Project Title:	Protected lettuce: to investigate nitrate levels in a range of butterhead and curly lettuce cultivars
Project number:	PC 297
Project Leader:	Julian Davies, Stockbridge Technology Centre
Report:	Final Report
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

Authentication

I declare that the work undertaken at Stockbridge Technology Centre was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

Signature…… Date……..……

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GROWER SUMMARY

Headline

Lettuce varieties had variable nitrate levels with lower levels demonstrated when harvesting delayed by one week following outer leaf trimming.

Background and expected deliverables

This work was initiated in response to current EC legislation concerning the maximum levels of nitrate in lettuce and in particular to undertake research for a continued derogation offered to UK Lettuce growers and to better understand nitrate variability within crops.

The aim of the work was to investigate if there were consistent differences between cultivars for butterhead and curly types when grown in the winter. Two harvest dates were used to determine if cultivars performed similarly when harvested early with minimal trimming and also 7 days later when more of the older leaves would be removed. Any differences in nitrate accumulation in cultivars could then be considered and exploited by growers. This information could be used to increase the understanding of nitrate accumulation in lettuce and how harvesting stage and trimming could be used to reduce the risk of crops exceeding the EC limits.

The second part involved intensive sampling and analysis to determine the head to head variation in trial plots. This would also help increase the understanding of nitrate levels in whole heads and compare levels in different parts of the head. If there was large head to head variation in lettuce grown adjacent to each other then the robustness of the sampling and reporting process would need to be considered and a variability factor built into any maximum nitrate levels set by the Authorities.

The third part involving intensive sampling and determining head to head variation in commercial crops would also increase the understanding of variability in nitrate levels in whole heads. If there was large head to head variation in adjacent heads grown in the soil and the deep water hydroponic

(DWH) system then the robustness of the sampling and reporting process would need to be considered and a variability factor built into any maximum nitrate levels set by the Authorities.

Summary of project and main conclusions

Lettuce was planted on 12 January 2009 into soil where nitrogen fertiliser had been applied to achieve 100ppm nitrogen. Conventional overhead irrigation was used. At the first harvest for the six butterhead cultivars on 30 March 2009 the heads were cut with only the lower soiled leaves removed, heads weighed and sent for nitrate analysis. A second harvest was taken 7 days later when the same plots were harvested but more of the older leaves were removed. This was carried out for the curly lettuce which was harvested on 6 April and also 8 days later.

The results showed that there were no consistent differences between the cultivars of butterhead lettuce. Mean nitrate levels ranged from 2985 to 3471ppm at the first harvest and 2268 to 2620ppm at the second harvest. For the curly types the mean nitrate levels ranged from 3148 to 3917ppm at the first harvest and 2391 to 2856ppm at the second harvest. Mean nitrate levels decreased between the first and second harvest date for both lettuce types. All samples were below the EC limit of 4500ppm.

The head to head variation study showed large variation between the 10 heads grown adjacent to each other. This confirms previous results where the variation between 3 samples of 10 heads taken from plots that were only $4m^2$ was considerably and could exceed 1000ppm. Where leaves had been cut in half and all the left hand side and right hand sides had been analysed separately as 2 samples per head there was variation in the nitrate levels. This again demonstrates that nitrate content is naturally very variable even within heads. Nitrate content in the ribs and the remaining leaf material was compared and found to be much higher in the rib material.

The final part of the project involved looking at head to head variation in soil and the deep water hydroponic grown lettuce on a commercial site in West

Sussex. The results showed that head to head variation was large. For the deep water hydroponic crop the range between the highest and the lowest was 50% of that observed in the soil grown crop. However, nitrate levels were higher in the deep water hydroponic crop and the reason for this requires further study as mean head weights were similar.

