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SHORT NOTE

Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants

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Abstract Two challenges frequently encountered in the production of ornamental plants in organic horticulture are: (1) the rate of mineralization of phosphorus (P) and nitrogen (N) from organic fertilizers can be too slow to meet the high nutrient demand of young plants, and (2) the exclusive use of peat as a substrate for pot-based plant culture is discouraged in organic production systems. In this situation, the use of beneficial soil microorganisms in combination with high quality compost substrates can contribute to adequate plant growth and flower development. In this study, we examined possible alternatives to highly soluble fertilizers and pure peat substrates using pelargonium (*Pelargonium peltatum* L'Her.) as a test plant. Plants were grown on a peat-based substrate with two rates of compost addition and with and without arbuscular mycorrhizal (AM) fungi. Inoculation with three different commercial AM inocula resulted in colonization rates of up to 36% of the total root length, whereas non-inoculated plants remained free of root colonization. Increasing the rate of compost addition increased shoot dry weight and shoot nutrient concentrations, but the supply of compost did not always completely meet plant nutrient demand. Mycorrhizal colonization increased the number of buds and flowers, as well as shoot P and potassium (K) concentrations, but did not significantly affect shoot dry matter or shoot N concentration. We conclude that addition of compost in combination with mycorrhizal inoculation can improve nutrient status and flower development of plants grown on peat-based substrates.

Keywords Arbuscular mycorrhiza · Compost · Organic horticulture · Pelargonium

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Introduction

Two critical factors in the commercial production of flowering ornamental plants are the choices of growth substrate and fertilization method. In Europe and North America, most pot-grown ornamental plants are produced and sold in peat-based substrates. In conventional production systems, these substrates are usually supplemented with soluble fertilizers to achieve optimal supply of nutrients such as nitrogen (N) and phosphorous (P).

In organic horticulture, however, the use of synthetic chemical fertilizers is discouraged. Moreover, the use of peat is viewed critically because peat is a limited natural resource (Joosten and Clarke 2002). The European Union (2004) and organic growers' associations of many countries support a reduction in the fraction of peat in growth substrates to a maximum of 70% in the next few years (George and Eghbal 2003). This generates problems for horticultural producers because high quality ornamental

plants require substrates with high nutrient availability during a short growth period for optimal development.

To allow the development of economically sustainable, ecological greenhouse horticulture, it is important (a) to characterize methods to improve nutrient supply from organic sources and (b) to find alternative substrates for peat in pot cultures without loss of plant quality.

To reduce peat at least partly in growth substrates, various alternative organic materials, such as compost (Veeken et al. 2004), have been tested. Compost as a nutrient source for plants may require amendments of other organic nutrient sources to meet the plant demand. In addition, soil or rhizosphere microorganisms that cannot be found within compost–peat substrates may help plants to mobilize and acquire nutrients from the substrate. A group of soil microorganisms living in very intimate contact with roots of most plant species are the arbuscular mycorrhizal (AM) fungi. These fungi are capable to assist the plant in the uptake of nutrients, such as P, N, zinc (Zn), copper (Cu), and sometimes potassium (K) (George 2000; Neumann and George 2005), and to increase plant dry weight (Lee and George 2005). Arbuscular mycorrhizal colonization may induce earlier flowering and increased flower numbers (Gaur and Adholeya 2005; Nowak 2004; Usha et al. 2005). This trait of AM fungi is of particular interest to horticultural production.

A number of studies have tested the interactive effects of nutrient supply and mycorrhizal colonization on flowering. On a peat substrate with organic NPK fertilizer, mycorrhizal pelargonium plants flowered earlier and had increased N, P, and K concentrations at low nutrient supply as well as increased P concentrations at high nutrient supply, while the number of flowers and the leaf dry weight were unaffected by mycorrhizal colonization (Nowak 2004). *Zinnia* and *Tagetes* plants had an increased number of flowers after mycorrhization, but final dry weight as well as K and P concentrations were unaffected (Aboul-Nasr 1996).

