EFFICACY OF "ABG-9008" AGAINST BURROWING NEMATODE (*RADOPHOLUS SIMILIS*) ON BANANAS

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ABSTRACT

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DiTera® is a biological nematicide of microbial origin with potential for managing several plant-parasitic nematodes in different crops. Efficacy of ABG-9008, an earlier formulation related to DiTera®, to control the burrowing nematode of bananas was evaluated using direct-contact and root-penetration assays, and in a greenhouse test. Nematodes were incubated for 48 hr at 25°C in 112 and 224 µg/ml suspensions of ABG-9008, corresponding to 112 and 224 kg/ha on a broadcast basis. Suspensions at 15 000 and 75 000 µg/ml also were included. Efficacy of ABG-9008 was assessed at the same rates on *Radopholus similis*-infected banana root segments in microtiter plates. Efficacy of the product was evaluated under greenhouse conditions by applications at day 0 and 5 after inoculation. Water and fenamiphos at 4.65 µg/ml (4.5 kg/ha) were included as standard controls in all experiments. Percentages of mortality in suspensions, which ranged from 16.8 to 24.6%, were not significantly different between those for 112 and 224 µg/ml and 15 000 µg/ml of ABG-9008. Percentages of mortality for fenamiphos (76.4) and 75 000 µg/ml ABG-9008 (69.8) did not differ ($P = 0.05$). Nematode recovery was greatly suppressed after 48 hr of incubation of infected banana roots exposed to 75 000 µg/ml ABG-9008 or fenamiphos, and mortality was significantly greater than the other ABG-9008 rates used. Numbers of *R. similis* from plants treated with 56 and 224 µg/ml of ABG-9008 were similar to those on untreated plants under greenhouse conditions with only fenamiphos giving effective control. Time of application of the products (0 and 5 days after inoculation) did not affect their efficacy on this nematode. ABG-9008 at 10 000 µg/ml was phytotoxic under greenhouse conditions.

Key words: ABG-9008, banana, burrowing nematode, chemical control, DiTera®, fenamiphos, *Musa* spp., *Radopholus similis*.

RESUMEN

Marin, D. H., K. R. Barker y T. B. Sutton. 2000. Eficacia de "ABG-9008" sobre el Nematodo Barrenador (*Radopholus similis*) en Banano. Nematrópica 30:1-8.

DiTera® es un nematicida biológico de origen microbial con potencial para el manejo de nematodos en diferentes cultivos. La eficacia de ABG-9008, una formulación temprana relacionada con DiTera®, para el combate del nematodo barrenador del banano se evaluó utilizando ensayos de contacto directo y de penetración de raíz. Se incubaron nematodos por 48 hrs a 25°C en 112 suspensiones y 224 µg/ml de ABG-9008, que correspondieron a aplicaciones comerciales de 112 y 224 kg/ha. A la vez, se incluyeron suspensiones a 15 000 y 75 000 µg/ml. Los ensayos de penetración de raíz se realizaron en platos microtiter con secciones de raíz de banano utilizando las mismas dosis de ABG-9008. La eficacia del producto fue evaluada bajo condiciones de invernadero haciendo aplicaciones a los 0 y 5 días después de la inoculación. Tratamientos con agua y fenamiphos 4.65 µg a.i./ml (4.5 kg/ha) fueron incluidos como testigo en todos los experimentos. El porcentaje de mortalidad en las suspensiones, el cual se mantuvo en un rango de 16.8 a 24.6%, no presentó diferencias significativas entre los tratamientos de 112 y 224 µg/ml y 15 000 µg/ml de ABG-9008. Los porcentajes de mortalidad para fenamifos (76.4) y ABG-9008 a 75 000 µg/ml (69.8) no presentaron diferencias significativas ($P = 0.05$). La recuperación de nemato-

dos fue altamente suprimida en las raíces de banano inoculadas durante 48 hrs y expuestas a ABG-9008 a 75 000 µg/ml o fenamifos, y la mortalidad fue significativamente mayor que en las otras dosis de ABG-9008 utilizadas. Bajo condiciones de invernadero, el número de *R. similis* en las plantas tratadas con 56 y 224 µg/ml de ABG-9008 fue similar al obtenido en las plantas no tratadas siendo, fenamifos el único tratamiento que presentó un combate efectivo. El tiempo de aplicación de los productos (0 y 5 días después de la inoculación) no tuvo efecto sobre la eficacia de éstos para el combate de este nematodo. Se observó fitotoxicidad bajo condiciones de invernadero cuando se utilizó ABG-9008 a 10 000µg/ml.

