THE EFFECTS OF CROPGUARD® ON THE MOTILITY, ULTRASTRUCTURE, AND RESPIRATION OF TWO *MELOIDOGYNE* SPECIES

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ABSTRACT

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The progressive withdrawal of Class I nematicides from world markets necessitates investigation and exploitation of more environmentally friendly products with nematicidal or nematostatic properties. Scientific, baseline knowledge about the biology, ultrastructure, and physiology of *Meloidogyne* spp. pertaining to the mode of action of CropGuard®, with furfural as the active ingredient, is lacking. The product was evaluated over time (1, 24, 48, 72, and 96 hr) for its effects on *M. incognita* and *M. javanica* second-stage juveniles (J2) motility, ultrastructure, and specific oxygen consumption rate. Four CropGuard® concentrations (0.01, 0.1, 1, and 10%) and a tap water control were used for all experiments. All four product concentrations significantly inhibited the movement of J2 of both *Meloidogyne* spp. Scanning electronmicroscopy (SEM) results indicated that the cuticle surfaces of J2 suspended in the CropGuard® concentrations were not visibly damaged, but that their lateral line structures showed a "sunken" appearance. Specific oxygen consumption rate (O_2) measurements confirmed that the respiratory physiology and motility of the J2 were adversely affected by the nematicide. Partial respiratory recuperation of *M. incognita* J2 occurred when they were transferred to sterile tap water after being suspended in the lower (0.01 and 0.1%) CropGuard® concentrations.

Key words: furfural, oxygen consumption rate, respiration, root-knot nematodes, second-stage juvenile motility.

RESUMO

Fourie, H, W. J. Van Aardt, C. Venter, and L. R. Tiedt. 2014. Eefeitos do CropGuard® sobre a mobilidade, ultra-estrutura e respiração de duas espécies de *Meloidogyne*. Nematropica 44:85-92.

A retirada progressiva de nematicidas Classe I dos mercados mundiais exige investigação e exploração de produtos mais ecológicos, com propriedades nematicidas ou nematostáticas. O conhecimento da base científica sobre a biologia, ultra-estrutura e fisiologia de *Meloidogyne* spp., com relação ao modo de ação do produto CropGuard®, que tem o furfural como ingrediente ativo é inexistente. O produto foi avaliado ao longo do tempo (1, 24, 48, 72 e 96 hr) por seus efeitos sobre a mobilidade, ultra-estrutura e taxa de consumo de oxigênio específico em juvenis de segundo estádio (J2) de. *M. incognita* e *M. javanica*. Quatro concentrações de CropGuard® (0,01, 0,1, 1 e 10%) e um controle com água de torneira foram usadas, em todos os experimentos. Todas as quatro concentrações do produto inibiram significativamente o movimento dos J2s de *Meloidogyne* spp. Resultados ao Microscópio Eletrônico de Varredura (MRV) indicaram que as superfícies da cutícula dos J2 tratados com o CropGuard® nas diferentes concentrações, não foram visivelmente danificada, mas que as suas estruturas de linhas laterais apresentaram uma aparência 'afundada'. Medições da taxa de consumo de oxigênio específico confirmaram (O_2)que a fisiologia respiratória e mobilidade do J2s foram negativamente afetadas pelo nematicida. Ocorreu recuperação respiratória parcial dos J2s de *M. incognita*, quando eles foram transferidos para a água de torneira esterilizada, depois dos tratamentos com CropGuard® nas concentrações mais baixas (0,01 e 0,1 %).

Palabras clave: furfural, oxigênio, taxa de consumo, respiração, nematoide das galhas, mobilidade do juvenil de segundo estádio.

Root-knot nematodes (*Meloidogyne* spp.) are an important constraint in the production of food crops worldwide (Moens *et al.*, 2009). In South Africa *Meloidogyne* spp. cause yield and quality losses to a wide range of staple food crops such as maize (McDonald and Nicol, 2005), potato (Keetch, 1989)

and tomato (Fourie *et al*., 2012). These pests also parasitize and limit yields of soybean (Keetch, 1989; Fourie *et al*., 2001; 2010) and sunflower (Bolton *et al*., 1989; Keetch, 1989).

Although synthetically derived nematicides have been and still are used as a major strategy to manage plant-parasitic nematodes on food crops in South Africa (CropLife, 2011), many products have been phased out locally due to the risk of toxic effects on the environment, humans, and animals (Anonymous, 2012; Verdoorn, 2012). Therefore, the use of alternative, environmentally friendly products is being investigated and exploited for their nematicidal or nematostatic properties. Such products include those that are derived from plants or other biological sources such as bacteria (Mendoza *et al*., 2008; Hallmann *et al*., 2009), fungi (Hallmann *et al*., 2009) and other micro-organisms (Coyne *et al*., 2009).