The effect of compost addition on mycorrhizal and non-mycorrhizal plant seedlings has been investigated in a few studies only. Compost containing substrates may be appropriate for mycorrhizal plants (Linderman and Davis 2001) if the quality of the compost is adequate (Perner et al. 2006). However, Sáinz et al. (1998) pointed out that compost amendment may suppress mycorrhizal colonization and therefore the activity of AM fungi. In addition, the chemical, physical, and biological properties of peat can have a strong impact on the AM colonization rate (some peat qualities may even be incompatible with a functioning AMF colonization) (Linderman and Davis 2003; Estaun et al. 1999). Thus, until now, it is not clear whether mycorrhizal root colonization and compost addition are complementary in increasing yield and flower production in organic management systems.

Therefore, we utilized pelargonium as test plant in an experiment studying whether (a) increasing the rate of compost application contributes to increased plant dry weight and N, P, and K supply, (b) a peat-compost substrate supports AM fungus colonization of plants, (c) AM fungus colonization is beneficial to plants on this substrate with regard to dry weight and N, P, K, and Zn supply, and (d) AM fungus colonization increases production of flowers and buds. The aim was to increase the understanding of the role of AM fungi in plant growth on organic substrates.

Materials and methods

Plant cultivation

In the experiment, single-rooted cuttings of pelargonium (*Pelargonium peltatum* 'Balcon Imperial Compact', Silze, Weener Halte, Germany) were placed in separate 250-ml pots filled with a peat substrate with an addition of 20 or 40% compost (see below). Drip irrigation (40 $ml \cdot min^{-1}$) was used every second day (total of 40 ml) to maintain favorable water conditions in the substrate. Additionally, every third or fourth day, the pots were weighed, and irrigation water was added to equalize the water content of the pots. The experiment was carried out from 11 Sep. to 23 Oct. 2002 (6 weeks) in a greenhouse facility of the Institute for Vegetable and Ornamental Crops at Großbeeren (longitude, 13°19′60′E; latitude, 51°22′0′N), Germany. Average air temperature in the greenhouse during this time was 23°C (minimum of 17°C and maximum of 27°C) during the day and 18°C (minimum of 17°C and maximum of 25°C) at night. Relative humidity was on average 66% during the day and 77% at night. The daily (10.5 h) mean light intensity (photosynthetically active radiation) was 8 $mol \cdot m^{-2}$ (maximum, 662 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Pots were rearranged at regular intervals.

Substrate preparation and characterization

The substrates used in this study were suitable for organic production. The compost was prepared from yard waste and shredded trees and bushes (Bruns 1998). The material used had a wide C/N ratio (40:1) at the beginning of the composting process. After 3 months of composting, the extractable nutrient concentration in the compost was: N, 150 $mg \cdot l^{-1}$; P, 360 $mg \cdot l^{-1}$; and K, 1,535 $mg \cdot l^{-1}$ (extraction by $CaCl_2$ [N] and CAL [P, K]). The compost had a salt concentration of 2.2 $g \cdot l^{-1}$ and a pH ($CaCl_2$) of 7.1. In the present study, this compost was mixed with sphagnum peat from the Baltic region (light peat H3, Bakulama, Sienlaukis, Lithuania) to obtain a compost substrate with 20 or 40% compost by volume. The substrates were limed with

CaO (Vereinigten Kreidewerke Dammann KG, Söhlde) to a pH of 6.2 and sieved to 10 mm. In addition, N fertilizer was added to the substrate 1 day before the start of the experiment. The N fertilizer (a mixture of 33% horn meal 0–2 mm, containing 10% N, and 66% horn meal 2–6 mm, containing 14% N) was uniformly mixed into the substrate (20%, 6,700 $mg \cdot l^{-1}$ and 40%, 5,500 $mg \cdot l^{-1}$).

In previous and unpublished work with this substrate and fertilizer, we observed that 25% of the added N became available to plants within 2 weeks after planting, and that within eight weeks after planting, 85% of the added N was available. Therefore, the plant-available N concentration of the compost substrate together with the horn meal fertilizer added by calculation up to 200 $mg \cdot l^{-1}$ in the first 2 weeks after planting in both compost addition treatments.

