Research Article

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Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.)

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Abstract

BACKGROUND: Garden sage (*Salvia officinalis* L., Lamiaceae) has gained importance during the last decade as a natural antioxidant, mainly owing to the antioxidant secondary plant metabolites rosmarinic acid (RA) and diterpenes such as carnosic acid. The aim of this work was to study the biomass production, the concentrations of total phenolics and RA and the essential oil of garden sage in response to phosphorus (P) supply. The treatments included P fertilisation and inoculation with arbuscular mycorrhizal fungi (AMF), since AMF are the most efficient biotic factor promoting the P uptake of plants.

RESULTS: The P concentration in the plant was highest in P-fertilised plants, intermediate in AMF-inoculated plants and lowest in non-inoculated/non-P-supplied plants. The leaf biomass increased only in P-fertilised plants. AMF-inoculated plants, in contrast, did not show an increased leaf biomass, but their root biomass was enhanced. The total phenolic and RA concentrations were affected differently in leaves and roots, with the highest concentration and yield being found in leaves of P-fertilised plants. In none of the treatments were the essential oil concentration and composition affected.

CONCLUSION: Optimal P fertilisation improves the antioxidant potential of garden sage and increases its biomass yield, thus optimising agronomic production of this plant for antioxidant use.

Keywords: *Salvia officinalis*; phosphorus; arbuscular mycorrhiza; secondary plant metabolites; rosmarinic acid; total phenolics; essential oil

INTRODUCTION

In plants, phosphorus (P) is required in relatively large amounts for the biosynthesis of primary and secondary metabolites,[1] since P has essential functions as a constituent of nucleic acids and phospholipids (biomembranes) and plays a key role in the energy metabolism of cells. From this viewpoint, factors affecting the P balance of plants are of interest. Besides P fertilisation, the most important biotic factor affecting the P status of plants is arbuscular mycorrhizal fungi (AMF), forming the respective arbuscular mycorrhizal (AM) symbiosis. The AM symbiosis refers to an association between plants (80% of all vascular plants) and soil-borne fungi that colonise the cortical tissue of roots during active plant growth. The AM symbiosis is characterised by fungal structures inside the roots, namely hyphae, arbuscules (highly branched structures) and vesicles (drop-shaped storage organs, not always present). Outside the roots a huge hyphal net spreads in the soil and is responsible for the absorption and translocation of P to the plant. The strong effect of AM hyphae on P acquisition results mainly from their greatly increased absorptive surface area and their narrow diameter, allowing P acquisition in capillaries not accessible to the roots.[2] However, the contribution to P uptake varies among AMF species.[3] The overall advantage for the plant associated with AMF is the improved supply of P, while the fungus gets plant-produced carbohydrates for growth and maintenance. Besides these functions, AM root colonisation often leads to increased biomass production[4] and/or alterations in the biosynthesis of secondary plant metabolites (SPMs) such as flavonoids, phenolics and terpenes.[5–9] Phenolic compounds are metabolised in the phenylpropanoid pathway. Enzymes such as phenylalanine ammonia-lyase and chalcone isomerase involved in the biosynthesis of phenolics were found to be affected by AM colonisation in legumes as a result of defence reactions.[10] Interestingly, some SPMs that protect plants against antimicrobial infections enhance the nutritional, antioxidant and medicinal value of plants for human consumption. Since bioactive SPMs are of specific importance for culinary herbs, spices and medicinal

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plants, even minor quantitative and/or qualitative changes in these compounds in plants could have a significant effect on their production. Therefore the AM symbiosis or P fertilisation could be of practical relevance and economic interest.

The genus *Salvia* is one of the most widespread members of the Lamiaceae family. Garden sage (*Salvia officinalis* L.), belonging to one of the most known species within this genus,[11] is used as a culinary herb and spice or for medicinal purposes against inflammations and infections of the mouth and throat. The plant is used mainly because of the high antioxidant activity of its essential oil constituents[11] and the polyphenols rosmarinic acid (RA) and carnosic acid.[12] In some species of Lamiaceae, e.g. oregano and basil, enhancements in biomass and/or the content of bioactive compounds as well as other types of modifications in secondary metabolism due to mycorrhization have been reported that were not mediated by P.[13–16] In contrast, in *Lavandula vera* cell suspension culture a positive effect of P on the RA content has been reported.[17]

The objective of the present study was to evaluate the effect of increased P supply, either through P supplementation or by inoculation with different AMF, in garden sage on parameters such as biomass, total phenolic and RA concentrations and essential oil quantity and quality.

