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The results and conclusions in this report are based on a single series of experiments. The conditions under which the experiments were carried out and the results have been reported with detail and 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 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 ..........................................................................................................

Dr I Burns
Report Editor
Horticulture Research International
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Date ..............................

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## PRACTICAL SECTION FOR GROWERS

### Objectives and background

Concerns about high nitrate levels in vegetable produce have led the EU to introduce limits on nitrate concentrations in some salad crops (European Commission, 1997a; 1999). For protected lettuce, maximum nitrate levels must not exceed 4500 ppm in fresh heads harvested in winter (between October and March) and 3500 ppm for summer crops (harvested between April and September). These limits are likely to create problems for lettuce growers in northern European countries such as the UK, because poor light quality can restrict the energy available for assimilation of nitrate by glasshouse crops. In recognition of this, the EU have granted UK growers a temporary derogation from the maximum levels, to allow time to adapt their existing N fertiliser and other growing practices. In the meantime growers must demonstrate that they are adhering to existing Codes of Good Agricultural Practice, and they are obliged to analyse representative samples of their produce regularly to confirm that nitrate concentrations are not excessive.

This project was designed to examine nitrogen fertiliser management and other practices likely to influence nitrate accumulation in protected lettuce with the aim of updating advice to growers. The aim was to provide a reliable strategy to ensure that nitrogen fertiliser is applied in such a way as to optimise growth and product quality, while avoiding excessive plant uptake or leaching through the soil profile. A number of parallel approaches have been adopted to this aim.

Scientists at HRI have developed a highly sophisticated computer-based system (WELL_N) which incorporates a model (N_ABLE) to accurately predict nitrogen requirements for outdoor vegetable crops. The model uses various site, crop and cultural details together with the expected weather data to predict the nitrogen fertiliser requirements. The model can also substitute actual weather data to predict ongoing changes in top dressing or irrigation requirements during growth. It therefore provides an ideal framework from which to develop an equivalent nitrogen fertiliser management recommendation system for protected crops that could provide site-specific advice for both base dressings and for supplementary fertiliser applications by top dressing or liquid feeds. A key objective of this project is to adapt the model to provide accurate nitrogen fertiliser recommendations for protected lettuce growers and evaluate its performance in N response experiments carried out at both HRI and on two commercial sites.

The project will also examine ways of manipulating the management of nitrogen fertiliser practice in order to optimise product quality and uniformity. This will include studies of the effects of different types of N fertiliser, and the method and timing of their application. For soil-grown crops such as lettuce there is known to be inherent variability in nitrogen uptake and accumulation between plants. This makes it difficult to draw conclusions on the nitrate content of the whole crop from the analysis of crop samples. Trials will therefore be carried out to identify the reasons for plant to plant variability with the aim of determining whether fertiliser management can be used to improve crop uniformity. Variability between cultivars will also be investigated on both summer and winter grown crops.

A key feature in the application of any nitrogen fertiliser management strategy is the need to provide reliable information on the nitrate status of the plants during growth before any supplementary nitrogen dressings or liquid nitrogen feeds are applied. Existing laboratory methods used to determine nitrate concentrations are laborious, complex and are unsuitable for on-site use. An additional objective of this project is therefore to develop and evaluate a reliable nitrate testing system suitable for use on the nursery, that is as accurate as laboratory methods, and is cost effective, fast and easy to use.

Once harvested, a lettuce crop is bagged and vacuum cooled to remove field heat on the nursery. It is then placed on a cool chain transport lorry and delivered to the supermarket depot. From here the crop is distributed to the point of sale where it is displayed, purchased and then ultimately consumed in the household. This project includes an investigation of the extent to which nitrate concentration in lettuce may change during these stages. In co-operation with a major retail outlet samples of commercial butterhead lettuce will be taken from key points within the marketing chain to enable any changes in nitrate content to be quantified.

The overall aim of the project is to use all of this information to develop a general management strategy for nitrogen fertiliser application and culture of protected soil-grown lettuce crops which will allow growers to optimise production and product quality whilst minimising excessive fertiliser usage. The project deals primarily with lettuce as a model crop, but opportunities will be identified for extending this approach to other glasshouse soil-grown crops such as celery or cut flowers in follow-up projects.

As information from the project becomes available it will be used as appropriate to amend the code of Good Agricultural Practice and will be made available to growers in order that methods can be changed if necessary.

## Summary of Results

The overarching conclusion of this work is that, despite using best management practices, none of the treatments examined in this project can be guaranteed to consistently meet the EU limits for nitrate in protected lettuce, currently set at 3500 ppm for summer crops and 4500 ppm for winter crops because of uncertainty about light levels in the last 10-14 days before harvest. However, there are a number of actions that can be taken to reduce the risk of exceeding these limits, including improved N fertiliser advice, more careful fertiliser management, and more frequent monitoring of nitrate in crops on the nursery.

