FINAL REPORT

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Project Leader:	M Hardgrave
Location of Project:	Horticulture Research International Stockbridge House Cawood Selby North Yorkshire YO8 OTZ Tel: 01757 268275 Fax: 01757 268996
Project Co-ordinators:	G Hayman, G Ward
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A REPORT TO THE HORTICULTURAL DEVELOPMENT COUNCIL
18, LAVANT STREET, PETERSFIELD, HANTS, GU32 3EW

GLASSHOUSE LETTUCE: REDUCTION OF NITRATE RESIDUES

Authentication

I declare that this work 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 .... M Hardgrave
Project Leader

Date ..6/12/94......

Report authorised by ....
(signature)

M R Bradley
Head of Station
HRI Stockbridge House
Cawood
Selby
North Yorkshire
YO8 OTZ

Date ....8.12.94....

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SUMMARY

It has been suggested that ingesting large amounts of nitrate can be detrimental to human health, although the medical evidence is unclear. Because a high percentage of dietary intake comes from vegetables, there is a European Community (EC) initiative to set maximum residue levels for nitrate in vegetables.

Because of disagreements between EC member states limits have not been finalised. At suggested levels of 2500 ppm in May to October, and 3500 from November to April UK growers would be unable to comply, particularly in summer. It is proposed that these limits will apply from May 1997 but there is likely to be a period before that date with temporary interim levels.

This work carried out at HRI Stockbridge House aimed to collate data currently available on measured levels in commercial crops and to evaluate cultural techniques designed to minimise residue levels without influencing crop productivity.

Data Collection

The results of nitrate tests on commercial crops from all over the UK were collected to give an overall view of UK grower ability to comply with the proposed legislation. Figures for 1993 show that only 19% of samples achieved the limits proposed for 1997 onwards and 54% did not meet the interim levels proposed for 1995.

Experimental Work

Experimental work evaluated the effects of cultivar, fertiliser, time of harvest and maturity at harvest on nitrate residues and crop productivity. Three trials were carried out between August 1993 and April 1994.

Testing throughout a uniform crop showed there to be a considerable variation in nitrate residues from one head to another. Therefore, sampling procedures must be carefully specified.

There were significant differences in nitrate residues between cultivars. In summer, residues in Titania were lower than those in Diego. It is important that residue testing is incorporated into all variety trials and comparative data is provided for growers.

Where a crop was more mature at harvest and extra trimming was required nitrate residues were reduced but quality also suffered. Removing extra leaves can help to reduce total nitrate levels but this technique is not always commercially acceptable.

Harvesting in the afternoon, even on sunny days did not influence nitrate residues compared with morning harvests. Nitrate levels will, however, tend to be lower after bright periods than after dull weather.

In order to control fertiliser applications soil sampling before every crop is important. There is a relatively reliable "on farm" method for nitrate testing in soil solution. Very low nitrogen rates can be applied with no detriment to the crop if residual levels are high.

Low base rates followed by sap testing and liquid fertiliser gave good crops but maintained residues at standard levels. The technique needs improvement but offers potential for those who want a controlled way of adding nitrogen as liquid feeds.

Low base rates applied to the planting rows only, successfully reduced residues without affecting crop quality. The method is fairly complicated to use but shows that low base rates without further liquid applications can be useful in certain situations.

The organic content of the soil must be considered when making fertiliser decisions. Nitrification from organic matter will provide nitrogen for the crop but the rate of release is unpredictable. This makes fertiliser management more difficult for those on organic soils and after incorporation of organic material.

Nitrate Testing Methods

Sap Testing was useful in calculating the need for liquid fertiliser applications but was not suitable for determining nitrate residues in whole heads.

The 'Kitchen Method' gave a much more reliable method of determining total nitrate residues at home. This method requires careful use to minimise errors but has been tested successfully on one commercial nursery. Tests show good correlation with laboratory analysis.

Conclusions

1. UK growers are unable to consistently meet the proposed EC maximum residue levels for nitrate in lettuce.

2. Even within a uniform crop there is variation between heads.

3. There is significant variation in nitrate residues between some varieties, but no variety offers a consistent way of achieving the proposed levels.

4. Extra trimming reduces total nitrate residue in the head, but may not be commercially acceptable.

5. Time of day when the crop was harvested did not affect residues in this trial.

6. Sap testing and liquid fertiliser applications maintained quality but did not reduce residues, at the rates used in the trial.

7. Application of half rate fertiliser in the planting rows maintained quality and reduced nitrate residues.

8. Sap testing did not offer a reliable indication of nitrate residue in the head at harvest.

9. The Kitchen Method of nitrate testing gave a reasonable indication of total nitrogen content.

SCIENCE SECTION

INTRODUCTION

It has been reported that ingesting large amounts of nitrate can be detrimental to the health of the consumer although medical evidence to support this is unclear. It is estimated that over 60% of dietary nitrate intake comes from vegetables and there is therefore a European Community (EC) initiative to reduce accepted maximum residue levels in certain vegetables in order to reduce dietary intake.

Lettuce has a greater capacity to accumulate nitrate in it's foliage than most other widely consumed vegetables and is therefore a high priority in the crops listed for the reduction of nitrate.

The EC currently aim to impose maximum residue limits of 2500 ppm nitrate in fresh material from May - October and 3500 ppm from November - April from May 1997. As an interim measure, from January 1995, levels of 4500 ppm nitrate from November - February, 3500 in March, April, September and October, and 2500 from May - August are proposed. With current growing practices these levels would be impossible to achieve and earlier MAFF funded work at HRI Stockbridge House confirms this view (Richardson & Hardgrave, 1992). Due to objections from several member states, particularly the UK, a working group was set up in July 1994 and the final decision will probably be delayed.

There is therefore a need to establish realistic year round targets for the U.K. and to develop cultural techniques whereby levels can be minimised.

The work is to be carried out over three years including an initial survey of literature and currently available data on nitrate levels.

OBJECTIVES

(i) To collate data currently available on measured nitrate levels in commercial crops.

(ii) To evaluate cultural techniques designed to reduce nitrate levels in plant material without sacrificing crop quality.

MATERIALS AND METHODS

1. Collection of data

1.1 Literature search

A computer search of the Commonwealth Agricultural Bureau Abstracts was carried out for the years 1984 - 1994, using the keywords 'lettuce', 'Lactuca sativa', and 'nitrates'.

1.2 Discussions with Dutch and Danish researchers

Close links have been formed with Wietse Post of PTG at Naaldwijk, a soil scientist specialising in studying nitrates in lettuce. His main field of work is NFT cropping but regular exchanges of views between Mr Post and Mrs Hardgrave ensure no duplication of work and that no opportunities are missed. In Denmark, Jans Willumsen is only just beginning trials work on nitrates in lettuce, working on soil grown crops. He is very keen to work closely with the UK on this issue and links have been established.

1.3 Liaison with U.K. lettuce growers and collection of currently available data

1.3.1 The subject of nitrate residues in lettuce was discussed at meetings of the Lettuce Technology Group in September 1993 and May 1994.

1.3.2 Mrs Hardgrave attended a meeting of Northern EC member states in Brussels with grower representatives and researchers to discuss the issue.

1.3.3 Mrs Hardgrave met MAFF officials in London in August 1993 to brief them on the current situation.

1.3.4 HRI Stockbridge House hosted a fact finding mission by MAFF officials in December 1993.

1.3.5 Trials were on view and results were discussed at a lettuce growers walk on 17 August 1994.

1.3.6 Currently available data from lettuce growers was collected and collated. This data is presented in Appendix II. The information has been used to regularly update MAFF on the current situation.

2. Experimental Work

Three experiments were carried out on the same site throughout the year to evaluate the treatment effects in a continuous cropping situation and to record season differences.

2.1 Treatments

2.1.1 Fertilizers

Each fertilizer treatment was applied to the same plots throughout the three trials to assess the affect of a build up of that treatment.

(i) Nil Control - No nitrogen (N) fertilizer applied to the plots, even at low indices.

(ii) Standard recommended base dressing (MAFF Reference Book 209) - Nitrogen fertilizer applied according to N index, evenly broadcast on the soil and raked in before planting.

(iii) 1/2 rate base dressing, monitoring with test strips and liquid feeding as necessary - Half the N recommendation according to N index applied to soil as above. Sap analysis, twice weekly, from the 7 true leaf stage using nitrate test strips. If the nitrate content of the plants fell below the pre-designated targets then liquid feed was applied through seephose. Note - when this treatment received liquid feed all other treatments received the same amount of water.