EXPERIMENTAL

Biological material, growth conditions and experimental set-up

To obtain genetically homogeneous plant material, green cuttings from a single *S. officinalis* plant (breeding line ATF10124) were rooted in a sterile substrate mixture of peat and expanded clay. After 4 weeks the rooted plantlets were transferred into 20 cm diameter pots (three plantlets per pot) containing an autoclaved (20 min at 120 °C) substrate mixture of sand, soil and expanded clay (1 : 1:1 v/v/v). The substrate mixture contained 1.12 mg PO_4^{2-} L^{-1}. Plants were grown in a random design in the greenhouse with a day/night cycle of 16 h at 22 °C and 8 h at 19 °C (relative humidity 50–70%). The experimental set-up consisted of six different treatments:

Symbivit without additional P,
Glomus mosseae without additional P,
Glomus intraradices without additional P,
Control without AMF and without additional P,
Half phosphorus (68 mg KH_2PO_4 L^{-1}) without AMF and
Full phosphorus (136 mg KH_2PO_4 L^{-1}) without AMF

The experiment was done with three replicates per treatment.

In the mycorrhizal treatments, 5 g of AM inoculum was added to each plantlet when the plantlets were transferred to the sterile substrate mixture. Three different AM inoculi were used. The pure inoculi of *G. mosseae* (BEG 12) and *G. intraradices* (BB-E) were purchased from Biorize/Agrauxine (Quimper, France). They consisted of lyophilised mycorrhizal roots containing sporocarps, spores and hyphae of the particular fungus blended with silica sand. The inoculum Symbivit® was purchased from Symbio-M (Lanskroun, Czech Republic) and consisted of six different *Glomus* species (*G. mosseae, G. intraradices, G. cladoideum, G. microagregatum, G. caledonium* and *G. etunicatum*).

During the growth period of 4 months, plants were watered with a nutrient solution (475 mg $Ca(NO_3)_2$, 256 mg K_2SO_4, 136 mg $MgSO_4$, 70 mg MoO_3, 8 mg NH_4NO_3, 50 mg $Fe_6H_5O_7 \cdot 3H_2O$, 1.3 mg $Na_2B_4O_7 \cdot 4H_2O$, 1.5 mg $MnSO_4 \cdot 4H_2O$, 0.6 mg $ZnSO_4 \cdot 7H_2O$, 0.54 mg $CuSO_4 \cdot 5H_2O$, 0.028 mg $Al_2(SO_4)_3$, 0.028 mg $NiSO_4 \cdot 7H_2O$, 0.028 mg $Co(NO_3)_2 \cdot 6H_2O$, 0.028 mg TiO_2, 0.014 mg $LiCl_2$, 0.014 mg $SnCl_2$, 0.014 mg KI and 0.014 mg KBr L^{-1}) containing full phosphorus (plus 136 mg KH_2PO_4 L^{-1}), half phosphorus (plus 68 mg KH_2PO_4 L^{-1}) or no phosphorus depending on the treatment.

Estimation of mycorrhization

At harvest time, roots were gently washed free from soil, and roots and shoots were separated. The degree of mycorrhization was estimated on defined fresh root segments of 1 cm length, starting 5 cm down the shoot, according to the staining method with ink (jetblack, Sheaffer, Fort Madison, IA, USA)[18] and the counting procedure of McGonigle *et al*.[19] Shoots and remaining roots were dried at 35 °C. Dried shoots were separated into stems and leaves. Leaves and roots were taken for estimation of all other parameters.

P concentration

Leaves and roots were ground, oven dried for 4 h at 105 °C and solubilised with a triple acid mixture for the analysis of P by the ammonium vanadate/molybdate method.[20] Values are given in g P kg^{-1} dry matter.

