Crop Protection 106 (2018) 125–131

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Volatile organic molecules from *Fusarium oxysporum* strain 21 with nematicidal activity against *Meloidogyne incognita*

Willian César Terra[a,*], Vicente Paulo Campos[a], Samuel Julio Martins[b], Lilian Simara Abreu S. Costa[c], Júlio Carlos Pereira da Silva[a], Aline Ferreira Barros[a], Liliana Estupiñan Lopez[a], Thaisa Conrado Nunes Santos[a], Geert Smant[d], Denilson Ferreira Oliveira[e]

[a] Department of Plant Pathology, Federal University of Lavras, 37200-000, Lavras, MG, Brazil
[b] Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, PA, 16802, United States
[c] Embrapa Environment, 13820-000, Jaguariúna, SP, Brazil
[d] Laboratory of Nematology, Wageningen University and Research, 6708 PD, Wageningen, The Netherlands
[e] Department of Chemistry, Federal University of Lavras, 37200-000, Lavras, MG, Brazil

ARTICLE INFO

Keywords:
Bioprospecting
Volatiles
Plant-parasitic nematodes
Fusarium oxysporum

ABSTRACT

Volatile organic compounds (VOCs) produced by microorganisms are potential alternatives for the development of new nematicides. In a previous study, we identified VOCs produced by *Fusarium oxysporum* strain 21 (*F.o–21*). In this study, we tested the eight most abundant VOCs produced by *F.o-21* against *Meloidogyne incognita.* Compounds 2-methylbutyl acetate **(1)**, 3-methylbutyl acetate **(2)**, ethyl acetate **(7)**, and 2-methylpropyl acetate **(8)** led to *in vitro* mortality of 100%, 91%, 100%, and 82%, respectively, in second-stage juveniles (J_2) of *M. incognita* at a concentration of 500 µg/mL. The lethal concentration (LC_{50}) for compounds **1**, **2**, **7**, and **8** in *M. incognita* J_2, was 236, 198, 213, and 218 µg/mL, respectively. Under the same conditions, the commercial nematicide called carbofuran (2,3-dihydro-2,2-dimethyl-1-benzofuran-7-yl N-methyl carbamate) showed an LC_{50} of 191 µg/mL. Eggs exposed to compounds **2** and **7**, for 72 h showed up to a 90% reduction in hatching, and the compounds **1**, **2**, **7**, and **8** reduced *M. incognita* infectivity by 52%, 52%, 36% and 41%, respectively. When the compounds were applied in tomato seedlings infested by *M. incognita,* compound **1** reduced the number of galls per root gram by 22% when compared to the negative control (without the application of nematicide). The compound 2-methylbutyl acetate **(1)** showed potential to be used in the field after improvements in the application technology.

1. Introduction

Plant-parasitic nematodes (PPN) are responsible for a reduction of 10% in the world's food production, representing a considerable limiting factor for global agriculture (McCarter, 2008). Among the most devastating PPN are the sedentary root-knot nematodes (RNKs) *Meloidogyne* spp. *Meloidogyne incognita* is the most widely spread species from the genus *Meloidogyne,* and it parasitizes more than 2000 plant species (Sasser, 1980).

Currently, commercial nematicides based on chemical products are one of the foremost tools used to control PPNs. However, because of their extremely harmful potential impact to the environment, these products have faced increasingly restrictive regulations (Noling, 2002). To make matters worse, it is particularly difficult to find new molecules with nematicidal activity for commercial purposes. For instance, since 1970 there have been no commercial releases of efficient nematicides (McCarter, 2008). A new nematicide, Nimitz®, belonging to a new chemical class (Fluoro-alkenyle) was registered from the United States Environmental Protection Agency (EPA) in March 2015.

