Reducing the nitrate content of protected lettuce

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Summary

A research project was carried out jointly between Teagasc, Kinsealy Research Centre and University College Dublin, Department of Crop Science, Horticulture and Forestry which studied the effects of cultivar, nitrogen fertilisation and light intensity on the nitrate content of protected butterhead lettuce.

In a series of cultivar trials of winter and summer butterhead lettuce, significant differences in the nitrate content of the lettuce between cultivars were found only in one experiment. In this instance, the differences were not consistent between successive harvests. It was concluded that screening lettuce cultivars for tissue nitrate level is unlikely to contribute to an overall reduction of nitrate levels.

The application of N in a liquid feed throughout the cropping period resulted in higher nitrate levels in lettuce plants grown in soil filled containers compared with a similar amount of N applied to the soil before planting. Withdrawing N for the final 10 days of the cropping period did not affect the nitrate content of the lettuce.

In an experiment studying nitrogen source and rate on lettuce grown in containers, the use of calcium cyanamide as a N source resulted in lower nitrate levels in the lettuce and gave a reduced head weight compared with calcium ammonium nitrate (CAN) or ammonium sulphate. Increasing the rate of CAN or ammonium sulphate gave higher lettuce nitrate levels. A nitrification inhibitor reduced the soil nitrate levels especially with sulphate of ammonia as the N source but did not affect the plant nitrate levels significantly. The addition of chloride to the soil reduced nitrate levels in the lettuce.

In a further fertilisation study using containers, calcium cyanamide again resulted in lower plant nitrate levels than CAN. Increasing the rate of CAN increased soil nitrate levels, lettuce head weight and plant nitrate levels. The relationship between soil nitrate levels, lettuce head weight and plant nitrate level indicates that the level of 100-150 $mg \cdot L^{-1}$ of nitrate N in the soil, advocated in the Code of Good Practice, is a compromise between maximising plant growth and minimising lettuce nitrate content.

A comparison between CAN and calcium cyanamide in a border soil experiment again showed that the latter N source resulted in lower lettuce nitrate levels. In this experiment the addition of chloride to the soil did not affect plant nitrate levels.

Lettuce was grown, in late summer, in small tunnels using a range of polyethylene cladding materials. Head weight correlated well with the

overall light transmission of the materials. In one of the materials that had a low light transmission, lettuce nitrate content was doubled compared with those grown under the materials with high light transmission.

Under both winter and summer conditions, the nitrate content of lettuce heads was not influenced by the time of day at which harvest took place. In experiments in which multiple harvests were carried out there was no consistent trend in nitrate content as the heads developed and matured. Within individual heads of lettuce there was a steep concentration gradient with the older outer leaves having much higher concentrations of nitrate than the younger inner leaves. Herbicides commonly used in protected lettuce production did not influence the nitrate content of the lettuce.

Introduction

There has been concern in some EU countries over the possible health risks associated with high dietary intakes of nitrates. Vegetables are the group of foods which makes the greatest contribution to nitrate consumption and leafy crops such as lettuce and spinach are particularly likely to contain high levels of nitrates. An EU regulation has set limits of 4,500 $mg{\cdot}kg^{-1}$ of nitrate in the fresh product for lettuce harvested from November 1 to March 31 and 3,500 $mg{\cdot}kg^{-1}$ from April 1 to October 31.

Ireland requested a derogation for lettuce and spinach which meant that for a transitional period the maximum concentrations did not apply. This was allowed for under the EU regulation provided that codes of good practice were drawn up and applied to achieve gradual progress towards the limits. A draft code of good agricultural practice for the production of protected lettuce in Ireland with respect to nitrate content has been drawn up and circulated to growers.

Nitrate levels in Irish lettuce

Samples of protected lettuce grown in Ireland have been analysed by the State Laboratory for the Department of Agriculture, Food and Rural Development since 1994. This has shown that some of the produce contains levels higher than the maximum concentrations allowed. An overall view of the results obtained in 1994, 1995 and 1996 graphed against the time of year is shown in Figure 1. The fitted curve shows the overall trend. The results show a clear pattern for lettuce nitrate levels to be higher in the winter/spring and autumn/winter periods of the year than in the summertime. This pattern is well established in many countries. It also shows that in the January to March period (Day 0 to 90) and again in

November and December (Day 305 on), some of the samples exceed the limit of 4,500 mg·kg^{-1}.

