Project title:	Baby leaf lettuce: N response studies to maximise yield and manage nitrate levels
Project number:	FV 418
Project leader:	Dr Richard M. Weightman, ADAS UK Ltd.
Report:	Final report
Previous reports:	None
Key staff:	Angela Huckle (contract manager and report author) Daniel Munro, Senior Research Technician Chris Dyer, Statistician
Location of project:	Growers' holdings in Dorset, Kent, Shropshire & Wiltshire
Industry representative:	Dr Graham Clarkson, Vitacress Salads
Date project commenced:	1 April 2013
Date project completed (or expected completion date):	7th February 2014

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

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Richard Weightman
Senior Research Scientist
ADAS UK Ltd.

Signature ..... Date .....

Angela Huckle
Consultant, Field Vegetables
ADAS UK Ltd.

Signature ..... Date .....

Report authorised by:

Barry Mulholland
Head of Horticulture
ADAS UK Ltd.

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

- Mean crop nitrogen (N) offtake of baby leaf lettuce was estimated at 63 kg N/ha which should be used as a baseline guide for determining crop N requirement,
- If background soil mineral nitrogen (SMN) prior to drilling is > 60 kg N/ha, further N applications are unlikely to be necessary.

Background

Nitrate is viewed as a contaminant of leafy vegetables, and limits for its control are set in EU legislation. In northern Europe including the UK where the growing season is short and the weather is often dull during crop growth, nitrate levels tend to be higher than in southern European states. Recent work in the UK funded by HDC has examined the nitrogen (N) responses of baby leaf rocket and spinach, and led to development of N recommendations for optimising yield, and minimising tissue nitrate concentration (TNC) in baby leaf salad crops (HDC Factsheet 08/13). In addition to its effects on TNC, the recent studies showed that over-supplying baby leaf rocket and spinach crops with fertiliser N can actually cause a reduction in yield, when the crop has adequate supplies of N from the soil.

The project aims were to gather robust and independent data on nitrate levels in commercial baby leaf lettuce crops, and determine the yield response to nitrogen fertiliser, taking into account varietal types (red vs green), soil mineral nitrogen prior to drilling, soil type and previous cropping.

The objectives were to:

- quantify yield responses to N on growers sites (representative soil types across the season) and determine optimum N needed to produce a marketable crop while remaining below the proposed EC limit for TNC of 3,000 mg/kg fresh weight,

- evaluate the extent to which yield response to fertiliser N can be predicted from soil mineral nitrogen (SMN) measurements taken prior to drilling,

- critically assess N offtake as a way to measure crop N demand, and for the grower provide a better way of estimating fertiliser N requirement.

This work therefore provides a basis for recommendations for N fertiliser for baby leaf lettuce, as there are currently none in the Fertiliser Manual (RB209; Defra, 2010).

Summary

Yield response

Five sites were chosen for the N response studies to represent the geographical spread of UK baby leaf lettuce growers. Six individual N responses for red (one Red Cos and two Red Batavia) and green baby leaf (Green Cos, Green Tango and Green Batavia) lettuce varieties (three of each colour/variety type) were obtained. Sampling was carried out through the summer into early autumn, representing the full duration of the UK growing season and covering both first and second crops. Topsoil (pH, P, K, Mg) and SMN samples at 0-30 cm depth were taken prior to drilling and prior to application of fertiliser N at each site. Nitrogen fertiliser as calcium ammonium nitrate was applied at six rates of 0, 40, 80, 120, 170 and 220 kg N/ha post drilling.

Significant yield responses to applied N were only seen at two locations (Sites 13/1 and 13/2) where initial background SMN was at or below 30 kg N/ha prior to applying N treatments (Figure 1). At these two sites, further applications of N above 40 kg/ha (Site 13/1) or 80 kg N/ha (Site 13/2) had no significant effect on yield.

In the remaining four response data sets (Sites 13/3, 13/4 & 13/5) where the initial SMN was 116 – 265 kg N/ha, yield declined with addition of fertiliser N indicating that N was becoming toxic to the crop at these levels (Figure 1).

Tissue nitrate concentration

The 3,000 mg/kg limit for TNC was exceeded at a number of the sites, with modest amounts of N applied as follows:

- 40 kg N/ha applied at Site 13/2,
- 80 kg N/ha at Site 13/3,
- 120 kg N/ha at Sites 13/1 & 13/4.

Of the two sites which showed a significant yield response to applied N, Site 13/2 had the additional problem that at 40 and 80 kg N/ha applied, its TNC levels were 3,090 and 3,221 mg/kg respectively. Hence, it could not be recommended that fertiliser N be applied to this crop, given this exceedance of the legal limit.

The only site therefore at which an N application for yield could be recommended, and at which the crop would also stay below the limit for TNC, would be Site 13/1 (with 40 kg N/ha).

Differences between red and green baby leaf types

Based on the fresh weight yields at the recommended N rates for yield and TNC (recommended N rates, 40 kg/ha for Site 13/1, and zero for the remaining sites), the average yield for the red types was just under half the yield at 17.9 t/ha, of the green types at 31.6 t/ha. This average assumed only 50% of the Green tango type would have been harvested; if the gross yield of this variety was used, the average yield of the green types was 43.2 t/ha).

TNC levels were similar overall between colour types: Red; 2,549 & 2,633 mg/kg and green; 2,344 & 2,938 mg/kg, at 40 and 80 kg N/ha respectively of applied N fertiliser averaged across the six data sets. However at one site (13/5) where Green and Red varieties were grown side by side in the same experiment, the Red Cos had significantly lower TNC overall than the Green Batavia type (1,921 vs 2,547 mg/kg).

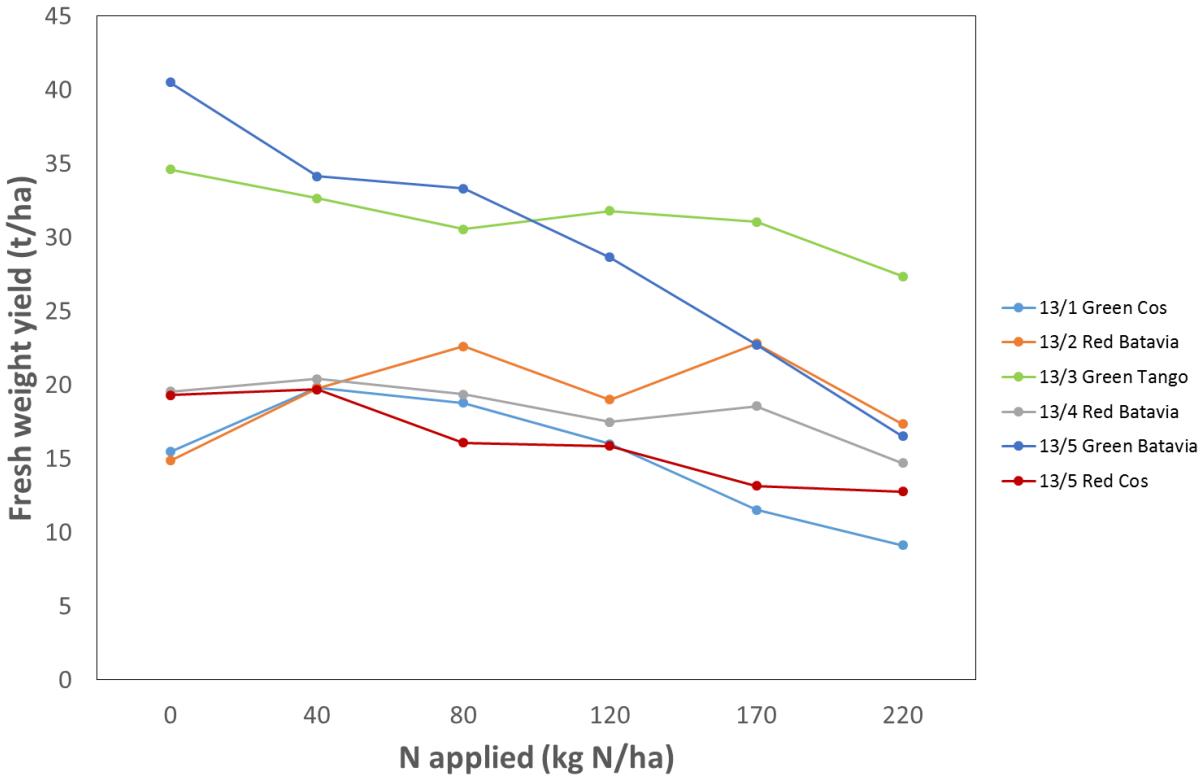

Figure 1. Effects of applied N fertiliser on fresh weight yield at four commercial production sites in 2013

N Recommendations

A key factor in determining an appropriate N recommendation is an estimate of total N uptake. Prior to this study there were no such estimates of this for baby leaf lettuce.