In addition, the information on the sources of variation in nitrate concentrations gained in this research will be useful for defining more rigorous protocols for the measurement and interpretation of nitrate data in lettuce. Further details of each of these aspects are given below in the description of the Scientific Conclusions and later in the Action Points arising from the work.

*Model development and evaluation.* Detailed examination and evaluation of the model WELL_N for soil-grown protected lettuce has revealed that many of the underlying principles used in its derivation are inappropriate for use in glasshouse environments. This evaluation also indicated that it would not be cost effective to continue to modify the existing versions of WELL_N because there would be no guarantee that such an approach would overcome these difficulties satisfactorily. A decision was therefore taken to construct a new model from scratch, incorporating only those components of WELL_N relevant to protected conditions, together with new relationships developed from the experiments carried out during the course of this project. A prototype version of this new model (to be known as LET_N) has now been completed. This has been designed to integrate seamlessly with a user-friendly interactive interface created on an MS-Excel spreadsheet. The interface provides a number of options, including simulation and optimisation routines. The simulation option solves the model and provides a graphical comparison of the predictions from the experimental data, allowing the effects of different N fertiliser levels to be evaluated quickly. The optimisation routine gives a rapid indication of the recommended application rate of N fertiliser which minimises the risk of excessive nitrate accumulation without loss of yield. An additional Liquid Feeding routine also allows the effects of up to five supplementary applications of fertiliser to be simulated. Preliminary tests have shown that the model is capable of providing a more accurate description of independent response data than previously modified versions of WELL_N in a limited number of experiments at three sites (two on commercial nurseries of the participating growers). However, further tests will be needed at a wider range of sites to provide an independent validation. This next phase of the work will need to be carried out in a subsequent project.

*Nitrogen fertiliser management.* The effect of type of nitrogen fertiliser on the head weight and nitrate content of glasshouse butterhead lettuce was determined within a year-round production programme. Fertiliser was applied to achieve N-min at planting of 100 mg/l all year or N-min 70 mg/l for the winter programme. Mean marketable head weight was not affected by fertiliser type or by rate of application. The amendment of urea and urea + calcium nitrate (UCAN) fertilisers with the nitrification inhibitor dicyandiamide (DCD) did not significantly reduce nitrate concentrations compared to other fertiliser types. In these trials the *mean* nitrate concentrations were below the legal limit for the harvesting period 1 April to 30 September (3500 ppm) and 1 October to 31 March (4500 ppm). However the *variation* in nitrate concentrations recorded between individual 10-head samples was large, with up to 15% of the samples exceeding the limit in winter and early summer crops. As a result, none of the treatments in this series of trials can guarantee that UK growers can consistently achieve nitrate levels below those presently in the EU regulation of 4500 and 3500 ppm.

Additional measurements showed that the timing of N applications via liquid feeds post planting had no significant effect on mean marketable head weight. However there was a suggestion that applications of N between 10 and 14 days prior to harvest could increase the risk of high nitrate concentrations in the lettuce.

*Variability.* Measurements on a butterhead lettuce crop grown hydroponically with a uniform nutrient supply indicated that the inherent variability in nitrate concentration between heads ranged from 10 to 15 %. Similar levels of variability (8 to 14 %) were also observed in a soil-grown crop harvested in winter. However, the variability increased dramatically during the summer growing period, with coefficients of variation reaching as much as 44 % between individual heads. This was probably because of the greater variation in light levels across a glasshouse during the summer.

Guidelines for laboratories carrying out the determination of nitrate in lettuce state that all analyses should be done on bulked samples of ten heads. Studies carried out as part of the evaluation of N fertiliser practices showed that variation between 10-head samples from crops grown in soil ranged from 8 to around 20% in year round production. Evidence from these experiments (and others used to test the LET_N model) also indicate that variability was greater in summer compared to winter grown crops.

Current UK Guidelines do not specify clear protocols for the extraction of nitrate from fresh lettuce samples and, as a result, a number of different methods are currently being used by commercial laboratories. Our results show that there can be large differences in the amounts of nitrate released from lettuce tissues by different extraction methods. For example, cold water extraction of lettuce heads (used by many laboratories) only removes about half of the nitrate recovered in hot water extracts. This is likely to provide an additional source of variation when nitrate concentration data from laboratories using different methods are compared.