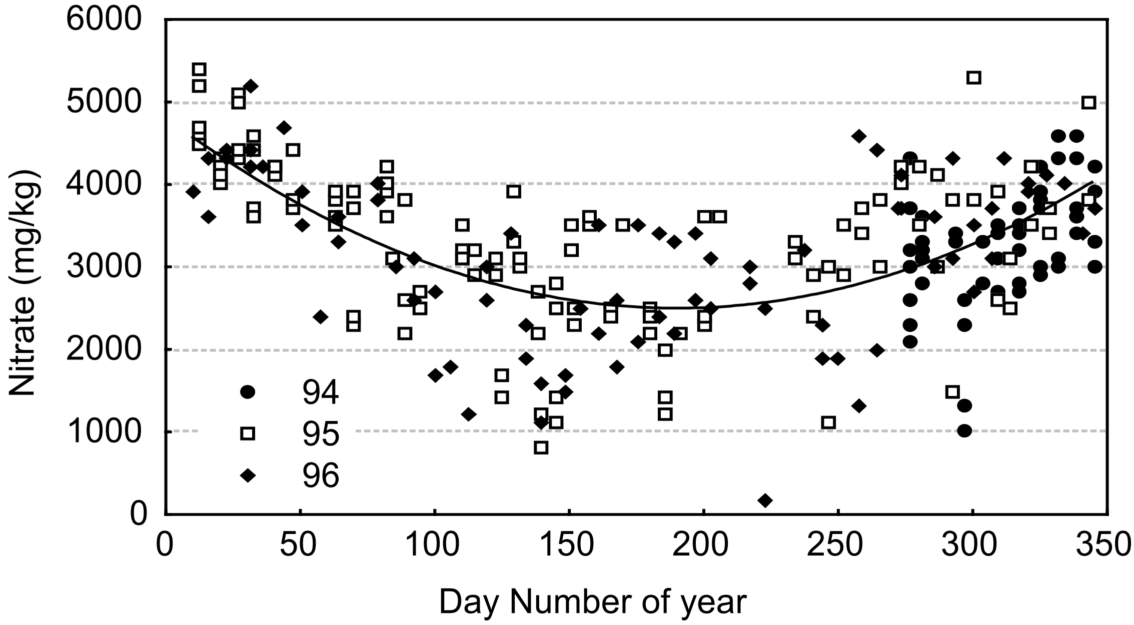

Figure 1 : Results of nitrate analysis of Irish protected lettuce from 1994 to 1996 (Source: Department of Agriculture, Food and Rural Development).

The same is true in the April 1 to October 31 period (Day 90 to 305) in that some of the samples are above 3,500 mg·kg^{-1} which is the permitted maximum nitrate content for this part of the year. What is also clear is that there is great variability in the nitrate content of lettuce with both high and low levels being found at the same period of the year. If we plot the average nitrate content found in each month of the year then the annual cycle becomes a lot more obvious (Figure 2).This shows that the highest nitrate contents occur in winter/spring and that January is the most critical month in terms of the average content being closest to the allowed maximum level.

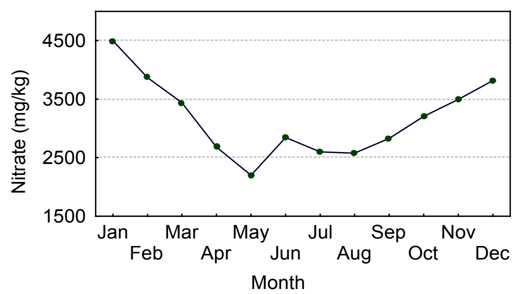

Figure 2. Monthly average of nitrate content in Irish protected lettuce, 1994-1996 (Source : Department of Agriculture, Food and Rural Development).

Project aim and scope

The purpose of this research was to examine nitrate accumulation in lettuce. Findings in the literature were at times conflicting in terms of their conclusions and often not relevant to the prevailing environmental conditions in Ireland. After careful consideration three key objectives were arrived at.

The first objective was to gain insight into and understanding of the inherent qualities of lettuce and identify possible cultivar differences. Experiments were set up to study nitrate distribution within the plant, nitrate accumulation during plant growth and nitrate accumulation in different lettuce cultivars.

The second objective was to investigate possible straightforward solutions to the problem of high nitrate levels. The focus was on fertilisation regimes and the experiments studied fertiliser types, usage rates and modes of application.

The third objective was to examine the effects of the environmental factors of light, temperature and carbon dioxide in order to comprehend the role each was playing in terms of nitrate accumulation. Timing of the lettuce harvest was also considered.

In conclusion consideration is given to the prevailing environmental conditions in Ireland and the possibility of successfully applying the findings of this research to Irish protected lettuce production.

The research project commenced at Kinsealy Research Centre in January 1998 and a series of glasshouse, field and controlled environment experiment were carried out over a 30-month period both in the research centre and the university.

Cultivar experiments

Introduction

Cultivar selection was examined in a series of three cultivar experiments carried out during the winter of 1997/1998, the summer of 1998 and the winter of 1998/1999. A total of 21 cultivars, including both winter and summer varieties, were selected for the experiments and assessed for their nitrate content at harvest.

The three experiments were grown in border soil in a glasshouse. Each experiment was laid out as a randomised block design with either three or five replications. Each plot was theoretically divided in four and a random number generator determined which quarter was to be cut on each of the harvest dates. Guard plants surrounded each plot and there were sufficient plants to maintain guard rows between the first and subsequent harvests. Fresh head weights were recorded at each harvest and a lettuce sample taken for nitrate analysis. Soil samples were taken before, during and after growing the lettuce.

