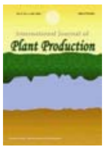

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Diurnal variation in the nitrate content of parsley foliage

S. A. Petropoulos[a,*], E. Constantopoulou[b], I. Karapanos[a], C. A. Akoumianakis[a], H. C. Passam[a]

[a]*Agricultural University of Athens, Laboratory of Vegetable Production, 75 Iera Odos, 11855 Athens, Greece.*
[b]*Technological Educational Institute of Messolonghi, Department of Greenhouse Crops and Floriculture, Nea Ktiria, 30200 Messolonghi, Greece.*
*Corresponding author. E-mail: fangio57gr@gmail.com

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Abstract

The diurnal fluctuation of nitrate concentration in the foliage of three parsley subspecies (plain-leafed, curly-leafed and turnip rooted parsley) cultivated in the spring and autumn of two consecutive years was examined. Nitrogen was applied at 30 (Control), 75, 150, 300 mg kg^{-1} in both years, but with the addition of 450 mg kg^{-1} in year 2. Harvest was carried out at 08:30, 12:30 and 17:30 on a single day for each sowing. The results showed that the nitrate concentration of the foliage was lowest when harvest was carried out at midday (12:30), although not always to a statistically significant level in the low N-application rates (≤ 75 mg kg^{-1}). In addition, increasing the rate of N application from 30 to 450 mg kg^{-1} resulted in a progressive increase in the nitrate concentration of the foliage irrespective of the time of day at which the plants were harvested. The concentration of nitrate differed between subspecies and was higher in the older, outer leaves in the case of turnip-rooted parsley than in the younger, inner leaves. It is concluded that although the nitrate concentration of the foliage of all three parsley subspecies increased with increasing rates of N-application, even the implement of the highest nitrogen rate (< 400 mg kg^{-1} fresh weight), did not resulted in nitrate content that exceeded the maximum level recommended by the E.U., irrespective of the time of harvest, subspecies, age of the leaves and the season of cultivation.

Keywords: Curly-leafed parsley; *Petroselinum crispum*; Plain-leafed parsley; Turnip-rooted parsley; Hamburg parsley; Diurnal variation of nitrates.

Introduction

Parsley (*Petroselinum crispum* [Mill.] Nyman) is a herb that is widely used throughout the world in fresh salads, as a condiment or garnish and as a constituent of cooked food. Parsley is also a source of essential oils for the pharmaceutical and cosmetics industries. Plain-leafed parsley (*P. crispum* [Mill.] Nym. ssp. *neapolitanum* Danert) is the most commonly cultivated subspecies within the Mediterranean region, although recently in Greece there have been attempts to introduce curly-leafed (*P. crispum* ssp. *crispum* L.) and turnip-rooted (*P. crispum* ssp. *tuberosum* [Bernh.] Crov.) parsley, which are crops primarily of central and northern Europe (Petropoulos et al., 2005).

Despite its beneficial properties parsley, like many other leafy crops, tends to accumulate nitrates under certain conditions, such as low light intensity and high nitrate application (Maynard and Barker, 1972; Maynard et al., 1976). Although nitrates may help to decrease blood pressure and support cardiovascular function when consumed according to DASH diet (Dietary Approaches to Stop Hypertension) (Hord et al., 2009), they have also been implicated in a number of human health disorders (e.g. methaemoglobinaemia) and possibly chronic diseases, such as cancer (Manassaram et al., 2006). Previous studies of factors affecting the nitrate concentrations in leafy vegetables have concentrated largely on lettuce and spinach since these are consumed in relatively larger amounts than most other plants in this category and therefore constitute a potentially greater health threat when their nitrate content is high. However, since the total intake is critical for human health, other species (e.g. parsley) should also be considered so that the total daily intake does not exceed the maximum intake level, which is currently set at ≤ 3.65 mg Kg^{-1} body weight (MAFF, 1998).

Within the low altitude, warm areas of the Mediterranean Basin, parsley is mainly grown from autumn to spring. In comparison with northern Europe, the winters are mild with a relatively high light intensity that favors the production of leafy vegetables low in nitrate. Despite this, a survey of nitrate levels in vegetable produce in northern Greece indicated that parsley had a higher nitrate content than most of the other local produce examined, including lettuce, spinach and endive (Siomos and Dogras, 1999).

