RESPONSE OF PHYTOPARASITIC NEMATODES AND MICROBIAL SOIL COMMUNITY STRUCTURE TO HIGH-TEMPERATURE TENT SOLARIZATION FOR DISINFESTING CONTAINER NURSERY SOIL

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ABSTRACT

Tent solarization is an alternative to chemical fumigation or steam treatment of soils for pest eradication. Diurnally-pulsed thermal inactivation using passive solar heating is the primary mode of action. However, biological and chemical processes are also involved, and the microbial community structure of the recolonized soil may play a role in long-term pest control. The goal of this work was to estimate recolonization processes, using soil phospholipid fatty acid analysis and respiration analyses, as related to nematode population dynamics and soil temperature regimen. Microbial community structure shifts during recolonization were much greater for treated soil at a higher temperature (single pulse at 73°C maximum) compared to moderately heated soil (single pulse at 46°C maximum). The soil recolonization rate as estimated by respiration rate was not different between the two heated treatments, indicating that the heating regimens tested did not reduce overall microbial activity or delay recolonization by the surviving community. The 73°C heat treatment completely eradicated both phytoparasitic and freeliving nematodes, while the 46°C treatment only reduced population densities. In general, freeliving nematode taxa were better able to tolerate the 46°C heating treatment than phytoparasites.

INTRODUCTION

Protecting the health of young plants during their establishment period is key to production of mature crops with optimal growth, quality, and yield. In many cases, vigorously established plants can tolerate far more pest or environmental stress later in their lifecycles than those attacked by pests during their initial growth. For that reason, protection of nursery plants from soilborne pests should be taken very seriously.

Treatments using soil fumigant chemicals (e.g., methyl bromide, 1,3-dichloropropene (Telone II^{R}), and metam sodium) are approved for nursery use, but they have issues regarding safety, environmental pollution, and for some, broad-spectrum efficacy. In light of these constraints, nursery operators will continue to need usable alternatives for soil disinfestation that are capable of complete elimination of soilborne pest populations with minimal toxicity and environmental risk. Also, organic nursery production, operations in developing countries, and home horticulturists require non-chemical and inexpensive soil disinfestation treatments. Certain adaptations of soil solarization (Stapleton, 2000), a non-chemical, passive, hydrothermal technique that can capture and accumulate solar heat energy under plastic film, are capable of heating soil to 60-70+°C, and so are good candidates for nursery applications (Stapleton, 2000). Although the primary mode of action of solarization is the physical heating of soil, research has shown that important biological and chemical elements also come into play (Stapleton, 2000).

Tent soil solarization is an approved method for eradication of phytoparasitic nematodes in horticultural applications (CDFA, 2004). For the approved treatment, disinfestation is accomplished by heating soil for one hour at 60°C, or 30 minutes at 70°C. An additional benefit may be recolonization of the soil by microorganisms that are antagonistic to subsequently invading nematodes, pathogens, or weed propagules. Little work has been done, however, to document biotic activity and recolonization in soils exposed to such high-temperature, short-exposure solarization. The goals of this research were to examine and compare microbial community activity and structural changes in soil that had been exposed to tent solarization at high temperatures.

MATERIALS AND METHODS

Soil Heating Treatments

Experimental soil was collected from a formerly agricultural field near Oakdale, CA, which had lain fallow with no pesticide or fertilizer applications for the previous 5 years. The soil was biologically active, receiving periodic precipitation and irrigation water, and having a mixed summer weed cover at the time of sampling. The soil was naturally infested with several phytoparasitic nematode species, including *Xiphinema, Paratrichodorus*, *Meloidogyne,* and *Mesocriconema*; as well as a variety of freeliving nematode taxa. Soil was sieved (10 mm mesh) to remove debris and root fragments, and placed into black polyethylene nursery bags (3.8 l capacity). Bags were watered to saturation and allowed to drain to field capacity overnight. Nine wooden cargo pallets (ca. 1.3 m x 1.3 m x 12.7 cm) were placed 0.7 m apart in a 3 x 3 orientation in the field, over sheets of 0.037 mm (1.5 mil)-thick black polyethylene film laying on the soil surface. Two of the prepared nursery bags were then placed near the center of each of the pallets. Soil heating treatments were then imposed by covering three pallets with a single layer of 0.05

mm transparent polyethylene film ("single tent"), leaving three pallets with bags uncovered, and placing control bags in deep shade. The pallets were arranged and covered in a completely randomized plot design. On each pallet, one of the two nursery bags were used for soil data collection, while the other was fitted with an external thermistor in the center of the soil mass, which was connected to a Hobo 'XT' micrologger (Onset Computer Corp., Pohasset, MA, USA) for continuous monitoring of soil temperatures at one minute intervals. The experiment was started at 1115 hours and incubated for one diurnal heating pulse. Air temperature and solar radiation data were taken from nearby California Irrigation Management Information System (CIMIS) meteorological station 194 ('Oakdale.A'; Stanislaus County), located about 5 km from the field site.