Soil nutrient levels were tested before planting. In each experiment the soil was flooded to reduce the nitrate level prior to adding fertiliser at the chosen rate. Seeds were sown into peat blocks and subsequently planted out at a spacing of 22 x 22cm. In all cases Basilex was applied two days before planting for control of Rhizoctonia. Kerb 50W was applied seven days after planting for weed control, and Rovral was applied for the control of Botrytis. Irrigation was carried out using an overhead sprinkling system.

Winter cultivar experiment

The first cultivar trial, grown in the Winter of 1997/1998, included nine at cultivars in a randomised block with three replications. After flooding the soil, a base dressing of CAN was applied to bring the nitrate level to 100ppm. Sulphate of potash and superphosphate (8% P) were also applied at a rate of $60g/m^2$ and $100g/m^2$ respectively. In this trial there were three harvest dates, Feb 23, Mar 2 and Mar 9. At each harvest a block of sixteen plants was cut from each plot, weighed and eight of the sixteen randomly selected and quartered. One quarter from each of the eight plants was taken, finely chopped with a knife and a 30g sample taken. It was then frozen prior to laboratory analysis.

The fresh weights of the nine cultivars and the nitrate contents at the three harvest dates are shown in Table 1. There were significant differences between the cultivars in head weight at the first and second harvests. Scott and Wendel tended to be the slowest developing cultivars. However by the final harvest the range of fresh weight differences was quite small with a mean of 181 g/head for Scott and 210 g/head for Kathy and Oskar.

There were no significant differences in level of nitrate between the cultivars at any of the three harvests. In addition there was no trend of increasing or decreasing level of nitrate as the heads matured, the mean level at the three harvests being 4132 on Feb 23, 4007 on Mar 2 and 4007 on Mar 9.

Table 1. Head weight and nitrate content of nine lettuce cultivars in the winter 1997/98 cultivar experiment (Values without a common suffix are significantly different).

Cultivar	Head weight (g)			Nitrate content ($mg \cdot kg^{-1}$)		
	Feb 23	Mar 2	Mar 9	Feb 23	Mar 2	Mar 9
Beverly	101^{bc}	147^{ab}	194	3591	4051	4114
Kathy	117^{ab}	163^{a}	210	4162	4073	4416
Lianne	103^{abc}	154^{ab}	194	4194	3789	3567
Nixon	100^{c}	143^{abc}	200	4146	3658	3948
Oskar	114^{abc}	155^{ab}	209	4251	3984	4303
Scott	97^{c}	109^{c}	181	4093	4019	3785
S7196	113^{abc}	126^{bc}	198	4308	4381	4199
S7198	118^{a}	155^{ab}	191	4285	4080	4162
Wendel	97^{c}	135^{abc}	184	4156	4030	3568
F-test	*	*	NS	NS	NS	NS
S.E.	5.2	9.2	7.5	286.4	252.1	207.8

Spring-summer cultivar experiment

During the summer of 1998 eight summer cultivars, Clinton, Flandria, Kennedy, Vegas, Yetta, E11.5924, LM1210 and S7253 were studied. A base dressing of CAN was applied to bring the nitrate level of the soil to 100ppm after flooding. There were five replications and two harvest dates, Jun 4 and Jun 16. At harvest, a block of eight heads was cut from each plot and quartered. An amalgam of one quarter from each of the eight heads was mechanically shredded using a food blender. A 30g sample of shredded lettuce was taken, frozen and subsequently analysed for nitrate.

The head weights and nitrate content of the cultivars are shown in Table 2. There were differences in head weight between the cultivars at both harvests. Flandria was heaviest on both dates followed by Vegas and LM1210 at the final harvest. SA7253 produced the lightest heads at both harvests.

As with the previous experiment, no significant differences in nitrate content were found between the cultivars. The average nitrate content at the first harvest was 3,542 $mg \cdot kg^{-1}$ and at the final harvest it was 3,531 $mg \cdot kg^{-1}$. This is in agreement with the previous experiment showing no trend in the nitrate level as the heads mature.

Table 2. Head weight and nitrate content of eight lettuce cultivars in the spring-summer cultivar experiment (Values without a common suffix are significantly different).

Cultivar	Head weight (g)		Nitrate content (mg·kg^{-}	
	Jun 4	**Jun 16**	**Jun 4**	**Jun 16**
Clinton	245^{bc}	340^{bcd}	3502	3321
Flandria	333^{a}	404^{a}	3430	3542
Kennedy	246^{bc}	333^{d}	3626	3782
Vegas	275^{b}	389^{abc}	3342	3455
Yetta	222^{cd}	329^{d}	3904	3714
E11.5924	279^{b}	339^{bcd}	3524	3666
LM1210	266^{b}	388^{abc}	3538	3163
S7253	194^{d}	275^{d}	3472	3602
F-test	***	***	NS	NS
S.E.	11.4	18.5	159.9	256.7

Autumn-winter cultivar experiment

Eight cultivars were grown in the Autumn of 1998 and harvested on January 14 and 25, 1999. As nitrate levels in the soil were very high prior to planting the soil was flooded to reduce nitrate N levels to between 100 and 200 mg·L^{-1} and no fertiliser was added. There were five replications and a block of eight heads harvested from each plot at both harvests. Unlike the previous two cultivar trials, in this experiment one quarter from each of the eight heads was randomly selected and the eight quarters frozen together as one sample. Each sample was subsequently analysed for nitrate.

