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Effects of substrate type on plant growth and nitrogen and nitrate concentration in spinach

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Abstract

The effects of three commercial substrates (a mixture of forest residues, composted grape husks, and white peat, black peat and coir) on plant growth and nitrogen (N) and nitrate (NO_3) concentration and content were evaluated in spinach (*Spinacia oleracea* L. cv. Tapir). Spinach seedlings were transplanted at 45 days after emergence into Styrofoam boxes filled with the substrates and were grown during winter and early spring in an unheated greenhouse with no supplemental lighting. Each planting box was irrigated daily by drip and fertilized with a complete nutrient solution. The NO_3 content of the drainage water was lower in coir than in the other substrates. However, shoot NO_3 concentration was not affected by substrate type, while yield and total shoot N and NO_3 content were greater when plants were grown in peat than in the mixed substrate or the coir. Leaf chlorophyll meter readings provided a good indication of the amount of N in the plants and increased linearly with total shoot N.

Introduction

The use of substrates and soilless culture systems for production of horticultural crops is increasing worldwide. Substrates often increase plant growth and yield in many crops, reduce the incidence of soil-borne diseases, and, when combined with collection of drainage water, increase the efficiency of water and nutrient use.[1-4] Despite these many benefits, there is currently very little information available concerning the influence of substrate type on plant growth and nutrient uptake in many crops, including leafy vegetables. Physical and chemical properties such as bulk density, water holding capacity, pH, cation exchange capacity, and nutrient content vary considerably among substrates and, therefore, likely have a considerable influence on plant development and nutrition.

Tissue nitrate (NO_3) concentrations tend to be higher when plants are grown in soilless culture systems,[5,6] and leafy vegetables such as spinach can accumulate levels that may be harmful to human health.[7,8] In many cases, the concentration of NO_3 in the plant tissues increases due to low light conditions and reduced photoperiod in these systems.[9-13] For example, a reduction of light from 800 to 200 $mol \times m^{-2} \times s^{-1}$ increased total shoot NO_3 concentration in spinach by more than 200%.[14] Nitrate accumulation also varies with the season, where it is often higher during the autumn and winter months than during the spring and summer.[15-17]

The objective of this study was to evaluate the influence of different substrate types on plant growth and shoot nitrogen (N) and NO_3 concentrations of spinach grown in an unheated greenhouse during the winter and early spring.

Materials and Methods

Growth conditions and substrates

The experiment was conducted in a greenhouse located at the *Herdade Experimental da Mitra* (38º31 52 N; 8º01 05 W), University of Évora, Portugal. The greenhouse was covered with thermal polyethylene and had no supplemental lighting. Air temperatures inside of the greenhouse ranged from 5 to 26ºC, and solar radiation ranged from 34 to 248 $W \cdot m^{-2} \cdot d^{-1}$. The experiment comprised three different commercial substrates: a mixture of forest residues, composted grape husks, and white peat (Substrato Universal Agriloja); a black peat blend (Super Terra Torfkultursubstrat 1; Hawita Flor, Germany); and a coir blend (Pelemix España S.L., Spain). Physical and chemical characteristics of the substrates, according manufacturer, are shown in Table 1. Mass wetness, moisture content, and bulk density were determined following the methods described by Fonteno and Harden (Table 2).[18]

Spinach (*Spinacia oleracea* L. cv. Tapir) seedlings were transplanted at 45 days after emergence into to Styrofoam planting boxes (100-cm long × 25-cm wide × 10-cm high) filled with 16 L of substrate. The seedlings were spaced 8-cm apart in three rows per box and 10-cm apart between rows. Treatments were arranged in a randomized complete block design with five replicate boxes per substrate treatment.

