

The Impact of Ammonium to Nitrate Ratio on the Growth and Nutritional Status of Kale

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Abstract

The effect of four $\text{NH}_4:\text{NO}_3$ ratios in the nutrient solution (0:100; 25:75; 50:50 and 75:25) on growth and nutrient concentrations of four kale (*Brassica oleraceae* L. var. *acephala*) hybrids: 'CN KAL 1029', 'Redbor', 'Winnetou', 'Reflex' and one indigenous cultivar: 'Ntopia Mytilinis' was investigated. In the first four weeks of cultivation none of the NH_4/NO_3 ratios applied induced adverse effects on most growth characteristics determined whereas plants grown with 75% $\text{NH}_4\text{-N}$ accumulated higher leaf N, P, K, Fe, Mn, Zn and Cu concentrations. After an eight week period, plants under 75% $\text{NH}_4\text{-N}$ showed significant reductions in many growth parameters suggesting a probable preference of kale plants grown for a prolonged period towards either a complete exclusion of NH_4 from the nutrient solution or a 25:75 or a 50:50 NH_4/NO_3 ratio. Among the genotypes tested, 'Ntopia Mytilinis' produced the greatest yield with the lowest leaf nitrate accumulation.

Keywords: *Brassica*; N form; nitrates; $\text{NO}_3:\text{NH}_4$ ratio; nutrient elements; yield

Introduction

Agriculture is changing in ways that closely link food production to human health and nutritional requirements (Emongor *et al.*, 2004; Assimakopoulou 2006). Moreover, plant foods provide most of the nutrients that feed the developing world. Brassicaceous plants represent one of the major vegetable crops grown worldwide, comprising a dietetic nutrient that has long been the object of many investigations. Kale (*Brassica oleraceae* L. var. *acephala*) is the one of the oldest forms of the cabbage family, originating in the Eastern Mediterranean, having been used as a food crop as early as 2000 BC, whereas Theophrastus described a savoyed form of kale in 350 BC (Balkaya and Yanmaz, 2005). In the last few years, kale has spread quickly in Greece once again, showing increasing economic potential because of its short biological cycle, spicy taste and nutritive value, commonly used as a salad for fresh consumption or as soup in different ways. It comprises a whole food as it can provide significant quantities of daily essential minerals and prebiotic carbohydrates. Relevant studies (Ayaz *et al.*, 2006, 2008; Sikora and Bodziarczyk, 2012) contributed to the knowledge of the nutritional properties and pro-healthy potential of the plant, as well as of the antioxidant and antibacterial activities of phenolic fractions isolated from the leaves and seeds (Lefsrud *et al.*, 2007; Kopsell *et al.*,

2013). Lotti *et al.* (2018) studied the diversity of kale in Apulia, Southern Italy, because of its great popularity, especially in the US, as a "superfood" due to its health benefits. Although Thavarajah *et al.* (2016) reported that kale nutritional quality could be further enhanced to benefit North American consumers; Šamec *et al.* (2018) supported the aspect that kale can be considered as a superfood, but the same as another cruciferous.

With regard to plant inorganic nutrition and hence plant growth, unexceptionably nitrogen plays a pivotal role. However, the application of high concentrations of N not only contaminates the environment, but also causes nitrate accumulation in the leaves of vegetable crops reducing their quality. Given that ammonium and nitrate are the two major N sources taken up by the roots of higher plants, the N form may have significant effect on both the growth and chemical composition of plants, including vegetables (Therios and Sakellariadis, 1988; Abu-Rayyan *et al.*, 2004; Wang and Li, 2004). The effect of these two forms on plant growth is dependent not only on the plant species, but also on their ratios and concentrations (Marschner, 1997). Specifically, the form of N supply, to a great extent, controls the uptake ratio of cations and anions and thus, influences dry matter production and root rhizosphere and apoplastic pH (Mengel *et al.*, 1994; Marschner, 1997). However, few vegetables actually perform well when $\text{NH}_4\text{-N}$ is provided as the only N source (Santamaria and Elia, 1997); in such a

case, plants may develop some symptoms of ammonia toxicity (Findenegg, 1987; Guo *et al.*, 2002), suggesting that normal growth needs $\text{NO}_3\text{-N}$ nutrition as well (Smiciklas and Below, 1992a, 1992b; Rideout *et al.*, 1994; Palaniswamy *et al.*, 2002). The appropriate fertilization is one of the most practical and effective ways of controlling and improving the yield and nutritional quality of crops for human consumption. In crops whose commercial yields are the leaves, such as lettuce, spinach, endive, cabbage etc., a great number of studies have been done on the influence of N fertilization (rate and form) on yield, nitrate accumulation and ion composition (Santamaria and Elia, 1997; Wang and Tadashi, 1997; Simonne *et al.*, 2001; Wang and Li, 2004; Gorenjak and Cencič, 2013). Although considerable information is available on the effects of various ratios of $\text{NH}_4\text{:NO}_3$ on growth for different plants, there is limited or no information on kale. Therefore, the objective of our experiment was to assess the impact of $\text{NH}_4\text{:NO}_3$ ratio on kale growth, nutrient element and leaf nitrate accumulation.

Materials and Methods

Plant culture and nutrient determinations

On February 1st, 2016, seeds of the four hybrids 'CN KAL 1029 F1', 'Redbor F1', 'Winnetou F1' and 'Reflex F1' and the indigenous cultivar 'Ntopia Mytilinis' ('Ntopia') of kale (*Brassica oleracea* L. var. *acephala*) were germinated and grown in sand culture for one month by receiving half strength nutrient solution for macronutrients and full strength for micronutrients (Hoagland and Arnon, 1938). Afterwards, the more uniform seedlings were transplanted to individual 4 L plastic pots (one seedling per pot), filled with medium grade silica sand and perlite (1:1 v/v) and placed in a glasshouse without supplementary heating and lighting at the Technological Educational Institute of Peloponnese (longitude 37.062° E; latitude 22.062° N). The pots were arranged in a completely randomized block factorial design (five genotypes x four N ratios) with 6 replicates. Four treatments (Tr0, Tr25, Tr50 and Tr75) were applied to the plants, with the same total N content (10 mmol L^{-1}), but with the following percent molar nitrate nitrogen ($\text{NO}_3\text{-N}$) to ammonium nitrogen ($\text{NH}_4\text{-N}$) ratios in the nutrient solutions: Tr0: 0% $\text{NH}_4\text{-N}$ +100% NO_3 , Tr25: 25% $\text{NH}_4\text{-N}$ +75% $\text{NO}_3\text{-N}$, Tr50: 50% $\text{NH}_4\text{-N}$ +50% $\text{NO}_3\text{-N}$, Tr75: 75% $\text{NH}_4\text{-N}$ +25% $\text{NO}_3\text{-N}$. In the present study, the effect of an all- NH_4 treatment (100% NH_4 ratio+0% NO_3) was not examined, because of expected poor growth or no growth under the specific treatment and the possibility of missing data while analyzing the growth and nutrient status characteristics. The composition of the macronutrients (mM) of the nutrient solutions applied were in the case of Tr0: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 2.0, NaNO_3 2.0, KNO_3 4.0, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.0, K_2HPO_4 1.0; Tr25: KCl 3.0, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 2.0, KNO_3 1.0, NH_4NO_3 2.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.0, K_2HPO_4 1.0; Tr50: KCl 5.0, $(\text{NH}_4)_2\text{SO}_4$ 1.0, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2.0, KNO_3 1.0, $(\text{NH}_4)_3\text{PO}_4 \cdot 3\text{H}_2\text{O}$ 1.0, MgCl_2 1.0; Tr75: KCl 6.0, $(\text{NH}_4)_2\text{SO}_4$ 1.0, NH_4NO_3 2.5, $(\text{NH}_4)_3\text{PO}_4 \cdot 3\text{H}_2\text{O}$ 1.0, CaCl_2 2.0, MgCl_2 1.0. Micronutrients applied in all treatments were the same according to the aforementioned

Hoagland and Arnon (1938) solution.

On March 23rd, the four aforementioned nutrient solutions were applied to the plants. The pH of every nutrient solution was monitored at 1-2 day intervals and maintained within the range 6.0-6.5 by the addition of 0.5 mol L^{-1} HCl or NaOH when needed. The relevant electrical conductivity (EC) of the solutions ranged from 1.8 to 2.2 dS cm^{-1} . Each plant was irrigated three times daily with 0.08 L of the appropriate nutrient solution. The mean temperature from 21-31/3/2016 in the glasshouse recorded was $17.4 \text{ }^\circ\text{C}$, from 1-30/4/2016 $21.1 \text{ }^\circ\text{C}$ and from 1-20/5/2016 $20.7 \text{ }^\circ\text{C}$.

The first 60 plants were harvested four weeks after the beginning of the treatments (harvest 1) when kale leaves had reached the marketable size whereas the other 60 plants were harvested 4 weeks later than the first harvest and eight weeks from the beginning of the treatments (harvest 2). At each harvest, the chlorophyll content of younger fully mature leaves was recorded by using a chlorophyll-meter (SPAD-502, Tokyo, Minolta, Japan). Every plant was separated into the upper plant part (stem and leaves) and the root. The root was washed carefully, three times with deionised water and the fresh weight (FW) of the stem, leaves and root, the stem length as well as the number of the leaves per plant were recorded. Then, the aforementioned plant material was dried to constant weight in a forced draught air oven at $80 \text{ }^\circ\text{C}$ and the relevant dry weights (DW) were recorded as well. Then, the dry plant material was either wet-ashed (Kjeldahl method) or dry-ashed in a furnace at $500 \text{ }^\circ\text{C}$. The concentrations of N were determined by using the indophenol-blue method in the wet digest, P by the molybdenum-blue method and K, Ca, Mg, Fe, Mn, Zn, Cu by using a Varian A220 atomic absorption spectrometer, in the dry digest (Allen, 1989). The nitrate content of the leaves was determined by the colorimetric determination of nitrate in plant tissues by nitration of salicylic acid (Cataldo *et al.*, 1975).