Table 3. Head weight and nitrate content of eight lettuce cultivars in the autumn-winter 1998/99 cultivar experiment (Values without a common suffix are significantly different).

	Head weight (g)		Nitrate content (mg·kg^{-}	
Variety	**Jan 14**	**Jan 25**	**Jan 14**	**Jan 25**
Beverly	116^{abc}	132^{bc}	4114^{ab}	3513^{bc}
Hilary	104^{c}	118^{c}	3815^{bc}	3317^{c}
LM1130	115^{bc}	138^{ab}	3558^{c}	3473^{bc}
Oskar	124^{ab}	130^{bc}	4010^{ab}	3674^{abc}
S7196	127^{ab}	150^{a}	3990^{ab}	3664^{abc}
Troubadour	107^{c}	141^{ab}	4085^{ab}	3412^{c}

Tzigane	131^{a}	143^{ab}	4301^{a}	3801^{ab}
Wendel	117^{abc}	142^{ab}	4210^{ab}	4004^{a}
F-test	**	**	*	**
S.E.	4.9	5.0	130.3	118.4

Tzigane had the largest head weight (131g) on Jan 14 and S7196 (150g) on Jan 25. The cultivar LM1130 had the lowest nitrate content (3558 $mg \cdot kg^{-1}$) at the first harvest. Tzigane, which had a high head weight, also had the highest nitrate content (4301 $mg \cdot kg^{-1}$). In the second harvest it was the cultivar Hilary that had the lowest nitrate content (3317 $mg \cdot kg^{-1}$) and Wendel that had the greatest (4004 $mg \cdot kg^{-1}$). The mean nitrate content of all cultivars at the first harvest was 4010 and at the second 3607 $mg \cdot kg^{-1}$.

Mode of fertiliser application

Lettuce plants of cultivar 'Clinton' were grown in soil filled containers. Three modes of fertiliser application were compared, advance soil application, liquid feeding every alternate day until harvest and liquid feeding ceasing 10 days before harvest. Two rates of N to supply 57 and 115 mg of N per litre of soil were included to give a factorial design of six treatments with seven replications.

Table 4. Effect of mode of application and rate of N on head weight and nitrate content of lettuce.

	Head weight (g)	Nitrate content ($mg \cdot kg^{-1}$)
Mode of application		
Base fertiliser	124	2037
Liquid feed to harvest	113	2462
Liquid feed to 10 days before harvest	113	2582
F-test	NS	***
s.e.	6.3	91.8
Rate of N ($mg \cdot L^{-1}$)		
57	117	1907
115	116	2813
F-test	NS	***
s.e.	5.2	75.0

The square 22 cm pots were set apart on a glasshouse bench at 35cm centres. Each pot was an independent unit and any leachate from the pots was trapped in a saucer and re-cycled. The nutrient content of the soil was determined prior to the experiment and this was taken into account when making up the nitrate treatments. The lettuce was planted out in July and harvested in September. The results are shown in Table 4.

The method of fertiliser application had no significant effect on the lettuce head weight but it had a very significant effect on the nitrate content of the harvest lettuce, with lowest nitrate levels being obtained where the fertiliser was applied to the soil in advance. Stopping the liquid feed applications 10 days before harvest did not reduce the nitrate content. The importance of fertiliser rate is clearly seen with significantly lower nitrate levels being obtained with the lower rate of nitrogen application.

Type and rate of N fertiliser, nitrification inhibitor and chloride

Three N fertilisers, calcium ammonium nitrate (CAN), sulphate of ammonia and calcium cyanamide were applied to the soil at three rates to supply 50, 100 and 200 $mg \cdot L^{-1}$ of N. The effect of adding potassium chloride to the soil and the use of a nitrification inhibitor (dicyandiamide) were also studied, giving a factorial design of 36 treatments with 6 replications. The nitrate content of the soil was allowed for when making up the treatments.

Each lettuce plant, cv. Clinton, was grown under glass in a square 22 cm container. The pots were placed side by side and a row of guard plants in containers surrounded the treatments. This glasshouse experiment was grown during summer 1998 and harvested in September.

The results (Table 4) indicate that using calcium cyanamide as the N source reduced the nitrate content of the lettuce but also gave a slight reduction in head weight. This indicates that crops grown with calcium cyanamide may require a slightly longer growing period.

Table 5. Effect of N fertiliser type and rate of N on the head weight and nitrate content of lettuce.