Each planting box was irrigated using 4 $L \cdot h^{-1}$ pressure-compensating drip emitters. Irrigation was controlled by a timer and averaged 20 to 30% drainage (leaching fraction) at each application. Nutrient solution was applied daily by fertigation, from transplanting to the day before harvest. The solution was made from fresh tap water [electrical conductivity (EC) of 0.3 $dS \cdot m^{-1}$; pH 7; and 0.10-0.30 $mmol \cdot L^{-1}$ NO_3] and initially contained 4.78 $mmol \cdot L^{-1}$ NO_3, 1.16 $mmol \cdot L^{-1}$ NH_4, 0.43 $mmol \cdot L^{-1}$ P, 4.29 $mmol \cdot L^{-1}$ K, 1.40 $mmol \cdot L^{-1}$ Ca, 0.49 $mmol \cdot L^{-1}$ Mg, 0.54 $mmol \cdot L^{-1}$ S, 46 $\mu mol \cdot L^{-1}$ B; 7.86 $\mu mol \cdot L^{-1}$ Cu, 8.95 $\mu mol \cdot L^{-1}$ Fe, 18.3 $\mu mol \cdot L^{-1}$ Mn, 2.60 $\mu mol \cdot L^{-1}$ Mo, and 7.64 $\mu mol \cdot L^{-1}$ Zn. The concentration was adjusted for plant growth at 21 days after transplanting (DAT) to 8.62 $mmol \cdot L^{-1}$ NO_3, 1.43 $mmol \cdot L^{-1}$ NH_4, 1.70 $mmol \cdot L^{-1}$ P, 4.45 $mmol \cdot L^{-1}$ K, 1.95 $mmol \cdot L^{-1}$ Ca, 0.49 $mmol \cdot L^{-1}$ Mg, 0.54 $mmol \cdot L^{-1}$ S, 46 $\mu mol \cdot L^{-1}$ B, 7.86 $\mu mol \cdot L^{-1}$ Cu, 8.95 $\mu mol \cdot L^{-1}$ Fe, 18.3 $\mu mol \cdot L^{-1}$ Mn, 2.60 $\mu mol \cdot L^{-1}$ Mo, and 7.64 $\mu mol \cdot L^{-1}$ Zn. The final pH of both solutions was 5.9.

Measurements

The pH, EC, and the concentration of NO_3 of the drainage water from each box was measured weekly using a potentiometer (pH Micro 2000 Crison), a conductivity meter (LF 330 WTW, Weilhein, Germany), and an ion-specific electrode and meter (Crison Instruments, Barcelona, Spain), respectively, following the

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procedures outlined by Prazeres.[19] A portable chlorophyll meter (Minolta SPAD-502: Soil Plant Analysis Development, Minolta Co., Osaka, Japan) was used to measure leaf greenness at 24 and 36 DAT. Two recently expanded leaves were selected from three plants in each box for the chlorophyll readings, and three measurements were taken on each leaf on both dates.

The plants were harvested at 36 DAT. The shoots of the plants were cut off at 1 cm above the substrate surface. Four representative plants (shoots) from each box were washed, oven-dried at 70°C for 2-3 days, weighed, ground, and analyzed for total N using a combustion analyzer (Leco Corp., St. Joseph, MI, USA). Additional leaf samples were stored at –80°C for NO_3 determination.[20] The samples were oven-dried at 65°C for 48 h, weighed (0.1000 g), macerated in a mortar, homogenized in a test tube with 10 mL of distilled water, agitated in a vortex, and incubated for 1 h at 45°C in a shaking water bath. Filtrated extract was then mixed with salicylic acid in 5% sulphuric acid (1:4), incubated for 20 min at room temperature, and mixed with 9.5 mL of 2 M sodium hydroxide. The concentration of NO_3 in the solution was then determined using UV-VIS spectrophotometer (Thermo Scientific, Genesys 10S) at 338 and 440 nm.

Data analysis

Data were analyzed by analysis of variance using SPSS Statistics 21 software (Chicago, IL, USA). Means were separated at the 5% level using Duncan's new multiple range test.

Results and Discussion

Drainage water

The pH in the drainage water was influenced by substrate type (Figure 1A). In general, pH was greater in the drainage water collected from the peat substrate than from the other two substrates. The pH also increased over time in each treatment, which was likely due to the differential uptake of ions from the nutrient solution. For instance, when N is supplied in the NO_3 form, there is an increase in hydroxide ion ($OH^–$) concentration in the drainage water.[21] The nutrient solution used in the present study provided ≈80% of the N as NO_3. On average, the pH increased at a rate of 0.14 to 0.19 units per week in the three drainage solutions.

The EC and concentration of NO_3 in the drainage water were also affected by substrate type (Figure 1B,C). In the former case, EC was initially greater with coir than with the other two substrates or in the nutrient solution (1.5 $dS.m^{–1}$). Coir often has high levels of Na and Cl.[1,2,22] Coir also resulted in lower NO_3 in the drainage water than the other two substrates, which could have been related to lower N availability in the substrate (Table 1) and to the ability of coconut fiber's to immobilize soluble N in the mix.[23-26]

Table 1. Physical and chemical characteristics of three commercial substrates.