Across five of the data sets, based on averages of the three highest levels measured across the N rates tested, total N uptake varied between 48 and 79 kg N/ha, with an average across five data sets of 63 kg N/ha. The exception was Site 13/3, the crop of Green Tango with an N uptake of 140 kg N/ha reflecting its higher gross yield.

A total uptake of 63 kg N/ha is just over half that of the wild rocket crop, and explains in part why there was such a small response to applied N in these studies. Four of the sites had SMN (0-30 cm) at drilling greater than 100 kg N/ha, which was more than sufficient to satisfy a crop N requirement of this magnitude.

The two sites with lower SMN at drilling (27-30 kg N/ha) would be expected to require only around 55 kg N/ha as fertiliser N, assuming all the SMN was recovered by the crop, and 60% fertiliser recovery. This agrees broadly with the actual responses seen at 40 and 80 kg N/ha applied at sites 13/1 and 13/2 respectively, the early season crops.

Financial Benefits

The area of baby leaf lettuce has increased from 79 ha in 2007 to 274 ha in 2012, worth £10M at retail level and the area grown is expanding year on year. There is a requirement for continued vigilance on nitrate levels, and the industry as a whole needs to demonstrate to the Food Standards Agency and the European Commission that it is endeavouring to reduce nitrate in leafy salad crops. Failure to address the problem may result in increased losses due to rejection of crop, and potentially loss of this and other leafy salad crop species in the UK if growers cannot comply with Commission (and/or retailers) limits.

Action Points

- Baby leaf lettuce crops grown in the summer of 2013 were at risk from exceeding the limits set for TNC in outdoor lettuce of 3,000 mg NO_3^-/kg,
- As found in previous HDC-funded work on wild rocket and baby leaf spinach, the risk was particularly high for the late season crops, when SMN builds up in the rotation due to soils warming, plus mineralization of N from soil organic matter, and residues from previous crops in the current and past seasons,

- A major finding is that with the exception of the Green Tango ‘multi-leaf’ type there was an average crop requirement for N of only 63 kg N/ha, approximately half that of wild rocket, meaning that recommended amounts of N for baby leaf lettuce are likely to be lower than those defined in HDC Factsheet 08/13,
- Based on such a low N requirement, in most cases, with a low SNS e.g. index zero (30-60 kg N/ha, 0-30 cm) at drilling, the crop is only like to need a maximum of 60 kg N/ha applied as fertiliser N,
- With SMN levels above 60 kg N/ha at drilling, it is likely that no further N will be required by baby leaf lettuce crops,
- Recommendations may be adjusted upwards if the grower has their own higher estimates of typical yields and N uptake for crops in their land, and can therefore justify a higher N requirement.

SCIENCE SECTION

Introduction

Nitrate is viewed as a contaminant of leafy vegetables, and limits for its control are set in EU legislation. In northern Europe including the UK where the growing season is short and the weather is often dull during crop growth, nitrate levels tend to be higher than in southern European states. Monitoring by the authorities has been carried out since 1998 (Weightman et al., 2013).

Recent work in the UK funded by HDC has examined the nitrogen (N) responses of baby leaf rocket and spinach (FV 340, 340a, 340b), and led to development of N recommendations for optimising yield, and minimising tissue nitrate concentration (TNC) in baby leaf salads crops (HDC Factsheet 08/13). While the early days of research on nitrate in salad crops focussed on whole head lettuce (particularly protected crops, where nutrition could be more closely controlled) there has been no work on baby leaf lettuce, a growing market in the bagged salads sector.

Notwithstanding effects on TNC, the recent studies have shown with rocket and spinach that over-supplying the crop with N can actually cause a reduction in yield, when the crop has adequate supplies of N from the soil (Weightman et al., 2013).

Legislation

In 2012, limits for tissue nitrate concentration (TNC) were revised (Commission Regulation (EU) No 1258/2011) and in general for lettuce were increased (i.e. it became easier in most cases for lettuce growers to comply with the limits). For the baby leaf crops studied in this report which are all outdoor crops grown in the UK, the relevant limit is 3,000 mgNO_3^-/kg for non-iceberg type lettuce grown in the 'open air' and harvested between 1st April and 30th September (other limits for TNC apply for iceberg-type lettuces, protected crops and for crops harvested outside these periods; see Factsheet 08/13 for further details).

However the one factor which could conceivably be particularly important for baby leaf crops, and contrasts with traditional lettuce production, is that all the leaves are harvested, and there is therefore no opportunity for trimming. Trimming is important because with larger whole head type lettuces, it is feasible to grow to a slightly higher than marketable head weight, and then trim off the outer leaves, because these are known to contain higher TNC. Thus the whole head product as marketed tended to be lower TNC than it would

otherwise be, if measured in the field. It is conceivable therefore that baby leaf crops where no such trimming occurs, would be at higher risk of exceeding the limits for TNC set in the legislation.

Factors affecting nitrate content in the crop

There are a number of factors affecting TNC in the plant including incident levels of radiation in the 5 to 20 days prior to harvest (Weightman et al., 2006), soil N supply (SNS; including soil mineral N prior to drilling plus N mineralised during crop growth) and amount of fertiliser N applied during growth. These factors have recently been reviewed by Weightman and Hudson (2013).

Project Aims and Objectives

The project aims were to gather robust and independent data on nitrate levels in commercial baby leaf lettuce crops, and determine the yield response to nitrogen fertiliser, taking into account varietal types (red vs green), soil mineral nitrogen prior to drilling, soil type and previous cropping.

The objectives were to:

- quantify yield responses to N on growers sites (representative soil types across the season) and determine optimum N needed to produce a marketable crop while remaining below the proposed EC limit of 3,000 mg NO_3^-/kg fresh weight,

- evaluate of the extent to which yield response to fertiliser N can be predicted from soil mineral nitrogen measurements taken prior to drilling,

- critically assess N offtake as way to measure crop N demand, and for the grower provide a better way of estimating of fertiliser N requirement.

This work therefore provides a basis for recommendations for N fertiliser for baby leaf lettuce, as there are currently none in the Fertiliser Manual (RB209; Defra, 2010).

Materials and Methods

Two separate studies were carried out in 2013. The first comprised four individual N response experiments at different locations, to quantify the yield responses of either red or green varieties of baby leaf lettuce (individual varieties grown at each site) to applied fertiliser N. The second study (a single experiment) was designed to examine the relative

yield responses of red and green lettuce types to applied N in a randomised factorial design.

Study One: Individual N rate experiments at four commercial sites

Four sites (13/1 – 13/4) were chosen for the nitrogen (N) response experiments to represent the geographical spread of UK baby leaf lettuce growers (Table 1).

Study 2 – Variety x Nitrogen response experiment

A site (13/5) was identified in Wiltshire, and seed of two contrasting varieties was drilled by the grower using neighbouring beds (Green Batavia and Red Cos). The beds effectively acting as main plots within a split plot design, and the N rate treatments were then fully randomised within the variety main plots.

Site selection, sampling and treatment applications

Field experiments were established on grower holdings. Experimentation was carried out through the summer into early autumn of 2013, representing the full duration of the UK growing season and covering both first and second crops. Variety, sowing and harvest dates for the sites are shown in Table 1. All sites were on sandy loam soil types. The initial aim was to select sites with low soil nitrogen supply (SNS) indices at which strong responses to applied N would be expected. Representative topsoil (for pH, P, K & Mg) and SMN samples at a 0-30 cm depth were taken prior to drilling and application of fertiliser N using a standard 12 point W-shaped sampling pattern, and soil samples were bulked to provide a single analysis for the whole trial for background SMN.

Table 1. Previous cropping details and sowing and harvest dates of four N response experiments on individual baby leaf lettuce varieties, and one variety x N rate experiment on commercial growers' premises in 2013.