	Head weight (g)	**Nitrate content ($mg \cdot kg^{-1}$)**
N Fertiliser		
CAN	303	2815
Sulphate of ammonia	302	2756
Calcium cyanamide	272	2483
F-test	*	***
s.e.	6.6	38.5
Rate of N ($mg \cdot L^{-1}$)		
50	305	2587
100	299	2716
200	273	2752
F-test	*	**
s.e.	10.9	38.5

As in the previous experiment increasing the rate of N added to 100 $mg \cdot L^{-1}$ increased the nitrate content of the lettuce but did not affect the head weight. A further increase to 200 $mg \cdot L^{-1}$ of N added reduced the head weight but did not affect the nitrate content.

The addition of potassium chloride to the soil reduced the average nitrate content of lettuce from 2,650 to 2,450 $mg \cdot kg^{-1}$. Soil analysis showed that the nitrification inhibitor did reduce the nitrate level in the soil where CAN and sulphate of ammonia were the N fertilisers. However this did not result in lower nitrate content of the lettuce which was not affected by addition of the inhibitor. The head weight was reduced from 307 to 278 g by the inhibitor implying a requirement for a slightly longer growing period.

N source and rate

This container experiment studied the effect of two N fertilisers, calcium ammonium nitrate (CAN) and calcium cyanamide, applied at rates of 25, 50, 100, 200 and 400 $mg \cdot L^{-1}$ of N to lettuce grown in peat and in mineral soil. This gave a factorial design of 20 treatments with 7 replications.

The mineral soil was analysed in advance and found to have little or no nitrate so the full rate of CAN and calcium cyanamide was applied to both the peat and soil mixes. In addition to the CAN or calcium cyanamide, sulphate of potash and di-calcium phosphate were applied to the soil, each at a rate of 200 mg·L^{-1}. Dolomitic lime, sulphate of potash, superphosphate, di-calcium phosphate and fritted trace elements were also added to the peat. All fertilisers were incorporated into the growing medium prior to filling the pots and planting out. For this experiment, seeds of the summer lettuce cultivar 'Recital' were sown on July 12. The plants were planted in 22 cm square containers on July 27 and harvested September 1, 1999.

The effects of both fertilisers on the head weight of the lettuce and the nitrate content of the lettuce are shown in Figures 3 and 4. Both fertilisers increased the lettuce head weight up to a rate of addition of 200 mg·L^{-1} (Figure 3). Using calcium cyanamide at rates higher than this reduced head weight whereas with CAN the response flattened out but did not decrease. As in previous experiments, CAN tended to produce heavier heads than calcium cyanamide. This was especially marked at high rates of N addition while at lower rates the difference between the two fertilisers was small.

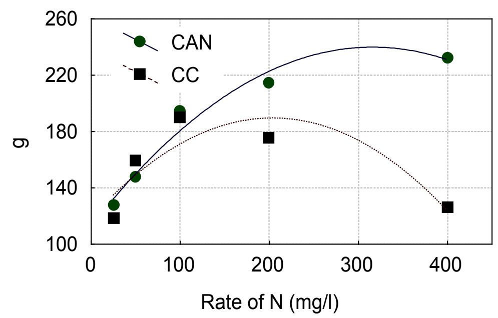

Figure 3. Effect of rate of calcium ammonium nitrate (CAN) and calcium cyanamide (CC) on the head weight of lettuce

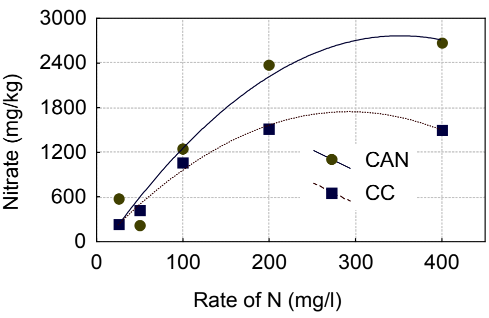

Figure 4. Effect of rate of calcium ammonium nitrate (CAN) and calcium cyanamide (CC) on the nitrate content of lettuce.

Increasing the rate of N also increased the nitrate content of the lettuce with both fertilisers but the rise in nitrate content was greater with CAN than with calcium cyanamide (Figure 4). This is in line with previous experiments.

Soil nitrate levels were measured at planting time. Nitrate levels were much higher in the soil than in the peat treatments indicating that nitrification occurred quickly in the soil. Where calcium cyanamide was used, soil nitrate levels were low. The relationship between the soil nitrate level and head weight, with CAN as the N source, is shown in Figure 5. As the soil nitrate rises above 100 mg·L^{-1} the response of head weight tends to level off.

The relationship between soil nitrate level and nitrate content of lettuce is shown in Figure 6. In this experiment the lettuce nitrate content varied widely between 575 mg·kg^{-1} at low levels of N to 2,680 mg·kg^{-1} at the high rate. The nitrate content is still increasing rapidly at a soil nitrate-N level of 100 mg·L^{-1}.

