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Influence of crop cycle and nitrogen fertilizer form on yield and nitrate content in different species of vegetables

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Abstract: Research was carried out in Latina province (Italy) on rocket, radish and zucchini grown under tunnel. Ten treatments, obtained by the factorial combination of two crop cycles (autumn-winter and winter-spring) and six nitrogen fertilizer forms (organic, organic-mineral, mineral in three modes, control with no nitrogen fertilization) were compared. The effects of these treatments were evaluated in terms of yield and nitrate content in the edible organs. In rocket, no significant difference in yield was detected between the autumn-winter and winter-spring crop cycles, although the former cycle resulted in a higher leaf nitrate content. The organic fertilizer treatment and the N-unfertilized control gave the lowest yields, but the mineral fertilizers caused the highest leaf nitrate accumulation. Radish yield did not vary between the two crop cycles, but the hypocotyl nitrate content was higher in the autumn-winter cycle. The crops fertilized with the two highest mineral supplies produced the highest yields, compared with the organic or organic-mineral treatments. In the autumn-winter crop, the mineral N fertilization resulted in the highest hypocotyl nitrate content, whereas in the winter-spring crop only the highest mineral N dose caused a higher nitrate content compared with the organic fertilizer. The highest zucchini yield was obtained from the winter-spring cycle at the two highest mineral fertilizer supplies. In the autumn-winter crop the highest mineral nitrogen dose resulted in the highest fruit nitrate content, while in winter-spring the two highest supplies caused this effect.

1. Introduction

An adequate supply of nitrogen fertilizers is generally needed to achieve high yield and quality performance of vegetable crops (Hochmuth, 1992), although a positive correlation between nitrogen availability and production has not always been shown (McCall and Willumsen, 1998). The ratio between nitric and ammonium nitrogen in N fertilizers also affects yield: in a study on zucchini, Chance *et al*. (1999) reported that a ratio between 1:0 and 1:3 is more effective than 3:1 in terms of production. On the other hand, an excessive supply of N fertilizer may have negative effects both on the quality of vegetables and on the environment (Beretta *et al*., 1990).

Excessive nitrogen fertilization may also result in nitrate accumulation, especially in leafy vegetables and a high food nitrate content is considered to be potentially dangerous to human health. This occurs because 5-10% of the ingested nitrate is reduced by bacterial enzymes in saliva and the gastrointestinal tract into the more toxic nitrite ion (Walters and Smith, 1981) which can, in turn, react with amines and amides giving rise to carcinogenic N-nitrose compounds (Hill, 1999). A positive correlation between drinking water nitrate levels and diabetes mellitus incidence in northern England was also reported (Parslow *et al*., 1997). However, some beneficial effects of nitrate on human health were also recognized (Duncan *et al*., 1997; Addiscott and Benjamin, 2000), while the high content in antioxidant compounds of some vegetables, like wild rocket (Martinez-Sanchez *et al*., 2008), can inhibit the formation of carcinogenic compounds (Steinmetz and Potter, 1991). In this respect, a contrasting report was published by Vermeer *et al*. (1998) who found that despite the low

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nitrate content of cauliflower, peas, beans and carrots, the presence of vitamin C and other antioxidants in the same vegetables could not prevent nitrosamine formation. Therefore, the nitrate content alone of vegetable foods cannot be considered as a quality indicator.

Genetic, environmental and cultural factors affect nitrate uptake and accumulation in plants (Bonasia *et al*., 2002). Nitrate in the soil is readily taken up by the plant where it is reduced to nitrite and ultimately converted into organic compounds by the nitrate-reductase enzyme complex, whose presence in the leaves increases in response to light and to nitrate availability in the soil (Guerrero *et al*., 1981). When the rate of nitrate uptake is faster than its assimilation rate, it is mainly accumulated in the plant cell vacuoles where is not toxic, differently from the ammonium ion (Crawford and Glass, 1998).