Site	Variety	Sowing & harvest dates 2013	Previous crop in 2012 season	N applied to 2012 crop (kg N/ha)	Previous crop(s) in current season (2013)	N applied to previous crop(s) in 2013 (kg N/ha)
Study 1						
13/1 Wilts	Green Cos	7 May - 13 June	Wholehead Lettuce	220	N/A	N/A
13/2 Dorset	Red Batavia	23 May - 2 July	Babyleaf Lettuce, Spinach, Babyleaf Lettuce, Babyleaf Brassicas	140 90	N/A	N/A

13/3 Kent	Green Tango	1 July - 8 Aug	Babyleaf Spinach, Wholehead Lettuce	135 120	N/A	N/A
13/4 Shrops	Red Batavia	17 July - 27 Aug	Wild Rocket Babyleaf Lettuce	96 96	Babyleaf Lettuce	96
Study 2						
13/5 Wilts	Green Batavia & Red Cos	7 Aug - 10 Sept	Wholehead Lettuce	220	Babyleaf Lettuce	100

N treatments and experimental design

Post drilling, plots for the six N treatment rates (0, 40, 80, 120, 170 and 220 kg N/ha) were marked out and calcium ammonium nitrate (CAN) fertiliser was applied by hand. For Study 1, the trials were carried out using a fully randomised block design with 4 replicates, to give a total of 24 plots at each site (6 N rates x 4 replicates). In Study 2 there were a total of 48 plots (6 N rates x 2 varieties x 4 replicates). Plot dimensions in all experiments were 5 m x 1.8 m, with a whole bed around the outside of each experiment providing guard plots, and to minimise the risk of overspreading by neighbouring commercial field operations. With the exception of N applied, the crop was otherwise grown using standard commercial practice with pesticides and irrigation applied as necessary. The CAN applied by ADAS was the only N-based fertiliser applied to any of the experimental plots after drilling. A typical trial following CAN treatment applications is illustrated in Figure 2, and during growth in Figure 3.

Figure 2. Typical N response trial set up, post-treatment application of CAN.

Figure 3.Typical N response trial at harvest: L: Single variety trial showing differences between plots early season; R red/green trial with varieties drilled in adjacent beds.

Sampling at harvest

As close to the intended harvest date as possible, samples of plant tissue and soil were taken for determination of Total N, TNC in the crop, and SMN on the nil N plots. Fresh weight, dry weight, leaf greenness, insect damage and disease were also assessed. Leaf tissue samples were taken before mid-day at each site using a 0.25 m^2 quadrat to collect

four samples of approx 500 g of foliage for Total N and TNC analysis from the cropped plots. A length of plot of at least 0.5m between adjacent N treatment plots was left un-harvested as a guard area. The samples were dispatched immediately, for receipt by NRM laboratories within 24 hours of collection, and remained chilled during transportation and storage. Leaf greenness was measured on each plot *in situ* using a Minolta SPAD meter. A separate sample was taken using a 0.25 m^2 quadrat from each cropped plot for analysis of fresh and dry weight on return to ADAS Boxworth (samples also chilled during transportation and storage).

Total N (% w/w dry basis by DUMAS combustion method) and tissue nitrate concentration (TNC, mg NO_3^-/kg fresh weight) were determined by NRM. Soil mineral nitrogen (SMN, kg N/ha to 30cm depth) was determined on the nil N plots. By combining the total N concentration with dry weight yield, an estimate could be derived of total crop N offtake (kg N/ha).

Statistical analysis

SMN, TNC, N offtake, yield, leaf greenness, insect damage and disease were analysed by analysis of variance (ANOVA). ANOVA and other analyses were carried out using the statistical software *GenStat* (12th Edition). Yield response curves could not be fitted to any of the data, therefore it was not possible to determine the economic optimum N rate interpolated between actual applied N rates in this study.

Results

Study One - Individual site summaries

In the following section, the results are reported for individual sites to make it easier for the reader to understand the background and the reasons for the responses at each site. For each site the initial SMN is presented as well as the measured parameters of crop growth. The key responses to applied N (fresh weight yield and TNC) are presented graphically. The remaining data and the statistical analyses are presented in tables.

Site 13/1 Wiltshire (Green Cos)

- Initial SMN low (30 kg N/ha) due to high rainfall in the west during autumn/winter 2012/13,
- Crop sown early in season (7 May 2013) therefore less time available for mineralisation of N from organic matter compared to other sites, especially with the cool, damp spring,
- Moderate yielding site for green variety (yield potential >19 t/ha fresh weight; Table 2; Figure 4),
- Significant increase in yield up 40 kg N/ha, followed by a significant decline in yield, in response to applied N fertiliser from 80 to 220 kg N/ha,
- Significant effect of applied N on leaf greenness or chlorophyll content up to 80 kg N/ha,
- TNC very low at zero N applied as SMN at drilling was low, but a significant increase in TNC to applied N, with a rapid increase from 54 to 2,760 mg/kg between 0 and 80 kg N/ha applied,
- TNC close to limit for outdoor lettuce at 80 kg N/ha and exceeded limit (3,350 mg/kg) with 120 kg N/ha applied),
- Maximum N offtake around 60 kgN/ha, with a decline at the highest N rates as yield declined.

Table 2. Effects of applied fertiliser nitrogen on yield, chlorophyll concentration, TNC and N offtake at harvest for a Green Cos crop in Wiltshire (site 13/1, harvested 13 June 2013).

Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**Probability (F value)**	**LSD (5%)**
N Rate (kg/N ha)	0	40	80	120	170	220		
Fresh Weight (t/ha)	15.5	19.8	18.8	16.0	11.5	9.1	<.001	3.26
Leaf chlorophyll (SPAD)	33.1	35.4	37.5	36.2	38.2	37.7	0.002	2.18
TNC (mg/kg)	54	1,652	2,760	3,350	3,716	3,918	<.001	346.3
N offtake (kg/N ha)	35.7	61.5	60.5	60.3	46.1	37.0	0.002	14.46

Initial SMN = 30 kg/N ha K index = 3 P index = 5

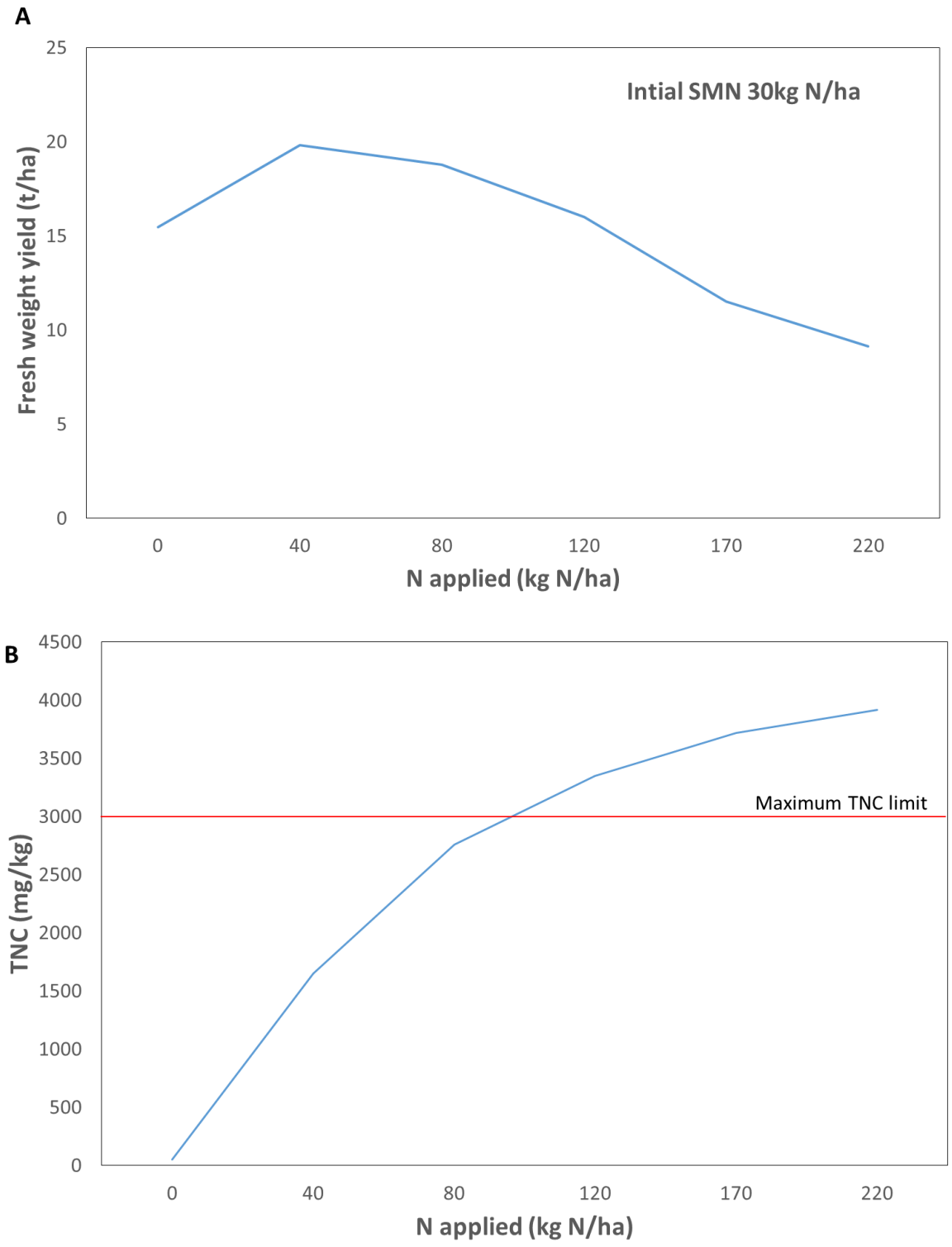

Figure 4. Effects of N rate on Green Cos at site 13/1 on A: Fresh Weight; B: Tissue Nitrate Concentration (TNC). Wiltshire 2013.