Soil Sampling and Handling

The morning after treatment, nursery bags were removed from the pallets and placed in a shaded screen house prior to 0800 hours to preclude additional solar heating. Aliquots of soil from each nursery bag were taken for nematode survival and microbial survival and activity testing. For nematode assays, 250 ml aliquots were taken; while for estimations of microbial activity, 100 ml aliquots were taken and immediately placed on ice for transfer to the laboratory. To prevent cross-contamination, each bag was sampled with a separate spatula and placed in sealable plastic storage bags. The nursery bags were then maintained in the shaded screen house for subsequent sampling in the same manner 7, 14, 28, and/or 42 days after treatment.

Effects of Treatments on Populations of Phytoparasitic and Freeliving Nematodes

Bags were sampled as before and 250 ml aliquots of soil were taken for nematode analysis one day and 42 days (6 wk) after treatment. Nematode samples were assayed microscopically following sieving and mist extraction. Voids in the bagged soil masses created by sampling were filled with silica sand to maintain homogeneous soil moisture and temperature conditions.

Effects of Treatments on Soil Respiration

Soil respiration measurements, calculated as CO_2 evolution rate, were made within 4 hours of soil sampling. The 160 g samples were placed into 250 ml reactors (May and VanderGheynst, 2001), aerated continuously with humidified air at 20 ml/min to avoid oxygen limitations, and incubated at 35°C. Carbon dioxide concentration was measured on the influent and effluent air of the reactors every 5 hours using an infrared CO_2 sensor (Vaisala, Suffolk, UK) and recorded using a data acquisition system. Carbon dioxide evolution rate (CER) was calculated from a mass balance on each reactor and cumulative respiration was calculated by integration of CER data with respect to time.

Effects of Treatments on Phospholipid Fatty Acid (PLFA) Composition

Prior to analysis, samples were stored at -20°C. PLFA analysis methods and nomenclature were adapted from Bossio et al. (1998). For each sample, total lipids were extracted from 8 dry g soil using 23 mL of a monophasic extraction mixture. The extraction mixture contained chloroform, methanol, and phosphate buffer at a ratio of 1:2:0.8 (v:v:v), with the volume of phosphate buffer adjusted to account for soil moisture content. After 2 h of extraction, the sample was centrifuged at 2500 rpm for 10 min and the supernatant decanted into a separatory funnel. The extraction was repeated for 30 min and the supernatant added to the separatory funnel along with 12 ml phosphate buffer, and 12 ml chloroform. Each sample was shaken for 2 min and then allowed to settle overnight, forming two phases. The chloroform layer containing the lipids was recovered and dried under N_2 at 32°C.

Lipid fractionation was performed using solid phase extraction columns with 500 mg silica gel (Fisher Scientific, Pittsburgh, PA). Columns were conditioned with 4 mL chloroform. Lipids were transferred to the column using 300 µl chloroform, followed by 700 µl chloroform. Neutral lipids were eluted with 6 ml chloroform, then glycolipids with 12 ml acetone, and finally polar lipids with 6 ml methanol. The polar lipids, including phospholipids, were recovered and dried under N_2 at 32°C. Transesterification of polar lipids was then carried out by mild alkaline methanolysis. Polar lipids were dissolved in 1 ml 0.2M KOH and 1 ml 1:1 (v:v) methanol:toluene, heated at 37°C for 15 min, and combined with 2 ml distilled H_2O and 0.3 ml acetic acid. Fatty acid methyl esters (FAMEs) were recovered by extracting twice with 2 ml hexane and then drying under N_2 at room temperature.

FAMEs were redissolved in 200 µl hexane containing 40 ng/µl methyl nonadecanoate (Sigma, St. Louis, MO), as an internal standard. Samples were analyzed using a Hewlett Packard 6890 Gas Chromatograph with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA). The injection was 2 µl with a 1:50 split, the flow rate was constant at 0.4 ml/min, and the temperature was 170°C followed by a 2°C/min ramp to 260°C. Peaks were identified using fatty acid calibration standards and microbial identification system software (MIDI, Inc., Newark, DE). Peaks that were not clearly resolved were reported as sums of possible fatty acids.

PLFA profiles revealed 16 fatty acids found in all samples. Other fatty acids, which were not consistently detected, accounted for less than 10% of total extracted PLFA and were removed from the analysis. Principal components analysis (PCA) was performed on mole percentage PLFA data, with moisture and organic matter content as covariables, using Canoco 4.5 (Microcomputer Power, Ithaca, NY) to reveal differences in microbial

community structure. Total PLFA provided a measure of microbial biomass (Zelles et al., 1992). Due to logistical constraints, respiration and PLFA analyses were done to compare only the tent and bagged heat treatments.