	Forest residues, husks, and peat	Peat	Coir
Organic matter (%)	60	90	94-98
Organic C (%)	-	-	40-50
Lignin + hemicellulose	-	-	85-90
N ($mg·L^{–1}$)	200-400	50-300	-
P ($mg·L^{–1}$)	100-200	35-131	-
K ($mg·L^{–1}$)	150-300	66-332	-
pH	5.5-6.5	6.1	5.5-6.5
EC ($mS·cm^{–1}$)	1-3	0.8	<1
C/N ratio	<20	53	80

Table 2. Mass wetness, moisture content, and bulk density of three commercial substrates.

Substrate	Mass wetness (g water × g substrate)	Moisture content (%)	Bulk density ($g·cm^{–3}$)
Forest residues, husks, and peat	2.40[c]	70.6[c]	0.27[a]
Peat	7.29[a]	87.8[a]	0.10[b]
Coir	4.75[b]	82.5[b]	0.14[b]

[a,b,c]Means followed by different letters within a column are significantly different at P<0.05.

Table 3. Effects of three commercial substrates on shoot dry weight and fresh yield of spinach.

Substrate	Shoot dry weight (g/plant)	Yield ($kg·m^{–2}$)
Forest residues, husks, and peat	1.28[b]	3.96[b]
Peat	1.71[a]	4.51[a]
Coir	1.20[b]	3.88[b]

[a,b]Means followed by different letters within a column are significantly different at P≤0.05.

Table 4. Effect of three commercial substrates on leaf chlorophyll (SPAD meter readings) and shoot nitrogen (N) and nitrate (NO_3) concentration and content in spinach.

Substrate	Chlorophyll 24 DAT	Chlorophyll 36 DAT	Shoot N concentration ($g\ kg^{–1}$)	Shoot NO_3 concentration ($mg\ g^{–1}$ DW)	Shoot NO_3 concentration ($mg\ g^{–1}$ FW)	Shoot N content (mg/plant)	Shoot NO_3 content (mg/plant)
Forest residues, husks, peat	39.0[b]	38.5[b]	32.2[b]	35.8[a]	3.63[a]	41.2[b]	45.7[b]
Peat	43.1[a]	43.8[a]	35.9[a]	42.3[a]	4.57[a]	61.4[a]	73.9[a]
Coir	39.0[b]	40.1[b]	37.9[a]	36.8[a]	3.83[a]	46.0[b]	44.1[b]

DAT, days after transplanting; DW, dry weight; FW, fresh weight. [a,b]Means followed by different letters within a column are significantly different at P≤0.05.

Plant growth and yield

Plants grown in peat had greater shoot dry weight and more yield (fresh weight) than those grown in other two substrates (Table 3). The yields were similar to those obtained when spinach was grown in a floating system[8] and greater than those obtained in soil.[27,28]

Leaf chlorophyll and shoot nitrogen and nitrate

Plants grown in peat were greener and had higher chlorophyll meter readings at 24 and 36 DAT than those grown in the other substrates (Table 4). The readings increased linearly with shoot N content and were within the range reported by others (Figure 2).[8,29,30]

None of the plants in the treatments showed visual symptoms of N deficiency. However, plants grown in the mix of forest residues, husks, and peat had lower shoot N concentration than those grown in the other substrates (Table 4). In general, shoot N concentrations were higher than those reported in Florida (<30 g kg^{-1}) but on the low end of the level considered to be sufficient for spinach at this stage of development (35-55 g kg^{-1}).[31,32]

Shoot NO_3 concentration was not affected by substrate type (Table 4). In each case, the values were higher than allowed by Regulation (EU) n°1258/2011 of the European Commission for fresh spinach (3.5 mg g^{-1} fresh weight). Therefore, these substrates do not appear to be a means of preventing high shoot NO_3 concentrations in spinach. Leaf NO_3 concentrations of spinach in a greenhouse, whether grown in soil or soilless culture systems, often exceed the value allowed by the EU. Siomos and colleagues found that plants from a soilless culture system had greater NO_3 and total N, P, and K content than plants harvested from soil.[33]