The nitrate content of plant tissues may be reduced by adjusting the nitrogen application rate, choosing the appropriate nitrogen (N) form, interrupting N fertilization a few days prior to harvest, supplementing Cl^{-1} as an antagonist to nitrate ion absorption, selecting varieties with a lower tendency to accumulate nitrates, as well as by fertilizer form and N:K ratio (Kotsiras et al., 2002; Demšar et al., 2004; Abu-Rayyan et al., 2004; Khah and Arvanitoyiannis, 2003). Increasing day length, light intensity and air temperature affect nitrate accumulation by promoting the activity of nitrate reductase in the leaves (Cantliffe, 1973; Quinche, 1982; Fallovo et al., 2009; Chadjaa et al., 1999; Gaudreau et al., 1999). Diurnal variations in the leaf nitrate concentration of spinach have also been reported (Umar et al., 2007), but in lettuce adjusting the harvest time did not achieve a reduction in nitrate levels (Siomos, 2000).

In this study, we examined some of the factors (the time of harvest during the day, the N-application rate and the sowing date) that influence nitrate accumulation in the foliage of three parsley subspecies with a view to establishing the best cultivation practices to obtain high quality produce.

Materials and Methods

Parsley (*Petroselinum crispum* [Mill.] Nym. ex A.W. Hill ssp. *neapolitanum* Danert cv. plain-leafed [G. Fytotechniki, Greece], *P. crispum* ssp. *crispum* cv. curly-leafed [G. Fytotechniki, Greece] and *P. crispum* ssp. *tuberosum* [Bernh.] Mart. Crov., turnip-rooted cv. Fakir [Bejo Zaden b.v., Holland]) seeds were sown in seed trays containing peat (KTS2, Klasmann-Deilman Gmbh, Geeste, Germany) and washed, riverbed sand in a ratio of 2:1 (v/v) on 3 October 2004 (year 1) and on 4 October 2005 and 15 January 2006 (year 2). The seed trays were placed on benches in an unheated greenhouse. When the plants had developed two true leaves, they were transplanted to 10 L plastic pots containing the same compost to which a base dressing of 150 g superphosphate (0-46-0), 90 g potassium nitrate and 900 g marble dust m^{-3} was added. Three plants were retained per pot and spaced to achieve a plant density of 27 plants m^{-2}. Each treatment was replicated three times, using ten pots per replicate (30 pots per treatment in total). For each cultivation, new pots and new substrate were implemented in order to avoid a fertilizer residue effect. Plants from the two October sowings were grown in an unheated greenhouse, whereas plants from the March sowing were transferred outdoors in April. Irrigation and fertilization was carried out in the form of liquid feeds with 30 (Control, bore water), 75, 150, 300 in year 1 plus 450 mg N kg^{-1} in year 2 (in the form of ammonium nitrate) with a frequency which varied from once a week (November-March) increasing to thrice a week in late April-May. The quantity of liquid feed per irrigation ranged from 0.3 L per pot and application for the first two weeks after transplantation, increasing progressively to 1.5 L per pot by the end of the growth cycle. Irrigation was applied manually with volumetric flasks, in order to apply the same volume to every pot. Plastic dishes were placed beneath the pots in order to avoid drainage loss. The mean daily temperature and solar radiation were recorded throughout the experiments by means of Hobo H_8 data loggers (Onset Computer Corp., Pocasset, MA, USA) for the indoor cultivation, whereas the respective data for the outdoor cultivations were obtained from the meteorological station of the Laboratory of General and Agricultural Meteorology of the Agricultural University of Athens. The mean total daily solar radiation throughout the three weeks before harvest day was 862.2 (harvest on 25-3-2005), 859.12 (harvest on 8-3-2006) and 1115.2 Watts m^{-2} (harvest on 11-5-2006), whereas the mean daily temperature was 9.4, 11.3 and 16.0 ^oC for the respective harvest days.

