Compost Science & Utilization, (2010), Vol. 18, No. 2, 111-118

Assessment of Plant Growth Promotion by Vermicompost in Different Progenies of Maritime Pine (*Pinus pinaster* Ait.)

Cristina Lazcano[1*], Luis Sampedro[2], Rafael Zas[3] and Jorge Domínguez[1]

1. Departamento de Ecología y Biología Animal, Universidad de Vigo, Vigo, Spain

2. Centro de Investigación Forestal de Lourizán - Unidad Asociada MBG-CSIC, Pontevedra, Spain

3. Misión Biológica de Galicia. Consejo Superior de Investigaciones Científicas, Pontevedra, Spain

**E-mail contact: cristina@uvigo.es*

The feasibility of incorporating vermicompost as a plant growth promoter into pine (*Pinus pinaster* Ait.) nurseries was investigated. Pines were grown in conventional peat-based nursery potting media where the peat was substituted by 0%, 2.5%, 5%, 10% and 25% solid vermicompost. In addition, in order distinguish between possible physical and biochemical mechanisms, the effects of solid vermicompost and vermicompost extracts on pine seedling growth were compared. Five different open-pollinated pine progenies were used in order to evaluate the possible genotype-dependent effects of vermicompost. All pots were provided with adequate mineral fertilization in order to avoid nutrient limitations and arranged in the greenhouse following a bifactorial randomized block design. Twenty weeks after sowing, seedlings were harvested and their aerial and root growth were measured, as well as biomass partitioning and seedling maturity. Amendment with solid vermicompost at 2.5% and 10% significantly stimulated pine seedling height, but not aerial biomass. Vermicompost also produced seedlings with greater shoot:root biomass ratios than the control. Besides, we noticed a significant inhibition of aerial and root biomass with the higher dose of solid vermicompost (25% substrate substitution). No effects, either positive or negative, were detected in plant growth due to the vermicompost extracts. All the reported results were the same for all the different progenies assayed, and therefore no genotype dependent effects were detected.

Introduction

Vermicompost is a nutrient rich microbiologically-active organic amendment which results from the interactions between earthworms and microorganisms in the breakdown of organic matter. It is a stabilized, finely-divided peat-like material with a low C:N ratio and high water-holding capacity that constitutes a source of plant nutrients which are released gradually, through mineralization, as the plant needs them (Domínguez 2004). Incorporation of vermicompost into growing media has shown to significantly improve plant growth, since it constitutes a slow-release source of nutrients that also modifies the physical properties of the potting substrates (Hidalgo *et al.* 2002; Chaoui *et al.* 2003; Hidalgo *et al.* 2006). Furthermore, several pot and field trials have shown that vermicompost produces significant positive effects on plant growth and yield at relatively low proportions (up to 20% of the growing media), even when plants are adequately supplied with mineral fertilizers, therefore suggesting the existence of non-nutrient mediated mechanisms of plant growth promotion (see revision by Edwards *et al.* 2004). These observations would seem to be reinforced by the presence of biologically active metabolites such as plant growth regulators (Tomati *et al. 1987*; El Harti *et al*. 2001) and humates (Muscolo *et al.* 1999; Atiyeh *et al*. 2002; Canellas *et al*. 2002) found in some vermicomposted materials.

Up to now, the potential of vermicompost as a plant growth promoter has seldom been studied in forest species. Alves and Passoni (1997) reported that vermicompost increased the germination index of the Brazilian tree *Licania tomentosa*, and in a previous study we observed that the use of vermicompost and vermicompost water extracts could enhance *Pinus pinaster* germination by 15% (Lazcano *et al*. 2010). Therefore, vermicompost could constitute an attractive alternative to mineral fertilizers in forest nurseries, since it appears to increase seedling growth significantly. Nevertheless, there are several plant-dependent factors that determine the appearance of differential responses to fertilization. Specifically, plant genotype is responsible for important differences in nutrient use efficiency (Kamau *et al.* 2008a) and resource allocation (Kamau *et al.* 2008b), as well as for varying responses to certain nutrient additions (Bonser *et al.* 1996; Zas *et al.* 2006). In addition, it has been demonstrated that different plant genotypes can produce different root exudates and therefore establish different relationships with the microbial community at the rhizosphere level (Rengel and Marschner 2005; Appuhn and Joergensen 2006). In this respect, Donald and Viser (1989) found that vermicompost had contrasting effects in the growth of the nursery species *Acacia mearnsii*, *Pinus patula* and *Eucaliptus grandis*, which shows that the effects of vermicompost might depend greatly on the species studied. Furthermore, these effects can vary even among varieties of the same species, as shown recently by Zaller (2007), evidencing that plant genotype might play a key role in the response to organic fertilizers and more specifically to vermicompost.

