Project title:	Wild Rocket: N response studies to manage and reduce nitrate levels									
Project number:	FV 370a									
Project leader:	Dr. Richard M. Weightman, ADAS UK Ltd.									
Report:	Final report, December 2011									
Previous reports:	None									
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Location of project:	Growers' holdings in Dorset, Kent, Norfolk, Sussex & Wiltshire									
Industry representative:	Graham Clarkson, Vitacress									
Date project commenced:	1 May 2011									
Date project completed	31 December 2011									
(or expected completion date):										

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

# AUTHENTICATION

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.

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# CONTENTS

Grower Summary	1
Headline	1
Background	1
Summary	2
Financial Benefits	4
Action Points	5
Science Section	6
Introduction	6
Legislation	6
Factors affecting nitrate content	6
Project objectives	7
Materials and methods	8
Study 1 - Nitrogen response experiments at six commercial sites	8
Study 2 - Rotational effects and interaction between crop residues and o	crop N
response at one site	11
Results	13
Study 1 - N response experiments at six commercial sites	13
Study 2 - Rotational effects and interaction between crop residues and o	crop N28
response	28
Discussion	31
Conclusions	35
Knowledge and Technology Transfer	37
Glossary	37
References	37

# **GROWER SUMMARY**

# Headlines

- Use of high levels of nitrogen fertiliser applied to wild rocket significantly increased tissue nitrate concentration (TNC) at harvest and hence the risk of exceeding the 6,000 mgNO<sub>3</sub>/kg limit set by the European Commission;
- Mean crop nitrogen (N) offtake of wild rocket was estimated at 110 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 > 65 kg N/ha, further N applications are unlikely to be necessary;
- In this study, at only 80 kgN/ha fertiliser applied, 25% of samples exceeded the 6,000 mgNO<sub>3</sub>/kg limit.

# Background

The preceding HDC funded project (FV 370), conducted in 2010 indicated that 25% of commercial wild rocket crops would exceed the 6,000 mg NO<sub>3</sub>/kg limit to be implemented by the European Commission in 2012. Since there had been no scientific studies at this point to quantify the response of wild rocket to N fertiliser, work was needed to determine an optimum N rate for recommendation to growers. FV 370 also indicated that many fields had very high levels of soil mineral nitrogen (SMN) prior to sowing (>300 kg N/ha) and it was not clear to what extent these should be taken into account by growers. Estimating crop N requirements can be difficult as soil N supply is determined by a combination of soil type, previous crop and N applications to it, the amount of over-winter rainfall, mineralisation of N from soil organic matter (OM) and the amount of N applied to preceding crops and their N offtakes, and finally how much N is mineralised from crop residues within the current season.

Taking into account currently observed best practice by working with commercial growers, this project aimed to gather robust and independent data on nitrate levels in wild rocket, and how they are affected by previous crop residues, soil type and different rates of N fertiliser.

The project was divided into two specific objectives:

 To gain information on N responses on grower's sites (representative soil types across the season) and determine optimal N to produce a marketable crop while remaining below the EC limit of 6,000 mg NO<sub>3</sub>/kg fresh weight,  To determine whether residues from incorporated previous crops affect subsequent N response and to what extent this can be predicted from SMN measurements.

# Summary

## Yield responses

Six sites were chosen for the N response experiments to represent the geographical spread of UK wild rocket growers. 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, 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.

Significant yield responses to applied N were only seen at two sites (numbers 11/1 and 11/6) where initial background SMN was at or below 65 kg N/ha prior to applying N treatments (Figure 1). At these two sites, further applications of N above 40 kg/ha had no significant effect on yield.

No significant effect of N applied on leaf greenness (measured by a SPAD meter) was seen, showing that reducing application rates of nitrogen should not affect the quality of the crop in terms of colour.

Across the six sites, based on averages across the three highest levels of applied N at each site, N offtake varied between 37 and 169 kg N/ha, with an average across all six sites of 108 kg N/ha. At three of the sites (11/1, 11/3 and 11/6) there was a significant response of N offtake to applied N, broadly mirroring the trend in yield responses.