Palabras claves: ABG-9008, banano, control químico, DiTera®, fenamiphos, Musa spp, nematodo barrenador, *Radopholus similis*.

INTRODUCTION

Plant-parasitic nematodes are widespread and are the most important pests of banana (*Musa* spp.) (Gowen, 1995). Crop losses associated with nematodes in bananas have been estimated to average 19.7% worldwide (Sasser and Freckman, 1987). The burrowing nematode, *Radopholus similis* (Cobb) Thorne, is the most damaging nematode attacking this crop (Gowen and Quénéhervé, 1990). Nematode management on banana encompasses a combination of practices, mainly propping and/or guying, and use of organophosphate and carbamate nematicides (Gowen, 1994; Gowen and Quénéhervé, 1990; Stover and Simmonds, 1987). Alternatives to nematicides for nematode management in banana are needed because of several problems including accelerated biodegradation of some nematicides, rising costs of the compounds, possible resistance of nematodes to nematicides, side effects on non-target organisms, and contamination of ground and/or surface water (Jaramillo, 1988; Quénéhervé, 1993).

Although the burrowing nematode is the main parasite in banana roots, its population levels in the soil are usually low, regardless of soil texture (Quénéhervé, 1988). Because low population densities of the free-living stage of *R. similis* occur in the soil and the parasite completes its life cycle inside the roots, potential products will likely need some systemic activity.

DiTera®, a product of fungal origin, has shown effective control of several important plant-parasitic nematodes of vegetables and fruit crops (Abbott Laboratories, 1996; Grau *et al.*, 1996; Hafez *et al.*, 1996; and Warrior *et al.*, 1999) and may provide an alternative for nematode management. The overall objective of this research was to evaluate the efficacy of ABG-9008, an earlier formulation related to DiTera®, for control of the burrowing nematode of bananas. Specific objectives included: 1) to assess the efficacy of ABG-9008 in direct-contact in vitro assays; 2) to determine effects of this product on nematodes in infected roots; and 3) to assess efficacy on *R. similis* on banana plants in a greenhouse.

MATERIALS AND METHODS

Direct contact in vitro *assay*: Burrowing nematodes, originally obtained from banana roots from Costa Rica were extracted from carrot-disc cultures (Kaplan and Davis, 1990) and quantified. For this purpose, a modified 'Mason Jar' procedure was used. This involved the placement of infected carrot tissues in glass beakers, barely covering them with water and incubating them at room temperature (~22°C) for at least 3 days. Nematodes were separated from the tissues by decanting-sieving, using a combination of 420-µm and 25-µm sieves.

Aliquots of 50-µL of sterile de-ionized water containing ~100 nematodes were placed in 2.5-cm glass dishes ("Beltsville dishes"), and 250 µL of the different test suspensions were added to complete a total volume of 300 µL. Nematodes were incubated for 48 hr in a moist chamber (100% relative humidity [RH] and 25°C). Mortality was evaluated based on nematode activity. Experiments were designed and analyzed as a completely randomized design with three replicates and were repeated once over time. Mortality (in percent) was calculated, and data were transformed to square root before statistical analyses.

Treatments included ABG-9008 at 112, 224, 15 000 and 75 000µg/ml, fenamiphos at 4.65 µg/ml (equivalent to 4.5 kg/ha) as a nematicide standard, and a water control. The low rates of ABG-9008 are equivalent to treatments of 112 and 224 kg/ha; the high rates of ABG-9008 in this test and others were suggested by the manufacturer to determine if the product had any activity on *R. similis*. In all studies, ABG-9008, a 100% active wettable powder formulation of *Myrothecium* sp. was tested. All soluble materials in ABG-9008 were retained in the test containers, and nonsoluble residue was discarded.