Nitrate accumulation rate depends on the vegetable species and on the plant organs, the highest concentrations being found in leafy/petiole species while lower nitrate contents are found in root/hypocotyl (Meah *et al*., 1994, Santamaria, 1999 b) or in fruit crops (Quince and Dvorak, 1980). The nitrate content also varies among cultivars within the same species: large differences were found in spinach (Cantliffe, 1972), celeriac (Delorez and Vulsteke, 1985), lettuce (Reinink *et al*., 1987), endive (Reinink *et al*., 1994) and carrot (Gutezeil and Fink, 1999).

In rocket, the nitrate content was found to increase under low solar radiation, as did the flavonoid content and antioxidant activity (Jin *et al*., 2009). However, when this crop is grown under high light intensity, more rapid plant growth requires high organic nitrogen availability, which consequently prevents nitrate accumulation. In fact, according to Padgett and Leonard (1993), organic nitrogen compounds can replace nitrate as osmolyte and adjust the plant nitrate absorption. Temperature also affects the uptake, translocation and assimilation of nitrate: nutrient solution heating stimulates nitrate absorption (Malorgio *et al*., 1995) but excessive air temperature favours its accumulation in the plant tissues (Behr and Wiebe, 1992).

As regards fertilization, ammonium and nitrate ions are the main nitrogen sources for plants. Even though nitrate assimilation is energetically rather expensive, it is usually the plant-preferred form (Salsac *et al*., 1987). In fact, a rapid ammonium uptake rate, exceeding its assimilation rate, would result in ammonium accumulation and toxicity (Maynard and Barker, 1969), while nitrate accumulation in plant tissues does not cause negative consequences. However, the preference between the two nitrogen forms depends on several factors: plant species, plant age, cultural method and the ratios between the concentrations of nitrogen/ammonium and other nutrients in the growth medium. In fact, while celery and fennel prove indifferent to the inorganic nitrogen form, chard is inhibited by ammonium nutrition (Santamaria *et al*., 1999 b). In lettuce, nitrate content can be reduced by distributing a part of the whole nitrogen supply as ammonia form, without changing the overall yield results (van der Boon *et al*., 1990) while in endive the exclusively ammonia form allows for no nitrate heads (Elia and Santamaria, 1997). Moreover, Rouphael and Colla (2005) reported that a greater nitrate content in zucchini was found in soil-grown plants compared to a soilless cultural system. In the latter context, it was found that interrupting the nitrogen supply at an advanced stage of the crop cycle, the fruit nitrate content was significantly reduced (Santamaria *et al*., 1998). This reduction appears to be related to the plant's ability to use the nitrate previously accumulated in vacuoles for protein synthesis (Blom-Zandstra and Lampe, 1983), in order to ensure growth when the substrate resources decrease (Koch *et al*., 1988). Finally, nitrapirine, a nitrification inhibitor, did not improve productive results in radish (Mills *et al*., 1976).

The present study was carried out to evaluate the effects of different nitrogen fertilization forms on yield and nitrate content of wild rocket, radish and zucchini in the Pontina plain (Latina, Italy), grown under tunnel in two cultural cycles, autumn-winter and winter-spring.

2. Materials and Methods

Wild rocket, radish and zucchini were grown on sandy soil (Table 1) in Fondi (Pontina plain, Latina province, Italy) in 2003-2004. The crops were grown under a thermal PE tunnel equipped with anti-freeze irrigation, activated at a temperature of 5°C. The structural unit was 7.20 m wide, 40.00 m long and 2.00 to 3.50 m tall, respectively, from wall to roof. Temperature and solar radiation values of the trial environment are reported in figure 1.

The experimental protocol was planned in order to compare 10 treatments, which originated from the factorial combination of two crop cycles (autumn-winter and winter-spring) with six nitrogen fertilizer forms (organic, organic-mineral, mineral in three modes, control not fertilized with nitrogen). For the treatment distribution within each crop cycle, a randomized block

Table 1 - Soil characteristics Fondi (Latina, Italy), 2003-2004

Soil characteristics		
Sand	%	86.00
Silt	%	4.00
Clay	%	10.00
Organic matter	%	2.06
Total nitrogen - Kjeldhal method	%	0.14
Available phosphorus - Olsen method	ppm	69.00
Available potassium - ammonium acetate method	ppm	245.00
Total lime	%	not detected
pH		6.40
Electrical conductivity (1:5) at 25°C	dS·m-1	0.74