Inoculation with AM fungi

Inoculation with AM fungi was carried out with three different commercially available inocula: TerraVital Hortimix (Pla; contains *Glomus mosseae*, *G. intraradices*, *G. claroideum*, and *G. microaggregatum*, >50 infective units per ml inoculum; PlantWorks, Heeley Close, Sittingbourne, Kent, UK), Endorize-Mix (Bio; contains *G. mosseae*, *G. intraradices*, *Glomus* sp., infective units not specified; Biorize, Rue Sainte Anne, Dijon, France), and AMYkor (Tri; contains *G. mosseae*, *G. intraradices*, and *G. etunicatum*, 50 infective units per ml inoculum; Triton, AMYkor GmbH, Wolfen, Germany). The inocula were mixed uniformly into the potting substrate before planting the seedlings. Addition rates were used according to the suppliers' recommendation and were: Pla, 5% (*v*/*v*); Bio, 5% (*v*/*v*); and Tri, 3% (*v*/*v*). Non-mycorrhizal (NAM) treatments were supplied with autoclaved (121°C for 20 min) Pla inoculum. In addition, a filtrate (589/3 blue ribbon paper filter, Schleicher and Schuell Bioscience GmbH, Dassel, Germany) of non-sterilized Pla inoculum was added to NAM pots in an effort to supply similar amounts of nutrients and microorganisms other than AM fungi to all treatments.

Harvest and plant analysis

Pelargonium buds and flowers were counted and removed three times during the experiment, and the individual counts were combined. At the end of the experiment, shoots were separated from roots, and shoot fresh weight (FW) was recorded. Shoots were then dried at 80°C for 2 days, and dry weight (DW) was recorded. The shoots were ground in a centrifugal grinder using a 0.25-mm sieve. Shoot samples were dry-ashed and dissolved in 18.5% HCl. Potassium and Zn were analyzed with an atomic absorption spectrophotometer (Perkin Elmer 3300, Überlingen, Germany). Phosphorus was analyzed photometrically with an EPOS-analyzer 5060 (Eppendorf, Hamburg, Germany). Nitrogen was determined after dry oxidation by the Dumas method (Elementar Vario EL, Hanau, Germany).

For the investigation of root DW, the substrate of the whole pot was washed to separate the roots from the substrate with running cold water using a set of sieves (smallest sieve size, 1 mm). The root FW and DW were recorded, and a representative subsample for assessment of mycorrhizal fungus colonization was taken and stored in 10% isopropanol. Mycorrhizal fungus colonization of roots was determined following the method of Koske and Gemma (1989) with slight modifications. Roots were cleared with 10% KOH, acidified with 2 N HCl, and stained with 0.05% trypan blue in lactic acid. Percentage root length colonization in cleared parts of the roots was determined with a microscope (Zeiss, Stemi2000, Göttingen, Germany) at 100× using the grid line intersection method (Giovannetti and Mosse 1980).

Statistics and experimental design

Pots were arranged in a completely randomized design. Data were analyzed by a two-factorial analysis of variance, with compost addition rate and mycorrhizal inoculation as experimental factors (n=4). Mean separation was carried out with the Tukey test (P<0.05). Individual treatment differences were subjected to t tests. Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK, USA) software.

Results

Colonization of pelargonium roots by AM was not significantly different between the two compost addition levels. Average root length colonization across both compost addition levels for the different inocula was (±SE): Pla, 36±4%; Bio, 34±4%; and Tri, 15±5%. Non-inoculated plants remained free of mycorrhizal colonization, although the substrate had not been sterilized before use.

Shoot DW was significantly higher on 40% compost than on 20% compost substrate and was not significantly affected by inoculation with AM (Table 1). Root DW was not influenced by compost treatment but was significantly enhanced in the Tri treatment compared to the NAM treatment (Table 1).

The number of buds and flowers was not significantly influenced by compost addition rate (Table 1). The Pla treatment increased significantly the number of buds and flowers compared to the NAM treatment. Comparison of the individual treatment combinations (Fig. 1) showed that on the 40% compost substrate, the number of buds and

Table 1 Shoot and root dry weight, number of buds and flowers, and shoot N, P, and K concentrations of pelargonium plants six weeks after planting

	Dry weight (g pot^{-1})		Buds and flowers (*n* pot^{-1})	Element concentration g (kg DW)$^{-1}$		
	Shoot	Root		N	P	K
Inoculum						
NAM	2.39±0.09a	0.19±0.02a	8.6±3.0a	26.1±0.8a	2.08±0.05a	9.2±0.7a
Pla	2.28±0.14a	0.16±0.03a	18.2±3.1b	26.4±0.4a	2.59±0.15b	21.0±1.6c
Bio	2.47±0.11a	0.20±0.04ab	15.8±1.7ab	25.8±0.9a	2.73±0.10b	14.9±1.0b
Tri	2.47±0.11a	0.27±0.02b	14.9±4.2ab	24.3±1.7a	2.24±0.06a	9.9±0.9a
Compost						
20%	2.27±0.08a	0.21±0.02a	13.4±2.5a	25.1±0.8a	2.01±0.06a	14.2±1.0a
40%	2.54±0.14b	0.20±0.03a	15.4±3.4a	26.2±1.1a	2.80±0.12b	13.3±1.1a
P value						
P (m)	0.362	0.009	0.039	0.281	<0.001	<0.001
P (c)	0.003	0.444	0.387	0.176	<0.001	0.259
P (m×c)	0.845	0.087	0.967	0.311	0.923	0.396

