World J Microbiol Biotechnol (2010) 26:161–170
DOI 10.1007/s11274-009-0155-7

ORIGINAL PAPER

Application of thymol and iprodione to control garlic white rot (*Sclerotium cepivorum*) and its effect on soil microbial communities

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Received: 6 May 2009 / Accepted: 5 August 2009 / Published online: 15 August 2009

Abstract The effect of different dosages of thymol alone, iprodione alone and combinations of thymol and iprodione on white rot disease of garlic and its impact on soil microbial community structure were investigated under greenhouse conditions. Thymol alone or in combination with the fungicide iprodione did not appear to reduce either white rot incidence or soil sclerotia density as compared to an infected control. However, iprodione alone or in combination with thymol reduced soil fungal biomass. In addition, iprodione alone decreased soil microbial activity as estimated by fluorescein diacetate (FDA). Soil bacterial community structure as estimated by phospholipid fatty acid (PLFA) profiles was also was affected by both thymol and iprodione applications. The correlation biplot of the individual PLFAs and biocide treatment indicated that the treatments with thymol alone increased cyclopropyl fatty acid (cy17:0 and cy19:0), while the treatments with iprodione alone increased some saturated and branched fatty acids (principally i16:0, a15:0 and 18:0). In addition, taking into account PLFA biomarkers, thymol applications reduced Gram-negative bacteria in soil. To our knowledge, this research is the first report about the effect of a monoterpene (thymol) on soil microflora.

Keywords Thymol · Iprodione · Garlic white rot · Soil microbial communities · Argentina

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Introduction

White rot of garlic and onion, caused by the soilborne fungi *Sclerotium cepivorum* Berk, is a continuing concern for worldwide garlic production. An inoculum density with few soil sclerotia in a litter of field soil can result in great crop losses (Davis et al. 2007). The longevity of sclerotia in the soil, the potential to infect the host over long periods of time, and the location of infection sites under the soil surface are among the most important limitations for control (Pinto et al. 1998). Several control methods have been studied including fungicide application (Davies and Savineli 1994; Zewide et al. 2007), soil fumigants (Entwistle 1990) and soil solarization (Melero-Vara et al. 2000).

Microorganisms are vital for soil fertility and for the degradation of organic matter and pollutants in soils. The study of the characteristics of the soil microbial community of an agroecosystem may provide information necessary to implement management practices to control diseases caused by soil microorganisms. Modern agriculture depends on a wide variety of synthetically produced chemicals, including

insecticides, fungicides, herbicides and other pesticides. Continuous widespread use and release of such synthetics may alter soil microbial communities and affect soil biological equilibrium (Martínez-Toledo et al. 1998).

Fungicide iprodione, 3-(3,5-dichlorophenyl)-*N*-iso-propyl-2,4-dioxoimidazoli dine-1-carboximide is commonly used to control a broad spectrum of crop diseases, including white rot caused by *S. cepivorum* in garlic and onion. There are many reports that iprodione may alter soil microbiota (Duah-Yentumi and Johnson 1986; Wauchope et al. 1992; Wang et al. 2004). Several authors studied the effect of repeated applications of iprodione on soil microbial parameters, and concluded that this fungicide had little impact on microbial biomass (Duah-Yentumi and Johnson 1986). Wang et al. (2004) however, by using (applying) DGGE analysis, observed that fungicide iprodione may alter soil microbial communities.

Recently, it was suggested that pesticide combinated with other natural products may reduce ecological impacts. Bioactive compounds derived from microbial, plant, or other natural sources are a largely untapped source of new pesticides. They are also widely considered to have characteristics conferring reduced risk to environment and a high potential for use in modern integrated pest management strategies (Thompson and Kreutrweiser 2007).

An extensive body of research has demonstrated that several essential oils and their main components possess a wide spectrum of biological activity. Essential oils have been studied for their antimicrobial activity against a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria and fungi (Cristani et al. 2007). Isman (2000) reported that some plant essential oils not only repel insects, but have contact and fumigant insecticidal actions against specific pests, and fungicidal actions against some important plant pathogens. In particular, the antimicrobial properties of thymol has been described extensively, mostly based on observations from semi-quantitative diffusion techniques or microtiter plates assays (Michiels et al. 2007). Other authors have studied the effect of thymol on airborne microbes (bacteria, yeasts and moulds), and they concluded that several microbial populations in air declined due to the effect of thymol application.

Although we demonstrated in previous studies that thymol may alter lipid composition and sclerotial development of *S. cepivorum* Berk (Lucini et al. 2006), we do not know of experiments conducted to evaluate the influence of thymol on selected soil-borne fungi and soilborne microorganisms under greenhouse conditions. Thus, the aim of the present study was to assess the impact of different dosages of thymol alone and in combination with the fungicide iprodione on *S. cepivorum* in soil and its effects on soil microbial communities under greenhouse conditions.

