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Nematodes as indicators of fumigant effects on soil food webs in strawberry crops in Southern Spain

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ABSTRACT

The phase-out of methyl bromide due to concerns regarding ozone depletion in the stratosphere has imposed the need of developing alternatives less aggressive to the environment. The use of 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) has extended in the last years, and has become essential to maintain strawberry production in Southern Spain, the main producer within the EU. However, their uncertain effects on the environment have become a new obstacle for their future use, and scientific evaluation of their toxicity is necessary to assess their impact on the environment. In this paper, we use the nematode assemblage as indicator of the effects of 1,3-D, Pic and 1,3-D + Pic on non-target soil fauna, and to infer their effects on soil food web functioning in two commercial strawberry farms in Southern Spain. Although affected, the abundance of bacterial-feeding nematodes did not differ among treatments due to compensatory growth of opportunistic nematodes. Fungal-feeding nematodes were strongly reduced by the fumigants, probably due to direct fumigants toxicity and to alterations on the fungal decomposition channel. Taxa richness and soil food web indices were also affected by the treatments. The ratio fungal to bacterial-feeding nematode abundances is proposed as the best indicator of the short and medium term effects of fumigants on non-target soil organisms. Implications of such findings on soil food web functioning and recovery are discussed.

1. Introduction

Spain is the main strawberry (*Fragaria ananassa* Duch.) producer within the European Union. In 2006, 330,485 tons of strawberry fruit were produced in Spain, 90% of which was destined for export. Spanish strawberry crops generate 290,400 millions euro a year, and cover 8296 ha (MARM, 2008). The most important area of strawberry production in Spain is located in Andalucía (Southern Spain), with 85% of the strawberry cultivation surface of the country and 97% of the production (MARM, 2008).

Since the phase-out of methyl bromide (MeBr) by the Montreal Protocol due to the concerns regarding ozone depletion in the stratosphere, multiple efforts have been carried to develop alternatives more respectful with the environment. With the end of the grace period conceded to Spain for critical uses, such as strawberry crops, soil fumigants as 1,3-dichloropropene (1,3-D; $C_3H_4Cl_2$) and chloropicrin (Pic; CCl_3NO_2), which have been proved to be effective MeBr alternatives, have emerged as likely candidates for substitution in strawberry crops (Porter et al., 2006; Fennimore et al., 2008; García-Méndez et al., 2008; Gilreath et al., 2008). Soil fumigants are necessary to maintain soil fungi (*Phytophthora*, *Fusarium*), plant-parasitic nematodes (*Meloidogyne*, *Pratylenchus*) and weeds under damage levels in strawberry crops (De Cal et al., 2005; García-Méndez et al., 2008; Schneider et al., 2008). However, new evidences of the environmental effects caused by 1,3-D and Pic are jeopardizing again commercial strawberry production. Decision 2007/619/CE established the non-inclusion of 1,3-D in the Annex I of Directive 91/414/EEC (The Council of the European Communities, 1991). According the Article 4 of such Directive, European Member States shall ensure that a plant protection product (PPP) is not authorized unless its active substance is listed in Annex I, and therefore the inclusion of the active substances of a PPP in such Annex is mandatory to place them on the European market. Decision 2007/619/CE recognized that 1,3-D is currently used as a substitute of MeBr, and that its withdrawn would produce an increase of the application of MeBr. Thus, a 18 months period was granted to Member States for selling and using PPP with 1,3-D, which can be extended for another 18 months depending on the effect of the non-inclusion decision on the use of MeBr. Indeed, until final decisions on the inclusion of Pic and 1,3-D on Annex I of the European Directive 91/414/EEC are available, it is important to gather as much scientific information as possible regarding their behaviour and effects on the environment.

The use of virtually impermeable films (VIF) has significantly reduced the amount of 1,3-D and Pic required for soil disinfestation (Porter et al., 2006). Impermeable films retain volatile soil

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fumigants in the soil during the first hours after application, and by increasing soil temperature, accelerate the degradation rate of the product. Ten days after the application, little chloropicrin should remain in the soil (Wang et al., 2006). Monitoring programs of 1,3-D contamination of ground waters in areas subjected to continuous use of fumigants have been developed in several European countries, and although 1,3-D residues above permitted levels were almost inexistent, further studies are required to evaluate fumigant effects on the environment (Terry et al., 2008).

Although economic viability and effectiveness of methyl bromide alternatives have been extensively studied, few information is available regarding medium- and long-term effects of 1,3-D and Pic on non-target soil organisms. Soil fumigants may reduce the potential nitrification rates in more than 55%, microbial C up to 23%, and alter soil respiration. In general, fungal populations are more affected by fumigation than microbial biomass (Stromberger et al., 2005; Collins et al., 2006).

European Directive 91/414/EEC establishes the criteria that have to be used to evaluate the likely effects of using active substances and PPP on the environment, including the assessment of using such products on soil micro- and macro-organisms. Such evaluations do not consider, sometimes, the especial and specific characteristics (climatic, edaphic, agricultural, and cultural) of agroecosystems in Mediterranean areas. The new directive on Sustainable Use of Pesticides establishes, indeed, the necessity of using risk indicators of pesticide use, and support Member States in the development of new specific national indicators. Within this context, studying the effect of conventional management on biodiversity presents a large scientific and social relevance.

