

Compost from forest cleaning green waste and *Trichoderma asperellum* strain T34 reduced incidence of *Fusarium circinatum* in *Pinus radiata* seedlings*

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Abstract

Fusarium circinatum is a quarantine pathogen in numerous countries that causes important economic losses in forest nurseries and mature pine trees. Alternatives to chemical control of plant diseases such as suppressive composts and the use of biological control agents in growth media can reduce the incidence and spread of disease. In this work, four gorse composts obtained from forest cleaning green wastes and reference compost prepared from the organic fraction of solid urban waste were studied as environmentally sustainable peat substitutes. Their ability to suppress the effects of mating types M1 and M2 of the pathogen *F. circinatum* on *Pinus radiata* was evaluated in the presence or absence of the biocontrol agent *Trichoderma asperellum* strain T34. One of the gorse composts was more suppressive than the reference compost (15% and 55% of disease incidence, respectively). The former had a low EC, moderate amounts of P, Ca, Mg and K, and high levels of NH_4^+ , as well as moderate levels of Ca, B and oligotrophic actinomycetes, the highest levels of total fungi, moderate levels of *Trichoderma* spp. and the lowest levels of *Fusarium* spp. Adding T34 to the moderately suppressive and non-suppressive composts decreased pre- and post-emergence incidence of the disease by up to 50%.

In conclusion, one gorse compost decreased the incidence of *F. circinatum* disease when used as a growth medium for *Pinus radiata* seedlings. *T. asperellum* strain T34 further reduced the incidence of disease and improved the health of pine seedlings grown in moderately non-suppressive composts.

Key words: Cattle slurry, *Gibberella circinata*, gorse, pine, poultry manure.

1. Introduction

Pitch canker, which is caused by the fungus *Gibberella circinata* (anamorph *Fusarium circinatum*) Nirenberg & O'Donnell, is a serious disease affecting pine plantations and nurseries around the world. Cankers are often associated with conspicuous, occasionally spectacular resin exudates ("pitch"). Multiple-branch infections may cause severe crown dieback and eventually lead to tree death (Dwinnell et al., 2001; Storer et al., 2002). The pitch canker pathogen can also infect seedlings and cause them to exhibit pre- and post-emergence damping off (Storer, 1998). Pitch canker was first reported in North Carolina, United States (Hepting and Roth, 1946) but has since spread throughout the southeast of the country and within California (Dwinnell et

al., 2001), Japan (Muramoto and Dwinell, 1990), Mexico (Guerra-Santos, 1999), South Africa (Viljoen et al., 1994), Chile (Wingfield et al., 2002) and Spain (Landeras et al., 2005) —the latter country was the first to report the presence of this pathogen in Europe. The European and Mediterranean Plant Protection Organization (EPPO, 2012) considers *F. circinatum* a quarantine pest that is present in the Mediterranean region but not widely distributed there; the disease is also officially controlled. Pitch canker infects 57 species of pines (*Pinus* spp.) and Douglas fir (*Pseudotsuga menziesii*) (Quesada et al., 2010; Wingfield et al., 2008; Gordon et al., 2006). Among the pine species, *Pinus radiata* D. Don is considered to be one of the most susceptible to this pathogen (Gordon et al., 2001), the presence of which in nurseries and plantations results in crop and yield losses, high monitoring and control costs, and bans on exports (Pérez-Sierra et al., 2007). Barnard and Blakeslee (1987) recommended growing the *Pinus* species and provenance that is best suited for a specific site. For sites with historically high levels of pitch canker, a more resistant *Pinus* species or genetically resistant genotype can be planted.

Sphagnum peat is the most widely used growth medium in tree nurseries, either alone or as a component of mixed media (Bunt, 1988; Heiskanen, 2013; Landis et al., 1990). However, finding effective alternatives is desirable because peat is obtained from peatlands, which are being rapidly depleted (Chong, 2005; Guerrero et al., 2002; Ingelmo et al., 1998; Ostos et al., 2007; Wilson et al., 2006). The increasing costs (particularly transportation costs) and declining availability of imported sphagnum peat in many countries have promoted a search for organic substitutes worldwide (Heiskanen, 2013). Thus, seedling growers are searching for more local growth medium components such as compost (Landis and Morgan, 2009). Also, there is a political and environmental drive to reduce the environmental burden of waste production and to increase the use of composts in growth media. In the EU, for example, the eco-label has established a quality assurance system that promotes the use of compost as a soil improver and a component of growth media.

One of the most important incentives to using composts as growth media is their commonly observed suppressive effect against a variety of soil-borne pathogens, as first suggested by Hoitink et al. (1975) and later confirmed by many authors (Hoitink and Fahy, 1986; Cotxarrera et al., 2002; Litterick et al., 2004; Noble and Coventry, 2005; Borrero et al., 2006; Castaño et al., 2013). This property is especially important for organic farming, where the use of synthetic pesticides is banned. The use of

microorganisms, whether individually or in combination with other measures of disease control, to replace pesticides has grown steadily in recent years (Moraga-Suazo et al., 2011). Biological control provides another effective alternative to chemicals and helps to minimize their adverse impact on human health and the environment (Kim et al., 2003). Moreover, EU Directives (Regulation EC 1107/2009) promote the use of integrated pest and disease management measures against plant diseases.

Trichoderma spp. are free-living fungi, the effectiveness of which has been ascribed to various modes of action including competition with other soil microorganisms for nutrients (Chet et al., 1987), and production of antibiotics and lytic enzymes to parasitize or inhibit other fungi (Harman et al., 1981; Zimand et al., 1996; Howell, 2003). Recent studies have shown that *Trichoderma* spp. can also colonize plant roots and trigger plant defence mechanisms (Olson and Benson, 2007; Segarra et al., 2007 and 2009; Trillas and Segarra, 2009). Moraga-Suazo et al. (2011) found that three *Trichoderma* spp. strains reduced mortality in *P. radiata* seedlings inoculated with *F. circinatum* to less than 50% if the antagonists were incorporated into the substrate prior to inoculation. Martínez-Álvarez et al. (2012) also succeeded in reducing *F. circinatum* disease by inoculating *Trichoderma viride* *in vitro* a few days before the pathogen.

Whereas mature compost can sustain biological control agents, immature compost does not support them, so may have an adverse impact on growth in crop plants and may even contain pathogen populations (De Ceuster and Hoitink, 1999; Litterick et al., 2004). This makes using mature, stable compost to test suppressiveness against major pathogens such as *F. circinatum* essential. Compost from gorse (*Ulex europaeus* L.) is a stable material with good physical and chemical properties that make it an effective substitute for peat (Gomez et al., 2009; Iglesias-Diaz et al., 2009). Furthermore, harvesting gorse bush material is essential to prevent forest fires (Badia et al., 2002).