Materials and methods

Experimental design

The study was conducted at Córdoba National University, Córdoba province, Argentina. The soil used was collected from a field cropped with garlic which had the following characteristics: sand 57%, silt 17%, loam 26%, organic matter 1.81%, and organic carbon 1.05%. The soil was homogenized by sieving (5 mm), remoistened to 50% of maximum water holding capacity, and filled into plastic containers (50 × 32 × 25 cm; length, width, and height, respectively) that contained small holes in the bottom for drainage. The containers were filled with soil to a level of 20 cm, slightly compacted to reach the original bulk density, and then were incubated at ambient greenhouse temperature (18–25°C). The *S. cepivorum* strain used throughout this study was isolated from infected garlic growing in an agricultural research farm in a centre of Córdoba city, Argentina. The sclerotia used in the experiment was generated on an artificial medium and conditioned prior to use. In order to obtain fresh subcultures, 3-mm PDA disks overgrown by mycelium of 7 days were collected from the outer circumference of a colony and transferred centrally to new plates, which were maintained in the same conditions until used. Fifteen-day-old garlic seedlings were inoculated with sclerotia (100 sclerotia 100 g^{-1} soil) by mixing with soil, and were then applied to the top soil of the pots in each treatment except to control. Containers with the soil with no sclerotia were included to determine the effect of thymol and iprodione on plant growth and soil microflora. The experimental design was an array of randomized blocks with four replications. Forty-five days after planting (DAP) ten treatments were applied. Treatments consisted of CO (uninoculated control), CS (control with sclerotia), TY375 (thymol at 375 g ha^{-1}), TY750 (thymol at 750 g ha^{-1}), IP400 (iprodione at 400 g a.i. ha^{-1}), IP600 (iprodione at 600 g a.i. ha^{-1}), TY375 + IP400 (thymol at 375 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}), TY375 + IP600 (thymol at 375 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}), TY750 + IP400 (thymol at 750 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}), TY750 + IP600 (thymol at 750 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}). The treatments with IP400 and IP600 contained the recommended dosages of iprodione, 40 and 60% respectively. Each replicate consisted of a plastic container with 28 garlic plants distributed in four rows. Greenhouse temperature was in the range of 19–24°C, with approximately a 14-h photoperiod.

Test substances

Thymol [5-methyl-2-(1-methylethyl)-phenol; 99.5% purity] was obtained from Sigma Chemical Co., St Louis,

Missouri, USA. A commercial formulated grade of the fungicide iprodione (Rovral, 50% wettable powder) was used. All other chemicals used in the study were analytical grade from Merck Co, Sintorgan Argentina, Cicarelli Co or Aldrich Chemical Co.

Incidence of white rot and soil sclerotial density

During the garlic growing season, the incidence of the disease (diseased plants as the percentage of initial stand) was evaluated for each container. A plant was considered diseased if it had typical symptoms of white rot (yellowing, wilt) and mycelium or sclerotia were seen around the base of the plant or on the bulbs. Disease incidence was estimated at 60, 75, 90, 105, 120 and 135 DAP. In addition, soil sclerotial density was estimated at 30 and 140 DAP. For the evaluation of sclerotial viability, portions of the soils were wet sieved, the retrieved sclerotia were surface disinfected, and placed on PDA supplemented with an appropriate antibiotic (Coventry et al. 2006). Sclerotial germination was assessed after 6 days of incubation of PDA as a measure of viability.

Soil microbial analysis

To obtain representative samples, three soil sub-samples regularly distributed were taken from each container (replicate) to make a composite sample at 90 DAP. The samples were then appropriately packed, labelled and stored at 4°C until analysis. These soil composite samples were then used to determine microbial activity, fungal biomass and microbial community structure.

Microbial activity

Microbial activity was measured by hydrolysis of fluorescein diacetate (FDA) according to a modified procedure of Adam and Duncan (2001). Each soil sample (2 g dry weight) was suspended in 15 ml phosphate-buffered saline; 200 µl FDA (0.05 g FDA in 50 ml chloroform/methanol 2:1) was added. Control contained 200 µl of distilled water. The soil suspension was incubated in water bath at 30°C for 30 min. Once removed from the incubator, 15 ml of the chloroform/methanol (2:1 v/v) was added immediately to terminate the reaction. Aliquot (2 ml) was centrifuged and the optical density of the supernatant was measured at 490 nm. Reference values for FDA hydrolysis were obtained using a calibration curve.

Fungal biomass

Soil ergosterol content was estimated according to a microwave-assisted protocol (Montgomery et al. 2000). Each soil sample was treated with 2 ml of MeOH and 0.5 ml of 2 M NaOH and then irradiated at 2,450 MHz and 900 W maximum output. Ergosterol was extracted two times with hexane, filtered, and evaporated under nitrogen flow. Samples were reconstituted with 200 µl of MeOH and analyzed with a Perkin Elmer HPLC equipped with a UV detector and reverse-phase column (Microsorb-MV C18, Varian) at 282 nm.

Microbial community structure

The soil microbial community structure was determined by analyzing the phospholipid fatty acid composition of the soil using a modification of Bossio and Scow method (1998). Each soil sample (10 g dry soil) was extracted overnight with 23 ml of one-phase buffer containing 1:2:0.8 ratio of chloroform, methanol, and phosphate buffer (8.7 g K_2HPO_4 l^{-1}, pH 7.4). The total lipid extract was fractioned into neutral lipids, glycolipids, and polar lipids by silicic acid chromatography (Guckert et al. 1985; McKinley et al. 2005) and the polar lipid fraction containing the phospholipids was isolated and transesterified into fatty acid methyl esters using a mild acid methanolysis reaction. Fatty acid methyl esters were analyzed by capillary gas chromatography with flame ionization detection on a PerkinElmer (Clarus 500 GC) using a 30 m non-polar column (Col-Elite-5) with both injector and detector maintained at 290°C. The column temperature was programmed to start a 180°C for 4 min and then ramp up at a rate of 4–280°C. Methyl nonadecanoate was used as a quantitative internal standard. The separated fatty acid methyl-esters were identified and quantified by chromatography retention time, using standard bacterial acid methyl ester mix (Supelco, Supelco UK, Poole, Dorset, UK). For each sample the abundance of individual fatty acid methyl-esters was expressed as nmol PLFA g^{-1} dry soil. The fatty acid nomenclature used is as follows: total number of carbon atoms:number of double bonds, followed by the position (ω) of the double bond from the methyl end of the molecule. Cis and trans configurations are indicated by c and t, respectively. Anteiso- and isobranching are designated by the prefix a or i. 10Me is a methyl group on the 10th carbon atom from the carboxyl end of the molecule. Cy indicates cyclopropane fatty acids. Br indicates a branched fatty acid with unknown branching configuration.