Determination of total phenolic and rosmarinic acid concentrations

Ground samples were extracted with methanol/water (50 : 50 v/v) in the ratio 50 mg sample/8 mL solvent at 25 °C for 1 h in an ultrasonic bath. Filtered extracts were used for the determination of total phenolic and rosmarinic acid concentrations. Total phenolics were determined according to the Folin–Ciocalteu method.[21] The results are expressed as caffeic acid equivalents (CAE). Rosmarinic acid was analysed by high-performance liquid chromatography (HPLC) according to the method of Cuvelier *et al*.[12] with some minor modifications. The HPLC analysis was performed on a reverse phase Symmetry® C_{18} column (4.6 mm × 150 mm, 5 µm pore size) equipped with a Symmetry® C_{18} guard column (Waters, Milford, MA, USA). The mobile phase was a linear gradient programme which started with 90% solvent A (840 mL of deionised water with 8.5 mL of acetic acid and 150 mL of acetonitrile) and 10% solvent B (methanol) and changed within 30 min to 100% solvent B at a flow rate of 1.5 mL min^{-1}. Methanolic samples of 20 µL of were injected. Detection was performed with a photodiode array detector (Waters 996 PDA) at 330 nm.

Chemicals

Folin–Ciocalteu's phenol reagent was obtained from Merck (Darmstadt, Germany), caffeic acid from Sigma Aldrich (Vienna, Austria) and rosmarinic acid from Roth (Karlsruhe, Germany). Solvents for extract preparation and HPLC analysis were of analytical grade and HPLC grade respectively.

Essential oil analysis

A 5 g portion of dried leaves was hydrodistilled with 200 mL of deionised water for 1 h. The essential oil was diluted with dichloromethane (1 : 100 v/v) for further analysis. Identification of the oil components was carried out using an HP 6890 gas chromatograph/mass spectrometer coupled with an HP 5972 mass-selective detector (Hewlett Packard, Palo Alto, CA, USA) and fitted with a DB-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). Analytical conditions were helium as carrier gas (average velocity 42 cm s^{-1}), an injection

temperature of 250 °C, a split ratio of 50 : 1 and a temperature programme of 60 °C for 4 min followed by 60–280 °C at 5 °C min^{-1}. Components were identified by comparison of Kovats retention indices[22] and mass spectra from the Wiley database.[23] Quantification of components was performed using an HP 6890 gas chromatograph/flame ionisation detector (Agilent) fitted with a DB-5 narrow-bore column (10 m × 0.1 mm, 0.17 µm film thickness; Agilent). Analytical conditions varied only in split ratio (150 : 1) and temperature programme (60–85 °C at 6 °C min^{-1} followed by 85–300 °C at 10 °C min^{-1}).

Statistical analysis

Treatment effects were determined by one-way analysis of variance (ANOVA). Significant differences between treatments (indicated by different letters) were confirmed by Tukey's highest significant difference (HSD) test at the 5% level of significance. Pearson's correlation coefficients were calculated at the level of $P = 0.05$ or $P = 0.01$. All statistical analyses were performed with SPSS for Windows Release 11.5.2.1 (SPSS, Chicago, IL, USA).

RESULTS

AM colonisation and biomass parameter

In this study we investigated the effect of P uptake of garden sage on growth and SPMs, including AMF as a biotic promoter for P uptake. A well-established mycorrhizal colonisation of the roots is an absolute requirement to correctly consider AMF. The degree of root colonisation was highest in the *G. mosseae* and Symbivit treatments (~32%) and significantly lower in the *G. intraradices* treatment (23%). No mycorrhizal structures were observed in the control and phosphorus treatments (Table 1).

The total biomass (leaves, stems and roots) did not show any differences between treatments (data not shown). The leaf biomass was significantly increased by 1.2-fold in the full and half phosphorus treatments compared with all other treatments (Fig. 1). The root biomass was lowest in the control treatment and increased slightly in the full phosphorus (1.1-fold), *G. intraradices* (1.6-fold) and *G. mosseae* (1.8-fold) treatments. These differences, although quite large, were found not to be significant owing to the high standard errors. A significant (2.1-fold) increase was observed in the Symbivit treatment (Fig. 1). A high correlation between AM colonisation and root biomass ($r = 0.70$, $P < 0.01$) and an even higher correlation between AM colonisation and root/shoot ratio ($r = 0.81$, $P < 0.01$) were found. Compared with the control and half phosphorus treatments, the root/shoot ratio was significantly increased by 1.7-fold in the *G. intraradices* and *G. mosseae* treatments and by 2.1-fold in the Symbivit treatment (Table 1).