(iv) 1/2 rate base dressing applied to the plant rows only - Half N recommendation according to N index. This was then placed on the soil in rows which corresponded to the planting rows of the crop and raked in carefully to maintain the rows. This method of application meant that 1/2 the recommended amount of N was applied to 1/2 the planting area and therefore in the planting area the N concentration was at full rate.

All other fertilizers were applied according to the index, following soil analysis.

2.2 Maturity

(i) As soon as plants reached marketable weight.

(ii) Latest acceptable harvest date.

2.3 Harvest time

(i) Harvest before 9am

(ii) Harvest after 3pm

2.4 Varieties

The choice of variety depended on the season.

	Summer Harvest	Winter Harvest	Spring Harvest
(i)	Titania (Enza Zaden)	Rachel (Enza Zaden)	Rachel (Enza Zaden)
(ii)	Diego (Rijk Zwaan)	Sano* (Yates)	Sano* (Yates)

* 'Low nitrate' variety

3. Cultural Notes

All crops were grown in a sandy loam soil in a 900 m^2 Venlo glasshouse at HRI Stockbridge House

	Summer Harvest	Winter Harvest	Spring Harvest
Sowing Date:	14 Jul 93	1 Sep 93	6 Jan 94
Planting Date:	9 Aug 94	30 Sep 93	24 Feb 94
First Harvest:	6 Sep 93	14 Dec 93	21 Apr 94
Second Harvest:	13 Sep 93	22 Dec 93	28 Apr 94
Plant Spacing:	20cm x 20cm	20cm x 20cm	20cm x 20cm
Population (plants/m^2)	25	25	25
Plot size:	56 plants	56 plants	56 plants
Temperature Setpoints:	Day 8°C Night 6°C	Day 7°C Night 7°C	Day 4°C Night 4°C
Ventilation:	10°C	Day 13°C Night 15°C	8°C
Carbon Dioxide:	500 vpm	500 vpm	500 vpm

4. Design

The experiment was of the split-split-plot type with fertilizer treatments applied to main plots, variety and maturity treatments applied to split-plots within main plots and time of harvest treatments applied to split-split-plots within split-plots. The main plots were complete beds and the four main plot treatments were arranged in four completely randomised blocks of four plots. The four split-plot treatments were arranged as randomised latin squares within the four main plot treatments. The split-split-plot treatments were completely randomised within split-plots. See Appendix I for plans.

5. Explanation of Statistical Terms

Throughout the report a number of statistical terms are referred to; these are:

SED = The standard error of the difference when comparing two means in that column of data.

A statistical term easier to interpret:

LSD 5% = The least (minimum) difference when comparing any two figures within a given column that is required for those figures to be statistically different.

A number of common notations are also used to indicate the degree to which values are significantly different.

NS = Not significant.

* = P <0.05, ie. the probability of this result occurring by chance is equal to or less than 1 in 20 (0.05 = 5%).

** = P <0.01, ie. the probability of this result occurring by chance is equal to or less than 1 in 100 (0.01 = 1%).

*** = P <0.001, ie. the probability of this result occurring by chance is equal to or less than 1 in 1000 (0.001 = 0.1%).

6. Records

Head Weight (g)
Grade (Class I, II, Waste)
Percentage Trimmings
Head Quality - Heart, Colour, Base
Head size
Nitrate content

7. Soil Analysis

Soil analysis was carried out before and after each trial at 0-15 cm and 15-30 cm depth for N, P, K, Mg, EC, and pH

8. The Snappy Sap Test

Sap tests were carried on 10 plants when 7 true leaves were visible. A lower leaf from the lettuce was taken and sap from the petiole squeezed onto the test strip. The time taken for the reaction strip to reach a pre-determined colour was measured and this allowed the nitrate concentration in the plant to be determined. If the nitrate level was below 5000, 5g Ammonium Nitrate in 1 l water per m^2 was applied. Further sampling was carried out at 4 day intervals when 5g Ammonium nitrate /m2 was applied if the nitrate level dropped below 2500. The tests were carried out using Nitrate test strips (BDH Reagents division).

After the first two trials the method was amended to apply less nitrate at each liquid feed. 1g Ammonium nitrate was applied per m^2, in 2 l water.

9. The 'Kitchen' Test

This method was developed by soil scientists at HRI Wellesbourne and tested at Stockbridge House.

Ten lettuce were sampled from the crop, cleaned, and cut into segments. Approximately one eighth of each lettuce was selected to make a total weight of 200 g. This 200 g of lettuce was liquidised with 200 ml water, then the solution filtered through a funnel filled with cotton wool. 20 ml of the filtrate was added to 80 ml of water (100 ml in total) and mixed.

Using a Nitracheck test strip and refloctometer the nitrate content of the solution was measured. The test strip was dipped in the solution then placed in the meter to obtain a reading of the nitrate concentration in ppm, multiplied by 10 this gives the mean nitrate content of the lettuce sampled.

RESULTS

1. **Literature Search**

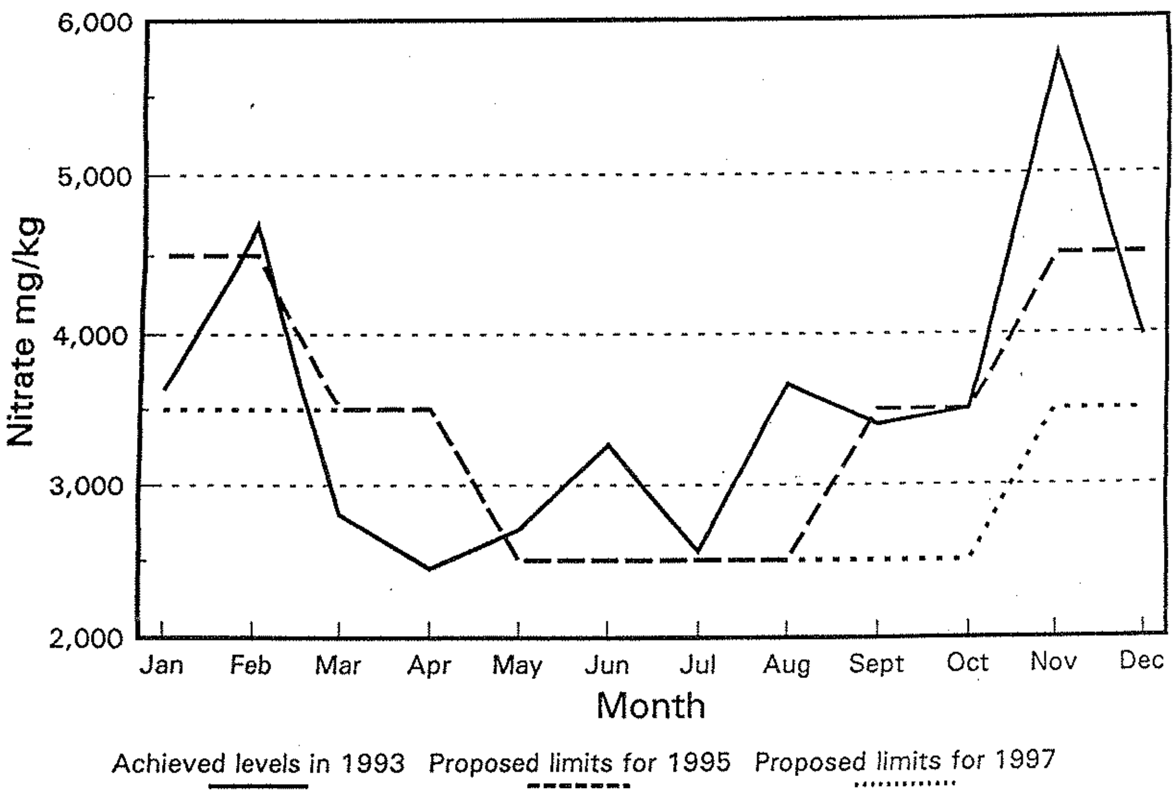
106 references and summaries were retrieved, these are presented in a separate appendix (Appendix VI).

2. **Data Collection**

The results of nitrate tests on commercial crops, were sent to Stockbridge House by growers from all over the country and collated to give an overall view of the UK glasshouse lettuce industries ability to meet proposed limits.

The results are presented in Fig 1 and Appendix II.

Fig 1: Achieved nitrate levels in 1993.



The figures for 1993 show that the average residue levels often exceeded the proposed final and interim limits.

Fig 1 shows average levels each month. The great variability between samples means that even when the mean was acceptable there were many samples that exceeded the limit.

In total 54% of 1993 samples did not meet the suggested interim levels (January 1995), rising to 97% in August.