Here we investigated the feasibility of incorporating vermicompost as a plant growth promoter into the potting media of pine (*P. pinaster*), a forest species with a high economic importance in the south-west of Europe. In order to investigate the possible physical or biochemical effects of vermicompost we also compared the performance of solid vermicompost and vermicompost extracts on pine seedling growth. The influence of plant genotype was investigated through the assessment of the effects of vermicompost treatments on five different pine progenies.

Material and Methods

Plant Material

Open pollinated pine seeds were obtained from five *P. pinaster* clones (A, B, C, D, E) randomly selected in a first generation clonal seed-orchard (Sergude, 42.82º N, 8.45º W). This seed-orchard provides seeds of high genetic quality for reforestation in the Atlantic region of Galicia (NW Spain). Following commercial procedures and in order to stimulate germination before sowing, seeds were introduced in a water bath with aeration at 24ºC for 24 hours; afterwards seeds were allowed to dry at room temperature, individually labeled and weighed in a precision balance.

Experimental Design

A randomized bifactorial experiment was performed including the factors *progeny* (five levels) and *vermicompost treatment* (seven levels). The basic potting mixture consisted of 4 L plastic pots filled with peat and perlite (1:1 by volume), commonly used in forestry nursery. Peat was characterized by pH of 6.18 ± 0.01 and electrical conductivity of 0.12 ± 0.00 mS cm^{-1}. Peat was substituted on a volume basis by the following treatments: (i) 0% vermicompost (control treatment), (ii) 2.5 % vermicompost (13.7 dw g pot^{-1}); (iii) 5% vermicompost (27.4 dw g pot^{-1}); (iv) 10% vermicompost (54.9 dw g pot^{-1}); (v) 25% vermicompost (137.3 dw g pot^{-1}). In addition, we compared the growth of the seedlings with vermicompost extracts to that of those with solid vermicompost. To this end, the amounts of vermicompost equivalent to the lowest and highest doses assayed (2.5 and 25% peat substitution) were provided as water extracts and administered during the 20 weeks of the experiment.

Mineral nutrition of the pine seedlings was ensured by supplementing all pots with 11.2 g of a slow-release fertilizer (Multicote® 15:7:15 +2MgO) according commercial practices. This resulted in an addition of 1.67, 0.78 and 1.67 g of N, P and K respectively to each pot. Pots were covered with a 1-cm layer of sand, and seven seeds belonging to the same progeny were sown in each pot. Pots were arranged in the greenhouse following a complete randomized block design with 4 replications and the pots, containing progeny-vermicompost combinations, were randomized within each block. They were watered daily with spray irrigation and the temperature was regulated to 25°C during daytime and 15°C at night. Twelve weeks after sowing, when the number of newly germinated seeds was stabilized, the number of seedlings per pot was homogenised at five, eliminating, where necessary, the two seedlings showing the extreme sizes in the pot.

Vermicompost and Vermicompost Extract Preparation

Vermicompost was produced from rabbit manure by the earthworm *Eisenia fetida* in 1 m^3 vermirreactors at the facilities of the vermicomposting company TODOVERDE (Ourense, Spain). For the vermicompost extracts preparation, the same total amount of vermicompost assayed in the solid state for the 2,5% and 25% doses (328.8 and 3288 g dw respectively), was divided into 20 applications which were stored at 4ºC until used. Each week during the 20 weeks of the experiment, one portion of the 2.5% and 25% doses was diluted in an amount of distilled water equal to the sum of the field capacity of the pots treated with the 2.5% or 25% extracts (1200 ml). This volume was chosen in order to avoid leaching of the extract from the pots. The solution was aerated for 24 hours at 20ºC using an air pump and then filtered through a 0.05 mm sieve and subsequently poured onto the pot's surface to field capacity (50 ml per pot). The main physicochemical characteristics of the vermicompost and the vermicompost extracts used in this experiment are summarized in Table 1.

TABLE 1.
Physicochemical characteristics of the vermicompost and vermicompost extracts used in this experiment. Values are means of three replicates.