Plants were grown on compost–peat substrate with 20% compost or 40% compost and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with one of three mycorrhizal inocula [TerraVital Hortimix (Pla), Endorize-Mix (Bio), AMYkor (Tri)]. Effects of the treatments [mycorrhizal inoculation (m); compost addition rate (c)] were tested with a two-way ANOVA. Different letters denote significant differences between means within one factor determined by the Tukey test ($P<0.05$). Values are means of four observations±standard error of the mean (SE).

flowers was higher also in the Bio and Tri treatments than in the NAM treatment.

Shoot N and K concentrations were not significantly different between the 20 and 40% compost substrates (Table 1). Shoot P concentration was significantly higher on 40% than on 20% compost substrate. Inoculation did not induce significant differences in shoot N concentration (Table 1). Shoot P and K concentrations were significantly increased by the Pla and Bio treatments on both substrates. The concentration of K in shoots was especially high in the Pla treatment. The shoot Zn concentration was not influenced by AM or compost addition rate (data not shown).

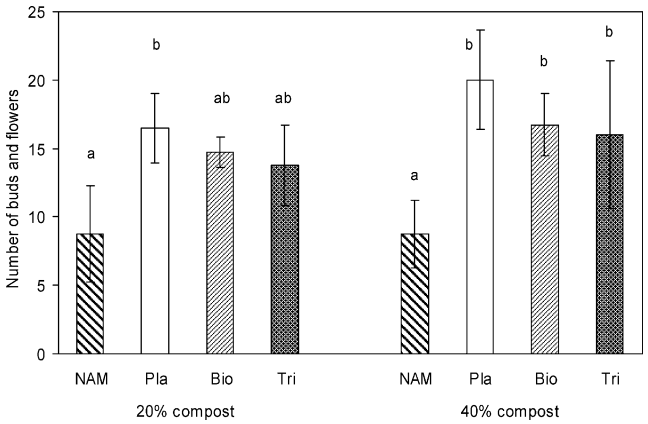

Fig. 1 Number of buds and flowers of pelargonium plants 6 weeks after planting in compost–peat substrates. Plants were either non-inoculated with mycorrhizal fungi or were inoculated with one of three mycorrhizal inocula [TerraVital Hortimix (Pla), Endorize-Mix (Bio), AMYkor (Tri)]. *Different letters* denote significant differences between means of mycorrhizal inoculation treatments determined by a *t* test ($P<0.05$). Means of four observations±SE (*vertical lines intersecting bars*)

Discussion

Roots of inoculated pelargonium plants were well colonized with mycorrhizal fungi. This was true at both compost addition rates (20 and 40%) and for all three commercial inocula. The observation that the horticultural substrate used in this study did not support spontaneous mycorrhizal colonization is of high practical significance. Horticultural producers must use inoculation and relatively low nutrient addition rates if they intend to grow mycorrhizal plants on these substrates.

Increasing the rate of compost amendment of the substrate from 20 to 40% had a supporting effect on the growth of pelargonium plants. This effect may be due to increased P supply, as evidenced by the increased shoot P concentration of plants grown in the 40% compost treatment. Another reason could have been the higher water-holding capacity of peat-based substrates with higher compost addition rate (Perner et al. 2006).

Inoculation with mycorrhizal fungi did not result in an increased pelargonium shoot DW. On soils deficient in P, mycorrhizal colonization supports plant development by supplying the plant with additional P and sometimes with N, K, or Zn (George 2000; Nowak 2004). Although we found low P concentrations in shoot tissue, substrate P availability may still have been too high to allow an AM-dependent shoot enhancement effect. Alternatively, on

organic substrates, some mycorrhizal fungi may be less effective in P uptake than on mineral soils (Perner et al. 2006).