Statistics

Data were subjected to analysis of variance (ANOVA). Where a significant F-test was observed, significant differences in mean values between treatments were evaluated by the ANOVA, Least Significant Difference (LSD) test at $P < 0.05$.

Results

Ammonium toxicity symptoms

Although plants, especially under the treatments Tr50 and Tr75, were grown with a high quantity of $\text{NH}_4\text{-N}$ in the nutrient solution, no visual symptoms of ammonium toxicity were observed in the aboveground plant part till the end of the experiment. However, a number of lateral roots of plants grown under Tr75 were brown and dead at the end of the experiment. Contrary to our results, Simonne *et al.* (1993) reported symptoms of NH_4 toxicity (reduced growth and curly leaves with dark-green areas surrounding yellow spots) in the leaves of turnip plants (*Brassica rapa* L.) grown in sand culture when NH_4 was the dominant nitrogen form.

Plant growth

Data concerning the main effects of cultivar/hybrid and N form and their interaction on several plant growth parameters are shown in the Tables 1 and 2 (1st and 2nd harvest respectively) whereas some leaf and stem characteristics are shown in Table 3 (harvests 1, 2).

At the 1st harvest, the main effects of the nitrogen form and ratio in the nutrient solution on several plant growth parameters like plant leaves, upper plant part and total plant FWs, were their non-significant differentiation despite the high presence of NH₄-N in the nutrient solution with 75% NH₄-N (Table 1). At the 2nd harvest, two months from the beginning of the treatments and one month after the 1st harvest, plants under Tr25 and Tr50 compared to plants under Tr0 did not present any significant differentiation of the aforementioned growth parameters whereas plants grown with 75% NH₄-N did. The latter ones had significantly (sign.) decreased leaves, stem, upper plant part and total plant FWs compared to plants grown under the other three ammonium to nitrate ratios (Table 2). However, at both harvests, by increasing the NH₄-N concentration in the nutrient solution, the root FW presented the trend to increase; i.e. at harvest 1, plants under Tr75 presented significantly greater root FW compared to plants under Tr0 (Tables 1, 2). The RT/SHT ratios increased by increasing the NH₄-N concentration in the nutrient solution, as well; at harvest 1, plants under Tr50 showed significantly higher RT/SHT ratio compared to plants under Tr0 whereas at harvest 2, plants under Tr75 showed significantly higher RT/SHT ratio compared to the other three treatments. With regard to the upper plant part, root and total plant DWs, there were no significant differences among plants grown under Tr0, Tr25 and Tr50, at both harvests; only plants grown with 75% NH₄-N showed the aforementioned parameters significantly decreased. The same trend was observed in the case of leaves DW (Table 2). Leaf water content, at harvest 1, was found to be significantly higher in plants under Tr75 compared to that of Tr0, Tr25 and Tr50 but not significantly differentiated among the four treatments at harvest 2. The plant leaf number and the leaf chlorophyll content (as expressed in SPAD units) did not differ among the four treatments, at both harvests (Table 3).

Contrary to the results of the main effects of nitrogen form on plant growth parameters determined, the main effect of the cultivar/hybrid showed significant differentiations in most cases. The results of the plant leaf biomass, which comprises the edible plant part, showed that the indigenous cultivar 'Ntopia' presented the significantly highest one, the hybrid 'CN KAL 1029' intermediate values (31.1% lower than 'Ntopia') whereas the hybrid 'Redbor' the lowest ones (41.9% lower than 'Ntopia'). 'Ntopia' presented the highest upper plant part and whole plant FW whereas the hybrid 'Redbor' the lowest ones (Table 1). Similar trends were observed at harvest 2 (Table 2). Although the root FW of 'Ntopia' followed by 'Winnetou' was significantly greater compared to the root of the other hybrids at harvest 1, the RT/SHT ratio of the cultivar presented lower values, significantly lower compared to the relevant ratio of 'Winnetou' (Table 1). With regard to leaf water content (LWC) at harvest 1, 'Ntopia' showed the significantly highest LWC, 'CN KAL 1029' and 'Redbor'

intermediate, whereas 'Winnetou' and 'Reflex' showed the lowest ones (Table 3). At harvest 2, the root FW, RT/SHT ratio and LWC were not significantly differentiated among the five genotypes tested (Table 2, 3). The main effect of the cultivar/hybrid on plant leaf number was that 'Ntopia' showed the lowest one, 'Reflex', 'CN KAL 1029' and 'Redbor' intermediate and 'Winnetou' the greatest one (Table 3); at this point, it must be reported that the leaves of 'Ntopia' are much wider compared to the four hybrids. The leaf chlorophyll content of 'Ntopia' was found to be the greatest one at both harvests while that of 'Reflex' the lowest one at the end of the experiment (Table 3). The relevant growth parameter results expressed on DW basis were similar to FW trends, at both harvests. Specifically, 'Ntopia' followed by 'Winnetou' and 'Reflex' showed significantly highest total plant, upper plant part and root DWs compared to 'CN KAL 1029' and 'Redbor' (Tables 1, 2). The stem length of 'CN KAL 1029' followed by 'Ntopia' was significantly lower compared to the other three hybrids; similar results were taken in the case of the stem FW (Tables 1, 2, 3). The interaction between hybrid/cultivar and N form was not found to be significant in any growth parameter determined, at any harvest (Tables 1, 2).

Elemental concentration

Data concerning the main effects of cultivar/hybrid and N form and their interaction on leaf and root nutrient element concentrations are shown in Tables 4, 5, 6 and 7 whereas the leaf nitrate content is in Table 8 and Fig. 1.

Leaf Nitrogen-Phosphorus-Potassium-Calcium Magnesium concentrations

Regarding the leaf N concentration at both harvests, the main effect of N form in the nutrient solution was that plants grown either with 0, 25 or 50% NH₄-N in the nutrient solution presented similar N level; however, plants grown with 75% NH₄-N showed the sign. highest N concentration. Leaf P concentration increased gradually by increasing NH₄-N in the nutrient solution at both harvests, whereas leaf K did not vary among plants under Tr0, Tr25 and Tr50, but increased under Tr75. On the contrary, leaf Mg decreased gradually by increasing NH₄-N in the nutrient solution, at both harvests. Leaf Ca increased by increasing NH₄-N in the nutrient solution till 50% but decreased by increasing NH₄-N in the nutrient solution at 75%, at both harvests (Tables 4, 5).

The main effects of the cultivar/hybrid on leaf nutrient concentrations were significant in most cases. At harvest 1, leaf N was higher in 'Reflex' and lower in 'Ntopia' whereas at harvest 2, it remained higher in 'Reflex' but lowered in 'CN KAL 1029' and 'Winnetou' whereas 'Ntopia' and 'Redbor' presented intermediate N values. Leaf P at harvest 1, was sign. higher in 'CN KAL 1029' and 'Redbor' compared to 'Reflex' and 'Ntopia' whereas 'Winnetou' presented intermediate values; at harvest 2, 'Reflex' presented the highest P. At both harvests, 'CN KAL 1029' presented the highest leaf K, Ca and Mg whereas 'Reflex' and 'Ntopia' presented the lowest ones (Tables 4, 5). The interaction between hybrid/cultivar and N form was found to be significant only in the case of K and Ca concentrations at harvest 1 and in the case of N, P, K, Mg, at harvest 2 (Tables 4, 5).

Table 1. Growth parameters of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1