The aim of the study was to evaluate the natural suppressiveness against *F. circinatum* in *P. radiata* seedlings of (i) different gorse composts and compost from the organic fraction of solid urban waste (SUW) mixed with peat and perlite, and (ii) the effect of the presence of *T. asperellum* strain T34, which was previously shown to be effective against *F. oxysporum*.

2. Materials and methods

2.1. Physical, chemical and physico–chemical properties of the composts

Four different gorse composts were studied, namely: compost produced on a small scale and containing poultry manure (C1), compost obtained on a small scale without additions (C2), compost produced on a large scale and containing cattle slurry (C3) and compost obtained on a large scale and allowed to ferment in the forest, where it was collected for transfer to the composting plant to end the process (C4). Additional compost obtained from the organic fraction of SUW containing 25% perlite and 25% peat (C5) was also studied.

All composts were characterized in physical, chemical and physico–chemical terms. Water retention was measured at different suction levels (1, 2.5, 5, 7.5 and 10 kPa) in a sandbox (De Boodt et al., 1974). Bulk density was calculated from volume, and dry weight measurements were made after oven-drying at 105 °C. Particle density was calculated from the organic matter (OM) and ash contents (EN-13039, 1999) as determined by ashing at 540 °C. Total porosity was calculated from particle and bulk densities (EN-13041, 1999 and EN-13040, 2007). Electrical conductivity (EC), pH and soluble macronutrients were determined in a 1:5 (v/v) extract (EN-13652, 2001); nitrate and ammonium with an ion-selective electrode; P by UV spectrophotometry at 880 nm (Olsen and Sommers, 1982); and K, Ca, and Mg by atomic absorption/emission spectrophotometry (USEPA, 1995). Three samples (replicates) were used per compost.

2.2. Microbial characterization of the composts

Cellulolytic actinomycetes (CA), *Bacillus* spp. (B), oligotrophic actinomycetes (OA) and total fungi (F) were determined by dilution plating on semi-selective media, using the procedure described by Tuitert et al. (1998) as modified by Borrero et al. (2004). Populations of *Fusarium* spp. (FUS) were measured using a semi-selective medium (Komada, 1975). Three samples (replicates) were studied per compost. Two plates were used for each medium and dilution of each sample.

2.3. Microorganisms used

T. asperellum strain T34 was grown in an agitated liquid medium containing 10 g/L malt for 5 days at 30°C. The culture was filtered to remove the mycelium and centrifuged at 10 000 g. The pellet was washed twice in distilled water to obtain medium-free conidia.

One isolate (the most pathogenic) of each mating type (MAT1 and MAT2) of *Fusarium circinatum* were obtained from the Areeiro Phytopathological Station (Pontevedra, Spain) and cultivated in the same way as T34.

2.4. *Trichoderma* spp. concentration in the composts

To study the population density of *Trichoderma* spp. at the beginning and end of the experiments, two samples from each compost were randomly selected from each of the three blocks (see following section) and subjected to dilution plating on semi-selective media designed for *Trichoderma* spp. (Chung et al., 1990).

2.5. Assessment of disease incidence

Disease suppression by the composts was assessed via a *F. circinatum* (Fc) bioassay. In order to standardize the initial moisture conditions, the composts were saturated in culture trays for 24 h and then drained for 3 days. The trays were covered to prevent evaporative loss during the process. Subsequently, the composts were placed in plastic bags for inoculation with the pathogens or biocontrol agent in accordance with the experimental design.

The bioassay was established to test the effects of three factors, namely: composts, pathogen inoculum and biocontrol agent. The Fc strains MAT1 (M1) and MAT2 (M2) were used as pathogens and *T. asperellum* strain T34 (T34) as the biocontrol agent. Thus, each compost was subject to six different treatments, namely: (1) no pathogen or biocontrol agent (Control, -T34); (2) no pathogen but T34 (Control, +T34); (3) M1 but no T34 (M1, -T34); (4) M1 and T34 (M1, +T34); (5) M2 but no T34 (M2, -T34) and (6) M2 and T34 (M2, +T34). First, T34 was inoculated at a rate of 10^4 conidia ml⁻¹ of compost⁻¹. Then, M1 or M2 were inoculated at 10^3 conidia ml⁻¹ one day later. Inoculated composts were stored in bags and shaken every day for a week.

Seeds were supplied by the official tree nursery of Xunta de Galicia and imbibed in distilled water for 48 h before planting. Then, one seed per cell was placed in forest size trays (220 cm³ each). For each treatment, 10 seeds were planted in each tray cell. The bioassay was replicated three times per treatment and treatments were arranged in a randomized block design.

Trays remained in an EGC walk-in chamber under controlled conditions of temperature (25 °C), relative humidity (60%) and photoperiod (12 h, 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) throughout the bioassay. Plants were irrigated on a daily basis with Peters

Professional® solution [(27:15:12) + B, Cu, Fe, Mn, Mo and Zn] supplemented with CaCl₂ and MgSO₄.

Disease incidence was monitored at 1-day intervals for 51 days after sowing. Germination was assumed to end 30 days after sowing, which signalled the end of pre-emergence and start of post-emergence. Disease incidence was scored on a symptom scale with 0 for asymptomatic plants and 1 for symptomatic or dead plants. Those plants that had not germinated by the end of the pre-emergence stage were considered to be dead.

2.6. Biomass and physiological analysis of plants

At the end of the study, two healthy plants per treatment and block (six plants per treatment) were randomly selected for assessment of photosynthesis, stomatal conductance and transpiration. These parameters were determined on an LI-6400 infrared gas analyzer from Li-Cor, Inc. (Lincoln, NE, USA) equipped with a special camera to measure conifers (LI-05-6400). Twelve plants were used to estimate total plant leaf area before drying using a GT-5000 flat-bed scanner from Epson (Nagano, Japan) to obtain images that were processed with imaging software (Nogués et al., 2001). Plant height and root length measurements were followed by measurements of dry weight of the aerial parts and roots of all seedlings in combination. Plants were dried to constant weight in an oven at 105 °C. Leaf, shoot and root dry weights were measured in two plants per block per treatment.