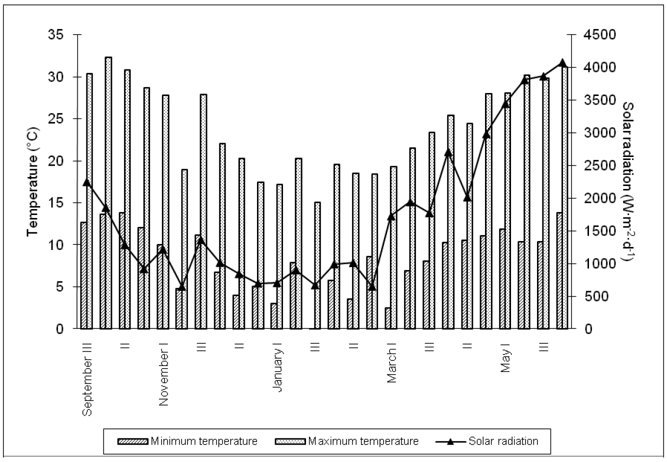

Fig. 1 - Trend of temperature and solar radiation. Fondi (Latina), 2003-2004.

design with three replicates was followed.

For each crop, the local standard agricultural practices were respected: plowing, hoeing, ridging, pre-planting chemical weed control, drip irrigation and parasite control. The fertilization plans were scheduled according to the average nutrient requirements of the crops (Tesi and Lenzi, 2005) and to the expected production levels.

Crops were harvested at midday in all of the plots and random representative samples of the edible plant parts were collected. The samples were transferred to the laboratory, where nitrogen content was determined by high-performance liquid chromatography (HPLC) using a Waters 600E chromatographic system, managed through the Millennium software version 3.05.01. The analytical column was a Dionex anion exchange column (model AS11, 4×250 mm, code P/N 044076) with a Dionex pre-column (4×50 mm, code P/N 044078). The eluent was 21 mmol l^{-1} NaOH at a flow rate of 1 ml min^{-1}. The detector was a Dionex pulsed electrochemical detector together with an anion self-regenerating suppressor.

Statistical data processing was performed by ANOVA, using the Duncan test for mean separation.

Wild rocket (Diplotaxis tenuifolia (L.) D.C.)

Pre-planting fertilization was applied with 50 kg·ha^{-1} of P_2O_5 from mineral superphosphate 18/20 and 150 of K_2O from potassium sulphate 48/50. With the exception of the N-unfertilized control, in all plots 90 kg·ha^{-1} of N were supplied as follows: organic N fertilization was applied at pre-planting as roasted leather; mixed organic-mineral N fertilization was supplied with 45 kg·ha^{-1} at planting from roasted leather and 45 kg·ha^{-1} at dressing from ammonium nitrate 26/27; mineral N fertilization was supplied as 25 kg·ha^{-1} at planting from ammonium sulphate 20/21 plus 65 kg·ha^{-1} from ammonium nitrate during the crop cycle. Organic-mineral N fertilization was applied in two phases, at six and three weeks before the expected second harvest, while the three mineral N treatments differed with respect to the application time at one, two or three weeks before the expected second harvest date.

Alveoli containing 10 plants each were transplanted on 25 September 2003 and on 15 January 2004 in the first and the second crop cycle, respectively. In plots of 12.60 m^2 (3.00 x 4.20 m) three ridges were made 0.40 m apart from each other, which included six rows with spacing 0.20 m, with a density of 215 plants per m^2.

Rocket was harvested in two phases, between 13 November and 9 January (2003-2004) in the first cycle and from 14 March to 28 April 2004 in the second cycle, according to the fertilization treatment. This crop was hand harvested by cutting the plants at about 2 cm above the soil surface when they reached 18 cm in height; crop weight was measured. At the second harvest, a random sample of 100 plants was collected in each plot and transferred to the laboratory for nitrate determination.

