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# Shifts in ascomycete community of bisolarizated substrate infested with *Fusarium oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *basilici* by PCR-DGGE

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**Abstract**

Substrate samples were artificially infested with *Fusarium. oxysporum* f. sp. *conglutinans* (FOC) and *F. oxysporum* f. sp. *basilici* (FOB) in order to evaluate the shift in fungal population by using culture dependent and culture independent methods. Solarization was carried out with transparent polyethylene film during a summer period on a greenhouse located in Northern Italy, in combination or not with *Brassica carinata* defatted seed meals and/or compost. Biosolarization treatment was carried out in a growth chamber by heating the substrate for 7 and 14 days at optimal (55 to 52°C for 6 h, 50 to 48°C for 8 h and 47 to45°C for 10 h/day) and sub-optimal (50 to 48°C for 20 h, 45 to 43°C for 8 h and 40 to 38°C for 10 h/day) temperatures. Plate counts and polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analyses were performed to evaluate the effect of biosolarization on the microbial population. The abundance of FOC and FOB were reduced as a consequence of biosolarization approach, while bacterial population (total aerobic mesophilic bacteria and *Pseudomonas* spp.) were higher compared to control samples during the experiment. PCR-DGGE fingerprints of the ascomycete community obtained from DNA directly extracted from infested substrate samples showed that the use of organic amendments increased the similarity of the fungal population.

**Keywords:**

Biosolarization

PCR-DGGE

Fungal diversity

Organic amendments

**1. Introduction**

Plant pathogens need to be managed in order to maintain high quality, safety and extended shelf-life of products. There is considerable interest in non–chemical methods for disease management, such as solarization or soil treatments with organic amendments, for controlling soil-borne pathogens to prevent serious production losses in different agricultural systems (Noble et al., 2005; Hadar and Papadopoulos, 2012). Soil solarization is an approach to soil disinfestations, involving covering soil with plastic film in order to accumulate heat, the effect is an increase in temperature lethal for many soil-borne plant pathogens (Gamliel and Katan, 2012). The integration of solarization with other control methods, such as biofumigation with Brassica plants or organic waste is desirable for the maximal efficiency in pathogen control (Oka et al., 2007; Núñez-Zofío et al., 2011, Pane et al., 2012; Motisi et al., 2013; Simmons et al., 2013). The approach of combining solarization and organic matter application is defined as biological solarization or biosolarization (Ros et al., 2008). Most of the research into controlling various soil-borne pathogens has been focused on the use of species belonging to the Brassicaceae (Cruciferae) family (Pane et al., 2012; Motisi et al., 2013). Isothiocyanates, that have shown fungitoxic proprieties able to control or inhibit the development of soil-pathogens, are developed from the reduction of glucosinolates contained in Brassica plants during the shredding process (Motisi et al., 2013). Culture dependent analysis alone may not be exhaustive to understand the shifts in the microbial ecology of soil in response to different plant pathogen control methods. The application of molecular techniques based on the analysis of the soil's nucleic acids provided useful information on the structure and diversity of soil microbial communities (Bonanomi et al., 2008; Gelsomino and Cacco, 2009). However, organic amendments have been reported to have either positive (Drinkwater et al., 1995; Sun et al., 2004) or no effect (Lawlor et al., 2000; Franke-Snyder et al., 2001) on microbial diversity. The analysis of the microbial community by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) is one of the best tools in soil microbial ecology, used to study complex microbial communities in many different habitats without cultivation steps on cultural media (Muyzer and Smalla, 1998). In a previous paper, Gilardi et al., (2013) showed that

organic amendments combined with solarization treatment of substrate infested by *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *basilici*, not providing any improvement to the level of disease management, positively affected yield of rocket and basil grown in the treated substrate. *F. oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *basilici* are a pathogens causing rocket (*Eruca sativa*) and basil (*Ocimum basilicum*) wilt, respectively (Gullino et al., 2012). *F. oxysporum* f. sp. *conglutinans* was recently observed in Italy on brassica crops such as cultivated (*E. sativa*) and wild (*Diplotaxis tenuifolia*) rocket (Garibaldi et al., 2006), while basil wilt, caused by *F. oxysporum* f. sp. *basilici* has long been known in Italy (Grasso, 1975).

In this study we investigate how biosolarization could affect the structure of the microbial populations evaluated by a culture dependent method and an independent method using DGGE of PCR-amplified 18S-ITS genes-coding fragments from DNA extracted directly from infested substrate.

## 2. Materials and methods

### *2.1. Inoculum preparation*

Isolates of *Fusarium oxysporum* f. sp. *basilici* (FOB009RB) (FOB) (Agroinnova collection, Torino) and *Fusarium oxysporum* f. sp. *conglutinans* (ATCC16600RB) (FOC) were used and cultured in Erlenmeyer flasks containing potato dextrose broth (PDB, Difco) plus 25 mg $l^{-1}$ streptomycin (AppliChem) at 25 °C for 10 days. The fungal conidia and mycelia were recovered by centrifugation for 20 min at 6000 x g at 15 °C. The fungal biomass from each strain was dried by mixing with sterile talc to avoid chlamydospore development (Locke and Colhoun, 1974) and stored at room temperature for further use.

### *2.2 Experimental design*

Two experimental trials were carried out. Plastic tanks (50 x 40x 20 cm) containing 20 l of a mixture (70:30 v/v) of sandy loam soil (sand, 58% ± 5 ; silt, 32% ± 5; clay, 10% ± 5; pH, 6.0-7.5;

organic matter content, 2.4%; cation exchange capacity, 5.7 meq100 $g^{-1}$ soil) and peat substrate (Tecno2, 70% white peat and 30% clay, Turco Silvestro Terricci, Bastia d'Albenga, SV, Italy) were artificially infested with the strains FOB and FOC resistant to benomyl (Lu et al., 2010) to reach a final concentration of $5\times10^4$ CFU $g^{-1}$ of substrate. The characteristics of the final substrate were: sand, 68.8% ± 5 ; silt, 6.8% ± 5; clay, 26% ± 5; pH, 7.1; organic matter content, 2.4%; cation exchange capacity, 5.7 meq100 $g^{-1}$ 98 soil). A total of 36 tanks of 20 l filled with the previously described substrate were infested with each pathogen, while a non-infested substrate was used as a control. Solarization was carried out in a series of samples under growth chamber conditions by covering the substrate with 50 μm thick transparent PE plastic film for 7 or 14 days. Two temperature conditions were chosen as previously reported (Tamietti and Garibaldi, 1987; Gullino et al., 1998): optimal (55-52°C for 6 h, 50-48°C for 8 h and 47-45°C for 10 h) and sub optimal temperature conditions (50-48°C for 6 h, 45-43°C for 8 h and 40-38°C for 10 h). Substrate temperature was monitored at a depth of 10 cm using a Digital Data Logger EM50 (Decagon Devices, USA). The untreated control substrate was kept between 25°C and 28°C under greenhouse conditions. At the end of the thermal treatment, each tank was kept in a greenhouse at temperatures ranging from 25°C to 28°C and 70-80% of Relative Humidity. Subsequently, 360 pots (2 l each) were prepared from the infested treated and non-treated substrates and 20 plastic pots were prepared from the non-infested substrate as a control. Organic amendments were applied alone or combined with solarization as follows: (i) 2.5g $L^{-1}$ of *Brassica carinata* defatted seed meals (Biofence, N organic 3%, P 2.2%, K 2%, organic C 52%, Triumph, Italy); (ii) 4 g $L^{-1}$ of a municipal compost (Acea Pinerolese, Pinerolo, Italy), prepared from the organic fraction of municipal solid and biodegradable wastes; (iii) a combination of *B. carinata* and compost. A series of substrate samples were mixed with the organic amendments before starting solarization while another series of substrate samples were mixed after solarization treatments. Inoculated untreated substrate was used as a control. Main information on the trials carried out are shown in Table 1. Samples were taken 15

(1st trial) and 60 (2st trial) days after artificial infestation of the substrate. The experiment was carried out in duplicate.

*2.3 Sampling and enumeration of microbial populations*

Substrate samples (100 g) were taken (at 1-15 cm depth) from each pot and mixed together in sterilized polyethylene bags using a sterilized spatula. Sub-samples (5g) were sieved (1.7 mm mesh) to remove debris and vegetation, and mixed at room temperature for 30 min with 45 ml of Ringer's solution (Merck, Germany) plus 5 µl of Tween 20 (Sigma, Italy) in sterilized glass bottles. Decimal dilutions in quarter-strength Ringer's solution (Merck) were prepared, and aliquots of 0.1 ml of the appropriate dilutions were spread in triplicate on the following media: *Pseudomonas* agar with cetrimide-fucidin-cephalosporin (CFC) supplement (Oxoid, Italy), incubated at 25 °C for 48 h for *Pseudomonas* spp.; for FOC and FOB detection, the Fusarium selective medium was used (Komada, 1975) plus 10 mg $l^{-1}$of benomyl, incubated at 20°C for 4 days; the total fungal populations were evaluated by using Potato Dextrose Agar (PDA, Merck) plus 25 mg $l^{-1}$ of streptomycin sulphate (BioChemica, Germany),incubated at 20°C for 4 days; Luria Bertani (LB) agar (Merck) (Bertani, 1951) was used for the total aerobic mesophilic bacteria, incubated at 20°C for 48 h. Results of plate counts were calculated as the mean of Log counts for three independent plates.