The foliage of the plants (leaves and petioles) was sampled on 25 March 2005 (year 1), 8 March 2006 (first sowing of year 2) and 11 May 2006 (second sowing of year 2) at three specific times (08:30, 12:30 and 17:30) during the day. Leaves were selected at random from 12 plants of each treatment and divided into three subsamples (every four plants constituting one subsample). In a separate experiment to examine possible variations in nitrate concentrations between leaves within the canopy of the same plant, fully mature outer leaves and developed leaves from the centre of the rosette were harvested at random from 12 plants of turnip-rooted parsley at midday (12:30) in parallel with the samples taken as indicated above and using the same procedure. After harvest, all samples were weighed, dried at 72 ± 1 ^oC for 3 days and ground in a MF 10 Basic Mill (IKA-Werke, GmbH & Co. KG, Staufen, Germany). The dry matter content ranged from 7-12 g 100 g^{-1} of fresh weight. For nitrate extraction, 100 mg of ground samples were suspended in 10 ml deionized water and incubated at 45 ^oC for 1 hour, followed by centrifugation at 5000 g for 15 minutes. The supernatants were put in plastic bottles and saved for analysis. The nitrate concentration within each sample was determined colorimetrically by the nitration of salicylic acid as described by Cataldo et al. (1975) using a Perkin Elmer Model Lambda 1A spectrophotometer (Perkin Elmer, Waltham, Mass.).

The experimental design was a split, split plot, each plot consisting of one harvest time of day, with fully randomized sub-plots comprising the N-fertilization levels and sub-sub plots the subspecies. Number of replicates was n=3. Statistical analysis was conducted with the aid of Statgraphics 5.1 plus (Statistical Graphics Corporation). Data were evaluated by multifactor analysis of variance for the main effects (ANOVA), whereas the means of values were compared by the LSD test (P=0.05).

Results

The climatic data recorded at half-hourly intervals throughout the experiment showed that solar radiation each day was maximal from about 11:00 to 14:30 whereas the air temperature continued to rise until about 15:00-16:00.

Statistical analysis of the nitrate content of leaves showed a significant interaction between the three factors examined (harvest time, N-application rate and subspecies) in the three sowings (Table 1). In all three crops, increasing the rate of N application from 30 to 450 mg kg^{-1} resulted in a progressive increase in the nitrate concentration of the foliage irrespective of the time of day at which the plants were harvested (Tables 2-4). The timing of harvest affected the nitrate content in all the crops, with foliage harvested at midday (12:30) having a lower nitrate concentration than that harvested in early morning (08:30) or late afternoon (17:30). At the higher N application rates (150-450 kg^{-1}) the highest and lowest concentrations of nitrate in the foliage were recorded in plants at 17:30 and 12:30 respectively, irrespective of the subspecies and season, and these differences were statistically significant ($P<0.05$). At the lower N application rates (30-75 mg kg^{-1}), nitrate concentrations were again higher in the late afternoon than at midday, but the differences were not always statistically significant (Tables 2-4). In addition, in year 2 the subspecies varied significantly in nitrate content regardless of N-application rate and harvest time, with turnip-rooted parsley having the lowest nitrate content and differing significantly from curly-leafed parsley in all cases (Tables 3 and 4).

Table 1. Analysis of variance of the nitrate content data of the three experiments.

Source of effect	1st year	2nd year	
	1st sowing	1st sowing	2nd sowing
Main effects	(P>F)	(P>F)	(P>F)
N (N level)	*	*	*
C (Cultivar)	*	*	*
T (Time)	*	*	*
Interactions			
N×C	*	*	*
T×C	ns	ns	ns
T×N	ns	*	*
T×N×C	ns	*	*

[*] Significant at P<0.05.
[ns] not significant at P<0.05.

Table 2. The mean values of nitrate content (n=3) of the foliage of three parsley subspecies (expressed in mg kg^{-1} fresh weight) sown in the autumn of year 1 in relation to the n-application rate and the time of day at harvest.