Effects of ABG-9008 on R. similis infection of root segments: Host-root penetration tests were performed using the methodology described by Kaplan and Davis (1991). Each of 96 flat-bottom wells (7-mm diam and 10-mm deep) of a microtiter plate were filled with 0.60 g of 60-mesh (254-µm) sterile fine sand (autoclaved for 1 hr at 121°C on 2 consecutive days).

Root segments (4.0-mm in length) were excised from the zone of elongation of banana plants (*Musa* AAA, Cavendish subgroup, cv. Grande Naine) grown in the greenhouse. Twenty-five µL of test suspensions were dispensed into each well before the root segments were placed horizontally on the surface of the substrate, and covered with an additional 0.28 g of dry 254-µm sand.

Burrowing nematodes extracted from carrot-disk cultures (Kaplan and Davis, 1990) were quantified, concentrated, and resuspended in sterile de-ionized water at a concentration of 8 000 nematodes/ml of which 25 µL of the nematode suspension (~200 nematodes) were added to each well. Microtiter plates were covered with plastic lids and placed in a moist chamber (100% RH at 25°C) for 24 hr. Root pieces were then collected, and active nematodes were extracted by incubation in 300 µL of sterile de-ionized water in separate wells of a 96-well, flat-bottom microtiter plate. Plates were incubated in a moist chamber (100% RH at ca. 25°C) for 48 hr. Extracted nematodes were quantified using an inverted-light microscope. To detect nematodes remaining in roots, the segments were incubated in 1% NaOCl for 10 min, rinsed under running tap water for 15 min, and stained for 30 sec in a boiling aqueous acid fuchsin solution (Byrd *et al.*, 1983). Root segments were placed between two microscope slides (25 × 75 mm), and all nematodes counted.

The experiment was designed and analyzed as a completely randomized design with 10 replicates, and was repeated once over time. The test consisted of four rates of ABG-9008 (112, 224, 15 000, and 75 000 µg/ml), and two controls (fenamiphos, 4.65 µg/ml, and water). Calculations of rates of nematicidal products were based on 600 µL, the total volume of sand used. Data were transformed with $\log_{10}$ (x + 1) before analyses.

Greenhouse experiment: Banana plants (*Musa* AAA, Cavendish subgroup, cv. Grande Naine) from tissue culture were grown in a 414-ml Styrofoam cups (8.9-cm top diam; 13.6-cm high). A mixture of 1:1

of sterile coarse river sand and 254-µm sand (autoclaved for 1 hr at 121°C) was used as substrate. A complete nutrient solution (Chem-Gro, Hydro-Gardens, Inc., Colorado Springs, CO) was added twice weekly. Before inoculation, plants were grown for 4 wk at 27°C and 80% RH in the greenhouse.

Burrowing nematodes extracted from carrot-disk cultures (Kaplan and Davis, 1990) were quantified, concentrated, and resuspended in sterile de-ionized water (40 nematodes/ml). Five ml of the nematode suspension (~200 nematodes) were added to the base of each plant. A mixture of juveniles and adults was used.

The experiment was designed as a 2 × 4 factorial (time of application: 0 and 5 days after inoculation; and chemical: ABG-9008 at 56, and 224 and 10 000 µg/ml, and fenamiphos at 4.65 µg/ml; plus two controls (noninoculated and inoculated). The 10 000 µg/ml rate of ABG-9008 was phytotoxic, leaving the final analysis as a 2 × 3 factorial in a randomized complete block design with 10 replicates.

Suspensions of ABG-9008 and a solution of fenamiphos were delivered in 40-ml aliquots added to the plants at 0 and 5 days after inoculation. Solutions and suspensions were kept under continuous agitation during application to ensure homogeneity of the products. Inoculated plants were maintained for 8 wk at ~27°C and 80% RH in the greenhouse.