Radish (Raphanus sativus L. Subsp. parvus)

Radish cv. Suprella, a commonly grown variety in the experimental area, was sown on 24 November 2003 in the first crop cycle and on 2 March 2004 in the second. The plots had a 5.20 m^2 (2.60 x 2.00 m) surface area including 0.80 m wide beds, with 0.10 x 0.05 m spacing between plants.

Pre-planting fertilization was applied with 80 kg·ha^{-1} of P_2O_5 from mineral superphosphate 18/20 and 100 kg·ha^{-1} of K_2O from potassium sulphate 48/50. In addition, 100 kg·ha^{-1} N, corresponding to the crop requirement, was supplied to all plots with the exception of the N-unfertilized control: organic N fertilization was applied at pre-planting as roasted leather; mixed organic-mineral N fertilization was applied in two phases as 50 kg·ha^{-1} N as roasted leather at pre-planting plus 50 kg·ha^{-1} N as ammonium nitrate at 20 days after sowing. The mineral N fertilization treatments supplied 70, 100 or 130 kg·ha^{-1} N: one-half of the total N supply was given at pre-planting as ammonium sulphate while the remaining 50% was given as ammonium nitrate in two applications, at 12 and 24 days before the expected harvest date. Therefore, the range of mineral N fertilization treatments supplied the intermediate amount (100 kg·ha^{-1}) +/- a 30% increase or reduction.

Radish crops were harvested 16-22 January and 6-11 April in 2004, depending on the crop cycle and fertilization treatment. Radish plants were manually harvested when the hypocotyls reached the 30-40 mm caliber: weight, number and average weight data were recorded. Moreover, a sample of 100 units was collected in each plot and transferred to the laboratory for nitrate assessment.

Zucchini (Cucurbita pepo L.)

Zucchini cv. Velvia, a commonly grown variety in the experimental area, was used for this study. The plants were transplanted on 23 September 2003 in the first cultural cycle and on 20 January 2004 in the second. Each plot was 35.10 m^2 (5.40 x 6.50 m) and plant density was 1.71 plants per m^2 (1.80 m between double rows and 0.65 m between plants along the row).

The fertilization doses were adjusted to the expected yields, which changed with the crop cycle: 60 $kg{\cdot}ha^{-1}$ of P_2O_5 from mineral superphosphate 18/20 and 240 of K_2O from potassium sulphate 48/50 were supplied at planting for the autumn-winter cycle; while 120 $kg{\cdot}ha^{-1}$ of P_2O_5 and 480 of K_2O were supplied at planting for the winter-spring cycle. In addition, nitrogen fertilization supplied 150 or 300 $kg{\cdot}ha^{-1}$ N for the autumn-winter or the winter-spring cycle, respectively. N fertilization was supplied as follows: organic N fertilization was applied at pre-planting as roasted leather; organic-mineral N fertilization was applied in two phases (50% at planting as roasted leather and 50% at dressing as ammonium nitrate). Mineral N fertilization treatments supplied 105, 150 and 195 $kg{\cdot}ha^{-1}$ or 210, 300 and 390 $kg{\cdot}ha^{-1}$ to the autumn-winter or winter-spring crop, respectively. One-third of the total N dose was applied at planting (35, 50, 65 $kg{\cdot}ha^{-1}$ as ammonium sulphate in the autumn-winter cycle or 70, 100, 130 $kg{\cdot}ha^{-1}$ in the winter-spring crop) while the remaining two-thirds of the total N dose was applied during the crop cycle (70, 100, 130 or 140, 200, 260 $kg{\cdot}ha^{-1}$ as ammonium nitrate). Therefore, the range of mineral N fertilization treatments supplied an intermediate rate (150 or 300 $kg{\cdot}ha^{-1}$) +/- a 30% increase or reduction. The N fertilization at dressing was evenly distributed in three applications with a 21-day interval.

Fruits were hand harvested when the corolla was open, from 23 October to 22 December 2003, and from 29 March to 27 May 2004, depending on the crop cycle and fertilization treatment. Fruit weight, fruit number and average fruit weight were recorded. In addition, 10 days after the last fertilizer application a sample of 20 marketable fruits was collected from each plot and transferred to the laboratory for nitrate assessment.