Subspecies (C)	Time (T)	N-Application rate (mg kg^{-1}) (N)				$LSD_{0.05}$
		30	75	150	300	
	8:30	101.0	152.0	201.0	265.0	10.6
Curly-leafed	12:30	96.0	141.0	187.0	254.0	8.3
	17:30	112.0	162.0	212.0	271.0	11.3
$LSD_{0.05}$		4.0	14.8	12.4	8.7	
	8:30	81.0	110.0	141.0	188.7	5.5
Turnip-rooted	12:30	79.0	107.0	133.0	176.0	6.0
	17:30	86.0	121.0	151.0	196.0	6.5
$LSD_{0.05}$		2.4	6.7	8.0	7.4	
	8:30	90.0	145.0	163.0	215.0	6.7
Plain-leafed	12:30	78.0	131.0	152.0	203.0	8.3
	17:30	96.0	153.0	168.0	223.0	10.8
$LSD_{0.05}$		7.3	8.1	11.3	9.9	

Mean separation in columns and rows by LSD (P=0.05).

Table 3. The mean values of nitrate content (n=3) of the foliage of three parsley subspecies (expressed in mg kg^{-1} fresh weight) sown in the autumn of year 2 in relation to the n-application rate and the time of day at harvest.

Subspecies (C)	Time (T)	N-Application rate (mg kg^{-1}) (N)					$LSD_{0.05}$
		30	75	150	300	450	
	8:30	80.0	123.7	171.0	230.0	310.3	4.3
Curly-leafed	12:30	78.0	110.0	153.3	217.7	290.3	4.4
	17:30	83.7	132.0	182.7	244.0	327.0	2.5
$LSD_{0.05}$		5.4	3.1	5.2	3.1	3.6	
	8:30	60.0	73.0	129.7	179.3	233.0	3.0
Turnip-rooted	12:30	57.0	69.0	112.7	161.3	217.0	3.4
	17:30	62.0	79.0	144.7	201.0	248.7	9.3
$LSD_{0.05}$		3.3	2.8	5.0	9.5	8.9	
	8:30	61.7	89.0	159.0	216.0	267.0	4.2
Plain-leafed	12:30	62.7	75.0	149.0	202.7	243.3	4.1
	17:30	66.0	110.7	173.0	233.0	284.0	3.5
$LSD_{0.05}$		6.6	2.4	3.5	3.1	4.8	
	(C) × (T)						
$LSD_{0.05}$	C × T_1	7.5	2.4	3.1	3.1	3.1	
	C × T_2	4.4	2.8	5.5	3.0	5.5	
	C × T_3	2.9	3.1	5.0	9.5	8.7	

Mean separation in columns and rows by LSD (P=0.05).

Table 4. The mean values of nitrate content (n=3) of the foliage of three parsley subspecies (expressed in mg kg^{-1} fresh weight) sown in the winter of year 2 in relation to the n-application rate and the time of day at harvest.

Subspecies (C)	Time (T)	N-Application rate (mg kg^{-1}) (N)					$LSD_{0.05}$
		30	75	150	300	450	
	8:30	58.0	88.7	140.3	202.7	256.0	3.6
Curly-leafed	12:30	62.0	72.0	125.0	188.0	237.3	4.8
	17:30	66.0	98.7	155.0	220.3	278.0	8.2
$LSD_{0.05}$		4.7	5.2	6.6	9.5	4.9	
	8:30	54.0	72.3	98.7	141.7	182.7	5.3
Turnip-rooted	12:30	57.0	61.7	87.0	127.3	159.7	4.4
	17:30	62.0	79.0	116.7	159.0	197.0	4.7
$LSD_{0.05}$		2.6	3.8	5.6	4.1	8.4	
	8:30	56.3	89.0	132.0	161.3	220.7	4.6
Plain-leafed	12:30	52.3	73.3	114.0	147.3	197.7	4.5
	17:30	61.3	98.7	148.0	180.0	238.0	5.0
$LSD_{0.05}$		3.8	6.7	5.5	3.9	5.4	
	(C) × (T)						
$LSD_{0.05}$	C × T_1	3.1	5.2	5.6	4.1	6.3	
	C × T_2	5.1	4.6	4.5	3.8	6.7	
	C × T_3	2.9	6.2	7.4	9.5	6.2	

Mean separation in columns and rows by LSD (P=0.05).