Site 13/2, Dorset (Red Batavia)

- Initial SMN low (26.9 kg N/ha) due to high rainfall in the south west during autumn/winter 2012/13,
- Crop sown early in season as a first crop (23 May) therefore less time available for mineralisation of N from any remaining organic matter from previous cropping in 2012,
- High yielding site (yield potential >22 t/ha fresh weight),
- There was no overall response to N in fresh weight yield, but yield increased from 14.9 to 22.6 t/ha in response to applied N fertiliser rates between 0 and 80 kg N/ha (Table 3; Figure 5),
- TNC was very low at zero N applied but always above the limit where any N was applied (>3000 mg/kg with >80 kg N/ha applied)
- Significant increase in leaf greenness at the highest rates of applied N (170 – 220 kg N/ha) although at these levels, TNC was above the limits (>3,464 mg/kg).

Table 3. Effect of applied fertiliser nitrogen on yield, chlorophyll concentration, TNC and n offtake at harvest for a crop of Red Batavia in Dorset (site 13/2, harvested 2nd July 2013).

Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**Probability (F value)**	**LSD (5%)**
N rate (kg N/ha)	0	40	80	120	170	220		
Fresh Weight (t/ha)	14.9	19.8	22.6	19.0	22.8	17.4	N.S	6.13
Leaf chlorophyll (SPAD)	27.1	27.6	28.5	29.9	33.7	33.6	0.01	4.11
TNC (mg/kg)	40	3,090	3,221	3,405	3,464	3,952	<.001	559.1
N offtake (kg/N ha)	32.6	63.9	83.1	73.1	81.1	74.1	<.001	19.88

Initial SMN = 26.9 kg/N ha K index = 2+ P index = 5

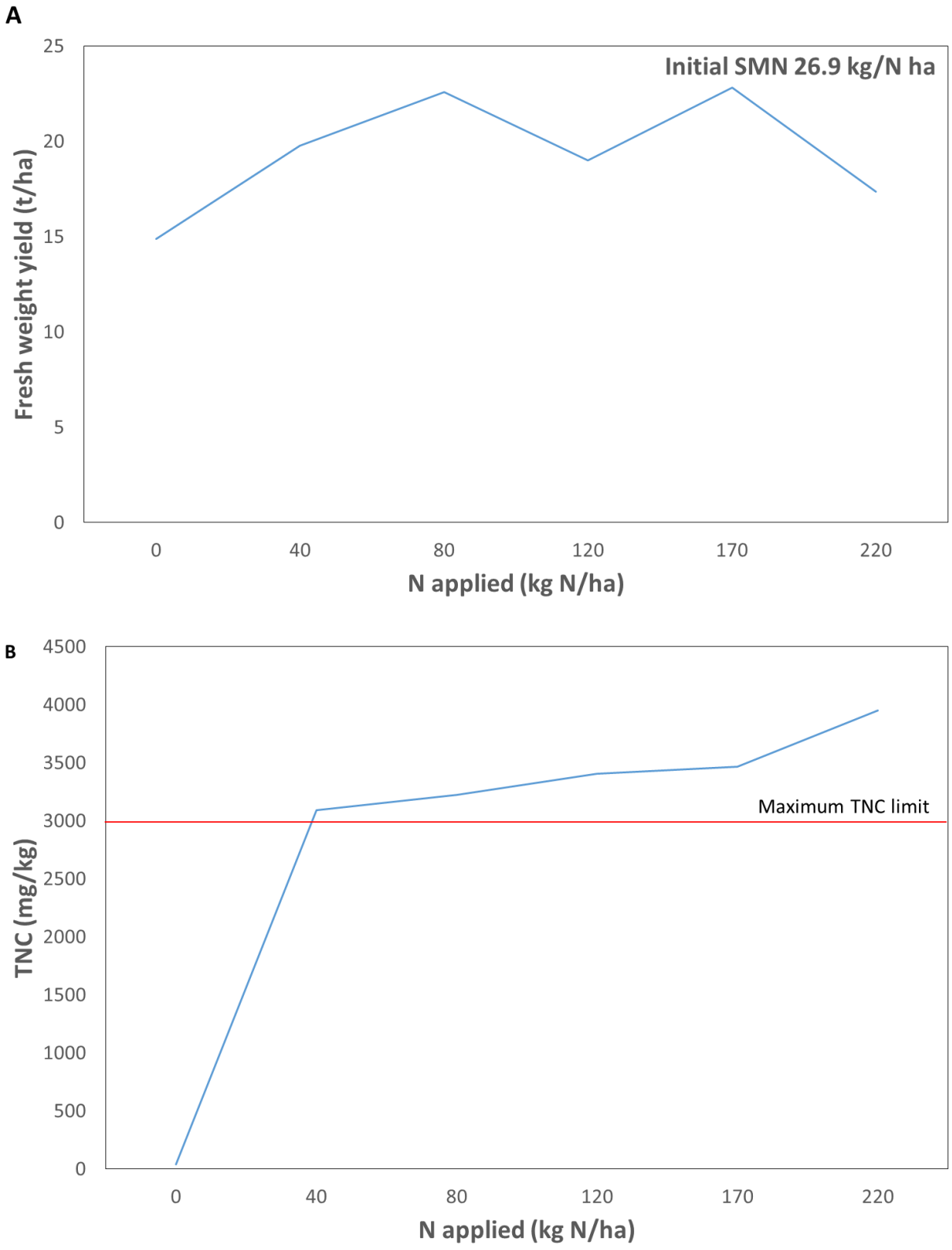

Figure 5. Effects of N rate on red Batavia at site 13/2 on A: Fresh Weight; B: Tissue Nitrate Concentration (TNC). Dorset 2013.

Site 13/3 Kent (Green Tango)

- Initial SMN high (166 kg N/ha) due to mineralisation of previous crop residues through early summer (sowing date 1st July),
- There was no significant response to N in fresh weight yield, but yield decreased with increasing N applications,
- Very high yielding crop (yield potential >34 t/ha fresh weight) although Green Tango is strictly a 'multi leaf' Apollo type, and only a fraction (ca. 50%) of the crop would be harvested as baby leaf (Table 4, Figure 6),
- No significant effect of applied N on leaf greenness/chlorophyll,
- TNC was already quite high at zero N applied (2,187 mg/kg),
- TNC showed a significant response to applied N, and was above the TNC limit for outdoor lettuce where N fertiliser was applied at or above 80 kg N/ha,
- Total N uptake high (up to 190 kg N/ha) due to high yield potential.

Table 4. Effect of applied fertiliser nitrogen on yield, chlorophyll concentration, TNC and N offtake at harvest for a Green tango crop in Kent (site 13/3, harvested 8th August).

Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**Probability (F value)**	**LSD (5%)**
N rate (kg/N ha)	0	40	80	120	170	220		
Fresh Weight by quadrat harvested (t/ha)	69.2	65.3	61.1	63.6	62.1	54.7	NS	15.87
Fresh Weight 50% (t/ha)*	34.6	32.6	30.5	31.8	31.1	27.4	-	-
Leaf chlorophyll (SPAD)	24.1	25.4	25.0	27.2	23.7	26.5	NS	5.55
TNC (mg/kg)	2,187	2,827	3,438	3,553	3,582	3,790	<.001	294.3
Total N uptake (kg/N ha)	140	193	185	161	187	168	NS	72.9
N offtake based on 50% yield (kg/N ha)**	69.9	96.5	92.7	80.5	93.5	84.1	-	

Initial SMN = 166.2 kg/N ha K index = 2- P index = 4

*, estimated from total fresh weight yield

**, estimated from measured total N uptake

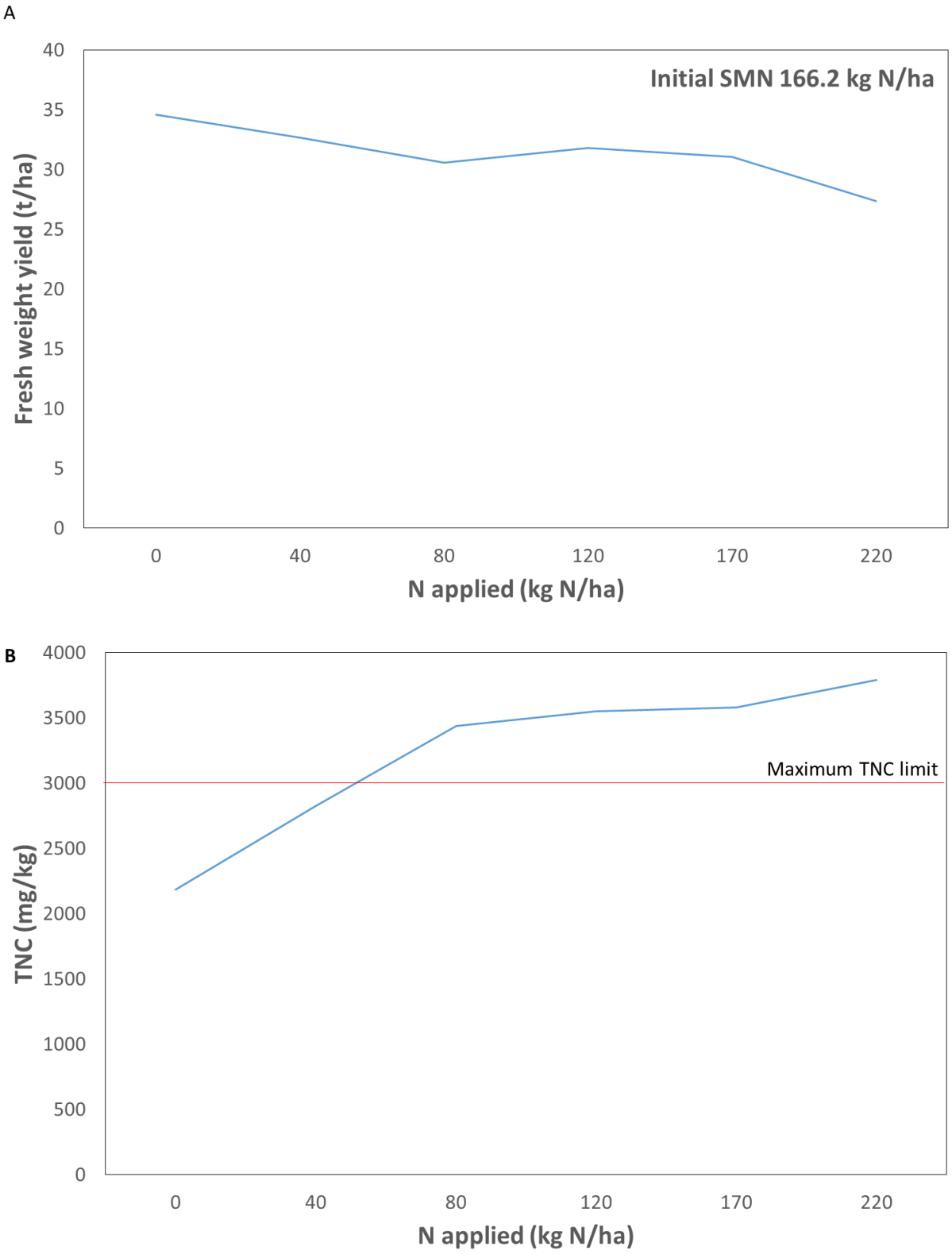

Figure 6. Effects of N rate on Green Tango crop at site 13/3 on A: Fresh Weight (50% of gross measured yield); B: Tissue Nitrate Concentration (TNC). Kent, 2013.

Site 13/4 Shropshire (Red Batavia)

- Initial SMN moderate (116 kg N/ha) due to mineralisation and crop residues from a previous baby leaf lettuce crop,
- Crop sown mid-season (drilled 7 July 2013) therefore more time available for mineralisation of N from organic matter from residues,
- High yielding site (yield potential >20 t/ha fresh weight),
- There was no significant response to N in fresh weight yield, and the yield declined with N applied above 40 kg N/ha (Table 5, Figure 7),
- No significant effect of applied N on leaf greenness (chlorophyll concentration index),
- TNC already quite high at zero N applied due to high SMN,
- TNC showed a significant response to N applied and remained close to limit or slightly above for outdoor lettuce from 80 kg N/ha to 220 kg N/ha (<3000 mg/kg with up to 80 kg N/ha applied),
- Total crop N offtake maximal at around 70 kg N/ha.

Table 5. Effect of applied fertiliser nitrogen on yield, chlorophyll concentration, TNC and N offtake at harvest for a Red Batavia crop in Shropshire (site 13/4, harvested 27th August).

Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**Probability (F value)**	**LSD (5%)**
N rate	0	40	80	120	170	220		
Fresh Weight (t/ha)	19.5	20.4	19.4	17.5	18.6	14.7	NS	6.174
Leaf chlorophyll (SPAD)	27.1	26.9	25.1	27.0	28.3	27.0	NS	2.41
TNC (mg/kg)	2,076	2,625	2,717	3,126	2,886	2,764	<.001	297
N offtake (kg/N ha)	55.1	65.8	62.1	63.2	70.0	48.6	NS	21.71

Initial SMN = 115.9 kg/N ha K index = 2+ P index = 4

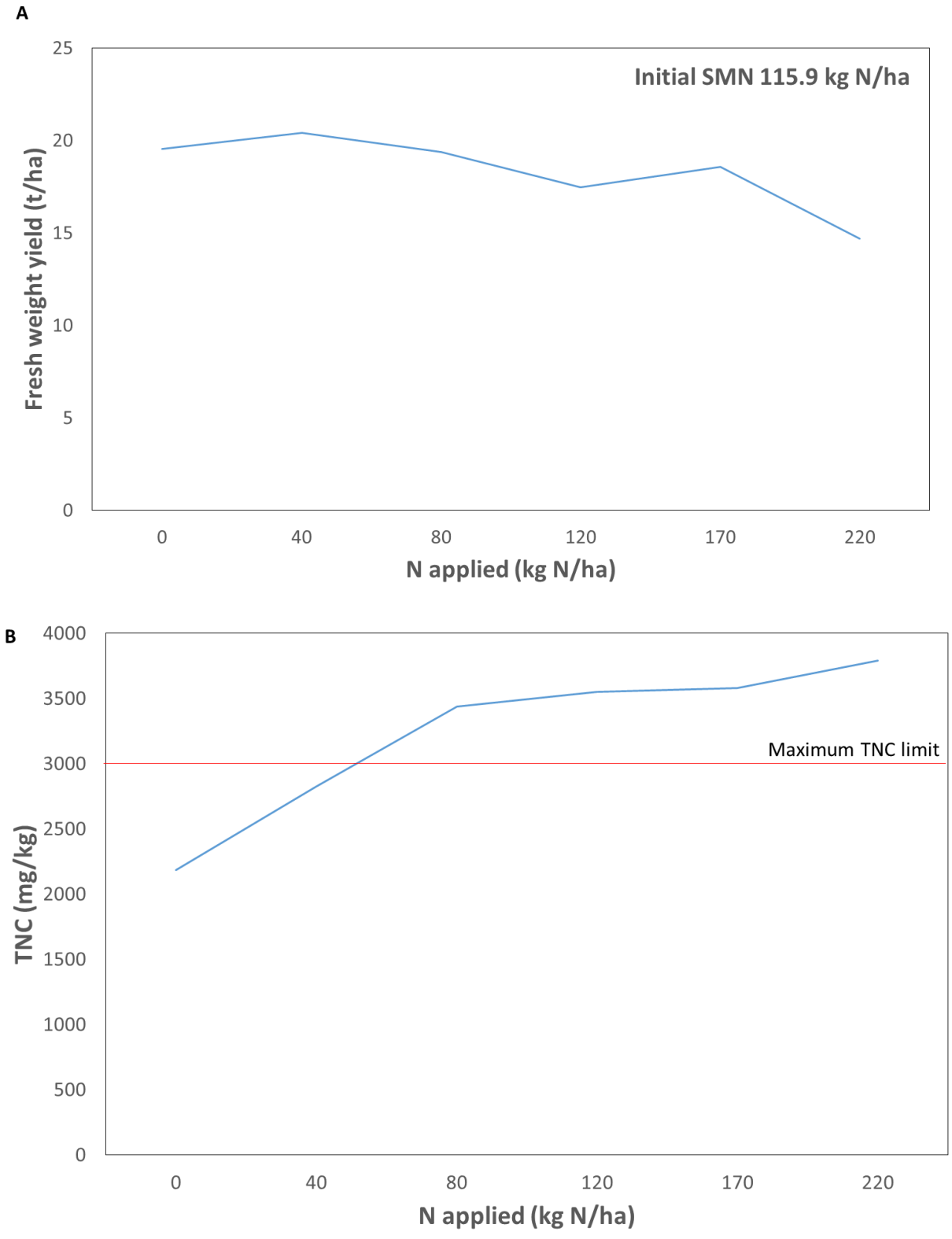

Figure 7. Effects of N rate on Red Batavia at site 13/4 on A: Fresh Weight; B: Tissue Nitrate Concentration (TNC). Shropshire 2013.