At site 11/2 the crop failed to emerge where treatment 6 (220 kg N/ha) was applied in addition to the initial background SMN of 183 kg N/ha. Higher fertiliser nitrogen treatments applied at sites 11/3 and 11/5 where initial the SMN was > 100 kg N/ha also caused a yield decrease to occur, indicating that N is becoming toxic to the crop at these levels.

In a second, separate study specifically designed to investigate the effects of incorporating residues from a previous wild rocket crop SMN was also very high and as a results there was no significant effect of applied fertiliser N, and yields apparently declined in response to increasing N (as at site 11/5).



**Figure 1.** Effects of applied N fertiliser on fresh weight yield at six commercial production sites in 2011.

#### **Tissue nitrate concentration**

Applied N had a significant effect on tissue nitrate concentration (TNC). At five of the six sites in Study 1, mean TNC exceeded the 6,000 mg NO<sub>3</sub>/kg EU limit at the highest levels of N applied. At three of the six sites, mean TNC exceeded the limit with 120 kg N/ha applied.

At four sites (11/3, 11/4, 11/5 & 11/6) mean TNC was within about 500 mg NO<sub>3</sub>/kg of the limit (5,442, 6,393, 5,804 & 5,862 mg NO<sub>3</sub>/kg respectively) with only 80 kg N/ha applied. Importantly, individual measurements of TNC from these sites showed that 25% of samples harvested exceeded the 6,000 mg NO<sub>3</sub>/kg limit at this 80kg/ha N application rate. Furthermore, in Study 2, which had very high levels of initial SMN, all N treatments exceeded the 6,000 mg NO<sub>3</sub>/kg limit.

#### Residual soil mineral nitrogen

Reflecting the relatively poor response of crop yield to applied N, at all sites a significant positive relationship was seen between N applied, the SMN remaining after harvest and

also TNC at harvest. In Figure 2, final SMN at harvest is plotted against total available N (applied fertiliser N plus initial SMN). This shows that where more than 200 kg N/ha is available to the crop, much of it remains in the soil post-harvest.



N applied plus initial SMN (kgN/ha)

**Figure 2.** Effects of N fertiliser applied and background SMN, on final SMN at harvest in N response experiments at six commercial growers' sites in 2011.

#### **Financial Benefits**

The UK market for rocket is worth ca. £52M at retail level, based on the annual volume of 3,619 tonnes of crop grown in 2010.

This study suggests that current typical commercial practice of applying 120 kg N/ha may result in 50% of crops exceeding the 6000 mg  $NO_3$ /kg limit if they are managed similarly to those we looked at.

This work is therefore of major strategic importance in protecting this market, and underlines the need for a better understanding of soil N supply in soils used for fast growing leafy salad crops such as wild rocket. For growers in Nitrate Vulnerable Zones, the results also demonstrate a clear risk of nitrate pollution which could also threaten the industry with respect to the Nitrate Directive, and the need to maintain nitrate in drinking water at less than 50 mg/litre.

# **Action Points**

- When determining fertiliser N application rates, take into account the potential background SMN from soil type, over winter rainfall and previous cropping.
- Also take into account applications of N to preceding crops within the current season, as well as mineralisation of N from soil organic matter between soils warming in spring and actual drilling of the crop.
- Where background soil mineral nitrogen (SMN) prior to drilling is > 100 kg N/ha, no further N applications are likely to be necessary.
- With SMN pre-drilling of up to 65 kg N/ha, an application of ca. 40-80 kg N/ha may be sufficient to meet crop N demand.
- At very low SMN and on light soils, with low levels of soil organic crop matter or crop residues, applications of up to 120kgN/ha may be justified for maximizing crop yield, but there may still be a risk of exceeding the limits for TNC.
- Consider recording SMN, crop yields and N offtakes for your particular location and cropping system to provide an evidence base for improved fertiliser N recommendations in the future.
- Where possible, avoid growing rocket at sites with SMN over 120-150 kg N/ha, particularly when continued mineralisation of residues is likely to contribute to soil N supply, as crops run a high risk of exceeding the TNC limit of 6,000 mg NO<sub>3</sub>/kg.
- Avoid supplying more than 200 kg N/ha in total (SMN + fertiliser N) to the wild rocket crop, as this will significantly increasing the risk of nitrate leaching and hence environmental pollution after harvest.

