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Efficacy of dimethyl disulfide (DMDS) against *Meloidogyne* sp. and three formae speciales of *Fusarium oxysporum* under controlled conditions

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ABSTRACT

The efficacy of a soil disinfectant, dimethyl disulfide (DMDS), was assessed on known populations of different formae speciales of *Fusarium oxysporum*: *F. oxysporum* f. sp. *radicis-lycopersici*; *F. o.* f. sp. *radicis-cucumerinum* and *F. o.* f. sp. *lycopersici* and *Meloidogyne* nematodes. Two trials were carried out over two years (2012–2013), in a greenhouse under semi-controlled environmental conditions and in a growth chamber, in 3-L volume pots filled with sterile vermiculite. Two doses of DMDS (400 L ha^{-1} and 600 L ha^{-1}) and two plastic covers: VIF (virtually impermeable film) and PE (transparent polyethylene) were tested. The results showed a full nematicidal effect for the two doses, without differences between the two types of plastic. The inoculum density of *F. oxysporum* was significantly decreased by DMDS, but expression of the disease was not prevented. The assessment of the phytotoxic effect on seed germination of lettuce showed that residual vapors of DMDS have a negative effect on seed germination in vermiculite.

1. Introduction

Dimethyl disulfide (DMDS) is found in several natural sources, especially in plants belonging to the Alliaceae and Brassicaceae families. This compound has been demonstrated to control nematodes, soil-borne pathogens and weeds (Coosemans, 2005; Fritsch, 2005). The mode of action of DMDS primarily affects mitochondrial respiration through blocking the activity of the enzyme cytochrome oxidase (Auger and Charles, 2003). Dimethyl disulfide has been considered as a chemical intermediate in refineries and the chemical industry for a long time, and its biological properties have been characterized and the compound was finally patented in December 2012 by Arkema (France) in Europe after decades without new soil fumigants. Dimethyl disulfide (Paladin/Accolade™) is nowadays commercially available in the USA, Israel, Morocco, Turkey, Jordan, and Egypt, to control nematodes and other soil-borne pathogens of vegetable crops (Arnault et al., 2013). Nematodes of the genus *Meloidogyne* and two formae speciales of *Fusarium oxysporum*: *F. oxysporum* f. sp. *radicis-lycopersici* (Forl), *F. oxysporum* f. sp. *radicis-cucumerinum* (Forc) are among the main diseases of tomato and cucumber crops.

Changes in modern agriculture have increased the importance of *Meloidogyne* in recent years, due to a high dissemination rate and a high frequency of infestation (Elling, 2013) and the incidence of *Meloidogyne* sp. in protected horticultural crops of Southeastern Spain constitutes a key factor (Greco and Esmenjaud, 2004). It is difficult to obtain accurate data on yield losses caused by nematodes, because they depend on different factors, such as populations in the soil at the beginning of the crop, the growing season, environmental conditions, resistant cultivars, and the use of rootstocks. Some authors have reported that this range fluctuates between 20% and 40% yield losses, and the mean yield loss is estimated to be about 30.8% of total production (Verdejo-Lucas

Abbreviations: Dimethyl disulfide, DMDS; transparent polyethylene of 50-μm thickness, PE; virtually impermeable film, VIF; *Fusarium oxysporum* f. sp. *radicis-lycopersici*, Forl; *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, Forc; *Fusarium oxysporum* f. sp. *lycopersici*, Fol; days after treatment or planting, dat.

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et al., 2002; Talavera et al., 2012).

F. oxysporum f. sp. *radicis-lycopersici* (Forl) causes a disease known as "tomato crown and root rot". This forma specialis was described for the first time in 1978 (Jarvis and Shoemaker) and has extended to several countries in the world including those in Europe (Leary and Endo, 1971; Jarvis et al., 1975; Krikun et al., 1982; Couteaudier et al., 1984; Malathrakis, 1985). In Spain, it was reported for the first time in tomato crops in greenhouses in the region of Murcia in 1985 (Tello and Lacasa, 1988).

F. oxysporum f. sp. *radicis-cucumerinum* (Forc) causes a disease known as "root and stem rot" of cucumber, which was first observed in Greece (Vakalounakis, 1996) and has subsequently been observed in different countries (Punja and Parker, 2000; Cerkauskas and Brown, 2001; Tesoreiro, 2003; Vakalounakis et al., 2004). In Spain, this disease was noted in Almeria in cucumber crops for the first time in 2001 (Moreno et al., 2001).

The causal agent of Fusarium wilt of tomato, *F. oxysporum* f. sp. *lycopersici* (Fol), has been extensively studied, and was shown to be a significant disease in tomato crops in greenhouses and outdoors in Southeaster Spain (Tello, 1984).

These pathogens are routinely controlled through chemical soil disinfection. Currently, few disinfectants are authorized in the European Union (Metam-Na, Dazomet), and the withdrawal of the most effective soil fumigants (Methyl bromide, 1,3-Dichloropropene, Chloropicrin, 1,3-Dichloropropene and Chloropicrin) against fungi and nematodes has led to deficiencies in the control of soil diseases (Elling, 2013). Grafting onto tomato varieties is a generalized practice in greenhouses in Southern Spain (Hoyos, 2007). Although chemical soil disinfection is useful to control Forl and Fol, and Forc (Añaños et al., 2009; Palmero et al., 2011), it has shown a low efficacy in controlling soil populations of *Meloidogyne* (López-Pérez et al., 2006). The control of *Meloidogyne* sp. in horticultural crops is based mainly on the application of soil fumigants before planting, on nematicides after planting, as well as on the use of crop varieties carrying the resistance gene *Mi* (Talavera et al., 2012).