The nitrate concentration measured in the foliage of the turnip-rooted subspecies varied with leaf age at the time of harvest. At N-application rates of 75 mg kg^{-1} or higher, the outer, older leaves invariably had a higher nitrate concentration than the younger inner leaves within the canopy, irrespective of season. The effect of leaf age was more pronounced as the rate of N application increased and was higher in the autumn sowing of year 1 than in the other two sowings probably due to lower temperatures and solar radiation during the winter of year 2 that delayed plant development at the early growth stages and therefore nitrogen uptake (Table 5).

Table 5. The mean values of nitrate content (n=3) of the foliage of turnip-rooted parsley (expressed in mg kg^{-1} fresh weight) in relation to n-application rate and the position of the leaves within the canopy.

Leaf position	N-application rate (mg N kg^{-1})					$LSD_{0.05}$
	30	75	150	300	450	
Autumn sowing (year 1)						
Internal	80.0	91.3	118.0	163.0		9.8
External	79.0	107.0	133.0	176.0		6.0
$LSD_{0.05}$	3.2	8.7	11.5	12.1		12.1
Autumn sowing (year 2)						
Internal	56.7	60.0	101.7	141.7	198.0	12.8
External	57.0	69.0	112.7	161.3	217.0	10.4
$LSD_{0.05}$	2.9	2.3	4.7	3.5	5.3	
Winter sowing (year 2)						
Internal	57.0	57.3	71.7	113.7	141.0	12.5
External	57.0	61.7	87.0	127.3	159.7	11.4
$LSD_{0.05}$	2.2	4.1	4.0	8.3	9.2	

Mean separation in columns and rows by LSD (P=0.05).

Discussion

Although N application in one form or another is essential for satisfactory crop growth, there is increasing concern about the effects of indiscriminate use of synthetic fertilizers, particularly nitrates, on the environment and human health. Leafy vegetables, such as parsley, are known to accumulate nitrate in large amounts (Maynard et al., 1976; MAFF, 1998) and a maximum application rate of 150 mg N kg^{-1} has been proposed for parsley in the Mediterranean Basin (Petropoulos et al., 2008), although this level is clearly lower than that adopted by many producers within the region. Therefore additional measures to reduce the nitrate content of the harvested product must also be considered, and these may be viewed as a means of improving quality (Santamaria, 2006).

Nitrate accumulation in plant tissues is particularly high during the winter when temperatures, light intensity and day length are less favorable for assimilation (Cantliffe 1972; Quinche, 1982; Fallovo et al., 2009; Chadjaa et al., 1999; Gaudreau et al., 1999; Blom-Zandstra, 1989). In previous studies, diurnal variations in the leaf nitrate concentration of spinach were reported depending on the genotype (Umar et al., 2007), but not in lettuce (Siomos, 2000). In parsley the lowering of foliar nitrate concentration at midday was observed in all three subspecies (Tables 2-4) irrespective of the cultivation season. It therefore appears that for parsley, harvest at midday offers a practical means of reducing nitrate concentration and thereby improving product quality. However, it is advisable that the leaves should be transferred immediately to a cool place or pre-cooled, since under conditions of high temperatures foliage may soon start to wilt after harvest if left exposed to the sun.

Similar to lettuce (Demšar et al., 2004; Siomos et al., 2002), the older, outer leaves of parsley tend to accumulate nitrate more than the younger, inner leaves (Table 5). This may relate to the lower metabolic activity of the older leaves, which are then used by the plant as nitrate sinks. However, when harvest was carried out 2-3 weeks earlier than in the present experiments, the outer leaves still showed a higher concentration of nitrates than the inner leaves (data not presented). The shading of leaves within the canopy may also play a role, the older outer leaves being displaced by the younger inner ones. In celery, shading is known to increase leaf nitrate content (Wojciechowska and Siwek, 2006).

In conclusion, relatively high temperatures and light intensity in the Mediterranean region even during winter favour the production of parsley with a nitrate content that is significantly lower than the limit imposed by E.U. legislation (Siomos and Dogras, 1999). This may be obtained not only by limiting N-application rates (e.g. to 150 ppm, as recommended by Petropoulos et al., 2008), but also by harvesting at midday. Since such low nitrate levels are difficult to achieve in northern Europe, particularly during the winter months (MAFF, 1998) low nitrate-containing produce from Mediterranean producing countries such as Greece could be promoted as a healthy alternative and thus fill a niche within the fresh vegetable market of northern countries.

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