Financial benefits

Cultivar selection appears to offer growers only very limited opportunity to minimise crop nitrate levels. Nitrate levels in high risk winter planted crops could be lowered by delaying harvesting by a week so that 40-60g of the older leaves can be removed during trimming. However, this will affect cropping timetables and reduce productivity in the glasshouse. The work has highlighted the high head to head and within head variability in nitrate levels and this needs to be considered by the Authorities when setting maximum nitrate levels. Growers should therefore continue to closely follow the current Code of Good Agricultural Practice to minimise the risk of crops exceeding the EC levels.

Action points for growers

- Continue to adhere to the Code of Good Agricultural Practice and in particular the guidance on sampling.

- For high risk crops delay harvesting until heads weigh at least 220-240g allowing for at least 2 or 3 of the oldest leaves to be removed whilst still achieving heads that are above the 160-180g minimum weight.

- During trimming remove those leaves which contain a high proportion of rib material as this contains more nitrate than the leaf material.

SCIENCE SECTION

Introduction

Despite voluntary adherence to the code of Good Agricultural Practice (GAP), the UK Lettuce industry currently has no proven system that can guarantee nitrate levels in the harvested crop will be below EC regulation number 563/2002 (Table 1).

Table 1: Summary of maximum nitrate levels in European Commission Regulation (EC) No. 563/2002.

Product	Harvest Period	Maximum nitrate levels mg/kg fresh product
Spinach (Fresh)	1st November - 31st March	3000
	1st April – 31st October	2500
Lettuce (protected and open-grown lettuce)	1st October – 31st March	4500
	1st April – 30th September	3500

The derogation awarded to UK Lettuce growers was reviewed in January 2005 and the Commission agreed to extend this based on evidence that codes of practice were currently applied, that UK growers have ongoing difficulties in keeping nitrate below the maximum levels, and that there are current or planned investigations to help identify ways to lower these levels (FSA update, July 2005). Exceedances of nitrate concentration in lettuces mainly occur after periods of low light levels, particularly in the winter. Low rates of photosynthesis in these instances result in slower plant growth that does not appear to be matched by a decrease in nitrate uptake from the soil.

Nitrate uptake into the xylem of plants is a process that requires energy and it has also been shown that nitrate itself can stimulate its own uptake (Taiz & Zeiger, 1992). Once inside the plant cell, nitrate is converted to ammonia before assimilation into organic compounds. The enzyme that is responsible

for the initial conversion of nitrate to nitrite is nitrate reductase (NR). This enzyme is therefore extremely important in the prevention of accumulation of excess nitrate in the vacuoles of plant cells. Genetic or environmental factors that decrease NR activity will affect the levels of nitrate accumulated in leaves.

HDC report, PC 88, highlighted that there was a great deal of variation within heads of the same cultivar and that there were no obvious differences between cultivars tested. The timing of harvest did not affect nitrate levels, even on sunny days. However, there was a tendency for lower nitrate residues in lettuce after bright days than after dull weather.

Byrne e*t al* (2001) looked at the distribution of nitrate within the plant and found that the lettuce heart contained the least nitrate (2880 mg/kg), surrounding leaves contained moderately high levels (4703 mg/kg) and outer leaves the highest levels (6000 mg/kg). This agrees with other work and highlights the importance of removing older leaves as a means of decreasing nitrate in the product at point of sale.

An HDC-funded project, PC 245, commissioned in summer 2005 looked at the effect of spectrally modifying plastics on the harvest nitrate content of baby leaf and lettuce. The project evaluated a range of photo-selective plastics covering Haygrove tunnels to identify whether nitrate content at harvest could be reduced by propagating lettuce in these structures before planting in the field. A second study looked at the potential of using these covers post planting to reduce nitrate content following standard glasshouse propagation. Results showed that the nitrate content of Lollo bionda plants at harvest was not affected by the film cover material used during propagation prior to field planting. Nitrate levels in butterhead lettuce propagated under glass and then planted in 5 tunnels covered with a range of photo-selective plastics was not affected by the post-planting regime.

