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Combination of biosolarization and grafting to control *Meloidogyne incognita* in greenhouse pepper crops

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ABSTRACT

The efficacy of combining biosolarization and grafting as integrated control of *Meloidogyne incognita* was evaluated in greenhouse pepper crops in south-east Spain. Field trials were conducted over a three consecutive year period in four greenhouses with soils naturally infested with *M. incognita* populations, virulent (two greenhouses) or avirulent (two greenhouses) to the *Me3* gene. The soils were biosolarized (for 6 weeks from August) using fresh sheep manure as organic amendment. Susceptible varieties were grafted onto Atlante (heterozygous *Me3*) rootstock. The combined use of grafted plants + biosolarization was found to reduce the severity and incidence of the nematode disease more effectively than biosolarized soil only strategies, in greenhouses with populations virulent to *Me3*, as measured by the gall index (0.3 *vs.* 5.3 or 1.5 *vs.* 3.3) and the percentage of infected plants (16.6% *vs.* 96.6% or 43.3% *vs.* 71.1%). However, in cases of greenhouses containing populations avirulent to *Me3* no differences were observed. In the greenhouses with populations virulent to *Me3*, marketable yield obtained from grafting + biosolarization treatment was 1.1 kg m^{-2} higher than that obtained from biosolarization treatment alone in four of the six trials. However no increase in production in greenhouses with populations avirulent to *Me3* was observed. Grafting + biosolarization proved to be an effective and durable method for controlling *M. incognita* in greenhouse pepper crops. Moreover, biosolarization reduces the risk of the development of populations capable of overcoming resistance conferred by *Me3* gene.

1. Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are a major problem in most intensive horticultural crops, leading to significant losses in yields (Collange et al., 2011; Rudolph et al., 2015) and arising associated costs from the complementary treatments required to decrease root damage. Even low population densities of root-knot nematodes before transplanting can cause serious damage (Ploeg and Phillips, 2001), particularly in crops with a long growing season, such as tomatoes or peppers. To reduce or prevent this potential damage, soil disinfection treatments during the pre-planting period are recommended, even when juveniles are not detected in pre-planting soil samples.

Since the phasing out of methyl bromide as a soil disinfectant in 2005, *Meloidogyne incognita* has become a growing problem in over 40% of greenhouses in south Spain, even with the use of 1,3-dichloropropene + chloropicrin as alternative (Guerrero et al., 2006, 2013; Ros et al., 2014a,b; Talavera et al., 2012). Restrictions on the use of soil fumigants have driven researchers to seek alternative pest management strategies (Bonanomi et al., 2007; Colla et al., 2012; Kokalis-Burelle et al., 2009; López-Pérez et al., 2005). Such is the case with root-knot *M. incognita*, especially in organic crops (Oka et al., 2007). Crop rotations with non-host cover crops of Brassicas for biofumigation or biosolarization, and biosolarization using different amendments (animal manures, urban waste or residues, agricultural by-products, green manures, etc.) have been studied in recent years as alternatives to chemical soil disinfection (Basallote-Ureba et al., 2016; Melero-Vara et al., 2012; Oka, 2010; Ros et al., 2016; Rudolph et al., 2015).

In attempts to control *Phytophthora* in greenhouse pepper crops in Murcia, biosolarization methods have proven highly efective and long lasting (Lacasa et al., 2015). However, attempts to control *M. incognita* using biosolarization over periods of several years were observed as deficient in some greenhouses (Guerrero et al., 2006, 2013; Ros et al., 2011). Moreover, the initiation of biosolarization in August means that the finalization of the previous crop cycle must be brought forward by

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two months, resulting in yield losses, raising concerns among farmers. However, initiating biosolarization with amendments at times more compatible with the crop cycle were found to be less effective (Guerrero et al., 2013; Núñez-Zofío et al., 2013), causing deficiencies to appear at an earlier stage in the following crop. In crops with a long growing season, such as peppers, chemical treatments with general/broad spectrum or specific fumigants, whether combined with solarization or not, may also be deficient (Cenis and Fuchs, 1988; Stapleton and DeVay, 1983). In both cases, any deficiencies in disinfection can be offset with specific treatments during the crop cycle in order to prevent increases in the nematode population and subsequent damage to crops.

The use of genetic resistance to control *Meloidogyne* in pepper has been evaluated by grafting onto resistant rootstocks, since introducing resistance into commercial varieties is both laborious and complex. At present there are three known genes that confer resistance to *M. incognita* in pepper: the N gene, known since 1957 (Fery et al., 1998; Thies and Fery, 2000) and genes *Me1* and *Me3* (= *Me7*), known since 1985 (Castagnone-Sereno et al., 2001; Djian-Capolarino et al., 2001, 2007; Fazari et al., 2012; Hendy et al., 1985). Several commercial and experimental rootstocks carrying these genes, whether associated or not with *Phytophthora* spp. resistance, are widely available and are well adapted to the systems and conditions of pepper cultivation in greenhouses in south-east Spain. They also are compatible with commonly used varieties of pepper, and demonstrate good agronomic performance and production.

When plants grafted on rootstocks that are carriers of *Me3* gene are cultivated in a greenhouse naturally infested by *M. incognita*, resistance is normally overcome as from the second year of cultivation (Robertson et al., 2006; Ros et al., 2011, 2014), with rootstocks behaving there after in the same way as susceptible varieties. The ease with which *Me3* resistance can be overcome in greenhouses, where pepper is cultivated for 8–10 months, is reflected in laboratory studies in which isolates of *M. incognita* virulent to *Me3* gene evolved from avirulent isolates (Castagnone-Sereno et al., 1996, 2007). This requires the establishment of strategies pyramiding several resistance genes in the same rootstock (Djian-Caporalino et al., 2014), co-cultivation of plants with different genes, or a combination of grafting (of varieties carrying resistance genes) and partial soil disinfection during the pre-planting stage.