3. Results

No significant difference in rocket yield was found between the autumn-winter and winter-spring crop cycles (data not shown). In the first cycle, however, the leaf nitrate content was as much as 64.3% higher than in the winter-spring.

Table 2 shows that in the autumn-winter cycle there was no yield difference between the mineral and mixed organic-mineral N fertilization forms. However, the mineral and organic-mineral treatments produced better results compared with the organic N fertilization and with the N-unfertilized control. In rocket, mineral N fertilization resulted in the highest leaf nitrate content compared with the other treatments, while the N-unfertilized control resulted in the lowest.

In the winter-spring cycle (Table 3), the production trend was similar to that observed in the autumn-winter cycle. However, organic-mineral fertilization did not produce significantly different results from the organic N fertilization treatment. The mineral and organic-min-

Table 2 - Wild rocket under tunnel: yield results and leaf nitrate content in the autumn-winter cycle, as influenced by nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable yield ($t{\cdot}ha^{-1}$)	Leaf nitrate content ($mg{\cdot}kg^{-1}$ of fresh weight)
Nitrogen fertilizer form		
Non-fertilized control	10.3 c	2706.7 c
Organic	13.9 b	3866.7 b
Organic-mineral	15.4 a	4240.3 b
Mineral 1: one week before the second harvest	16.0 a	5032.0 a
Mineral 2: two weeks before the second harvest	16.3 a	5150.0 a
Mineral 3: three weeks before the second harvest	16.4 a	5317.7 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

Table 3 - Wild rocket under tunnel: yield results and leaf nitrate content in the winter-spring cycle as influenced by nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable yield ($t{\cdot}ha^{-1}$)	Leaf nitrate content ($mg{\cdot}kg^{-1}$ of fresh weight)
Nitrogen fertilizer form		
Non-fertilized control	11.0 c	1626.0 c
Organic	15.1 b	2322.8 b
Organic-mineral	16.8 ab	2838.3 a
Mineral 1: one week before the second harvest	17.3 a	2842.2 a
Mineral 2: two weeks before the second harvest	17.4 a	3083.3 a
Mineral 3: three weeks before the second harvest	17.6 a	3300.0 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

eral N fertilization treatments resulted in higher leaf nitrate content compared with the organic N fertilizer application or with the N-unfertilized control.

Radish yield did not vary between the two crop cycles: the hypocotyl number per unit surface area and their average weight were unaffected by the crop cycle factor (Table 4). In contrast, N fertilizer form significantly affected the edible organ production: mineral fertilization at the two highest doses was more effective than the organic and the organic-mineral N fertilizer forms. The organic N supply had better effects on yield, compared to N-unfertilized control, but it was less effective than the other treatments. These results were mainly affected by the average hypocotyl weight, which changed significantly in response to the different N fertilization forms while only the unfertilized control resulted in a reduced hypocotyl number.

Table 4 - Radish under tunnel: hypocotyl yield as a function of crop cycle and nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable hypocotyl yield Weight ($t\cdot ha^{-1}$)	No. per m^2	Mean weight (g)
Crop cycle			
Autumn-winter	26.9	139.9	19.2
Winter-spring	27.4	137.0	20.0
	NS	NS	NS
Nitrogen fertilizer form			
Non-fertilized control	20.8 D	128.2 B	16.2 E
Organic	25.4 C	138.0 A	18.4 D
Organic-mineral	27.9 B	140.0 A	20.0 C
Mineral 1: 70 $kg\cdot ha^{-1}$	28.5 AB	140.2 A	20.4 BC
Mineral 2: 100 $kg\cdot ha^{-1}$	30.0 A	142.2 A	21.1 AB
Mineral 3: 130 $kg\cdot ha^{-1}$	30.5 A	142.3 A	21.5 A

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

Table 5 - Radish under tunnel: hypocotyl nitrate content as a function of crop cycle and nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Nitrate content ($mg\cdot kg^{-1}$ of fresh weight) Autumn-winter	Winter-spring
Nitrogen fertilizer form		
Non-fertilized control	1020.3 d	478.3 c
Organic	1229.7 c	1038.3 b
Organic-mineral	1431.7 b	1258.3 ab
Mineral 1: 70 $kg\cdot ha^{-1}$	1670.0 a	1311.7 ab
Mineral 2: 100 $kg\cdot ha^{-1}$	1697.0 a	1357.3 ab
Mineral 3: 130 $kg\cdot ha^{-1}$	1743.0 a	1571.7 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