# SCIENCE SECTION

# Introduction

# Legislation

Rocket is a fast growing leafy vegetable which is valued for its strong, hot, peppery flavour and is frequently used in bagged salads, showing rapid and sustained growth in sales in recent years. However, nitrate levels are often high in rocket and HDC project FV 370, (Roques and Weightman, 2010) found that 50% of crops grown in 2010 would have exceeded the 5,000 mg NO<sub>3</sub>/kg limit proposed at the time for tissue nitrate concentration (TNC). Eventually, a 6,000 mg NO<sub>3</sub>/kg limit was agreed by the Commission (Anon., 2011) which will come into force in 2012. However, 25% of crops grown in 2010 would still have exceeded this higher limit. This therefore poses a significant threat to the industry in terms of the cost of abandoned crops and/or the threat of legal action by the authorities.

This work also showed that nitrate levels are often higher towards the end of the season when prolonged dull conditions are experienced. Previous surveys such as the FSA retail salads survey in 2004, found that average nitrate concentrations in rocket were more than 60% higher than in any of the other salad vegetables tested. A broader European Food Safety Authority (EFSA) study in 2008 similarly noted that rocket has a higher nitrate concentration than any other vegetable, such that the ADI (Acceptable Daily Intake) of nitrate can be exceeded by eating just 47 g rocket at median tissue nitrate content (TNC) (EFSA, 2008).

The new limits for nitrate relate to all species of salad rocket (*Eruca sativa*), garden (*Eruca vesicaria*) and wall/wild rocket (*Diplotaxis tenuifolia*) and these are currently included within the annual surveillance sampling.

# Factors affecting nitrate content

While there has been little research into nitrate levels in rocket, there is a more extensive body of literature on nitrate in other salad vegetables such as lettuce, some of which is applicable to rocket. The major environmental factors influencing nitrate content in vegetables are nitrogen fertilisation and light intensity (Santamaria, 2006). It is not easily distinguished whether previous crop residues or seasonal light levels contribute most to the higher nitrate levels later in the cropping season, because these factors occur together in later crops (e.g. if there is a wet summer, later sown crops drilled on previously incorporated residues in warm conditions are conducive to mineralisation of soil organic matter, coincident with dull, wet conditions).

Inevitably, there is a link between the level of N supplied to the crop and its N offtake. The total N available to the crop is comprised of any fertiliser N applied by the grower, plus the soil N supply. In the case of rocket, this is a combination of the soil available N, typically soil mineral nitrogen (SMN), a sum of nitrate and ammonium N measured prior to drilling, and a contribution from N mineralised by the soil organic matter in warm and wet conditions. The survey in 2010 indicated that some growers may not be aware of the appreciable amount of SMN often available in these vegetable crops, particularly when successive crops are grown, and crop residues are incorporated.

Notwithstanding the effects of fertiliser N supply, nitrate accumulation is highest at low light levels, such as during winter and periods of dull weather (Premuzic *et al.*, 2002). This is because the enzyme nitrate reductase, which converts nitrate to nitrite, is activated by light energy. Previous research with lettuce by Burns (2000) and on rocket in HDC project FV 370 (Roques and Weightman, 2010) has shown that dull weather in the period 5 days prior to harvesting can increase nitrate levels. However it is not clear how critical these periods are compared to, for example, high levels of SMN.

There are currently no guidelines on appropriate levels of nitrogen fertiliser for rocket in the fertiliser recommendations. Very little work on nitrate in rocket has been done and despite the fact that many of the larger growers appear to be managing nitrogen use well, there is a need to understand the N requirements of rocket better and to spread best practice to the wider industry.

# **Project objectives**

This project aimed to gather robust and independent data on nitrate levels in wild rocket, and how they are affected by previous crop residues and different rates of nitrogen fertiliser. The work took into account best practice by working with commercial growers, and investigated factors such as crop residues, soil type and fertiliser on nitrate levels in rocket. The project was divided into two specific objectives:

- To gain information on N responses on growers' sites (representative soil types across the season) and determine optimum N needed to produce a marketable crop while remaining below the EC limit of 6,000 mg NO<sub>3</sub>/kg fresh weight.
- To determine whether residues from incorporated previous crops affect subsequent N response and to what extent this can be predicted from SMN measurements.