Furfural (CropGuard®: Illovo Sugar South Africa Limited, Merebank, Durban, South Africa) is an example of a plant-derived product. It is a by-product of the sugarcane refining process and is registered as a contact nematicide on a variety of food crops in several countries including South Africa (Haydock *et al*., 2006; Coyne *et al*., 2009; CropLife, 2011). Locally, the product is registered as CropGuard® 900 EC with furfural (aldehyde) as the active ingredient at 900 g/L (CropLife, 2011). It has no known residual, and therefore no withholding period, after application in soils where a wide range of food crops are grown (CropLife, 2011; Anonymous, 2014;). Although the product is used on a relatively wide scale by local commercial crop producers, baseline information about its mode of action on the biology, ultrastructure, and physiology of economically important *Meloidogyne* spp. is lacking.

In vitro techniques have been used to study the effect of products with nematicidal or nematistatic characteristics on the motility of *Meloidogyne* spp. second-stage juveniles (J2) by various researchers (Nordmeyer and Dickson, 1989; Mendoza *et al*., 2008; Terefe *et al*., 2009). However, limited research has been focused on physiological processes such as respiration (Chitwood and Perry, 2009). Nordmeyer and Dickson (1989) studied the oxygen consumption rates ($\dot{M}O_2$) of *Meloidogyne* spp. J2 after they were suspended in nematicides. The $\dot{M}O_2$ of plant-parasitic nematodes is a valuable expression of their biological activity, and inhibition of the respiratory functions of an organism has a major effect on its energy metabolism (Kämpfe, 1978) as was demonstrated for *Meloidogyne* spp. J2 in the study by Nordmeyer and Dickson (1989). Oxygen consumption rate inhibition of other plant-parasitic and free-living nematodes has been documented when exposed to nematicides (Bhatt and Rhode, 1970; Ritzrow and Kämpfe, 1971; Reversat, 1977).

Although Nordmeyer and Dickson (1989) investigated the effect of various synthetic and biologically derived nematicides on the motility and $\dot{M}O_2$ of *Meloidogyne* spp. J2, no information is available on the effect of furfural. Therefore, to obtain a better understanding of the mode of action of furfural against *Meloidogyne* spp., the objectives of this study were to investigate the effect of four concentrations of CropGuard® on: i) the motility inhibition of *M. incognita* and *M. javanica* J2, ii) the ultrastructure of J2 of these species by means of scanning electron microscopy (SEM), and iii) the $\dot{M}O_2$ of J2 of these two species.

Local populations of *M. incognita* race 2 and *M. javanica* were reared in vivo in roots of tomato (cv. Floradade) in 20-L plastic pots in separate greenhouses (25 ± 1°C maximum temperature, 20 ± 1°C minimum temperature and 14-hr light:10-hr dark photoperiod). These populations were originally established from *M. incognita*-infected groundnut roots and pods (Northern Cape Province; 27.95° S, 24.85° E) and *M. javanica*-infected pumpkin roots (Limpopo Province; 25.88° S, 29.89° E), respectively. Species identification of both populations was done using the SCAR-PCR method (Zijlstra *et al*., 2000). Although *M. incognita* is not listed as one of the three major root-knot nematode species being associated with groundnut (Dickson & De Waele, 2005), this species has been listed as a parasite of the crop in South Africa (Keetch & Buckley, 1984; Kleynhans *et al*., 1996). Twenty-four-hour-old, hatched J2 from these two respective species were used for motility, SEM, and $\dot{M}O_2$ experiments. To obtain freshly hatched J2 of the two *Meloidogyne* spp., infected tomato roots of 30-d-old seedlings were removed from the pots in which they were grown and washed under running tap water. Eggs of each respective species were extracted separately using an adapted NaOCl method (Riekert, 1995). Second-stage juveniles of each of the two species were subsequently obtained by hatching the extracted eggs according to the method by Moura *et al*. (1993) at 25 ± 1°C. Second-stage juveniles that were recovered after the first 24 hr were discarded and only those collected after the next 24 hr were used for all the experiments.