Biosolarization is used in over 100 ha of organically grown greenhouse pepper crops in Murcia. Grafting on certain rootstocks has the added interest of improving the behaviour of the scion variety against abiotic stresses, particularly salinity (Calatayud et al., 2010; Penella et al., 2013), temperature, and solar radiation (López-Marín et al., 2012).

The aim of this study was to assess the efficacy of combining biosolarization and grafting on rootstocks with resistance to *M. incognita* in greenhouses used for pepper cultivation in south-east Spain in order to establish strategies for long-term and sustainable resistance management.

2. Material and methods

2.1. Site and soil

Field trials in four experimental greenhouses were conducted at the "Torre-Blanca" Experimental Station of IMIDA in Murcia, south-east Spain (latitude 37°45′ N, longitude 0°59' W), over three consecutive years. The clay-loam soils (Haplic calcisols, Haplocalcids) hada pH of 7.5–7.8, an OM content of 2–3% and C/N ratio of 10.1–10.5. Before starting the trials, pepper crops had been cultivated in the greenhouses for over six years in non-disinfected soils, which were naturally infested with *M. incognita* race 2, (Robertson et al., 2006). In greenhouses 1 and 3 (GH1 and GH3), *M. incognita* populations had overcome the resistance conferred by *Me3* gene as a result of the repetitive cultivation of plants grafted on C25 rootstock before the beginning of the present assay (Atlante of Ramiro Arnedo S.A., heterozygous *Me3*) prior to starting our trials (Ros et al., 2014a,b). Susceptible varieties had been cultivated in greenhouses 2 and 4 (GH2 and GH4) and the nematode populations were avirulent to the *Me3* gene when the trial began (Ros et al., 2014a,b).

Nematode densities from each greenhouse were determined at the first year of the trials, before soil treatments for each whole greenhouse, and after soil treatments for each microplots, by taking composite soil samples (five cores from 10 to 30 cm depth from each plot), extracting nematodes (Flegg, 1967) and counting RKN juveniles.

2.2. Experimental design

Soil treatments in each greenhouse were arranged in a completely randomized design with three replications per treatment. The soil treatments were: i) biosolarization (BS) with 2.5 kg m^{-2} of fresh sheep manure (FSM) (initiated in August and covered with 0.05 mm thick transparent polyethylene film (PE); ii) Methyl Bromide (MeBr) 98:2 (Brom-o-gas, supplied by Dead Sea Bromide) at 30 g m^{-2} and covered with Virtually impermeable plastic film (VIF) 0.04 mm thick (Sotrafa S.A.); iii) non-disinfected soil. MeBr treatment was unavailable for GH3 and GH4 due to the phase out of MeBr in Europe in 2005.

The plots measured 60 m^2 in greenhouses GH1 and GH3, with three rows of 50 plants, and 48 m^2 in greenhouses GH2 and GH4, with three rows of 40 plants. The plants in all the plots were spaced at 0.40 m within the row and 1.00 m between rows (2.5 plants $\cdot m^{-2}$). One row (subplot of 20 m^2 in GH1 and GH3 and 16 m^2 in GH2 and GH4) consisted of grafted plants and another row was planted with ungrafted plants. One row of susceptible ungrafted plants acted as buffer between plots. The soil treatments were repeated in the same plots for three consective years and grafted or ungrafted plants were planted in the same row every year.

2.3. Soil treatments

Biosolarization was initiated during the third week of August each year. After removing the debries of the previous crop, each plot was tilled independently from the others, longitudinally, to avoid the mixing of soils, and prepared for disinfection. Fresh sheep manure (2.5 kg m^{-2}) was incorporated using a rototiller. Drip irrigation lines were installed at 0.50 m intervals between lines and 0.40 m between emitters (3 L$\cdot h^{-1}$) in the same line. Each plot was covered by PE film. Irrigation was applied two consecutive days for 4 h every day. The plastic film was removed after 6 weeks (Núñez-Zofio et al., 2013). During this period, non-disinfected plots remained dry and weed-free. The MeBr 98:2 was applied by cold fumigation the first week of November, retaining the plastic covers for two weeks. Composted sheep manure (2 kg m^{-2}) was added to the soil before planting in both MeBr-treated and non-disinfected plots.

2.4. Grafting, planting, and cultivation

The Japanese top graft procedure was used for grafting (Lee, 1994). Rootstock and scion hybrids were cut at a 45° angle below and above the cotyledons, respectively, and the rootstock and scion were fixed with a clip. Atlante (carrier *Me3*; Ros et al., 2014a,b) was grafted onto the following hybrid scions: cvs. Almuden (Syngenta Seeds, in GH1 and GH3), Ribera (De Ruiter, in GH2) and Pedrosa (De Ruiter, in GH 4). Each year of the respective trials, 38 day old plants were planted in the third week of December in GH1 and GH2 and the first week of January in GH3 and GH4. The trials were finished during the last week of July in GH1 and GH2 and the first week of August in GH3 and GH4. A drip system was used for irrigation and fertilisation, according to crop requirements. Pests were controlled according to Integrated Pest Management (CAARM, 2010).