*2.4 DNA extraction from substrate samples and PCR–DGGE analysis of the ascomycete community*

Substrate samples (1g) as used for the plate count were taken at the end of the experiment and stored at -20 ºC until examination. Total DNA extraction was carried out using the NucleoSpin® Soil (Macherey-Nagel, Germany) kit, according to manufacturer's instructions. DNA was quantified using the Nanodrop 1000 (Thermo Scientific, Milano, Italy) and standardized at 50 ng/µl. The primers NS1/ ITS4A and ITS1/ITS2 were used to amplify the fungal 18S-ITS genes (Danon et al., 2010). PCR products were analyzed by denaturing gradient gel electrophoresis

(DGGE) using Bio-Rad Dcode apparatus, and bands to be sequenced were purified as previously reported (Ferrocino et al., 2013). To determine the closest known relatives of the partial 18S-ITS gene sequences obtained, searches were performed in public data libraries (GenBank) with the Blast search program (http://www.ncbi.nlm.nih.gov/blast/). A database of fingerprints was created by using the software Bionumerics version 5.1 (Applied Maths, Sint Marten Latem, Belgium). A dendrogram of similarity was retrieved by using the Dice coefficient and Unweighted Pair Group Method using Arithmetic average (UPGMA) clustering algorithm (Vauterin and Vauterin, 1992).

*2.5 Data analysis*

Each trial was carried out in duplicate and all the data obtained were analyzed using one-way analysis of variance (ANOVA) for each individual date, with treatments being the main factor. ANOVA analyses were performed with the SPSS 18.0 statistical software package (SPSS Inc., Cary, NC, USA). The Tukey HSD test was applied when ANOVA revealed significant differences (P=0.05).

## 3. Results

*3.1 Microbial analysis*

The results of the plate counts of substrate infested with FOB on specific media are shown in Table 2 (panel A and panel B). Load of FOB on *Komada* medium plus benomyl was reduced in order of approximately 1 Log cycle if compared with control samples in all the conditions adopted in the first trial. The same behavior was observed in the second trial. Under optimal thermal treatment, the effect of biosolarization on the reduction of FOB is clear, on the other hand there is no significant difference in the use of tested amendments alone, or combined with, the thermal treatment at different times of application. Total fungal populations seem to be unaffected by the use of solarization, with or without the amendments, under sub optimal and optimal thermal condition in both of the trials. The population count of total aerobic mesophilic bacteria, under sub

optimal temperature conditions, appears to not be affected by any of the treatments applied. On the other hand, under optimal temperature conditions, only the samples after 1 week of biosolarization for 7 days with *B. carinata*, and the samples after biosolarization for 14 days with compost showed a load decrease (Tab.2, panel B). For *Pseudomonas* spp. it was not possible to find any significative difference in each of the treatments used (Tab.2, panel B).

The results of the plate counts of substrate infested with FOC on specific media are shown in Table 3 (panel C and panel D). Load of FOC on Komada medium in the first trial was reduced by approximately 2 log cycles after biosolarization, and in the second trial by approx. 1 log cycle under sub optimal temperature conditions (Tab.3, panel C). In contrast, statistical values of FOC in the substrate samples remained constant throughout the whole experiment (Tab. 3, panel C). The same behaviour was observed under optimal thermal treatment. No significant differences in pathogen counts were observed when using the treatments applied at both 7 and 14 days. Load of total fungal populations monitored on PDA medium was generally similar to the control samples in both of the trials (Tab.3, panel C). No significant differences were observed when using the two temperature conditions and the two applications of treatment at 7 and 14 days. Counts of *Pseudomonas* spp. in the first trial were generally lower if compared with the inoculated non-treated control. Moreover, in some cases especially after 14 days of thermal treatment, the counts were higher when compared with the inoculated non-treated control. In addition, the same trend was observed in both of the thermal treatment conditions tested. The same behaviour was shown by the total bacterial count (Tab.3, panel D). Application of amendments before or after thermal treatment did not affect the plate count in both trials.

*3.2 PCR–DGGE analysis of the ascomycete community*

The total DNA extracted from substrate samples was employed to amplify the 18S-ITS genes, giving PCR products of approximately 300 bp that were analyzed by DGGE. A data matrix of the fingerprints was obtained and the dendrograms of similarity are presented in Figure 1 (FOB)

and Figure 2 (FOC), while the results of the band sequencing are shown in Table 4 (FOB) and Table 5 (FOC). Repeated DNA extraction and PCR-DGGE analysis of the samples confirmed the fingerprinting obtained (data not show); therefore, the band sequencing was performed on only one set of samples. The PCR–DGGE analysis of FOB infested substrate showed that most of the samples were contaminated by *Fusarium oxysporum* and *Pseudallescheria boydii* (Table 4). *P. fimeti* and *Curvularia* spp. were only found in samples treated with compost. *Aspergillus versicolor*, *Hypocreales* spp. and *Peziza ostracoderma* were identified among the bands in the fingerprints in few samples, while *Myrothecium roridum* was only found in two samples treated with *B. carinata* after 2 weeks of solarization (Table 4). Cluster analysis of the FOB infested substrate (Fig. 1) showed a lower similarity between the profiles obtained from the DNA analyses. Few sub-clusters with a high percentage of similarity ( >50%), and grouping of samples with the same treatment, were detected (Fig. 1).

The results of the band sequencing from the PCR–DGGE fingerprints obtained from DNA directly extracted from FOC infested samples are shown in Table 5. The PCR–DGGE analysis showed that most of the samples were contaminated by *Cochliobolus* spp., *Thielaviopsis basicola*, *Scedosporium prolificans, F. oxysporum,* while *Alternaria brassicae* was identified among the band in the fingerprints of samples treated with *B. carinata*. Control samples were contaminated with *Pseudallescheria boydii* and *Candida subhashii* (Table 5). Cluster analysis of the FOC infested substrate (Fig. 2) showed several groups with a high percentage of similarity ( >60%) between samples treated with the same amendments (Fig. 2). It is possible to find a sub-cluster of grouped samples treated with *B. carinata* alone or in combination with compost. It is also possible to find a sub-cluster of grouped compost treated samples. In general FOC infested samples treated with composts after and before solarization had the same fingerprints.

**4. Discussion**

In recent years several studies have used the potential of soil solarization as an alternative method to control soil-borne pathogens (Núñez-Zofío et al., 2011). The success of soil solarization is based on the fact that different fungi have different temperature sensitivities due to modes of survival (Bollen, 1969; Saremi et al., 2011). Lu et al., 2010 showed that a temperature of 60°C for 15 minutes was lethal for chlamydospores of *F. oxysporum* f. sp. *raphani* and *F. conglutinans* mixed in soil. The combination of solarization with organic amendments such as *B. carinata* or compost is desirable for the maximal efficiency in pathogen control (Oka et al., 2007; Pane et al. 2011). Biosolarization offers a safe and environmentally sustainable means to protect plants from soil borne pathogens, moreover, the combination with organic amendments such as Brassica plants or organic waste can reduce levels of fungicide (integrated control), promoting a reduction of disease similar to that achieved with fungicide treatment (Bonanomi et al., 2008). Combination of several interrelated processes occurring during biosolarization can give a lower pathogen incidence with an improved health, growth rate, and quality of crop plants (Katan, 1987; Stapleton and DeVay, 1995). Disease suppression by organic amendments could be related to specific microorganisms involved in mechanism of predation, parasitism and competition (Weller et al., 2002; Bonamomi et al., 2007 Hadar and Papadopoulos, 2012). It can also be correlated with metabolic activity produced by more groups of microorganisms (Hoitink and Boehm, 1999) and the pathogen responses, as well as the microbial population of the soil can be differently affected (Termorshuizen and Jeger, 2008). The result of our study showed that the use of biosolarization can reduce *Fusarium oxysporum* f. sp. *basilici* and *F. oxysporum* f. sp. *conglutinans* population while having no effect on the total aerobic mesophilic bacteria. Recent reports (Coates-Beckford et al., 1997; Shukla et al., 2000) have shown similar results. In most cases, the combined treatments were not statistically significantly different from each treatment alone, probably due to the high efficacy of thermal treatments.

In addition, our results showed that bacterial population (total aerobic mesophilic bacteria and *Pseudomonas* spp.) were higher compared to control samples during the biosolarization

experiment. Other authors (Bonanomi et al., 2008) reported that solarization increased the total numbers of *Pseudomonads*. Biological and chemical changes in soil during solarization can produce microbial community alterations, for this reason the fungal diversity developing during the biosolarization was also assessed in this study. The PCR-DGGE approach used has been also employed with good results in other studies (Pingsheng et al., 2012; Motisi et al., 2013**).** We found in substrate samples infested with FOC that *Fusarium oxysporum*, *Alternaria brassicae* and *Cochliobolus* spp. were only present in substrate samples treated with *B. carinata*. *Alternaria* spp. is an important pathogen of oilseed Brassicas (Chavan et al., 2013) while the genus *Cochliobolus* comprises major fungal pathogens of gramineous crops and weeds (Tsuchiya and Taga, 2001). In most of the samples was also detected *Thielaviopsis basicola* a pathogen causing the black root rot of tobacco (Stutz et al.,1986, Keel et al.,1992) and more than 100 species were identified as susceptible (Johnson, 1916). We also only found *Scedosporium prolificans* in samples treated with compost. In general we found that biosolarization for two weeks reduces the number of fungal species if compared with control samples. On the other hand, no differences in species diversity were found between the two temperature conditions used.