Four concentrations (0.01, 0.1, 1, and 10% CropGuard®) and a tap water control were used in all experiments. CropGuard® concentrations were prepared by diluting the 10% stock product, which is recommended for commercial application (Anonymous, 2014), with sterile tap water. These concentrations were chosen since it is expected that dilution of the product would occur after irrigation or rainfall following product application. Two ml of each product concentration/treatment were transferred to plastic well-plates, and approximately 150 actively moving J2 of the two respective *Meloidogyne* spp. contained in 50-µl sterile tap water were added to each of the five different treatments in each well. The well-plates were subsequently incubated in a dark, temperature-regulated cabinet (25°C ± 1). Motility of the J2 was recorded 1, 24, 48, 72, and 96 hr after

exposure to the treatments by counting the number of motile and non-motile J2. (Ntalli *et al*., 2010). Second-stage juveniles were considered non-motile when no movement occurred after being observed for 30 s to 1 min using a stereo microscope at 100× magnification. Recuperation of the J2 was determined by washing them free from the various CropGuard® concentrations and the sterile tap water control and recording their motility after they had been incubated in freshly prepared, sterile tap water for 24 hr (Wuyts *et al*., 2006).

SEM studies on J2 of the *Meloidogyne* spp. were done after they were exposed to the same CropGuard® concentrations and the sterile tap water control as described for motility studies, with the exception that they were exposed to the five treatments for 96 hr only. Second-stage juveniles were prepared for microscopic evaluation by transferring them to a 70% ethanol solution for 12 hr, then dehydrating them twice in an ethanol series of 80, 90, and 100% for 15 min each. The J2 were then dried with liquid carbon dioxide in a critical-point-drying apparatus, mounted on SEM stubs with double-sided carbon tape, and sputter-coated with gold/palladium (67/33%). Ultimately, the body surfaces of the J2 were viewed under a FEI Quanta 250 ESEM (Electron Microscopy ETH, Zurich, Switzerland) operating at 10kV under high-vacuum mode.

To determine the $\dot{M}O_2$ of *M. incognita* and *M. javanica* juveniles, measurements were made in two separate experiments using a Strathkelvin Clark-type polarographic oxygen electrode (Model SI130) with an Oxygen meter (Model 782) in combination with two MT200A Mitocells (Strathkelvin Instruments, Scotland). The Mitocells consisted of a respiration cell and an oxygen electrode equipped with a polystyrene membrane at the bare electrode tip. The oxygen electrodes were calibrated at 25°C and at a specific partial oxygen pressure at the onset of each experiment as recommended in the instruction manual of Strathkelvin Instruments. All measurements were performed at 25ºC for 20 min. One Mitocell was used as the experimental and the other as the control cell. Second-stage juveniles of the respective *Meloidogyne* spp. were obtained after hatching the eggs as described above for the motility and SEM experiments. The J2 were contained in 500 µl of either a sterile tap water control or the same four CropGuard® concentrations used for the preceding experiments. Two thousand five hundred J2 that were exposed to the different treatments were subsequently pipetted into the experimental respiration chamber. Directly after each treatment sample was washed with sterile tap water using an 8µm-pore filter to reduce bacterial contamination before determining their $\dot{M}O_2$ (Nordmeyer and Dickson, 1989). No antibiotics were used during $\dot{M}O_2$ since it was reported by Nordmeyer and Dickson (1989) to affect the oxygen uptake by *Meloidogyne* spp. J2.

A plunger was inserted into each of the respiration chambers that displaced 200 µl of excess liquid along with about 1,000 J2 into the capillary and open top part of the vertical plunger. It was found after testing, that the nematodes that were confined in the plunger capillary did not affect or interfere with the J2 $\dot{M}O_2$ values of the 1,500 J2 left in the 300-µl experimental respiration chamber. Oxgen consumption rate measurements were subsequently done on J2 in the experimental respiration chamber that were left in the 300-µl respiration liquid that filled the chamber.

The exact volume of the liquid in each of the two respiration chambers was determined after the total body volume of the 1,500 J2, each represented as a cylinder, was calculated. This was done by determining the mean length and width for 20 J2 of each of the two *Meloidogyne* populations. A Nikon Eclipse 50*i* light microscope and the Nikon, NIS Elements Software programme (Nikon, Tokyo, Japan) were used for such measurements. The total J2 body volume, expressed in microlitre was subsequently subtracted from the 300-µl liquid that filled each respiration chamber. A mean body volume value of 0.677 µl for 1,500 *M. javanica* J2 was obtained. *Meloidogyne incognita* J2, which were generally longer than *M. javanica* J2, had a calculated body volume of 0.685 µl for 1,500 J2. The oxygen concentration (µMol l^{-1}) in the respiration chambers at the specific oxygen air saturation, temperature, and barometric pressure for *M. javanica* J2 and for *M. incognita* J2 was calculated (Gnaiger and Forstner,1983; Garcia and Gordon, 1992). The $\dot{M}O_2$ for J2 of the two respective species was measured as indicated in the following paragraph.