	Vermicompost	Vermicompost Extract (2.5%)	Vermicompost Extract (25%)
pH	7.32	7.96	7.74
EC (mS cm^{-1})	0.29	0.20	0.38
DOC	4967 µg g dw^{-1}	133 µg ml^{-1}	1180 µg ml^{-1}
DON	2241 µg g dw^{-1}	56 µg ml^{-1}	352 µg ml^{-1}
N-NH_4^+	15 µg g dw^{-1}	0.42 µg ml^{-1}	1.81 µg ml^{-1}
N-NO_3^-	1303 µg g dw^{-1}	131 µg ml^{-1}	176 µg ml^{-1}

Chemical Analysis

The vermicompost was sieved (2 mm) and moisture and organic matter content were calculated gravimetrically after drying at 60ºC for 24 h and ashing at 450ºC for 6 h. The pH and electrical conductivity (EC) of the vermicompost extracts were determined directly, and the pH and EC of the solid vermicompost was determined in water-diluted samples (1:20). Inorganic nitrogen (NH_4^+ and NO_3^-) in 0.5 M K_2SO_4 extracts (1:10 w/v) of the solid vermicompost and in the aqueous vermicompost extracts was determined by the modified indophenol blue technique (Sims *et al*. 1995), with a microplate reader (Bio-Rad Model 550). Total extractable N was determined after oxidation with $K_2S_2O_8$ as described by Cabrera and Beare (1993) and the dissolved organic nitrogen (DON) content was calculated as (total extractable N)–(NH_4^+ –N +NO_3^- –N) both in the K_2SO_4 and water extracts. Dissolved organic carbon (DOC) was determined colorimetrically at 590 nm after moist digestion ($K_2Cr_2O_7$ and H_2SO_4) of aliquots of the water and 0.5 M K_2SO_4 extracts (1:10 w/v).

Evaluation of Seedling Growth

All pots were monitored twice a week and the date of germination of each seedling was recorded. The number of days of growth was calculated based on the germination and harvest dates. The growth of the seedlings was estimated 20 weeks after sowing by measuring the height and the stem diameter at 0.5 cm above the root collar. Seedlings were clipped off at the root collar and the different aboveground components (young needles, mature needles and shoot) were separated for subsequent dry matter determination by drying at 60ºC for 48 hours. The ratio of young to mature needles was used to estimate seedling maturity.

The root system was carefully separated from the substrate under a gentle water jet using a sieve to collect any root fragments detached; subsequently lateral roots (d<0.2 mm) were separated from the taproot and both were dried at 60ºC for 48h for biomass determination. Shoot to root ratio was calculated using the sum of the above- and belowground plant fractions respectively.

Data Analysis

All statistical analyses were performed using SAS software program (version 9.3, SAS Institute, USA). All the aboveground growth parameters were analyzed on a seedling basis (shoot height, shoot diameter, volume index, biomass of mature and young needles, and number of mature needles). However, parameters involving root measurements (root biomass, taproot biomass, lateral root biomass, shoot:root ratio) were analyzed on a pot-mean basis due to the difficulty of separating individual seedlings in the same pot.

Two types of analyses were carried out: i) the effects of the incorporation of the different doses of solid vermicompost in pine seedling growth were evaluated by linear mixed models (MIXED procedure in SAS) and generalized linear mixed models (GLIMMIX procedure in SAS) in case of parameters with Poisson distribution (number of mature needles). The dose of vermicompost (0%, 2.5%, 5%, 10%, 25%) and the progeny (A, B, C, D, E), as well as their interaction, were introduced in the model as fixed factors while pot and block were introduced as random factors. When the main effects were significant, differences among factor levels were tested for significance using the LSMEAN statement at p = 0.05. Data are shown as least square means ± s.e.

ii) In order to analyze the effects derived from applying solid or water-extractable vermicompost, we compared the effects of vermicompost extracts to the effects of equivalent doses of solid vermicompost using a linear mixed model and generalized linear mixed models as described previously. The progeny (A, B, C, D, E), the nature of vermicompost amendment (solid vermicompost or vermicompost extracts), and the dose (2.5% and 25%) were introduced as fixed factors, while pot and block were introduced as random factors. Specific comparisons among factor levels were tested for significance using the LSMEAN statement at p = 0.05.

In both analyses the effects were corrected by two covariates: the number of days of growth of each seedling and seed weight, since both parameters had a significant influence on seedling growth (mean values per pot were used in case of root growth analysis). Given that the seed weight also entails strong progeny effects, the residual seed weight of the pinions within each progeny was used as a covariate instead of the whole values in order to control the within-progeny variation without losing the variability due to the progeny effect on seed weight and therefore on seedling growth and development (Sorensen and Campbell 1993). In all cases, parameter distributions were analyzed for normality by the Kolmogorov-Smirnov criterion and homogeneity of variances was checked by Levene's test.

Results

Effects of Incorporating Different Doses of Solid Vermicompost on Pine Seedling Growth

Incorporation of small amounts of vermicompost into the potting media significantly affected the growth of the pine seedlings (Table 2). Seedling height was significantly stimulated by vermicompost addition, independently of plant genotype. Addition of 2.5% and 10% vermicompost produced an increase of 2 and 1.8 cm in shoot height respectively as compared to 0% vermicompost, while the rest of the doses assayed did not produce significant differences, as shown in Table 3. The incorporation of vermicompost influenced also the morphology of the seedlings independently of plant genotype (Table 2). The substitution of 2.5% of the peat in the potting media by vermicompost produced a 15% increase in the shoot:root ratio of the seedlings. However, this parameter was most affected by 25% peat substitution by vermicompost which produced a 23% increase as compared to the unamended control potting media, as shown in Table 3.