2.7. Statistical analysis

The three principal components (independent variables) examined were (a) type of compost, (b) presence or absence of *T. asperellum strain* T34 and (c) presence or absence of *F. circinatum* inoculum. Data were processed by multivariate analysis of variance (MANOVA) for statistical analysis and the LSD test for post-hoc analysis. Differences between data pairs where $F < 0.05$ were assumed to be significant. Data normality was previously confirmed via the Kolmogorov–Smirnov test and homogeneity of variance via Levene’s comparison test. All statistical calculations were performed using the software PASW Statistics v. 18.

3. Results

3.1. Characterization of the composts

There were significant differences in physical, chemical and physico-chemical properties between gorse composts and the compost obtained from the organic fraction of USW, and also between composts obtained on a small and large scale (Table 1). Thus, gorse composts had lower BD values than USW compost (particularly that produced on a large scale). Gorse composts also had higher AC and OM values, but lower pH values, than USW compost. Small scale-produced composts (C1, C2 and C5) had higher EC and Ca concentrations than large scale-produced composts (C3 and C4). The best composts (C4, C2 and C3) had higher TP values than the others. Small scale-produced gorse composts had the highest NO_3^- and Mg contents, whereas C3 had the lowest NH_4^+ content, and C1 and C5 the highest K contents. Compost C5 had the highest pH and AW values, but the lowest OM, NO_3^- , P and Mg contents. P levels, however, were widely variable and could not be ranked by composition or production scale. C4 had a moderate pH and low EC values, in addition to moderate levels of P, Ca, Mg and K, and high levels of NH_4^+ (Table 1).

Based on microbial populations, C1 and C5 had the highest levels of CA (Table 2). All gorse composts contained similar levels of *Bacillus* spp. that were significantly lower than those in C5. OA levels were lower for small scale-produced than for large scale-produced gorse composts and peaked in C5. Finally C4 had the highest levels of (F) and lowest of *Fusarium* spp., whereas C5 and C2 had the highest levels of *Fusarium* spp. (Table 2).

Table 3 illustrates the correlations between physico-chemical/chemical and microbial properties in the composts. As can be seen, OM correlated negatively with the populations of actinomycetes, B and FUS; EC correlated with increased CA and negatively with F populations and pH correlated positively with the populations of actinomycetes and B and correlated negatively with T. On the other hand, NO_3^- levels correlated negatively with B, OA and F and correlated positively with T. NH_4^+ levels favoured overall fungal growth. Also, high levels of Ca correlated negatively with populations of fungus; Mg correlated negatively with B and OA and positively with T; K correlated positively with the populations of actinomycetes and B and P correlated negatively with B, OA and FUS.

3.2. Assessment of disease incidence

There were significant differences ($P < 0.05$) in disease incidence between pine seedlings at the pre-emergence stage, particularly according to factors Fc and T34 (Fig. 1). Isolate M2 (30%) caused more deaths than M1 (25%) at the pre-emergence stage (Fig. 1a), but the differences were not significant; it also led to increased disease incidence in the composts receiving no T34 inoculum (Fig. 1b).

There were also significant differences ($P < 0.05$) in disease incidence between pine seedlings at the post-emergence stage, especially according to factors Fc and T34, and also between composts (C) (Fig. 2). Isolates M1 and M2 led to similar levels of incidence at this stage; also, such levels were significantly higher than those in the control (uninoculated) composts (Fig. 2a). Post-emergence deaths were also significantly greater in number in those composts receiving no T34 inoculum (Fig. 2b). C4 was the most suppressive compost against Fc (15% post-emergence deaths), followed by C2 (25%), C3 (30%) and C5 (35%); on the other hand, C1 (55%) was the most conducive (Fig. 2c).

The highest correlations of pre- and post-emergence deaths with physical properties of the composts were those for bulk density, which influenced total porosity and aeration in the composts, and high UW levels, which were directly correlated with a high pre-emergence incidence of disease (Table 4). Moreover, high EC levels correlated positively with disease incidence but only at the post-emergence stage. NH_4^+ correlated negatively with disease incidence in *P. radiata* at pre- and post-emergence. Finally, Ca correlated positively with disease incidence at the post-emergence stage while K correlated positively with disease both at pre- and post-emergence (Table 4).

3.3. Plant biomass and physiological analysis

There were significant differences ($P < 0.05$) in aerial height and dry weight (Fig. 3); thus, plants grown in composts receiving no T34 inoculum were on average 20 mm longer than those grown with T34 (Fig. 3a). There were also significant differences ($P < 0.05$) in aerial and root biomass between seedlings at the end of the study depending on the compost used (C) (Fig. 3). Thus, plants grown on C4, C3 and C2 had significantly greater height, dry weight and root length than those grown on C5 or C1 (Figs. 3b and 3c). The interactions Fc *T34 and Fc *T34 *C also had significantly different effects ($P < 0.05$) on aerial dry weight (Fig. 3).

The physiological factors examined varied significantly ($P < 0.05$) according to factor (Fc) and photosynthesis in *P. radiata* seedlings, as well as factor C (Fig. 4). Thus, there were significant differences in photosynthesis-related measurements between healthy *P. radiata* seedlings inoculated with the pathogen (M1 or M2) and the control plants at the post-emergence stage (Fig. 4a). Seedlings grown on C4 had the highest photosynthetic rates and stomatal conductance (Figs. 4b and 4d), and, together with those grown on C2, the highest transpiration rates (Fig. 4c). Composts C2 and C3 had intermediate values of the other physiological properties studied, and C1 and C5 the lowest (Figs. 4b–4d).

Disease incidence in pine seedlings was significantly correlated, both pre- and post-emergence, with aerial and root growth, and also with all physiological properties (results not shown).

3.4. Evolution of *Trichoderma* spp.

Composts C2 and C3, which contained the highest levels of natural *Trichoderma* spp., exhibited the lowest levels upon inoculation with T34 (Table 5). Conversely, the composts with the lowest levels of natural *Trichoderma* spp. had the highest levels of *Trichoderma* spp. upon inoculation with T34 (particularly C5). In the presence of pathogen (M2), *Trichoderma* spp. levels were maintained throughout the study in the composts with the largest initial populations. Worth special note here were the low levels of natural *Trichoderma* spp. in compost C4, which increased to the highest levels upon inoculation with M2 and T34.