The control respiration chamber was filled with sterile tap water (without J2 added) and the background $\dot{M}O_2$ measured without stirring the liquid. The experimental chamber was then filled with 1,500 of *Meloidogyne* spp. J2 that were also suspended in sterile tap water and the $\dot{M}O_2$ was measured, also without stirring. Subsequently, the $\dot{M}O_2$ for J2 of the respective species that were exposed to the four CropGuard® concentrations were measured as described above by conducting the following three experiments as listed in the following paragraph.

One thousand five hundred *M. jacvanica* and *M. incognita* J2 were exposed for 1 hr (Experiment A) and 96 hr (Experiment B) to each of the four CropGuard® concentrations and the sterile tapwater control treatment and their $\dot{M}O_2$ was recorded. For Experiment C, to determine recovery after exposure to the CropGuard® treatments, 1,500 J2 from the two respective *Meloidogyne* spp. were first exposed for 96 hr to the four CropGuard® concentrations and the sterile tap water control treatment and then transferred to sterile tap water for 24 hr before recording their $\dot{M}O_2$ as described above.

Trial layouts for the motility and SEM studies were randomized complete block designs (RCBD). Five replicates were included for each treatment of the motility and SEM experiments and three for the

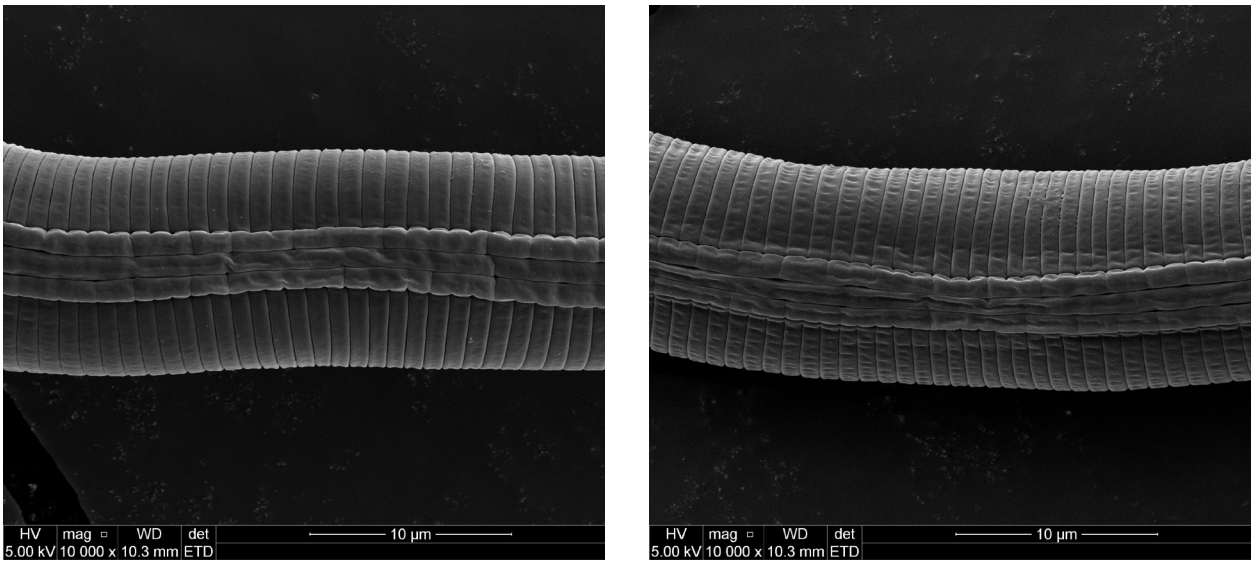

Fig. 1A and B. Scanning electron microscope micrographs of the cuticle surfaces of second-stage juveniles of *Meloidogyne incognita* (A) and *Meloidogyne javanica* (B) after being suspended in the sterile tap water for 96 hr.