Seedling biomass was significantly influenced by vermicompost addition independently of the progeny considered (Table 2) but no effects on aerial and root biomass were observed at the smaller doses (2.5%, 5% and 10%). In contrast, we observed a reduction of 12% in aerial biomass with 25% vermicompost as compared with the 0% dose (Table 3). Similarly, 25% peat replacement by vermicompost produced a significant decrease in the shoot diameter of the seedlings (Table 3).

Total root biomass was also reduced by 26% by the addition of 25% vermicompost (Table 3). Among the root fractions analyzed, 25% vermicompost produced a significant decrease in the biomass of the secondary roots, as shown in Table 3, while tap root was not affected (Table 2).

In addition, adverse effects with high doses of vermicompost were also observed in the maturity of the seedlings. We observed a significant decrease in the number of mature needles in seedlings grown with 25% vermicompost as compared to those treated with 0% vermicompost., probably due to their lower biomass Nevertheless, the ratio of mature to young needle biomass did not show any difference among the seedlings grown with the different vermicompost proportions (Table 3).

Solid Vermicompost Versus Vermicompost Extracts

The type of vermicompost amendment significantly influenced the growth and maturity of the seedlings, and notable differences were observed in most of the parameters studied according to whether solid vermicompost or vermicompost extracts had been applied, although these effects depended on the dose used (Table 4).

Seedling shoot height was significantly affected both by the type and dose of vermicompost amendment applied. The observed increase in seedling height with 2.5% solid vermicompost amendment was not produced with vermicompost extracts, resulting in significant differences between solid vermicompost and vermicompost extracts at the 2.5% dose, as shown in Figure 1. Furthermore, 25% vermicompost extracts did not produce significant changes in seedling height as compared to the unamended

TABLE 2.
Results of the mixed models and generalized mixed models of the effect of progeny, dose of vermicompost, interaction between progeny and dose, seed weight residuals and days of growth on the growth parameters measured in the pine seedlings.

	Progeny		Dose		Progeny x Dose		Days of Growth		Seed Weight	
	F	p	F	p	F	p	F	P	F	p
Shoot Height	6.09	<0.01	4.84	<0.01	n.s.	n.s.	1181.7	<0.01	4.38	0.04
Shoot diameter	3.79	<0.01	5.96	<0.01	n.s.	n.s.	659.7	<0.01	28.95	<0.01
Aerial biomass	2.85	0.03	3.88	<0.01	n.s.	n.s.	582	<0.01	30.41	<0.01
Root biomass	9.58	<0.01	3.31	<0.01	n.s.	n.s.	4.89	0.03	13.33	<0.01
Shoot:Root ratio	0.84	0.50	4.63	<0.01	n.s.	n.s.	0.44	0.50	0.37	0.54
Biomass of the taproot	8.41	<0.01	2.01	0.09	n.s.	n.s.	3.32	0.07	16.76	<0.01
Biomass of secondary roots	8.88	<0.01	3.31	0.01	n.s.	n.s.	3.42	0.07	8.8	<0.01
Mature:young needle biomass	2.64	0.03	1.76	0.13	n.s.	n.s.	189.95	2.35	19.52	1.23
Number of mature needles per seedling	2.89	0.03	3.77	<0.01	n.s.	n.s.	975.26	<0.01	18.7	<0.01

TABLE 3.
Growth parameters of the pine seedlings grown with 0%, 2.5%, 5%, 10% and 25 % solid vermicompost. Values are least square means ± standard error and different letters in each row indicate significant differences at p < 0.05.

	0%	2.5%	5%	10%	25%
Shoot height (cm)	29.68 bc	31.79 a	30.76 ab	31.53 a	28.36 c
Shoot diameter (cm)	0.25 a	0.26 a	0.25 a	0.25 a	0.23 b
Aerial biomass (g)	1.35 a	1.47 a	1.36 a	1.40 a	1.19 b
Root biomass (g)	0.31 a	0.28 a	0.30 a	0.29 a	0.22 b
Shoot:root ratio	4.29 c	4.96 ab	4.95 ab	4.75 b	5.30 a
Number of mature needles	1.62 ab	1.79 a	1.58 bc	1.58 bc	1.43 c
Mature:young needle ratio	0.10 ab	0.11 a	0.09 b	0.11 ab	0.09 ab
Biomass of the secondary roots (g)	0.20 a	0.18 a	0.19 a	0.19 a	0.14 b