4. Discussion

Most of the physical, chemical and physico-chemical properties studied were correlated with the source of the raw materials, whether primary (gorse and USW) or secondary (poultry manure, cattle slurry), as well as with the scale of the composting process (small or large) (Zamora-Nahum et al., 2007). Fisher et al. (1981) and Blakeslee et al. (1999) previously found that high levels of fertilizer made plants more susceptible to pitch canker disease. Our results are consistent with theirs: disease incidence was correlated with K, Ca and EC levels. Also, high pH values have been associated with large populations of bacteria and actinomycetes (Waksman, 1927), and used as a predictive factor against *Fusarium* wilt in tomatoes grown on various kinds of compost (Borrero et al., 2004). Our results are consistent with a link between pH and microbial populations, but not with suppression of *F. circinatum*; in fact, the most conducive

compost (C5) exhibited the highest levels of actinomycetes and bacillus. Also, *F. circinatum* disease could not be predicted from the $\text{NH}_4^+/\text{NO}_3^-$ ratio because the most suppressive and conducive composts (C4 and C5, respectively) had similar ratios — which contradicts previous results for *F. oxysporum* disease (Borrero et al., 2012).

The levels of *Fusarium* spp. found in C2, C3 and C5 cannot be assumed to correspond to non-pathogenic saprophytic *Fusarium* spp. exclusively because the control plants exhibited a low incidence of disease even though they were not inoculated with *F. circinatum*. The incidence of disease was significantly reduced in the control plants treated with T34 but receiving no pathogen. For example, composts C3 control –T34 and +T34 had a disease level of 0.33 ± 0.03 and 0.00 ± 0.0 , respectively, and composts C5 control –T34 and + T34 one of 0.13 ± 0.07 and 0.07 ± 0.03 , respectively. These composts may have contained other pathogenic *Fusarium* spp. besides *F. circinatum* or other soil-borne plant pathogens not removed during the thermophilic phase of the composting process (Noble and Roberts, 2004). The presence of disease in the control plants may also have been originated in the seeds (Wingfield et al., 2008).

C4 was the most suppressive compost pre- and post-emergence with both M1 and M2. Although T34 failed to reduce disease incidence in this compost, it proved effective in moderately and non-suppressive composts (up to 50% in C2 and C5 post-emergence with M2). Similar results were obtained previously for T34 against *Rhizoctonia solani* using composts of variable suppressiveness (Trillas et al., 2006). Other studies also found that strains UDC-280, UDC-351 and UDC-404 of *Trichoderma* spp. reduced death in *P. radiata* seedlings by up to 50% after enrichment of the growth media prior to inoculation with *F. circinatum* (Moraga-Suazo et al., 2011). *Trichoderma asperellum* strain T34 also proved effective against *F. oxysporum* f. sp. *dianthus* (Sant et al., 2010), f. sp. *lycopersici* (Cotxarrera et al., 2002; Segarra et al., 2010; Borrero et al., 2012) and other soil pathogens (Segarra et al., 2013). There have been many studies on the application of antagonistic microbes for biological control of *Fusarium* wilts including *Bacillus* spp., non-pathogenic *Fusarium* spp., *Pseudomonas* spp., *Penicillium* spp, *Trichoderma* spp., *Paenibacillus* spp. etc (Fuchs et al., 1999; Sabuquillo et al., 2006; Hervas et al., 1998; Dijksterhuis et al., 1999).

With regards microbial populations in the composts, C4 exhibited the highest levels of total fungi, the lowest concentrations of *Fusarium* spp. and moderate levels of natural *Trichoderma* spp. compared to the other composts. As previously found in other

suppressive composts (Hoitink et al., 1993), allowing C4 to remain in the forest during the first stage of the composting process may have facilitated natural colonization by beneficial antagonistic microorganisms. This compost exhibited the highest levels of *Trichoderma* spp. —not necessarily T34— in the presence of pathogen (M2). However, the presence of T34 in C2 and C5 reduced disease incidence but increased the population of *Trichoderma* in C5. In previous studies on *F. oxysporum* disease, the presence of the pathogen also increased T34 populations (Borrero et al., 2012; Sant et al., 2010). The populations of *Trichoderma* spp. found in C5 must have been exclusively or largely due to T34. In C2, however, *Trichoderma* populations changed very little, whether the pathogen M2 was present or absent.

Some *Trichoderma* spp. strains not only trigger plant defence responses, but also enhance growth through rhizosphere competition (Shoresth et al., 2005; Verma et al., 2007). This was echoed by the current results: plants grown on composts containing T34 exhibited greater aerial growth in terms of dry weight and vigour —pines exhibited greater dry weights but lower heights (results not shown). Hohmann et al. (2012) obtained similar results for *P. radiata* seedlings inoculated with *Trichoderma hamatum* strain LU592, which increased the height of aerial shoots and stem diameter by 16% and 8%, respectively. In this work, C4 led to the greatest aerial and root length, and also the heaviest weight. These results are consistent with all physiological parameters measured in pine seedlings grown on Fc inoculated compost C4 as control plants. Surviving plants grown on compost C4 were healthier than those grown on the other composts. Finally, plants grown on C5, which had the lowest rate of photosynthesis, exhibited some metabolic deficiencies suggesting that they might have been overcoming the disease.

5. Conclusions

All studied composts exhibited suitable physical and physico–chemical properties for use as nursery growth media for *P. radiata*. Furthermore, gorse compost produced on a large scale exhibited natural suppressive properties against *F. circinatum* disease. The naturally suppressive compost had high AC, low EC, high NH_4^+ , and low Ca and K levels. The biological control agent *T. asperellum* strain T34 reduced pre- and post-emergence incidence of the disease in the moderately suppressive composts, which testifies to its potential as an effective alternative to chemical treatment against *F. circinatum* in *P. radiata* seedling production.

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TABLES

Table 1. Physical, physicochemical and chemical properties of plant growth media evaluated in bioassays. Mean \pm (S.E.) values. Within each column, values with different letters are significantly different ($P < 0.05$) according to DMS's test.