Only 19% of samples achieved the proposed limits for May 1997 onwards.

3. Experimental Work

A series of three trials was carried out, with harvests in September, December and April to enable seasonal effects to be examined.

3.1 Nitrate Content

In the summer period no 'low nitrate' varieties were available and therefore two standard varieties were chosen. In the winter and spring the low nitrate variety Sano was available for testing.

Table 1 shows the nitrate content of the heads at harvest. In summer the levels achieved were slightly above the target level of 2500 ppm in the fresh material. In winter nearly all samples exceeded the limit of 4500 ppm but in spring all residue levels were acceptable.

3.1.1 Varieties

In summer there were significant differences in nitrate residues between the two standard varieties, showing the importance of including nitrate testing in variety trials. In both winter and spring no differences in nitrate content were recorded between Rachel and Sano.

3.1.2 Fertiliser

In summer, the first time the site was used for growing lettuce, no differences were recorded between the four fertiliser treatments, although the standard rate treatment had the highest residues. Where no nitrogen fertiliser was applied the levels in the soil were sufficient to grow a good crop and nitrate residues in the heads exceeded 2000 ppm (Table 1).

In the winter and spring trials differences between fertiliser treatments were recorded.

Where no nitrogen was applied plant nitrate content fell substantially but this was associated with severe deterioration in head quality.

In both winter and spring applying nitrogen fertiliser into the planting rows significantly reduced nitrate residues without affecting head weight or quality when compared with the control.

In both summer and winter the sap testing procedures indicated that liquid feeding was required. Analysis at harvest showed that the method was maintaining levels at the standard level rather than reducing them.

The planned amount of fertiliser applied at each application was therefore reduced for the spring trial. In this trial testing indicated that no liquid feeding was required due to high soil residues after the winter crop. A very good crop with reduced nitrate residues was produced. This technique has potential but needs further refinement before final recommendations can be made.

3.1.3 Maturity

In summer, delaying harvest by one week increased trimming by approximately 10%, but significantly reduced the nitrate content in the marketable heads.

In winter and spring, delaying the harvest date increased trimming by approximately 17% and 5% respectively and had no significant impact on nitrate residues.

This technique increased the amount of waste heads.

3.1.4 Time of Harvest

In all three trials the time of day that the crop was harvested had no effect on plant nitrate residues.

3.2 Head Weight and Quality

The greatest differences in head weight were between varieties. In summer Titania was heavier than Diego and in winter/spring Rachel was heavier than Sano (Table 2).

There was no clear relationship between head weight and nitrate content.

Putting the nitrogen fertiliser in rows gave slightly reduced head weights in the first trial but had no effect thereafter. In the third trial where no liquid feed was applied head weights were slightly lower in that treatment. Compared with varietal differences, the effects of the fertiliser treatments on head weight were small.

Delaying harvesting increased head weights in summer and spring but not in winter. The time that the crop was harvested did not affect head weight.

The quality of variety Sano was low in winter due to glassiness but in spring quality from both Rachel and Sano was good (Table 3).

The nil fertiliser treatment had a gradually increasing effect on head quality. In the first trial it was as good as the other treatments but by the third trial 65% of the heads were unmarketable due to their reduced size.

Delaying harvest generally reduced quality and increased waste (Tables 3-5).

The results for detailed quality assessments are given in Appendix III, Tables A to D.

Table 1: Nitrate content (ppm in fresh matter).

	Summer Harvest	**Winter Harvest**	**Spring Harvest**
<u>Varieties</u>			
Titania/Rachel/Rachel	2381	5134	1782
Diego/Sano/Sano	2637	5246	1605
SED (33 df)	73.0	198.0	110.6
LSD (P = 0.05)	149	-	-
Significance	***	NS	NS
<u>Fertilisers</u>			
Nil	2042	3788	397
Standard	2793	5704	1939
½ Base + liquid	2687	6019	1703
½ Base in rows	2514	5298	1439
SED (9 df)	130.5	278.6	99.6 +
LSD (P = 0.05)	-	569	243.7
Significance + to compare 3 fertilizer treatments	NS	* 8%	**
<u>Maturity</u>			
Minimum	2680	5068	1799
Latest	2338	5312	1589
SED (33 df)	73.0	198.0	110.6
LSD (P = 0.05)	149	-	-
Significance	***	NS	NS
<u>Time</u>			
a.m.	2491	5284	1592
p.m.	2527	5096	1795
SED (50 df)	56.2	150.6	119.5
LSD (P = 0.05)	-	-	-
Significance	NS	NS	NS

Table 2: Mean head weights (g).

	Summer Harvest	**Winter Harvest**	**Spring Harvest**
Varieties			
Titania/Rachel/Rachel	254	141	265
Diego/Sano/Sano	232	126	239
SED (33 df)	2.87	2.04	5.09
LSD (P = 0.05)	5.9	4.2	10.5
Significance	***	***	***
Fertilisers			
Nil	211	113	113
Standard	264	138	254
½ Base + liquid	256	138	247
½ Base in rows	241	144	256
SED (9 df)	6.66	4.75	2.14 +
LSD (P = 0.05)	13.6	-	5.2
Significance + to compare 3 fertilizer treatments	*	NS	**
Maturity			
Minimum	223	132	226
Latest	262	134	278
SED (33 df)	2.87	2.04	5.09
LSD (P = 0.05)	6.0	-	10.5
Significance	***	NS	***
Time			
a.m.	244	136	255
p.m.	241	130	249
SED (50 df)	3.39	1.72	4.50
LSD (P = 0.05)	-	7.4	-
Significance	NS	***	NS

Table 3: Percentage Class I Heads

	Summer Harvest	Winter Harvest	Spring Harvest
Varieties			
Titania/Rachel/Rachel	99.1	96.1	97.9
Diego/Sano/Sano	98.6	66.1	96.9
SED (33 df)	0.67	3.94	1.02
LSD (P = 0.05)	-	8.0	-
Significance	NS	***	NS
Fertilisers			
Nil	99.7	81.3	35.9
Standard	98.9	79.4	96.6
½ Base + liquid	98.5	87.8	99.0
½ Base in rows	98.2	76.0	96.6
SED (9 df)	1.01	6.30	0.84 +
LSD (P = 0.05)	-	-	2.1
Significance + to compare 3 fertilizer treatments	NS	NS	*
Maturity			
Minimum	99.5	78.8	99.5
Latest	98.2	83.5	95.3
SED (33 df)	0.67	3.94	1.02
LSD (P = 0.05)	1.4	-	2.1
Significance	* 7%	NS	***
Time			
a.m.	98.7	80.7	97.6
p.m.	99.0	81.5	97.2
SED (50 df)	0.59	3.25	0.93
LSD (P = 0.05)	-	-	-
Significance	NS	NS	NS

Table 4: Percentage Class II heads.

	Summer Harvest	Winter Harvest	Spring Harvest
<u>Varieties</u>			
Titania/Rachel/Rachel	0.5	0.4	0.5
Diego/Sano/Sano	0.2	18.0	0.5
SED (33 df)	0.38	2.52	0.48
LSD (P = 0.05)	-	5.1	-
Significance	NS	***	NS
<u>Fertilisers</u>			
Nil	0.2	3.9	0.0
Standard	0.0	9.4	0.8
½ Base + liquid	0.5	8.6	0.0
½ Base in rows	0.8	14.8	0.8
SED (9 df)	0.47	3.22	0.59 +
LSD (P = 0.05)	-	-	-
Significance + to compare 3 fertilizer treatments	NS	NS	NS
<u>Maturity</u>			
Minimum	0.0	14.7	0.2
Latest	0.8	3.7	0.9
SED (33 df)	0.38	2.52	0.48
LSD (P = 0.05)	0.8	5.1	-
Significance	*	***	NS
<u>Time</u>			
a.m.	0.8	10.8	0.4
p.m.	0.0	7.6	0.6
SED (50 df)	0.36	1.70	0.48
LSD (P = 0.05)	0.7	7.3	-
Significance	*	* 6%	NS

Table 5: Percentage unmarketable heads.