Harvest 1 Cultivar	Plant leaf FW	Stem FW	Shoot FW	Shoot FW	Root FW	Plant FW (g)	Root/ Shoot FW	Shoot DW	Root DW	Plant DW								
'CN KAL 1029'	343.3	b*	19.4	a	362.7	a	80.4	a	443.2	ab	0.23	ab	35.4	a	7.2	a	42.6	a
'REDBOR'	289.6	a	44.1	bc	333.7	a	78.3	a	412.1	a	0.24	ab	35.1	a	7.5	a	42.6	a
'WINNETOU'	335.4	ab	45.4	c	380.8	a	101.1	bc	482.0	b	0.27	b	43.7	b	11.2	c	55.0	b
'REFLEX'	324.5	ab	40.8	bc	365.2	a	85.8	ab	451.0	ab	0.24	ab	43.3	b	9.3	b	52.6	b
'NTOPIA'	498.4	c	38.0	b	536.4	b	103.9	c	640.3	c	0.20	a	47.7	b	10.7	bc	58.4	b
<i>N form (% NH₄-N)</i>																		
0% NH ₄ -N	363.2	a	40.2	bc	403.4	a	82.2	a	490.8	a	0.22	a	44.1	b	9.1	ab	53.2	b
25% NH ₄ -N	347.6	a	42.7	c	390.3	a	87.4	ab	480.1	a	0.23	ab	42.7	b	9.6	b	52.4	b
50% NH ₄ -N	357.2	a	34.2	ab	391.4	a	89.8	ab	491.7	a	0.27	b	40.9	ab	9.9	b	50.8	b
75% NH ₄ -N	364.9	a	33.1	a	398.0	a	100.3	b	480.2	a	0.21	a	36.5	a	8.0	a	44.5	a
<i>Cultivar x N form (% NH₄-N)</i>																		
'CN KAL' 1029 x 0%	398.5	a	22.9	a	421.3	a	92.0	a	513.3	a	0.22	a	42.3	a	7.7	a	50.0	a
x 25%	313.0	a	20.3	a	333.4	a	76.1	a	409.5	a	0.23	a	35.5	a	7.5	a	43.1	a
x 50%	278.3	a	16.2	a	294.5	a	85.3	a	379.8	a	0.30	a	30.2	a	7.6	a	37.9	a
x 75%	383.4	a	18.3	a	401.7	a	68.3	a	470.1	a	0.17	a	33.7	a	5.9	a	39.6	a
'REDBOR' x 0%	276.2	a	49.4	a	325.6	a	64.7	a	390.3	a	0.20	a	38.3	a	6.9	a	45.2	a
x 25%	255.6	a	46.9	a	302.5	a	73.6	a	376.1	a	0.25	a	32.1	a	7.3	a	39.5	a
x 50%	319.0	a	41.4	a	360.4	a	106.5	a	466.9	a	0.30	a	38.1	a	9.5	a	47.6	a
x 75%	307.5	a	38.9	a	346.4	a	68.6	a	415.0	a	0.20	a	32.0	a	6.1	a	38.1	a
'WINNETOU' x 0%	341.7	a	52.0	a	393.7	a	97.8	a	491.5	a	0.25	a	45.7	a	11.5	a	57.3	a
x 25%	352.2	a	50.8	a	402.9	a	106.6	a	509.5	a	0.27	a	45.5	a	11.5	a	57.0	a
x 50%	318.4	a	38.2	a	356.6	a	98.5	a	455.1	a	0.29	a	43.5	a	11.2	a	54.7	a
x 75%	329.2	a	40.8	a	370.0	a	101.7	a	471.7	a	0.28	a	40.2	a	10.7	a	50.9	a
'REFLEX' x 0%	343.0	a	44.4	a	387.4	a	87.9	a	475.4	a	0.23	a	47.9	a	9.4	a	57.3	a
x 25%	338.4	a	46.4	a	384.8	a	84.1	a	469.0	a	0.22	a	49.5	a	9.6	a	59.1	a
x 50%	334.6	a	41.3	a	375.9	a	94.4	a	470.2	a	0.25	a	42.0	a	10.1	a	52.1	a
x 75%	281.8	a	30.9	a	312.7	a	76.8	a	389.5	a	0.24	a	33.8	a	8.3	a	42.0	a
'NTOPIA' x 0%	456.5	a	32.5	a	488.9	a	94.7	a	583.6	a	0.19	a	46.4	a	9.9	a	56.3	a
x 25%	478.9	a	49.1	a	528.0	a	108.5	a	636.5	a	0.21	a	51.0	a	12.3	a	63.2	a
x 50%	535.7	a	33.8	a	569.5	a	116.9	a	686.5	a	0.21	a	50.6	a	11.3	a	61.9	a
x 75%	522.4	a	36.7	a	559.1	a	95.6	a	654.7	a	0.17	a	42.7	a	9.3	a	52.0	a

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 2. Growth parameters of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 2

Harvest 2 Cultivar	Plant leaf FW	Stem FW	Shoot FW	Shoot FW	Root FW (g)	Plant FW	Root/ Shoot FW	Plant leaf DW	Shoot DW	Root DW	Plant DW	Root/ Shoot DW										
'CN KAL 1029'	992.8	b*	71.6	a	1064.4	ab	116.0	a	1180.4	ab	0.11	a	95.2	ab	104.4	a	16.0	a	120.3	a	8.5	a
'REDBOR'	793.1	a	158.2	c	951.3	a	115.7	a	1067.0	a	0.12	a	86.1	a	108.5	a	16.8	ab	125.3	a	8.8	a
'WINNETOU'	933.2	b	173.3	c	1106.4	b	122.0	a	1228.5	b	0.11	a	100.3	bc	124.8	b	19.2	b	144.0	b	9.0	a
'REFLEX'	986.6	b	167.0	c	1153.6	b	116.9	a	1270.5	b	0.10	a	106.0	cd	125.3	b	18.6	ab	146.9	b	9.0	a
'NTOPIA'	1199.6	c	123.0	b	1322.7	c	129.6	a	1452.3	c	0.10	a	110.2	d	128.2	b	19.3	b	144.6	b	9.8	a
<i>N form (% NH₄-N)</i>																						
0% NH ₄ -N	1026.0	b	161.8	c	1187.8	b	109.5	a	1297.3	b	0.09	a	103.5	b	124.3	b	18.1	ab	142.4	b	8.1	a
25% NH ₄ -N	1010.4	b	148.5	bc	1158.9	b	125.1	a	1284.0	b	0.11	a	110.9	b	130.7	b	19.8	b	150.5	b	8.9	ab
50% NH ₄ -N	1055.1	b	138.6	b	1193.7	b	121.7	a	1315.4	b	0.10	a	105.1	b	124.3	b	17.7	ab	142.0	b	9.4	b
75% NH ₄ -N	801.9	a	111.8	a	913.8	a	121.7	a	1035.5	a	0.13	b	78.7	a	93.6	a	16.4	a	110.0	a	9.6	b
<i>Cultivar x N form (% NH₄-N)</i>																						
'CN KAL 1029' x 0% NH ₄ -N	1074.4	a	88.1	a	1162.5	a	98.2	a	1260.7	a	0.08	a	86.5	a	96.4	a	14.2	a	110.6	a	4.6	a
x 25% NH ₄ -N	1082.1	a	75.5	a	1157.6	a	103.6	a	1261.2	a	0.09	a	115.8	a	125.2	a	15.2	a	140.4	a	9.3	a
x 50% NH ₄ -N	1010.3	a	59.8	a	1070.1	a	125.7	a	1195.8	a	0.12	a	98.4	a	106.9	a	17.1	a	124.1	a	10.0	a
x 75% NH ₄ -N	831.6	a	68.6	a	900.1	a	130.6	a	1030.7	a	0.15	a	80.1	a	88.9	a	17.4	a	106.4	a	10.2	a
'REDBOR' x 0% NH ₄ -N	825.7	a	184.7	a	1010.4	a	92.6	a	1103.0	a	0.09	a	92.7	a	116.8	a	15.2	a	131.9	a	8.7	a
x 25% NH ₄ -N	813.5	a	148.6	a	962.1	a	145.8	a	1107.9	a	0.15	a	92.8	a	115.5	a	20.3	a	135.8	a	8.3	a
x 50% NH ₄ -N	823.1	a	171.3	a	994.4	a	121.9	a	1116.4	a	0.12	a	86.0	a	111.1	a	17.7	a	128.8	a	8.9	a
x 75% NH ₄ -N	709.9	a	128.3	a	838.2	a	102.6	a	940.8	a	0.12	a	72.9	a	90.7	a	14.0	a	104.7	a	9.3	a
'WINNETOU' x 0% NH ₄ -N	1008.4	a	199.0	a	1207.5	a	120.3	a	1327.8	a	0.10	a	109.4	a	138.5	a	20.7	a	159.2	a	8.7	a
x 25% NH ₄ -N	961.8	a	190.4	a	1152.2	a	116.4	a	1268.6	a	0.10	a	106.6	a	133.3	a	21.3	a	154.6	a	8.6	a
x 50% NH ₄ -N	995.6	a	184.7	a	1180.3	a	127.8	a	1308.1	a	0.11	a	102.7	a	127.9	a	17.9	a	145.8	a	9.2	a
x 75% NH ₄ -N	791.9	a	127.6	a	919.5	a	122.9	a	1042.4	a	0.13	a	82.7	a	99.4	a	17.0	a	116.4	a	9.3	a
'REFLEX' x 0% NH ₄ -N	1060.9	a	196.4	a	1257.3	a	102.6	a	1359.9	a	0.08	a	114.7	a	140.8	a	19.0	a	159.8	a	8.9	a
x 25% NH ₄ -N	1032.5	a	174.1	a	1206.6	a	124.1	a	1330.7	a	0.10	a	119.8	a	141.4	a	20.3	a	161.7	a	8.6	a
x 50% NH ₄ -N	1098.0	a	164.9	a	1262.9	a	119.0	a	1381.9	a	0.09	a	112.2	a	135.1	a	17.3	a	154.4	a	9.3	a
x 75% NH ₄ -N	755.0	a	132.4	a	887.5	a	121.9	a	1009.3	a	0.14	a	77.4	a	95.7	a	18.0	a	113.6	a	9.3	a
'NTOPIA' x 0% NH ₄ -N	1170.9	a	128.4	a	1299.4	a	133.8	a	1433.1	a	0.12	a	114.3	a	129.0	a	21.5	a	150.5	a	9.8	a
x 25% NH ₄ -N	1237.9	a	156.7	a	1394.6	a	141.1	a	1535.7	a	0.10	a	119.7	a	138.3	a	21.8	a	160.1	a	10.2	a
x 50% NH ₄ -N	1348.7	a	112.1	a	1460.8	a	114.1	a	1574.9	a	0.08	a	126.3	a	140.5	a	18.5	a	159.0	a	10.4	a
x 75% NH ₄ -N	981.0	a	97.5	a	1078.5	a	135.2	a	1213.6	a	0.13	a	80.6	a	93.6	a	15.4	a	108.9	a	8.8	a

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 3. Leaf characteristics of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvests 1 and 2