C ^x	Properties of plant medium ^y														
	BD	TP	AC	EAW	WBC	UW	O.M.	pH	EC	NH ₄ ⁺	NO ₃ ⁻	P	Ca	Mg	K
C1	0.27 b	85.34 c	43.20 b	11.40 b	2.03 c	28.70 c	65.18 c	4.70 b	1.66 a	107.51 c	3,834.41 b	98.35 a	641.70 a	193.60 a	945.00 a
C2	0.18 c	90.85 b	45.81 b	11.91 b	3.05 b	30.08 c	52.61 d	3.50 c	1.03 c	135.02 b	4,405.30 a	39.30 d	318.35 b	188.55 a	545.00 b
C3	0.14 d	91.33 b	37.06 c	12.35 b	4.98 a	37.01 a	84.04 a	4.02 c	0.53 d	31.05 d	1,054.33 c	59.80 c	49.85 e	58.05 b	475.00 b
C4	0.13 e	92.82 a	56.35 a	7.88 c	2.85 b	25.74 d	68.92 b	4.68 b	0.35 e	206.50 a	1,030.10 c	70.36 b	87.35 d	61.90 b	487.00 b
C5	0.32 a	84.86 c	25.67 d	19.47 a	5.14 a	34.59 b	34.20 e	7.95 a	1.10 b	103.95 c	543.91 d	20.45 e	225.70 c	28.75 b	1010.00 a

^x C1, gorse compost produced on a small scale with poultry manure; C2, gorse compost produced on a small scale; C3, gorse compost produced on a big scale with cattle slurry; C4, gorse compost produced on a big scale; C5, 50% compost of organic fraction of USW+ 25% perlite + 25% peat.

^y BD, bulk density (g·cm⁻³); TP, total porosity (%); AC, aeration capacity (%); EAW, easily available water (%); WBC, water buffering capacity (%); UW, unavailable water (%); OM, total organic matter (%). pH; EC, electrical conductivity (dS·cm⁻¹); NH₄⁺, NO₃⁻, P, Ca, Mg, K (mg·L⁻¹ substrate) in 1:5 (v/v) extract. Media values \pm (E.S.) and DMS post hoc. Within each column, values followed by different letters are significantly different based on DMS test at $P < 0.05$.

Table 2. Microbiological properties of plant growth media evaluated in bioassays.

C ^x	x 10 ⁵ CFU/cm ³ of plant growth medium ^y					
	CA	B	OA	F	T	FUS
C1	70.313 a	18.805 b	1.7169 c	1.7169 b	0.4088 ab	0.1308 c
C2	2.0969 d	26.185 b	6.8081 c	0.3025 c	0.5237 a	0.6958 a
C3	11.490 c	32.238 b	46.004 b	2.0677 bc	0.5558 a	0.3446 b
C4	32.390 b	4.7263 b	37.279 bc	19.780 a	0.1536 bc	0.0001 c
C5	124.83 a	110.86 a	98.899 a	6.2186 b	0.0015 c	0.7441 a

^x C1, gorse compost produced on a small scale with poultry manure; C2, gorse compost produced on a small scale; C3, gorse compost produced on a big scale with cattle slurry; C4, gorse compost produced on a big scale; C5, 50% compost of organic fraction of USW+ 25% perlite + 25% peat.

^y CA = cellulolytic actinomycetes, B = Bacillus spp., OA = oligotrophic actinomycetes, F = fungi, T= Trichoderma spp., FUS = Fusarium spp. Within each column, values followed by different letters are significantly different based on DMS test at $P < 0.05$.

Table 3. Dry weight in aerial part of seedlings.

DRY WEIGHT ^y							
x	CONTROL		M1		M2		T
	-T34	+T34	-T34	+T34	-T34	+T34	
C1	37.50 b (9.62) A	61.00 c (6.42) A	51.50 c (8.08) A	46.00 b (6.57) A	50.50 c (8.65) A	51.00 c (13.07) A	49.79 c (3.73)
C2	98.17 a (8.39) A	79.67 ab (11.41) A	68.33 ab (6.93) A	93.33 a (7.89) A	74.83 ab (8.08) A	89.40 a (7.10) A	83.80 ab (3.66)
C3	79.33 a (8.37) B	95.00 a (5.12) A	83.50 a (11.39) AB	99.50 a (11.39) A	83.67 a (6.90) AB	93.67 a (5.65) A	89.11 b (3.31)
C4	99.67 a (5.03) AB	87.67 a (2.55) AB	81.50 a (7.92) B	111.20 a (5.98) A	95.00 a (6.17) AB	98.33 a (5.10) AB	95.11 a (3.09)
C5	49.00 b (9.93) A	57.50 bc (3.89) A	55.67 bc (5.05) A	58.00 b (18.17) A	45.50 bc (0.82) A	54.17 b (8.25) A	53.76 c (2.38)
T	71.97 (5.01) A	76.33 (5.20) A	68.10 (4.93) B	83.15 (5.20) A	71.64 (5.10) A	76.90 (5.01) A	

^x C1, gorse compost produced on a small scale with poultry manure; C2, gorse compost produced on a small scale; C3, gorse compost produced on a big scale with cattle slurry; C4, gorse compost produced on a big scale; C5, 50% compost of organic fraction of USW+ 25% perlite + 25% peat; T, total.

^y Dry weight (mg) in aerial part of the seedlings. Control= without *Fusarium circinatum*; M1 and M2= with *Fusarium circinatum* MAT 1 and MAT 2 respectively at 10^3 microconidia·cm⁻³; -T34= without *Trichoderma* T34; +T34= with *Trichoderma* T34 at 10^4 microconidias·cm⁻³ and T=total. Media values ± (E.S.) and DMS post hoc. Within each row, values followed by different capital letters; and within each column, values followed by different small letters, are significantly different based on DMS test at P < 0.05.

Table 4. Trichoderma spp. concentration of five plant growth at the end of the bioassay.

C ^x	x 10 ⁴ CFU/cm ³ of plant growth medium ^y							
	CONTROL				M2			
	-T34	+T34	-T34	+T34	-T34	+T34	-T34	+T34
C1	40.880 ab D	116.46 ab A	1.8480 b C	8.3549 d B				
C2	52.370 a A	41.882 b B	39.600 ab B	51.774 c B				
C3	55.583 a A	35.578 b B	57.557 a B	165.94 b B				
C4	15.361 bc B	121.06 ab AC	71.347 a C	395.79 a A				
C5	0.151 c A	224.14 a B	1.0714 b A	83.760 bc A				

^x C1, gorse compost produced on a small scale with poultry manure; C2, gorse compost produced on a small scale; C3, gorse compost produced on a big scale with cattle slurry; C4, gorse compost produced on a big scale; C5, 50% compost of organic fraction of USW+ 25% perlite + 25% peat.

^y Control= without *Fusarium circinatum*; M1 = with *Fusarium circinatum* MAT 1 at 10³ microconidia cm⁻³; -T34= without *Trichoderma* T34; +T34= with *Trichoderma* T34 at 10⁴ microconidias cm⁻³. Media values and DMS post hoc. Within each row, values followed by different capital letters; and within each column, values followed by different small letters, are significantly different based on DMS's test at P < 0.05.