Phosphorus concentration and amount

The P concentration was generally higher in leaves than in roots, with one exception in the full phosphorus treatment. The leaf P concentration was significantly increased by 1.4-fold in the *G. mosseae*, Symbivit and half phosphorus treatments and by 1.7-fold in the full phosphorus treatment. The root P concentration was again highest in the full phosphorus treatment but still significantly increased in the Symbivit and half phosphorus treatments compared with the control treatment (Table 1). The P amounts in leaves and roots were calculated by multiplying the leaf or root P concentration by the leaf or root dry matter for each plant. The P amounts were higher in leaves than in roots. The leaf P amounts showed almost the same trend as the leaf P concentrations in the full phosphorus treatment, with a 1.9-fold increase compared with the control treatment. The root P amount was lowest in the control treatment, slightly enhanced in the half phosphorus

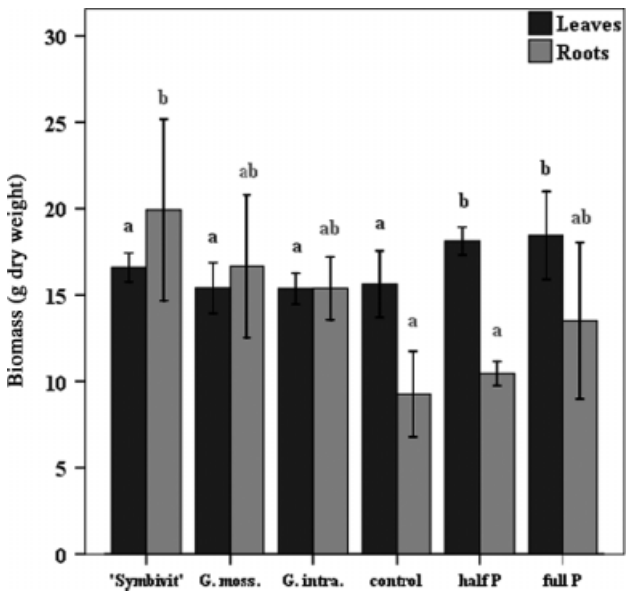

Figure 1. Leaf (dark bars) and root (light bars) biomass parameters (mean ± standard error). Different letters within a parameter denote significant differences according to Tukey's HSD test ($P = 0.05$).

Table 1. Degree of mycorrhization, root/shoot ratio and P concentrations and amounts in leaf and root fractions of garden sage (mean ± standard error)

Treatment	Degree of mycorrhization (%)	Root/shoot ratio	Leaf P concentration (g kg^{-1})	Root P concentration (g kg^{-1})	Leaf P amount (mg per plant)	Root P amount (mg per plant)
Symbivit	32.7 ± 4.0c	0.65 ± 0.07c	2.4 ± 0.1c	2.2 ± 0.1b	39.1 ± 1.1cd	37.2 ± 0.4bc
G. mosseae	32.5 ± 3.0c	0.57 ± 0.06bc	2.3 ± 0.0bc	2.0 ± 0.1ab	34.9 ± 1.2bc	33.2 ± 2.2bc
G. intraradices	23.3 ± 4.5b	0.52 ± 0.03bc	1.9 ± 0.1ab	1.8 ± 0.1ab	29.6 ± 0.7ab	26.8 ± 1.6b
Control	0a	0.32 ± 0.02a	1.7 ± 0.0a	1.3 ± 0.2a	26.1 ± 1.5a	12.3 ± 2.3a
Half phosphorus	0a	0.31 ± 0.02a	2.4 ± 0.1c	2.2 ± 0.1b	42.7 ± 1.8d	23.1 ± 1.4ab
Full phosphorus	0a	0.38 ± 0.05ab	2.8 ± 0.1d	3.5 ± 0.2c	50.5 ± 1.9e	46.5 ± 5.8c

Different letters within a column denote significant differences according to Tukey's HSD test ($P = 0.05$).

treatment and significantly enhanced in all other treatments. The significantly highest root P amount was observed in the full phosphorus treatment. In general, the full phosphorus treatment gave the highest P concentration and amount compared with the other treatments. The Symbivit treatment showed the highest effect on P uptake from the substrate among the group of different mycorrhizal treatments, followed by the *G. mosseae* treatment with a slightly lower concentration and amount. The *G. intraradices* treatment had the least effect on P uptake (Table 1).