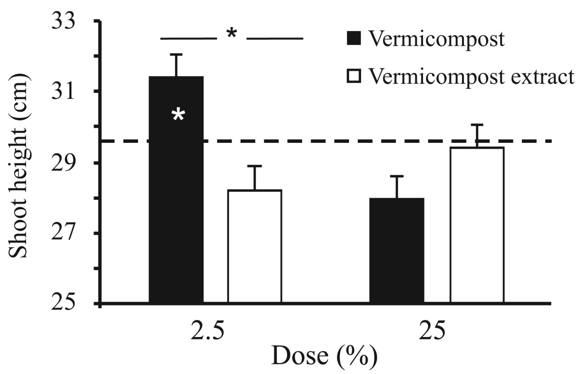

FIGURE 1. Shoot height of the pine seedlings grown with the different vermicompost treatments: solid vermicompost (black bars) and vermicompost extracts (white bars), at 2.5% and 25% doses. Bars represent least square means ± standard error. Asterisks between the bars indicate significant differences between them. The spotted line represents the unamended control. Asterisks inside the bars indicate significant differences with the control.

control. Similarly, there were no significant changes in the shoot:root biomass of the seedlings with 2.5% and 25% vermicompost extracts (4.4 ± 0.16 and 4.7 ± 0.16 respectively) as compared to the control (4.3 ± 0.17) although there were significant differences with the solid vermicompost at the 25% dose.

There was also a significant effect of the type of vermicompost amendment on the aerial and root biomass of the seedlings, which depended, as before, on the dose considered (Table 4). The use of vermicompost extracts did not produce significant changes in the aerial biomass of the seedlings as compared to the unamended control and therefore the decrease in this parameter with 25% solid vermicompost was not observed with the extracts, as shown in Figure 2. Similar results were observed in the biomass of the two root fractions analyzed although the most affected was the secondary roots fraction; again, root biomass fractions were not affected by the vermicompost extracts, but there were significant differences with the solid vermicompost at the 25% dose.

The type of vermicompost amendment and the dose applied were also observed to have a significant effect on the number of mature needles per seedling (Table 4). Although incorporation of 2.5% and 25% vermicompost extracts did not modify the number of mature needles in relation to the control, there were

TABLE 4.
Results of the mixed models and generalized mixed models of the effect of the dose of vermicompost (2.5% and 25%), type of vermicompost amendment (solid, extracts), seed weight residuals and days of growth on the different growth parameters measured in the pine seedlings.

	Progeny		Dose		Type		Dose x Type		Days of Growth		Seed Weight	
	F	p	F	p	F	p	F	p	F	P	F	p
Shoot height	7.1	<0.01	13.78	<0.01	8.95	0.03	16.56	<0.01	629.83	<0.01	0.30	<0.01
Shoot diameter	4.07	<0.01	10.77	<0.01	4.59	0.03	20.39	<0.01	526.41	<0.01	22.20	<0.01
Aerial biomass	1.3	0.269	10.55	0.01	0	0.97	13.04	<0.01	325.61	<0.01	21.16	<0.01
Total root biomass	5.19	<0.01	1.28	0.26	5.02	0.03	4.79	0.03	2.91	0.09	6.12	0.01
Biomass of the taproot	7.59	<0.01	2.98	0.08	2.05	0.16	5.32	0.02	4.85	0.03	16.11	<0.01
Biomass of the secondary roots	3.69	<0.01	0.49	0.48	4.77	0.03	3.91	0.05	1.74	0.19	1.95	0.16
Shoot:root ratio	0.77	0.55	4.33	0.04	12.45	<0.01	n.s	n.s	0.24	0.63	0.69	0.41
Mature:young needle biomass	1.01	0.62	2.28	0.37	0.00	0.96	n.s	n.s	64.89	<0.01	8.18	<0.01
Number of mature needles	2.88	0.03	28.28	<0.01	0.04	0.84	29.49	<0.01	910.67	<0.01	21.78	<0.01

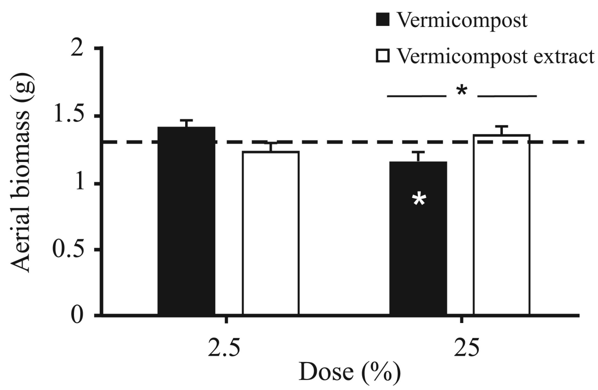

FIGURE 2. Aerial biomass of the pine seedlings grown with the different vermicompost treatments: solid vermicompost (black bars) and vermicompost extracts (white bars), at 2.5% and 25% doses. Bars represent least square means ± standard error. Asterisks between the bars indicate significant differences between them. The spotted line represents the unamended control. Asterisks inside the bars indicate significant differences with the control.