Statistical analyses

All data were analyzed using INFOSTAT/Professional 2005p.1 (F.C.A.-Universidad Nacional de Córdoba, Argentina). The response variables of white rot incidence, sclerotial density, microbial activity, and fungal biomass were analyzed by using standard analyses of variance

(ANOVA). In all cases, residuals were tested for normality via Shapiro–Wilks' test, and non-normal data were appropriately transformed when required. Means were compared using Fisher's protected least significant differences (LSD) procedure. Principal component analysis (PCA) was performed on individual PLFAs (nmol g^{-1} dry soil) to assess changes in soil microbial community structure. All samples were analyzed for PLFA profiles using a set of 26 fatty acids that were present in most of the samples. Non-transformed means are reported in figures, tables, and text.

Results and discussion

White rot incidence

The effects of thymol alone or in combination with iprodione on while rot incidence at 60, 75, 90, 105, 120 and 135 DAP are shown in Fig. 1. In our work design, thymol was selected because it exhibited the highest antifungal activity against various pathogenic fungi as compared with other monoterpenes (Bendahou et al. 2008). Thymol dosages used in this experiment were selected considering previous works about herbicide effect of thymol and red thyme essential oil on selected weeds under greenhouse conditions (Tworkoski 2002; Kordali et al. 2008). However, several garlic plants treated with thymol and thymol + iprodione showed different phytotoxicity symptoms (data not shown). In consequence, these plants were discarded in order to optimize disease incidence estimation. Except from CO, disease incidence ranged from 0 to 8.3%, 0 to 32.1%, 20.5 to 75.0%, 46.6 to 96.4%, 52.4 to 96.8% and 91.7 to 100.0% at 60, 75, 90, 105, 120, and 135 DAP, respectively. At 60 and 135 DAP, there were no significant differences in white rot incidence among treatments. At 75, 90, 105 and 120 DAP iprodione alone (IP400 and IP600) tended to have the lowest values of white rot incidence compared with the rest of the treatments. However, thymol alone (TY375 and TY750) and thymol + iprodione treatments (TY375 + IP400, TY357 + IP600, TY750 + IP400 and TY750 + IP600) did not significantly decrease white rot incidence compared to CS. Except to iprodione alone (IP400 and IP600 at 120 DAP), none of the biocide combination treatments improved the health of the garlic plants compared with CS. In contrast with these findings, potent fungitoxic properties of carvacrol and thymol against various plant pathogens were previously documented (Sokovic et al. 2002). Kordali et al. (2008) reported that thymol completely inhibited mycelial growth of 17 phytopathogenic fungi and their antifungal effects were higher than commercial fungicide benomyl. Our results suggest that application of thymol alone or in combination with a broad spectrum fungicide iprodione is poorly effective to control white rot disease progress under greenhouse conditions. These differences may be due to the fact that the previous studies were done under in vitro conditions. In relation to this, other authors have found a strong negative correlation between the growth of several fungal species under in vitro conditions and their occurrence in soil with biocide amendment. Thus, the fungal species most strongly inhibited by biocides on culture agar plates were among those least suppressed and even stimulated under greenhouse or field conditions (Lévesque and Rahe 1992). To our knowledge, this is the first report on the effect of thymol on a phytopathogenic fungus under greenhouse conditions.

Sclerotial density

A number of alternative methods have been investigated to attempt to control soilborne diseases and soil sclerotia. These methods include chemical control, biological agents and sclerotia germination stimulants as di-allyl disulphide, but there have been several problems with inconsistent control (Pinto et al. 1998; Coventry et al. 2002; Davis et al. 2007). In our work, total sclerotia and viable sclerotia were similarly affected by both biocide treatments thymol and iprodione (Fig. 2). Except for CO, sclerotial density ranged from 12 to 91 sclerotia g^{-1} soil and 28 to 277 sclerotia g^{-1} soil at 30 and 140 DAP, respectively. Total sclerotial density was increased during the garlic growing season. At both 30 and 140 DAP, there was little difference in sclerotial density among treatments. Except to TY750 + IP400 at 30 DAP, the lower values of total sclerotial and viable sclerotial density were observed under iprodione treatments (IP400 and IP600). At 140 DAP, sclerotial density (both total and viable sclerotia) under IP600 was lower than TY375. In addition, none of the biocide treatments with thymol alone or in combination with iprodione significantly decreased sclerotial density. The little efficacy of iprodione on soil sclerotia found in our work may be due to microbial degradation (Coventry et al. 2002). This is supported by Walker et al. (1986), who measured residues of iprodione and vinclozolin following repeated application of the fungicides to sandy loam soil under laboratory conditions.