A further HDC-funded project, PC 243, commissioned in summer 2005 looked at the effects of irrigation method and partial root drying on crop nitrate

levels at harvest using 2 planting dates in the autumn. The results showed that high quality lettuce could be produced by using trickle irrigation and where 20% less water was applied there were higher residual soil nitrogen levels. Neither the irrigation method nor using 20% less water affected the nitrate levels in lettuce at harvest. Levels were below the EC limit but there was considerable variability, up to 1045ppm between sub-samples of lettuce harvested from the same plot.

HDC-funded project, PC 263, commissioned in summer 2007 looked at the effect of soil nitrogen fertiliser levels and shading. Nitrogen fertiliser had been applied to achieve 30, 75, 100 and 200ppm nitrogen using conventional overhead irrigation and trickle system. The results showed no effect of soil nitrogen levels on the nitrate content of the lettuce at harvest. Even where soil levels had been increased to 200ppm the lettuces were below the EC limit of 4500ppm. Residual soil nitrogen levels at harvest did increase as the rate of nitrogen fertiliser was increased. Soil nitrogen levels decreased by 50% in the 2½ weeks before harvest.

The second part of PC 263 looked at the effect of shading with soil nitrogen levels increased to 100ppm following the Good Agricultural Practice guidelines. The shading treatments involved suspending either 1 or 2 layers of non-woven fleece (18g/m^2) to create a 15 or 30% reduction in light levels. These were applied to the crop at 4, 3, 2 or 1 week before harvest (15, 22 or 29 January and 5 February). At harvest there was no effect of shading treatment on nitrate levels in the heads with some samples exceeding the 4500ppm EC limit even where no shading had been applied over the crop. The reasons for the lack of effect are unclear.

Objectives

The aim of this work was to compare a range of cultivars of both butterhead and curly lettuce types for their nitrate levels when harvested at an early maturity stage and a week later when more of the outer leaves would be removed during normal trimming. The variation in nitrate levels between

individual heads and within different parts of the head was also studied for both lettuce types to identify whether the large differences observed between lettuce samples could be better understood.

Trial details

Site

The trial was undertaken at Stockbridge Technology Centre, Cawood, Selby, North Yorkshire, YO8 3TZ. The crops were grown in Venlo glasshouses with a 3.3m ridge height with a floor area of 148m^2.

Part 1: Comparing the nitrate content in a range of butterhead and curly lettuce cultivars.

Treatments

A. Cultivars

1. Edgar (Nunhems)
2. Sibel (Nunhems)
3. Brian (Nunhems)
4. Wiske (Rijk Zwaan)
5. Abeba (Rijk Zwaan)
6. E115055 (Enza)
7. Jillis (Enza)
8. Mirata (Nunhems)
9. Gatwick (Rijk Zwaan)

1-6 are butterhead types and 7-9 are curly types

B. Harvest stage

1. Marketable weight of approx 180-200g with minimal trimming
2. 7 or 8 days later after the first harvest

Details

Seed of both lettuce types was sown into peat blocks on the 4 November 2008 and propagated in a standard glasshouse. Nitrogen fertiliser was added to the soil based on soil analysis, to increase the level to 100ppm using ammonium nitrate.

Lettuces were planted on 12 January 2009 at a spacing of 20 x 20cm, with 4 replicates of each treatment. Each plot had 3 rows of plants with 15 plants in each row.

At the first harvest 12 heads from one row on each plot were carefully cut with just the very outer soiled leaves removed to produce a marketable head of about 180g. At the second harvest either 7 or 8 days later another 12

heads from the adjacent row were cut with the outer 3 or 4 leaves removed to produce a marketable head.

Records and assessments

Leaf nitrate analysis at harvest

At each harvest one box of 12 heads was cut from each replicate plot and sent to NRM Laboratories for nitrate analysis. Each set of 12 heads was harvested from a single row 2.2m long.

Glasshouse Environmental Monitoring

The number of sun hours and radiation levels were monitored throughout the trial. The data are presented in Appendix I.