Harvests 1,2 Cultivar	Harvest 1 Leaf/Water Content %	Harvest 2 Leaf/Water Content %	Harvest 1 Plant Leaf Number	Harvest 2 Plant Leaf Number	Harvest 1 Chlorophyll Content (SPAD units)	Harvest 2 Chlorophyll content (SPAD units)	Harvest 2 Stem length (cm)							
CN KAL 1029	90.1	b*	89.1	a	7.8	b	30.8	b	46.2	a	53.3	b	16.6	a
REDBOR	89.4	b	89.2	a	7.7	b	32.5	bc	45.9	a	49.3	ab	38.1	b
WINNETOU	88.4	a	89.3	a	8.9	c	38.4	d	45.3	a	51.0	ab	35.4	b
REFLEX	88.2	a	89.3	a	8.1	b	34.9	c	46.6	a	46.8	a	36.5	b
NTOPIA	91.1	c	88.6	a	6.9	a	25.2	a	51.0	b	65.1	c	19.0	a
<i>N form (% NH4)</i>														
0% NH4-N	88.9	a	88.6	a	8.0	a	32.1	a	46.5	a	52.6	a	32.0	b
25% NH4-N	88.9	a	89.0	a	7.9	a	33.1	a	48.3	a	56.5	a	31.7	b
50% NH4-N	89.2	a	90.0	a	8.0	a	33.4	a	46.9	a	51.7	a	27.6	ab
75% NH4-N	90.6	b	88.7	a	7.6	a	30.9	a	46.3	a	51.5	a	27.3	a
<i>Cultivar x N form (% NH4-N)</i>														
CN KAL 1029 x 0% NH4-N	89.9	a	86.7	a	7.7	a	29.7	a	45.0	a	51.2	a	19.0	a
x 25% NH4-N	89.3	a	89.1	a	7.3	a	30.3	a	50.8	a	62.2	a	17.2	a
x 50% NH4-N	89.4	a	90.2	a	8.2	a	31.7	a	42.6	a	50.4	a	12.5	a
x 75% NH4-N	91.6	a	90.4	a	7.8	a	31.7	a	46.3	a	49.5	a	18.7	a
REDBOR x 0% NH4-N	88.3	a	88.8	a	7.3	a	32.3	a	46.1	a	47.0	a	37.3	a
x 25% NH4-N	89.3	a	88.6	a	8.0	a	35.0	a	46.7	a	51.0	a	40.0	a
x 50% NH4-N	89.4	a	89.5	a	7.8	a	29.0	a	47.9	a	52.7	a	38.0	a
x 75% NH4-N	90.7	a	89.8	a	7.5	a	33.7	a	43.0	a	46.3	a	37.2	a
WINNE x 0% NH4-N	88.4	a	89.0	a	9.3	a	39.3	a	44.5	a	54.7	a	39.5	a
x 25% NH4-N	88.7	a	88.9	a	9.0	a	41.3	a	45.7	a	52.0	a	38.0	a
x 50% NH4-N	87.6	a	89.6	a	9.0	a	41.0	a	45.1	a	45.1	a	38.3	a
x 75% NH4-N	89.1	a	89.6	a	8.3	a	32.0	a	45.8	a	52.0	a	27.2	a
REFLEX x 0% NH4-N	87.6	a	89.1	a	8.3	a	36.3	a	46.2	a	43.9	a	40.7	a
x 25% NH4-N	87.1	a	88.4	a	8.2	a	34.3	a	45.8	a	48.0	a	37.8	a
x 50% NH4-N	88.7	a	89.8	a	8.2	a	36.7	a	49.1	a	46.5	a	34.0	a
x 75% NH4-N	89.2	a	89.7	a	7.8	a	32.3	a	45.3	a	48.9	a	33.3	a
NTOPIA x 0% NH4-N	90.5	a	89.7	a	7.3	a	23.0	a	50.5	a	66.3	a	21.8	a
x 25% NH4-N	90.3	a	90.6	a	6.8	a	24.3	a	52.4	a	69.3	a	22.5	a
x 50% NH4-N	91.1	a	90.6	a	6.8	a	28.7	a	49.7	a	64.0	a	15.3	a
x 75% NH4-N	92.3	a	88.3	a	6.7	a	24.7	a	51.3	a	61.0	a	16.5	a

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 4. Leaf nutrient element concentrations of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1

Harvest 1 Cultivar	N	P	K g kg ⁻¹ leaf dw	Ca	Mg	Fe	Mn ppm leaf dw	Zn	Cu	B										
CN KAL 1029	54.6	ab*	6.3	c	57.8	c	15.8	d	4.76	c	49.8	a	39.3	b	28.0	c	4.8	b	48.0	b
REDBOR	56.3	b	6.6	c	50.3	b	12.1	bc	4.19	b	47.3	a	36.6	b	28.3	c	4.6	b	50.5	bc
WINNETOU	55.9	b	5.9	bc	47.9	b	10.8	ab	4.42	bc	43.9	a	34.8	ab	26.3	bc	4.8	b	40.9	a
REFLEX	64.5	c	5.2	ab	38.9	a	10.2	a	3.47	a	48.0	a	29.8	a	24.9	ab	3.6	a	49.5	b
NTOPIA	50.9	a	4.7	a	39.1	a	12.6	c	4.33	b	42.6	a	30.5	a	23.3	a	3.2	a	54.4	d
<i>N form (% NH4)</i>																				
0% NH4-N	55.2	a	3.6	a	44.8	a	9.2	a	4.88	c	41.8	a	26.9	a	22.6	a	3.7	a	49.6	a
25% NH4-N	55.7	a	4.7	b	41.8	a	12.8	b	4.54	b	46.8	b	29.1	ab	23.1	a	4.0	ab	48.2	a
50% NH4-N	54.2	a	5.8	c	44.6	a	15.5	c	4.36	b	47.1	b	32.9	b	22.5	a	4.2	b	49.7	a
75% NH4-N	60.3	b	8.9	d	55.9	b	11.6	b	3.15	a	49.2	b	47.9	c	36.3	b	4.8	c	46.8	a
<i>Cultivar x N form (% NH4-N)</i>																				
CN KAL 1029 x 0%	56.0	a	3.8	a	52.4	ij	11.6	c-f	5.52	a	43.4	a	31.3	bc	25.3	a	4.9	f-i	48.5	d-g
x 25%	53.2	a	5.1	a	52.5	ij	15.9	h	4.97	a	48.6	a	34.7	bc	24.2	a	3.8	b-f	44.9	b-e
x 50%	50.9	a	6.6	a	55.2	jk	19.8	i	4.81	a	48.8	a	37.3	d	22.4	a	4.2	d-g	48.8	d-g
x 75%	58.5	a	9.9	a	71.3	l	15.7	h	3.74	a	58.2	a	53.9	c	39.9	a	5.8	i	49.6	efg
REDBOR x 0%	50.5	a	4.1	a	49.4	g-j	7.8	ab	4.95	a	43.7	a	24.5	ab	23.0	a	3.9	def	51.5	e-h
x 25%	53.8	a	5.2	a	47.5	f-i	13.8	fgh	4.73	a	49.8	a	35.2	d	24.2	a	4.4	d-h	50.0	efg
x 50%	54.9	a	6.6	a	41.6	b-f	15.2	h	4.15	a	54.0	a	36.3	d	25.3	a	4.7	e-h	56.4	gh
x 75%	66.2	a	10.7	a	62.7	k	11.6	c-f	2.92	a	48.4	a	50.3	e	40.6	a	5.4	hi	40.7	a-d
WINNETOU x 0%	57.4	a	4.7	a	46.3	e-i	9.4	a-d	4.99	a	38.1	a	27.8	abc	23.6	a	4.5	d-h	47.7	c-f
x 25%	57.3	a	4.8	a	44.1	d-h	12.0	d-g	5.11	a	46.5	a	27.9	abc	23.9	a	4.6	e-h	35.8	a
x 50%	52.1	a	5.9	a	44.4	d-h	13.0	e-h	4.36	a	46.9	a	32.7	bc	21.6	a	5.1	ghi	39.6	ab
x 75%	56.8	a	8.3	a	56.6	jk	8.8	abc	3.20	a	44.0	a	50.9	e	36.2	a	4.9	f-i	40.3	abc
REFLEX x 0%	66.6	a	2.7	a	40.7	b-f	9.3	a-d	4.33	a	43.9	a	30.0	abc	21.0	a	2.3	a	48.4	def
x 25%	63.0	a	4.7	a	34.1	ab	10.5	b-e	3.68	a	49.7	a	27.4	abc	20.8	a	4.4	d-h	51.1	efg
x 50%	62.3	a	5.6	a	42.4	c-g	13.9	fgh	3.67	a	50.5	a	29.9	abc	22.7	a	3.7	b-e	50.2	efg
x 75%	66.9	a	7.8	a	38.3	a-d	6.9	a	2.21	a	46.7	a	31.8	bc	34.9	a	3.9	c-f	48.4	def
NTOPIA x 0%	45.7	a	2.4	a	35.1	abc	8.0	ab	4.41	a	40.5	a	20.6	a	20.1	a	2.8	ab	51.8	e-h
x 25%	51.4	a	3.9	a	30.8	a	11.8	c-f	4.24	a	40.7	a	20.4	a	22.2	a	2.8	abc	59.1	h
x 50%	51.1	a	4.4	a	39.5	b-e	15.7	h	4.80	a	45.9	a	28.3	abc	20.6	a	3.4	bcd	53.6	fgh
x 75%	55.4	a	8.0	a	50.95	hij	15.0	gh	3.66	a	43.3	a	52.5	e	30.1	a	4.0	def	53.0	fgh

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 5. Leaf nutrient element concentrations of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 2