# Materials and methods

# Study 1 - Nitrogen response experiments at six commercial sites

#### Sampling and treatment application

Six sites were chosen for the nitrogen (N) response experiments to represent the geographical spread of UK wild rocket growers. 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, 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. Variety, sowing and harvest dates for the sites are included below (Table 1). All soils were a sandy loam.

Post drilling, plots for the six nitrogen 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. The trial was carried out using four-fold replication and a fully randomised block design to give a total of 24 plots at each site (6 N rates x 4 replicates). Plot dimensions were 5 m x 1.8 m and with the exception of N applied, the crop was 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. A typical trial set up post CAN treatment application is illustrated in Figure 3.

**Table 1.** Previous cropping details and sowing and harvest dates for the six N responsesites on commercial growers' premises in 2011

Site	Var	Sowing date	Harvest date	Previous crop in 2010 season	N applied to 2010 crop (kg N/ha)	Previous crop(s) in current season (2011)	N applied to previous crop(s) in 2011 (kg N/ha)
11/1	F	2 June	4 July	Wheat	215	None	NA
11/2	Е	26 May	6 July	Grass/clover ley	0	2 x Rocket	100 to each crop
11/3	Е	11 June	9 July	Wheat	275	None	NA
11/4	F	6 July	9 Aug	Spinach, lettuce, beet leaves	not known	Lettuce	170
11/5	F	9 Aug	15 Sept	Lettuce, spinach, wild rocket	not known	Lettuce and brassicas	170 100
11/6	G	9 Aug	20 Sept	Wheat	275	Rocket	None



Figure 3. Typical N response trial set up, post-treatment application of CAN.

As close to the intended harvest date as possible, samples of plant tissue and soil were taken for the determination of the following parameters:

**Crop:** Total N (% w/w dry basis by DUMAS combustion method); tissue nitrate concentration (TNC, mg NO<sub>3</sub>/kg fresh weight); fresh and dry weight per unit area; leaf greenness (or more strictly chlorophyll concentration index, using the Minolta SPAD meter); insect damage and disease.

Soil: Soil mineral nitrogen (SMN, kg N/ha to 30 cm depth) on each plot.

Leaf tissue samples were taken before mid-day at each site using a 0.25 m<sup>2</sup> quadrat to collect 24 crop samples of approx 500 g for Total N and TNC analysis. Samples were dispatched to NRM laboratories within 24 hours of collection and chilled during transportation and storage. A separate sample was taken using a 0.1 m<sup>2</sup> quadrat from each plot for analysis of fresh weight, dry weight, insect damage and disease on return to ADAS Boxworth. These samples were also chilled during transportation and storage. Leaf greenness was measured on each plot *in situ* using a Minolta SPAD meter. Figure 4 shows equipment used for sampling and assessment. By combining the Total N measurement with dry weight yield, an estimate could be derived of total crop N offtake (kg N/ha).



a Plant tissue sampling



**b** Soil corer



c SPAD meter measurements

Figure 4. Equipment used for sampling and assessment.

# Study 2 - Rotational effects and interaction between crop residues and crop N response at one site

This part of the work was carried out at one grower site on a brick earth soil type (medium soil) using a first and second crop of wild rocket grown in the exactly the same position in continuous rotation. Three baby leaf lettuce crops were grown on the trial site in 2010, and two previous crops of rocket had been grown on the trial site prior to the first trial crop being sown in 2011 with approximately 100 kg N/ha applied to each crop. Rotational effects were tested by establishing an early crop, but leaving blocks of ground undrilled within each bed to leave 20 cropped and 20 un-cropped plots. Following harvest these plots were then drilled with a second crop to give main plots of either first or second crop, within a split plot experiment with four-fold replication. A nitrogen response experiment was carried out using five rates within each of the main treatments, with treatments allocated randomly between the two main plots to give a total of 40 plots. The un-cropped plots were kept weed-free so that no plant residues would be incorporated in the second part of the experiment.

Representative topsoil (for pH, P, K & Mg) and SMN samples at a 0-30 cm depth were taken prior to drilling of the first crop and prior to application of fertiliser N for each main plot to give eight samples of background SMN for each main plot. Fertiliser was then applied to the cropped plots at grower recommended rate for a first crop of 100 kg N/ha.