Soil microbial activity and fungal biomass

FDA is widely accepted as an accurate and simple method for measuring total microbial activity in soil (Adam and Duncan 2001). Perucci et al. (2000) suggested that FDA might be considered as a suitable tool for measuring the early impact of pesticides on soil microbial biomass, as it is a sensitive and non-specific test able to depict the hydrolytic activity of soil microbes. FDA is mediated

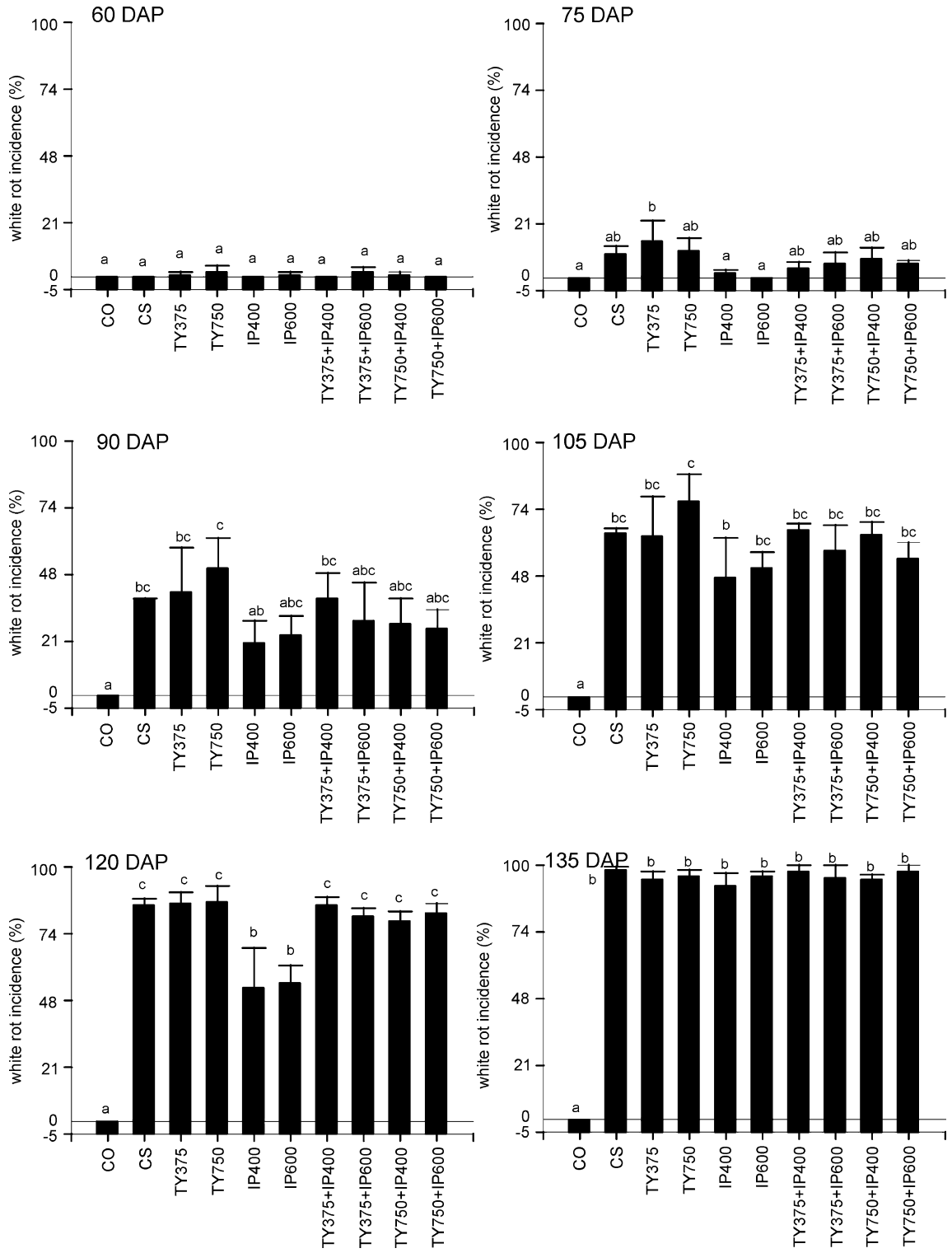

Fig. 1 Effect of different dosages of thymol alone, iprodione alone and combinations of thymol and iprodione on disease incidence of white rot (*S. cepivorum*) in garlic plants at 60, 75, 90, 105, 120 and 135 days after planting (DAP). A *different letter* indicates significant differences between treatments ($P \leq 0.05$). *CO* uninoculated control, *CS* control with sclerotia, *TY375* thymol at 375 g ha^{-1}, *TY750* thymol at 750 g ha^{-1}, *IP400* iprodione at 400 g a.i. ha^{-1}, *IP600* iprodione at 600 g a.i. ha^{-1}, *TY375 + IP400* thymol at 375 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY375 + IP600* thymol at 375 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}, *TY750 + IP400* thymol at 750 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY 750 + IP600* thymol at 750 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}