Harvest 2 Cultivar	N	P	K	Ca g kg ⁻¹ leafdw		Mg	Fe	Mn	Zn ppm leafdw	Cu	B										
CN KAL 1029	465	a	4.0	a	47.8	d	12.9	c	3.8	d	43.7	bc	29.4	a	30.9	ab	3.8	b	38.5	b	
REDBOR	50.7	b	3.9	a	40.8	bc	8.9	b	2.6	b	44.9	c	35.2	ab	29.7	a	3.8	b	38.4	b	
WINNETOU	45.6	a	3.8	a	42.4	c	11.6	c	3.3	c	37.4	a	40.3	b	36.3	b	3.1	a	31.2	a	
REFLEX	61.8	c	8.9	c	36.6	b	9.7	b	2.4	b	39.2	ab	41.4	b	30.7	a	2.7	a	33.0	a	
NTOPIA	52.9	b	8.0	b	25.1	a	6.0	a	1.9	a	44.6	c	41.5	b	27.3	a	2.7	a	35.2	ab	
<i>Nform (% NH4)</i>																					
0% NH4-N	47.7	a	4.71	a	37.79	b	9.3	ab	3.5	c	43.2	a	24.9	a	17.8	a	2.7	a	32.0	a	
25% NH4-N	49.1	a	4.63	a	33.35	a	10.4	b	2.9	b	40.2	a	25.4	a	20.3	a	2.8	a	34.1	ab	
50% NH4-N	50.3	a	5.50	b	37.15	ab	10.6	b	2.3	a	44.0	a	47.9	b	35.1	b	3.1	a	38.6	c	
75% NH4-N	59.1	b	8.14	c	45.81	c	9.0	a	2.5	a	40.3	a	52.1	b	50.7	c	4.2	b	36.6	bc	
<i>Cultivar x Nform (% NH4-N)</i>																					
CN KAL 1029	x 0%	40.0	a	3.02	ab	47.13	fg	10.93	a	4.81	k	46.2	a	25.6	a	18.3	ab	3.3	a	37.3	a
	x 25%	40.0	a	3.56	abc	42.23	def	12.92	a	3.94	j	37.5	a	26.7	abc	23.3	abc	3.8	a	36.5	a
	x 50%	45.8	abc	2.99	ab	45.57	efg	14.76	a	3.02	e-i	41.3	a	52.9	ef	27.8	bcd	3.5	a	36.9	a
	x 75%	60.3	fg	6.24	de	56.31	h	13.10	a	3.27	hi	49.7	a	60.7	fg	54.1	f	4.6	a	42.8	a
REDBOR	x 0%	43.2	ab	3.74	abc	41.16	c-f	7.83	a	2.90	e-i	45.4	a	26.5	ab	19.1	ab	2.8	a	34.7	a
	x 25%	51.6	cde	3.42	abc	40.94	c-f	9.51	a	3.18	ghi	43.3	a	27.2	abc	20.3	abc	3.3	a	36.3	a
	x 50%	53.4	c-f	4.06	abc	41.67	gh	9.93	a	2.47	cde	51.1	a	55.1	ef	31.1	cd	4.1	a	48.0	a
	x 75%	55.6	def	4.49	bc	39.26	c-f	8.17	a	2.02	abc	39.7	a	52.5	def	48.2	ef	4.9	a	34.7	a
WINNETOU	x 0%	40.2	a	3.18	ab	37.60	cde	11.18	a	4.10	j	36.0	a	23.8	a	17.2	ab	2.6	a	26.9	a
	x 25%	46.1	abc	2.69	a	34.10	bcd	12.57	a	3.49	ij	33.8	a	24.8	a	21.1	abc	2.8	a	30.5	a
	x 50%	46.2	abc	4.29	abc	46.05	efg	12.57	a	2.74	d-h	41.7	a	69.9	g	52.4	f	3.1	a	30.1	a
	x 75%	49.9	b-e	5.04	cd	51.70	gh	9.93	a	2.79	e-h	37.9	a	47.3	de	54.6	f	3.9	a	35.8	a
REFLEX	x 0%	63.9	gh	7.35	e	36.01	cd	10.60	a	3.11	fi	40.3	a	26.5	ab	16.4	a	2.1	a	27.6	a
	x 25%	56.3	efg	6.95	e	32.64	bc	12.17	a	2.54	c-f	38.7	a	25.9	a	16.9	ab	2.0	a	34.2	a
	x 50%	58.0	efg	9.14	f	35.46	bcd	9.00	a	1.84	ab	41.6	a	48.8	def	38.4	de	2.8	a	37.0	a
	x 75%	68.9	h	12.25	g	42.35	def	7.16	a	2.12	bcd	36.3	a	39.7	cd	51.1	f	3.7	a	33.3	a
NTOPIA	x 0%	51.2	b-e	6.23	de	27.07	b	5.88	a	2.56	e-g	48.2	a	22.1	a	18.2	ab	2.9	a	32.0	a
	x 25%	51.6	cde	6.54	de	16.83	a	4.85	a	1.59	ab	47.8	a	22.2	a	19.9	ab	2.2	a	32.9	a
	x 50%	47.9	a-d	6.54	de	16.98	a	6.53	a	1.49	a	44.5	a	33.7	abc	25.7	abc	1.9	a	40.2	a
	x 75%	60.9	fgh	12.66	g	39.44	c-f	6.64	a	2.11	a-d	38.0	a	39.5	bcd	45.6	ef	3.8	a	36.2	a

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 6. Root nutrient element concentrations of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1

Harvest 1 Cultivar	P	K	Ca G kg ⁻¹ root dw		Mg	Fe	Mn ppm root dw	Zn	Cu	B									
CN KAL 1029	5.4	a'	6.5	c	12.5	a	2.58	c	264.6	b	35.2	c	42.6	a	15.3	a	21.8	a	
REDBOR	5.4	a	7.7	d	11.8	a	2.23	ab	252.4	ab	33.1	bc	42.4	a	17.0	a	22.4	a	
WINNETOU	5.3	a	4.8	ab	11.2	a	2.06	a	216.9	ab	29.5	abc	35.4	a	14.6	a	24.9	a	
REFLEX	5.8	a	5.3	b	11.2	a	2.42	bc	203.2	a	25.8	ab	40.5	a	16.7	a	28.4	b	
NTOPIA	5.4	a	3.9	a	11.2	a	2.18	ab	205.4	a	25.2	a	34.3	a	14.3	a	28.0	b	
<i>Nform (% NH4)</i>																			
0% NH4-N	4.0	a	4.6	a	14.8	c	2.96	d	224.3	ab	23.9	a	31.0	a	11.7	a	25.3	a	
25% NH4-N	4.9	b	5.1	ab	13.2	bc	2.46	c	260.3	b	25.8	a	34.5	ab	13.5	ab	24.2	a	
50% NH4-N	7.1	d	5.7	b	11.6	b	2.07	b	194.1	a	41.8	b	39.0	b	14.8	b	25.1	a	
75% NH4-N	5.8	c	7.1	c	6.8	a	1.69	a	226.6	ab	27.5	a	51.6	c	22.2	c	26.0	a	
<i>Cultivar x Nform (% NH4-N)</i>																			
CN KAL 1029	x 0%	3.7	a	4.4	a	15.9	a	3.29	a	260.4	a	24.8	a	33.4	a	14.9	a	21.2	a
	x 25%	4.8	a	5.6	a	15.6	a	2.71	a	272.2	a	29.0	a	36.4	a	11.4	a	22.2	a
	x 50%	6.3	a	6.7	a	10.2	a	2.22	a	262.7	a	49.6	a	47.2	a	15.7	a	22.0	a
	x 75%	6.6	a	9.2	a	8.5	a	2.09	a	262.6	a	37.6	a	53.4	a	19.2	a	21.6	a
REDBOR	x 0%	3.4	a	6.7	a	16.1	a	3.03	a	236.1	a	25.3	a	35.5	a	11.8	a	18.0	a
	x 25%	4.6	a	7.4	a	12.4	a	2.40	a	270.7	a	24.5	a	37.6	a	17.8	a	19.3	a
	x 50%	8.2	a	8.0	a	13.5	a	2.06	a	238.9	a	54.5	a	41.6	a	12.9	a	25.8	a
	x 75%	5.3	a	8.7	a	5.1	a	1.45	a	259.4	a	28.1	a	55.0	a	25.7	a	25.2	a
WINNETOU	x 0%	3.8	a	3.6	a	13.0	a	2.52	a	232.0	a	24.5	a	25.7	a	10.0	a	25.8	a
	x 25%	4.6	a	5.0	a	11.9	a	2.01	a	252.7	a	28.7	a	35.1	a	14.1	a	23.5	a
	x 50%	6.8	a	4.9	a	11.1	a	1.95	a	210.4	a	35.8	a	33.5	a	14.7	a	21.6	a
	x 75%	5.9	a	5.6	a	8.6	a	1.75	a	170.5	a	28.8	a	47.2	a	19.4	a	28.8	a
REFLEX	x 0%	4.9	a	4.8	a	15.7	a	3.23	a	223.9	a	23.6	a	32.8	a	11.7	a	26.5	a
	x 25%	6.1	a	4.6	a	13.3	a	2.65	a	224.7	a	26.5	a	36.7	a	13.2	a	27.2	a
	x 50%	7.0	a	5.2	a	10.2	a	2.16	a	179.8	a	33.7	a	42.3	a	17.2	a	29.0	a
	x 75%	5.3	a	6.4	a	5.7	a	1.62	a	191.2	a	19.4	a	50.3	a	24.5	a	31.0	a
NTOPIA	x 0%	4.2	a	3.5	a	13.2	a	2.72	a	169.3	a	21.2	a	27.7	a	10.3	a	32.7	a
	x 25%	4.5	a	3.8	a	12.6	a	2.54	a	281.1	a	20.4	a	26.8	a	11.1	a	28.5	a
	x 50%	6.9	a	3.6	a	12.9	a	1.95	a	122.0	a	35.4	a	30.4	a	13.5	a	27.2	a
	x 75%	6.0	a	4.81	a	6.1	a	1.52	a	249.2	a	23.7	a	52.2	a	22.5	a	23.7	a