Secondary metabolites

Our study focused on concentrations and yields of total phenolics and RA in both leaves and roots as parameters for the antioxidant capacity. Furthermore, we estimated the essential oil quantity and composition of the volatile fraction of garden sage leaves.

The concentrations of total phenolics and RA showed opposite trends in leaves and roots (Fig 2). The total phenolic concentration in leaves ranged between 39 and 43 g CAE kg^{-1} dry matter in the AM and control treatments and was slightly (1.1-fold) but not significantly increased in the half phosphorus treatment, while the full phosphorus treatment gave a significant 1.2-fold increase in total phenolics. A highly significant correlation between total phenolic and P concentrations in leaves ($r = 0.66$, $P < 0.01$) was found. The total phenolic concentration in roots showed no significant differences between treatments, with the full phosphorus treatment resulting in the lowest value (Fig. 2A). The RA concentration was generally higher in leaves than in roots. The RA concentration in leaves ranged between 9.4 and 10.6 g kg^{-1} for the AM and control treatments. The half phosphorus treatment gave 11.7 g RA kg^{-1} and the full phosphorus treatment 14.2 g RA kg^{-1}. A positive correlation between leaf RA and P concentrations ($r = 0.62$, $P < 0.01$) was observed. The RA concentration in roots was also not significantly different between treatments owing to the high variation between replicates (Fig. 2B). The correlation between RA concentrations in leaves and roots was highly significant ($r = -0.62$, $P < 0.01$). The total phenolic and RA yield parameters were calculated by multiplying the total phenolic or RA concentration by the biomass dry matter fraction of the parameter. The total phenolic yield in leaves was significantly increased in the full phosphorus treatment but only slightly increased in the Symbivit and half phosphorus treatments. In roots we found the significantly highest effect of total phenolic yield in the Symbivit treatment and slightly enhanced yields in the *G. mosseae, G. intraradices* and full phosphorus treatments (Table 2). The higher total phenolic yields in AM-treated roots compared with phosphorus-treated roots were due to the higher root biomass rather than the total phenolic concentration. The total phenolic yield per plant did not show any significant differences between treatments, but the Symbivit treatment gave the highest amount (data not shown). The leaf RA yield (Table 2) showed exactly the same significant differences as the RA concentration in leaves (Fig. 2B) for all treatments. The root RA yield was significantly increased in the *G. intraradices* and Symbivit treatments compared with the phosphorus and control treatments. The total RA yield per

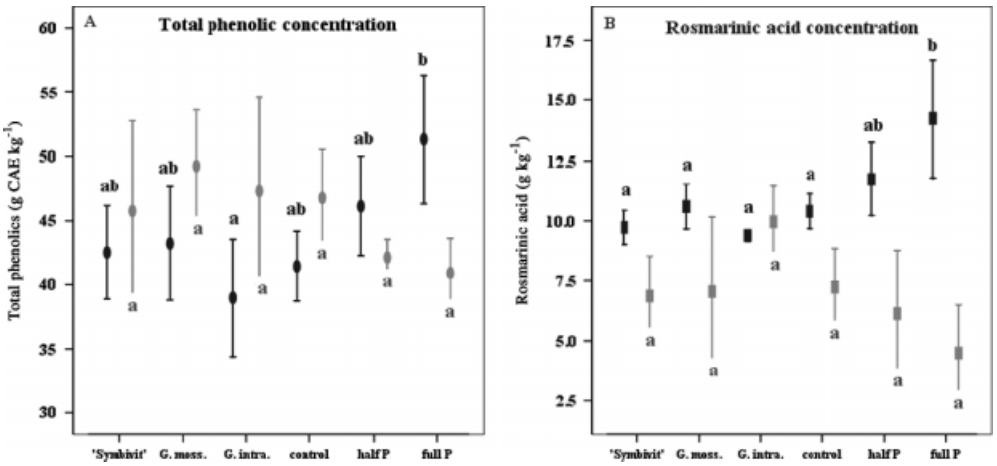

Figure 2. (A) Total phenolic and (B) rosmarinic acid concentrations in leaf (dark symbols) and root (light symbols) fractions of garden sage (mean ± standard error). Different letters within a parameter and fraction denote significant differences according to Tukey's HSD test ($P = 0.05$).