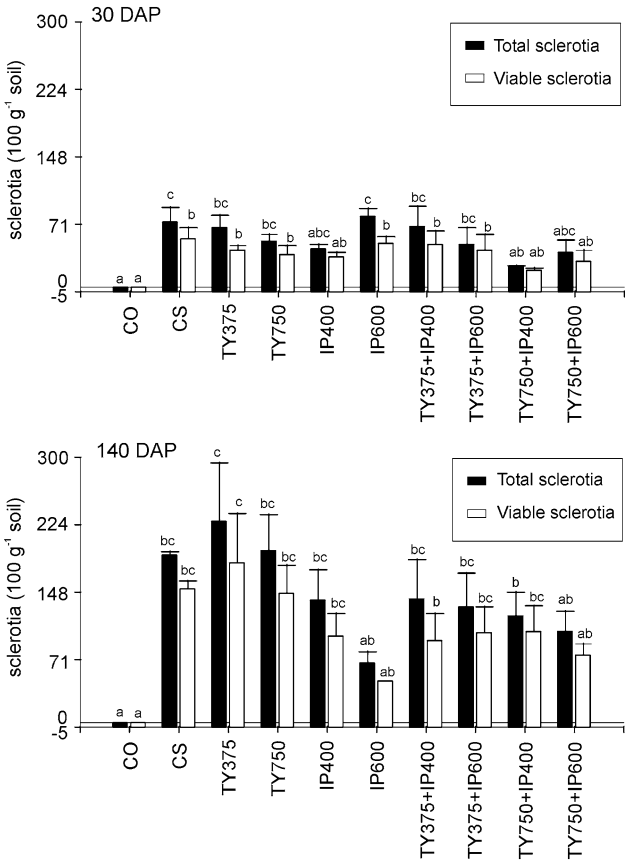

Fig. 2 Effect of different dosages of thymol alone, iprodione alone and combinations of thymol and iprodione on total and viable sclerotia of *S. cepivorum* in soil. A *different letter* indicates significant differences between treatments within either viable or total sclerotia ($P \leq 0.05$). *CO* uninoculated control, *CS* control with sclerotia, *TY375* thymol at 375 g ha^{-1}, *TY750* thymol at 750 g ha^{-1}, *IP400* iprodione at 400 g a.i. ha^{-1}, *IP600* iprodione at 600 g a.i. ha^{-1}, *TY375 + IP400* thymol at 375 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY375 + IP600* thymol at 375 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}, *TY750 + IP400* thymol at 750 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY750 + IP600* thymol at 750 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}

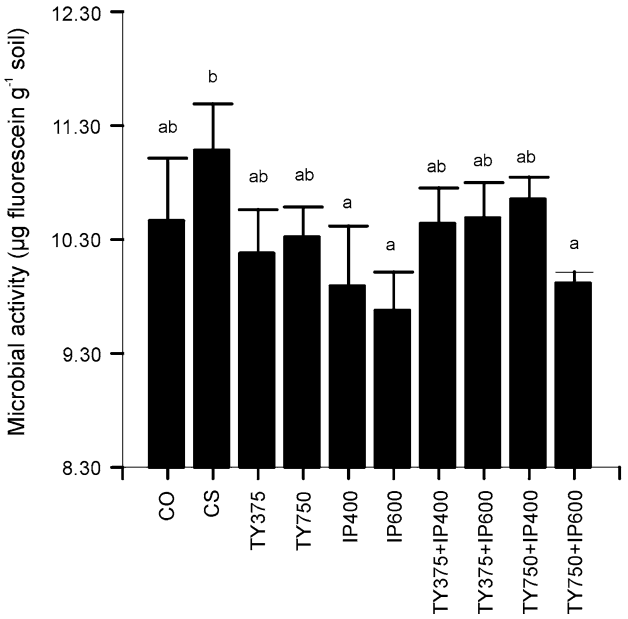

Fig. 3 Effect of different dosages of thymol alone, iprodione alone and combinations of thymol and iprodione on soil microbial activity as estimated by FDA reaction. A *different letter* indicates significant differences between treatments ($P \leq 0.05$). *CO* uninoculated control, *CS* control with sclerotia, *TY375* thymol at 375 g ha^{-1}, *TY750* thymol at 750 g ha^{-1}, *IP400* iprodione at 400 g a.i. ha^{-1}, *IP600* iprodione at 600 g a.i. ha^{-1}, *TY375 + IP400* thymol at 375 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY375 + IP600* thymol at 375 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}, *TY750 + IP400* thymol at 750 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY750 + IP600* thymol at 750 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}

simultaneously by protease, esterase and lipase (Pal et al. 2005). In this study, it was evident that there was a significant effect of iprodione on microbial activity, as determined by FDA hydrolysis (Fig. 3). Soil microbial activity of treated soils ranged from 8.5 to 12.5 µg fluorescein g^{-1} soil. Microbial activity was higher in CS soil, and no statistical differences were detected in thymol alone treatments (TY375 and TY750). However, soil treated with iprodione in a combination with thymol (TY750 + IP600) had also lower microbial activity as shown in Fig. 3. The decrease in FDA due to iprodione application may be related to the reduction in soil microbial biomass as well as the inhibitory effect of iprodione on the synthesis of the enzymes involved in FDA hydrolysis. These results are in agreement with other studies showing either increases or decreases in microbial activity in soil treated with different broad spectrum fungicides (Podio et al. 2008).

Fungal biomass, measured as ergosterol content, also varied considerably between treatments (Fig. 4). Ergosterol content ranged from 2.1 to 11.2 µg ergosterol g^{-1} soil. Similar to FDA response, thymol alone (TY375 and TY750) also did not affect soil ergosterol content. All treatments with iprodione alone or in combination with thymol showed a decrease in soil fungal biomass. These results suggest that iprodione can alter fungal microbial communities. Similarly a number of studies using culture-dependent methods, ergosterol content and fatty acid profiles have indicated that a variety of pesticides can affect soil fungal biomass at recommended application rates, with direct rather than indirect effects responsible (Pal et al. 2005; Klose et al. 2006; Chirnside et al. 2007). However, ergosterol content does not discriminate between species and only provides a surrogate measure of the total live fungal biomass (Feeney et al. 2006). Recently, Ludley et al. (2009) reported that monoterpenes can affect ectomycorrhizal and saprotrophic fungal activity under natural substrata. In our results, the fungal biomarker 18:1ω9c is clearly missing in all treatments with thymol alone or in