These results indicate that the range of nitrate-N advocated in the Code of Good Practice of 100-150 mg·L^{-1} is a compromise between maximising head weight and minimising the lettuce nitrate content. Above this range there will be only a small response in head weight but still a relatively large increase in the nitrate content. Below this range the head weight will be affected to a greater degree.

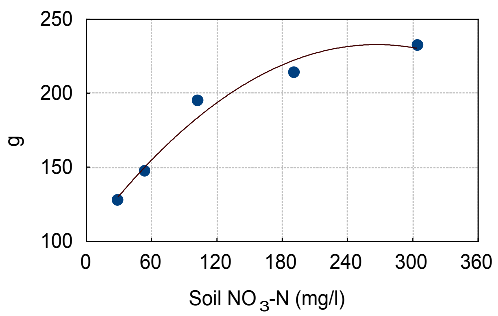

Figure 5. Relationship between soil nitrate level at planting and the head weight of lettuce with CAN.

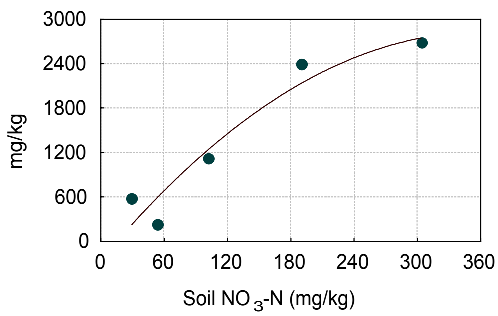

Figure 6. Relationship between soil nitrate level at planting and the nitrate content of lettuce with CAN.

N fertiliser and chloride in border soil

Results from the container trials have indicated that the type of N fertiliser and the addition of chloride to the soil significantly affect the amount of nitrate in the harvested lettuce. The aim of this experiment was to study whether a similar effect could be found with a crop of lettuce grown in the glasshouse border soil.

. Calcium ammonium nitrate and calcium cyanamide were each applied at rates of 100 mg·L^{-1} of N. Both fertiliser types were applied with and without chloride (100 and 0 mg·L^{-1} of Cl) to give a factorial design of four treatments and six replications. Chloride was applied in the form of potassium chloride. The fertilisers were evenly distributed over each marked plot and then rotovated into the soil.

Each plot was seven plants wide and 16 plants long and the lettuce was planted at 22cm centres. There were four plots, one of each treatment, across the width of the glasshouse and five blocks running north south down the length of the house. Each plot was separated from the next by guard plants and a path, and there were six guard lines between the blocks.

This was a winter trial. Seeds of lettuce cultivar 'Wendel' were sown on September 21, planted on October 20, 1999 and harvested on February 26, 2000.. At harvest, 24 heads of lettuce were cut from the centre of each plot and the fresh weight recorded. From the 24 heads, three whole plant samples were selected at random for subsequent analysis.

Table 6. Effect of N fertiliser and chloride addition to the soil on head weight and nitrate content of lettuce grown in border soil.

	Head weight (g)	Nitrate content ($mg \cdot kg^{-1}$)
N fertiliser		
CAN	120.1	4453
Calcium cyanamide	113.0	4130
F-test	NS	*
s.e.	3.11	92.7
Chloride		
Control	116.1	4401
Chloride added	117.0	4180
F-test	NS	NS
s.e.	3.11	92.7

The results are shown in Table 6. In this experiment using calcium cyanamide as the N source did not adversely affect the weight of the heads of lettuce. Calcium cyanamide did give a reduction in nitrate content of the lettuce which agrees with the results of the container experiments. In this experiment the addition of chloride had no effect on either head weight or nitrate content.

The nitrate levels in this experiment were high. This reflects the time of year in which the experiment was carried out but may also be a function of a high level of nitrification activity in the soil. Flooding with water was carried out to lower the soil nitrate level before the experiment.

Effect of polyethylene cladding materials

The object of this experiment was to compare the performance of eight different polythene films in terms of their suitability for growing lettuce with a low nitrate content. The films chosen were selected for a range of light transmitting properties. The design was a randomised block of eight treatments with eight replications. Each block was composed of eight tunnels running east-west. This allowed ventilation on the north side with minimum light interference. Each tunnel measured 1 x 1 m and three wire hoops, approximately 60cm high at the centre, supported the polythene. In each tunnel there were 16 plants, including guard plants, in a 4 x 4 arrangement. Plants are spaced at 20cm centres.

Table 6. The effect of a range of cladding materials on the head weight and nitrate content of lettuce grown in polyethylene tunnels (Values without a common suffix are significantly different).