Statistical Analysis

Analysis of variance was undertaken on the raw data set. Comparisons were made between means based on the least significant difference (LSD) and a 95% confidence interval was used for all analyses.

Part 2: Determining head to head and within head variability in nitrate content in butterhead and curly types.

A block of lettuce (6 rows x 22 plants (butterhead) or 6 rows x 44 plants (curly)) was planted on 12 January 2009 and grown adjacent to the main trial area. At harvest 2 (curly) or 3 samples (butterhead) of 10 adjacent heads from the centre of each plot were harvested and sent to NRM Laboratories for nitrate analysis.

The samples were analysed as follows:

Butterhead type cultivar Brian

Sample A – 10 individual heads were harvested with minimal trimming. Ten nitrate analyses were undertaken on the whole heads.

Sample B – 10 individual heads were harvested with minimal trimming and at NRM each leaf was carefully cut into 2 down the mid rib. All the left hand halves were bulked together as sample 1 and the right hand halves bulked together as sample 2. Twenty nitrate analyses were undertaken.

Sample C – 10 individual heads were harvested with minimal trimming and at NRM each leaf was carefully dissected to remove the mid rib from the surrounding leaf material. All the mid ribs were bulked together to form one sample and the rest of the leaf material bulked together to form another sample. Twenty nitrate analyses were undertaken.

Curly type cultivar Gatwick

Sample A – 10 individual heads were harvested with minimal trimming. Ten nitrate analyses were undertaken on the whole heads.

Sample B – 10 individual heads were harvested with minimal trimming and at NRM each leaf was carefully cut into 2 down the mid rib. All the left hand halves were bulked together as sample 1 and the right hand halves bulked together as sample 2. Twenty nitrate analyses were undertaken.

Part 3: Sampling of commercial lettuce crops

This part of the project was undertaken by Colin Bloomfield on a West Sussex nursery.

Lettuces were cut and 10 heads sent to NRM laboratories for individual nitrate analysis.

The sample details are given below:

Sample number	Harvest date	Growing method	Cultivar	Number of heads
1	Week 45	DWH	Brian	10
2	(2008)	Soil	Edgar	10

3	Week 4 (2009) Week 4 (2009)	DWH	Edgar	10

Each head was carefully trimmed and weighed before sending for analysis.

Results

The results for each part of the project are presented separately. Nitrate results for each replicate can be found in Appendix II.

Part 1: Comparing the nitrate content in a range of butterhead and curly lettuce cultivars.

Establishment after planting was excellent. All plots looked similarly vigorous throughout the growing period.

The results for the butterhead types are presented in Tables 2 and 3.

Table 2: Nitrate levels in butterhead types – harvested on 30 March 2009 (ppm)

Cultivar	Nitrate level (ppm)	Range (ppm)	Mean head weights (g)	Range (replicates)
Edgar	3065	2795-3424	182	159 – 193
Sibel	3305	3087-3490	186	148 – 208
Brian	3471	3029-4022	180	174 – 200
Wiske	3219	2897-3410	195	179 – 206
Abeba	2985	2817-3122	186	147 – 202
E115055	3040	2777-3215	190	161 - 207
SED (24df) for comparing cultivars	188.5			
LSD (5%)	389.1 (*)			

There were significant differences between the six cultivars. Brian had significantly higher nitrate levels than Edgar, Abeba and E115055.

However, the nitrate content was well within the EC limits for all cultivars despite harvesting the lettuce at an early maturity stage and removing only the minimum number of leaves with soil on them. There was large variation between the 4 replicates.

Table 3: Nitrate levels in butterhead types – harvested 7 days later on 6 April 2009 (ppm)

Cultivar	Nitrate level (ppm)	Range (ppm)	Mean head weights (g)	Range (replicates)
Edgar	2544	1927-3052	215	207 – 224
Sibel	2620	2303-3105	222	209 – 238
Brian	2579	2454-2764	217	208 – 224
Wiske	2539	2237-2719	225	214 – 241
Abeba	2268	1652-2671	224	214 – 239
E115055	2618	2179-2848	219	206 - 229
SED (24df) for comparing cultivars	237.4			
LSD (5%)	490.0 (NS)			

Delaying harvesting by 7 days allowed more of the older leaves to be removed whilst still meeting the minimum weight of 180g. There were no significant differences in the nitrate content between the six cultivars. Nitrate levels were generally at least 400ppm lower at this second harvest. There was again large variation between the 4 replicates.