Substrate samples infested with FOB showed the presence of *Pseudallescheria fimeti* and *Curvularia* spp. in most of the samples derived from compost. In this case the use of *B. carinata* alone or in combination with compost reduced the number of species in substrate samples. *Pseudallescheria fimeti* and *Curvularia* spp. can infect animals including humans (Gilgado et al., 2007; Cunha et al., 2013). There was no difference between temperature conditions and days of solarization. This was probably due to the relatively short duration of the trials. DGGE fungal patterns among treatments were very similar: cluster analysis was not able to differentiate between the use of *B. carinata* and compost treatments, as reported in other studies (Klamer et al., 2002; Costa et al., 2006). These fungal richness reductions appear surprising because organic amendments are responsible for an increase in the microbial richness (Sun et al., 2004). The loss of diversity caused by the use of organic amendments could be due to the increase of a few dominant microbial

species, such as *Pseudallescheria boydii*, *Cochliobolus* spp. and *Thielaviopsis basicola* that may rapidly exclude other species by competition.

As reported (Motisi et al., 2010) in some cases, soil amendments with Brassica or compost can add some pathogens to the soil treated. Though this negative effect of biofumigation with Brassica, our data confirm the effectiveness of biosolarization against FOB and FOC.

In a parallel study the combination of organic amendments with a short period of soil solarization (7 or 14 days) did not provide any improvement in the control of Fusarium wilt of rocket and basil when compared from s soil solarization treatment alone (Gilardi et al., 2013).

## 5. Conclusions

The results of this experiment show the potential of combining soil solarization with organic amendments, however, the observed changes in the composition of the soil fungal communities in response to biosolarization may be of great importance in understanding the potential impact of biosolarization on soil ecosystem.

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## References

Bertani, G., 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. 62, 293-300.

Bollen, GJ., 1969. The selective effect of heat treatment on the microflora of a greenhouse soil. Neth. J Plant Pathol. 75,157-163.

Bonanomi, G., Antignani, V., Pane, C., Scala, F., 2007. Suppression of soilborne fungal diseases with organic amendments. J. Plant Path. 89, 311-324.

Bonanomi, G., Chiurazzi, M. , Caporaso, S., Del Sorbo, G., Moschetti, G., Scala, F., 2008. Soil solarization with biodegradable materials and its impact on soil microbial communities. Soil Biol. Biochem. 40, 1989-1998.

Chavan, V., Bhargava, S., Kamble, A.,2013. Temporal modulation of oxidant and antioxidative responses in *Brassica carinata* during beta-aminobutyric acid-induced resistance against *Alternaria brassicae.* Physiol. Mol. Plant Path. 83, 35-39.

Coates-Beckford, P.L., Cohen, J.E., Ogle, L.R., Prendergast, C.H., Riley, D.M., 1997. Effects of plastic mulches on growth and yield of cucumber (*Cucumis sativus L.*) and on nematode and microbial population densities in the soil. Nematropica 27. 191-207.

Costa, R., Salles, J.F., Berg, G., Smalla, K., 2006. Cultivation-independent analysis of *Pseudomonas* species in soil and in the rhizosphere of field-grown *Verticillium dahliae* host plants. Environ. Microbiol. 8, 2136-2149.

Cunha, K.C., Sutton, D.A., Fothergill, A.W., Gene, J., Cano, J.,Madrid, H.de Hoog, S., Crous, P.W. Guarro, J., 2013. In vitro antifungal susceptibility and molecular identity of 99 clinical isolates of the opportunistic fungal genus *Curvularia*. Diagn. Microbiol. Infect. Dis. 76, 168-174.

Danon, M., Yona, C., Yitzhak, H., 2010. Ascomycete communities associated with suppression of *Sclerotium rolfsii* in compost. Fungal Ecol. 3, 20-30.

Drinkwater, L.E., Letourneau, D.K., Workneh, F., vanBruggen, A.H.C., Shennan, C., 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. Ecol. Appl. 5, 1098-1112.

Ferrocino, I., Chitarra, W., Pugliese, M., Gilardi, G., Gullino, M.L., Garibaldi, 2013. Effect of elevated atmospheric $CO_2$ and temperature on disease severity of *Fusarium oxysporum* f.sp. *lactucae* on lettuce plants. Appl. Soil Ecol. 72, 1-6.

Franke-Snyder, M., Douds, D.D., Galvez, L., Phillips, J.G., Wagoner, P., Drinkwater, L., Morton, J.B., 2001. Diversity of communities of arbuscular mycorrhizal (AM) fungi present in conventional versus low-input agricultural sites in eastern Pennsylvania. USA. Appl. Soil Ecol. 16, 35-48.

Gamliel, A., Katan, J., 2012. Soil and substrate disinfestation in protected structures, in Gullino, M.L., Katan, J., Garibaldi, A. (Eds), Fusarium Wilts of Greenhouse Vegetable and Ornamental Crops. APS Press, St. Paul, MN. 121-131.

Garibaldi, A., Gilardi, G., Gullino, M.L., 2006. Evidence for an expanded host range of *Fusarium oxysporum* f. sp. *raphani*. Phytoparasitica 34, 115-121.

Gelsomino, A., Cacco, G., 2006. Compositional shifts of bacterial groups in a solarized and amended soil as determined by denaturing gradient gel electrophoresis. Soil Biol. Biochem. 38 91-102

Gilardi, G., Demarchi, S., Gullino, M.L., Garibaldi, A., 2013. Effect of simulated soil solarization and organic amendments on Fusarium wilt of rocket and basil under controlled conditions. J. Phytopatol.(in press).

Gilgado, F., Gene, J., Cano, J., Guarro, J., 2007. Reclassification of *Graphium tectonae* as *Parascedosporium tectonae* gen. nov., comb. nov., *Pseudallescheria africana* as *Petriellopsis africana* gen. nov., comb. nov and *Pseudallescheria fimeti* as *Lophotrichus fimeti* comb. Nov. Int. J. Syst. Evol. Microbiol. 57, 2171-2178.

Grasso, S., 1975. Un avvizzimento del basilico da *Fusarium oxysporum*. Infor. Fitopatol 25, 5-7.

Gullino, M.L., Minuto, A., Garibaldi, A., 1998 Improved method of bench solarization for the control of soilborne diseases in basil. Crop Prot. 17, 497-501.

Gullino, M.L., Katan, J., Garibaldi, A., 2012. Fusarium wilt of sweet basil, In: Gullino, M.L., Katan, J., Garibaldi, A., (Eds.), Fusairum wilts of greenhouse vegetable and ornamental crops. APS Press, St. Paul, MN. pp. 185-190.

Hadar, Y., Papadopoulos, K.K., 2012. Suppressive composts: microbial ecology links between abiotic environments and healthy plants. Annu. Rev. Phytopathol. 50, 133-153.

Hoitink, H.A.J., Boehm, M.J., 1999. Biocontrol within the context of soil microbial communities: a soil dependent phenomenon. Ann. rev. Phytopathol. 37, 427-466.

Katan, J., Gamliel, A., 2012. Soil solarization for the management of soilborne pests: the challenges, historical perspective, and principles, In: Gamliel, A., Katan, J. (Eds), Soil Solarization: Theory and Practice. APS Press, St. Paul, MN. pp. 45- 52.

Keel, C., Weller, D.M., Natsch, A., Défago, G., Cook, R.J., Thomashow, L.S., 1996. Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. Appl. Environ. Microbiol. 62, 552-563.

Klamer, M., Roberts, M.S., Levine, L.H., Drake, B.G., Garland, J.L., 2002. Influence of elevated $CO_2$ on the fungal community in a coastal scrub oak forest soil investigated with terminal-restriction fragment length polymorphism analysis. Appl. Environ. Microbiol. 68, 4370-4376.

Komada, H., 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8, 114-125.

Lawlor, K., Knight, B.P., Barbosa-Jefferson, V.L., Lane, P.W., Lilley, A.K., Paton, G.I., McGrath, S.P., O'Flaherty, S.M., Hirsch, P.R., 2000. Comparison of methods to investigate microbial populations in soils under different agricultural management. FEMS Microbiol. Ecol. 33, 129-137.

Locke, T., Colhoun, J., 1974. Contributions to a method of testing oil palm seedlings for resistance to *Fusarium oxysporum* Schl. f. sp. *elaeidis* Toovey. Phytopathol. 79, 77-92.

Lu, P., Aimonino Ricauda, D., Gilardi, G., Gullino, M.L., Garibaldi, A., 2010. Efficacy of different steam distribution systems against five soilborne pathogens under controlled laboratory conditions. Phytoparasitica, 38, 175-189.

