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# **Growing spinach in a floating system with different volumes of aerated or non aerated nutrient solution**

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*Key words*: hydroponics, hypoxia, leafy vegetables, nitrate accumulation, oxygen, *Spinacia oleracea* L.

**Abstract: Vegetables grown in a floating system may encounter problems of hypoxia at root level, especially in the summer when temperature is high. Depending on the species, oxygen deficiency may cause a lower yield due to a reduction in water and mineral uptake by the plants. On the other hand, plants under oxygen stress may reduce nitrate accumulation, thus ameliorating produce quality. In the present work spinach was grown in summer and autumn in a floating system in different volumes (252, 126 and 60 l per m2 of cultivated area) of aerated or non aerated nutrient solution. Aeration kept oxygen concentration at 7-8 mg l-1 while in the non aerated** solution oxygen decreased gradually reaching at harvest, on average, values of  $1.92$  mg  $1<sup>1</sup>$  and  $2.83$  mg  $1<sup>1</sup>$  in sum**mer and autumn respectively. Such levels of hypoxia did not affect yield and did not reduce nitrate accumulation either. On the contrary, in the summer cycle leaf nitrate content was significantly lower when the nutrient solution was aerated. Reduction of the volume of the solution to 60 l m-2 of cultivated area induced a decrease in nitrate accumulation without negative effect on yield. No significant aeration x volume interaction was observed.**

#### **1. Introduction**

A floating system is a soilless cultivation technique where plants are grown on alveolate or fissured polystyrene panels floating in tanks containing the nutrient solution (Lazzarin *et al.*, 2001; Tesi, 2002). The system is particularly suitable for growing small-size, shortcycle species like leafy vegetables, especially if aimed at producing ready-to-use salads (Nicola *et al.*, 2007). As in other hydroponic systems, plants grown in a floating system may encounter problems of oxygen deficiency (hypoxia) at root level, as roots themselves gradually consume the oxygen dissolved in the nutrient solution. Oxygen deficiency may cause a reduction in water and mineral uptake by the plants, with consequences on the development of their aerial part and therefore on yield (Morard and Silvestre, 1996). The problem is surely more urgent in the summer, since with higher temperatures the quantity of oxygen dissolved in a solution decreases and root respiration rate increases (Boisseau *et al.*, 1988). In a floating system the stillness of the solution, that may favour the occurrence of hypoxia, is compensated for by the large volumes normally adopted  $(150-250)$  l per m<sup>2</sup> of cultivated area) and the shortness of the growing cycles. In any case, in order to avoid any risk, growers aerate the nutrient solution to enrich it with oxygen, thus incurring additional costs. On the other hand, plants under oxygen stress are known to increase the activity of nitrate reductase (Garcia-Novo and Crawford, 1973; Lambers *et al.*, 1978; Veen, 1988), the key enzyme for nitrate utilization by plants (Campbell, 1988). Therefore, the reduction of oxygen concentration in the nutrient solution could actually control nitrate accumulation in hydroponically-grown vegetables, as suggested by Ferrante *et al.* (2003). In the present work oxygen trend during summer and autumn cultivation of spinach in a floating system equipped or not with a device to aerate the nutrient solution was studied and the possible repercussions on spinach yield and quality (namely nitrate content) were investigated. The aim of the work was to verify if, when the nutrient solution was not aerated (thus achieving a cost reduction), oxygen level was kept high enough to ensure spinach yield but at the same time decreased enough to control its nitrate accumulation. Furthermore, different volumes of solution were tested in order to verify if a cost saving may be achieved in the floating system by reducing water and nutrient consumption without negative effects on yield and produce quality, at least in spinach, and if such eventuality may be limited by oxygen concentration when the nutrient solution is not aerated.

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### **2. Materials and Methods**