Similarly to rocket, also in radish (Table 5) nitrate content was higher in the autumn-winter cycle, on average as much as 25.3% compared to the winter-spring cycle. In the autumn-winter cycle, leaf nitrate content was higher in response to organic-mineral and mineral N fertilization. Instead, during the winter-spring cycle the highest mineral nitrogen supply caused a nitrate concentration increase only in comparison with the organic form. Moreover, in the winter-spring cycle, the maximum mineral fertilizer dose (130 $kg\cdot ha^{-1}$ equally distributed before and after planting) resulted in a three-times higher leaf nitrate content compared with the unfertilized control, whereas in the autumn-winter cycle a 71% increase was recorded between the same two treatments.

In both crop cycles, the highest zucchini production was obtained with the two highest mineral fertilizer supplies (Tables 6 and 7). However, in the autumn-winter cycle (Table 6) the intermediate dose was not different from the lowest one and the latter was as effective as the organic-mineral treatment in both crop cycles. The control treatment resulted in lower fruit number and average weight while the mineral N fertilization produced the highest values.

The winter-spring yield (Table 6 and 7) was more than double the autumn-winter yield, as a consequence of the increased fruit number (+100%), while their

Table 6 - Zucchini under tunnel: yield results and fruit nitrate content in the autumn-winter cycle as a function of nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable fruit yield Weight ($t\cdot ha^{-1}$)	No. per plant	Mean weight (g)	Fruit nitrate content ($mg\cdot kg^{-1}$ of fresh weight)
Nitrogen fertilizer form				
Non-fertilized control	13.8 e	9.3 c	87.1 c	496.7 e
Organic	19.9 d	12.0 b	97.5 b	638.7 d
Organic-mineral	23.1 c	13.0 ab	103.8 ab	723.7 d
Mineral 1: 105 $kg\cdot ha^{-1}$	23.9 bc	13.3 ab	105.7 ab	850.0 c
Mineral 2: 150 $kg\cdot ha^{-1}$	25.8 ab	14.0 a	107.5 a	1022.0 b
Mineral 3: 195 $kg\cdot ha^{-1}$	26.9 a	14.3 a	110.6 a	1186.0 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

Table 7 - Zucchini under tunnel: yield results and fruit nitrate content in the winter-spring cycle as a function of nitrogen fertilizer form. Fondi (Latina, Italy), 2003-2004

Treatment	Marketable fruit yield Weight ($t \cdot ha^{-1}$)	No. per plant	Mean weight (g)	Fruit nitrate content ($mg \cdot kg^{-1}$ of fresh weight)
Nitrogen fertilizer form				
Non-fertilized control	30.3 d	19.3 c	91.3 d	331.7 c
Organic	39.9 c	23.3 b	100.3 c	426.7 bc
Organic-mineral	47.0 b	25.7 ab	107.6 b	443.0 bc
Mineral 1: 210 $kg \cdot ha^{-1}$	48.3 b	25.7 ab	109.7 ab	536.3 ab
Mineral 2: 300 $kg \cdot ha^{-1}$	54.5 a	28.0 a	113.6 ab	606.0 a
Mineral 3: 390 $kg \cdot ha^{-1}$	55.0 a	28.0 a	115.5 a	678.3 a

Means followed by different letters are significantly different according to the Duncan test at $p \leq 0.05$.

average weight was not significantly different.

In the autumn-winter cycle (Table 6), the maximum mineral N rate and the unfertilized control resulted in the highest and lowest fruit nitrate concentrations (1186 vs 497 $mg \cdot kg^{-1}$), respectively. Furthermore, no difference was detected between the organic and organic-mineral N fertilization treatments. In the winter-spring cycle (Table 7), the mineral N treatments led to a greater fruit nitrate accumulation compared with the control and the organic N fertilization treatment.