Motisi, N., Dorè, T., Lucas, P., Montfort, F., 2010. Dealing with the variability in biofumigation efficacy through an epidemiological framework. Soil Biol. Bioch. 42, 2044-2057.

Motisi, N., Poggi, S., Filipe, J.A.N., Lucas, P., Dore´, T., Montfort, F., Gilliganb, C.A., Baileya, D.J., 2013. Epidemiological analysis of the effects of biofumigation for biological control of root rot in sugar beet. Plant Pathol. 62, 69-78.

Muyzer, G., Smalla, K., 1998. Application of DGGE and TGGE in microbial ecology. Antonie van Leeuwenhoek 73, 127-141.

Noble, R., and Coventry, E., 2005. Suppression of soil-borne plant diseases with compost: a review. Biocontrol Sci. Technol. 15, 3-20.

Núñez-Zofíoa, M., Larreglaa, S., Garbisua, C., 2011. Application of organic amendments followed by soil plastic mulching reduces the incidence of *Phytophthora capsici* in pepper crops under temperate climate. Crop Prot. 30, 1563-1572.

Oka, Y., Shapira, N., Pinchas F., 2007. Control of root-knot nematodes in organic farming systems by organic amendments and soil solarization. Crop Prot. 26 1556-1565.

Pane, C., Villecco, D., Pentangelo, A., Lahoz, E., Zaccardelli, M., 2012. Integration of soil solarization with *Brassica carinata* seed meals amendment in a greenhouse lettuce production system. Acta Agricul. Scand. 62, 291-299.

Pingsheng, J., Kon, D., Yin, J., Jackson, K.L., Csinos, A., 2012. Soil amendments with *Brassica* cover crops for management of *Phytophthora* blight on squash. Pest Manag. Sci. 68, 639-644.

Ros, M., Garcia, C., Hernandez M.T., Lacasa, A., Fernandez, P., Pascual, J.A., 2008. Effects of biosolarization as methyl bromide alternative for Meloidogyne incognita control on quality of soil under pepper. Biol. Fertil. Soils 45, 37-44.

Saremi, H., Okhovvat, S.M., Ashrafi, S.J., 2011. *Fusarium* diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in Iran. African J. Biotech. 10, 18391-18398,

Shukla, A.K., Mishra, R.R., 1996. Response of microbial population and enzyme activities to fungicides in potato field soil. Proc. Ind. Nat. Sci. Acad. Biol. Sci. 62, 435-438.

Simmons, C.W., Guo, H., Claypool, J.T., Marshall, M.N., Perano, K.M., Stapleton, J.J., Vander Gheynst, J.S., 2013. Managing compost stability and amendment to soil to enhance soil heating during soil solarization. Waste Manag. 33, 1090-1096.

Stutz, E.W., Défago, G., Kern, H., 1986. Naturally-occurring fluorescent *Pseudomonas* involved in suppression of black root-rot of tobacco. Phytopathology 76, 181-185.

Sun, H.Y., Deng, S.P., Raun, W.R., 2004. Bacterial community structure and diversity in a century-old manure-treated agroecosystem. Appl. Environ. Microbiol. 70, 5868-5874.

Tamietti, G., Garibaldi, A., 1987. Effectiveness of soil solarization against *Rhizoctonia solani* in northern Italy. Proc. Group Meet. Int. Pest Manag. Prot. Veg. Crops 193-197.

Termorshuizen, A.J., Jeger, M.J., 2008. Strategies of soilborne plant pathogenic fungi in relation to disease suppression. Fung. Ecol. 1, 108-114.

Tsuchiya, D., Taga, M., 2001. Cytological karyotyping of three *Cochliobolus* spp. by the germ tube burst method. Phytopathology 91, 354-360.

Vauterin, L., Vauterin, P., 1992. Computer-aided objective comparison of electrophoreticpatterns for grouping and identification of microorganisms. Europ. Microbiol. 1, 37–41.

Weller, D.M., Raaijimakers, J.M., McSpadden Gardener, B.B., Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Rev. Phytopathol. 40, 309-348.

Johnson, J., 1916. Host plants of *Thielaviopsis basicola*. J. Agr. Res. 7, 289-300.

**Figure 1 -** Dendrogram of similarity generated by the digitized PCR-DGGE fingerprints of DNA directly extracted from substrate infested with *F. oxysporum* f. sp. *basilici* (FOB).

**+** *Brassica carinata* (B); Compost (C); combination of *B. carinata* and compost (B+C).

**++** S-O (sub-optimal temperature 50°C for 6 h, 45°C for 8 h and 40°C for 10); O (optimal temperature 55°C for 6 h, 50°C for 8 h and 47°C for 10 h).

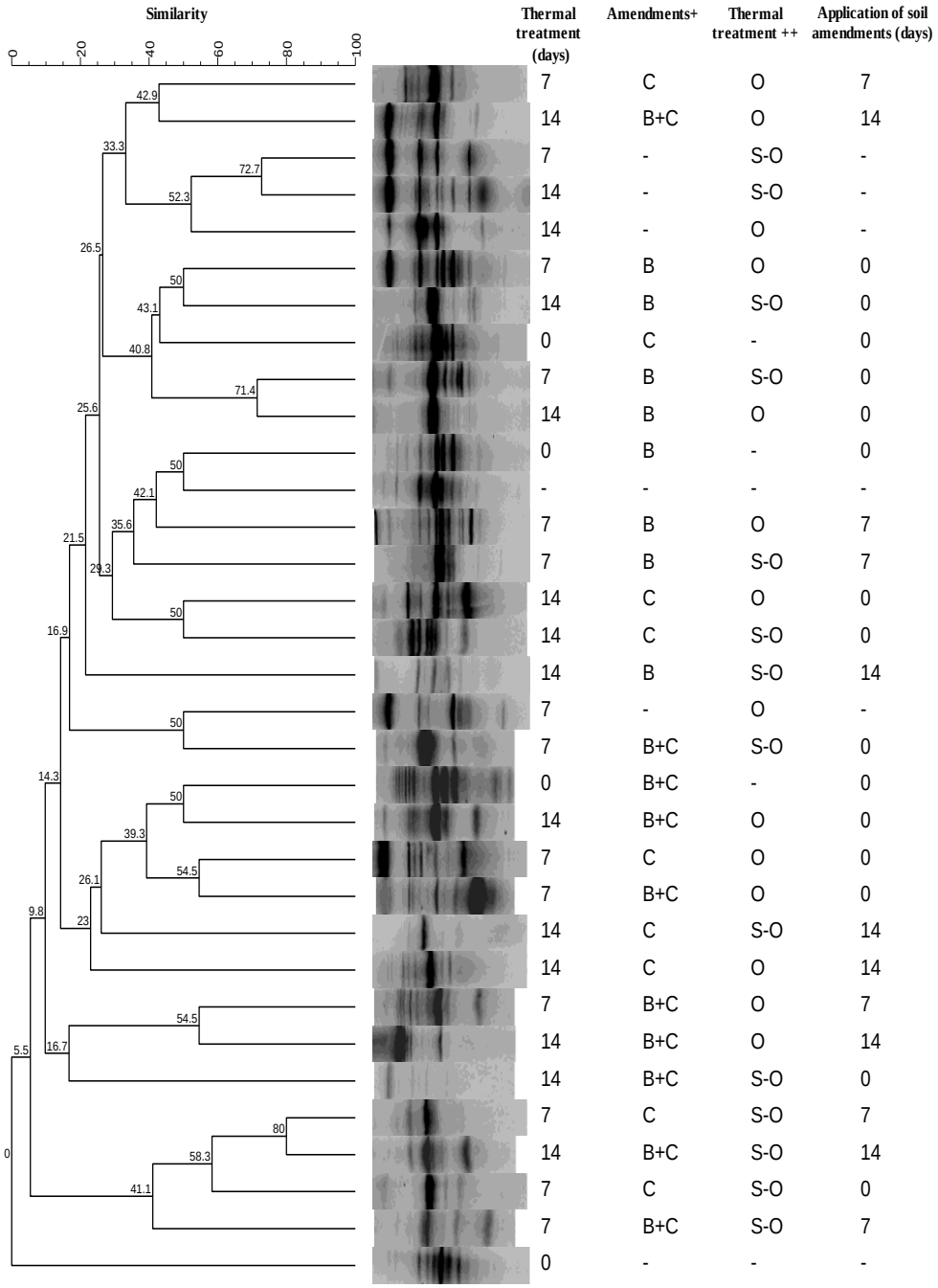

**Figure 2 -** Dendrogram of similarity generated by the digitized PCR-DGGE fingerprints of DNA directly extracted from substrate infested with *F. oxysporum* f. sp. *conglutinans* (FOC).

**+** *Brassica carinata* (B); Compost (C); combination of *B. carinata* and compost (B+C).

**++** S-O (sub-optimal temperature 50°C for 6 h, 45°C for 8 h and 40°C for 10); O (optimal temperature 55°C for 6 h, 50°C for 8 h and 47°C for 10 h).