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 7. Root nutrient element concentrations of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 2

Harvest 2 Cultivar	P	K	Ca g kg ⁻¹ root dw	Mg	Fe	Mn ppm root dw	Zn	Cu	B										
CNKAL1029	6.6	a	3.5	a	10.0	bc	1.8	b	304.3	c	59.1	c	59.0	b	22.8	a	22.6	c	
REDBOR	8.1	b	6.3	c	11.7	c	1.7	b	241.4	b	61.2	c	60.3	b	18.6	a	26.1	d	
WINNETOU	6.6	a	5.1	b	8.4	b	1.5	a	190.6	a	40.2	ab	47.7	a	20.4	a	36.2	e	
REFLEX	7.2	ab	4.9	b	5.1	a	1.4	a	196.1	a	32.1	a	43.6	a	22.8	a	17.6	b	
NTOPIA	7.1	ab	4.3	ab	10.3	bc	1.9	b	214.6	ab	50.3	bc	47.0	a	18.9	a	14.1	a	
<i>N form (% NH₄)</i>																			
0% NH ₄ -N	5.9	a	3.7	a	14.1	d	2.1	d	233.9	bc	54.0	b	41.7	a	14.0	a	19.5	a	
25% NH ₄ -N	7.0	b	3.9	a	11.4	c	1.7	c	267.0	c	60.8	b	43.4	a	15.1	a	22.7	b	
50% NH ₄ -N	7.5	bc	5.5	b	6.7	b	1.4	b	193.6	a	54.6	b	60.7	b	16.9	a	25.3	b	
75% NH ₄ -N	8.1	c	6.1	b	4.1	a	1.3	a	225.0	ab	24.9	a	61.2	b	36.9	b	25.1	b	
<i>Cultivar x N form (% NH₄-N)</i>																			
CNKAL1029	x 0%	6.6	a	3.8	a	15.7	gh	2.6	h	285.4	a	72.4	a	55.0	a	16.9	a	22.4	e-i
	x 25%	6.7	a	2.8	a	12.6	efg	1.8	d-g	378.4	a	85.6	a	57.0	a	15.4	a	21.7	e-h
	x 50%	8.4	a	3.4	a	8.3	b-e	1.5	a-d	255.0	a	57.2	a	72.2	a	25.9	a	26.9	h-k
	x 75%	6.3	a	4.1	a	3.4	ab	1.3	ab	298.3	a	21.1	a	52.0	a	33.2	a	20.7	d-h
REDBOR	x 0%	5.3	a	5.6	a	14.7	fgh	2.0	g	245.6	a	56.5	a	45.5	a	10.4	a	23.3	f-i
	x 25%	8.3	a	4.1	a	16.6	gh	1.8	c-g	264.4	a	80.6	a	49.7	a	14.2	a	29.0	jk
	x 50%	8.7	a	7.3	a	9.8	c-f	1.5	a-d	200.4	a	72.7	a	72.6	a	17.0	a	27.4	ijk
	x 75%	10.1	a	8.2	a	5.9	abc	1.4	abc	255.3	a	35.1	a	73.3	a	32.7	a	24.7	gj
WINNETOU	x 0%	5.3	a	3.3	a	13.3	efg	1.9	efg	231.9	a	43.7	a	34.7	a	13.1	a	19.7	b-g
	x 25%	6.9	a	5.1	a	9.6	c-f	1.6	b-g	207.3	a	46.6	a	38.5	a	12.2	a	31.5	k
	x 50%	6.2	a	6.2	a	5.8	abc	1.3	ab	141.2	a	42.0	a	52.7	a	14.5	a	41.5	l
	x 75%	8.1	a	5.8	a	4.8	abc	1.1	a	181.8	a	28.5	a	65.0	a	41.9	a	46.5	l
REFLEX	x 0%	4.5	a	3.5	a	7.0	a-d	1.6	b-f	193.8	a	28.5	a	29.7	a	12.1	a	17.1	a-f
	x 25%	7.4	a	3.8	a	6.5	abc	1.5	a-e	231.3	a	42.7	a	36.5	a	22.2	a	15.6	a-e
	x 50%	8.0	a	6.3	a	4.1	ab	1.5	a-e	173.8	a	38.6	a	52.2	a	13.8	a	17.6	a-e
	x 75%	7.0	a	6.0	a	2.6	a	1.2	ab	185.5	a	18.8	a	56.1	a	43.1	a	19.4	c-g
NTOPIA	x 0%	6.4	a	1.4	a	19.9	h	2.7	h	202.6	a	68.9	a	43.6	a	17.4	a	15.3	a-d
	x 25%	5.7	a	3.4	a	11.8	d-g	1.9	fg	253.4	a	48.5	a	35.5	a	11.5	a	13.3	a
	x 50%	7.3	a	4.5	a	5.7	abc	1.6	b-f	197.8	a	62.6	a	50.7	a	13.1	a	13.5	ab
	x 75%	9.1	a	6.73	a	3.7	ab	1.3	ab	193.7	a	21.3	a	59.3	a	33.7	a	14.2	abc

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 8. Leaf nitrate concentration of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates (0% NH₄-N, 25% NH₄-N, 50% NH₄-N and 75% NH₄-N) in the nutrient solution, at harvest 1 and harvest 2

Cultivar	Harvest 1 Nitrate ppm dw leaf	Harvest 2 Nitrate ppm dw leaf
CNKAL1029	260.6	354.9
REDBOR	245.9	324.4
WINNETOU	173.3	435.0
REFLEX	218.2	353.8
NTOPIA	190.9	149.8
<i>N form (% NH₄)</i>		
0% NH ₄ -N	338.2	462.2
25% NH ₄ -N	272.3	377.6
50% NH ₄ -N	144.8	312.4
75% NH ₄ -N	118.9	157.4
<i>Cultivar x N form (% NH₄-N)</i>		
CNKAL1029	x 0%	343.3
	x 25%	306.6
	x 50%	229.4
	x 75%	163.3
REDBOR	x 0%	350.0
	x 25%	355.0
	x 50%	138.0
	x 75%	140.6
WINNETOU	x 0%	289.2
	x 25%	235.6
	x 50%	106.5
	x 75%	100.4
REFLEX	x 0%	384.4
	x 25%	244.6
	x 50%	118.5
	x 75%	125.1
NTOPIA	x 0%	307.8
	x 25%	219.9
	x 50%	124.8
	x 75%	38.2

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

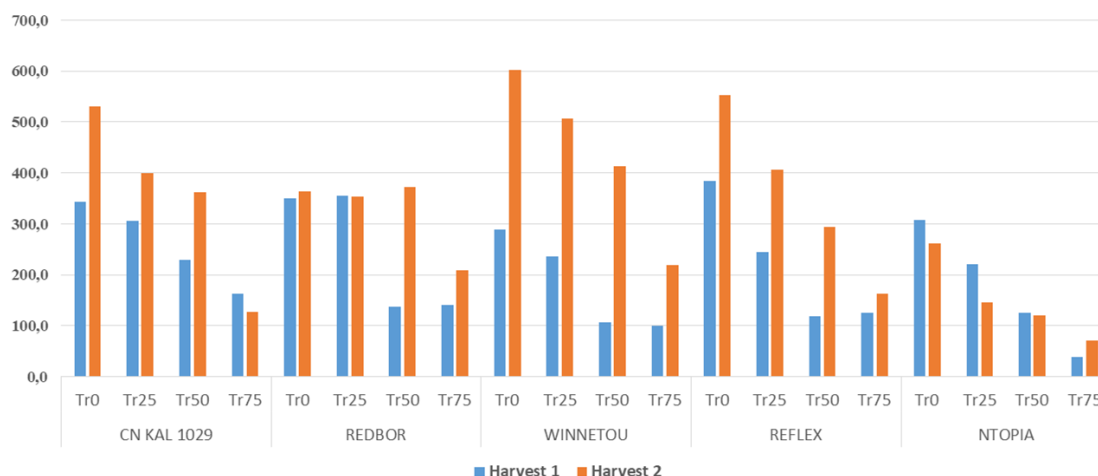


Fig. 1. Leaf nitrate concentration of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale grown with different N forms and rates (0% $\text{NH}_4\text{-N}$, 25% $\text{NH}_4\text{-N}$, 50% $\text{NH}_4\text{-N}$ and 75% $\text{NH}_4\text{-N}$) in the nutrient solution, at harvest 1 and harvest 2

Root Nitrogen-Phosphorus-Potassium-Calcium-Magnesium concentrations

At both harvests, the main effect of N form on root P and K concentrations was that they increased gradually by increasing $\text{NH}_4\text{-N}$ in the nutrient solution with the majority of the differences being significant; the only exception was the root P of plants under Tr75 which was found to be lower compared to the relevant one of plants grown under Tr50, at harvest 1. On the contrary, root Ca and Mg decreased by increasing $\text{NH}_4\text{-N}$ in the nutrient solution at both harvests, with the majority of the differences being significant, as well (Tables 6, 7). Regardless of the N form, the main effect of cultivar/hybrid on root P and Ca at harvest 1 was their non-significant differentiation; 'Redbor' presented the highest K and 'CN KAL 1029' high Mg level (Table 6). At harvest 2, root P, K and Ca concentrations were higher in 'Redbor' whereas 'Ntopia', and root Mg highest in 'CN KAL 1029' and 'Redbor' (Table 7). The interaction between hybrid/cultivar and N form was found to be significant only in the case of root Ca and Mg concentrations, at harvest 2.