The results for the curly types are presented in Tables 4 and 5.

Table 4: Nitrate levels in curly types – harvested on 6 April 2009 when heads were approx 180-200g with minimal trimming (ppm)

Cultivar	Nitrate	Range	Mean	Range

	level (ppm)	(ppm)	head weights (g)	(replicate)
Jillis	3149	2919-3481	214	199 – 230
Mirata	3917	3574-4070	200	176 – 221
Gatwick	3148	2728-3579	217	195 - 232

Nitrate levels in the curly types showed larger differences between the cultivars. Mirata had higher nitrate levels than the other 2 cultivars but this cultivar is not normally grown at this time of the year. Mirata had a paler green colour which would suggest potentially lower nitrate levels but this was not the case.

Table 5: Nitrate levels in curly types – harvested 8 days later on 14 April 2009 (ppm nitrate).

Cultivar	Nitrate level (ppm)	Range (ppm)	Mean head weights (g)	Range (replicate)
Jillis	2617	2547-2680	248	237 – 262
Mirata	2856	2573-3083	233	223 – 242
Gatwick	2391	2228-2547	257	250 - 270

Delaying harvesting by 8 days gave lettuce with lower nitrate content as more of the older leaves were removed during trimming. Nitrate levels were generally at least 500ppm lower at the second harvest.

Part 2: Determining head to head and within head variability in nitrate content in butterhead and curly types.

The results for the head to head variability study for butterhead are presented in

Table 6.

Table 6: Nitrate content in 10 individual heads and dissected leaves for Brian harvested on 29 March after minimal trimming (ppm)

Head number	Sample A		Sample B		
	Whole	Head	Left hand	Right hand	Head

	head nitrate (ppm)	weight (g)	side nitrate (ppm)	side nitrate (ppm)	weight (g)
1	3782	209	3548	3446	224
2	3401	188	3964	3831	222
3	3707	215	3517	3450	244
4	3286	184	3215	3490	249
5	3565	183	3322	3805	239
6	3649	184	3441	3149	221
7	3277	206	3459	3743	225
8	3920	186	3220	3180	228
9	3339	184	3623	3038	210
10	3406	235	3623	3583	196
Mean	**3533**	**197**	**3493**	**3472**	**226**

The results showed a 643ppm variation between the 10 butterhead lettuce grown adjacent to each other in the glasshouse. Despite careful trimming and similar head weights this variation again confirms the large natural variability between apparently similarly looking heads.

Dividing each leaf individually into 2 halves and analysing the 2 sets of leaves gave large differences with 5 samples having a difference of over 200ppm and 2 samples having a difference of over 600ppm. The mean nitrate content of the 10 whole heads and those where each leaf had been cut in half were very similar.

The results for the head to head variability for curly are presented in Table 7.

Table 7: Nitrate content in 10 individual heads and dissected leaves for Gatwick harvested on 6 April after minimal trimming (ppm)

Head number	Sample A		Sample B		
	Whole head nitrate (ppm)	Head weight (g)	Left hand side nitrate (ppm)	Right hand side nitrate (ppm)	Head weight (g)
1	3410	278	3849	3100	285
2	3680	264	3428	3189	285
3	3809	273	3114	3605	231
4	3486	223	3477	3326	263
5	3574	227	3375	3534	237
6	3791	297	3224	3525	270
7	3384	223	3676	3587	265

8	3751	233	3725	3260	281
9	3654	263	3264	3441	245
10	3587	261	3534	2954	255
Mean	**3613**	**254**	**3467**	**3352**	**262**

The results showed a 425ppm variation between the 10 heads of curly lettuce grown adjacent to each other in the glasshouse. Trimming the heads was more difficult than for the butterhead. The crop could have been harvested earlier so that the heads were closer to the 180g minimal weight, but this harvest was scheduled based on the maturity of the cultivar trial plots.