4. Discussion and Conclusions

In the present investigation rocket and radish did not show yield differences between the autumn-winter and winter-spring cycles. This is presumably due to the light and temperature requirements of these crops which allow for an equally good production in both seasons. In fact, the two crop periods are quite similar in terms of duration and day-length, though the latter decreases from the beginning to the end of the autumn-winter cycle while the opposite trend occurs in winter-spring. Our results are in accordance with those reported by Inada and Yabumoto (1989) who found that growth of a radish crop was promoted by increasing day-length, but it was less sensitive to variations in temperature regime. Differently from rocket and radish, zucchini yield was affected by the cultural cycle, as the winter-spring cycle resulted in a higher fruit production compared with the autumn-winter cycle. Presumably, the increasing day-length, light intensity and temperature of the second part of the winter-spring cycle played a crucial role in improving crop productivity. Similar results were reported by Rouphael and Colla (2005).

As regards N fertilization, the wild rocket yield was favourably affected by the mineral fertilizer treatment as well as the organic-mineral treatment. A contrasting report was published by Cavarianni *et al*. (2008) who found that an increasing mineral N supply caused the rocket yield to decrease.

Differently from rocket, radish showed a positive response to nitrogen increase, both in organic-mineral and in mineral form. Guven (2002) reported that radish yield benefited from an increase of inorganic nitrogen supply, while Fuke *et al*. (2000) reported a better effect of the mixed organic-mineral fertilization compared with the exclusively mineral treatment. In the present work, the organic N fertilization was less effective on radish production than the other examined treatments, although it produced better results than the unfertilized control. Yield was influenced almost exclusively by the hypocotyl average weight, which gradually decreased from the highest nitrogen rate to the unfertilized control. The number of marketable edible organs was conditioned instead only by the absence of N in the control treatment, which resulted in 9% deformed or undersized hypocotyls. The zucchini yield in both crop cycles was better affected by mineral nitrogen fertilization achieved with the intermediate or 30% increased rate, compared to the organic-mineral or organic fertilization forms. Nevertheless, in the autumn-winter cycle the intermediate mineral supply did not produce better yield results than the 30% reduced treatment. Zotarelli *et al*. (2008) reported that, in the same cultural cycle, a 50% increased N supply, compared to the crop requirement, did not modify zucchini yield but a 50% decrease was less effective. Moreover, the reduced mineral nitrogen application did not result in a better production than the organic-mineral supply while, as recorded also for wild rocket and radish, the organic N treatment accelerated zucchini plant development only compared to the control. However, it should be stressed that the lower yield in the organic N fertilization treatments resulted from a combination of the production factors. In fact, these treatments did not affect the fruit emission rate nor their size. Termine *et al*. (1987) reported that organic N fertilization did not condition the production level of leek and turnip.

In this study the tested species showed a different attitude toward accumulation of nitrate in their edible organs. In particular, rocket was the only crop that in the autumn-winter cycle displayed nitrate levels above the highest EC regulation limits (4500 mg of $NO_3^- \cdot kg^{-1}$ of fresh weight), confirming also the influence of cultivation time on vegetable nitrate content. A clear tendency to accumulate more nitrates in the autumn than in the spring was shown, the latter being characterized by low cloud cover and increasing photoperiod. This is in agreement with the reports of Rouphael and Colla

(2005) on zucchini and Elia *et al*. (1997) on broccoli rab grown in growth chamber and subjected to additional lighting. In the latter case, the leaf blade nitrate reduction was a result of nitrate-decreased absorption rather than its assimilation increase. In fact, in spinach (Steingröver *et al*., 1986 b), parallel to the leaf blade nitrate reduction, the net nitric ion adsorption was reduced while the nitrate-reductase activity did not change except at the end of the night period. This effect would not result from direct light inhibition, but from an adsorption feedback regulation achieved by the amino acids formed in the leaf blades and then transported to the roots. Moreover, it was found that under low solar radiation, typical of the winter season, the nitrate content is high even with a low nitrogen supply, whereas with high solar radiation the ion concentration increases only by supplying nitrogen (Roorda van Eysinga and van der Meijs, 1985; Santamaria *et al*., 1999 a). This indicates that solar radiation intensity and photoperiod length regulate the nitrate-reductase activity, the enzyme which reduces nitrate to nitrous ion (Steingröver *et al*., 1986 a). In particular, the solar radiation effect is multiple, as it gives input to the nitrate-reductase synthesis and induction, supplying also the reducing power (NADH) through photosynthesis (Behr and Wiebe, 1992). It is believed that in the vacuole, nitrate acts as the cell turgor osmotic regulation, as an alternative to sugars and organic acids, poorly synthesized under low radiation conditions (Blom-Zandstra and Lampe, 1985).