	Summer Harvest	Winter Harvest	Spring Harvest
<u>Varieties</u>			
Titania/Rachel/Rachel	0.4	3.0	1.6
Diego/Sano/Sano	1.1	15.6	2.6
SED (33 df)	0.50	2.64	1.04
LSD (P = 0.05)	-	5.4	-
Significance	NS	***	NS
<u>Fertilisers</u>			
Nil	0.0	14.6	64.8
Standard	1.0	10.4	2.6
½ Base + liquid	1.0	3.6	1.0
½ Base in rows	1.0	8.6	2.6
SED (9 df)	0.82	5.21	1.01 +
LSD (P = 0.05)	-	-	-
Significance + to compare 3 fertilizer treatments	NS	NS	NS
<u>Maturity</u>			
Minimum	0.5	6.1	0.4
Latest	1.0	12.5	3.8
SED (33 df)	0.50	2.64	1.04
LSD (P = 0.05)	-	5.4	2.1
Significance	NS	*	**
<u>Time</u>			
a.m.	0.5	7.8	2.1
p.m.	1.0	10.8	2.1
SED (50 df)	0.50	2.50	0.68
LSD (P = 0.05)	-	-	-
Significance	NS	NS	NS

3.3 Sample Variability

Samples of lettuce for analysis taken across a bed of lettuce (Figure 2) were assessed for variability in nitrate content. Figure 3 shows how the nitrate content changed along and across the bed. These variations may be due to variations in soil nitrogen, dry patches etc and indicate that care must be taken over the selection of lettuce for sampling.

Figure 2: Sampling Position

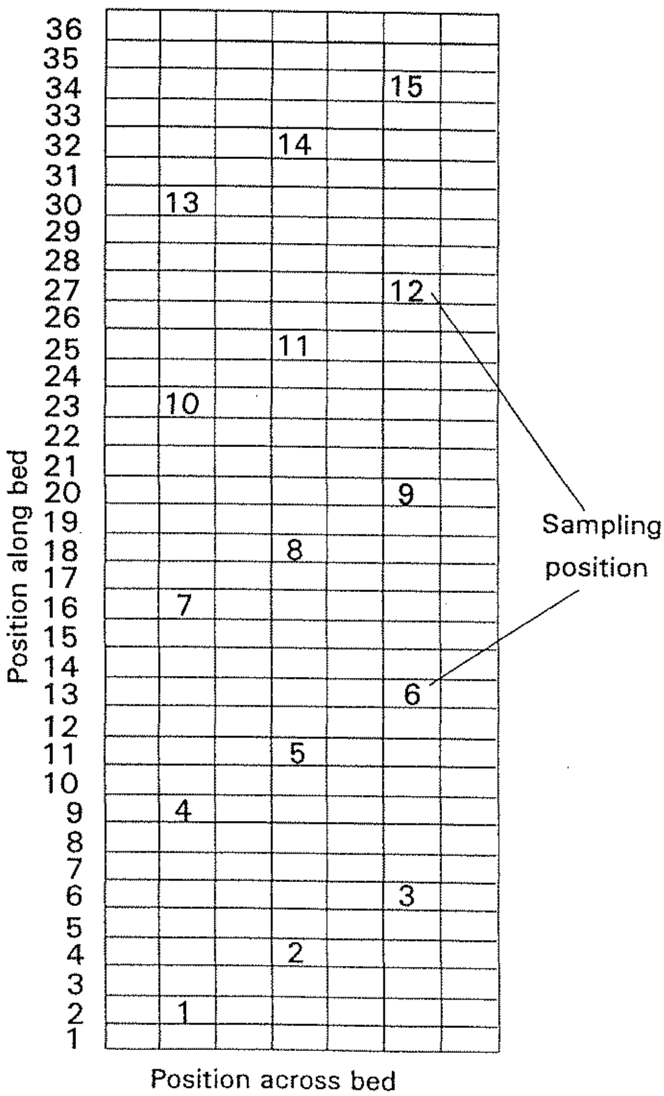
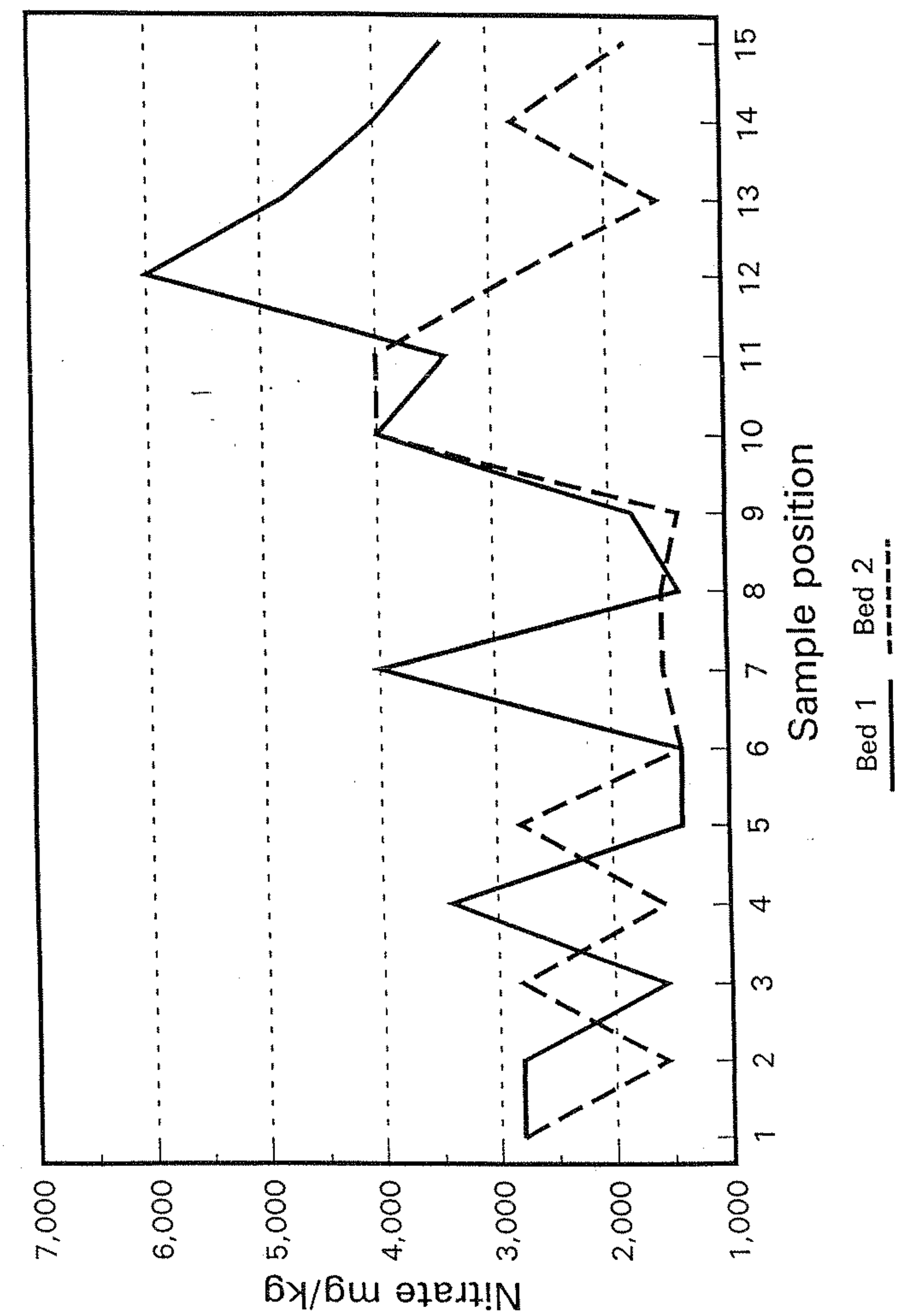


Figure 3: Variation in nitrate content with sampling position.



3.4 New Techniques for Field/Home Analysis

3.4.1 Snappy Sap Test

The snappy sap test is a nitrate measuring method developed for use in the field to obtain an instant result. A single leaf from a plant is removed and the sap squeezed from the petiole onto a nitrate test strip. This strip changes colour according to the amount of nitrate in the sap and the colour can be measured against a pre-calibrated colour chart to determine the concentration of nitrate in the leaf.

Table 6 shows the results of a test where for each sample 8 lettuce were analysed individually using the Snappy Sap Test on one leaf and then the rest of the heads were sent for laboratory analysis. The results are very variable with no correlation between the two sets of figures which is probably because although taken from the same head of lettuce the samples were taken from different areas of the head.

The Snappy Sap Test reading, taken from a single leaf will not be representative of the whole head, while the laboratory analysis will be a mean of the whole head.

Table 6: Sap testing and laboratory analysis of plant nitrate at harvest - September harvest.

	Test Strip	Lab Analysis
Varieties		
Titania	944	2381
Diego	935	2637
SED (33 df)	36.6	73.0
LSD (P = 0.05)	-	149
Significance	NS	***
Fertilisers		
Nil	877	2042
Standard	938	2793
½ Base + liquid	1008	2687
½ Base in rows	935	2514
SED (9 df)	57.2	130.5
LSD (P = 0.05)	-	-
Significance	NS	NS

3.4.2 The Kitchen Method of Nitrate Measurement

Method

This method was developed by soil scientists at HRI Wellesbourne and tested at Stockbridge House.