Dividing each leaf individually into 2 halves and analysing the 2 sets of leaves gave large differences with 6 samples having a difference of over 200ppm and 4 samples having a difference of over 400ppm. The mean nitrate content of the 10 whole heads and those where each leaf had been cut in half was overall quite similar when considering the normal variability observed between samples.

Table 8: Nitrate content in rib and leaf material for 10 individual heads for Brian harvested on 30 March 2009 after minimal trimming (ppm)

Head number	Nitrate content (ppm)		Head weight (g)
	Leaf	Rib	
1	3822	4061	213
2	3131	4278	223
3	3840	4230	222
4	3503	3729	204
5	2905	4092	175
6	3667	4637	186
7	3521	4101	210
8	3446	3548	189
9	3331	3844	205
10	3246	4447	183
Mean	**3441**	**4097**	**201**

Overall the rib material had higher nitrate levels than the green leaf material. However due to the lower proportion of the rib material relative to the leaf material it should not significantly affect the overall nitrate content of the heads. However, for crops harvested at an early maturity stage then on occasions the outer leaves which can have more rib might adversely affect the mean nitrate content of the sample.

Part 3: Sampling of commercial lettuce crops

The results obtained from samples taken from commercial crops are presented in Tables 9 and 10.

Table 9: Nitrate levels in a commercial deep water hydroponic grown lettuce crop of Brian harvested in Week 45 (2008) (ppm)

Head number	Nitrate levels (ppm)	Head weight (g)
1	4336	204
2	3915	196
3	3964	166
4	3862	192
5	3654	190
6	3720	194
7	3981	188
8	4194	176
9	4083	198

10	3888	218
Mean	**3960**	**192**

The nitrate content of the individual heads showed relatively large variability between the heads (682ppm). There did not appear to be any obvious correlation between nitrate content and head weight.

Table 10: Nitrate levels in commercial deep water hydroponic and soil grown lettuce crop of Edgar harvested in Week 04 (2009) (ppm)

Head number	Soil grown		DWH grown	
	Nitrate level (ppm)	Head weight (g)	Nitrate level (ppm)	Head weight (g)
1	3499	140	4624	156
2	3308	152	4309	146
3	3047	152	4664	140
4	3880	140	4154	194
5	3552	150	4278	164
6	3836	164	4181	152
7	3986	162	4518	154
8	4137	150	4686	166
9	3587	154	4646	156
10	3791	150	4389	150
Mean	**3662**	**151**	**4445**	**158**

Nitrate levels in the soil and deep water hydroponic grown crops which were planted on the same date and harvested at the same time showed that the soil grown crop had lower nitrate levels than the deep water hydroponic crop.

For the soil crop there was a variation of 1090ppm between the highest and lowest sample compared to 532ppm for the DWH crop.

Discussion

This project has confirmed that harvesting lettuce at an early maturity stage gives crops with a higher nitrate content than in crops where harvesting is delayed. At the early harvest more of the older leaves are retained and these contain more nitrate than the younger inner leaves.

Table 11: Mean nitrate levels in butterhead lettuce at early maturity and 7 days later and the decrease between harvests (ppm)

Cultivar	Harvest 1 (ppm)	Harvest 2 (ppm)	Decrease between H1 and H2	% decrease
Edgar	3065	2544	521	17
Sibel	3305	2620	685	21
Brian	3471	2579	892	26
Wiske	3219	2539	680	21
Abeba	2985	2268	717	24
E115055	3040	2618	422	14

When analysed over both harvest dates the cultivar Abeba had significantly lower nitrate levels than Sibel and Brian. The percentage decrease between Harvest 1 and 2 was generally similar for each cultivar. This suggests that nitrate accumulation in the older leaves was similar for all the cultivars.