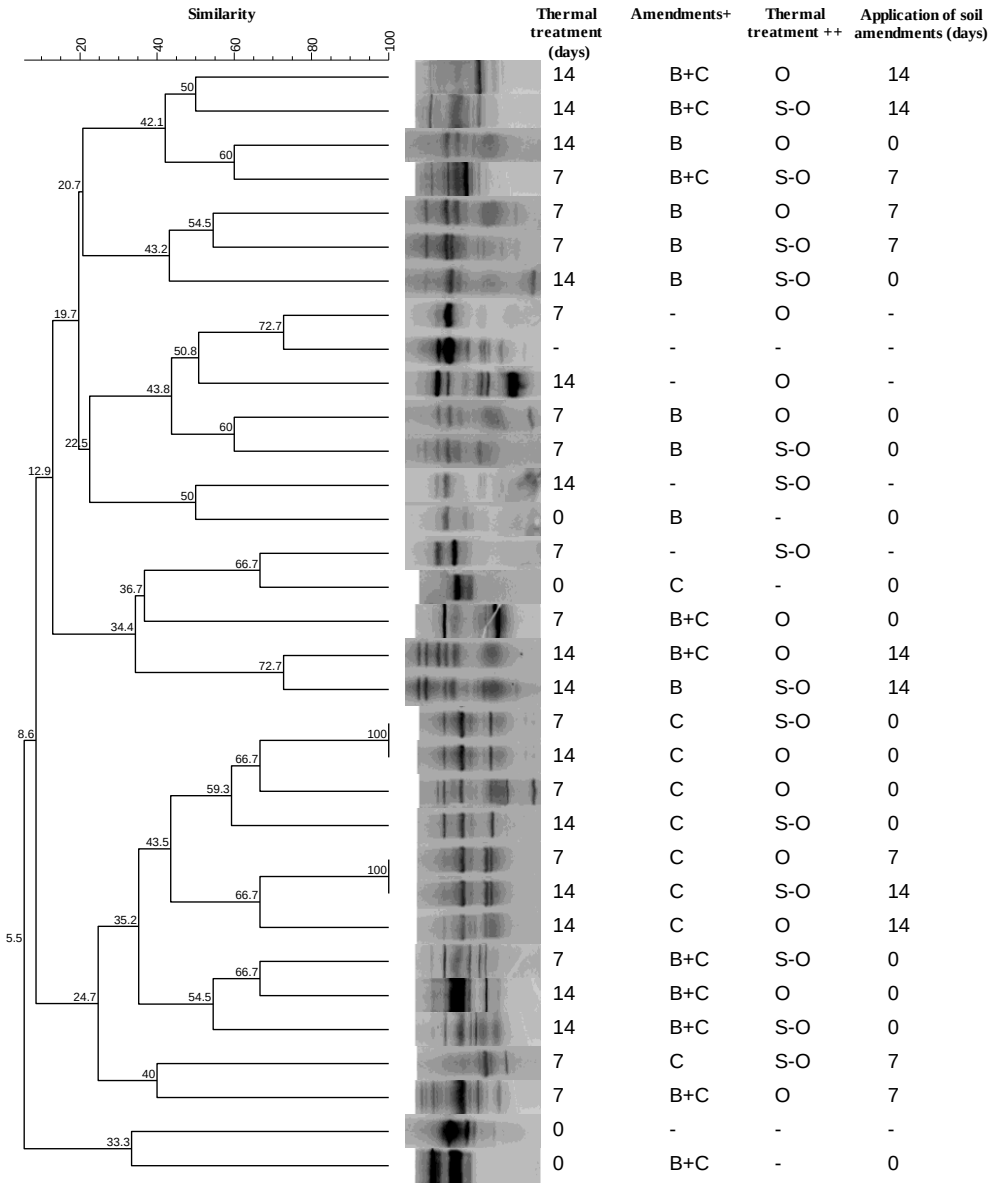

**Table 1**-Main information on the trials carried out

| Thermal treatment (days) | Amendments | Thermal treatment* | Application of soil amendments (days) | sample name |
|---|---|---|---|---|
| Inoculated control | - | - | - | 1 |
| 7 | - | Optimal temperature | 0 | 2 |
| 7 | - | Sub-Optimal temperature | 0 | 3 |
| 14 | - | Optimal temperature | 0 | 4 |
| 14 | - | Sub-Optimal temperature | 0 | 5 |
| - | *Brassica carinata* (2.5g $L^{-1}$ ) | - | - | 6 |
| - | Compost (4 g $L^{-1}$) | - | - | 7 |
| - | *Brassica carinata* (2.5g $L^{-1}$ ) + Compost (4 g $L^{-1}$) | - | - | 8 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ ) | Optimal temperature | | 9 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ ) | Sub-Optimal temperature | 0 | 10 |
| 7 | Compost (4 g $L^{-1}$) | Optimal temperature | 0 | 11 |
| 7 | Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 0 | 12 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ ) + Compost (4 g $L^{-1}$) | Optimal temperature | 0 | 13 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ ) + Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 0 | 14 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ ) | Optimal temperature | 0 | 15 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ ) | Sub-Optimal temperature | 0 | 16 |
| 14 | Compost (4 g $L^{-1}$) | Optimal temperature | 0 | 17 |
| 14 | Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 0 | 18 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ )+ Compost (4 g $L^{-1}$) | Optimal temperature | 0 | 19 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ )+ Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 0 | 20 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ ) | Optimal temperature | 7 | 21 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ ) | Sub-Optimal temperature | 7 | 22 |
| 7 | Compost (4 g $L^{-1}$) | Optimal temperature | 7 | 23 |
| 7 | Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 7 | 24 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ )+ Compost (4 g $L^{-1}$) | Optimal temperature | 7 | 25 |
| 7 | *Brassica carinata* (2.5g $L^{-1}$ )+ Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 7 | 26 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ ) | Optimal temperature | 14 | 27 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ ) | Sub-Optimal temperature | 14 | 28 |
| 14 | Compost (4 g $L^{-1}$) | Optimal temperature | 14 | 29 |
| 14 | Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 14 | 30 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ )+ Compost (4 g $L^{-1}$) | Optimal temperature | 14 | 31 |
| 14 | *Brassica carinata* (2.5g $L^{-1}$ )+ Compost (4 g $L^{-1}$) | Sub-Optimal temperature | 14 | 32 |
| Non-treated, non-inoculated control | - | - | - | 33 |

*Maximum temperature at 10 cm substrate depth in sub-optimal (50°C for 6 h, 45°C for 8 h and 40°C for 10) and optimal (55°C for 6 h, 50°C for 8 h and 47°C for 10 h) conditions.

**Table 2** (Panel A) Plate counts of different microbial groups in substrate infested with *F. oxysporum* f. sp. *basilici* (FOB)