Volatile organic compounds (VOCs), which are naturally present in the ecology of plants and microorganisms, are considered potential molecules for the development of commercial nematicides (Campos et al., 2010). VOCs have up to 20 carbon atoms and show high vapor pressure and lipophilic character (Effmert et al., 2012). In recent years, considerable progress has been made in understanding the role of VOCs produced by microorganisms (mVOCs) in the multitrophic interactions from the soil. *Muscodor albus* is an endophytic fungus that releases VOCs capable of killing a broad range of plant pathogens (Grimme

* Corresponding author. Federal University of Lavras, Department of Plant Pathology, Lavras, University Campus, 37200-000, MG, Brazil.
E-mail address: vpcampos@dfp.ufla.br (W.C. Terra).

https://doi.org/10.1016/j.cropro.2017.12.022
Received 20 September 2016; Received in revised form 27 November 2017; Accepted 22 December 2017

et al., 2007). Companies intend to use *M. albus* and its volatiles for soil sterilization (Alpha et al., 2015). Non-pathogenic isolates of *Fusarium oxysporum* release VOCs with antifungal, nematicidal and plant growth-promoting effects (Freire et al., 2012; Zhang et al., 2015; Bitas et al., 2015; Costa et al., 2015). Gu et al. (2007) demonstrated the nematicidal activity of nine VOCs of microbial origin in *Bursaphelenchus xylophilus* Steiner & Buhrer at a concentration lower than 2 mmol/L. These and other previous studies in the literature described around 1000 VOCs produced by 480 bacterial and fungal species and are gathered in a specific data base (Lemfack et al., 2014).

In general, studies regarding the activity of VOCs against nematodes use the second-stage juvenile (J_2) (Freire et al., 2012; Terra et al., 2017), which is the infective stage of the RKNs. Therefore, there is little knowledge on the effect of such compounds in eggs, which are the main survival source of nematodes in the soil between harvests. Moreover, nematicides are likely to be more effective against J_2 than eggs (Eisenback & Hunt, 2009).

Considering the potential that VOCs have to control PPN, there were studies that allowed us to observe the production of VOCs with considerable nematicidal activity by *F. oxysporum* strain 21 (*F.o-21*) (Freire et al., 2012; Terra et al., 2017). Using gas chromatography-mass spectrometry (GC-MS), these studies detected 50 VOCs produced by this fungus. In this study, we investigated the nematicidal activity of the eight most abundant VOCs produced by *F.o-21*. Our objectives were: (1) to evaluate the *in vitro* nematicidal activity of VOCs against *Meloidogyne incognita* J_2, in a single concentration; (2) to calculate lethal concentrations for 50% (LC_{50}) of *M. incognita* J_2; (3) to evaluate the effect of VOCs on the eclosion of *M. incognita* J_2; (4) to test the infectivity of *M. incognita* J_2 originated from eggs exposed to VOCs in tomato plants; and (5) to test the infectivity of *M. incognita* J_2 in tomato plants after the direct application of the compounds in the infested substrate.

2. Material and methods

2.1. Acquisition of VOCs

The eight VOCs used in these experiments were 2-methylbutyl acetate **(1)**, 3-methylbutyl acetate **(2)**, 1-phenylethanol **(3)**, (R,R)-butane-2,3-diol **(4)**, ethanol **(5)**, 3-methyl-1-butanol **(6)**, ethyl acetate **(7)**, and 2-methylpropyl acetate **(8)**. They were obtained from commercial sources and used as received, without any further treatment. These are the most abundant VOCs produced by *F.o-21* (Terra et al., 2017). Aspects such as commercial availability and costs were also considered to select them.

2.2. Collecting M. incognita eggs and J_2

Pure populations of *M. incognita* were multiplied in tomato plants and kept in a greenhouse for three months. Galled roots were then separated from the soil, washed in tap water, and cut in pieces of 0.5 cm length. Eggs were then extracted from tomato plant roots by placing the roots in 800-mL glass Mason jars with 0,5% NaOH for 2–3 min according to the process described by Hussey and Barker (1973). Eggs were used in the experiments or set in a hatching chamber. All J_2 hatched between the second and third days were used in the experiments.