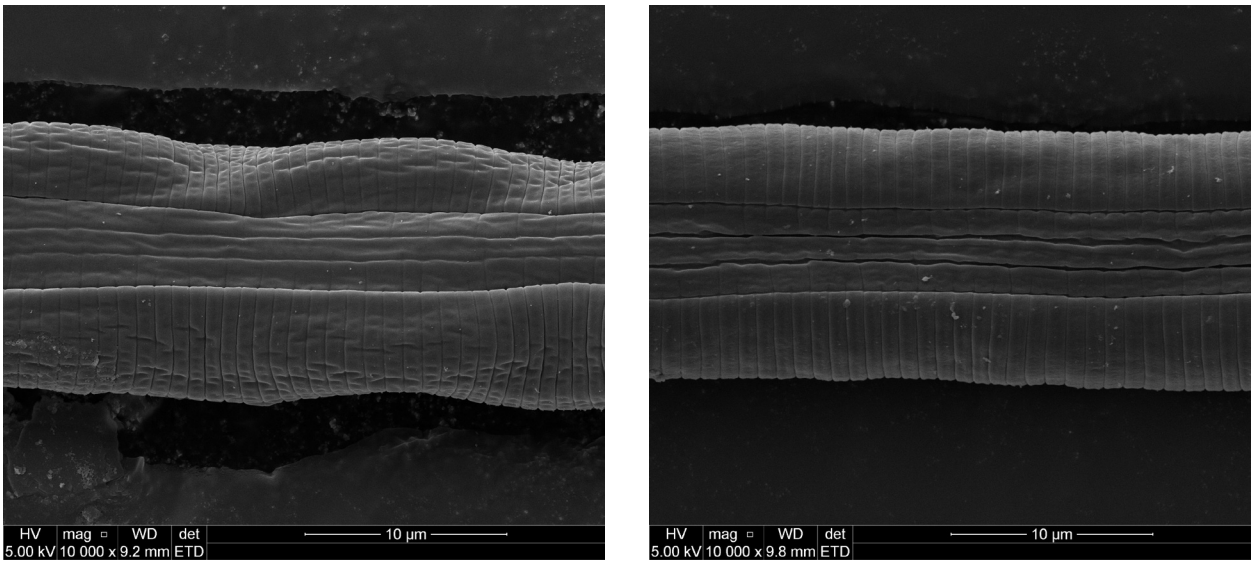

Fig. 2A and B. Scanning electron microscope micrographs showing the irregular appearance of the cuticle surfaces of *Meloidogyne incognita* (A) and *Meloidogyne javanica* (B) second-stage juveniles after being suspended in a 0.01% concentration of CropGuard® for 96 hr.

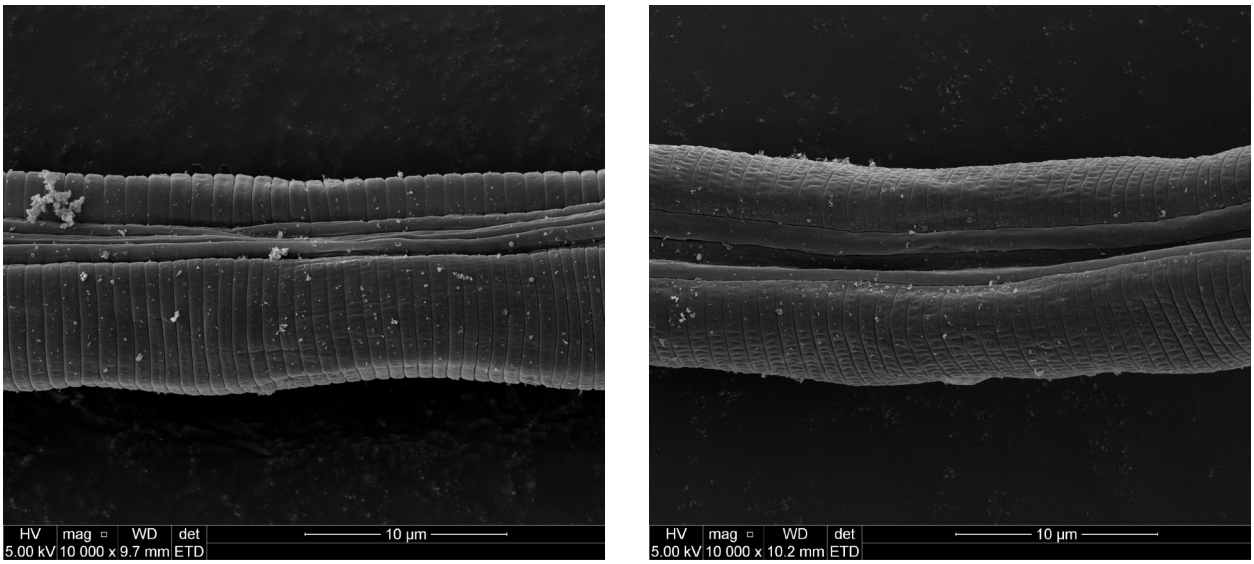

Fig. 3A and B. Scanning electron microscope micrographs showing the sunken lateral line structures on the cuticle surfaces of second-stage juveniles of *Meloidogyne incognita* (A) and *Meloidogyne javanica* (B) after being suspended in a 10% concentration of CropGuard® for 96 hr.

