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Effects of non-chemical soil fumigant treatments on root colonisation with arbuscular mycorrhizal fungi and strawberry fruit production

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ABSTRACT

The effects of biofumigation and soil heating on arbuscular mycorrhizal fungi (AMF) colonisation, strawberry growth and strawberry yield in pot experiments compared with untreated soil and chemical fumigation with dazomet were tested. Three different *Brassicaceae* species (*Brassica juncea*, *Eruca sativa*, *Sinapis alba*) were used as biofumigant plant green manure and soil heating was applied to simulate soil solarisation. Half of the plants were inoculated with indigenous arbuscular mycorrhizal fungi inoculum. With one exception (*E. sativa*) among the uninoculated plants, the treatments significantly decreased the mycorrhizal colonisation parameters compared with the untreated control. Dazomet displayed the greatest inhibitory effects on AMF establishment. In addition, the intensity and number of bands corresponding to *Glomus* spp. obtained with temporal temperature-gradient gel electrophoresis were lower for strawberry plants from biofumigant treatments than from the control. For the inoculated plants, there were almost no significant differences among the mycorrhizal colonisation parameters. The mass of leaves for the uninoculated and inoculated plants was higher for almost all non-chemical soil fumigant treatments compared with the control, except for heating of the uninoculated treatments. The number of strawberry fruits for the uninoculated biofumigant treatments was the highest, being higher than the values observed for the heating treatments, the chemical disinfection treatments and the control. There were no significant differences among the inoculated treatments. Biofumigation with *Brassicaceae* species resulted in higher soil organic matter and mineral nutrients and had a relatively small effect on AMF colonisation ($F\% = 59.0$, 80.3, 47.3 for Bj, Es and Sa, respectively) compared with uninoculated controls ($F\% = 84.3$). Despite the reduced AMF colonisation, biofumigation resulted in a higher fruit number and mass of leaves. Therefore, it represents a non-chemical soil fumigation method that should be applied in sustainable strawberry production.

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most intensively produced fruit crop. However, yields often suffer from soil-borne fungal pathogens (Mass, 1998). Therefore, chemical disinfection of the soil is widely used by growers. Methyl bromide was the most frequently used chemical disinfectant in the past (Martin and Bull, 2002; Shaw and Larson, 1999). In 2005, its use was prohibited due to its depletion of the ozone layer (U.S. Environmental Protection Agency, 1999; EC No, 2037/2000). Therefore, methyl bromide has been replaced by less harmful chemicals, such as 1,3-dichloropropene, which was phased out in 2009 in the European Union, chloropicrin (which will be phased out in 2013), dazomet, and others (Ajwa and Trout, 2004; García-Méndez et al., 2008; Mark and Cassells, 1999).

Therefore, the use of non-chemical control approaches has been encouraged as a sustainable alternative to chemical methods in agricultural plant defence. These approaches include various cultural practices (e.g., solarisation, biofumigation), induction of plant defence responses and application of biological control agents (Linderman, 1994; Charron and Sams, 1999; Fageria et al., 2005; Alabouvette et al., 2006). For soil solarisation, the soil is covered by a transparent plastic foil, which facilitates heating by solar radiation to temperatures that are detrimental to soilborne pathogens (Pinkerton et al., 2002). Biofumigation is used to suppress soilborne pests by taking advantage of toxic compounds that can be released from soil-incorporated tissues of plants. The biofumigant

Abbreviations: AMF, arbuscular mycorrhizal fungi; Bj, *Brassica juncea*; DSE, dark septate endophyte; Es, *Eruca sativa*; HPLC, high-performance liquid chromatography; Sa, *Sinapis alba*; TTGE, temporal temperature gradient gel electrophoresis.

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plants of the *Brassicaceae* family are characterised by high glucosinolate content. Glucosinolates are released into the soil, where they can influence microbial growth (Lazzeri and Manici, 2001; Seigies and Pritts, 2006). Isothiocyanates (glucosinolate hydrolysis products) are the most common *Brassicaceae* volatiles that have biofumigant properties (Gardiner et al., 1999; Schreiner and Koide, 1993), and they are chemically changed to the active degradation products of synthetic fumigants like dazomet, and other methylisothiocyanate generating active ingredients (Mattner et al., 2008). In addition, the incorporation of *Brassicaceae* residues can significantly modify the biological, physical and chemical properties of the soil. These plants act as green manure and thus enhance the fertilising effects of the soil and consequently affect the soil microbial community (Lazzeri and Manici, 2001; Seigies and Pritts, 2006). It has been demonstrated that the incorporation of different *Brassicaceae* biofumigant plants into the soil can decrease the populations of pathogenic fungi (Charron and Sams, 1999; Lazzeri and Manici, 2001; Martin and Bull, 2002).

Strawberry plants are generally colonised by mutualistic fungi: the arbuscular mycorrhizal fungi (AMF). A functional symbiosis enhances strawberry plant growth (Malusa et al., 2006; Vestberg et al., 2004) and increases resistance against pathogens in strawberries and other crops (Filion et al., 2003; Matsubara et al., 2004; Smith and Read, 1997). Thus, the use of current soil disinfection methods, including chemical control, biofumigant plants and solarisation, can affect the soil microbial population. However, little is known about the effects of these approaches on strawberry plant interactions with AMF. Therefore, the main aim of this study was to assess the effects of selected non-chemical soil fumigant treatments (biofumigation and heating) on AMF colonisation of strawberry plants and on strawberry plant growth and fruit production compared with untreated control plants and plants subjected to chemical fumigation. The diversity of the AMF associated with the strawberry roots was determined, and the species were identified using temporal temperature-gradient gel electrophoresis (TTGE) molecular technique and sequencing (Cornejo et al., 2004). In addition, the effects of indigenous AMF inoculum from a strawberry field on growth conditions and strawberry production were tested.