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 each plot as described above. 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<sup>2</sup> quadrat to collect 4 samples of approx 500 g of foliage for Total N and TNC analysis from the cropped plots. This sample was dispatched to NRM laboratories within 24 hours of collection and chilled during transportation and storage. A separate sample was taken using a 0.1 m<sup>2</sup> quadrat from each cropped plot for analysis of fresh weight, dry weight, insect damage and disease on return to ADAS Boxworth. These samples were also chilled during transportation and storage. A separate sample was chilled during transportation and storage. These samples were also chilled during transportation and storage. These samples were also chilled during transportation and storage. These samples were also chilled during transportation and storage. These samples were also chilled during transportation and storage. These samples were also chilled during transportation and storage. These samples were also chilled during transportation and storage. Leaf greenness was measured on each plot in situ using a Minolta SPAD meter.

Post harvest and prior to the second crop being drilled SMN was sampled again for each main plot at 0-30cm depth to give 8 representative samples for background SMN for the second part of the trial. The second crop was then drilled in all plots for the second part of the trial. Post drilling of this crop the trial was re-marked out for the N response treatments, and N treatments were applied as detailed in the treatment Table 2.

First Crop	N Treatment	N rate (kg N/ha)	Product applied (kg/ha)*
No	1	0	0
No	2	50	185
No	3	100	370
No	4	150	555
No	5	200	740
Yes	1	0	0
Yes	2	50	185
Yes	3	100	370
Yes	4	150	555
Yes	5	200	740

Table 2. Treatments applied to crop residue x N response study

\* Figures rounded as appropriate.

As close to the intended final harvest date as possible samples of plant tissue and soil were taken from each of the 40 plots for determination of Total N, TNC, and SMN on each plot. Fresh weight, dry weight, leaf greenness, insect damage and disease were also assessed using the same methods as described previously.

#### Weather data

Weather data for each site for both the N response studies carried out at six sites and the rotational study at one site, from sowing date to sampling date were sourced from the ADAS irrigation scheduling software *Irriguide*. *Irriguide* interpolates weather data for a defined site and altitude using a least squares distance model and real data from five surrounding Met Office weather stations (Bailey & Spackman, 1996). Data included daily maximum and minimum temperatures, daily rainfall and daily solar radiation.

#### 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* (12<sup>th</sup> Edition).

Yield response curves and fitted functions (e.g. linear, quadratic or a linear plus exponential) between N applied and potential factors such as yield and SMN level were fitted using *GenStat* (12<sup>th</sup> Edition). Economic optimum N (Nopt) was calculated for the lowest background SMN site (site no 11/1).

# Results

# Study 1 - N response experiments at six commercial sites

This section first summarises the overall effects, and compares responses across sites. The data for individual sites are presented on separate tables with site-specific comments on the following pages.

## Yield and N responses

Statistical analyses were completed for all six sites (Tables 3-8) and nitrogen applied (kg N/ha) was plotted against yield, with curves fitted as appropriate (Figures 6a-11a). Only one site (site 11/1; Table 3, Fig 6) with the lowest background SMN of 52.4 kg N/ha showed a typical sigmoidal N response curve where yield increases with SMN applied and then plateaued.

Significant yield responses to applied N were only seen at two sites (numbers 11/1 and 11/6) where initial background SMN was at or below 65 kg N/ha prior to applying the N treatments. At these sites, the significant difference was between the first two N treatments i.e. between zero and first level of N applied (40 kg/ha) based on the LSD. Further applications of N above 40 kg/ha had no significant effect on yield.

No significant effect of N applied on leaf greenness (measured by the SPAD meter) was seen, showing that reducing application rates of nitrogen is unlikely to affect the quality of the crop in this respect.

Crop N offtake should be used as a guide to fertiliser N responses. Across the six sites, based on averages across the three highest levels of applied N at each site, N offtake varied between 37 and 169 kg N/ha, with an average across all six sites of 108 kg N/ha. At three of the sites (11/1, 11/3 and 11/6) there was a significant response of N offtake to applied N, broadly mirroring yield responses.