Regarding soil biodiversity, and the effects of PPP on soil organisms, directive 91/414/EEC requires acute and long term studies on their effects on earthworms (and sometimes on the Collembola *Folsonia*). Earthworms have been selected as soil indicators to perform risk assessment evaluations, but their very low abundance in Spanish agricultural fields makes necessary the development on new evaluation criteria with more suitable organisms. This necessity has been highlighted by the European Commission (CSTEE, 2000) and the European Food Safety Authority (EFSA, 2007), which coordinate the peer review of PPP within the frame of the directive 91/414/EEC. As some of the most abundant metazoans inhabiting the soil system, nematodes have been extensively used as indicators of soil disturbance, health, and functioning (Bongers, 1990; Popovici, 1994; Ekschmitt et al., 2001; Neher, 2001; Ferris and Bongers, 2006), and may be considered as an useful tool in bioindicator development. Four out of five metazoans are nematodes, they play fundamental roles in soil functioning, and their permeable cuticle puts them in direct contact with soil pollutants. Their utility as bioindicators in agricultural systems has been recognized by the European Commission through the Bio-Bio project (Biagini and Zullini, 2006).

The objectives of this paper were to (1) evaluate the effects of 1,3-dichloropropene and chloropicrin on non-target nematodes, (2) to infer soil food web condition and recovery under different 1,3-D and Pic applications, and (3) evaluate the adequacy of different nematode indicators to assess the effects of soil fumigants on the soil food web and its recovery through a cropping cycle.

2. Material and methods

2.1. Experimental design

Nematodes were studied in two commercial strawberry farms in Southern Spain, located in Moguer and Cartaya (Huelva province, Southern Spain). Mean annual air temperature is 18.1 °C and mean annual precipitation is 490 mm. In both farms, the soil presents a sandy texture and was amended with animal manure before tilled.

Table 1
Summary of the treatments applied in Moguer and Cartaya. Formulation of the products, rate of application, number of plots, and number of replicates sampled per plot is indicated.

Treatments	Formulation	Rate	Plots/reps.
Moguer			
1,3-D + Pic/VIF	Pic 44% + 1,3-D 80.3%	30 g/m^2	3/3
Pic/VIF	96%	30 g/m^2	3/3
Control/VIF	–	–	3/3
Cartaya			
1,3-D + Pic	Pic 44% + 1,3-D 80.3%	50 g/m^2	1/5
Pic	96%	40 g/m^2	1/5
1,3-D	97.5%	13 g/m^2	1/5
Control	–	–	1/5

In Moguer, a 0.32 ha experimental plot was used to test the efficiency of 11 chemical pesticides in controlling soils pests and diseases. Each treatment had three replicates composed by three beds 30 m long randomly distributed. Effects of soil fumigants on non-target nematodes were studied in plots treated with 1,3-D + Pic (chloropicrin 44% (33.3% w/w) + 1,3-dichloropropene 80.3% (60.8 w/w)), Pic alone, and in a non-treated control.

Both PPP were applied at 30 g/m^2 by injection at 20 cm depth. Beds were covered with VIF at the same time that fumigants were applied. Non-treated controls went under the same process but did not receive any chemical treatment. Plastic irrigation tubing was situated in the top of the beds under the VIF cover.

In Cartaya, four treatments were established. 1,3-D (97.5% w/w 1.180 g/L), 1,3-D + Pic (chloropicrin 44% (33.3% w/w) + 1,3-dichloropropene 80.3% (60.8 w/w)), and Pic alone were applied by soil injection at 20 cm depth, and a non-treated control did not receive fumigants. Each treatment included three beds 75 m long (50 m in the control), separated by a 50 cm wide furrow.

A summary of the treatments applied and experimental design in both farms is shown in Table 1.

2.2. Soil sampling and nematode identification

Soil samples were taken at 0–20 cm depth at regular intervals along the three beds that composed each treatment. Three samples per replicate were collected in Moguer the day before and 31 days, 22 weeks and 35 weeks after the application of the treatments (September 10th, 2007, October 11th, 2007, February 12th, 2008, May 22nd, 2008). Camarosa strawberry plants were planted 3 weeks after soil fumigation, and then covered by open plastic greenhouses. Each greenhouse covered three beds in which one treatment was applied.

In Cartaya, five soil samples were collected in each treatment the day before and 30 days, 22 weeks and 31 weeks after the application of the treatments (October 9th, 2007, November 8th, 2007, March 11th, 2008, May 21st, 2008). Last sampling date was 2 weeks before the end of the harvest in both farms. Soil samples were stored at 4 °C in the laboratory until processing. Nematodes were extracted by a modification of Baermann funnels (Barker, 1985) and counted under a dissecting microscope. Then, 200 nematodes were identified at genus/family level after Bongers (1989).

Nematode taxa were classified into five main trophic groups: bacterial-feeders, fungal-feeders, plant-parasites/herbivores, omnivores, and predators (Yeates et al., 1993). Besides, they were classified along the colonizer–persiter (c–p) scale (Bongers, 1990). The c–p scale classifies nematode families into five groups from c–p 1 (enrichment-opportunistic nematodes, bacterial-feeders, with high reproduction rates and short life cycles) to c–p 5 (large nematodes with low reproduction rates and long life cycles, very sensitive to soil perturbation) (Bongers, 1990). The ratio of the