2. Materials and methods

2.1. Plant material, experimental design and management

The pot experiment was conducted in a greenhouse at the Agricultural Institute of Slovenia on strawberry plants *Fragaria* × *annanasa* Duch. var. 'Marmolada' obtained by micropropagation and acclimatised in a greenhouse at day/night temperatures of 22 °C/18 °C with a 14-h photoperiod for one month before transplantation in December 2003 (Berljak et al., 2003). The plants were transplanted into pots filled with treated soil and grown under greenhouse conditions with natural light at temperatures maintained above 20 °C for one year. The plants were watered manually according to their needs (approximately 3 l/pot/month).

Pots were arranged in a completely randomised experimental design with 15 replications (pots) and two factors: inoculation with AMF and soil treatment methods. The AMF inoculation group included uninoculated or inoculated soil. Inoculum was produced by growing the AMF for 4 months in maize (*Zea mays*) as host plants in soil collected from the organic strawberry field (Agricultural Institute of Slovenia). Inoculum potential was characterised using the level of AMF root colonisation of the host plant, where the mycorrhizal frequency (*F*%), mycorrhizal intensity (*M*%), and arbuscular density (*A*%) were assessed according to Trouvelot et al. (1986) (*F*% = 96.7; *M*% = 20.9; *A*% = 10.9) (Regvar et al., 2001).

The soil treatments included the following: 1. Indian mustard, *Brassica juncea* (L.) Czern. & Coss, 'Negro Caballo' (Bj); 2. arugula, *Eruca sativa* Miller, unknown variety (Es); 3. White mustard, *Sinapis alba* L., 'Asta' (Sa); 4. heating; 5. The chemical disinfectant dazomet and 6. untreated control.

The *Brassicaceae* plants were sown on 22 April (50 for Bj and Sa and 70 plants/m^2 for Es) and grown in the Agricultural Institute Experimental Field at Brdo pri Lukovici, Slovenia (latitude 47° 56′ 19″ N – longitude 11° 36′ 47″ E – altitude 350 m a.s.l.; soil characteristics in Section 2.2) for 44 days. The above-ground parts were collected just before flowering (11 June) and frozen at −20 °C. The average mass of plants in the field was 4.1 for Bj, 3.2 for Es and 2.3 kg/m^2 for Sa. For all treatments, soil from the experimental field was used. For the uninoculated first three treatments with *Brassicaceae* plants, 25 g of frozen and ground-up plants were added to 250 g of soil. Pots of uninoculated treatments 4, 5 and 6 were filled with 275 g of heated, disinfected or untreated soil. In the inoculated treatments, 100 g of soil was replaced with AMF inoculum. For the dry heat treatment, the soil was incubated at 37 °C for 200 h (Inkubator I 50, Kambič, Slovenia) to simulate the temperature regime of solarisation using transparent foil. In a preliminary soil disinfection field experiment, this regime was determined to be most effective under our climate conditions. For the chemical fumigation, the soil was manually treated with dazomet at 50 g m^{-2} of soil, according to the manufacturer's instructions (Basamid®, BASF, AG, Germany) and Lazzeri and Manici (2001). All of the treatments and the substrates were prepared 12 days before the strawberry plantlets transplanting, except for the treatment with dazomet, which was prepared two months in advance.

2.2. Soil and Brassicaceae plants analyses

The texture of soil used in experiment was silt loam (24.2% clay, 8.8% sand, 18.9% coarse silt, 48.1% fine silt) (Saxton et al., 1986). The supply of mineral nutrients in the soil, except P, was good according to the Slovene national soil classification (Mihelič et al., 2010). The soil parameters were measured before the experiment in July 2003 (pre-treatment) and after the experiment in October 2004 (post-treatment; ten months of strawberry growth in soil without added AMF inoculum). The organic matter content was determined according to ISO 14235:1998 Soil quality – determination of organic carbon by sulphochromic oxidation; the pH was determined according to ISO 10390:2005 Soil quality – determination of pH; and the total nitrogen was determined according to ISO 11261:1995 Soil quality – determination of total nitrogen-modified Kjeldahl method. The amount of available phosphorus (P_2O_5) and potassium (K_2O) in the soil was determined using the Egner–Riehm–Domingo method (Egner et al., 1960).

The total amount of glucosinolates in the plant dry mass was determined for the Bj, Es and Sa plants. Frozen plant samples were lyophilised, ground and analysed according to ISO 10633-1:1995 Oilseed residues – determination of glucosinolates content – Part 1: Method using high-performance liquid chromatography (HPLC). A Waters HPLC system was used (Milford, Massachusetts, USA), with a 600E pump, a 717 plus autosampler, and 996 diode-array detection.

2.3. Determination of strawberry root colonisation and plant growth

Root fungal colonisation was examined after seven weeks of growth for 10 randomly selected plants from the control and from