| Thermal treatment (days) | Amendments | Application of amendments (days) | *Komada medium* (Log CFU $g^{-1}$ ± SD[b]) | | | | PDA (Log CFU $g^{-1}$ ± SD) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sub-optimal [a] thermal treatment | | Optimal thermal treatment | | Sub-optimal thermal treatment | | Optimal thermal treatment | |
| | | | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial |
| Inoculated control | - | - | 3,1 ±1,7 bc | 4,1 ±0,3 d | 4,1 ±0,2 d | 4,1 ±0,3 c | 4,8 ±0,5 a-c | 4,9 ±0,1 a | 4,8 ±0,5 a-c | 4,9 ±0,1 ab |
| 7 | - | - | 2,5 ±0,5 bc | 2,5 ±0,3 bc | 2,2 ±0,3 b | 2,6 ±0,3 b | 4,4 ±1,3 a-c | 3,7 ±1,0 a | 4,2 ±0,9 a-c | 4,5 ±0,3 ab |
| 14 | - | - | 2,3 ±0,3 bc | 2,5 ±0,3 bc | 2,1 ±0,1 b | 2,4 ±0,5 b | 3,3 ±1,1 a | 4,5 ±0,2 a | 3,7 ±1,0 ab | 4,6 ±0,3 ab |
| - | *B. carinata* | 0 | 3,5 ±0,3 c | 3,6 ±0,2 d | 3,5 ±0,3 cd | 3,6 ±0,2 c | 4,9 ±0,2 a-c | 4,5 ±0,7 a | 4,9 ±0,2 a-c | 4,5 ±0,7 ab |
| - | Compost | | 2,4 ±1,8 bc | 3,7 ±0,1 d | 2,4 ±1,8 b | 3,7 ±0,1 c | 5,2 ±0,2 bc | 4,6 ±0,7 a | 5,2 ±0,2 bc | 4,6 ±0,7 ab |
| - | *B. carinata* + compost | 0 | 2,8 ±0,8 bc | 3,7 ±0,3 d | 2,8 ±0,8 bc | 3,7 ±0,3 c | 5,3 ±0,2 c | 4,4 ±0,8 a | 5,3 ±0,2 c | 4,7 ±0,3 ab |
| 7 | *B. carinata* | 0 | 2,3 ±0,4 bc | 2,7 ±0,1 bc | 2,1 ±0,2 b | 2,3 ±0,2 b | 4,0 ±0,7 a-c | 4,2 ±0,6 a | 4,4 ±1,3 a-c | 4,0 ±1,1 a |
| 7 | Compost | | 2,3 ±0,3 bc | 2,8 ±0,1 c | 2,2 ±0,3 b | 2,2 ±0,3 b | 3,8 ±0,7 a-c | 4,4 ±0,1 a | 4,6 ±0,6 a-c | 4,1 ±0,9 ab |
| 7 | *B. carinata* + compost | 0 | 2,0 ±0,0 b | 2,5 ±0,1 bc | 2,3 ±0,3 b | 2,4 ±0,6 b | 4,0 ±1,1 a-c | 3,9 ±0,8 a | 4,2 ±0,3 a-c | 4,2 ±1,0 ab |
| 14 | *B. carinata* | 0 | 2,3 ±0,4 bc | 2,5 ±0,4 bc | 2,4 ±0,5 bc | 2,3 ±0,5 b | 3,5 ±1,2 ab | 4,2 ±0,7 a | 3,5 ±0,8 a | 4,4 ±0,3 ab |
| 14 | Compost | | 2,2 ±0,3 bc | 2,5 ±0,4 bc | 2,4 ±0,4 b | 2,4 ±0,5 b | 3,9 ±0,8 a-c | 4,1 ±0,6 a | 4,3 ±0,4 a-c | 4,3 ±0,4 ab |
| 14 | *B. carinata* + compost | 0 | 2,1 ±0,2 b | 2,5 ±0,6 bc | 2,1 ±0,2 b | 2,2 ±0,2 b | 4,0 ±1,0 a-c | 3,5 ±0,7 a | 3,8 ±0,7 ab | 3,7 ±0,7 a |
| 7 | *B. carinata* | 7 | 2,3 ±0,4 bc | 2,5 ±0,3 bc | 2,1 ±0,2 b | 2,4 ±0,3 b | 3,9 ±0,9 a-c | 4,6 ±0,4 a | 4,7 ±1,4 a-c | 5,2 ±0,5 b |
| 7 | Compost | 7 | 2,4 ±0,5 bc | 2,6 ±0,3 bc | 2,4 ±0,5 bc | 2,1 ±0,2 b | 3,9 ±0,9 a-c | 3,9 ±0,8 a | 3,8 ±0,6 ab | 3,7 ±0,7 a |
| 7 | *B. carinata* + *compost* | 7 | 2,2 ±0,2 bc | 2,6 ±0,2 bc | 2,1 ±0,1 b | 2,0 ±0,0 b | 4,5 ±0,5 a-c | 4,4 ±0,3 a | 4,2 ±0,5 a-c | 4,6 ±0,3 ab |
| 14 | *B. carinata* | 14 | 2,4 ±0,5 bc | 2,3 ±0,3 bc | 2,4 ±0,4 b | 2,1 ±0,2 b | 3,9 ±0,8 a-c | 3,8 ±0,9 a | 4,1 ±0,9 a-c | 4,4 ±0,1 ab |
| 14 | Compost | 14 | 2,0 ±0,0 b | 2,2 ±0,2 b | 2,2 ±0,3 b | 2,0 ±0,2 b | 4,2 ±0,8 a-c | 4,2 ±0,7 a | 4,3 ±0,2 a-c | 4,2 ±0,2 ab |
| 14 | *B. carinata* + compost | 14 | 2,1 ±0,2 b | 2,3 ±0,4 bc | 2,2 ±0,2 b | 2,2 ±0,2 b | 4,5 ±0,4 a-c | 3,9 ±0,8 a | 5,0 ±0,9 a-c | 4,1 ±0,2 ab |
| Not treated not inoculated control | - | - | 0,0 ±0,0 a | 0,0 ±0,0 a | 0,0 ±0,0 a | 0,0 ±0,0 a | 5,2 ±0,1 bc | 4,5 ±0,4 a | 5,2 ±0,1 bc | 4,5 ±0,4 ab |

[a] Maximum temperature at 10 cm substrate depth in sub-optimal (50°C for 6 h, 45°C for 8 h and 40°C for 10) and optimal (55°C for 6 h, 50°C for 8 h and 47°C for 10 h) conditions.
[b] Results were calculated as the mean Log count for the three plate replicates. Values with different superscripts in the same column differ significantly (P<0.05).

**Table 2** (Panel B) Plate counts of different microbial groups in substrate infested with *F. oxysporum* f. sp. *basilici* (FOB)

| Thermal treatment (days) | Amendments | Application of amendments (days) | LB medium (Log CFU $g^{-1}$ ± SD[b]) | | | | *Pseudomonas* CFC medium ( Log CFU $g^{-1}$ ± SD) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sub-optimal [a] thermal treatment | | Optimal thermal treatment | | Sub-optimal thermal treatment | | Optimal thermal treatment | |
| | | | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial |
| Inoculated control | - | - | 9,8 ±0,2 d | 9,2 ±0,8 ab | 9,8 ±0,2 ab | 8,5 ±0,2 e-h | 7,8 ±0,7 a-c | 7,2 ±0,7 a-c | 7,8 ±0,7 b-e | 7,2 ±0,7 a-d |
| 7 | - | - | 10,3 ±0,0 e | 7,5 ±0,9 a | 9,8 ±0,0 a | 8,8 ±0,1 gh | 9,1 ±0,0 e | 6,1 ±1,2 a | 8,2 ±0,4 de | 7,9 ±0,1 b-d |
| 14 | - | - | 9,0 ±0,1 a | 9,5 ±0,8 b | 9,0 ±0,0 b | 7,2 ±0,5 ab | 8,9 ±0,0 e | 7,5 ±0,6 a-c | 7,5 ±0,4 a-d | 7,4 ±0,7 a-d |
| - | *B. carinata* | 0 | 11,0 ±0,0 f | 9,5 ±0,6 b | 11,0 ±0,0 b | 8,9 ±0,1 h | 8,8 ±0,1 de | 8,3 ±1,8 c | 8,8 ±0,1 e | 6,0 ±0,8 ab |
| - | Compost | | 10,1 ±0,0 e | 8,9 ±0,3 ab | 10,1 ±0,0 ab | 8,6 ±0,2 f-h | 8,1 ±0,1 b-c | 6,2 ±1,0 a | 8,1 ±0,1 c-e | 8,4 ±1,5 d |
| - | *B. carinata* + compost | 0 | 10,2 ±0,1 e | 9,5 ±0,8 b | 10,2 ±0,1 b | 8,8 ±0,1 f-h | 8,1 ±0,0 b-c | 8,3 ±1,7 bc | 8,1 ±0,0 c-e | 7,5 ±0,8 a-d |
| 7 | *B. carinata* | 0 | 10,2 ±0,1 e | 9,3 ±0,4 ab | 7,5 ±0,2 ab | 7,0 ±0,0 a | 9,1 ±0,1 e | 7,8 ±0,4 a-c | 6,6 ±0,3 a | 8,1 ±2,0 cd |
| 7 | Compost | | 11,0 ±0,0 f | 9,4 ±0,6 b | 9,8 ±0,0 b | 8,0 ±0,0 d-f | 9,1 ±0,0 e | 7,8 ±0,4 a-c | 6,9 ±0,1 ab | 7,4 ±0,5 a-d |
| 7 | *B. carinata* + compost | 0 | 9,2 ±0,1 ab | 9,1 ±1,1 ab | 8,8 ±0,1 ab | 7,2 ±0,2 ab | 8,4 ±0,4 c-e | 8,2 ±1,0 bc | 7,9 ±0,1 b-e | 5,8 ±0,8 a |
| 14 | *B. carinata* | 0 | 9,0 ±0,0 a | 9,7 ±1,5 b | 9,0 ±0,0 b | 7,1 ±0,2 a | 7,5 ±0,1 ab | 7,8 ±0,6 a-c | 7,7 ±0,0 a-d | 6,8 ±0,4 a-d |
| 14 | Compost | | 9,2 ±0,1 ab | 9,5 ±1,7 b | 8,5 ±0,2 b | 7,8 ±0,3 b-d | 8,1 ±0,2 b-c | 6,9 ±0,2 a-c | 7,1 ±0,3 a-c | 6,2 ±0,6 a-c |
| 14 | *B. carinata* + compost | 0 | 11,0 ±0,0 f | 8,3 ±0,3 ab | 8,3 ±0,1 ab | 7,9 ±0,1 c-e | 8,2 ±0,1 b-c | 6,3 ±1,3 ab | 7,4 ±0,8 a-d | 7,3 ±0,9 a-d |
| 7 | *B. carinata* | 7 | 9,3 ±0,0 bc | 8,5 ±0,5 ab | 9,1 ±0,0 ab | 7,9 ±0,1 cd | 7,3 ±0,1 a | 7,7 ±1,0 a-c | 8,0 ±0,3 c-e | 7,5 ±1,6 a-d |
| 7 | Compost | 7 | 10,2 ±0,1 e | 8,9 ±0,1 ab | 9,7 ±0,0 ab | 8,0 ±0,0 de | 8,4 ±0,4 c-e | 7,1 ±0,1 a-c | 7,8 ±0,2 b-e | 7,9 ±0,9 b-d |
| 7 | *B. carinata* + *compost* | 7 | 11,0 ±0,0 f | 9,4 ±0,6 b | 8,7 ±0,0 b | 7,3 ±0,4 a-c | 7,9 ±0,0 a-c | 7,3 ±0,3 a-c | 7,7 ±0,4 a-d | 7,8 ±1,2 b-d |
| 14 | *B. carinata* | 14 | 10,1 ±0,0 e | 9,3 ±0,8 ab | 9,0 ±0,1 ab | 8,2 ±0,0 d-g | 7,6 ±0,0 ab | 8,1 ±1,2 a-c | 7,6 ±0,1 a-d | 6,8 ±0,6 a-d |
| 14 | Compost | 14 | 9,1 ±0,0 ab | 9,2 ±0,9 ab | 9,0 ±0,1 ab | 7,4 ±0,3 a-c | 8,9 ±0,1 e | 6,9 ±0,6 a-c | 7,0 ±0,1 a-c | 6,6 ±0,3 a-d |
| 14 | *B. carinata* + compost | 14 | 10,3 ±0,0 e | 9,1 ±1,0 ab | 11,0 ±0,0 ab | 8,1 ±0,2 d-f | 7,8 ±0,1 a-c | 8,1 ±0,9 a-c | 7,8 ±0,1 b-e | 8,2 ±1,0 d |
| Not treated not inoculated control | - | - | 9,4 ±0,0 c | 8,9 ±1,2 ab | 9,4 ±0,0 ab | 7,9 ±0,1 cd | 7,9 ±0,5 a-c | 6,5 ±0,8 a-c | 7,9 ±0,5 b-e | 6,6 ±0,4 a-d |