### *Growing conditions*

Spinach (*Spinacia oleracea* L.) cv. Seven R was cultivated in a floating system in a glasshouse in Sesto Fiorentino (FI, Italy). Two cultivation cycles were carried out, one in the summer (sowing on 1 July 2008) and the other in autumn (sowing on 30 September 2008). Plants were grown in polystyrene 32.5x51.4x5 cm 160-alveoli trays (three seeds per alveolus). Trays floated in polypropylene 36x56x29 cm tanks, lined with a black (inside)/white (outside) polyethylene sheet, on 42, 21 or 10 l of continuously aerated or non aerated nutrient solution (252, 126 or 60 l of nutrient solution per  $m<sup>2</sup>$  of cultivated area, respectively). The bottom of the tanks containing 10 and 21 l of nutrient solution was raised in order to bring the cultivated panels at the same height as panels floating on  $252 \text{ m}^{-2}$  of nutrient solution. Aeration was provided by aquarium aerators (air flux: about  $2 \cdot 1$  hr<sup>-1</sup> per 1 of nutrient solution). The nutrient solution was composed as follows (macronutrients in mmol  $1<sup>-1</sup>$ , micronutrients in µmol  $1<sup>-1</sup>$ <sup>1</sup>): 12 N-NO<sub>3</sub>, 3.8 N-NH<sub>4</sub>, 2.8 P, 8.4 K, 3.5 Ca, 1.4 Mg, 40 Fe, 10 Mn, 40 B, 5 Zn, 1 Cu, 1 Mo.

During the cultivation cycles the nutrient solution consumed by the plants was restored once (after 10 days and 14 days of cultivation in the floating system for the summer and autumn cycle, respectively) by adding tap water. Tap water was composed as follows (macronutrients in mmol  $l^{-1}$ , micronutrients in µmol  $l^{-1}$ <sup>1</sup>): 0.08 N-NO<sub>3</sub>, <0.0027 N-NH<sub>4</sub>, traces of P, 0.9 K, 1.8 Ca, 0.57 Mg, 0.14 Fe, <0.04 Mn, 10.17 B, 0.09 Zn, 0.36 Cu, 0 Mo. At harvest, the overall consumption was on average 79.9 l m-2 in summer and 74.0 l m-2 in autumn, without differences due to the imposed treatments (aeration/volume). Plants were harvested on 23 July and 30 October 2008 for the summer and autumn cycle, respectively, when their developmental stage was suitable for ready-to-use salads (about 12 cm height). During the cultivation period, maximum, minimum and mean air temperature were 38.3, 19.9 and 27.5 $\degree$ C, and 34.3, 15.6 and 21.6 $\degree$ C, respectively in the summer and autumn.

#### *Data collection*

During the cultivation period, every two-three days oxygen concentration and temperature of the nutrient solution were measured using a portable dissolved oxygen meter HI 9146 (Hanna Instruments, Padova, Italy). At the same time, pH and electric conductivity (EC) were also checked by the portable pH and conductivity meter HI 991300 (Hanna Instruments, Padova, Italy).

At harvest, yield and produce quality were evaluated by collecting the following data: leaf and root fresh weight (FW, determined by weighing the whole production from each tank), leaf dry weight (DW, by ovendrying one sample of 100 g of fresh leaves per tank at 80°C until constant weight), leaf area (measured on

five plants per tank using the leaf area meter LI-3000 by Li-Cor, Lincoln, NE, USA), leaf colour (estimated on 10 plants per tank using the Chlorophyll Meter SPAD-502, Konica Minolta, Tokyo, Japan) and nitrate content, which was measured spectrophotometrically on two samples per tank using the salicylic-sulphuric acid method (Cataldo *et al.*, 1975).

#### *Statistics*

The treatments were arranged in a two-way randomized block design with three replications (one replication=one tank). Data were subjected to analysis of variance (ANOVA) and means were compared using the SNK test at P=0.05 level of significance.

#### **3. Results and Discussion**

#### *Nutrient solution*

Aeration of the nutrient solution affected pH and oxygen concentration, while it did not show any effect on EC and temperature (Fig. 1 and 2). In the aerated nutrient solution pH increased more than in the non aerated one, although without exceeding well-tolerated values for the species. Aeration kept oxygen concentration at 7-8 mg  $I^{-1}$  while in the non aerated solution oxygen decreased gradually reaching values below 5 mg  $1^{-1}$ after 17 days in summer and after 21 days in autumn,



Fig. 1 - Effect of aeration (left) and volume (l per  $m<sup>2</sup>$  of cultivated area) (right) of the nutrient solution on its  $O<sub>2</sub>$  concentration, temperature, pH and EC during the summer cultivation cycle of spinach. Error bars (±SE) are shown when larger than symbols. Arrows indicate the date when the consumed solution was restored with tap water.



Fig. 2 - Effect of aeration (left) and volume (l per  $m<sup>2</sup>$  of cultivated area) (right) of the nutrient solution on its  $O<sub>2</sub>$  concentration, temperature, pH and EC during the autumn cultivation cycle of spinach. Error bars ( $\pm$ SE) are shown when larger than symbols. Arrows indicate the date when the consumed solution was restored with tap water.

that is five and nine days before harvest, respectively. Therefore, when the nutrient solution was not aerated, plants were subjected to sub-optimal oxygen concentrations only for a short period of their cultural cycle. At harvest oxygen level in non aerated tanks was on average  $1.92 \text{ mg } l^{-1}$  in summer and  $2.83 \text{ mg } l^{-1}$  in autumn.