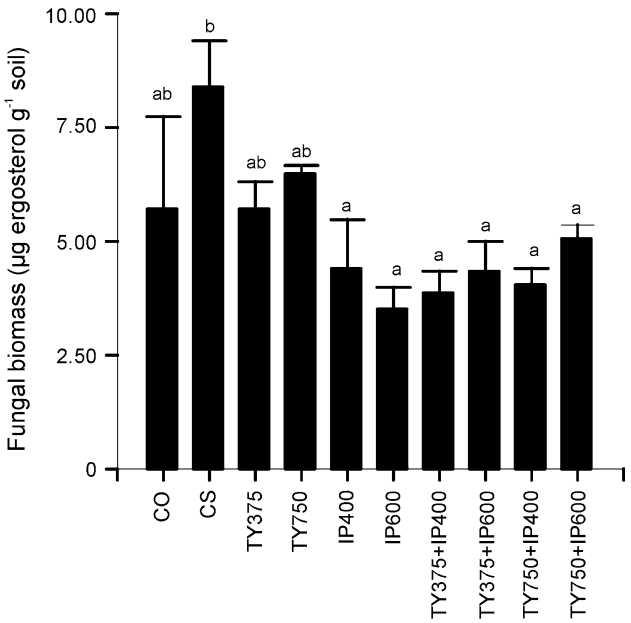

Fig. 4 Effect of different dosages of thymol alone, iprodione alone and combinations of thymol and iprodione on fungal biomass as estimated by soil ergosterol content. A *different letter* indicates significant differences between treatments ($P \leq 0.05$). *CO* uninoculated control, *CS* control with sclerotia, *TY375* thymol at 375 g ha^{-1}, *TY750* thymol at 750 g ha^{-1}, *IP400* iprodione at 400 g a.i. ha^{-1}, *IP600* iprodione at 600 g a.i. ha^{-1}, *TY375 + IP400* thymol at 375 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY375 + IP600* thymol at 375 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}, *TY750 + IP400* thymol at 750 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY750 + IP600* thymol at 750 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}

combination with iprodione (Table 1). This result was inconsistent with respect to fungal biomass as estimated by ergosterol content. Discrepancies between ergosterol and phospholipid fungal biomarkers have been previously reported (Högberg 2006). Considering that fungal species may differ in their sensibility to terpenes, more work is required to characterise specific fungal species affected by thymol applications.

Soil microbial community

The mean of 26 individual PLFAs, averaged across each biocide treatments, is represented in Table 1. One way ANOVAs for most of the individual PLFA variables showed a significant effect of the thymol and iprodione applications. Biocide treatment highly affected most of the fatty acids analyzed. Palmitic acid (16:0), a ubiquitous PLFA in all samples at the highest concentration, and another four fatty acids (10ME 16:0, 18:1w9c, 18:1w7c and 18:0) at the highest relative abundance were the biomarker fatty acid in the samples (Table 1).

The PCA conducted on changes in composition of the specific PLFAs showed that the percentage of variance explained by PC1 was 32%, while PC2 was 27%. The main two axes extracted by the PLFA explained 59% of the variance in the sample (Fig. 5). Moreover, loadings along PC1 were mostly saturated-chain and hydroxylated PLFAs (13:0, 2OH 14:0, 3OH 12:0, 20:0 and 17:0). PC2 was weighted by branched PLFAs (i17:0, i15:0 and i16:0). PCA clearly discriminated thymol, iprodione and CS treatment. The thymol alone treatments (TY375 and TY750) fell to the right on Axis 1, while the iprodione alone treatments (IP400 and IP600) fell to the left Axis 2. However, except for the highest dosages of treatment combination (TY750 + IP600), there was an unclear separation among biocide treatment combinations (TY375 + IP400, TY375 + IP600, TY750 + IP400 and TY750 + IP600).

The correlation biplot of the individual PLFAs and biocide treatment indicated that the treatments with thymol alone (TY375 and TY750) increased cyclopropyl fatty acid (cy17:0 and cy19:0), while the treatments with iprodione alone (IP400 and IP600) increased some saturated and branched fatty acids (principally i16:0, a15:0 and 18:0) (Fig. 5). It is widely accepted that branched fatty acids are Gram-positive biomarkers. However, the relative abundance of Gram positive and Gram negative bacteria may also influence the proportion of cyclopropyl fatty acids, which are mainly found among Gram negatives. Wang et al. (2004) reported that a high dosage of iprodione can alter soil microbial community structure and change biodiversity indexes; however, these authors observed that its impact was transient and reversible. According to our data, Actinomyces and Gram-positive bacteria were not consistently affected by either iprodione or thymol treatments (Table 1). However, Gram-negative bacteria biomarkers were significantly reduced with TY375 and TY750 applications. These results are partially consistent with those reported by Liolios et al. (2009), who studied in vitro antimicrobial effect of thymol on different species of Gram-negative and Gram-positive bacteria. The antimicrobial activity of thymol observed in our results can be explained by the lipophilic character of its chemical structure. The monoterpenes act by disrupting microbial cytoplasmic membrane, which thus loses its high impermeability for protons and larger ions (Gill and Holley 2006). It is interesting to mention that bacteria predominantly respond to environmental conditions and particular antimicrobial agents such as thymol and carvacrol by modulating membrane fluidity. However, the specific mechanism involved in the antimicrobial action of monoterpenes has been poorly studied (Cristani et al. 2007).