The high NO_3 concentrations in the present study were likely related to the environmental conditions in the greenhouse, nutritional factors, and the cultural techniques used. Light intensity was low in the greenhouse, not only due to the time of year (winter and early spring), but also due to the fact that the plastic film on the greenhouse was not totally transparent, and because a high planting density (64 plants/m^2) led to a considerable amount of leaf shading. As previously mentioned, plants often accumulate more NO_3 under low light conditions and during reduced photoperiods.[9-12] Peet and colleagues cited by Gruda found that the amount of daylight received was reduced by 30% or more by the glasshouse structure, while the other environmental factors, including the availability of water and nutrients, were usually at optimal levels.[34] The high ratio of NO_3:NH_4 (≈80) may have also led to high leaf NO_3 concentrations in our study. It has been reported that spinach accumulates more NO_3 when grown with solutions containing high NO_3:NH_4 ratios.[8] However, in that case, the total amount of N applied was 12 mmol L^{-1} and greater used in the present study (5.9 mmol L^{-1} from planting to 20 DAT and 10 mmol L^{-1} from 21 DAT until the day before harvest).

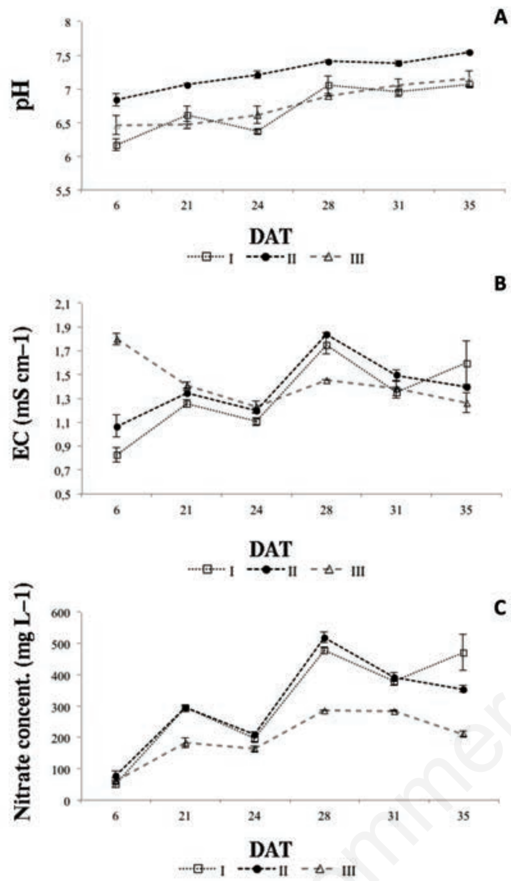

Figure 1. Effects of three commercial substrates (I, forest residues, husks, and peat; II, peat; III, coir) on pH (A), electrical conductivity (B) and concentration of nitrate (C) in the drainage water. Each symbol represents the mean of four replicates, and the error bars represent ±1 standard error.

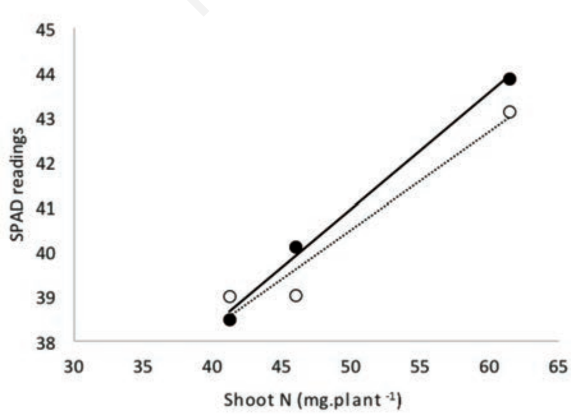

Figure 2. Relationship between shoot N (mg×plant–1) and leaf chlorophyll (SPAD meter readings) at 24 (○) and 36 (●) days after transplanting (DAT) in spinach. SPAD readings (24 DAT)=0.22 mg/plant N+29.5 (r2=0.9513, P<0.0001); SPAD readings (36 DAT)=0.26 mg/plant N+27. 9 (r2=0.9943, P<0.0001).

Conclusions

Black peat substrate produced more yield and a higher content of N and NO_3 in the shoots than the mix of forest residues, composted grape husks, and white peat or the coir substrate. SPAD meter readings provided a good indication of the amount of N in the plants.

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