No significant differences were noticed in temperature and oxygen concentration due to the volumes of the solution, while pH and EC showed variations that were greater when the volume was lower (Fig. 1 and 2). Those variations (a rise in pH and a decrease in EC) were due to the water added to the tanks to restore the solution consumed by the plants (about 8 l per tank in summer and 7 l in autumn), whose effect was inversely proportional to the starting volume of the solution.

#### *Spinach yield and quality*

In summer, temperature conditions unfavourable to germination in the first week after sowing (29.5°C as mean temperature) led to a lower plant density, justifying the lower yield in the summer cycle compared to the autumn one (on average 1.6 kg m<sup>-2</sup> and  $2.\overline{7}$  kg m<sup>-2</sup> of fresh leaves, respectively). On the other hand, in summer the single plants showed a larger leaf area and more developed roots (Table 1 and 2).

Leaf and root production and leaf area did not show any significant differences due to the treatments in both the summer and the autumn cultivation cycles (Table 1 and 2).

Table 1 - Effect of aeration and volume (1 per  $m<sup>2</sup>$  of cultivated area) of the nutrient solution on leaf production (leaf FW and leaf DW), leaf area and root production (root FW) in spinach grown in a floating system in the summer season

Treatments	Leaf FW	- - Leaf DW	Leaf area	Root FW
	$(kg \, m^{-2})$	$(g m^{-2})$	$(cm2 plant-1)$	$(g m^{-2})$
Aeration	1.6 a	85.9 a	55.3 a	162.7a
No aeration	1.7a	91.6 a	53.7 a	138.0 a
Volume 1252	1.5a	94.5a	50.5 a	134.0a
Volume 1 126	1.8 a	91.7 a	59.5 a	169.0 a
Volume 160	1.6 a	80.0a	53.6 a	148.0 a
Interaction aeration x volume	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>

For each factor, values in each column followed by the same lower-case letter are not statistically different (SNK Test, P≤0.05).  $NS = not significant.$ 

Table 2 - Effect of aeration and volume (1 per  $m^2$  of cultivated area) of the nutrient solution on leaf production (leaf FW and leaf DW), leaf area and root production (root FW) in spinach grown in a floating system in the autumn season

Treatments	Leaf FW	ັ Leaf DW	Leaf area	Root FW
	$(kg \, m^{-2})$	$(g m^{-2})$	$(cm2 plant-1)$	$(g m^{-2})$
Aeration	2.5a	112.0a	53.2 a	93.3 a
No aeration	2.8a	127.7 a	45.2 a	84.0 a
Volume 1252	2.5a	109.7a	47.1a	85.0 a
Volume 1 126	2.9a	130.2 a	50.9 a	93.0 a
Volume 160	2.6a	119.7 a	49.6 a	88.0 a
Interaction aeration x volume	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>

For each factor, values in each column followed by the same lower-case letter are not statistically different (SNK Test, P≤0.05). NS = not significant.

Spinach tolerance to hypoxia in a floating system was previously observed in spring (Lenzi *et al.*, 2008; Baldi *et al.*, 2009), in summer (Lenzi *et al.*, 2008) and in autumn (Tesi *et al.*, 2003 b); on the contrary, when cultivated in summer, spinach showed a reduction in yield due to oxygen depletion (Tesi *et al.*, 2003 b) but in this case the length of the cycle was 40 days and plants were exposed to hypoxia conditions for almost twice as long compared to the present work, as well as with respect to the work described in Lenzi *et al.* (2008).

No effect on crop yield of root hypoxia in a floating system was detected in rocket (Ferrante *et al.*, 2003; Lenzi *et al.*, 2008; Baldi *et al.*, 2009) and lamb lettuce (Ferrante *et al.*, 2005). In head lettuce, roots were exposed to oxygen concentration of 2.1 mg  $1^{-1}$  without any significant effect on plant growth (Goto *et al.*, 1996), while oxygen levels close to 0 mg  $1^{-1}$  during the last two weeks of cultivation caused an evident decrease in yield (Tesi *et al.*, 2003 a).

