. In agricultural soils, biological products may degrade rapidly. In addition, biological products evaluated in this study were effective at the higher concentrations tested, which may be greater than concentrations in agricultural soils following applications at labeled rates and may not be economical. Application of these biologically-derived products at recommended rates demonstrated no efficacy against six genera of plant-parasitic nematodes in a wine grape vineyard in Washington State (Riga and Pinkerton, unpublished data). Research needs to be directed to adjusting application timing and concentrations for specific application methods and nematode pathosystems.

#### LITERATURE CITED

- Abu-Gharbieh, W., E. H. Verney, and W. R. Jenkins. 1962. Relationship of meadow nematode to verticillium wilt of strawberry. *Phytopathology* 52:921.
- Akhtar, M., and I. Mahmood. 1994. Potentiality of phytochemicals in nematode control: A review. *Bioresource Technology* 48:189-201.
- Ayoub, S. M. 1981. *Plant Nematology*, An agricultural training aid. NemaAid Publications, Sacramento, CA. 195 pp.
- Birch, N. A. E., W. M. Robertson, and L. E. Fellows. 1993. Plant Products to control plant parasitic nematodes. *Pesticide Science* 39:141-145.
- Brown, D. J. F., A. Dalmasso, and D. L. Trudgill. 1993. Nematode pests of soft fruits and vines. Pp. 427-462 in K. Evans, D. L. Trudgill, and J. M. Webster (eds.). *Plant Parasitic nematodes in temperate agriculture*. CAB International, U.K.
- Chitwood, D. J. 1993. Naturally occurring nematocides. Pp. 300-315 in S. O. Duke, J. J. Menn, and J. R. Plimmer (eds.), *Pest Control with enhanced environmental safety*. Amer Chem Soc.
- Chitwood, D. J. 2002. Phytochemical based strategies for nematode control. *Annual Review Phytopathology* 40:221-249.
- Esnard, J., and B. M. Zuckerman. 1998. Small fruits. Pp. 685-725 in K. R. Barker, G. A. Pederson, and G. L. Windham (eds.). *Plant and Nematode Interactions*. American Society of Agronomy, Madison, WI.
- Fernandez, C., R. Rodriguez-Kabana, P. Warrior, and J. W. Kloepper. 2001. Induced soil suppressiveness to a root-knot nematode sp by a nematocide. *Biological Control* 22:103-114.
- Ferris, H., and L. Zheng. 1999. Plant sources of Chinese herbal remedies: Effects on *Pratylenchus vulnus* and *Meloidogyne javanica*. *Journal of Nematology* 31:241-263.
- Grau, P. A., R. Hopkins, J. D. Radewald, and P. Warrior. 1996. Efficacy of DiTera biological nematocide for root-knot nematode suppression on carrot. *Nematropica* 26:270.
- Ji, P., M. T. Momol, S. M. Olson, P. M. Pradhannang, and J. B. Jones. 2005. Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. *Plant Disease* 89:497-500.
- Kurppa, S., and T. C. Vrain. 1989. Effects of *Pratylenchus penetrans* on the infection of strawberry roots by *Gnomonia comari*. *Journal of Nematology* 21:511-516.
- LaMondia, J. A. 1999. Effects of *Pratylenchus penetrans* and *Rhizoctonia fragariae* on vigor and yield of strawberry. *Journal of Nematology* 31:418-423.
- LaMondia, J. A. 2002. Seasonal populations of *Pratylenchus penetrans* and *Meloidogyne hapla* in strawberry roots. *Journal of Nematology* 34:409-413.
- Marin, D. H., K. R. Barker, and T. B. Sutton. 2000. Efficacy of "ABG-9008" against burrowing nematode (*Radopholus similis*) on bananas. *Nematropica* 30:1-8.
- McElroy, F. D. 1992. A plant health care program for brambles in the Pacific Northwest. *Journal of Nematology* 24:457-462.
- McKenry, M. V., and S. A. Anwar. 2003. Nematicidal activity of walnut extracts against root-knot nematodes. *Journal of Nematology* 35:358.

- National Agricultural Statistics Service. 2006. Non-citrus fruit and nuts 2005 preliminary summary. United States Department of Agriculture. Fr Nt 1-3 (06).
- Oka, Y., S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv, and Y. Spiegel. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology* 90:710-715.
- Perez, E. E., and E. E. Lewis. 2006. Use of entomopathogenic nematodes and thyme oil to suppress plant-parasitic nematodes on English boxwood. *Plant Disease* 90:471-475.
- Pinkerton, J., and C. E. Finn. 2005. Responses of strawberry species and cultivars to the root lesion and northern root-knot nematodes. *HortScience* 40:33-38.
- Soler-Serratos, A., N. Kokalis-Burelle, R. Rodríguez-Kábana, C. F. Weaver, and P. S. King. 1996. Allelochemicals for control of plant-parasitic nematodes I. Thymol. *Nematropica* 26:57-71.
- Szczygiel, A. and H. Profic-Alwasiak. 1989. Studies on the interaction between nematodes and fungi infecting strawberry plants. *Acta Horticulturae* 265:561-568.
- Twomey, U., R. N. Rolfe, P. Warrior, and R. N. Perry. 2000. Effects of the biological nematicide, DiTera, on movement and sensory responses of second stage juveniles of *Globodera rostochiensis*, and stylet activity of *G. rostochiensis* and fourth stage juveniles of *Ditylenchus dipsaci*. *Nematology* 4:909-919.
- Twomey, U., R. N. Rolfe, P. Warrior, and R. N. Perry. 2002. Effect of the biological nematicide, DiTera, on hatching of *Globodera rostochiensis* and *G. pallida*. *Nematology* 2:255-362.
- Vrain, T. C., and R. J. Coopeman. 1987. Interaction between *Agrobacterium tumefaciens* and *Pratylenchus penetrans* in the root of two raspberry cultivars. *Canadian Journal of Plant Pathology* 9:236-240.
- Vrain, T. C., T. A. Forge, and R. De Young. 1997. Population dynamics of *Pratylenchus penetrans* parasitizing raspberry. *Fundamental and Applied Nematology* 20:29-36.
- Wilson, C. L., J. M. Solar, A. El Ghaouth, and M. E. Wisniewski. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Disease* 81:204-210.

---

*Received:*

11/V/2006

*Accepted for publication:*

21/VII/2006

*Recibido:*

*Aceptado para publicación:*