Product Name	**Light transmission (%)**			**Head weight (g).**	**Nitrate content ($mg \cdot kg^{-1}$)**
	Total	**Direct**	**Diffuse**		
Hyticlear	87	58	29	226.5[ab]	637[b]
Hytilux	87	40	47	215.0[ab]	665[b]
Hytifite	86	54	32	240.1[a]	827[b]
Hytithermic	86	26	60	234.2[ab]	690[b]
Astrolux	70	56	14	208.3[b]	712[b]
Superclear	90[+]			225.7[ab]	795[b]
Supergreen	65[+]			171.0[c]	866[b]
Superwhite	30[+]			81.0[d]	1671[a]
F-test				***	***
S.E.				9.2	74.2

Irrigation was provided via an ooze pipe system with two Poritex hose pipes running the length of each block. They were all connected to the one tap and uniform irrigation was provided when required. The field site was rotovated and the soil was nutrients analysed in advance. CAN, sulphate of potash and superphosphate were subsequently added at rates of 50, 50 and 25 g/m^2. The fertiliser was evenly distributed and lightly forked in to each $1m^2$ plot. This was a late summer trial. Seeds of the cultivar Recital were sown on July 28, planted out on August 12 and harvested on September 27.

At harvest the four centre plants in each treatment were cut and the fresh weight recorded. One plant was then chosen at random for nitrate analysis. This plant was homogenised with a known quantity of distilled water and a 10g sub-sample taken. This sub-sample was frozen prior to laboratory analysis for nitrate.

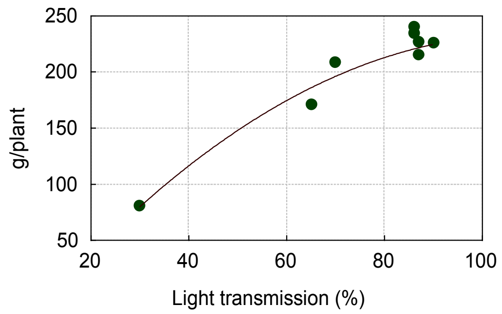

Figure 7. Relationship between light transmission of polyethylene film and lettuce head weight.

The head weights were significantly influenced by the type of polyethylene film (Table 6) and were much lighter under the white film. Most of the variation in head weight could be explained by the light transmission characteristics of the cladding materials and this relationship is shown in Figure 7.

The nitrate content of the lettuce was much higher under the white polyethylene with the low light transmission (Table 6). Differences between the other cladding materials were not significant.

Time of harvest

These experiments were carried out was to determine if there was a significant difference in the nitrate content of lettuce harvested at different times throughout a day. Seeds of lettuce cultivar Troubadour were sown into peat blocks on September 22, 1998, planted out on October 16 and harvested on February 4, 1999. The plot size was 4 x 13 lettuce plants, and include an outer ring of guard plants. Seven heads of lettuce were randomly selected and harvested at each of the three designated harvesting times, 8am, 12 noon and 4pm. Fresh weight was recorded and the lettuce plants frozen for subsequent nitrate analysis.

A summer experiment was also carried out. In this case the plants were grown in peat filled containers which were 22 cm square and there was an outer ring of guard plants. Eight plants were harvested at random at 8am, 12 noon and 4pm.. These square containers were placed sided by side giving a plant spacing of 24cm centres. Plants of the lettuce cultivar Recital were planted on July 30 and harvested on September 3, 1999. At harvest, fresh weights were recorded and the plants were put in a cold store. Each plant was subsequently homogenised with a known quantity of distilled water and a 10g sub-sample taken. This sub-sample was frozen prior to laboratory analysis for nitrate.

Table 7. Nitrate content of lettuce harvested at different times of the day (mg·kg^{-1})

Time of day	Month of harvest	
	February 4	September 3
8.00	3309	439
12 noon	3304	530
4.00 pm	3290	509
F-test	NS	NS
s.e.	184.5	54.4

The results are shown in Table 7. In neither experiment was there any significant effect of the time of harvesting.

A controlled environment experiment was also carried out to study the diurnal variation in nitrate content. Seeds of the lettuce cultivar 'Hilary' were sown into rockwool blocks on December 17, 1999, planted onto 11-cm pots of Shamrock potting compost on January 13 and harvested on March 7, 2000

The plants were grown in pots on two shelves in the centre of a growth chamber with a sixteen hour day and at a temperature of 18 ^{o}C. Light was provided by six fluorescent tubes (TL 33) over each shelf. Ambient carbon dioxide levels prevailed in the chamber. Plants were liquid fed every alternate day to begin with and everyday as their maturity progressed and their requirements increased. Feeding was manual, each plant received an equal measured volume on set days.

Each shelf contained 42 (6 x 7) plants at 20cm centres. There were 20 (4 x 5) treatment plants and guard rows on all four sides. Every treatment plant on each shelf was numbered from one to twenty. Each number occupied the same position on all three shelves. Two numbers were randomly selected for harvest each hour and both plants with that number were cut. Four lettuce plants were harvested every two hours from 6 am to 10 pm. In this way the nitrate content after 8 hours of darkness, and following two, four, six, eight, ten, twelve, fourteen and sixteen hours of light, was measured.

At harvest the fresh weights were recorded and whole plant samples frozen for subsequent nitrate analysis. The relationship between nitrate content and time of day is shown in Figure 8. The relationship was not significant.