In conclusion, thymol alone or in combination with iprodione does not seem to affect either white rot incidence in garlic plants or soil sclerotial density. These results do not support the initial hypothesis that thymol can be effective against a phytopathogenic fungus under greenhouse

Table 1 Fraction of individual PLFAs (nmol g^{-1} soil dry weight) and identified PLFA specific for bacteria (nmol g^{-1} soil dry weight) in all treatments with different dosages of thymol alone, iprodione alone and combinations of thymol and iprodione

PLFA	CO	CS	TY375	TY750	IP400	IP600	TY375 + IP400	TY375 + IP600	TY750 + IP400	TY750 + IP600
12:0**	0.46 ± 0.2	1.23 ± 0.4	1.62 ± 0.9	1.55 ± 0.4	1.23 ± 0.4	0.76 ± 0.5	2.21 ± 0.6	3.95 ± 0.1	1.52 ± 0.6	4.03 ± 0.1
13:0**	0.22 ± 0.1	0.05 ± 0	0.26 ± 0.1	0.19 ± 0.1	0.48 ± 0.2	0.54 ± 0.1	0.55 ± 0.1	0.59 ± 0.1	0.17 ± 0.1	0.61 ± 0.1
12:0 2OH**	0.18 ± 0.1	0.08 ± 0	0.14 ± 0	0.35 ± 0.1	0.47 ± 0.1	0.33 ± 0.1	0.64 ± 0.2	0.45 ± 0.1	0.04 ± 0	0.44 ± 0.1
12:0 3OH	0.22 ± 0.1	0.30 ± 0.1	0.22 ± 0.1	0.22 ± 0.1	0.43 ± 0.1	0.34 ± 0.1	0.28 ± 0.1	0.39 ± 0.1	0.11 ± 0	0.46 ± 0.1
14:0	0.72 ± 0.3	1.17 ± 0.8	0	0.31 ± 0.2	1.09 ± 0.6	0.75 ± 0.4	0.36 ± 0.3	0	0	0
i15:0*	1.79 ± 0.2	3.83 ± 0.1	2.5 ± 1.1	2.15 ± 0.5	2.33 ± 0.6	0.84 ± 0.3	2.08 ± 0.4	3.46 ± 0.1	2.57 ± 1.2	4.97 ± 1.4
a15:0***	1.95 ± 0.7	1.67 ± 0.5	0	0.43 ± 0.2	3.41 ± 1.0	1.33 ± 0.6	3.4 ± 0.6	0	0	0
15:0***	0.15 ± 0.1	5.79 ± 0.3	0.52 ± 0.1	0.46 ± 0.2	0.2 ± 0.1	0.25 ± 0.2	0.26 ± 0.2	0.89 ± 0.5	0.42 ± 0.2	0.89 ± 0.5
br16:0***	0.16 ± 0.1	0	0.72 ± 0.2	0	0	0	0.08 ± 0	0.85 ± 0.1	0.63 ± 0.2	0.89 ± 0.1
14:0 2OH	0.50 ± 0.2	0	0	0	1.1 ± 0.2	1.07 ± 0.1	0.62 ± 0.2	0	0	1.92 ± 1.9
14:0 3OH***	0.63 ± 0.3	2.39 ± 0.4	3.75 ± 1.3	3.35 ± 1.5	0	0.67 ± 0.6	0.02 ± 0	4.81 ± 0.1	3.34 ± 0.2	4.86 ± 0.1
i16:0**	1.27 ± 0.7	0	0	0	3.01 ± 1.5	1.63 ± 0.3	3.37 ± 0.9	0	0	0
16:1ω9c***	2.54 ± 0.6	17.21 ± 0.8	5.24 ± 1.5	3.58 ± 0.8	5.77 ± 1.2	1.89 ± 0.5	4.70 ± 0.8	5.68 ± 0.1	3.34 ± 1.0	5.58 ± 0.1
16:0***	28.45 ± 7.5	41.91 ± 1.5	19.81 ± 1.5	0.8 ± 0.5	28.64 ± 3.8	15.92 ± 3.2	22.28 ± 3.7	32.64 ± 0.2	32.72 ± 5.8	33.09 ± 0.2
10Me 16:0**	4.60 ± 1.4	7.33 ± 1.2	4.55 ± 1.1	7.24 ± 0.2	10.07 ± 0.2	6.65 ± 0.3	5.95 ± 1.1	8.39 ± 2.1	4.83 ± 0.8	8.86 ± 2.5
i17:0	0.27 ± 0.1	0.96 ± 0.2	0.66 ± 0.3	0.6 ± 0.3	0.44 ± 0.1	0.18 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.64 ± 0.3	0.73 ± 0.1
cy17:0**	0.65 ± 0.3	2.14 ± 0.4	0.09 ± 0	0.54 ± 0.2	0.25 ± 0.1	0.22 ± 0.1	0.6 ± 0.3	0.02 ± 0	0	0.04 ± 0
17:0	0.47 ± 0.2	0.26 ± 0.2	0.73 ± 0.1	0.64 ± 0.1	0.75 ± 0.3	0.42 ± 0.2	0.65 ± 0.1	1.62 ± 0.2	0.39 ± 0.2	1.25 ± 0.6
16:0 2OH	0.19 ± 0.1	0.16 ± 0.1	0.2 ± 0.1	0.34 ± 0.1	0.7 ± 0.2	0.84 ± 0.2	0.6 ± 0.4	0.76 ± 0.1	0.41 ± 0.2	0.71 ± 0.1
18:2	19.26 ± 4.7	31.05 ± 0.3	24.28 ± 3.6	8.77 ± 2.9	0	8.17 ± 1.8	4.12 ± 2.4	5.05 ± 0.3	4.39 ± 0.1	5.02 ± 0.4
18:1ω9c***	22.54 ± 0.5	3.66 ± 2.1	0	0	13.92 ± 0.5	8.82 ± 3.8	0	0	0	0
18:1ω7c***	10.01 ± 0.3	15.54 ± 0.7	14.89 ± 3.7	7.44 ± 2.6	21.27 ± 4.8	7.69 ± 0.5	21.73 ± 0.6	22.15 ± 5.2	9.25 ± 0.2	23.03 ± 6.0
18:0***	18.5 ± 1.4	1.15 ± 0.7	0.14 ± 0.1	0.43 ± 0.3	11.36 ± 3.79	12.46 ± 1.3	11.42 ± 2.5	0.41 ± 0.4	0.71 ± 0.5	0.46 ± 0.4
cy19:0	0.21 ± 0.2	0.17 ± 0.1	0	0.13 ± 0.1	0	0	0.11 ± 0.1	0.11 ± 0.1	0.03 ± 0	0.12 ± 0.1
20:0***	0.19 ± 0.1	0.49 ± 0.2	0.27 ± 0.1	0.18 ± 0.1	1.22 ± 0.7	0.47 ± 0.3	1.18 ± 0.3	2.64 ± 0	0.24 ± 0.1	2.64 ± 0.1
22:0***	0.64 ± 0.34	6.57 ± 1.3	1.13 ± 0.2	1.79 ± 0.6	0.76 ± 0.2	0.75 ± 0.4	0.48 ± 0.1	0.42 ± 0.1	0.61 ± 0.3	0.44 ± 0.1
Specific PLFAs										
Gram (+)	5.44 ± 1.6	6.46 ± 0.3	3.87 ± 1.6	3.19 ± 0.8	9.19 ± 3.0	3.97 ± 1.3	9.33 ± 1.88	3.02 ± 0.8	3.82 ± 1.7	6.58 ± 1.3
Gram (−)***	35.95 ± 1.0	38.73 ± 1.0	20.21 ± 5.2	11.7 ± 3.2	41.21 ± 5.7	18.62 ± 3.7	27.13 ± 0.9	12.62 ± 1.3	12.61 ± 0.9	28.76 ± 6.0
Actinomyces**	4.58 ± 1.4	7.33 ± 1.1	4.55 ± 1.1	7.24 ± 0.2	10.07 ± 0.2	6.65 ± 0.3	5.95 ± 1.18	3.14 ± 0.2	4.83 ± 0.8	8.86 ± 2.5