The method was tested on a summer crop of lettuce at Stockbridge House and by a local grower who was trained in how to use the technique by HRI staff.

Ten lettuce were sampled from the crop, cleaned, and cut into segments. Approximately one eighth of each lettuce was selected to make a total weight of 200 g. This 200 g of lettuce was liquidised with 200 ml water, then the solution filtered through a funnel filled with cotton wool. 20 ml of the filtrate was added to 80 ml of water (100 ml in total) and mixed.

Using a Nitracheck test strip and refloctometer the nitrate content of the solution was measured. The test strip was dipped in the solution then placed in the meter to obtain a reading of the nitrate concentration in ppm, multiplied by 10 this gives the mean nitrate content of the lettuce sampled.

The grower used three whole lettuce for the kitchen method and sent nine for analysis.

Figure 4 shows the results obtained at Stockbridge House. There is a correlation between the laboratory and kitchen results but the scatter shows that there is clearly considerable scope for error.

Figure 5 shows one set of growers results where excellent correlation was achieved.

Figure 4: Evaluation of Kitchen Method - Stockbridge House

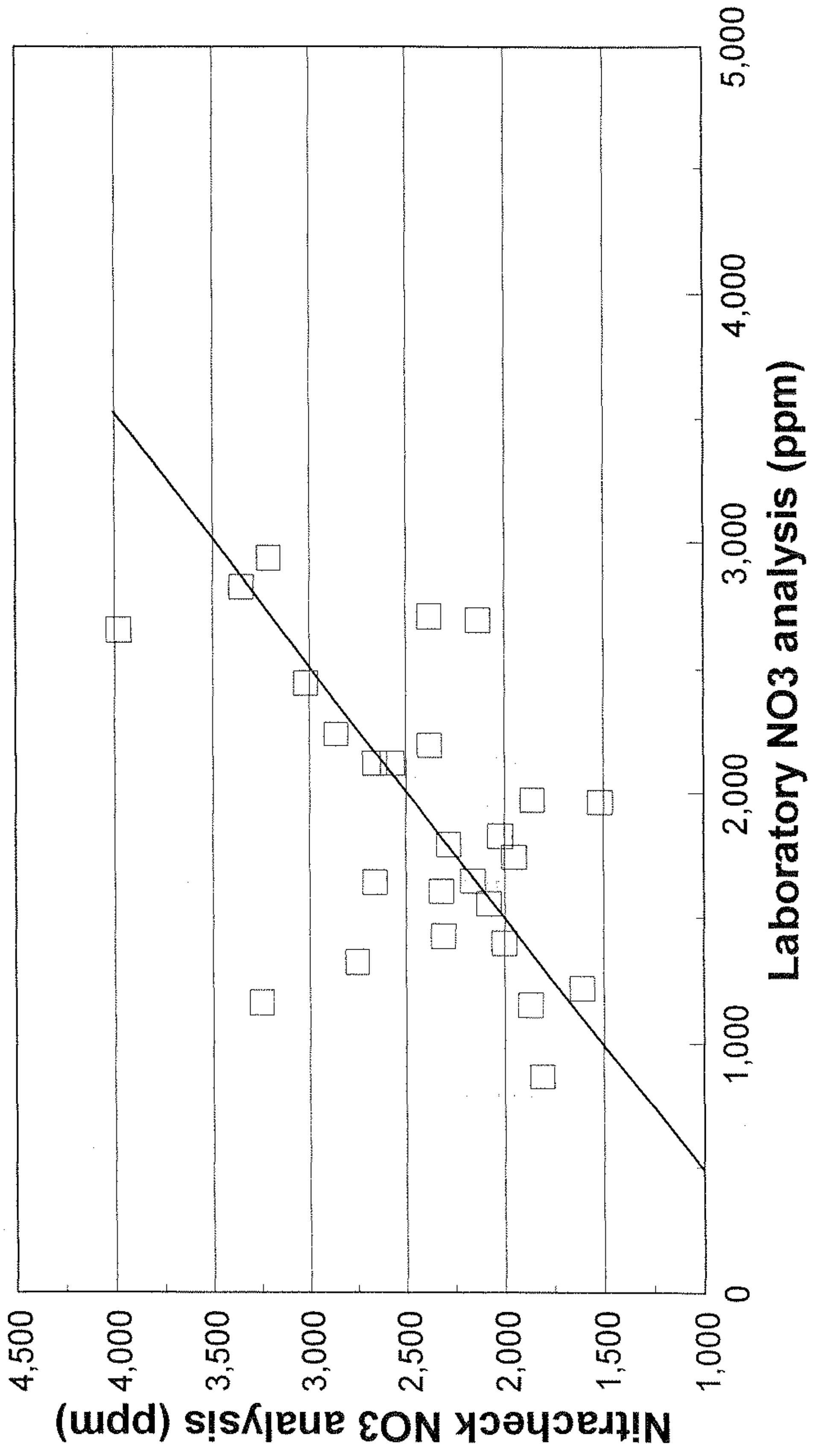
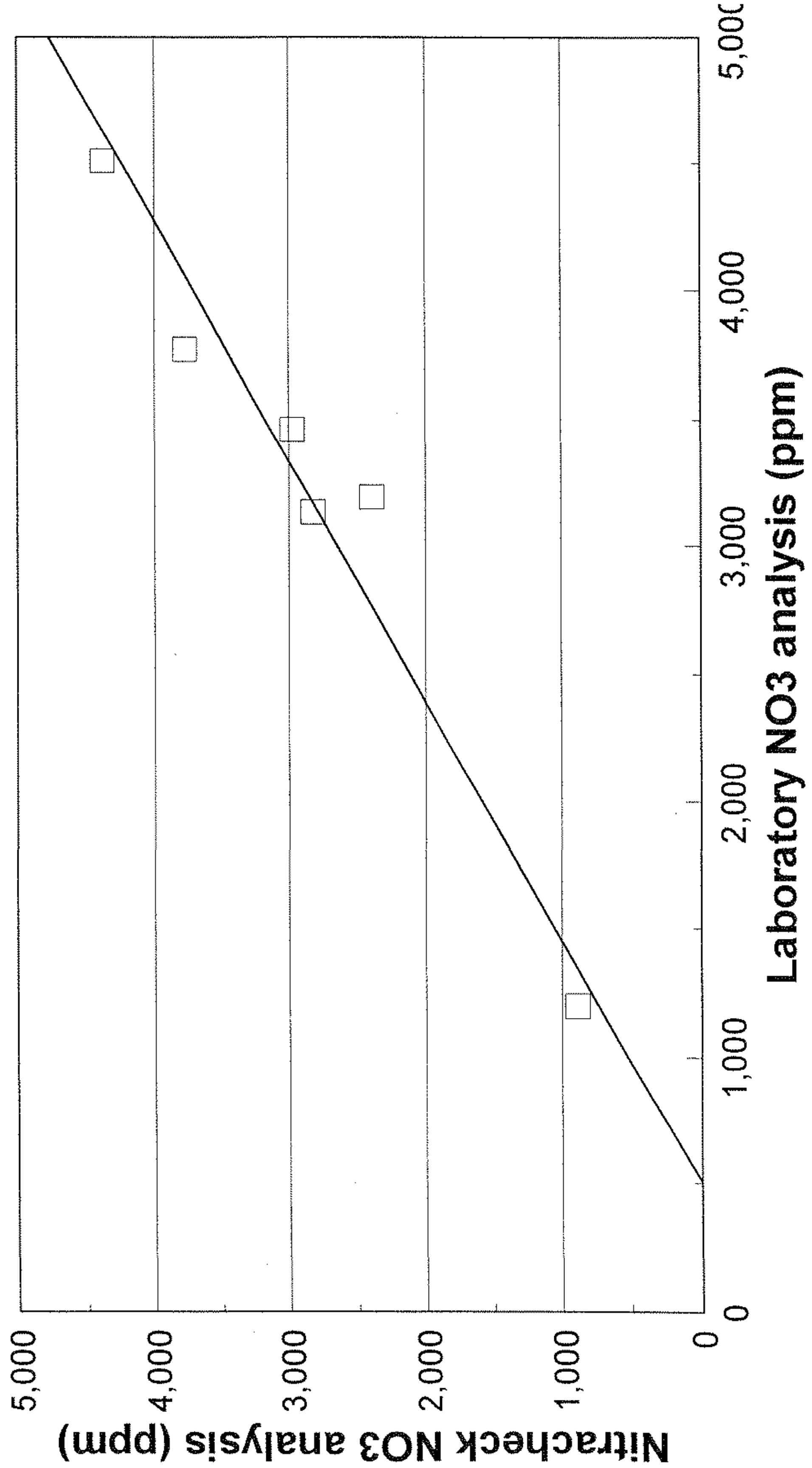


Figure 5: Evaluation of Kitchen Method - Grower Figures



DISCUSSION

The survey of data collected from growers shows that at present they would have severe difficulty in achieving the proposed maximum residue levels, particularly in summer. More data is being collected to update the picture. The proposed limits, and target dates are under review and it is likely that they will be changed before the legislation is finalised. All lettuce growers should ensure that they are aware of the changing situation and how it will affect them.