Table 12: Mean nitrate levels in curly lettuce at early maturity and 8 days later and the decrease between harvests (ppm)

Cultivar	Harvest 1 (ppm)	Harvest 2 (ppm)	Decrease between H1 and H2	% decrease
Jillis	3149	2617	532	17
Mirata	3917	2856	1061	27
Gatwick	3148	2391	757	24

A similar pattern of results was recorded for the curly lettuce.

Although nitrate levels were higher in Mirata at Harvest 1 this cultivar is not recommended for cropping at this time of the year.

The variability between 10 individual heads grown in close proximity to each other indicates the inherent variability that can be observed between heads. The results for both types are summarised below.

Table 13: Highest, lowest, mean and median values for the 10 individual heads of each lettuce type

Variant	Nitrate levels (ppm)	
	Butterhead	Curly
Highest value	3920	3809
Lowest value	3277	3384
Mean	3533	3613
Median	3486	3621

Considerable variation exists between individual heads and this needs to be taken into account when setting maximum nitrate levels. A measurement of uncertainty factor needs to be included when reporting nitrate results in a similar way to that used for determining whether a sample has exceeded a Maximum Residue Limit (MRL) for a pesticide.

The differences in the nitrate content of the right and left hand leaf samples prepared from each of the 10 heads was variable and is summarised below.

Table 14: Differences in nitrate content between the right hand and left hand samples from 10 individual heads of each lettuce type

Head number	Nitrate levels (ppm)	
	Butterhead	Curly
1	102	749
2	133	239
3	67	491
4	275	151
5	483	159
6	291	301
7	284	89
8	40	465
9	585	177
10	40	580
Mean	**230**	**340**

With differences of this magnitude between carefully prepared samples it is possible that the natural variability in nitrate content between heads could

potentially make a crop unmarketable by exceeding the 4500ppm nitrate limit. This suggests that a high number of heads should be used to increase confidence in the results.

Lettuce crops grown in deep water hydroponic systems would be expected to grow more uniformly than soil grown crops as head to head competition should be less as they are not competing directly for water and nutrients. The results for the commercial samples are summarised below.

Table 15: Highest, lowest, mean and median values for the 10 individual heads of each lettuce types from the commercial sites

Variant	Crop 1	Crop 2	
	DWH	Soil	DWH
Highest value	4336	4137	4686
Lowest value	3654	3047	4154
Mean	3960	3662	4445
Median	3940	3689	4453

The large variation in nitrate levels recorded in trial plots was also recorded in commercial crops. The reasons for the higher nitrate levels in the lettuces grown in deep water hydroponic systems for Crop 2 are unclear with large differences recorded between the highest and lowest values for all 3 sets of nitrate analysis.

Conclusions

1. The potential for growers to reduce nitrate levels in butterhead and curly types by using particular cultivars looks to be limited.

2. There can be a very significant reduction in nitrate levels by delaying harvesting by a week during March so that more of the outer leaves are removed during trimming.

3. Delaying harvesting by a week reduces the productivity of the glasshouse but it is an important way to help avoid the risk of crops exceeding the maximum EC nitrate levels.

4. Despite careful growing and harvesting procedures the natural variability in nitrate content in lettuce can be large. This is both between heads and within heads.

5. Head to head variability was lower in lettuce grown in a deep water hydroponic system compared to growing in the soil.

Recommendations

Further work should be undertaken to:

1. **Evaluate the effect of different shading regimes and the plants response to periods of lower light throughout different parts of the growing period** – although the results in a previous study showed little effect growers need further information on what the effects of reduced light levels and day length are on crop nitrate levels. A greater range of cover durations at different times of the year to simulate various light levels might enable a better understanding of how light affects nitrate content in plant material. This might then be used to decide whether to delay harvesting.

Technology Transfer

The results have been discussed with members of the industry at a Leafy Salads seminar at STC on 29 April 2009.