Both a reduction and an increase in root growth upon mycorrhizal colonization have been observed under favorable conditions (Liu et al. 2004; Martin and Stutz 2004). In the present experiment, the higher root DW in the Tri treatment had no apparent consequences for shoot DW or for the number of buds and flowers of pelargonium plants.

A comparison of the nutrient concentrations of the pelargonium shoots in the present study with literature values for adequately fertilized plants (Bergmann 1993) showed a sufficient supply of N and Zn to the plants in this study. At both rates of compost addition, shoot P and K concentrations were low in the NAM and Tri treatments, whereas the plants treated with Pla and Bio had higher P concentrations and adequate K concentrations (lower limit indicating sufficient supply: 3 mg kg^{-1} for P and 12 mg kg^{-1} for K; Bergmann 1993). Pelargonium plants grown at the higher rate of compost addition and inoculated with Pla or Bio inoculants thus had a shoot P concentration also indicating sufficiency.

The contribution of AM fungi to plant nutrient uptake is often particularly evident in plants that are deficient in a certain nutrient. Thus, it is not surprising that no mycorrhizal effect on pelargonium shoot N or Zn concentrations was found in the present study. In the case of pelargonium shoot P and K concentrations, a mycorrhizal effect was evident. These findings correspond with those of Nowak (2004) in a study of pelargonium provided with low NPK supply. Mycorrhizal fungi are well known for their efficient P uptake, but the contribution of K to plants by AM has been described more rarely and specifically on acidic soils (see e.g., Alloush and Clark 2001). It is possible that small aggregates of compost and peat remained acidic in the limed substrate, and that the hyphae entered these acidic aggregates and exploited additional K sources. Moreover, enhanced decomposition of organic material in the substrate due to microorganism activity may lead to increased release of humic acids, with the consequence of decreased pH and increased K availability. The pelargonium shoot P concentration was not increased when Tri inoculum was used. With this inoculum, AM-acquired P was likely incorporated preferentially in the roots, leading to a considerable increase in root DW in this treatment.

A significant finding of this study for practical horticulture was the increased number of buds and flowers with AM inoculation (Table 1; Fig. 1). A similar increase in buds and flowers with mycorrhizal colonization has been described previously for *Tagetes*, *Zinnia*, *Callistephus* and tomato plants (Aboul-Nasr 1996; Gaur and Adholeya 2005; Poulton et al. 2002). In the present experiment, the rate of compost amendment to the substrate had no influence on the number of buds and flowers, and the number of buds and flowers did not correspond with either shoot N or Zn concentration. Shoot concentrations of P and K were increased in the Pla and Bio treatments, but only shoot K concentration corresponded with the number of buds and flowers.

Potassium is involved in a wide range of functions in plants: photosynthesis, enzyme activation, protein synthesis, and osmotic potential. Potassium also acts as a counterion to inorganic ions and organic biopolymers (Marschner 1995). It has also been shown that K is a carrier ion in xylem and phloem, transporting solutes, assimilates, and hormonal stress signals such as abscisic acid (Peuke et al. 2002). Hormones, such as gibberellins that induce the bud production (Krizek and Fletcher 2005) could be transported in faster rates due to higher levels of K in the plant. Thus, mycorrhizal colonization may either directly influence plant hormonal balance or may indirectly affect plant hormone levels by altered plant nutrient status.

We conclude that (a) AM colonization was established in pelargonium plants on a horticultural substrate, irrespectively of varied rate of compost addition to the substrate; (b) increasing the rate of compost amendment moderately increased pelargonium shoot DW due to higher nutrient supply, but compost-peat substrates may still require additions of, for example, NPK sources to result in plant nutrient sufficiency; and (c) AM had no effect on shoot DW or shoot N concentration, but it increased shoot P and K concentrations on a compost–peat substrate low in P and K supply.

We also conclude that bud and flower production (d) was not affected by the rate of compost amendment of the substrate and (e) can be increased or accelerated by inoculation with a commercial mycorrhizal inoculum. Increase in bud and flower production may have been the result of AM-mediated increases in plant nutrient (especially K) concentrations in combination with a possible hormonal effect induced by the presence of mycorrhizal colonization. Mycorrhizal plants may accumulate nutrients in a shorter time span so that they are, earlier in life, sufficiently supplied with nutrients to initiate flower development.

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