*Rapid nitrate tests for nursery use.* Research has been carried out into the use of different procedures for simple extraction and analysis of nitrate from fresh plant material with the aim of developing a rapid test for use on commercial nurseries. Comparisons of simple test methods showed that the Nitrachek kit used in conjunction with Merckoquant nitrate test strips gives equivalent results to conventional laboratory based methods where the same extraction procedure is used. This test will therefore be suitable for use by growers on nurseries, as soon as current uncertainty about the best possible method for extracting nitrate from lettuce tissues is resolved. However, to ensure that the test is reliable, the users will need to be provided with a supply of pure water, together with standard solutions to calibrate the meter.

*Variety effects.* Four screening trials using a range of commercial lettuce varieties were conducted to compare their capacity for nitrate accumulation. Although no single variety was found to exhibit consistently lower nitrate concentrations, the butterhead types were generally found to have lower nitrate concentrations at harvest than novelty types of lettuce grown under the same conditions.

*Post-harvest effects*. Two separate studies to determine the changes in nitrate concentration in lettuce heads at different points in a simulated marketing chain (from harvest to use-by-date) carried out in conjunction with one of the commercial partners showed no detectable trends in the nitrate levels, although the mean values varied somewhat between sampling dates. This variability was greater for summer crops than for winter ones, possibly reflecting the softer nature of their tissues which may have been more vulnerable to water loss.

**Action points**

*For Growers and Advisors*

Modify the NFU Code of Good Agricultural Practice to include the following advice

- Measure the mineral N content of the top 20 cm of soil at least once during the winter and summer. Apply either ammonium or nitrate fertiliser before planting to raise the mineral N content of the soil to 70 mg/l for crops harvested in winter or 100 mg/l for crops harvested in summer

- Avoid using slow release fertilisers or nitrification inhibitors to control the N supply

- Avoid application of N in the overhead irrigation water within 10 to 14 days of harvest

- Differences in nitrate accumulation between the common comercial butterhead cultivars are small, so select your variety on the basis of other beneficial attributes, *eg* latest mildew resistance.

- Be aware that some red novelty types of lettuce often contain higher nitrate concentrations than butterhead varieties grown under the same conditions

- Use the Nitrachek meter to monitor the nitrate concentration of all crops harvested, and to provide guidance on adjusting N fertiliser applications for following crops

- Take particular care to ensure that summer crops are vacuum cooled as soon as possible after harvest to avoid water loss

- Use the LET_N recommendation system for more accurate site-specific advice when it becomes available after further testing

*For HM Government*

Use the following information to help revise existing EU legislation and to develop future protocols for sampling and analysis of nitrate in lettuce

- Low light levels in the 10 to 14 day period before harvest is likely to reduce assimilation of N and may lead to rapid increases in nitrate concentration to levels above the current EU limits for glasshouse lettuce, even when best practice is followed

- The variability in nitrate concentration in protected lettuce was found to range from

  - 10 to 15 % between individual heads due to inherent (mainly genetic) differences within a crop

  - 8 to 14 % between individual heads of soil-grown crops in winter

  - 17 to 44 % between individual heads of soil-grown crops in summer

  - 8 to 19 % between bulked 10-head samples (as recommended in the UK nitrate monitoring guidelines) of soil-grown protected lettuce in year-round production

- Nitrate data generated by different laboratories can vary by up to 100 %. This is due mainly to lack of specificity in methods of nitrate extraction from lettuce tissues.

**Practical and financial anticipated benefits**

By meeting the strict quality standards imposed by the EC the viability of lettuce businesses with a current value of almost £25 million will be maintained. Failure to comply with EC Regulation 194/97 (and its amendment 864/99) will lead to the failure of the UK glasshouse lettuce industry once the current derogation is lifted.

The largest benefits arising from this project will be achieved through

- updating the current Code of Good Agricultural Practice to provide growers with recommendations based on scientific studies of the value of different cultural and other practices in reducing the risk of excessive nitrate accumulation

- advising the UK Government and Food Standards Agency on appropriate scientific basis for implementing EU regulations on nitrates in soil-grown glasshouse lettuce.

- developing a new N fertiliser recommendation system which will avoid over-fertilisation of soil-grown glasshouse lettuce.

- devising standard operating procedures for a simple nitrate test for use by growers to monitor the nitrate status of their crops directly in the nursery.

With increasing environmental pressures all growers will face costs in ensuring that nitrate runoff from crops does not reach water courses. The economics of lettuce growing will not stand high investment to meet these pressures but by following a practice in which nitrogen fertiliser is applied only according to crop requirement (and not in excess) leaching will be minimised and hence costs kept to a minimum.

Accurate calculations of fertiliser requirements are likely to show some reduction on the current recommended levels, especially for winter grown crops, and there will therefore be a corresponding saving in fertiliser costs. For lettuce, a saving of 10% could be expected.