Each value is the mean ± SE of four replicates

Significant differences are indicated by * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$, respectively

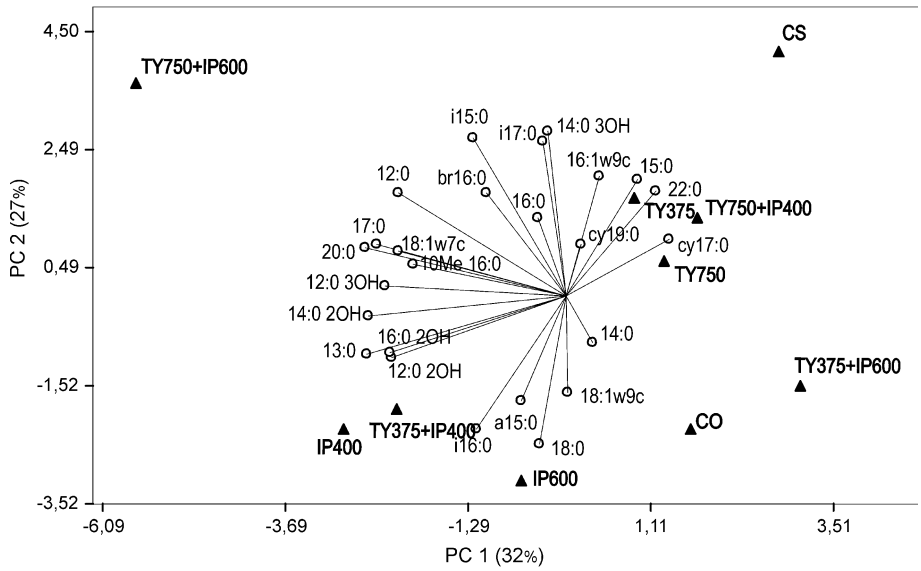

Fig. 5 Correlation biplot showing the relationship between the biocide treatments and individual PLFAs. *CO* uninoculated control, *CS* control with sclerotia, *TY375* thymol at 375 g ha^{-1}, *TY750* thymol at 750 g ha^{-1}, *IP400* iprodione at 400 g a.i. ha^{-1}, *IP600* iprodione at 600 g a.i. ha^{-1}, *TY375 + IP400* thymol at 375 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY375 + IP600* thymol at 375 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}, *TY750 + IP400* thymol at 750 g ha^{-1} + iprodione at 400 g a.i. ha^{-1}, *TY750 + IP600* thymol at 750 g ha^{-1} + iprodione at 600 g a.i. ha^{-1}

conditions. This hypothesis had been formulated based on preliminary data showing that thymol affected mycelial growth and sclerotial development of *S. cepivorum* under in vitro conditions. In contrast, these results appear to indicate that thymol applications reduced Gram-negative bacteria. To our knowledge, this research is the first report about the effect of a monoterpene on soil microbial community structure. Further work is needed to study the influence of selected monoterpenes on crop diseases and soil microflora.

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