The experimental trials aimed to determine how cultural techniques and varieties can affect nitrate residues and crop quality at harvest.

Cultivar

The cultivar chosen can play a large part in determining nitrate residues as well as yield, quality and speed of maturity. In this trial, although there were no 'low-nitrate' varieties available for summer testing, of the standard varieties Titania produced a larger head with lower nitrate residues than Diego.

In the winter and spring the low-N variety Sano was tested. Under the conditions of this trial the residues at harvest were not significantly different from those of the standard, Rachel.

All lettuce breeders are now putting considerable effort into incorporating low-nitrate accumulation properties into their breeding programmes. It is a slow process to add this factor, whilst also improving disease resistance and quality. It is important that in all variety trials nitrate residues are measured and comparative figures are available for use in selecting varieties.

Maturity

In spring and summer delaying harvest reduced nitrate residues, but also increased the trimming requirements and the number of unmarketable heads.

Because lettuce accumulate nitrate in their older leaves any extra trimming will decrease the overall nitrate content of the head.

In this trial, head weights increased with delayed harvest, despite the extra trimming required but as overall quality fell anyone considering the use of this technique to reduce high residues should consider the implications very carefully. It is probably not practical in many circumstances.

Harvesting Time

Work in the past has shown that extra light decreases nitrate residues in the leaves. This is the reason why nitrate levels are lower in summer than winter, and lower residues can be achieved in southern Europe.

For this reason this trial aimed to determine whether harvesting in the afternoon gave lower residues than harvesting in the morning. No significant differences were recorded at harvest but monitoring throughout the life of the crop showed that levels tended to go up and down according to bright or dull periods.

Fertilisers

Previous research has shown that there is potential to change nitrate residues in plants by adjusting nitrogen fertiliser applications. This must however be achieved without any detrimental affect on yield or quality.

This trial used ammonium nitrate applied in different manners.

In the first trial (in summer), despite flooding to reduce residual nitrate in the soil, there were no significant differences in nitrate residues or production between fertiliser treatments. Even where no nitrate fertiliser was applied production was good and nitrate residues exceeded 2000 mg/l.

Release of nitrogen from organic residues in the soil, nitrification, will always occur. Although the grower can control the inorganic fertiliser that he adds, rates of nitrification from the soil are still not fully understood and are therefore unpredictable. Growers on organic soils, and those that add organic material will have the least control over nitrogen release and availability for the crop.

Standard recommendations for nitrogen fertiliser for lettuce were made some time ago and are based on ensuring the crop will never run out. They are therefore probably excessive to the plants requirements.

In this trial applying nitrogen at half rate into the planting rows reduced nitrate residues without affecting quality. The plants had sufficient fertiliser early in their life but as the root system expanded they were in soil with a lower nitrate level and therefore contained lower residues at harvest.

It is fairly complicated to apply the fertiliser using this technique but a method could be devised if it is consistently successful. Applying half rate fertiliser over the whole area may offer the same benefit but differs in that the plants start off their growth with reduced nitrate availability. The two methods are being compared in further trials.

Applying fertiliser according to sap testing maintained good growth but also maintained nitrate residues at the same level as the standard fertiliser treatment. The technique has therefore been amended to apply lower rates at each application and will be tested further. It offers a method for those growers who prefer to liquid feed to show that they are applying fertiliser according to the crop requirements and not to excess.

Field Testing Techniques

Sap Testing can be used to determine when to apply fertiliser but is not a good indicator of total nitrate residue in the head. Because the level is variable throughout the head the same leaf must always be used. The test only represents the nitrate content in that particular leaf.

The Kitchen Method of nitrate testing offers a much more reliable method of testing residues in the whole head. The method requires some accuracy in weighing and measuring volumes and there is scope for error if not carried out carefully. The equipment required is not expensive and staff can easily be trained in the methods used.

Some discrepancy from laboratory analysis is likely if samples are not fresh when they reach the laboratory.

With some further testing and development to make routine testing simple and reliable this method offers potential for growers to carry out quick analysis on their crop before or at harvest.

CONCLUSIONS

1. UK growers are unable to consistently meet the proposed EC maximum residue levels for nitrate in lettuce.

2. Even within a uniform crop there is variation between heads.

3. There is significant variation in nitrate residues between some varieties, but no variety offers a consistent way of achieving the proposed levels.

4. Extra trimming reduces total nitrate residue in the head, but may not be commercially acceptable.

5. Time of day when the crop was harvested did not affect residues in this trial.

6. Sap testing and liquid fertiliser applications maintained quality but did not reduce residues, at the rates used in the trial.

7. Application of half rate fertiliser in the planting rows maintained quality and reduced nitrate residues.

8. Sap testing did not offer a reliable indication of nitrate residue in the head at harvest.

9. The Kitchen Method of nitrate testing gave a reasonable indication of total nitrogen content.

FURTHER WORK

Collating data on growers achieved residue levels will continue.

Residue testing must be incorporated into lettuce variety trials.

It is important to verify whether growers can carry out their own reliable nitrate testing at home.

Considerable work is required on optimum fertiliser applications.

ACKNOWLEDGEMENTS

This work was funded by the Horticultural Development Council.

The contribution of Alan Scaife at HRI Wellesbourne in developing the Sap Test and the Kitchen Method is acknowledged and thanks are also due to Clive Rahn for his help in devising the fertiliser treatments.

Special thanks go the Lesley Sykes of Snaith Salad growers for testing the Kitchen Method and providing her results for use in this report.

APPENDIX I: TRIAL PLANS

Reduction of nitrate residues in glasshouse lettuce Plan Trial 1

→ N

8 a	16 a	24 b	32 a	40 b	48 b	56 a	64 b	72 a	80 b	88 a	96 b	104 a	112 b	120 b	128 b
3	3	4	1	4	2	1	2	4	3	1	2	1	4	3	2
7 b	15 b	23 a	31 b	39 a	47 a	55 b	63 a	71 b	79 a	87 b	95 a	103 b	111 a	119 a	127 a
6 b	14 a	22 a	30 a	38 b	46 a	54 a	62 b	70 a	78 a	86 b	94 b	102 a	110 b	118 b	126 a
1	2	1	3	1	1	3	3	2	2	4	4	4	2	4	3
5 a	13 b	21 b	29 b	37 a	45 b	53 b	61 a	69 b	77 b	85 a	93 a	101 b	109 a	117 a	125 b
4 b	12 b	20 a	28 b	36 a	44 b	52 a	60 b	68 a	76 b	84 a	92 a	100 b	108 a	116 a	124 b
2	4	3	2	2	4	4	4	3	1	3	3	2	1	1	1
3 a	11 a	19 b	27 a	35 b	43 a	51 b	59 a	67 b	75 a	83 b	91 b	99 a	107 b	115 b	123 a
2 a	10 b	18 b	26 a	34 b	42 a	50 b	58 a	66 b	74 a	82 b	90 a	98 a	106 b	114 b	122 a
4	1	2	4	3	3	2	1	1	4	2	1	3	3	2	4
1 b	9 a	17 a	25 b	33 a	41 b	49 a	57 b	65 a	73 b	81 a	89 b	97 b	105 a	113 a	121 b
A	B	C	D	B	D	A	C	D	C	B	A	C	A	D	B
rep 1				rep 2				rep 3				rep 4			