[a] Maximum temperature at 10 cm substrate depth in sub-optimal (50°C for 6 h, 45°C for 8 h and 40°C for 10) and optimal (55°C for 6 h, 50°C for 8 h and 47°C for 10 h) conditions.
[b] Results were calculated as the mean Log count for the three plate replicates. Values with different superscripts in the same column differ significantly (P<0.05).

**Table 3** (Panel C) Plate counts of different microbial groups in substrate infested with *F. oxysporum* f. sp. *conglutinans* (FOC)

| Thermal treatment (days) | Amendments | Application of amendments (days) | *Komada medium* (Log CFU $g^{-1}$ ± SD[b]) | | | | PDA (Log CFU $g^{-1}$ ± SD) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sub-optimal [a] thermal treatment | | Optimal thermal treatment | | Sub-optimal thermal treatment | | Optimal thermal treatment | |
| | | | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial |
| Inoculated control | - | - | 4,5 ±0,5 d | 3,6 ±0,2 d | 4,1 ±0,5 d | 3,5 ±0,2 d | 4,9 ±0,3 de | 5,1 ±0,2 a | 4,9 ±0,3 ef | 5,1 ±0,2 a |
| 7 | - | - | 2,0 ±0,0 b | 2,1 ±0,2 b | 2,0 ±0,0 b | 2,4 ±0,3 b | 2,5 ±0,5 a | 5,0 ±0,4 a | 3,2 ±0,6 ab | 5,0 ±0,4 a |
| 14 | - | - | 2,0 ±0,0 b | 2,7 ±0,3 bc | 2,0 ±0,0 b | 2,5 ±0,2 bc | 2,7 ±0,3 ab | 4,8 ±0,2 a | 3,4 ±0,5 ab | 4,8 ±0,2 a |
| - | *B. carinata* | 0 | 4,2 ±0,7 d | 3,9 ±0,3 d | 3,6 ±0,7 d | 3,7 ±0,3 d | 4,7 ±0,1 b-e | 5,2 ±0,4 a | 4,7 ±0,1 d-f | 5,2 ±0,4 a |
| - | Compost | | 4,1 ±0,7 d | 3,7 ±0,3 d | 3,4 ±0,7 d | 4,0 ±0,3 d | 4,7 ±0,1 c-e | 5,1 ±0,4 a | 4,7 ±0,1 d-f | 5,1 ±0,4 a |
| - | *B. carinata* + compost | 0 | 3,0 ±0,6 c | 3,7 ±0,4 d | 3,5 ±0,6 c | 4,0 ±0,3 d | 5,1 ±0,4 e | 5,1 ±0,3 a | 5,1 ±0,4 f | 5,1 ±0,3 a |
| 7 | *B. carinata* | 0 | 2,0 ±0,0 b | 2,5 ±0,3 bc | 2,0 ±0,0 b | 2,4 ±0,3 b | 3,0 ±0,1 a-d | 5,1 ±0,4 a | 4,6 ±0,6 c-f | 5,1 ±0,4 a |
| 7 | Compost | | 2,0 ±0,0 b | 2,2 ±0,4 b | 2,0 ±0,0 b | 2,0 ±0,0 b | 3,3 ±1,5 a-e | 4,8 ±0,5 a | 3,6 ±0,3 a-c | 4,8 ±0,5 a |
| 7 | *B. carinata* + compost | 0 | 2,0 ±0,0 b | 2,1 ±0,2 b | 2,0 ±0,0 b | 2,6 ±0,4 bc | 4,2 ±2,4 a-e | 5,3 ±0,2 a | 3,7 ±0,3 bcd | 5,3 ±0,2 a |
| 14 | *B. carinata* | 0 | 2,1 ±0,1 b | 2,1 ±0,2 b | 2,0 ±0,0 b | 2,6 ±0,3 bc | 2,9 ±0,9 a-c | 5,2 ±0,3 a | 4,0 ±1,1 bcd | 5,2 ±0,3 a |
| 14 | Compost | | 2,0 ±0,0 b | 2,9 ±0,1 c | 2,0 ±0,0 b | 2,8 ±0,5 bc | 2,5 ±0,5 a | 4,9 ±0,5 a | 3,9 ±0,4 bcd | 4,9 ±0,5 a |
| 14 | *B. carinata* + compost | 0 | 2,0 ±0,0 b | 2,5 ±0,5 bc | 2,0 ±0,0 b | 2,4 ±0,1 bc | 2,9 ±1,1 a-c | 4,8 ±0,1 a | 2,7 ±0,4 a | 4,8 ±0,1 a |
| 7 | *B. carinata* | 7 | 2,0 ±0,0 b | 2,5 ±0,4 bc | 2,0 ±0,0 b | 2,4 ±0,2 b | 3,3 ±1,3 a-e | 5,2 ±0,3 a | 3,6 ±0,4 a-c | 5,2 ±0,3 a |
| 7 | Compost | 7 | 2,0 ±0,0 b | 2,6 ±0,3 bc | 2,0 ±0,0 b | 2,5 ±0,3 bc | 3,6 ±1,1 a-e | 4,9 ±0,3 a | 3,6 ±0,5 a-c | 4,9 ±0,3 a |
| 7 | *B. carinata* + *compost* | 7 | 2,0 ±0,0 b | 2,4 ±0,3 bc | 2,0 ±0,0 b | 2,5 ±0,3 bc | 4,0 ±1,2 a-e | 5,0 ±0,2 a | 4,2 ±0,7 b-f | 5,0 ±0,2 a |
| 14 | *B. carinata* | 14 | 2,0 ±0,0 b | 2,2 ±0,4 b | 2,0 ±0,0 b | 2,9 ±0,1 c | 2,7 ±0,4 ab | 5,0 ±0,2 a | 3,7 ±0,5 a-c | 5,0 ±0,2 a |
| 14 | Compost | 14 | 2,0 ±0,0 b | 2,2 ±0,3 b | 2,0 ±0,0 b | 2,3 ±0,2 b | 3,6 ±1,0 a-e | 5,2 ±0,4 a | 4,3 ±0,4 b-f | 5,2 ±0,4 a |
| 14 | *B. carinata* + compost | 14 | 2,0 ±0,0 b | 2,2 ±0,3 b | 2,0 ±0,0 b | 2,2 ±0,3 b | 3,6 ±0,9 a-e | 5,3 ±0,5 a | 4,0 ±0,5 bcd | 5,3 ±0,5 a |
| Not treated not inoculated control | - | - | 0,0 ±0,0 a | 0,0 ±0,0 a | 0,0 ±0,0 a | 0,0 ±0,0 a | 4,7 ±0,4 b-e | 5,2 ±0,1 a | 4,7 ±0,4 d-f | 5,2 ±0,1 a |

[a] Maximum temperature at 10 cm substrate depth in sub-optimal (50°C for 6 h, 45°C for 8 h and 40°C for 10) and optimal (55°C for 6 h, 50°C for 8 h and 47°C for 10 h) conditions.
[b] Results were calculated as the mean Log count for the three plate replicates. Values with different superscripts in the same column differ significantly (P<0.05).