Table 2. Yield parameters of leaf and root fractions for SPMs of garden sage (mean ± standard error)

Treatment	Leaf total phenolic yield (mg CAE per plant)	Root total phenolic yield (mg CAE per plant)	Leaf rosmarinic acid yield (mg per plant)	Root rosmarinic acid yield (mg per plant)	Leaf essential oil yield (mg per plant)
Symbivit	708 ± 48ab	901 ± 89b	162 ± 9a	132 ± 3b	210 ± 10a
G. mosseae	671 ± 65a	820 ± 107ab	164 ± 13a	105 ± 18ab	196 ± 9a
G. intraradices	599 ± 40a	653 ± 23ab	144 ± 4a	154 ± 19b	205 ± 9a
Control	651 ± 62a	431 ± 50a	162 ± 10a	65 ± 4a	197 ± 11a
Half phosphorus	836 ± 73ab	440 ± 10a	213 ± 18ab	63 ± 12a	241 ± 7a
Full phosphorus	942 ± 87b	556 ± 104ab	261 ± 25b	56 ± 3a	239 ± 18a

Different letters within a column denote significant differences according to Tukey's HSD test ($P = 0.05$).

plant was significantly increased in the full phosphorus treatment (0.32 ± 0.02 g per plant) compared with the control treatment (0.23 ± 0.01 g per plant), while the total RA yield in the other treatments varied between 0.28 and 0.30 g per plant.

The essential oil concentration did not differ between treatments (data not shown). The mean essential oil concentration for all treatments was 13.0 ± 0.8 g kg^{-1}. Furthermore, the essential oil yield did not show significant differences between treatments (Table 2), although the mean essential oil yield increased by 1.2-fold in the half and full phosphorus treatments compared with the AM and control treatments. The essential oil composition was not influenced at all by the P uptake of the plants (data not shown).

DISCUSSION AND CONCLUSIONS

With the present study we aimed to extend current knowledge about the effects of increased P acquisition of plants, either through P supplementation or by the establishment of an AM symbiosis, on the growth and SPM production of garden sage, a traditionally well-known culinary and medicinal plant.

P is required in relatively large amounts but is often poorly available in soil because of largely insoluble calcium, iron and aluminium phosphates or fixation to clay mineral surfaces.[24] The substrate used in our study contained low levels of a water-soluble P fraction (0.1–5 mg PO_4^{2-} L^{-1} soil solution)[1] to prevent severe P deficiency symptoms of control plants and allow good AM colonisation in the AM treatments. In plants, P has multifunctional roles as a constituent of nucleic acids or biomembranes. Furthermore, it is highly involved in the energy metabolism of cells and is therefore required for the biosynthesis of primary and secondary metabolites in plants.[1] Having this in mind, factors affecting the P balance in plants are of interest in sage production.

All sage plants grew well throughout the study period, without any visible P deficiency symptoms. The AM colonisation degrees were satisfactory, especially in the Symbivit and *G. mosseae* treatments. Both these treatments significantly increased the P concentration in leaves compared with the control treatment. The Symbivit treatment was similar in efficiency of P uptake to the half phosphorus treatment. In leaves and roots, *G. intraradices* enhanced the P concentration only slightly, which could be partly explained by the 10% lower root colonisation. Moreover, as P uptake is AMF-dependent, differences in P level in plants can be AMF species-specific and do not necessarily correlate with the percentage of colonisation.[3] Contradictory results concerning *G. intraradices* and P uptake were found after chickpea inoculation.[25] From our data we conclude that the efficiency of P acquisition in mycorrhizal symbiosis is dependent not only on the species of fungus but also on the host plant.

Highest P concentrations were expected in the full phosphorus-treated plants, since P was added continuously with the nutrient solution. In our case the full phosphorus-treated plants had the highest P concentration, similar to the finding in oregano.[14] However, in two independent studies performed with basil, neither additionally applied P nor AMF increased the P concentration in leaves and shoots.[13,16] This means that it is not self-evident that plants with higher P supply, either through P fertilisation or through AMF, will show increased P concentration in their tissues.