At harvest, plant height was measured from the base of the plant to the emerging youngest leaf. After severing, shoot weights were recorded. Roots were removed from the sand and washed. Root-necrosis (0-100%: 0 = healthy; 100 = entire root exhibiting root necrosis) was also determined by visually examining the root segments. Nematodes were first extracted by the modified 'Mason Jar' procedure which involved cutting the roots in 1-cm pieces and incubating them for 7 days at 25°C. The residual water and roots were then washed on a 420-µm sieve, and the sample was collected on a 25-µm sieve. Nematode suspensions were diluted to 100 ml and a 10-ml aliquot was taken to quantify the number of nematodes per plant. Additionally, the numbers of nematodes were divided by the total fresh root weights to determine the number of nematodes per gram of root. The remaining number of nematodes and eggs were determined by maceration and sieving the roots (Araya *et al.*, 1995). All nematode data were transformed using $\log_{10}(x + 1)$ before statistical analyses.

RESULTS

Direct-contact in vitro *assay*: Mortality of nematodes at 112, 224 and 15 000 µg/ml ABG-9008 was slightly higher, but not significantly different from the water control (Table 1). Numbers of *R. similis* in the ABG-9008 (75 000 µg/ml) and fenamiphos treatments were similar, and lower ($P = 0.05$) than those in the other treatments. Efficacy of the products was also tested at 16 and 24 hr in the first run of the experiment (with same number of replicates per harvest) and results did not differ from 48 hr ($P = 0.05$) which was selected as the standard time of exposure to the treatments (data not shown).

Effects of ABG-9008 on R. similis infection of root segments: Separate analyses were performed for nematodes recovered after incubation of infected host roots in sterile water for 48 hr and the nematodes that remained inside the roots after the incubation period. In both analyses, all treatments reduced ($P = 0.05$) nematode infection of roots, compared to the water control (Table 2). Numbers of nematodes remaining in roots were greater for ABG-9008 at 112 and 224 µg/ml than for the higher rates of ABG-9008 and for fenamiphos.

Table 1. Effects of four rates of ABG-9008 compared to fenamiphos and a water control on *Radopholus similis* in vitro and on rate of nematode infection of banana-root segments.

Test material and rate (µg/ml)	*In vitro* test mortality (%)	Root infection test	
		Residual nematodes in roots	Nematodes egressing from roots
ABG-9008:			
112	25 a[z]	29 b	52 b
224	23 a	29 b	52 b
15 000	17 a	9 c	28 c
75 000	70 b	7 c	13 c
Fenamiphos:			
4.65	77 b	7 c	28 c
Water control	0 a	48 a	86 a

[z]Numbers in columns with the same letter are not different, based on protected LSD ($P = 0.05$).

Numbers of nematodes that egressed from roots treated with ABG-9008 at 112 and 224 µg/ml were higher than those treated with 15 000 and 75 000 µg/ml ABG-9008 or fenamiphos. ABG-9008 at 15,000 and 75 000 µg/ml provided control similar to fenamiphos.

Greenhouse test: Root necrosis in noninoculated control plants was not different from plants treated with fenamiphos, but was less ($P = 0.05$) than in the ABG-9008 treatments or the inoculated control (Table 2). Time of application and the time × chemical interaction did not show any effect on root necrosis.

Nematode populations, whether determined by incubation or blending/sieving of the roots, provided similar results. Numbers of nematodes extracted (incubation + maceration/sieving) from roots in ABG-9008 treatments (56 and 224 µg/ml) were not different from those in the inoculated control, although they differed ($P = 0.05$) from those in the fenamiphos treatment. Number of eggs revealed trends similar to numbers of vermiform *R. similis*. Time of application did not affect the overall performance of the products. Fenamiphos efficiently controlled burrowing nematodes at both times of application.

Noninoculated plants were slightly taller than inoculated plants (data not shown). Height of plants treated with 56 µg/ml ABG-9008 was less ($P = 0.05$) than that of plants treated with 224 µg/ml ABG-9008 and fenamiphos. Time of treatment application did not affect plant height (data not shown). Fresh shoot weight in the noninoculated control was greater ($P = 0.05$) than that in the nematode inoculated controls and the other treatments (data not shown). Neither ABG-9008 rate nor fenamiphos resulted in a greater shoot weight than the inoculated control. Time of application was not significant, but the time × chemical interaction was significant at $P < 0.01$, because shoot weight of plants treated on day 0 after inoculation was slightly higher than those treated on day 5. A time × chemical interaction was the only

Table 2. Comparative effects of ABG-9008 and fenamiphos applied at the time of inoculation of banana with *Radopholus similus* or at 5 days post-inoculation.