Study Two – Variety x N Trial (single site)

This study was designed to compare red and green varieties drilled on the same date at the same location. Due to the limitations of commercial drilling operations, the two varieties could not be randomised within the field experiment, but instead were allocated to adjacent beds. Nevertheless, N rate treatments were fully randomised within the variety main plots, and the field was otherwise uniform (see Figure 3).

Site 13/5 Wilts (Green Batavia, Red Cos)

- Initial SMN was high (235 kg N/ha) as this was a late season crop drilled following two previous salad crops, late in season (7 August),
- Yield decreased significantly with any N applied, for both varieties, with the green variety showing the greatest reduction when compared to the red (Table 6, Figure 8),
- High yielding site (yield potential >19 t/ha fresh weight)
- No significant effect of applied N on leaf greenness or chlorophyll content
- A relatively low TNC for this site (average 2,234 mg/kg) and no significant response of TNC to N applied, despite high SMN at drilling.

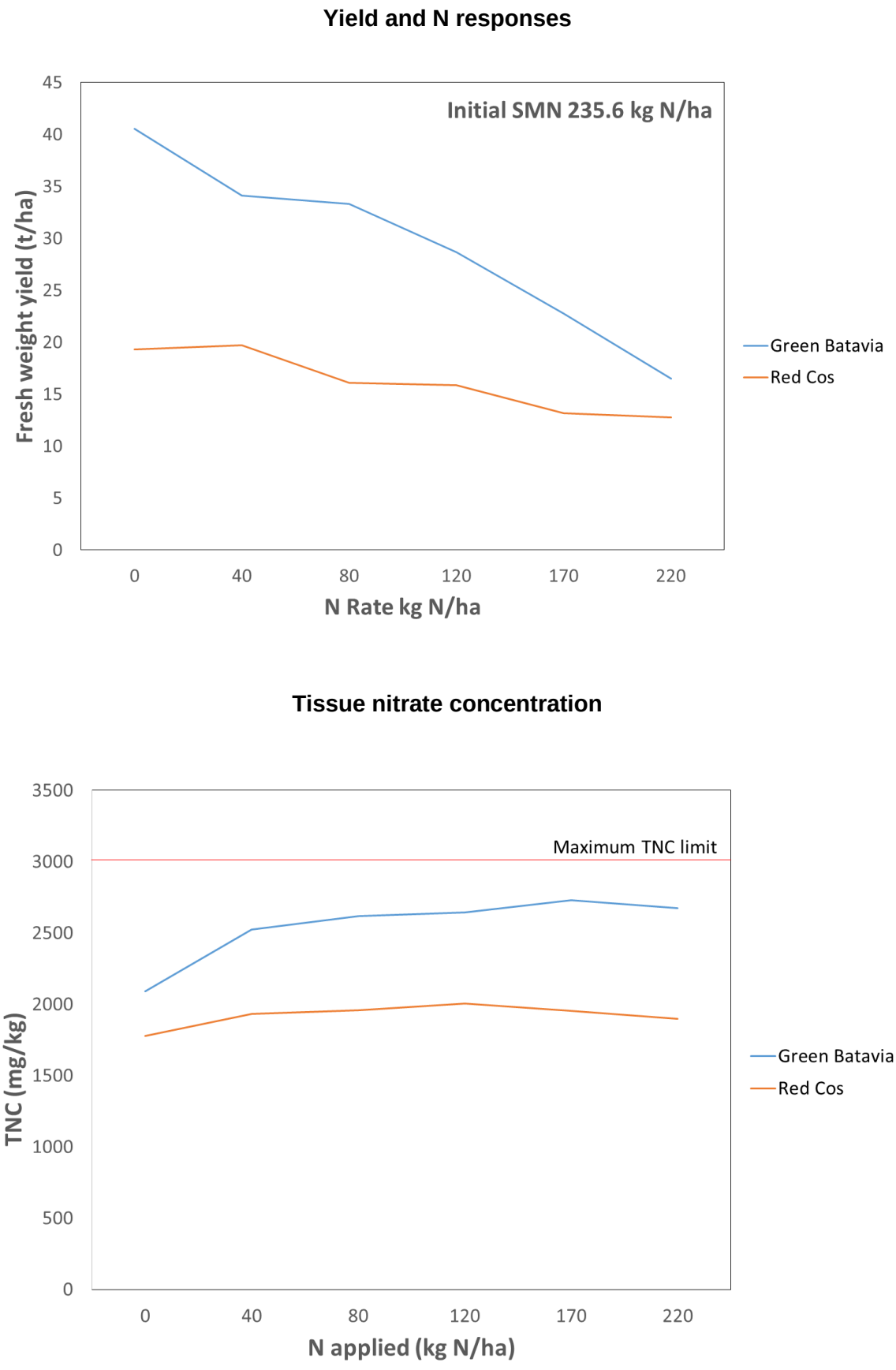

Figure 8. Effects of N rate on Red Batavia and Green Cos grown together at site 13/5. Wiltshire 2013.

Table 6. Effect of applied fertiliser nitrogen on yield, chlorophyll concentration, TNC and N offtake at harvest for Green Batavia and Red Batavia varieties grown on a single site in Shropshire (site 13/5, harvested 10th September 2013).

Treatment	1	2	3	4	5	6	Variety Means	Probability (F value) Variety	LSD (5%) Variety	Probability (F value) N Rate	LSD (5%) N rate
N Rate kg/N ha	0	40	80	120	170	220					
Yield (t/ha)											
Green	40.5	34.1	33.3	28.7	22.7	16.5	29.3				
Red	19.3	19.7	16.1	15.9	13.2	12.8	16.1	<.001	4.35	<.001	5.65
N Rate Means	29.9	26.9	24.7	22.3	18.0	14.6					
SPAD											
Green	29.4	30.8	30.7	31.0	31.4	32.1	30.9				
Red	32.1	32.8	31.8	31.9	33.0	33.4	32.5	0.011	1.06	N.S	1.95
N Rate Means	30.8	31.8	31.3	31.4	32.2	32.8					
TNC (mg/kg)											
Green	2,092	2,522	2,617	2,644	2,730	2,676	2,547	0.008	394.0	N.S	379.6
Red	1,779	1,932	1,960	2,007	1,952	1,896	1,921				
N Rate Means	1,935	2,227	2,288	2,325	2,341	2,286					
N Offtake (kg/N ha)											
Green	68.7	65.8	62.7	49.5	50.1	42.2	56.5	0.015	9.40	0.008	11.33
Red	46.3	48.9	47.8	42.0	39.2	36.8	43.5				
N Rate Means	57.5	57.3	55.3	45.7	44.7	39.5					

Initial SMN = 265 kg/N ha K index = 3 P index = 5

Residual soil mineral nitrogen

Table 7 shows the amount of SMN left in the soil (nil N plots) at harvest. It can be seen that the residual SMN increases through the season, as the initial SMN also increased as described in the individual site summaries

Table 7. Variety name, harvest date and residual SMN (0-30 cm) at harvest as measured in nil N plots, for 5 experiments carried out in 2013.