Fertilizers

A: Nil

B: Standard base

C: 1/2 Base + liquid

D: 1/2 Base in rows

Treatments

Titania		Diego	
Mat 1	Mat 2	Mat 1	Mat 2
1	2	3	4

a = 9 am

b = 3 pm

Reduction of nitrate residues in glasshouse lettuce Plan Trial 2

→ N

8 a	16 b	24 b	32 a	40 a	48 b	56 a	64 b	72 b	80 a	88 a	96 b	104 b	112 a	120 b	128 a
2	4	1	3	3	1	3	2	2	4	1	1	3	4	4	2
7 b	15 a	23 a	31 b	39 b	47 a	55 b	63 a	71 a	79 b	87 b	95 a	103 a	111 b	119 a	127 b
6 a	14 b	22 a	30 b	38 a	46 a	54 b	62 b	70 a	78 a	86 b	94 b	102 a	110 a	118 b	126 b
3	3	4	1	2	3	4	3	4	1	4	2	2	1	2	1
5 b	13 a	21 b	29 a	37 b	45 b	53 a	61 a	69 b	77 b	85 a	93 a	101 b	109 b	117 a	125 a
4 a	12 b	20 b	28 a	36 a	44 b	52 b	60 a	68 a	76 b	84 b	92 a	100 b	108 a	116 a	124 b
4	1	2	2	4	4	1	1	1	3	2	3	4	2	3	3
3 b	11 a	19 a	27 b	35 b	43 a	51 a	59 b	67 b	75 a	83 a	91 b	99 a	107 b	115 b	123 a
2 b	10 b	18 a	26 a	34 a	42 b	50 b	58 a	66 b	74 a	82 a	90 b	98 b	106 a	114 a	122 b
1	2	3	4	1	2	2	4	3	2	3	4	1	3	1	4
1 a	9 a	17 b	25 b	33 b	41 a	49 a	57 b	65 a	73 b	81 b	89 a	97 a	105 b	113 b	121 a
A	B	C	D	B	D	A	C	D	C	B	A	C	A	D	B
rep 1				rep 2				rep 3				rep 4			

Fertilizers

A: Nil

B: Standard base

C: 1/2 Base + liquid

D: 1/2 Base in rows

Treatments

Rachel		Sano	
Mat 1	Mat 2	Mat 1	Mat 2
1	2	3	4

a = 9 am

b = 3 pm

Reduction of nitrate residues in glasshouse lettuce Plan Trial 3

N →

8 b	16 a	24 b	32 a	40 a	48 b	56 a	64 b	72 a	80 b	88 a	96 b	104 a	112 b	120 a	128 b
1	2	4	3	3	4	4	2	1	3	4	3	1	2	2	1
7 a	15 b	23 a	31 b	39 b	47 a	55 b	63 a	71 b	79 a	87 b	95 a	103 b	111 a	119 b	127 a
6 a	14 b	22 b	30 b	38 b	46 a	54 a	62 a	70 a	78 a	86 a	94 a	102 b	110 b	118 a	126 b
3	1	2	4	4	2	2	3	3	1	3	1	4	4	1	2
5 b	13 a	21 a	29 a	37 a	45 b	53 b	61 b	69 b	77 b	85 b	93 b	101 a	109 a	117 b	125 a
4 b	12 a	20 b	28 b	36 a	44 a	52 b	60 a	68 b	76 a	84 b	92 a	100 b	108 a	116 b	124 a
2	3	3	1	1	3	3	1	2	4	2	4	2	1	4	4
3 a	11 b	19 a	27 a	35 b	43 b	51 a	59 b	67 a	75 b	83 a	91 b	99 a	107 b	115 a	123 b
2 b	10 a	18 a	26 a	34 b	42 a	50 b	58 a	66 b	74 a	82 b	90 a	98 a	106 b	114 b	122 a
4	4	1	2	2	1	1	4	4	2	1	2	3	3	3	3
1 a	9 b	17 b	25 b	33 a	41 b	49 a	57 b	65 a	73 b	81 a	89 b	97 b	105 a	113 a	121 b
A	B	C	D	B	D	A	C	D	C	B	A	C	A	D	B
rep 1				rep 2				rep 3				rep 4			

Fertilizers

A: Nil

B: Standard base

C: 1/2 Base + liquid

D: 1/2 Base in rows

Treatments

Rachel		Sano	
Mat 1	Mat 2	Mat 1	Mat 2
1	2	3	4

a = 9 am

b = 3 pm

APPENDIX II: NITRATE LEVELS IN COMMERCIAL CROPS

Nitrate levels in Protected Butterhead Lettuce

Date	Grower	NO3 mg/kg fresh weight	Mean	Range	Laboratory
JAN 1993					
18 January	Single	3460 3920 4040 1640 3730 4010 3940 3780 4480 3430	3643	1640-4480	NIAB
			3643		
FEB 1993					
2-17 Feb	S	5360 3863 4829	4684	3863-5360	Lancrop
			4684		
MARCH 1993					
11 March	S	2804	2804	2804	Lancrop
			2804		
APRIL 1993					
7-28 April	S	3088 2472	2780	2472-3088	Lancrop
20 April	S	2442 3278 1436 2062 3019 2164 2904 1979 2209 2282	2378	1436-3019	NIAB
			2445		
MAY 1993					
5-26 May	S	2702 3021 2246 2848	2704	2246-3021	Lancrop
			2704		
JUNE 1993					
2 June	S	3317 3741 3783	3614	3317-3783	NIAB
5 June	S	2800	2800	2800	-
4-16 June	S	2977 2667 3575	3073	2667-3575	Lancrop
			3266		
JULY 1993					
7-22 July	S	2676 2441	2559	2441-2676	Lancrop
			2559		
AUGUST 1993					
6 August	Group	3410 3240 3270 2800 8350 2950 2000 4430 4420 3450	3646	2000-8350	ADAS
		3120 2980 2890 2790 4790 3280 3840 3620			
6 August	S	3560 3630 3330 3830 4740 3260 2810 2730 3890 3050	3483	2730-4740	ADAS
13 August	S	3800	3800	3800	-
3-26 August	S	4563 3960 4784	4436	3960-4784	Lancrop
			3674		
SEPT 1993					
8 September	S	1885	1885	1885	Beverley Analytical
14 September	G	3020 3180 4230 2600 3450 3430 3880 3290 2890 3750	3389	2600-4510	ADAS
		3090 2850 4510 3280			
17 September	S	3584 3628 3544 2906	3416	2906-3628	Lancrop
16-29 Sept	S	3535 4740	4138	3535-4740	Lancrop
			3394		
OCT 1993					
13 October	S	3562	3562	3562	Lancrop
14 October		1781 4018 3659 3867	3331	1781-4018	Lancrop
21 October	S	3546 3581 2834 3953 3650 3448 4886 3369 2985 3137	3617	2834-4886	NIAB
		4395			
25 October	G	3770 3900 3890 3730 3340 3740 2750 4120 1910 3020	3455	1910-4120	ADAS
		3330 4020 3370 3480			
			3501		
NOV 1993					
3 November	S	3172	3172	3172	-
13-17 Nov	S	4652 5316	4984	4652-5316	Lancrop
18 November	S	6158 8284 4696 8018	6789	4696-8284	Lancrop
			5757		
DEC 1993					
1 December	G	4260 5610 2830 4210 3280 3850 2620 3880 4110 4640	3845	2620-5610	ADAS
		2980 3870			
21 December	G	4480 3782 4202 4577	4260	3782-4577	Beverley Analytical
December	S	5582 4177 3393 3260	4103	3260-5582	Lancrop
			3980		

Means The figures shown in the shaded boxes are the monthly means calculated by averaging the individual sample results.