At site 11/2 the crop failed to emerge where treatment 6 (220 kg N/ha) was applied in addition to the initial background SMN of 183 kg N/ha. Higher fertiliser nitrogen treatments applied at sites 11/3 and 11/5 where initial the SMN was > 100 kg N/ha also caused a yield decrease to occur indicating that too much N can have detrimental effect on crop growth.

#### **Tissue nitrate concentration**

Applied N had a significant effect on TNC at all six sites. At five sites, this was a highly significant effect (p<0.001) with TNC increasing rapidly in response to applied N and then plateauing, The fifth site (11/5) achieved a lower level of significance (p<0.05), however this site had a very high level of SMN (>200 kg N/ha) prior to application of the experimental N treatments, and hence the harvested crop had a TNC of 5,000 mg NO<sub>3</sub>/kg with zero N applied.

At five of the six sites, the average TNC exceeded the 6,000 mg NO<sub>3</sub>/kg limit at the highest levels of N applied. At four sites (11/3, 11/4, 11/5 & 11/6) at only 80 kg N/ha applied, average TNC was within about 500 mg NO<sub>3</sub>/kg of the limit (5,442, 6,393, 5,804 & 5,862 mg NO<sub>3</sub>/kg respectively). Looking at the same data again but considering the raw data points (i.e. the individual measurements of TNC recorded samples harvested from each replicate), 25% of the samples exceeded the 6,000 mg NO<sub>3</sub>/kg limit with only 80 kg N/ha applied.

#### Residual soil mineral nitrogen

Despite the relatively poor response of crop yield to applied N, at all sites a significant relationship was seen between N applied and SMN remaining after harvest and TNC at harvest. In Figures 6b -11b, total available N (applied fertiliser N plus initial SMN) is plotted against final SMN at harvest. This shows that at values higher than 200 kg N/ha for total available N, a large amount of nitrogen is left in the soil post-harvest since readily available N has exceeded crop N requirement.

#### Other environmental variables

Solar radiation can have an influence on TNC as discussed in FV 370. Figure 5 shows that TNC is reduced at higher levels of solar radiation but if N is applied the rate of N reduction is reduced, indicating that available N had a major influence.

Variety demonstrated little influence on TNC, SMN or N offtake as the same variety (E) was used at sites 11/2 and 11/3, and variety F was used at three sites (11/1, 11/4 and 11/5). In all cases no similarities in trends of TNC, N offtake or SMN were seen indicating that the amount of soil N supply was the major influencing factor.



**Figure 5.**Influence of solar radiation in last 5 days before wild rocket harvest on TNC at harvest, for data at two N treatment levels in Study 1.

Individual site summaries on next page

## Individual site summaries

## Site 11/1

- Initial SMN low (in range 0-65 kg N/ha) due to efficient recovery of N by preceding wheat crop in 2010
- Crop sown early in season (2 June) therefore less time available for mineralisation of N from organic matter compared to other sites
- Strong response to applied N fertiliser
- High yielding site (yield potential >30 t/ha fresh weight)
- No effect of applied N on leaf greenness
- TNC generally low at typical commercial N rates (<6000 mg/kg with 120 kg N/ha applied)
- Low levels of residual SMN at harvest (<32 kg N/ha) at typical commercial N rates indicating low leaching risk

			Treat	ment				
Variable	1	2	3	4	5	6	Probability (F value)	LSD (5%)
N rate (kg N/ha)	0	40	80	120	170	220	-	-
Yield (fresh weight t/ha)	12.2	23	26.9	31.5	23.4	30.9	<0.001	7.47
SPAD reading	32.81	33.97	31.31	32.43	33.22	29.72	NS	
TNC (mg/kg)	832	2,321	4,444	5,524	6,066	6,019	<0.001	1,147.6
N offtake (kg N/ha)	66.5	108.5	141.3	192	133.3	181.1	0.019	71.07
SMN at harvest (kg N/ha)	8	15	32	76	113	187	0.038	232.8
Initial SMN = 52.4 kg N/ha	P index	= 3, K ir	ndex =1					

**Table 3.** Effect of applied fertiliser nitrogen on yield, greenness, TNC and SMN at harvestfor site 11/1 (harvested 4 July 2011)

Initial SMN 52.4 kg N/ha

Figure 6a. Fresh weight yield response to nitrogen applied at site 11/1.