Site code	**13/1**	**13/2**	**13/3**	**13/4**	**13/5**	
County	Wilts	Dorset	Kent	Shrops	Kent	
Variety	Green Cos	Red Batavia	Green Tango	Red Batavia	Green Batavia	Red Cos
Harvest date	13 June	2 July	8 Aug	27 Aug	10 Sept	
Residual SMN (kg N/ha)	20.2	5.6	27.2	46.4	70.0	68.4

Discussion

Yield responses

In four out of six N response datasets in the present project (taking Studies 1 and 2 together), there was no requirement for additional fertiliser N based on the statistical analysis by ANOVA. With the exception of a Red Batavia crop (site 13/4) yields actually declined with increasing amounts of N (Figure 9). As a result, no curves could be fitted and estimation of economic optima became irrelevant, other than assuming that zero N application was the best option in those cases. Even with the high yielding Green Tango crop (site 13/3), because SMN was high prior to drilling (166 kg N/ha) there was no requirement for additional N after drilling.

At two sites (13/1 and 13/2) there was an apparent requirement of 40 to 80 kg N/ha for yield, but when TNC was also taken into account, only one crop (site 13/1, a Green Cos variety) still justified addition of a modest amount (40 kg/ha) of fertiliser N (Table 8).

The average yield of the red varieties was only 57% of that of the green varieties (even assuming 50% harvested yield for the Green Tango), but there was little overall difference in TNC between the crop types when averaged across sites, and N rates in the range 0 to 80 kg N/ha.

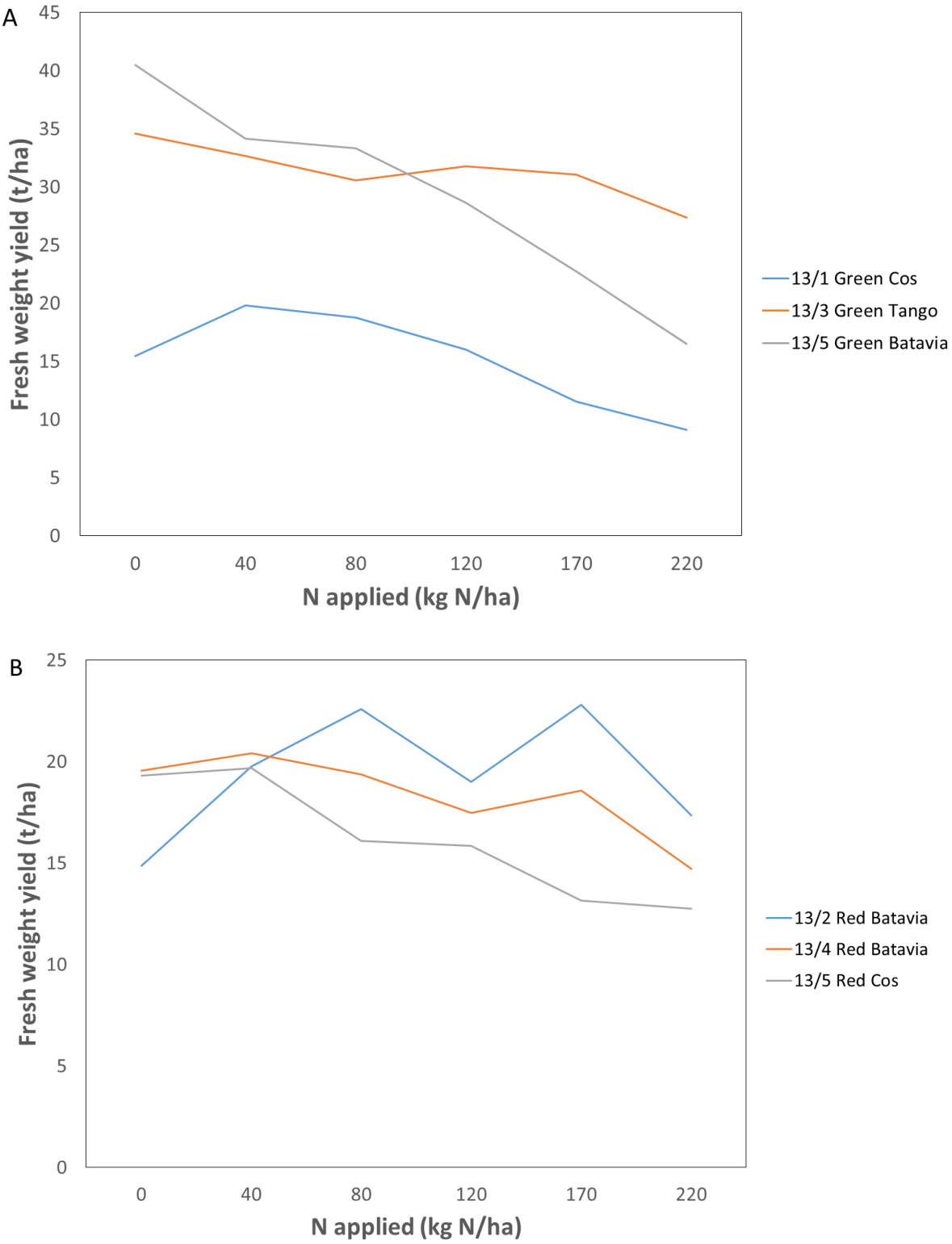

Figure 9. Effects of rate of N fertiliser applications on fresh weight yield of A: green varieties; B: red varieties at 5 sites in 2013 (Green Tango yields shown at 50% of measured yields).

Table 8. Summary of recommended N application rates based on data from 5 field experiments with different varieties of red and green baby leaf lettuce crops in 2013, where recommendation is based on either yield alone, or yield plus also maintaining tissue nitrate concentration (TNC) below the limit of 3000 mg/kg set in the regulations.

Site code	Variety	Rec N for yield only (kg N/ha)	TNC at nil N (mg/kg)	TNC at 40 kg N/ha (mg/kg)	TNC at 80 kg N/ha (mg/kg)	Rec N for yield and TNC (kg N/ha)	FW yield at Rec N (t/ha)
	Green types						
13/1	Green Cos	40	54	1,652	2,760	40	19.8
13/3	Green Tango	0	2,187	2,827	3,438	0	*34.6
13/5	Green Batavia	0	2,092	2,522	2,617	0	40.5
Average			*1,444*	*2,334*	*2,938*		*31.6*
	Red types						
13/2	Red Batavia	80	40	3,090	3,221	0	14.9
13/4	Red Batavia	0	2,076	2,625	2,717	0	19.5
13/5	Red Cos	0	1,779	1,932	1,960	0	19.3
Average			*1,298*	*2,549*	*2,633*		*17.9*

* Yield of Green Tango presented at 50% x measured gross yield

Tissue nitrate concentration

Table 8 above shows clearly that as the crops become later sown, the TNC in the crop on nil N plots increases. This reflects the higher SMN prior to drilling at these sites, as the soil warms up and in some cases, previous crop residues mineralize. In summary, the 3,000 mg/kg limit for TNC was exceeded at a number of the sites (Figure 10), with modest amounts of N applied as follows:

- 40 kg N/ha applied at Site 13/2,
- 80 kg N/ha at Site 13/3,
- 120 kg N/ha at Sites 13/1 & 13/4.

Interestingly at Site 13/5 (the variety x N trial), TNC did not exceed the limits set, even at the highest N rate applied. This was unexpected given that the site had a very high SMN at drilling (265 kg N/ha) and was also the latest crop, when day length would have been shortest and hence radiation levels low. There is no explanation for this based on parameters measured in this study, or incident solar radiation levels (data not presented here), and represents the sort of anomalous result that growers often report in commercial practice.