Leaf Iron, Manganese, Zinc, Copper and Boron concentrations

According to ANOVA results, the main effect of N form was that leaf Fe, Mn, Zn and Cu concentrations of kale plants increased by increasing $\text{NH}_4\text{-N}$ in the nutrient solution, at both harvests. In the case of Fe at harvest 1, the leaf Fe concentrations of plants grown under Tr25, Tr50 and Tr75 were found to be sign. greater compared to plants grown under Tr0, without being sign. differentiated among them (Table 4); however, at harvest 2, the concentration of the element did not sign. differ among the four N treatments (Table 5). Leaf Mn and Zn concentrations of plants grown under Tr75 were also found to be elevated compared to the three other treatments Tr0, Tr25 and Tr50, at harvest 1; however, at harvest 2, elevated leaf Mn and Zn concentrations were noticed not only in plants grown under Tr75 but also under Tr50 compared to plants grown under Tr0 and Tr25 (Table 4). Boron concentration at harvest 1, was not sign. differentiated

among the four N treatments whereas at harvest 2, it increased by increasing $\text{NH}_4\text{-N}$ in the nutrient solution (Tables 4,5). Regardless of the N form, the main effect of cultivar/hybrid on leaf Fe was that the concentration of the element at harvest 1 did not sign. vary among the five genotypes (Table 4); at harvest 2, 'Ntopia' and 'Redbor' followed by 'CN KAL 1029' presented the highest Fe level whereas 'Winnetou' presented the lowest one (Table 6). At harvest 1, 'CN KAL 1029', 'Redbor' and 'Winnetou' presented higher leaf Mn, Zn and Cu compared to 'Reflex' and 'Ntopia', and 'Winnetou' showed the lowest leaf boron concentration (Table 4). At harvest 2, 'CN KAL 1029' presented lower Mn level, 'Winnetou' higher Zn whereas 'CN KAL 1029' and 'Redbor' higher leaf Cu and B concentrations (Table 5). The interaction between hybrid/cultivar and N form was found to be significant in the cases of leaf Mn, Cu and B concentrations at harvest 1 and in the cases of Mn and Zn, at harvest 2 (Tables 4, 5).

Root Iron, Manganese, Zinc, Copper and Boron concentrations

The main effect of N form on root Fe concentration at both harvests, was that plants under Tr25 presented the highest Fe level while root Zn and Cu concentrations increased by increasing $\text{NH}_4\text{-N}$ in the nutrient solution. Mn concentration at harvest 1 increased by increasing $\text{NH}_4\text{-N}$ in the nutrient solution up to 50%, but under Tr75 it was found to be lower compared to plants under Tr50, at both harvests. B was not sign. differentiated among the four N form treatments at harvest 1, but increased by increasing $\text{NH}_4\text{-N}$ in the nutrient solution at harvest 2 (Tables 6, 7). Regardless of the N form, the main effect of cultivar/hybrid on root Fe, Mn and Zn concentrations at both harvests was that 'CN KAL 1029' and 'Redbor' presented the higher values

whereas root Cu concentration was not significantly differentiated among the five genotypes. 'Ntopia', at harvest 1, presented low root Fe, Mn, Zn and Cu but high B (Tables 6, 7). The interaction between hybrid/cultivar \times N form was found to be significant only in the case of root B concentration, at harvest 2 (Table 7).

Leaf nitrate concentration

Regardless of the genotype, the main effect of N form on leaf nitrate concentration was that by increasing $\text{NH}_4\text{-N}$ in the nutrient solution, the leaf nitrate concentration decreased gradually, at both harvests; all the relevant differences were significant, except the lower but non-sign. differentiated nitrate content of plants under Tr75 compared to plants under Tr 50, at harvest 1 (Table 8). Regardless of the N form, the main effect of genotype on leaf nitrate concentration was that 'CN KAL 1029' and 'Redbor' at harvest 1, as well as 'Winnetou' at harvest 2, showed the highest leaf nitrate contents whereas 'Ntopia' at both harvests presented the relevant lowest concentrations. It should be mentioned that four weeks after the beginning of the treatments (harvest 1) 'Winnetou' presented the lowest leaf nitrate concentration but eight weeks later the same hybrid presented the highest nitrate content (Table 8). Given that the Fpr of the interaction cultivar/hybrid and N form for leaf nitrate concentration at harvest 2 was found to be statistically significant ($P < 0.05$), it should be noticed that when 'Ntopia' was grown with 25% $\text{NH}_4\text{-N} + 75\% \text{NO}_3\text{-N}$ in the nutrient solution, the leaf nitrate concentration decreased by 45% compared to plants grown with 100% $\text{NO}_3\text{-N}$; when the same cultivar was grown with 50% $\text{NH}_4\text{-N}$, the relevant nitrate concentration decreased by 54% whereas when it was grown with 75% $\text{NH}_4\text{-N}$, the nitrate concentration of the cultivar decreased by 73%. The relevant decreases in leaf nitrate concentration of 'Redbor' were found to be 3%, -3% and 42%, respectively, whereas those of the other three hybrids presented intermediate decrease percentages (Fig. 1).

Discussion

The nutrient concentrations of kale leaves determined in the present work were found to be higher compared to those reported by the National Nutrient Database for Standard Reference Legacy Release for kale (Agricultural Research Service, United States Department of Agriculture 2018). Among the macronutrients determined, the concentration of N followed by that of K were the highest ones whereas the most abundant micronutrients were Fe and B followed by Mn and Zn (Tables 4, 5). Ayaz *et al.* (2006) analyzing kale leaves harvested from six different fields in Trabzon (Turkey) determined nutrient levels similar to ours, whereas Grace *et al.* (2000) reported deficient in Mn and Zn kale crops in New Zealand.

Our results indicated that when plants had reached their marketable size (four weeks from the beginning of the treatments-1st harvest), the presence of 25%, 50% and 75% ammonium to nitrate N in the nutrient solution did not affect leaf biomass produced (which comprises the edible plant part), upper plant part and total plant FWs of kale. The only traits that were affected were the root biomass

which increased and the stem FW that decreased by growing plants under 75% $\text{NH}_4\text{-N}$ in the nutrient solution (Table 1). Although the fresh matter of plant leaves, upper plant part and total plant were not affected up to 75% ammonium nitrogen at harvest 1, the relevant dry matter decreased; it may be related to the significant increase of leaf water content under 75% ammonium N (Table 3). At the 1st harvest, the Root/Shoot ratio increased by increasing ammonium up to 50% but decreased by the further ammonium increase. The imbalance between shoot and root growth indicated that partitioning in the plants is affected by the ammonium concentration even when the other growth parameters remained stable. The aforementioned results showed that kale leaves for at least a four week culture can tolerate even 75% $\text{NH}_4\text{-N}$ in the nutrient solution without showing any adverse leaf growth effects. Given that, the complete exclusion of NH_4 from the nutrient solution (plants under 100% $\text{NO}_3\text{-N}$) did not differentiate the growth of kale plants, it could be assumed that during this period there is no preference of the plant toward the N form and rate up to 75% $\text{NH}_4\text{-N} + 25\% \text{NO}_3\text{-N}$ ratio in the hydroponic solution.

With regard to nutrient concentrations, leaf N and K did not vary up to 50% $\text{NH}_4\text{-N}$ but increased in plants under 75% $\text{NH}_4\text{-N}$. Leaf P increased gradually from 0% to 75% $\text{NH}_4\text{-N}$ while leaf Mg decreased gradually. Leaf Ca increased by increasing $\text{NH}_4\text{-N}$ up to 50% $\text{NH}_4\text{-N}$ but decreased under 75% $\text{NH}_4\text{-N}$. Consequently, kale leaves harvested up to the first four weeks accumulate higher quantities of N, P and K when grown with 75% $\text{NH}_4\text{-N}$ compared to plants grown with 0, 25 and 50% $\text{NH}_4\text{-N}$; the same trend was observed in the case of leaf Fe, Mn, Zn and Cu contents whereas B was not differentiated because of the ammonium to nitrate ratio (Table 4). The results of the 2nd harvest indicated that after eight weeks from the beginning of the treatments and four from the 1st harvest, kale plants grown with 75% $\text{NH}_4\text{-N}$ compared to plants with 0, 25 and 50% $\text{NH}_4\text{-N}$ showed sign. reductions in most of the growth parameters determined (plant leaves, shoot, total plant FWs and DWs, stem length) (Tables 2, 3), as well as symptoms of ammonium toxicity in the root system. In particular, there was an approximately 25% reduction in the leaf fresh and dry matter of plants grown with 75% $\text{NH}_4\text{-N}$ compared with plants grown with either 0%, 25% or 50% $\text{NH}_4\text{-N}$, suggesting a probable preference of kale plants grown for a prolonged period towards either a complete exclusion of $\text{NH}_4\text{-N}$ from the nutrient solution or an ammonium to nitrate N ratio up to 50%. The opposing effect on the growth of kale plants fed with 75% $\text{NH}_4\text{-N}$ might be due to the unavailability of NO_3 as a N source and the higher demand for carbohydrates channelled for NH_4 assimilation and detoxification (Beevers and Hageman, 1969; Tabatabaei *et al.*, 2006). The present work showed that the ammonium threshold that kale plants can tolerate for normal growth for a prolonged period of cultivation is the presence of 50% $\text{NH}_4\text{-N}$. Similar findings were found by Zhang *et al.* (2007) for cabbage grown with five $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratios in the nutrient solution (1:0; 0.75:0.25; 0.5:0.5; 0.25:0.75; and 0:1); their results showed that cabbage growth was reduced much more (by 87%) when the proportion of $\text{NH}_4\text{-N}$ in the nutrient solution was more than 75% compared with the ratio of 0.5:0.5, 35 days after the transplanting suggesting a possible toxicity due to the

accumulation of a large amount of free ammonia in the leaves.