Table 5. Pearson correlation coefficients between physicochemical and chemical parameters and microbiological properties of plant growth media evaluated in bioassays.

	O.M.	EC	pH	NO ₃ ⁻	NH ₄ ⁺	Ca	Mg	K	P
CA	-0.497**	0.379*	0.723**	-0.191	0.062	0.280	-0.164	0.683*	-0.108
B	-0.599**	0.146	0.700**	-0.385*	-0.216	-0.102	-0.426*	0.498**	-0.598**
OA	-0.399*	-0.041	0.544**	-0.510**	-0.110	0.235	-0.506*	0.303*	-0.489*
F	0.107	-0.483**	0.002	-0.343*	0.586**	-0.361*	-0.293	-0.256	0.124
T	0.428/	0.043	-0.585**	0.412*	-0.312	0.108	0.423*	-0.341	0.238
FUS	-0.627**	0.187	0.318	0.045	-0.312	-0.058	-0.037	0.221	-0.768**

** Significant correlation at level p<0.01; * Significant correlation at level p<0.05.

OM, total organic matter (%). pH; EC, electrical conductivity (dS·cm⁻¹); NH₄⁺, NO₃⁻, P, Ca, Mg, K (mg·L⁻¹ substrate) in 1:5 (v/v) extract. CA = cellulolytic actinomycetes, B = *Bacillus* spp., OA = oligotrophic actinomycetes, F = fungi, T= *Trichoderma* spp., FUS = *Fusarium* spp.

Table 6. Pearson correlation coefficients between physical and physicochemical parameters of plant growth media evaluated in bioassays and disease incidence in pre- and post-emergence stage.

	BD	TP	AC	EAW	WBC	UW	OM	EC	pH	NO₃⁻	NH₄⁺	Ca	Mg	K	P
PRE	0.307*	-0.304*	-0.342**	0.277*	0.202	0.284*	-0.193	0.238	0.205	0.001	-0.271*	0.128	0.009	0.277*	-0.154
POST	0.425**	-0.501**	-0.299*	0.198	-0.049	0.170	-0.069	0.515**	0.143	0.182	-0.337**	0.456**	0.197	0.472**	0.196

** Significant correlation at level p<0.01; * Significant correlation at level p<0.05.
 BD, bulk density (g·cm⁻³); TP, total porosity (%); AC, aeration capacity (%); EAW, easily available water (%); WCB, water buffering capacity (%); UW, unavailable water (%); OM, total organic matter (%). pH; EC, electrical conductivity (dS·cm⁻¹); NH₄⁺, NO₃⁻, P, Ca, Mg, K (mg·L⁻¹ substrate) in 1:5 (v/v) extract.
 PRE= disease incidence in pre-emergence stage; POST= disease incidence in post-emergence stage.

Table 7- Pearson correlation coefficients between some physiological and biomass parameters of the Pinus radiata seedlings at the end of the bioassay.

	POST	DW.AP	H.AP	DW.RP	L.RP	PHOTOSYNTESIS	TRANSPIRATION	CONDUCTANCE
PRE	0.698**	-0.500**	-0.559**	-0.378**	-0.456**	-0.470**	-0.371**	-0.320*
POST		-0.625**	-0.591*	-0.565**	0.540**	-0.523**	-0.437**	-0.389**
DW.AP			0.770**	0.869**	0.742*	0.591*	0.438**	0.409**
H.AP				0.586**	0.665**	0.654**	0.465**	0.384**
DW.RP					0.741**	0.442*	0.311*	0.284*
L.RP						0.681**	0.536**	0.514**

** Significant correlation at level p<0.01; * Significant correlation at level p<0.05.
 PRE= disease incidence in pre-emergence stage; POST= disease incidence in post-emergence stage; DW.AP= dry weight of the aerial part; H.AP= height of the aerial part; DW. RP= dry weight of the root part; L.RP= length of the root part of the seedlings.

FIGURES

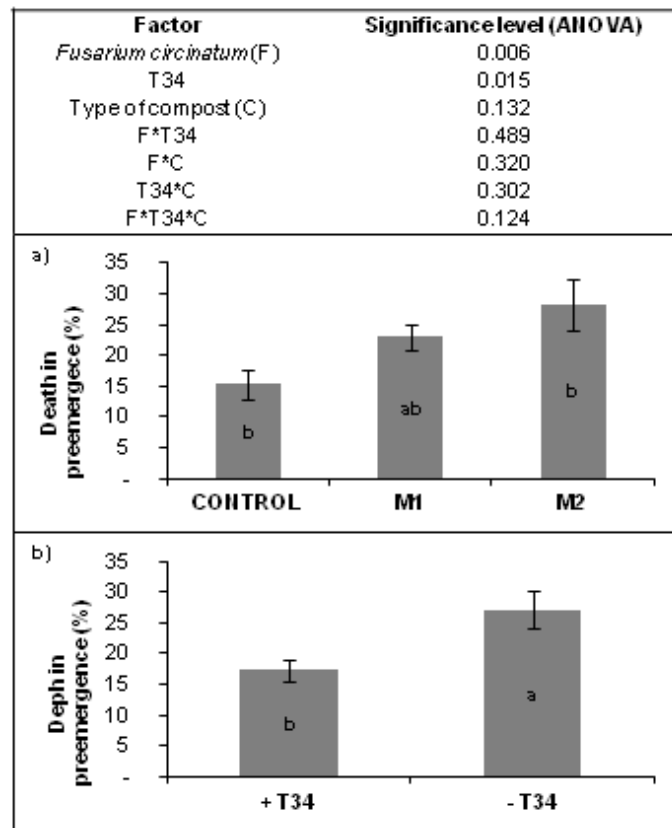


Fig. 1. Disease incidence at the pre-emergence stage in *Pinus radiata* D. Don seedbeds. Significance level (ANOVA) for *Fusarium circinatum* (Fc), *Trichoderma asperellum* (T34), type of compost (C) and their main interactions. (a) Pre-emergence deaths according to factor T34 (-T34= without Trichoderma and +T34= with Trichoderma at 10^4 conidia cm^{-3}); (b) Pre-emergence deaths according to factor Fc (Control = without Fc; M1 and M2= with *Fusarium circinatum* MAT 1 and MAT 2, respectively, at 10^3 microconidia cm^{-3}). Mean values, S.E. in error bars and post-hoc LSD. Different letters in each column are significantly different at $P < 0.05$ as per the LSD test.