Date	Grower	NO3 mg/kg fresh weight	Mean	Range	Laboratory
JAN 1994					
18 January	G	3720 4210 3860 5090 4560 3490 4820 4060 5380 3220 3330	4158	3220-5380	ADAS
24 January	S	827 1340 800 779 1200 2714 2375 3793 2769 1423 2639 2704 2969 4007 2115 2378 1168 2216 713	2049	713-4007	NIAB
January	S	6822 6778 6069	6556	6069-6822	Lancrop
			3162		
FEB 1994					
2 February	S	3106	3106	3106	Beverley Analytical
8 February	S	3655 4607 3956 4186 4297 3717 4917 3548 4355 5538 4696 5626 5006 5582	4549	3548-5626	Lancrop
10 February	S	5670 5538 7132	6113	5538-7132	
23 February	S	4133	4133	4133	Lancrop
			4698		
MARCH 1994					
2 March	S	4110	4110	4110	ADAS
3 March	S	2849	2849	2849	-
4 March	S	2715 3667	3191	2715-3667	Beverley Analytical
8-11 March	S	2800 3252	3026	2800-3252	Lancrop
16 March	G	3500 4360 2920 3370 3410 3800 3600 3670 3070 2550 1970 3210 3240	3282	1970-4360	ADAS
March	S	4784 6158 4146 4873	4990	4146-6158	Lancrop
			3666		
APRIL 1994					
19 April	S	3974 3872 4222	4023	3872-4222	Lancrop
	S	3710 (Novita)	3710	3710	ADAS
			3945		
MAY 1994					
JUNE 1994					
21 June	S	4102 3486	3794	3486-4102	Lancrop
			3794		
JULY 1994					
8 July	S	2930 3350 3070 1410	2690	1410-3350	-
14 July	G	2720	2720	2720	-
22 July	S	1639 1533	1586	1533-1639	Lancrop
			2379		
AUGUST 1994					
24 August	S	3460 3190 3770 4510 3130 1220	3213	1220-4510	ADAS
			3213		
SEPT 1994					
5 September	S	3813			
12 September	S	2642			
14 September	G	2500 3400 2300 3600 3700 2400 3100 3800 2500 3200 2500 3300 3100 3300 2200 2100	2938	2100-3800	Aspland & James
	S	3270 3960 3630 4750 3790	3880	3270-4750	ADAS
20 September	S	3630 4760 3790 3203 2193	3515	2193-4760	Lancrop & ADAS
26 September	S	4443	4443	4443	-
			3272		
OCTOBER 1994					
3 October	S	4183	4183	4183	-
10 October	S	4297	4297	4297	-
13 October	S	4257 3823 3517	3866	3517-4257	Lancrop
16 October	S	3923	3923	3923	-
			4000		

Means The figures shown in the shaded boxes are the monthly means calculated by averaging the individual sample results.

APPENDIX III: DETAILED QUALITY ASSESSMENTS

Table A: Heart (score 0-5, where 5 is good).

	Summer Harvest	Winter Harvest	Spring Harvest
Varieties			
Titania/Rachel/Rachel	3.50	2.01	3.74
Diego/Sano/Sano	3.82	1.70	4.07
SED (33 df)	0.074	0.065	0.071
LSD (P = 0.05)	0.15	0.13	0.15
Significance	***	***	***
Fertilisers			
Nil	3.40	1.58	0.93
Standard	3.76	1.90	3.95
½ Base + liquid	3.79	1.89	3.86
½ Base in rows	3.69	2.06	3.90
SED (9 df)	0.141	0.125	0.083 +
LSD (P = 0.05)	-	-	-
Significance + to compare 3 fertilizer treatments	NS	NS	NS
Maturity			
Minimum	3.40	1.80	3.23
Latest	3.43	1.92	4.58
SED (33 df)	0.074	0.065	0.071
LSD (P = 0.05)	0.15	0.13	0.15
Significance	***	* 6%	***
Time			
a.m.	3.90	2.06	3.72
p.m.	3.43	1.66	4.09
SED (50 df)	0.081	0.064	0.065
LSD (P = 0.05)	0.35	0.28	0.13
Significance	***	***	***

Table B: Colour (score 0-5, where 5 is dark).

	Summer Harvest	**Winter Harvest**	**Spring Harvest**
<u>Varieties</u>			
Titania/Rachel/Rachel	3.14	3.52	3.49
Diego/Sano/Sano	3.34	2.95	3.09
SED (33 df)	0.075	0.066	0.075
LSD (P = 0.05)	0.15	0.13	0.15
Significance	**	***	***
<u>Fertilisers</u>			
Nil	3.22	3.19	3.00
Standard	3.30	3.25	3.32
½ Base + liquid	3.28	3.25	3.28
½ Base in rows	3.16	3.25	3.27
SED (9 df)	0.94	0.083	0.122 +
LSD (P = 0.05)	-	-	-
Significance + to compare 3 fertilizer treatments	NS	NS	NS
<u>Maturity</u>			
Minimum	3.26	3.24	2.88
Latest	3.22	3.23	3.71
SED (33 df)	0.075	0.066	0.075
LSD (P = 0.05)	-	0.13	0.15
Significance	NS	NS	***
<u>Time</u>			
a.m.	3.47	3.07	3.09
p.m.	3.01	3.40	3.49
SED (50 df)	0.078	0.054	0.065
LSD (P = 0.05)	0.34	0.23	0.13
Significance	***	***	***

Table C: Base (score 0-5, where 5 is full).

	Summer Harvest	Winter Harvest	Spring Harvest
<u>Varieties</u>			
Titania/Rachel/Rachel	3.64	3.92	4.49
Diego/Sano/Sano	3.79	3.08	4.17
SED (33 df)	0.060	0.541	0.087
LSD (P = 0.05)	0.12	-	0.18
Significance	*	NS	***
<u>Fertilisers</u>			
Nil	3.91	3.38	4.11
Standard	3.66	3.15	4.20
½ Base + liquid	3.68	3.25	4.53
½ Base in rows	3.61	4.22	4.26
SED (9 df)	0.067	0.815	0.043 +
LSD (P = 0.05)	-	-	0.11
Significance + to compare 3 fertilizer treatments	NS	NS	***
<u>Maturity</u>			
Minimum	3.74	3.21	4.22
Latest	3.69	3.79	4.44
SED (33 df)	0.06	0.541	0.087
LSD (P = 0.05)	-	-	0.18
Significance	NS	NS	*
<u>Time</u>			
a.m.	4.10	3.88	4.48
p.m.	3.33	3.12	4.18
SED (50 df)	0.048	0.543	0.059
LSD (P = 0.05)	0.21	-	0.12
Significance	***	NS	***

Table D: Head size (score 0-5, where 5 is large).

	Summer Harvest	Winter Harvest	Spring Harvest
<u>Varieties</u>			
Titania/Rachel/Rachel	3.56	2.99	4.38
Diego/Sano/Sano	3.33	3.01	4.27
SED (33 df)	0.050	0.037	0.088
LSD (P = 0.05)	0.10	-	-
Significance	***	NS	NS
<u>Fertilisers</u>			
Nil	3.27	2.76	2.26
Standard	3.58	3.06	4.25
½ Base + liquid	3.54	3.08	4.38
½ Base in rows	3.39	3.10	4.34
SED (9 df)	0.101	0.085	0.052 +
LSD (P = 0.05)	-	-	-
Significance + to compare 3 fertilizer treatments	NS	NS	NS
<u>Maturity</u>			
Minimum	3.49	3.00	4.11
Latest	3.40	3.00	4.54
SED (33 df)	0.050	0.037	0.088
LSD (P = 0.05)	-	-	0.19
Significance	NS	NS	***
<u>Time</u>			
a.m.	3.72	3.01	4.43
p.m.	3.16	2.99	4.22
SED (50 df)	0.042	0.039	0.072
LSD (P = 0.05)	0.18	-	0.15
Significance	***	NS	**

APPENDIX IV: SOIL ANALYSIS

Soil Analysis

Soil Nitrate N (mg/l), (Index)

	23 July 1993		28 September 1993		15 February 1994		3 May 1994	
Nil N fertiliser								
0-15 cm	<10	(0)	10	(0)	22	(0)	4	(0)
15-30 cm	<10	(0)	12	(0)			4	(0)
30-45 cm	<10	(0)	11	(0)			5	(0)
Standard Base								
0-15 cm	<10	(0)	27	(0)	33	(1)	12	(0)
15-30 cm	<10	(0)	18	(0)			18	(0)
30-45 cm	<10	(0)	21	(0)			20	(0)
½ Base + Liquid								
0-15 cm	<10	(0)	9	(0)	81	(2)	5	(0)
15-30 cm	<10	(0)	10	(0)			14	(0)
30-45 cm	<10	(0)	12	(0)			19	(0)
½ Base in Rows								
0-15 cm	<10	(0)	12	(0)	21	(0)	7	(0)
15-30 cm	<10	(0)	19	(0)			7	(0)
30-45 cm	<10	(0)	17	(0)			10	(0)

Key

22 June 1993	Before Trial 1 (summer)
22 September 1993	After Trial 1, before Trial 2 (winter)
5 February 1994	After Trial 2, before Trial 3 (spring)
3 May 1994	After Trial 3

APPENDIX V: NITROGEN FERTILISER APPLICATIONS

Nitrogen Fertiliser Applications (g/m^2 Ammonium Nitrate)

	Tmt A Nil	Tmt B Standard	Tmt C ½ Base + Liquid	Tmt D ½ Base in rows
Trial 1 (Summer 1993)				
5 Aug	-	40	20	20
27 Aug	-	-	5	-
Trial 2 (Winter 1993)				
28 Sept	-	20	20	20
19 Oct	-	-	5	-
29 Oct	-	-	5	-
12 Nov	-	-	5	-
Trial 3 (Spring 1994)				
21 Feb	-	20	-	20