$\dot{M}O_2$ experiments. Second-stage juvenile motility data were subjected to repeated analyses of variance (MANOVA), and $\dot{M}O_2$ data were subjected to factorial analyses of variance using Statistica Version 11 (Statsoft Ltd, Bedford, United Kingdom). Means were separated by the Tukey test at $P \leq 0.05$. All experiments were repeated once to verify the results obtained.

Significant ($P \leq 0.05$) interactions existed between the four CropGuard® concentrations and exposure times for J2 motility for *M. incognita* (F ratio = 2) and *M. javanica* (F ratio = 23) (Table 1). Motility of J2 exposed to all the CropGuard® concentrations differed significantly ($P \leq 0.05$) from those of the sterile tap water control for all observation times. Motility in the four CropGuard® concentrations did not differ with either *Meloidogyne* spp. for the 24-, 48-, 72-, and 96-hr exposure times. However, for *M. javanica*, significantly more J2 were motile for the 0.01% CropGuard® after the 1 hr exposure time compared to the other three product concentrations. Motility was generally the highest for the 0.01% CropGuard® concentration and lowest for the 10% concentration for both *Meloidogyne* spp. during all exposure times. No recovery in terms of their motility was evident after transferring J2 to sterile tap water post exposure to the four CropGuard® concentrations and the sterile tap water control for 96 hr (data not shown).

SEM investigations of J2 suspended in the tap-water control and the four CropGuard® concentrations for 96 h showed no damage to the cuticle surface for either *M. incognita* (Fig. 1A) or *M. javanica* (Fig. 1B). However, an irregular appearance of the cuticle of J2 of both species was evident at the lowest (0.01%) CropGuard® concentration (Figs. 2A; 2B). The lateral lines of J2 suspended in the three higher concentrations (0.1, 1, and 10%) exhibited lateral line structures having a "sunken" appearance compared to those exposed to the sterile tap water control (Figs. 3A; 3B). Lateral lines of J2 exposed to the 0.1 and 1% CropGuard® treatments were similar to those for the 10% treatment and were therefore not included.

Oxygen consumption rates data for experiments A (Table 2), B (Table 2), and C (Table 3) showed significant ($P \leq 0.05$) differences among the different CropGuard® concentrations and the two *Meloidogyne* spp. for all three experiments with F ratio values of 4.4 (Experiment A), 12.58 (Experiment B) and 9.9 (Experiment C), respectively. For all three experiments, the $\dot{M}O_2$ of *M. javanica* was significantly ($P \leq 0.05$) higher than those of *M. incognita*, particularly for the sterile tap-water controls. The $\dot{M}O_2$ of *M. javanica* in the different CropGuard® concentrations were 67 to 200% higher than those of *M. incognita* (Table 2). Except for the sterile tap water control, the $\dot{M}O_2$ of the J2 of both species was highest in the 0.01% CropGuard® concentration and zero in the highest (10%) concentration for all three experiments.

The $\dot{M}O_2$ for *M. incognita* and *M. javanica* J2 that were suspended in sterile tap water was significantly ($P \leq 0.05$) higher than those of the four CropGuard® concentrations after 1 hr, except for the 0.01% treatment for *M. javanica* (Table 2). Results after 96 hr were similar to those for a 1-hr exposure although the $\dot{M}O_2$ for both species was ($P \leq 0.05$) lower. In contrast with *M. javanica*, the $\dot{M}O_2$ of *M. incognita* J2 exposed to the lowest (0.01%) CropGuard® concentration did not differ from those that were suspended in the sterile tap water control. This was contrary to those recorded for *M. javanica* where all CropGuard® concentrations differed significantly ($P \leq 0.05$) from the sterile tap water control.