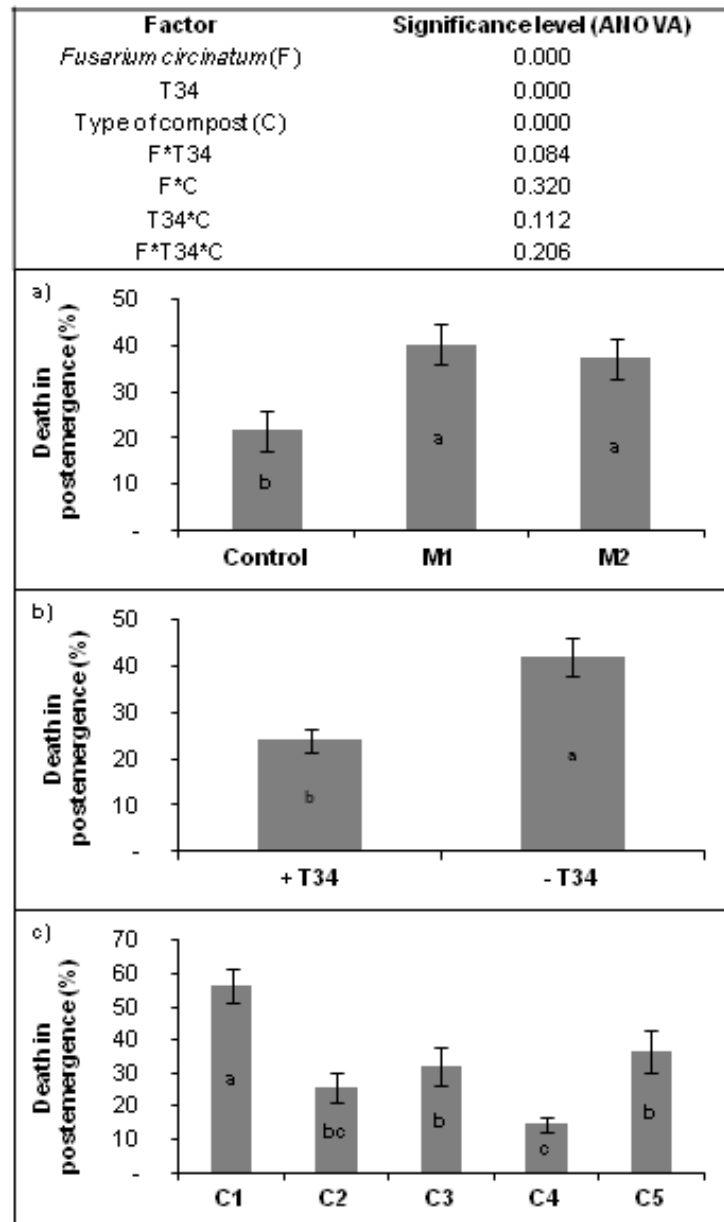


Fig. 2. Disease incidence at the post-emergence stage in *Pinus radiata* D. Don seedbeds. Significance level (ANOVA) for *Fusarium circinatum* (Fc), *Trichoderma asperellum* (T34), type of compost (C) and their main interactions. (a) Post-emergence deaths according to factor T34 (-T34 = without *Trichoderma*; +T34 = with *Trichoderma* at 10^4 conidia cm^{-3}); (b) Post-emergence deaths according to factor F (Control = without *Fusarium circinatum*; M1 and M2 = with *Fusarium circinatum* MAT 1 and MAT 2, respectively, at 10^3 microconidia cm^{-3}); (c) Post-emergence deaths according to factor C (C1 = gorse compost produced on a small scale and containing poultry manure; C2 = gorse compost produced on a small scale; C3 = gorse compost produced on a large scale and containing cattle slurry; C4 = gorse compost produced on a large scale; C5 = reference substrate consisting of 50% compost from organic fraction of USW (Urban Solid Waste) + 25% perlite + 25% peat. Mean values, S.E. in error bars and post-hoc LSD. Different letters in each column are significantly different at $P < 0.05$ as per the LSD test.