Plant nitrate concentration is known to be also subject to the available nitrogen amount and quality (Citak and Sonmez, 2010), and this explains why the unfertilized control plants always exhibited the lowest tissue nitrate levels. Only in the zucchini fruits harvested in spring, values were not different between the control and organic or mixed treatments, confirming this species' lower attitude. On the contrary, wild rocket displayed a remarkable tendency to accumulate nitrates in the aerial apparatus: in the unfertilized plants, a nearly triple (2.9) and more than five-fold (5.2) content was detected, compared with the corresponding control of radish and zucchini, respectively. According to Quinche and Dvorak (1980), the latter accumulate less nitrate because they receive organic nitrogen mainly through the phloem, which does not carry inorganic nitrogen forms. In other studies, rocket was reported to accumulate high nitrate levels both under reduced nitrogen availability (Bianco *et al*., 1998; Santamaria *et al*., l999 a) or increased supply (Santamaria *et al*., 2002).

Organic fertilization, compared to the other supply forms, caused the lowest nitrate content in the edible organs; it was only higher than the unfertilized control (as much as 29% in zucchini and 51% in radish on average). Similar results were reported for lettuce (Stopes *et al*., 1989) and radish (Ebid *et al*., 2008) crops fertilized with composted manure. The organic-mineral treatment resulted in nitrate content increases between 41% in zucchini and 80% in radish compared with unfertilized plants. Therefore, even in such circumstance zucchini demonstrated to be a refractory species to nitrate accumulation. The mineral nitrogen fertilization caused the highest nitrate content in the edible organs: compared to the non-fertilized control, the increase was +90.2, +108.0 and +96.3% on average, respectively in rocket, radish and zucchini. Santamaria *et al*. (1993) reported that mineral nitrogen caused a higher nitrate accumulation than the organic-mineral form in spinach. Nevertheless, in the autumn-winter cycle a 30% reduction of the mineral nitrogen dose resulted in a lower nitrate accumulation, compared to the maximum and to the intermediate supply, respectively in radish and in zucchini. Previously, other researchers found a direct relationship between the nitrogen rate supplied and the vegetable nitrate content (Maynard *et al*., 1976; Tesi *et al*., 1995; Cavarianni *et al*., 2008).

With regard to the time of fertilizer supply, in rocket, in both crop cycles, the latest N dressing application (one week before harvest) did not cause a higher leaf nitrate accumulation than the earliest one. Opposite findings were reported by Graifenberg *et al*. (1990) for lettuce: leaf nitrate content increased with the reduction of the interval between nitrogen fertilizer application and harvest.

In conclusion, the studies carried out in the Pontina plain (Latina, Italy) under tunnel, in 2003-2004, showed that the cultivation time produced a significant effect only on zucchini yield, which was better affected by the winter-spring cycle. Moreover, the autumn-winter crops exhibited higher nitrate content in the edible organs. As for the fertilization treatments, in rocket N fertilization in organic-mineral form was the most appropriate, as it gave yield values as high as the exclusively inorganic supply. In addition, the organic-mineral fertilizer resulted in a lower leaf nitrate accumulation in the autumn-winter cycle, which did not exceed the EC threshold. In contrast, in radish and zucchini the two mineral supplies corresponding to nitrogen crop requirement or to its 30% increase produced the best yield results. Moreover, they also caused the highest radish hypocotyl nitrate content, which was however much lower than the limits set by some European countries for vegetable trade (Santamaria, 2006).

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