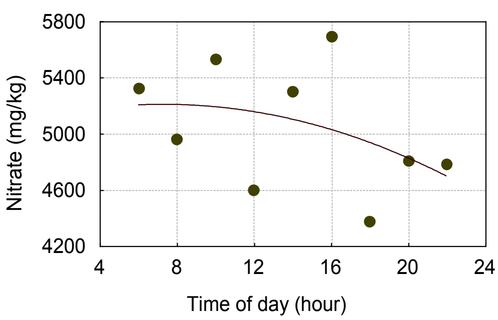

Figure 8 : The relationship between nitrate content and time of day in lettuce grown under controlled environment conditions.

The results of these experiments indicate that harvesting the lettuce at particular times of the day will not be successful in influencing the nitrate content of the lettuce.

Distribution of nitrate in the lettuce plant

Three heads of soil grown glasshouse lettuce were selected and samples of outer leaves, middle leaves and heart were taken. Each sample of leaves was weighed and frozen prior to laboratory analysis for nitrate.

Table 8. Nitrate content of different parts of the lettuce plant

Position	Nitrate content ($mg \cdot kg^{-1}$)
Outer leaves	6,099
Middle leaves	4,703
Heart	2,880
F-test	***
S. E. (df=2)	183.7

The results (Table 8) clearly indicates that the heart of the lettuce contains the least nitrate, (2,880 mg kg^{-1}), with levels increasing in the surrounding leaves to 4,703 mg kg^{-1} and reaching a maximum mean concentration in excess of 6,000 mg kg^{-1} in the outer leaves. This underlines the importance of stripping away the outer leaves as a means of reducing the nitrate level in the product being sold.

Effect of herbicides

In the past herbicides have been shown to have a significant effect on the crop nitrate content. The object of this experiment was to investigate the possibility that currently available herbicides may also have an effect on nitrate accumulation in lettuce.

Container grown lettuce, cv. Recital, was treated with four herbicides, propyzamide (Kerb flo), chlorpropham (Atlas CIPC 40), trifluralin (Treflan) and chlorpropham with cetrimide (Croptex Pewter). Each herbicide was applied to the soil, prior to planting, at twice the recommended application rate. A hand weeded control was included to give a randomised block design of five treatments and eight replications. Plants were grown in 24 cm square containers. Lines of guard plants surrounded the eight blocks.

The soil was analysed in advance and found to contain little or no nitrate. CAN was applied at a rate of 100 $mg \cdot L^{-1}$. All fertilisers were incorporated into the growing medium prior to filling the pots and planting out. Basilex was applied two days after planting for control of Rhizoctonia. Irrigation was provided via an overhead sprinkling system.

Seed was sown on July 12 and the seedlings planted out on July 27 and harvested on September 7, 1999. Fresh weights were recorded at harvest. Whole plant samples were put in short-term cold storage prior to being homogenised with known quantities of distilled water.

Table 8. The effect of herbicides applied at double recommended rate on the head weight and nitrate content of lettuce.

Herbicide treatment	Head weight (g)	Nitrate ($mg \cdot kg^{-1}$)
Propyzamide	167.3	1467
Chlorpropham	198.9	1716
Trifluralin	178.2	1816
Chloropropham+cetrimide	185.5	1715
Hand weeding	184.5	2003
F-test	NS	NS
s.e.	8.19	221.2

Sub-samples of approximately 10g each were weighed and frozen in miniature petri-dishes prior to nitrate analysis. Soil samples were taken prior to planting and at harvest.

The results (Table 8) indicate that herbicides have no significant effect on the nitrate content of lettuce. Nor was there any effect on the weight of the heads.

Conclusions

- Selection among presently used cultivars is not likely to contribute significantly to a reduction in lettuce nitrate level.
- Applying N in the form of liquid feed, as against a pre-planting dressing to the soil, increased nitrate level in the lettuce heads. Liquid feeding therefore should not be used as a routine.
- Increasing the N level in the soil consistently resulted in higher concentration of nitrate in lettuce. The level of soil nitrate N recommended in the Code of Good practice of 100-150 $mg \cdot L^{-1}$ is a compromise between maximising plant growth and minimising nitrate content. It may prolong crop duration by a few days compared with using higher levels.
- The use of calcium cyanamide as a nitrogen source consistently resulted in lower nitrate concentrations in the lettuce compared with calcium ammonium nitrate. There was a slight reduction in head weight which may result in a slightly longer cropping period.
- In a comparison of polyethylene cladding materials, lettuce growth was proportional to the light transmission of the material. One material with a light transmission coefficient of 30% resulted in a doubling of the nitrate concentration in the lettuce.
- There were no consistent trends in nitrate content of lettuce in successive harvests as the heads matured.

- Time of harvest of lettuce during the day did not affect the nitrate concentration in the heads.
- The outer leaves of the lettuce plant contained twice the nitrate level as the innermost leaves. Removal of the outer leaves is important in keeping the nitrate content the produce down.
- Herbicides did not affect the nitrate concentration in the lettuce.

Publications

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