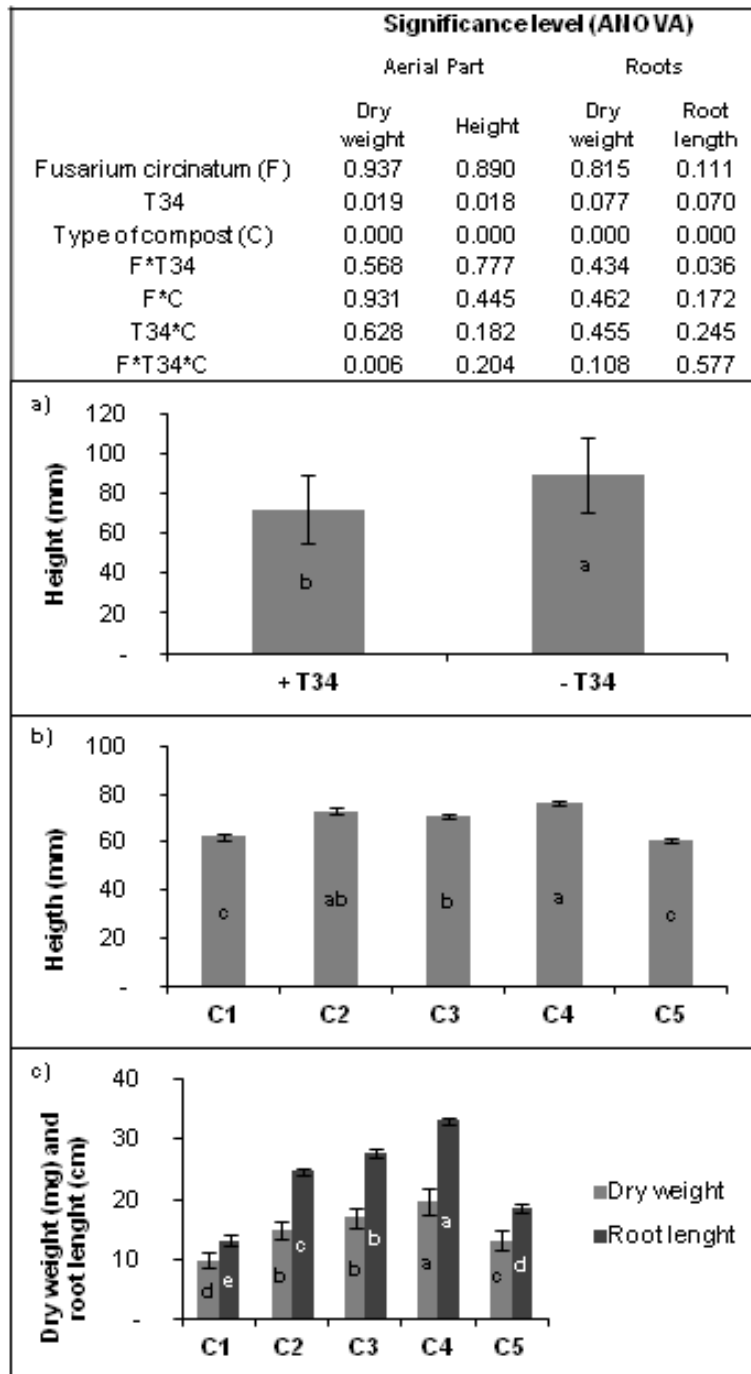


Fig. 3. Biomass measurements of *Pinus radiata* D. Don seedlings at the end of the bioassay. Significance level (ANOVA) for *Fusarium circinatum* (Fc), *Trichoderma asperellum* (T34), type of compost (C) and their main interactions. (a) Height of the aerial part of seedlings according to factor T34 (-T34= without Trichoderma; +T34 = with Trichoderma at 10^4 conidia cm^{-3}); b) height of the aerial part of seedlings according to factor C; (c) dry weight and root length of the root part of seedlings according to factor C (C1 = gorse compost produced on a small scale and containing poultry manure; C2 = gorse compost produced on a small scale; C3 = gorse compost produced on a large scale and containing cattle slurry; C4 = gorse compost produced on a large scale; C5 = reference substrate consisting of 50% compost from the organic fraction of USW (Urban Solid Waste) + 25% perlite + 25% peat. Mean values, S.E. in error bars and post-hoc LSD. Within each column of the same colour tone, different letters in each column of the same colour are significantly different at $P < 0.05$ as per the LSD test.

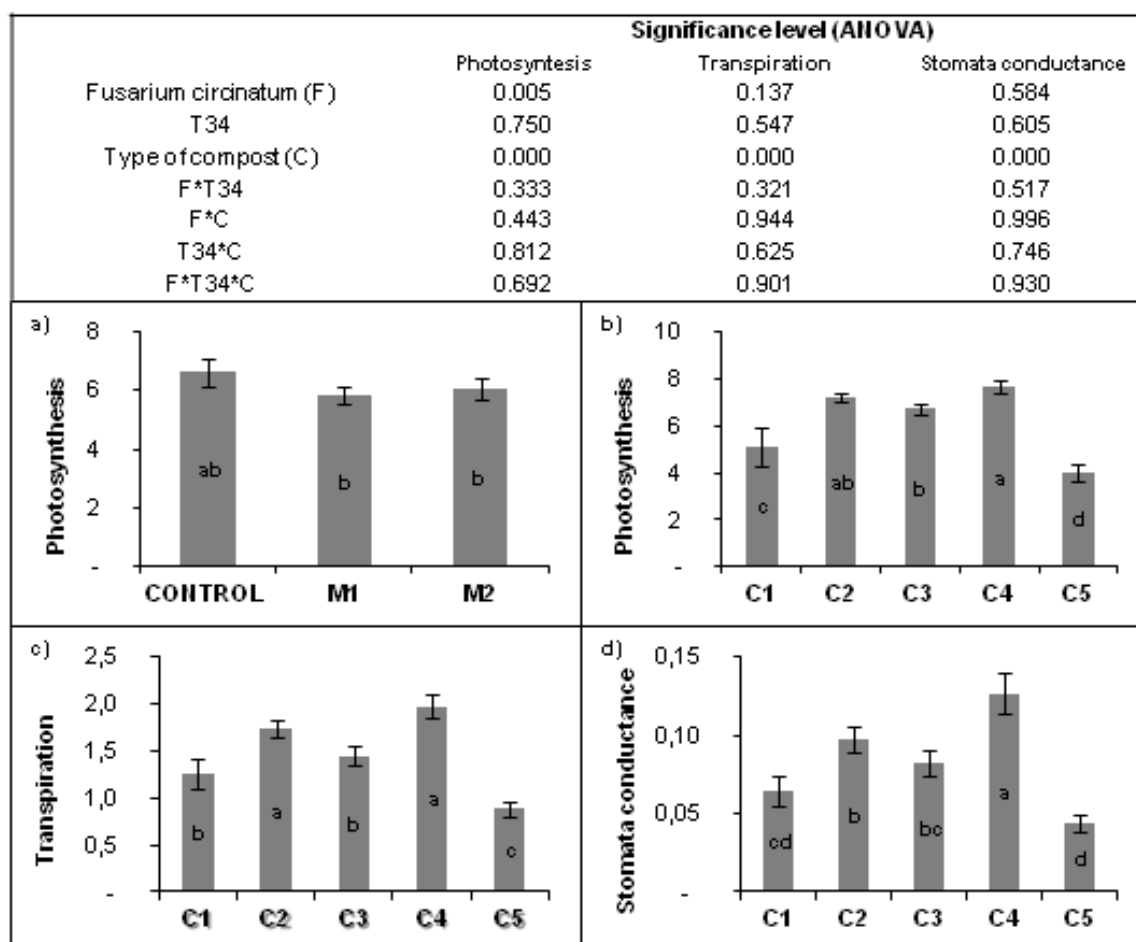


Fig. 4. Physiological properties of *Pinus radiata* D. Don seedlings at the end of the bioassay. Significance level (ANOVA) for *Fusarium circinatum* (Fc), *Trichoderma asperellum* (T34), type of compost (C) and their main interactions. (a) Rate of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) according to factor Fc (Control = without *Fusarium circinatum*; M1 and M2 = with *Fusarium circinatum* MAT 1 and MAT 2, respectively, at 10^3 microconidia cm^{-3}); and (b) rate of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (c) stomatal conductance ($\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and (d) transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) according to factor C (C1 = gorse compost produced on a small scale and containing poultry manure; C2 = gorse compost produced on a small scale; C3 = gorse compost produced on a large scale and containing cattle slurry; C4 = gorse compost produced on a large scale; C5 = reference substrate consisting of 50% compost from the organic fraction of USW (Urban Solid Waste) + 25% perlite + 25% peat. Mean values, S.E. in error bars and post-hoc LSD. Different letters in columns of the same colour are significantly different at $P < 0.05$ as per the LSD test.