The effects of the higher P concentrations in the phosphorus and AM treatments on the biomass parameter are contradictory. Applied P enhanced the leaf biomass, while increased P supply through AMF (especially in the Symbivit treatment) promoted mainly an increase in root biomass. A general assumption is that plants with increased P supply show increased photosynthetic rates, thus enhancing carbon acquisition and further growth. In both phosphorus treatments the leaf biomass increased significantly up to the same level. We assume that already the half phosphorus supply is sufficient for garden sage to reach the plateau of the yield response curve.[26] Interestingly, the increased P concentration due to mycorrhizal root colonisation did not stimulate leaf growth at all. The plant responsiveness to mycorrhiza, i.e. the difference in growth between plants with and without mycorrhiza,[27] was unaffected, since the total biomass did not show any differences between treatments. When discussing these results with the P response curve,[27] the P concentration in the tissues of mycorrhizal-treated plants increased more than the total biomass growth rate. Only the root growth was affected by mycorrhization, especially that of plants inoculated with Symbivit, the most effective treatment. In our study, when looking at the biomass, the AM symbiosis seems to be disadvantageous for the plant. In this context it should be noted that in several herbaceous and woody plants the carbon drain to mycorrhizal roots is about 4–14% higher than that to non-mycorrhizal roots.[24,28] This enhanced carbon drain could be an explanation for the mycorrhizal effect seen. Furthermore, we observed an increase in root biomass instead of an increase in leaf biomass in the AM treatments. The parameter which reflects this relation best is the root/shoot ratio. In general, lower root/shoot ratios were recorded for mycorrhizal than for non-mycorrhizal plants. Furthermore, Marschner[1] found that increasing nitrogen supply leads to a decrease in root/shoot ratio. In our study, all treatments were supplied with the same amount of nitrogen, so the enhanced root/shoot ratio found should be nitrogen-independent. P also could not be involved in the increase in root/shoot ratio, since the highest P concentrations were detected in leaves and roots of the full phosphorus-treated plants, but at the same time these plants showed a low root/shoot ratio. Our data indicate that the observed increase in root growth in AM plants is not mediated by P but rather mediated through a different fungus–plant effect.

The high correlation between the degree of root colonisation and the root biomass ($r = 0.70$) raises the question of how much the fungal weight (arbuscules, vesicles and hyphae) inside the roots contributes to the total root biomass. It has been reported that the fungal biomass associated with roots ranges between 3 and 16% of the root dry weight.[28] Even if we consider that about 20% of the root dry weight is due to the fungus, the results indicate a significant effect of AMF on the root biomass.

The yield parameter is a useful indicator of the output efficiency of a certain treatment. Owing to the high root biomass of the AM treatments, the root P amount reaches almost the same level as that of the full phosphorus treatment. However, looking at the leaf P amount, the AM treatments do not enhance the quantity of P per plant to the same extent as the phosphorus treatments. For the agronomic production of garden sage, we can conclude that P fertilisation yields a higher leaf biomass than AMF inoculation. However, it has been observed several times that plants can benefit from AMF through enhanced resistance against pathogens and modifications, depending on the quality and quantity of SPMs.[4] Alterations in the composition and quality of SPMs by AMF have been observed, especially for phenolic compounds and terpenoids.[13–16,29]

Garden sage as a traditionally used herb and spice is appreciated, among other things, for its high antioxidant capacity, based on phenolic compounds, particularly RA, and on its essential oil

composition.[11] Antioxidant compounds, especially polyphenols resulting from phenylpropanoid metabolism and terpenoids derived from the mevalonate pathway, play an important role in the protection of organisms, cells and cell membranes against harmful effects of oxygen radicals. In leaves of garden sage, medium correlations of high significance level between P and total phenolic concentrations and between P and RA concentrations were observed. Thus the highest total phenolic and RA concentrations were found in the full phosphorus treatment. The high correlation between P and phenolics including RA was previously reported only for an *in vitro* cell suspension culture of *L. vera*.[17] In basil shoots, P, total phenolic and RA concentrations did not increase in AM- and P-supplied plants.[16] These findings confirm our assumption concerning P uptake and phenolic compounds. In the study with basil an increase in the RA content in roots was observed,[16] but unfortunately the P concentration in roots was not estimated. Interestingly, in the present study we have the reverse effect between P and phenolic compounds, especially the RA concentration in roots compared with leaves (Fig. 2). This effect is clearly expressed in a negative correlation between the RA concentrations in leaves and roots. Taking a closer look at the P effect on the RA concentration in garden sage leaves, the Symbivit and *G. mosseae* treatments significantly increased the P concentration but not the RA concentration. Therefore AMF could play a suppressive role during RA biosynthesis. For the total RA yield per plant the full phosphorus treatment led to a significantly increased RA amount, which was the result of an active gain in biomass. For the yield of total phenolics per plant we observed no significant differences between treatments, indicating a shift in synthesis between roots and leaves.