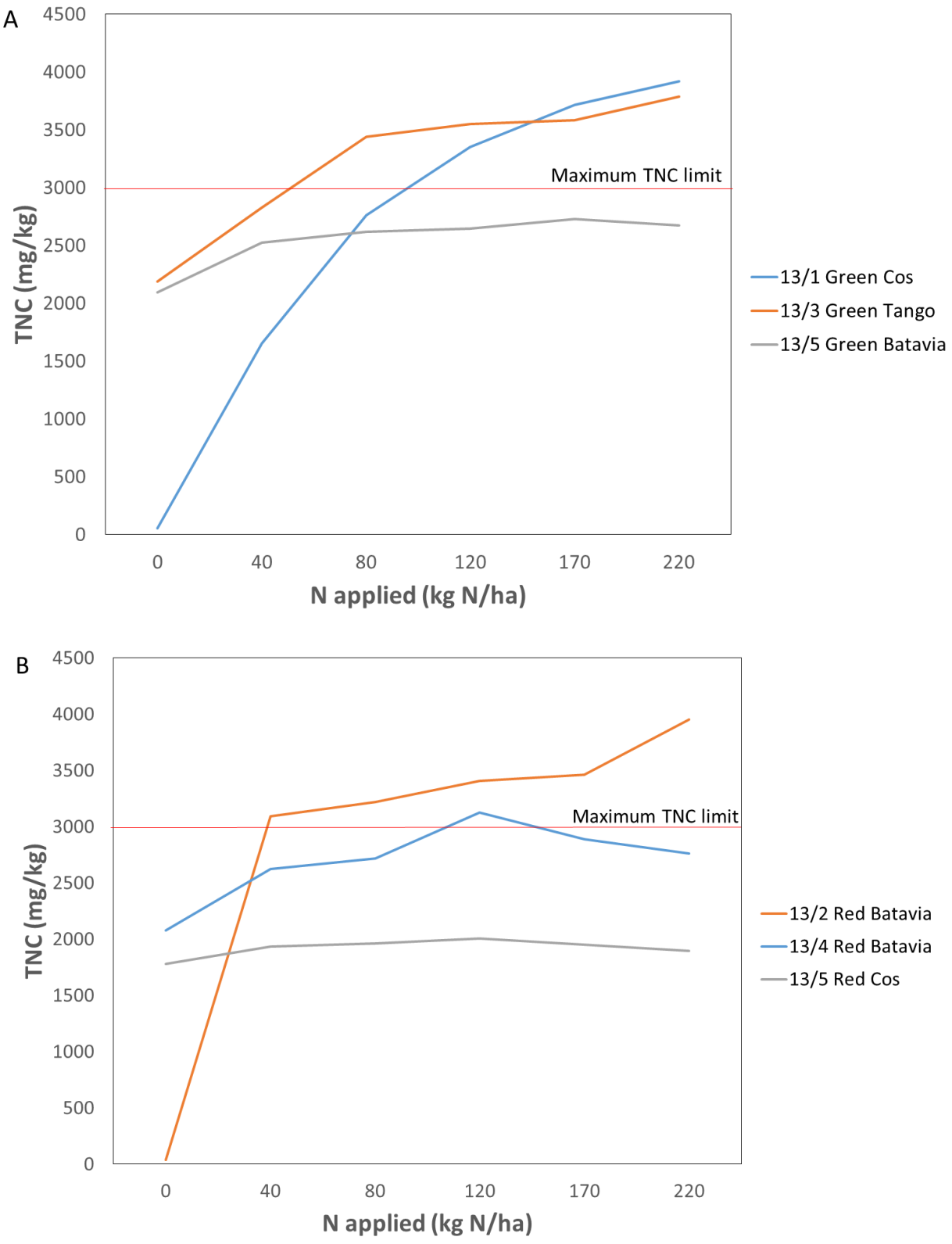

Figure 10. Effects of N rate on TNC on A: green varieties; B: red varieties, 2013.

N recommendations for baby leaf lettuce

In the previous work on wild rocket, a useful guide in devising N recommendations was that the total N uptake for such a baby leaf crop was 113 kg N/ha. Based on the SMN at 0-30 cm available to the crop prior to drilling, and the typical recovery of fertiliser N (ca. 60%), an appropriate fertiliser recommendation could then be devised (Factsheet 08/13) to meet this crop requirement.

A key factor in determining the N recommendation is therefore a measure of total N uptake, and prior to this project we had no such estimate of this for baby leaf lettuce.

With the exception of the Green Tango variety (site 13/3), the average uptake appears to be 63.4 kg N/ha averaged across green and red varieties (Table 9). This is just over half that of the wild rocket crop, and explains in part why there was such a small response to applied N. Four of the sites had SMN (0-30 cm) at drilling greater than 100 kg N/ha, which was more than sufficient to satisfy a crop N requirement of only 63 kg/ha. The two sites with lower SMN at drilling (27-30 kg N/ha) would only be expected to require only ca. 55 kg N/ha as fertiliser N, assuming all the SMN was recovered by the crop, plus 60% fertiliser recovery, and this agrees broadly with the actual responses seen at 40 and 80 kg N/ha applied at sites 13/1 and 13/2 respectively, the early season crops.

Table 9. Summary of initial and residual SMN (0-30 cm) at harvest and maximum N uptake and N uptake in nil N plots, based on data from 5 field experiments with different varieties of red and green baby leaf lettuce crops in 2013.

Site code	Variety	Initial SMN (kg N/ha)	Max N uptake (kg N/ha)	N uptake in nil N plots (kg N/ha)	Residual SMN in nil N plots (kg N/ha)
	Green types				
13/1	Green Cos	30	60.8	35.7	20.2
13/3	Green Tango	166	(139.8)	(140.0)	27.2
13/5	Green Batavia	265	65.7	65.7	70.0
	Red types				
13/2	Red Batavia	27	79.1	32.6	5.6
13/4	Red Batavia	116	63.7	55.1	46.3
13/5	Red Cos	265	47.7	46.3	68.4

Conclusions

- Baby leaf lettuce crops grown in the summer in the UK are often at risk from exceeding the limits set for TNC in outdoor lettuce of 3,000 mg NO_3^-/kg,
- As found in previous HDC-funded work on wild rocket and baby leaf spinach, the risk is particularly high for the late season crops, when SMN builds up in the rotation due to soils warming, plus mineralisation of N from soil organic matter, and residues from previous crops in the current and past seasons,
- A major finding is that with the exception of the Green Tango 'multi-leaf type there was an average crop requirement for N of only 63 kg N/ha, approximately half that of wild rocket (113 kg N/ha), meaning that Recommended amounts of N for baby leaf lettuce are likely to be lower than those defined in HDC Factsheet 08/13 ,
- Based on such a low N requirement, in most cases, with a low SNS e.g. index zero (30-60 kg N/ha, 0-30 cm) at drilling, the crop is only like to need a maximum of 60 kg N/ha applied as fertiliser N,
- Nevertheless, in some cases this amount will be sufficient to raise TNC above the limits set in the Regulations,
- These recommendations should be treated with caution as they only rely on one year's results,
- Recommendations may be adjusted upwards if the grower has his own higher estimates of typical yields and N uptake for crops in his land, and can therefore justify a higher N demand.

Further work

Further work is desirable to:

- Confirm the N requirements in another season, before these results can be used to form specific recommendations for baby leaf lettuce,
- Repeat the N response experiments on more and lower SNS sites, and with a lower maximum N rate applied, to achieve more sensitivity in estimating the N optima,
- Confirm the higher N requirement of the Green Tango 'multi-leaf' type, and to match this with robust measurements of crop N offtake and hence N residues returned to the field after harvest.

Knowledge and Technology Transfer

- Presentation to RDPE workshop on nitrate as a contaminant in fresh produce, at G's, December 2013.
- Article prepared for HDC News February 2014.

Glossary

ANOVA	Analysis of Variance
CAN	Calcium Ammonium Nitrate
DW	Dry weight
FW	Fresh weight
LSD	Least significant difference (from ANOVA, 95% probability)
N	Nitrogen
RB209	DEFRA Fertiliser manual
SMN	Soil Mineral Nitrogen (sum of ammonium and nitrate-N, kg/ha)
SNS	Soil Nitrogen Supply
SPAD	Arbitrary units relating to Chorophyll Concentration Index
TNC	Tissue nitrate concentration

Acknowledgements

The support of Graham Clarkson of Vitacress Salads, and the various UK fresh produce companies who hosted trials is gratefully acknowledged including PDM Produce, The Watercress Company, Intercrop and Vitacress.

References

Defra (2010). Fertiliser Manual (RB 209). 8th Edition.

Weightman, R. M., Dyer, C., Buxton, J. and Farrington, D. S. (2006). Effects of light level, time of harvest and position within field on variability of tissue nitrate concentration in commercial crops of lettuce (*Lactuca sativa*) and endive (*Cichorium endiva*). Food Additives and Contaminants, 23 (5); 462-469.

Weightman, R. M., Huckle, A.J., Roques, S.E., Ginsburg, D. & Dyer, C.J. 2012. Factors influencing tissue nitrate concentration in field grown wild rocket (*Diplotaxis tenuifolia*) in southern England. Food Additives and Contaminants, Volume 29, Issue 9, 2012; 1425-1435 http://dx.doi.org/DOI:10.1080/19440049.2012.696215.

Weightman R.M. and Hudson, E.M. 2013. Noxious or nutritious? Progress in controlling nitrate as a contaminant in leafy crop species. Food Energy and Security (open access) http://onlinelibrary.wiley.com/doi/10.1002/fes3.28/full.

Weightman, R.M., Smith, J.A., Huckle, A.J and Hudson, E.M. 2013. Monitoring and controlling levels of nitrate in UK fresh produce. Aspects of Applied Biology 116, Acrylamide, Furans and Other Food-borne Contaminants, from Plant Science to Food Chemistry, 19-25.