significant differences with the solid vermicompost at the 25% dose. However, no effects of the treatments were observed in the ratio of mature to young needles. Neither the solid vermicompost nor the vermicompost extracts produced significant differences in this parameter as compared to the control.

Discussion

The incorporation of small amounts of vermicompost into the potting media influenced significantly the growth of the pine seedlings. We observed a significant increase in seedling height with the lowest dose of solid vermicompost (2.5%) indicating a promotion of plant growth by vermicompost and suggesting a quadratic-like response of the plant to this type of amendment. Similar increases were observed by Atiyeh *et al.* (2001) in the shoot height of tomato plants after replacement of a commercial peat-based potting media by 5% vermicompost when additional mineral fertilization was provided to the plants. Such results would seem to indicate that other causes than nutrient supply - i.e. the presence of plant growth regulators - might be the cause for this growth enhancement.

In addition, seedling morphology was significantly altered by vermicompost incorporation to the potting media, resulting in an increase in the shoot:root ratio of the seedlings. This increase was due to different growth patterns depending on the dose of vermicompost: while 2.5%, 5% and 10% doses produced small and non-significant increases in aerial biomass and decreases in root biomass, 25% vermicompost produced significant decreases in both biomass fractions. These shifts in biomass allocation to shoots with small amounts of vermicompost indicate that the conditions in the potting media were better for the seedlings than without vermicompost addition, allowing the acquisition of enough resources that were allocated to aerial biomass instead of to root biomass (McCarthy and Enquist 2007).

In spite of this growth enhancement with the smaller doses of vermicompost, both aerial and root biomass were significantly reduced by 25% vermicompost incorporation. Root biomass was more affected by high doses of vermicompost than shoot biomass resulting in a higher shoot:root ratio than the control seedlings. Among the root fractions, the most influenced by vermicompost were the lateral roots, while the tap roots remained unaffected. These reductions in seedling growth should be taken into account by forest nursery producers since reduced plant biomass, especially root biomass, might also reduce post-transplant vigour, and hence survival of the seedlings in the field (Burdett *et al.* 1983). Reduced growth with high doses of vermicompost has also been reported elsewhere, (Roberts *et al*. 2007) and it has been attributed to the establishment of adverse physical and/or chemical conditions in the media due to vermicompost addition. A change in the pH of the potting media towards slightly alkaline conditions due to vermicompost addition could result in a proportional decrease in the availability of certain ions such as B, Zn, Fe, Mn, and Cu (Rengel 2002). Nevertheless no sign of nutrient deficiencies was observed in the seedlings at the end of this experiment thereby indicating other factors as causes of the decrease in seedling growth. Previous studies have shown that vermicompost has normally a higher bulk density and lower particle size than the peat-based potting media (Hidalgo *et al*. 2006). Therefore, increasing amounts of vermicompost result in proportional increases in bulk density and water holding capacity which might ameliorate potting media physical conditions to some extent but which could also result detrimental when the airspace in the pots is excessively reduced (Atiyeh *et al*. 2001).

In contrast to the solid vermicompost, the use of vermicompost extracts did not produce any effects on the seedlings, either positive or negative. Thereby confirming that the changes observed in pine seedling growth are due to the change in the physical properties of the potting media after solid vermicompost addition. Vermicompost extracts have been shown to enhance growth and suppress plant diseases in several plant species (Scheuerell and Mahaffee 2004, Arancon *et al*. 2007). A recent study by Spaccini *et al*. (2008) showed that aerated compost extracts contained most of the low-weight compounds associated to a compost matrix, most of them of microbial origin and therefore potentially bioactive substances. Moreover, a further study demonstrated that these extracts produced greater effects on plant physiology than the equivalent bulk compost (Puglisi *et al*. 2008). The presence of bioactive substances associated to the low molecular weight fraction of humic acids, capable of inducing changes in plant morphology and physiology, has also been reported in vermicompost (Canellas *et al*. 2002; Quaggiotti *et al*. 2004). However, no evidence of such bioactivity was found in our study with vermicompost extracts.