**Figure 6b.** Relationship between SMN at harvest and N applied plus initial SMN at site 11/1.

## Site 11/2

- Initial SMN high (>150 kg N/ha) as a result of following a crop of clover in 2010, and also preceded in the 2011 season by two rocket crops which also received 100 kg N/ha each
- No response to applied N fertiliser in terms of yield or N offtake
- Moderate yielding site (yield potential >20 t/ha fresh weight)
- Weak effect of applied N on leaf greenness (80 kg N/ha and higher N treatments had significantly greener leaves, very small amounts of purpling seen on zero N plots)
- TNC generally low at all N rates (<5,500 mg/kg at highest level of N applied)
- High levels of residual SMN at harvest (299 kg N/ha) at typical commercial N rates (120 kg/ha applied) indicating high leaching risk

			Treatm	nent				
Variable	1	2	3	4	5	6	Probability (F value)	LSD (5%)
N rate (kg N/ha)	0	40	80	120	170	220		
Yield (fresh weight t/ha)	19.8	23.5	24	23.8	23.7	20.6	NS	
SPAD reading	38.34	38.92	46.02	42.44	40.33	36.52	0.044	5.847
TNC (mg/kg)	1,200	3,117	3,975	4,426	4,770	5,153	<0.001	820.3
N offtake (kg N/ha)	108	135.9	141.5	139.9	143.6	133.7	NS	
SMN at harvest (kg N/ha)	24	88	87	299	242	596	<0.001	173.6

**Table 4**. Effect of applied fertiliser nitrogen on yield, greenness, TNC and SMN at harvest for site 11/2 (harvested 6 July 2011)

Initial SMN = 182.6 kg N/ha P index = 4, K index = 2+



Figure 7a. Fresh weight yield response to nitrogen applied at site 11/2.





## Site 11/3

- Initial SMN moderately high (ca.100 kg N/ha) due to high inputs to previous wheat crop (275 kg N/ha) and likely mineralisation in period between soil warming in spring, and rocket drilling in mid June
- No response to applied N fertiliser
- Low yielding site (yield potential <20 t/ha fresh weight)
- No effect of applied N on leaf greenness
- TNC in danger zone for exceedencies at typical commercial N rates (<5,500 mg/kg with 80 kg N/ha applied)
- High levels of residual SMN at harvest (<130 kg N/ha with 80 kg N/ha applied) hence high leaching risk

			Treat	ment				
Variable	1	2	3	4	5	6	Probabilit y (F value)	LSD (5%)
N rate (kg N/ha)	0	40	80	120	170	220		
Yield (fresh weight t/ha)	9.78	14.28	16.47	14.5	14.34	13.59	NS	
SPAD reading	31.56	30.7	28.59	29.64	29.52	30.91	NS	
TNC(mg/kg)	2,126	4,783	5,442	6,202	6,112	6,302	<0.001	924.5
N offtake (kg N/ha)	44.6	75.5	86	79	80.9	73	0.003	18.34
SMN at harvest (kg N/ha)	16	58	134	165	168	219	0.006	101

**Table 5.** Effect of applied fertiliser nitrogen on yield, greenness, TNC and SMN at harvest for site 11/3 (harvested 9 July 2011)

Initial SMN = 106.5 kg N/ha P index = 3, K index = 2-





Figure 8a. Fresh weight yield to nitrogen applied at site 11/3.



Figure 8b. Relationship between SMN at harvest and N applied plus initial SMN at site 11/3

## Site 11/4

- Initial SMN moderate (in range 65-150 kg N/ha) due to following an early season lettuce crop which received 170 kg N/ha.
- No response to applied N fertiliser
- Low yielding site (yield potential <20 t/ha fresh weight)
- No effect of applied N on leaf greenness
- TNC high at typical commercial N rates (<6,000 mg/kg with 80 kg N/ha applied)
- High levels of residual SMN at harvest (<100 kg N/ha) at typical commercial N rates indicating high leaching risk