Treatment and rate (µg/ml)	Associated root-necrosis (0-100 scale)		Nematodes/plant[y] Day 0		Day 5	
	0-time	5-days	Nematodes	Eggs	Nematodes	Eggs
ABG-9008:						
56	39 a[z]	30 a	2 900 a	3 900 a	4 100 a	5 400 a
224	31 a	33 a	2 400 a	3 800 a	2 900 a	3 900 a
Fenamiphos:						
4.65	20 b	20 b	20 b	50 b	20 b	50 b
Inoculated control	36 a		3 500 a	4 600 a	—	—
Uninoculated control	21 b					

[y]Roots evaluated for necrosis, and nematodes extracted from roots 8 weeks after inoculation.
[z]Numbers in columns with the same letter are not different, based on protected LSD ($P = 0.05$).

significant factor for fresh root weight. Differences in root weight were more affected by the initial size of the plants than by any specific treatments.

DISCUSSION

Due to the continuous cultivation of bananas in most production areas of the world, and the low proportion of the endoparasitic burrowing nematode population in the soil in infested sites, any potential product to be used in nematode management will likely require some systemic activity. ABG-9008 showed some activity in the direct-contact assay and in the infected banana-root tests; however, efficacy in the greenhouse test was very poor in comparison to fenamiphos. Thus, DiTera® apparently has very limited systemic activity. Rates of DiTera® or ABG-9008 higher than 224 µg/ml, but lower than 10 000 ug/ml, should be tested to determine if they are effective in controlling the burrowing nematode under longer-term greenhouse conditions. It is also important to use rates comparable to those that might be used in the field, and this needs to be determined for each individual crop. Recent results from California on walnuts (Westerdahl, B. B., personal communication) have been encouraging as they have confirmed the activity of ABG-9008 formulations against *Pratylenchus vulnus*, a related endoparasitic nematode, resulting in significant yield enhancement. The information on use directions, formulation, application rate and timing may be useful in developing DiTera® as a nematicide for banana.

Other greenhouse tests with ABG-9008, including *Heterodera schachtii* on sugar beet (Hafez *et al.*, 1996), have resulted in only slight-to-moderate nematode control. ABG-9008 at 56, 112, and 224 µg/ml rates suppressed cyst development at 45 days by 16, 33, and 57%, respectively. Field experiments on banana over longer periods of evaluation may provide useful information that cannot be obtained from controlled experiments. In contrast to our greenhouse

results, field studies conducted in Costa Rica, Ecuador, Honduras, Guatemala and other locations revealed that the related registered product DiTera® reduced populations of *R. similis* and other plant parasitic nematodes such as *Belonolaimus* spp., *Meloidogyne* spp., and cyst nematodes in turf, bananas, and/or field, fruit and vegetable crops (Warrior *et al.*, 1999). DiTera® was also reported to control of *Meloidogyne* spp. on carrot (Grau *et al.*, 1996).

The factors responsible for the differential efficacy of DiTera® reported from studies in the field versus the related ABG-9008 in the greenhouse warrant study. For example, tests on free eggs of *Globodera pallida* showed that DiTera® disrupted the normal change in eggshell permeability induced by potato-root diffusate (Twomey *et al.*, 1998). Exposure of cysts to 1% (10 000 µg/ml) and 10% (100 000 µg/ml) DiTera® irreversibly prevented egg hatch. Other detrimental effects of this biological nematicide may occur under long-term field conditions. The potential phytotoxicity of DiTera® or other products related to ABG-9008, especially at very high rates, on crops such as banana also should be considered in field tests. Also, based on the current information available on the mode of action of DiTera® and its active ingredients (technical information, Valent Biosciences), this product affects nematodes in multiple ways, both directly and indirectly, and thus could effect nematode control in a manner distinctly different from that of traditional chemical nematicides (Perry *et al.*, 2000).

A safe and environmentally acceptable nematicide is urgently needed for many crops, including banana. Biological nematicides and nematode antagonists/parasites, when integrated into pest/crop management systems could contribute in resolving this increasingly important problem and further work in this area is warranted.

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