SPMs resulting from phenylpropanoid metabolism are often involved in plant defence reactions and play crucial roles in overcoming a great diversity of biotic and abiotic stresses. A key step in phenylpropanoid metabolism is mediated by the enzyme phenylalanine ammonia-lyase (PAL), which converts L-phenylalanine into cinnamic acid, the precursor of several compounds associated with the defence response.[30] The enzyme cinnamic acid 4-hydroxylase (C4H) is the second important enzyme in the biosynthetic sequence of this pathway. It catalyses the hydroxylation of cinnamic acid into coumaric acid and is involved in the biosynthesis of RA.[31,32] There is some evidence that biotic stresses such as exposure to pathogens activate changes in the gene expression level.[30,32,33]

In AM-inoculated leek and gingko, even at maximum root AM colonisation, no increases in PAL activity and phenol formation as antimicrobial substances were found.[34] On the other hand, in the early stages of root AM colonisation a stimulation of the transcription and activity of PAL was reported in mycorrhizal plants, whereas PAL was suppressed in the later stages of root AM colonisation.[10,35] Although symbiotic and pathogenic interaction modifications in the SPM pathway seem to be different, in fact, common strategies are shared.[36] Until now it has been assumed that the metabolism of SPMs in AM-inoculated plants is modified mainly during the early stages of AM development. These modifications seem to occur to a much lesser extent than those in response to pathogen attack. Furthermore, many genes identified as being regulated differently in AM symbiosis (1–4%) turn out to be regulated by other factors as well, such as P or pathogen attack.[24,37]

Other interesting factors involved in plant growth and development with key regulator functions in response to pathogens are the naturally occurring plant hormones jasmonic acid and methyl jasmonate.[38] Low concentrations of jasmonate were found to induce genes encoding the enzyme PAL in flavonoid biosynthesis.[38] Another study reported an increase in RA in sweet basil by exogenous methyl jasmonate application.[31] One explanation could be that increased P supply leads to enhanced methyl jasmonate production, which in turn, in up-regulation of PAL, results in enhanced RA concentration in garden sage. However, in plants colonised by AMF, the biosynthesis of RA seems to be regulated differently. Another possibility is that gene expression is regulated differently in leaves and roots. The total phenolic and RA yield is highest for the full phosphorus treatment in leaves and for the Symbivit treatment in roots. However, both the phosphorus and AM treatments show approximately the same amount of antioxidants in terms of the phenolics and RA of leaves and roots together. Up to now, only the herb fraction has been used for culinary and pharmaceutical purposes. The consideration of both biomass fractions as a harvest product is economically useful only for annual plants and is actually not interesting for perennial plants such as garden sage delivering several cuttings per year over many years.

The essential oil quantity and quality make garden sage valuable as a food-flavouring additive and for therapeutic purposes. Neither the quantity nor the quality of the essential oil was significantly affected by the different treatments. Enzymes involved in the mevalonate pathway, which are important in the biosynthesis of terpenes[39] and thus the essential oil, seem not to be affected either by P fertilisation or by AMF in garden sage. In the food industry a stable quality of the essential oil composition is obligatory. Furthermore, for the agronomist it is important to know that increased P fertilisation has no negative effect on the composition of the product. A closer look shows that the essential oil yield increased by 1.2-fold as a result of P fertilisation compared with the other treatments, an increase due to the enhanced leaf biomass. From our data we can conclude that in garden sage the essential oil is not affected by root AM colonisation. This contrasts with other studies where at least one of the essential oil parameters, i.e. concentration, yield or composition, was altered by a non-P-mediated AMF effect.[13,14,29,40]

To summarise, we can conclude from the data presented here that P application to garden sage increases the leaf biomass, the total phenolic and RA concentrations and the RA yield in leaves. On the one hand, these changes could enhance the resistance of sage plants against pathogens; on the other hand, they could raise the market value of garden sage owing to its increased benefit for human health due to its enhanced antioxidant activity. This effect was not visible when garden sage was inoculated with different types of AMF, even though P concentrations were increased. Further studies are necessary to explain how P application leads to an increase in SPMs, probably by affecting the phenylpropanoid pathway.

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