An imbalance in Root/Shoot ratio occurred at harvest 2 as well, as it increased by increasing the ammonium concentration in the nutrient solution at 75% of the total nitrogen whereas the relevant ratios of plants grown under 0%, 25% and 50% $\text{NH}_4\text{-N}$ remained non-differentiated (Table 2). It has repeatedly been reported that an increased Root/Shoot ratio has physiological advantages because it may not only increase the quantity of nutrient element absorbing sites with a resultant higher source-sink ratio in the plant, but also renders lower the total requirement by decreasing the proportion of shoot, in which most of the nutrient element of the plant is utilized. Similar results were taken by Wei *et al.* (1994) and Hajiboland *et al.* (2003) in relevant experiments with susceptible and resistant to Fe-deficiency subclovers and rice cultivars, respectively, as the Root/Shoot ratio of those plants increased with increasing chlorosis resistance. However, despite the prolonged imposition of plants under 75% $\text{NH}_4\text{-N}$ there was no significant differentiation in the plant leaf number, the leaf water content and chlorophyll content (Table 3).

Leaf nutrient concentrations at harvest 2 presented similar trends compared to the relevant ones at harvest 1; leaf N and K did not vary up to 50% $\text{NH}_4\text{-N}$ but increased in plants under 75% $\text{NH}_4\text{-N}$; the N and K increase of plants grown under 75% $\text{NH}_4\text{-N}$ could be due to the greater accumulation of the elements in the smaller leaf biomass of those plants (Table 2). Leaf P increased gradually from Tr0 to Tr75; the leaf P gradual increase by increasing $\text{NH}_4\text{-N}$ in the nutrient solution could be due to the acidification of the rhizosphere because of the excretion of H^+ from plant roots fed with NH_4^+ . Leaf Ca increased gradually by increasing $\text{NH}_4\text{-N}$ up to Tr 50 but decreased under Tr75. The ratio 50% $\text{NH}_4\text{-N}/50\%$ $\text{NO}_3\text{-N}$ not only did not cause adverse effects in kale plants growth but also increased many leaf nutrient concentrations; Zhang *et al.* (2007) results were similar to ours as they reported higher leaf P, K, Ca and Mg concentrations in cabbage plants grown under 50% $\text{NH}_4\text{-N}/50\%$ $\text{NO}_3\text{-N}$. An appropriate NH_4/NO_3 ratio improves the absorption of many nutrients and maintains a suitable proportion of N assimilation and storage that should benefit the plant growth and quality of kale as a vegetable. Adversely to Zhang *et al.* (2007) results, our work showed that leaf Mg decreased gradually by increasing $\text{NH}_4\text{-N}$ in the nutrient solution. Reduced Ca and Mg concentrations in kale plants grown with high ammonium N in the nutrient solution may have been due to the reduced uptake of the elements as an antagonism effect to the high presence of the cation NH_4^+ in the nutrient solution; our results are in accordance with the findings of Pill and Lambeth (1977), Alan (1989), Mills and Jones (1996), Fageria (2001), Kotsiras *et al.* (2002) and Tabatabaei *et al.* (2006).

Leaf Mn, Zn, Cu and B contents increased by increasing $\text{NH}_4\text{-N}$ in the nutrient solution whereas Fe did not vary. The high pH in the rhizosphere owing to $\text{NO}_3\text{-N}$ supply reduced the shoot P concentration and markedly suppressed the Mn, Zn and Cu contents (Tables 4, 5); similar results were obtained by Savvas *et al.* (2003). The well-known effect of high rhizosphere pH on the uptake of P, Fe, Mn, Zn, Cu has also been referred to by Marschner (1997). P, K, Mn, Zn and Cu results similar to ours were obtained by Simonne *et al.* (1993) when working with

turnip plants (*Brassica rapa* L.) grown in sand culture under five $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratios (1:0, 3:1, 1:1, 1:3, 0:1).

Taking into consideration that brassicas are consumed in part for their nutritional values of K, Ca, Mg, Fe and Zn, Kopsell *et al.* (2004) have reported an average of a twofold difference in elemental accumulation among cultivars and selections measured by them. The present work showed a significant variability among the five genotypes tested for their leaf macro- and micronutrients contents, however these differences were lower than double; i.e. the leaf K, Ca and Mg concentrations of the hybrid 'CN KAL 1029' were higher by 33%, 35% and 27%, respectively, compared to 'Reflex' hybrid whereas the relevant micronutrient concentration differences were even lower (Table 4). Moreover, Kopsell *et al.* (2004) had reported that among the 22 kale and collard cultivars and selections tested, the hybrid with the highest leaf elemental accumulation was 'Redbor'. In our experiment 'Redbor' presented intermediate nutrient contents compared to the other genotypes included. The information on genotypic variability for elemental accumulation may be important for producers and consumers looking to select kale cultivars with higher nutritional levels of beneficial dietary elements.

Regarding leaf nitrate accumulation, leafy vegetables including kale, accumulate fairly large nitrate quantities. The data given in literature concerning nitrate content in kale range from 300 to 1283 mg kg^{-1} fresh matter (Chweya, 1988; Kim and Yoon, 2003) whereas our results showed that the nitrate content of kale leaves ranged from 700 to 6000 mg kg^{-1} fresh matter. Moreover, our study showed that the leaf nitrate content of kale plants decreased by increasing ammonium N in the nutrient solution. Zhang *et al.* (2007) reported lower leaf nitrate in cabbage plants grown with 50% $\text{NH}_4\text{-N}/50\%$ $\text{NO}_3\text{-N}$, as well. Our investigation showed also that when 'Ntopia' was grown with 25%, 50% and 75% $\text{NH}_4\text{-N}$ in the nutrient solution, the leaf nitrate concentration decreased by 45%, 54% and 73% respectively, as compared to plants fed with 100% $\text{NO}_3\text{-N}$; the relevant decreases of 'Redbor' were 3%, -3% and 42% and those of the other three hybrids presented intermediate values (Fig. 1). Despite the aforementioned large decreases of leaf nitrates of the indigenous cultivar 'Ntopia' when growing with increasing ammonium nitrogen in the nutrient solution, the growth of the same cultivar was not sign. affected even when the concentration of $\text{NH}_4\text{-N}$ was up to 50%.

Comparing the level of total nitrogen and nitrates not only in relation to the cultivar/hybrid but also to the time of harvest, we observed that leaf nitrate concentration between harvest 1 and harvest 2 (28 days interval) increased. In particular, 'Winnetou' increased nitrate concentration by 151%, 'Reflex' by 62%, 'CN KAL 1029' by 36% and 'Redbor' by 32% whereas the indigenous cultivar 'Ntopia' decreased its leaf nitrate content by 22% (Table 8). Adversely, the results of the comparison of leaf total nitrogen concentration between harvest 1 and harvest 2 showed that leaf N decreased. The correlation coefficient between leaf nitrates and leaf total nitrogen was found to be significantly negative ($r=-0.36$). Korus and Lisiewska (2009) study with kale plants grown in the field, showed an opposite trend; they reported that kale plants obtained at the 2nd harvest (14 weeks after the planting of the seedlings in the field) contained 9% more total nitrogen and 67%

fewer nitrates compared to the 1st harvest (10 weeks after the planting of the seedlings in the field). A similar to total N decrease from harvest 1 to harvest 2 was also shown in the cases of leaf K, Ca and Mg. Among the genotypes tested, 'Winnetou' and 'CN KAL presented the greatest N decreases from harvest 1 to harvest 2 whereas leaf P of 'Reflex' and 'Ntopia' increased. Leaf Fe, Cu and B concentrations at harvest 2 compared to those at harvest 1 decreased as well, whereas leaf Mn and Zn increased. Root Fe level was not differentiated between the two harvests whereas root Mn, Zn and Cu increased and root B decreased. The higher nutrient and the lower nitrate contents of kale leaves at the first harvest that took place four weeks after the treatments compared to the eight week harvest, is interesting from a nutritional point of view, because it is well known that fruit and vegetables usually contribute about 35 and 24% respectively to the total K and Mg dietary intake of humans (Levander, 1990).

Conclusions

The effect of four different ammonium to nitrate nitrogen ratios (0:100, 25:75, 50:50 and 75:25 NH₄/NO₃) in the nutrient solution on growth parameters, nutrient element and leaf nitrates accumulation of four kale hybrids 'CN KAL 1029 F1', 'Redbor F1', 'Winnetou F1', 'Reflex F1' and one indigenous Greek cultivar 'Ntopia Mytilinis' was that during the first four weeks of cultivation none of the four NH₄/NO₃ ratios applied adversely affected plant growth. Moreover, kale plants grown with 75% NH₄-N+25%NO₃-N accumulated higher N, P, K, Fe, Mn, Zn and Cu quantities and lower nitrates in their foliage. However, kale plants grown for longer than one month period could not tolerate 75% NH₄-N in the nutrient solution without adverse effects on plant development, suggesting their probable preference towards either a complete exclusion of ammonium from the nutrient solution or to 25:75 and 50:50 NH₄/NO₃ ratios. Among the genotypes tested, 'Ntopia Mytilinis' produced the greatest yield with the lowest leaf nitrate accumulation as compared to the four hybrids tested whereas the hybrid 'CN KAL 1029 F1' presented the highest leaf K, Ca, Mg concentrations. These results indicate that producers wishing to maximize yield and elemental uptake as well as minimize leaf nitrate content of kale plants need to consider the ratio of NH₄/NO₃ in their fertility programs in combination to the cultivar/hybrid used.

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