2.3. Culture of F. oxysporum

The isolate *F.o-21*, obtained from *Meloidogyne exigua* Goeldi egg's mass isolated from rhizospheres of coffee plants, was selected for this study because it has the capacity to produce toxic VOCs against *M. incognita* (Freire et al., 2012). This isolate was preserved in water, according to Castellani's method (Castellani, 1939), under the number (CML 3605) of the Coleção Micológica de Lavras from the Federal University of Lavras, MG, Brazil. In the experiments, from the stock culture, the *F. o-21* isolate was transferred to a malt agar medium (MA) (malt 20 g/L, agar 20 g/L) and incubated for 6 days at 25° C. A 10 mm plug was then taken from the border of the colonies and sub cultured to new plates with MA medium, where the fungus was kept at 25 °C until its use.

2.4. Nematicidal activity of VOCs in M. incognita

To evaluate the nematicidal activity of the eight selected VOCs against *M. incognita* J_2, an aqueous suspension (200 µL) with 250 J_2 and the VOC solution (1 mL) at a concentration of 600 µg/mL in an aqueous solution with Tween 80® at 0.01 g/mL were added to a polypropylene microtube (2 mL). This way, J_2 were directly exposed to solutions of different VOCs at a 500 µg/mL concentration. The microtubes were closed with a screw cap and sealed with a Parafilm® plastic film. A completely random design with six replicates per treatment was used. J_2 exposed to an aqueous solution of carbofuran (2,3-dihydro-2,2-dimethyl-1-benzofuran-7-yl N-methylcarbamate, 98%, Sigma-Aldrich) at 500 µg/mL was considered the positive control. The negative control was the solution used for compounds dissolution, Tween 80® at 0.01gm/L. After exposing the J_2 to the VOCs for 1 h or 24 h (independent assays), microtubes were opened, an aliquot (200 µL) containing approximately 40 J_2 was collected and was transferred to ELISA polypropylene micro-plates. To decide whether nematodes were alive or dead, one drop (10 µL) of 1 M NaOH was added into the well. The J2 that responded to NaOH by changing their body shape within 3 min were considered alive, and otherwise was considered dead, a technique described by Chen & Dickson (2000) and modified by Amaral et al. (2003). Only VOCs that resulted in the mortality of *M. incognita* J_2 above 80% were selected for the lethal concentration (CL_{50}) and the following assays. The experiments for both 1 and 24 h assays were carried out twice, and the two experiments were combined for the 24 h assay, since there was no significant interaction between treatment and experiment ($P = .2513$). For the 1 h assay the experiments were analyzed separately due to a significant interaction between treatment and experiment ($P \leq .001$).

To determine the corresponding lethal concentrations for 50% (LC_{50}) of the *M. incognita* J_2, the selected VOCs were evaluated once again at nine different concentrations. The compounds tested here were 2-methylbutyl acetate **(1)**, 3-methylbutyl acetate **(2)**, ethyl acetate **(7)** and 2-methylpropyl acetate **(8)**. J_2 nematodes were exposed to concentrations of 500, 400, 350, 300, 275, 225, 200, 150, and 100 µg/mL. A completely random design with five replicates per treatments was used. The exposure period of J_2 to the VOCs was 48 h. The values of LC_{50} to carbofuran were also determined.

2.5. Hatching of M. incognita J_2 from eggs exposed to VOCs

An aqueous suspension (2 mL) containing 5000 *M. incognita* eggs was placed in a hatching chamber, which consisted of a Petri dish 6 cm in diameter. Then, a VOC's solution of 10 mL at 600 µg/mL in aqueous solution of Tween 80® at 0.01 g/mL was added to the hatching chamber. Therefore, the final concentration of VOCs within the chamber was of 500 µg/mL. The tested compounds in this study were 2-methylbutyl acetate **(1)**, 3-methylbutyl acetate **(2)**, ethyl acetate **(7),** and 2-methylpropyl acetate **(8)**. The chamber was closed with a cover and sealed with a Parafilm® plastic film. The hatching chambers containing the eggs were then incubated at 28 °C in darkness. A completely random design with five replicates per treatment was used. The positive control consisted of carbofuran at 600 µg/mL (final concentration in the chamber was 500 µg/mL). The negative control was the solution used for compounds dissolution, Tween 80® at 0.01 g/mL. After 72 h, the hatching chamber was opened to identify hatched J_2. Eggs residue in the hatching chamber was washed with tap water and placed in the hatching chambers filled with tap water. After 72 h, the hatched J_2 were quantified again.