Acknowledgements

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Appendix I: Radiation and number of sun hours

Date	Radiation (Watts/m²)	Sun hours	Date	Radiation (Watts/m²)	Sun hours
16 Jan	718	0.2	2 Mar	3633	5.4
17	1614	3.4	3	1406	0
18	1958	5.3	4	4118	4.3
19	572	0	5	5660	8.2
20	1958	6.1	6	6396	8.9
21	1929	4.8	7	2348	0
22	1293	1.7	8	4044	5.5
23	1490	2.0	9	4645	6.6
24	1300	0.7	10	5078	6.1
25	1454	0.9	11	4192	1.6
26	433	0	12	3462	2.1
27	1165	0	13	3326	1.8
28	991	0	14	4568	3.7
29	1595	0	15	5034	4.1
30	1218	0	16	5222	2.1
31	2314	2.6	17	6283	5.8
1 Feb	736	0.1	18	5821	5.8
2	1668	1.1	19	3583	1.7
3	2736	6.1	20	6574	6.5
4	2083	2.8	21	7215	8.0
5	852	0	22	5290	4.4
6	3013	4.2	23	4761	5.5
7	2842	4.2	24	6039	5.5
8	2966	3.3	25	6738	9.6
9	2578	2.8	26	5413	6.5
10	3016	5.9	27	5838	7.5
11	1326	2.2	28	4308	3.0
12	816	0	29	9120	9.2
13	3738	7.1	30	2998	2.5
14	1894	0	31	5497	2.0
15	1690	1.2	1 April	5652	4.2
16	2078	0	2	5529	5.4
17	1737	0.2	3	4406	3.9
18	504	0	4	6506	6.7
19	1963	0	5	8231	8.5
20	2476	1.0	6	4309	0
21	3678	4.7	7	7801	6.8
22	2566	2.3	8	5366	5.9
23	1932	0	9	3394	0
24	2608	2.0	10	3693	1.2
25	1772	0.6	11	3866	0
26	950	0	12	4403	0.4
27	2022	0.3	13	6615	4.7
28	1628	0	14	4534	1.0
1 Mar	3391	3.8			

Appendix II: Nitrate results for each replicate

Table A: Nitrate levels in butterhead types – harvested on 30 March 2009 (ppm)

Cultivar	Rep 1	Rep 2	Rep 3	Rep 4	Mean
Edgar	3220	2821	2795	3424	3065
Sibel	3339	3490	3304	3087	3305
Brian	3574	4022	3029	3260	3471
Wiske	3202	2897	3366	3410	3219
Abeba	3100	2901	2817	3122	2985
E115055	3215	2777	3193	2976	3040

Sample size: 12 heads per plot

Table B: Nitrate levels in butterhead types – harvested on 6 April 2009 (ppm)

Cultivar	Rep 1	Rep 2	Rep 3	Rep 4	Mean
Edgar	2657	1927	2538	3052	2544
Sibel	2520	2303	2551	3105	2620
Brian	2454	2764	2564	2533	2579
Wiske	2719	2578	2237	2622	2539
Abeba	2423	1652	2671	2325	2268
E115055	2848	2662	2179	2781	2618

Sample size: 12 heads per plot

Table C: Nitrate levels in curly types – harvested on 6 April 2009 (ppm)

Cultivar	Rep 1	Rep 2	Rep 3	Rep 4	Mean
Jillis	3481	2919	3047	3149	3149
Mirata	3574	4057	3968	4070	3917
Gatwick	3273	3579	2728	3012	3148

Sample size: 12 heads per plot

Table D: Nitrate levels in curly types – harvested on 14 April 2009 (ppm)

Cultivar	Rep 1	Rep 2	Rep 3	Rep 4	Mean
Jillis	2680	2578	2662	2547	2617
Mirata	2990	2777	3083	2573	2856
Gatwick	2547	2401	2387	2228	2391

Sample size: 12 heads per plot