RESULTS

Soil and Air Temperature and Solar Radiation Accumulation

Soil temperature in the center of the soil mass in the single-tent treatment reached a maximum of 72.7°C. Soil temperature was above 70°C for 155 min; above 60°C for 527 min; above 50°C for 432 min; and above 45°C for 475 min. Soil temperature in the bag only treatment reached a maximum of 46.0°C, and the soil was above 45°C for 149 min. Soil temperature in the nonheated soil mass during the experimental period varied between 22-24°C. Air temperature during the one-day experimental time period at the nearby CIMIS meteorological station was 36.7°C maximum and 13.3°C minimum, and 282 watts per m^2 solar radiation were accumulated.

Effect of Soil Heating Regimens on Nematode Population Densities

The single pulse, 73°C maximum heat treatment eliminated all active phytoparasitic and freeliving nematode forms at the one day posttreatment assay. The 46°C maximum treatment reduced *Meloidogyne* sp. and *M. xenoplax* to undetectable levels, and *Xiphinema americanum* and *Paratrichodorus* sp. by 71.8% each. The total freeliving nematode soil population density was reduced by 36.3%. The assay taken 6 wk posttreatment from bags incubated in the screenhouse showed that the 73°C maximum treatment had completely eradicated the phytoparasitic taxa and virtually eliminated all freeliving nematodes as well. On the other hand, significant recolonization by all phytoparasitic taxa, except for *M. xenoplax,* and total freeliving nematodes had occurred in the bags treated at 46°C maximum.

Effect of Heating Regimens on Soil Respiration

There were no differences in moisture and organic matter content, or respiration between the two heating treatments, one day after exposure.

Effects of Soil Heating Treatments on PLFA Analysis

Total microbial biomass in the 73°C maximum soil treatment was 20% lower than the 46°C maximum treatment one day after exposure, and for the remaining 28 days of recolonization, as well. There were also differences in microbial community structure between soils. One day after exposure, the 73°C maximum soils were enriched in a mixture of iso and anteiso terminally branched and straight chain phospholipids, while 46°C maximum soils were enriched in iso and 10 Me branched saturated and monounsaturated phospholipids. Between days 1 and 14, 73°C maximum samples became more enriched in iso and anteiso branched saturated phospholipids, and then in Sum 9 (cy19:0ω10c/unk 18.846/unk 18.858) and Sum 7 (18:1ω7c/ω9t/ω12t) by day 28. In contrast, 46°C maximum samples were enriched in cyclopropyl and monounsaturated phospholipids 14 days after solarization, and in predominantly monounsaturated phospholipids by day 28.

DISCUSSION

Destruction of the nematodes tested in these experiments using heavily-infested soil indicated that tent solarization can provide eradication of phytoparasitic nematodes from containerized nursery soils without the risk or expense of chemical or steam treatments. Tent solarization may be used commercially in nursery operations in warmer climatic areas. It can be especially useful for nursery operations in developing countries, where steam generation may be technically or economically unfeasible, and safety precautions for use of highly toxic fumigant chemicals may be lacking. In general, freeliving nematode taxa were better able to tolerate the 46°C heating treatment than phytoparasites, as has been previously shown. Based on similar field results, along with the laboratory study of Ruiz et al. (2003), a simple, double-tent solarization method was developed, tested, modified, and approved by the California Department of Food and Agriculture (2004) as a nematicidal treatment for container nursery plants grown within the State. Tent solarization was also found to eliminate weed seed in soil and planting mixes (Stapleton et al., 2002).

Tent solarization significantly increased the soil temperature above the bag treatment and resulted in a larger shift in microbial community structure during recolonization. Enrichment of terminally branched iso and anteiso PLFAs has been observed in thermophilic compost communities and in gram-positive, spore forming bacteria (Marshall et al., 2004). The prevalence of these PLFAs in the tent treated soils one day after solarization and at 14 and 28 days suggests thermophilic communities survived high temperature solarization and persisted during recolonization. The shift in microbial community structure during recolonization of bag treated soils was small relative to tent treated soils. The PLFAs found in bag samples were predominantly monounsaturated fatty acids which are commonly associated with gram-negative bacteria and moderate, mesophilic ecosystems (Marshall et al., 2004). The presence of these markers and the negligible change in community structure suggest that temperature changes in these treatments were either too low or of too short duration to have a significant impact on

the microbial community. Rapid recolonization of the soil by members of the native microbial community may have implications on post-solarization pest control.

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