Partial $\dot{M}O_2$ recuperation occurred after J2 of both nematode species were removed from the CropGuard® at concentrations of 0.1 and 0.01% and transferred to sterile tap water for 24 hr (Table 3). No $\dot{M}O_2$ recuperation of J2 was recorded after suspension in the 1 and 10% CropGuard® concentrations. The $\dot{M}O_2$ of J2 was reduced from 110 $\mu Mol \cdot h^{-1} g^{-1}$ to 53 $\mu Mol \cdot h^{-1} g^{-1}$ for *M. incognita* and from 197 $\mu Mol \cdot h^{-1} g^{-1}$ to 87 $\mu Mol \cdot h^{-1} g^{-1}$ for *M. javanica* in the sterile tap water control from the onset of the first experiment to termination of the last experiment (Tables 2 and 3).

In vitro J2 motility studies showed that all four CropGuard® concentrations inhibited the movement of these life stages of both *M. incognita* and *M. javanica* within 1 hr after exposure. Suspension of J2 of both *Meloidogyne* spp. in the CropGuard® concentrations also resulted in significant non-motility for up to 96 hr after exposure, indicating the ability of this bionematicide adversely affects the biology of J2 of these species. Ntalli *et al.* (2010) obtained similar results with a furfural compound that they extracted from *Melia azedarach* fruits and reported that furfural was the most active bionematicidal compound that they have tested against *M. incognita* J2. Although no recuperation of J2 of both species occurred after being transferred to sterile tap water post exposure to all CropGuard® concentrations, it is not known whether lack of motility can be equated with death of the J2 since vital stains or other mechanisms to determine actual mortality of the nematodes were not employed.

Scanning electronmicroscopy studies indicated that CropGuard® at the four different concentration levels studied did not result in any visible damage to the cuticle surface of the J2 when compared to those exposed to the sterile tap water controls. The only visible effects of the product on the J2 body cuticle was the slight irregular appearance of the cuticle at the lowest CropGuard® treatment and the sunken appearance of the lateral line structures of those suspended in the 0.1, 1, and 10% CropGuard®.These irregularities to the cuticle cannot be explained, but could possibly be ascribed to an osmotic effect that might also be be linked to their reduced motility as indicated in the motility experiments.

Oxygen consumption rate measurements confirmed that the biology of the J2 was adversely

Table 1. Number of motile *Meloidogyne incognita* and *Meloidogyne javanica* second stage juveniles after suspension in four different concentrations of CropGuard® for 1, 24, 48, 72, and 96 hr at 25°C.

	1 hr		24 hr		48 hr		72 hr		96 hr	
Treatments	*M. incognita*	*M. javanica*	*M. incognita*	*M. javanica*	*M. incognita*	*M. javanica*	*M. incognita*	*M. javanica*	*M. incognita*	*M. javanica*
Sterile tap water	150 (± 0)a[z]	150 (± 0)a	149 (± 1) a	149 (± 0.7) a	149 (± 1) a	148 (± 0.5) a	148 (± 0.8) a	149 (± 0.6) a	148 (± 0.8) a	148 (± 0.7) a
0.01%	33 (± 8) b	14 (± 5) b	2 (± 0.8) b	1 (± 0.8) b	2 (± 0.8)b	0 b	1 (± 0.6) b	0 b	1 (± 0.6) b	0 b
0.1%	9 (± 3) b	4 (± 2) c	1 (± 0.9) b	0.3 (± 0.4) b	1 (± 0.7) b	0 b	0.3 (± 0.5) b	0 b	0.3 (± 0.5) b	0 b
1%	1 (± 0.7) b	1 (± 0.7)c	1 (± 0.8) b	0.3 (± 0.5) b	1 (± 0.8) b	0 b	0.2 (± 0.4) b	0 b	0 b	0 b
10%	0.2 (± 0.3) b	0 c	0.5 (± 1) b	0 b	0.5 (± 0.7) b	0 b	0.2 (± 0.4) b	0 b	0 b	0 b

[z]Means in a column followed by the same letter do not differ significantly by Tukey's HSD Test ($P \leq 0.05$); Standard deviations from the means are listed in parentheses.

Table 2. The oxygen consumption rate ($\dot{M}O_2$ in µmol $O_2 h^{-1} g^{-1}$) of *Meloidogyne incognita* and *Meloidogyne javanica* second stage juveniles suspended in sterile tap water and four different concentrations of CropGuard® for 1 and 96 hr (Experiment B) at 25°C.