Seedling growth was shown to be strongly dominated by genotype, as demonstrated by the highly significant differences found between progenies in most of the growth parameters evaluated. Moreover, the residual weight of the seeds, representing the variations from the mean seed weight of each progeny, and the number of days of growth, which depended on the date of germination and harvest, largely determined seedling growth, but, in accordance with results posed by other authors (e.g. Parker *et al.* 2006), did not affect the biomass partitioning of the pine seedlings. Accounting for these two important factors controlling early seedling performance is essential in order to correctly analyze the effects of vermicompost on plant growth, which would be otherwise not identified if these factors were ignored in the analysis.

Plant genotype has also been shown to play a key role in the response to vermicompost. Previous studies demonstrated big interspecific (Donald and Visser 1989) and even intraspecific (Zaller 2007) differences in the growth of plants cultivated with vermicompost. Further, in a previous study by Zas and Fernández-López, (2005), the same genetic material as the one used here revealed important differences between progenies in their responses to mineral fertilization supplemented through subirrigation. In our study all progenies responded equally to the addition of vermicompost, either solid or liquid, to the potting media, therefore suggesting that vermicompost has an impact on pine seedling growth which must be attributed to factors other than mere nutrient supply.

Conclusions

Peat replacement by vermicompost in a commercial potting media produced a significant increase in pine seedling shoot height and a significant change in plant morphology with increases in the shoot:root ratio. These changes in plant growth and morphology took place at very small doses (2.5%) and when plants were supplied with complete mineral nutrition thereby indicating the possible existence of nutrient-independent plant growth promoting effects. Nevertheless, the substitution of peat by higher doses of vermicompost (25%) produced detrimental effects on seedling growth, probably due to the creation of adverse physical conditions in the growing media. The observed effects were common for all the progenies studied indicating that, at least at this level of genotypic dissimilarity, no genotype dependent effects of vermicompost were detected. These detrimental effects of vermicompost on plant growth have seldom been signalled in the literature and must be taken into account in order to maintain the confidence of nursery producers in these types of organic potting amendments.

Acknowledgements

This work was financially supported by INIA-RTA07-100-00 and Xunta de Galicia (07MRU023383PR) grants. Cristina Lazcano was financially supported by a grant from “Fundación la Caixa”.

References

Alves, W.L. and Passoni, A.A. 1997. Compost and vermicompost of urban solid waste in *Licania tomentosa* (Benth) seedlings production to arborization. *Pesqui. Agropecu. Bras.*, 32: 1053-1058.

Appuhn, A. and Joergensen, R.G. 2006. Microbial colonisation of roots as a function of plant species. *Soil Biol. Biochem*., 38: 1040-1051.

Arancon, N.Q., Edwards, C.A., Dick, R. and Dick, L. 2007. Vermicompost tea production and plant growth impacts. *BioCycle*, 48 (11): 51-52.

Atiyeh, R.M., Arancon, N.Q., Edwards, C.A. and Metzger, J.D. 2002. The influence of earthworm-processed pig manure on the growth and productivity of marigolds. *Bioresource Technol*., 81:103-108.

Atiyeh, R.M., Edwards, C.A., Subler, S. and Metzger, J.D. 2001. Pig manure vermicompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth. *Bioresource Technol*., 78: 11-20.

Bonser, A.M., Lynch, J. and Snapp, S. 1996. Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. *New Phytol*., 132: 281–288.

Burdett, A.N., Simpson, D.G. and Thompson, C.F. 1983. Root development and plantation establishment success. *Plant Soil*, 71: 103–110.

Cabrera, M.L. and Beare, M.H. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Sci. Soc. Am. J.*, 57: 1007–1012.

Canellas, L.P., Olivares, F.L., Okorokova-Façanha, A.L. and Façanha, A.R. 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots. *Plant Physiol.*, 130: 1951-1957.

Chaoui, H.I., Zibilske, L.M., and Ohno, T. 2003. Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. *Soil Biol. Biochem*., 35:

295-302.
Domínguez, J. 2004. State of the Art and new perspectives on vermicomposting research. Edwards, C.A. (Ed.). Earthworm Ecology. 2nd ed. CRC Press, Boca Raton, FL, USA, pp 401-424.
Donald, D.G.M. and Visser, L.B. 1989. Vermicompost as a possible growth medium for the production of commercial forest nursery stock. *Applied Plant Science,* 3: 110-113.
Edwards, C.A., Domínguez, J. and Arancon, N.Q. 2004. The influence of vermicompost on plant growth and pest incidence. Shakir, S.H., Mikhail, W.Z.A. (Eds.). Soil Zoology for Sustainable Development in the 21st Century. Self-Publisher, Cairo, Egypt, pp.396-419.
El Harti, A., Saghi, M., Molina, J.-A.E. and Téller, G. 2001. Production d'une substance rhizogène à effet similaire à celui de l'acide indole acètique par le ver de terre *Lumbricus terrestris*. *Can. J. Zool.-Rev. Can. Zool.*, 79: 1911-1920.
Hidalgo, P.R. and Harkess, R.L. 2002. Earthworm castings as a substrate amendment for chrysanthemum production. *HortScience,* 37: 1035-1039.
Hidalgo, P.R., Matta, F.B. and Harkess, R.L. 2006. Physical and chemical properties of substrates containing earthworm castings and effects on marigold growth. *HortScience,* 41: 1474-1476.
Kamau, D. M., Spiertz J.H.J. and Oenem O. 2008 b. Carbon and nutrient stocks of tea plantations differing in age, genotype and plant population density. *Plant Soil*, 307: 29-39.
Kamau, D.M., Spiertz, J.H.J., Oenem, O. and Owuor, P.O. 2008 a. Productivity and nitrogen use of tea plantations in relation to age and genotype. *Field Crop. Res.*, 108: 60-70.
Lazcano, C., Sampedro, L., Zas, R., Domínguez, J. 2010. Vermicompost enhances germination of the maritime pine (*Pinus pinaster* Ait.). *New Forests*. 39: 387-400.
McCarthy, M.C. and Enquist, B.J. 2007. Consistency between an allometric approach and optimal partitioning theory in global patterns of plant biomass allocation. *Funct. Ecol.,* 21: 713-72.
Muscolo, A., Bovalo, F., Gionrtiddo, F. and Nardi, S. 1999. Earthworm humic matter produces auxin-like effects on *Daucus Carota* cell growth and nitrate metabolism. *Soil Biol Biochem.,* 31: 1303-1311.
Parker, W.C., Noland, T.L. and Morneault, A.E. 2006. The Effects of Seed Mass on Germination, Seedling Emergence, and Early Seedling Growth of Eastern White Pine (*Pinus strobus* L.). *New Forest.,* 32: 33-39.
Puglisi, E., Fragoulis, G., Del Re, A.A.M., Spaccini, R., Piccolo, A., Gigliotti, G., Said-Pulicino, D. and Trevisan, M. 2008. Carbon deposition in soil rhizosphere following amendments with compost and its soluble fractions, as evaluated by combined soil-plant rhizobox and reporter gene systems. *Chemosphere*, 73: 1292-1299.
Quaggiotti, S., Ruperti, B., Pizzeghello, D., Francioso, O., Vitaliano, T. and Nardi, S. 2004. Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). *J. Exp. Bot*., 55: 803-813.
Rengel, Z. and Marschner, P. 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. *New Phytol*., 168: 305-312.
Rengel, Z. 2002. Role of pH in Availability of Ions in Soil. Rengel, Z. (Ed.). Handbook of Plant Growth. pH as the Master Variable. Marcel Dekker, Inc. New York -Basel. pp. 331-358.
Roberts, P., Edwards, C.A., Edwards-Jones, G. and Jones, D.L. 2007. Responses of common pot grown flower species to commercial plant growth media substituted with vermicomposts. *Compost Sci. Util*., 15: 159-166.
Scheuerell, S.J. and Mahaffee, W.F. 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultim*um. *Phytopathology*, 94: 1156- 1163.
Sims, G.K., Ellsworth, T.R. and Mulvaney, R.L. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. *Commun. Soil. Sci. Plan*., 26.
Sorensen, F.C. and Campbell, R.K. 1993. Seed weight - seedling size correlation in coastal Douglas-fir: genetic and environmental components. *Can. J. Forest Res.,* 2: 275-285.
Spaccini, R., Baiano, S., Gigliotti, G. and Piccolo, A. 2008. Molecular characterization of a compost and its water-soluble fractions. *J. Agr. Food Chem.,* 56: 1017-1024.
Tomati, U., Grappelli, A. and Galli, E. 1987. The presence of growth regulators in earthworm- worked wastes. Bonvicini Paglioi, A.M., Omodeo, P. (Eds.). On earthworms. Mucchi Editore, Modena, Italy, pp. 423-436.
Zaller, J.G. 2007. Vermicompost as a substitute for peat in potting media: Effects on germination, biomass allocation, yields and fruit quality of three tomato varieties. *Sci Hortic –Amsterdam,* 112: 191-199.
Zas, R. and Fernández-López, J., 2005. Juvenile genetic parameters and genotypic stability of *Pinus pinaster* Ait. Open pollinated families under different water and nutrient regimes. *Forest Sci*., 51: 165-174.
Zas, R., Pichel, F., Martíns, P. and Fernández-López, J. 2006. Fertilization x genotype interaction in *Pinus radiata* open pollinated seedlings planted in three locations in Galicia (NW Spain). *New Forest*., 32: 253-263.