**Table 3** (Panel D) Plate counts of different microbial groups in substrate infested with *F. oxysporum* f. sp. *conglutinans* (FOC)

| Thermal treatment (days) | Amendments | Application of amendments (days) | LB medium (Log CFU $g^{-1}$ ± SD[b]) | | | | *Pseudomonas* CFC medium ( Log CFU $g^{-1}$ ± SD) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sub-optimal [a] thermal treatment | | Optimal thermal treatment | | Sub-optimal thermal treatment | | Optimal thermal treatment | |
| | | | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial | 1st trial | 2nd trial |
| Inoculated control | - | - | 6,5 ±0,3 abc | 7,8 ±0,2 b | 6,5 ±0,3 c | 7,8 ±0,2 bcde | 5,6 ±0,5 b | 6,4 ±0,4 abc | 5,6 ±0,5 ab | 6,4 ±0,4 bcdef |
| 7 | - | - | 6,0 ±0,0 a | 8,5 ±0,2 def | 8,8 ±0,1 f | 8,8 ±0,1 i | 5,0 ±0,0 a | 7,1 ±0,2 c | 5,0 ±0,0 a | 7,0 ±0,0 efg |
| 14 | - | - | 6,0 ±0,0 a | 8,0 ±0,1 bc | 6,0 ±0,0 a | 7,6 ±0,1 abcd | 5,0 ±0,0 a | 6,1 ±0,2 ab | 5,0 ±0,0 a | 6,7 ±0,0 cdefg |
| - | *B. carinata* | 0 | 7,9 ±0,1 de | 8,8 ±0,1 f | 7,9 ±0,1 e | 8,8 ±0,1 i | 5,0 ±0,0 a | 7,1 ±0,1 c | 5,0 ±0,0 a | 7,1 ±0,1 g |
| - | Compost | | 6,7 ±0,1 abc | 8,6 ±0,1 ef | 6,7 ±0,1 c | 8,6 ±0,1 hi | 5,0 ±0,0 a | 6,9 ±0,1 bc | 5,0 ±0,0 a | 6,9 ±0,1 efg |
| - | *B. carinata* + compost | 0 | 9,0 ±0,0 f | 8,1 ±0,1 bcde | 9,0 ±0,0 f | 8,1 ±0,1 efg | 8,0 ±0,1 e | 6,7 ±0,4 abc | 8,0 ±0,1 d | 6,7 ±0,4 cdefg |
| 7 | *B. carinata* | 0 | 6,7 ±0,2 abc | 7,2 ±0,3 a | 6,0 ±0,0 a | 7,1 ±0,1 a | 5,0 ±0,0 a | 6,5 ±0,0 abc | 5,0 ±0,0 a | 6,4 ±0,1 bcde |
| 7 | Compost | | 6,0 ±0,0 a | 8,8 ±0,2 f | 6,0 ±0,0 a | 8,5 ±0,4 ghi | 5,0 ±0,0 a | 6,4 ±0,1 abc | 5,0 ±0,0 a | 5,9 ±0,2 ab |
| 7 | *B. carinata* + compost | 0 | 6,0 ±0,0 a | 8,0 ±0,0 bcd | 6,1 ±0,2 ab | 7,8 ±0,3 bcde | 5,0 ±0,0 a | 6,5 ±0,0 abc | 5,3 ±0,2 ab | 6,4 ±0,2 bcdef |
| 14 | *B. carinata* | 0 | 6,2 ±0,3 a | 7,9 ±0,1 bc | 6,0 ±0,0 a | 7,5 ±0,2 abc | 5,0 ±0,0 a | 6,1 ±0,1 ab | 5,0 ±0,0 a | 6,9 ±0,2 efg |
| 14 | Compost | | 6,0 ±0,0 a | 7,8 ±0,3 bc | 6,0 ±0,0 a | 7,9 ±0,0 cde | 5,0 ±0,0 a | 6,5 ±0,4 abc | 5,0 ±0,0 a | 7,0 ±0,1 fg |
| 14 | *B. carinata* + compost | 0 | 7,0 ±0,2 bc | 8,6 ±0,1 def | 6,0 ±0,0 a | 7,3 ±0,1 ab | 5,0 ±0,0 a | 6,7 ±0,3 abc | 5,0 ±0,0 a | 5,6 ±0,5 a |
| 7 | *B. carinata* | 7 | 7,2 ±0,1 cd | 8,3 ±0,1 cdef | 7,2 ±0,0 d | 8,0 ±0,1 cdef | 6,9 ±0,0 c | 5,9 ±0,4 a | 5,0 ±0,0 a | 6,8 ±0,1 defg |
| 7 | Compost | 7 | 6,0 ±0,0 a | 8,2 ±0,1 bcde | 6,0 ±0,0 a | 7,9 ±0,1 cde | 5,0 ±0,0 a | 6,4 ±0,2 abc | 5,0 ±0,0 a | 6,8 ±0,1 defg |
| 7 | *B. carinata* + *compost* | 7 | 6,4 ±0,5 ab | 8,2 ±0,1 bcde | 7,1 ±0,1 d | 8,5 ±0,1 fghi | 7,2 ±0,1 c | 6,4 ±0,4 abc | 5,8 ±0,2 b | 6,1 ±0,1 abc |
| 14 | *B. carinata* | 14 | 9,4 ±0,0 f | 8,3 ±0,2 cdef | 7,1 ±0,1 d | 7,8 ±0,2 cde | 8,0 ±0,0 e | 6,9 ±0,2 bc | 5,8 ±0,7 b | 6,2 ±0,1 abcd |
| 14 | Compost | 14 | 8,1 ±0,2 e | 7,9 ±0,4 bc | 6,0 ±0,0 a | 8,0 ±0,1 def | 5,0 ±0,0 a | 6,9 ±0,5 bc | 5,8 ±0,2 b | 6,6 ±0,0 cdefg |
| 14 | *B. carinata* + compost | 14 | 6,6 ±0,6 abc | 8,1 ±0,1 bcde | 6,4 ±0,2 bc | 8,3 ±0,1 efgh | 7,3 ±0,0 cd | 6,4 ±0,4 abc | 7,0 ±0,3 c | 6,4 ±0,1 bcde |
| Not treated not inoculated control | - | - | 8,0 ±0,2 e | 8,1 ±0,2 bcde | 8,0 ±0,2 e | 8,1 ±0,2 efgh | 7,7 ±0,1 de | 6,7 ±0,3 abc | 7,7 ±0,1 cd | 6,7 ±0,3 cdefg |

[a] Maximum temperature at 10 cm substrate depth in sub-optimal (50°C for 6 h, 45°C for 8 h and 40°C for 10) and optimal (55°C for 6 h, 50°C for 8 h and 47°C for 10 h) conditions.
[b] Results were calculated as the mean Log count for the three plate replicates. Values with different superscripts in the same column differ significantly (P<0.05).

**Table 4.** Results of band sequencing from DGGE profiles of DNA directly extracted from substrate infested with *F. oxysporum* f. sp. *basilici* (FOB).

| Species identification and accession number § | Samples * | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
| *Aspergillus versicolor* JX160053 | | | x | | | | | | | | | | | | | x | x | | | | | | | | | | | | | | | | |
| *Fusarium* spp. JX160053 | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x |
| *Hypocreales* spp. JX179228 | | | x | | | | | | x | | | | | | | | | | | | | x | | | | | | | | | | | |
| *F. oxysporum* JX915255 | x | x | x | x | x | x | x | x | x | x | x | | x | | | | | | | | x | | | | | x | x | x | | | x | x | x |
| *Myrothecium roridum* HQ43334 | | | | | | | | | | | | | | | x | x | | | | | | | | | | | | | | | | | |
| *Pezizomycetes* spp. FN868473 | | | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | |
| *Pseudallescheria fimeti* AY879799 | | | | | | | x | | | | | x | | | | | | | | | | | x | x | | | | | x | x | | | |
| *Pseudallescheria boydii* EF151338 | | | | | | | x | x | | | x | x | | x | | | | x | x | | | | | x | x | x | | | | x | x | x | |
| *Curvularia* spp. JN207242 | | | | | | | | | | | | x | | | | | | | | x | | | | x | | | | | x | x | | | |
| *Peziza ostracoderma* U40472 | | | | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | | |

***** sample descriptions are reported in Table 1.
**§** Species identified from sequencing of bands in the profiles of each sample. The presence of the x indicates the presence of the bands in the DGGE profiles.

**Table 5.** Results of band sequencing from DGGE profiles of DNA directly extracted from substrate infested with *F. oxysporum* f. sp. *conglutinans* (FOC).

| | **Samples *** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species identification and accession number §** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
| *Phoma* spp. HE609795 | | | | | x | | | | x | | x | | x | | | x | | | | | | | | | x | | | | | | | | |
| *Scedosporium prolificans* AY228121 | x | | | | x | | | | | | x | x | | x | | | x | x | x | | | | x | x | x | x | | | x | x | | | |
| *Fusarium* spp. HQ631005 | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x |
| *Pseudallescheria boydii* GU566237 | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *F. oxysporum* JF79674 | x | | | x | x | | | | | | | | | x | | | | | | | | | x | x | x | | | | x | x | x | x | |
| *Candida subhashii* NR073356 | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Uncultured Oidiodendron* GQ338892 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x |
| *Alternaria brassicae* JX290150 | | | | | | | | | x | x | | | | | x | x | | | | | x | x | | | | | | | | | | | x |
| *Cochliobolus* spp. JQ717312 | | x | x | x | x | | x | | x | x | x | x | | x | x | x | x | x | | x | x | x | x | | | | x | x | x | x | | | |
| *Thielaviopsis basicola* GQ131877 | x | | | x | x | x | | x | x | x | x | x | x | x | | | x | x | x | x | x | x | x | | x | x | | x | x | x | x | x | x |

***** sample descriptions are reported in Table 1.

**§** Species identified from sequencing of bands in the profiles of each sample. The presence of the x indicates the presence of the bands in the DGGE profiles.