	Experiment A: 1 hr		Experiment B: 96 hr	
Treatments	*M. incognita*	*M javanica*	*M incognita*	*M javanica*
Sterile tap water	110 (± 12.2) aB[z]	197 (± 24.9) aA	12 (± 5.3) aB	25 (± 7.4) aA
0.01%	86 (± 19.5) bB	144 (± 36.2) abC	8.2 (± 1.3) abB	5.8 (± 3.6) bB
0.1%	47 (± 5.1) cB	98 (± 14.0) bcD	0 cB	4.1 (± 3.6) bB
1%	0 dE	53 (± 7.4) cdF	0 cB	0 bB
10%	0 dE	0 dE	0 cB	0 bB

[z]Means in the same columns followed by the same lower case letter do not differ at $P \leq 0.05$ by Tukey's HSD Test; Means for *Meloidogyne* spp. in the same row followed by the same upper case letter do not differ significantly at ($P \leq 0.05$) by Tukey's HSD Test; Standard deviations from the means are listed in parentheses.

Table 3. The oxygen consumption rate ($\dot{M}O_2$ in µmol $O_2 h^{-1} g^{-1}$) of *Meloidogyne incognita* and *Meloidogyne javanica* second-stage juveniles suspended in sterile tap water after being exposed to four different concentrations of CropGuard® for 96 hr (Experiment C) at 25°C.

Treatments	*M. incognita*	*M. javanica*
Sterile tap water	53 (± 11.0) aB[z]	87 (± 16.1) aA
0.01%	17.3 (± 6.8) bC	5.5 (± 3.6) bC
0.1%	3.3 (± 0.5) bcC	4.1 (± 1.1) bC
1.0%	0 cC	0 bC
10.0%	0 cC	0 bC

[z]Means in the same columns followed by the same lower case letter do not differ at $P \leq 0.05$ by Tukey's HSD Test; Means for *Meloidogyne* spp. in the same row followed by the same upper case letter do not differ significantly at ($P \leq 0.05$) by Tukey's HSD Test; Standard deviations from the means are listed in parentheses.

affected by the chemical. Inhibition of the respiration of the J2 of both *Meloidogyne* spp. occurred after they were suspended in the different CropGuard® concentrations for 1 and 96 hr, which was contrary to the higher $\dot{M}O_2$ of J2 suspended in sterile tap water controls for the same exposure period. Although some recuperation of *M. incognita* occurred after the juveniles were transferred to tap water post exposure to the four CropGuard® concentrations, $\dot{M}O_2$ results indicated that the respiration physiology of J2 of both species were inhibited to a large degree over the period of exposure.

Calculated $\dot{M}O_2$ for J2 of the same *Meloidogyne* spp. that were used in the studies of Nordmeyer and Dickson (1989) were respectively about 3 and 5 times lower at 28ºC than those recorded in our study at 25ºC. The most probable explanation for this discrepancy is that our methodology was slightly different. Nordmeyer and Dickson (1989) also used the Clark-polarographic oxygen electrode method, but they stirred the medium containing the J2 in the respiration chambers in their experiments. However, their stirring speed was not mentioned. We refrained from stirring because Marks and Sorenson (1971) and Moens *et al.* (1996) have shown that stirring at speeds above 60 rpm adversely affected respiration and mechanically killed nematodes. The higher $\dot{M}O_2$ obtained for J2 in our study compared with those obtained by Nordmeyer and Dickson (1989) could be that the specific gravity of nematodes, which is above 1.084 (Hooper, 1986), caused them to sink to the bottom of the respiration cell chamber where the Clark-polarographic oxygen electrode was installed. The resulting higher density of J2 directly around and above the oxygen electrode tip caused a higher rate of oxygen depletion of the liquid medium in the electrode vicinity. Thus, the $\dot{M}O_2$ obtained will be higher compared with actual $\dot{M}O_2$ values when no diffusion gradients occur in the respiration cell. The "inflated" $\dot{M}O_2$ values could still be used for testing products with nematicidal activity, however, by allowing sufficient time for all experiments for the nematodes to sink to the bottom of the respiration chamber in all the treatments. Other factors that could have caused higher $\dot{M}O_2$ obtained during our study could be differences in metabolic quality or activity between local *Meloidogyne* spp. populations or environmental conditions (Hussey and Janssen, 2002). It is advisable, in future work of this type, to include stirring of liquid medium in which the *Meloidoygne* spp. J2 are suspended using a slow baseline speed of 25 rpm as reported by Atkinson (1973) for the marine nematodes *Enoplus brevis* (Bastian) and *E. communis* (Bastian).

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