Dimethyl disulfide has been tested in the United States over two years (2010 and 2011) in microplots of vine crops, against *Pythium ultimum*, *Fusarium* spp. and nematodes (*Tylenchulus semipenetrans*, *Meloidogyne* spp.), and in field trials (Cabrera et al., 2014). In addition, DMDS has been tested against *Paratylenchus* spp. and *Mesocriconema xenoplax*). In China, Mao et al. (2014) studied the inoculum density of *Fusarium* sp. and *Phytophthora* sp., as well as the population of *Meloidogyne* sp. after treatment with DMDS. However, data regarding the inoculum density of fungi and the population of nematodes before the treatment are absent. Furthermore, DMDS was tested alone and in combination with chloropicrin to control Fusarium wilt disease in strawberry (Li et al., 2014). Moreover, several experimental field trials were conducted in Italy on tomato and melon (Leocata et al., 2014; Sasanelli et al., 2014) and in Spain on cucumber and pepper (Zanón et al., 2014). These trials were carried out in greenhouses that were naturally infested with root-knot nematodes. In all cases, the tested DMDS doses (from 300 to 400 kg ha^{-1}) showed low root-gall indices compared to the reference plants.

As has been cited, several studies on the nematicidal effect of DMDS on soils naturally-infested with *Meloidogyne* have been performed, but none under controlled conditions with artificial inoculations. Moreover, there is lack of information concerning the effect of DMDS on the above mentioned three different formae speciales of *F. oxysporum* (Forl, Fol and Forc). The aim of the present study was to assess the effect of DMDS on artificially inoculated populations of four main soil-borne pathogens: *Meloidogyne* and Forc, Forl, Fol, on bioassays carried out under controlled conditions.

2. Materials and methods

All trials included in this study were performed on pot bioassays containing an inert substrate: vermiculite (Termita, Spain) to avoid effects that might modify the efficacy of DMDS. The product was applied to the substrate by exposure to the gas for 14 days, and seeds were sown in the pots.

2.1. Trial conditions, experimental design and statistical analysis of data

Trials were carried out during two consecutive years (2012–2013). Each trial was subdivided into two parts. The first part consisted of inoculation (each pathogen was inoculated separately), colonization with the pathogen proper, and chemical treatments with DMDS. Greenhouse trials were carried out in one greenhouse in the experimental field of UAL-Anecoop of the University of Almería over 14 weeks. The second part involved bioassays with pathogen-susceptible plants in a growth chamber (14 h light, 16,000 lux) for 10 weeks. Each trial lasted for 6 months. Temperatures were recorded every 30 min with data loggers (HOBO Pro V2 Weatherproof, MA, USA). Each data logger had two sensors, which recorded environmental temperature (external sensor) and the temperature in the substrate (internal sensor).

Two different doses of DMDS were evaluated: 400 and 600 L ha^{-1} (9.8 and 14.7 g of active ingredient per kg of vermiculite, respectively), combined with two plastic films (PE: polyethylene) (Solplast, Spain) of 50 μm thickness or a virtually impermeable film cover (VIF) (Solplast) of 30 μm thickness. For each of the treatments of the control pots without DMDS application, four replicates (pots) were used. During the application of DMDS EC (94.1%) (Paladin®, Arkema, France), the product concentration never exceeded 0.25%, which is recommended for drip irrigation in protected crops. Immediately after applying the product, pots were individually sealed with the plastic cover. The fumigation period lasted 14 days and subsequently, plastic covers were removed to allow substrate ventilation and gas dissipation. Once phytotoxicity was evaluated (section 2.4), substrate samples were taken, to measure the inoculum density of formae speciales of *F. oxysporum* and the amount of mobile juveniles (J2) of *Meloidogyne* sp. Subsequently, four tomato seeds cv "Rio Grande" (Ramiro Arnedo, Spain) were sown in each pot for the Forl, Fol and nematode treatments, whereas cucumber seeds cv Marketmore (Ramiro Arnedo, Spain) were used for the Forc treatment. For *Meloidogyne* sp. trials, plants were monitored for 10 weeks and then were removed from the pots and were evaluated for their height (cm), root length (cm), biomass (g dry matter per plant), root galls and the number of juveniles (J2) of *Meloidogyne* sp. present in the substrate (explained below). The density of *F. oxysporum* in the substrates was measured after the treatments and the reduction in inoculum density was calculated as a percentage. The roots of sampled plants were also observed and an evaluation of symptoms was carried out in the Forc and Forl treatments on a scale of 0–4 (Parke and Grau, 1992). For Fol, which causes vascular wilt, cross-sections of the plant stem were taken along its length and these were disinfected and plated onto selective medium for *Fusarium* (Komada, 1975) to check vascular necrosis and subsequent *F. oxysporum* growth on the tissue. Differential disease expression was calculated with respect to the severity of the control test disease in each fungal pathogen according to Abbott's formula (Mao et al., 2012, 2014):

$$Y = \frac{Xi - Xf}{Xi} * 100$$

where Y is the percentage reduction in the density of inoculum or