As far as the quality aspect is concerned, the imposed treatments did not cause any differences in the colour of the product (SPAD values), while they showed some effects on nitrate accumulation in the summer cycle (Table 3). In previous experiments cultivation in a floating system without a device to oxygenate the nutrient solution reduced nitrate accumulation in lamb lettuce (Ferrante *et al.*, 2005) and head lettuce (Tesi *et al.*, 2003 a) but did not show any effect in spinach and rocket (Lenzi *et al.*, 2008; Baldi *et al.*, 2009). In the experiment of Ferrante *et al.* (2003) rocket showed a reduction in nitrate content when plants were subjected to anoxia conditions by bubbling nitrogen gas through the nutrient solution one week before harvesting.

In the present experiment spinach nitrate accumulation was even higher when the nutrient solution was not aerated, with statistically significant differences compared to the aerated nutrient solution in the summer season (Table 3).

Probably, to obtain a decreasing effect on nitrate accumulation by the onset of oxygen stress according to the mechanism suggested by Gracia-Novo and Crowford (1973) (nitrate used as an electron acceptor alternative to free oxygen), prolonged conditions of anoxia or severe hypoxia are necessary.

In experiment presented in this work, a decrease in leaf nitrate content was obtained also by reducing the volume of the nutrient solution, with statistically significant differences again in the summer cycle (Table 3). Such decrease, already observed both in spinach and rocket (Baldi *et al.*, 2009), was not correlated to oxygen, as demonstrated by the non-significant interaction aeration x volume and by the fact that oxygen concentration in the solution did not change due to its volume (Fig. 1 and 2). Instead, since the lower volume underwent a stronger diluting effect from the water added to restore plant consumption, the nitrate decrease was probably simply due to a lower availability of nitric ions for plants. A similar result was obtained in spinach and other fresh-cut vegetables grown in a floating system by using low-concentrated nutrient solutions (Alberici *et al.*, 2008; De Pascale *et al.*, 2008).

In autumn, when environmental conditions are more favourable to plant nitrate accumulation (Maynard *et al.*, 1976), nitrate content was on average higher than in the summer. As in the summer, nitrate was lower with aeration and with the lower volume, although the differences with respect to non aerated conditions and higher volumes, respectively, were not statistically significant (Table 3).

## **4. Conclusions**

From the literature it appears that when vegetables are grown in a floating system without devices to enrich the nutrient solution with oxygen, root hypoxia may have or not a negative effect on yield depending on the species, the season and the length of the growing cycle. The season and duration of the cycle result in different hypoxia levels and more or less prolonged exposure to it. In spinach, in the case of short cycles (20-30 days), both in summer and autumn, hypoxia was not severe enough to influence yield even with low volumes of nutrient solution (up to  $60 \text{ l}$  per m<sup>2</sup> of cultivated area).

Extent and duration of oxygen deficiency are prob-

Table  $3$  - Effect of aeration and volume (1 per m<sup>2</sup> of cultivated area) of the nutrient solution on SPAD and leaf nitrate content in spinach grown in a floating system in the summer and autumn seasons

Treatments	Summer cycle		Autumn cycle	
	<b>SPAD</b>	Leaf nitrate $(mg kg-1 FW)$	<b>SPAD</b>	Leaf nitrate $(mg kg-1 FW)$
Aeration	24.0a	3610 b	23.6a	4495 a
No aeration	26.2a	4085a	24.9a	4814 a
Volume 1 252	24.6a	4090a	23.9a	4822 a
Volume 1 126	25.1a	3901 ab	23.7a	4780 a
Volume 160	25.7a	3553 b	25.2a	4361a
Interaction aeration x volume	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>

For each factor, values in each column followed by the same lower-case letter are not statistically different (SNK Test, P≤0.05). NS = not significant.

ably the most important factors influencing also nitrate accumulation. While anoxia or severe hypoxia may reduce vegetable nitrate accumulation (Ferrante *et al.*, 2003; Tesi *et al.*, 2003 a), we found that in the case of short exposure to oxygen depletion up to 2-3 mg  $l^{-1}$  not aerating the nutrient solution may result in an even higher nitrate content in spinach leaves.

Therefore, although the necessity to aerate or not the nutrient solution in a floating system depends on the aim (yield/quality) and the specific situation, we support the use of aeration. Furthermore, reduction of the volume of the nutrient solution per  $m<sup>2</sup>$  of cultivated area, which did not cause any effect on spinach yield and reduced nitrate accumulation up to 60 l, appears an interesting strategy in order to save water and fertilizers and at the same time ameliorate produce quality.

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