			Trea	tment			_	
Variable	1	2	3	4	5	6	Probability (F value)	LSD (5%)
N rate (kg N/ha)	0	40	80	120	170	220		
Yield (fresh weight t/ha)	13.03	11.31	14.35	13.96	13.68	16.68	NS	
SPAD reading	35.05	34.38	35.52	35.71	36.11	36.18	NS	
TNC (mg/kg)	4,252	5,738	6,393	6,206	6,210	6,369	<0.001	742
N offtake (kg N/ha)	77.8	70	94.2	94.1	88.9	109.8	NS	
SMN at harvest (kg N/ha)	18	65	100	158	203	359	<0.001	111.7

**Table 6.** Effect of applied fertiliser nitrogen on yield, greenness, TNC and SMN at harvest for site 11/4 (harvested 9 August 2011)

Initial SMN = 83.9 kg N/ha P index = 4, K index = 3

Site 4 affected by sampling from poor crop establishment due to waterlogging



Initial SMN 83.9 kg N/ha

Figure 9a. Fresh weight yield to nitrogen applied at site 11/4.





## Site 11/5

- Initial SMN high (>200 kg N/ha) probably due to following early-mid season lettuce (170 kg N applied) and brassica crops (100 kg N/ha applied).
- Declining but non-significant yield response to applied N fertiliser
- Very low yielding site (yield potential <10 t/ha fresh weight) probably due to toxicity from high levels of available N
- No effect of applied N on leaf greenness
- TNC high at all N rates N rates (>5,000 mg/kg)
- High levels of residual SMN at harvest (<150 kg N/ha with 40 kg N/ha applied)

			Treat	ment				
Variable	1	2	3	4	5	6	Probability (F value)	LSD (5%)
N rate (kg N/ha)	0	40	80	120	170	220		
Yield (fresh weight t/ha)	8.02	6.89	9.94	7.60	5.82	2.97	NS	
SPAD reading	35.94	37.28	36.31	35.88	36.94	37.32	NS	
TNC (mg/kg)	5,060	5,413	5,804	5,475	5,569	5,928	0.027	488.3
N offtake (kg N/ha)	45.5	38.1	58.2	50.9	38.0	22.0	NS	
SMN at harvest (kg N/ha)	43	152	135	253	313	316	<.001	118.9

**Table 7.** Effect of applied fertiliser nitrogen on yield, greenness, TNC and SMN at harvest for site 11/5 (harvested 15 September 2011)

Initial SMN = 205.0 kg N/ha P index = 4, K index 2+







**Figure 10b.** Relationship between SMN at harvest and N applied plus initial SMN at site 11/5

## Site 11/6

- Initial SMN low (in range 0-65 kg N/ha) as crop followed wheat in 2010 then a preceding rocket crop in 2011, to which no N was applied
- Moderate response to applied N fertiliser
- Medium yielding site (yield potential >20 t/ha fresh weight)
- No effect of applied N on leaf greenness by SPAD but purpling observed on leaves of zero N plots
- TNC in danger zone at typical commercial N rates (5,862 mg/kg at 80 kg N/ha applied)
- Low levels of residual SMN at harvest (<55 kg N/ha) at typical commercial N rates indicating low leaching risk

			Treat	ment				
							Probability	LSD
Variable	1	2	3	4	5	6	(F value)	(5%)
N rate (kg N/ha)	0	40	80	120	170	220		
Yield (fresh weight t/ha)	9.97	14.72	16.18	18.58	17.22	22.93	0.029	6.980
SPAD reading	33.45	37.46	36.40	35.03	33.26	33.89	NS	
TNC (mg/kg)	654	3,015	5,862	6,968	8,396	8,111	<.001	1,149
N offtake (kg N/ha)	51.8	86.2	112.6	123.4	112.7	145.1	0.020	49.98
SMN at harvest (kg N/ha)	5.2	12.5	31.2	53.6	126.8	244.3	<.001	39.04

**Table 8.** Effect of applied fertiliser nitrogen on yield, greenness, TNC and SMN at harvest for site 11/6 (harvested 20 September 2011)

Initial SMN = 62.5 kg N/ha P index = 3, K index = 2-



Figure 11a. Fresh weight yield response to nitrogen applied at site 11/6.



Figure 11b Relationship between SMN at harvest and N applied plus initial SMN at site 11/6



Due to the initial SMN being greater than 100 kg N/ha, little response was seen for yield, leaf greenness or interactions between N applied and crop residues (Table 9). Crop residues did increase SMN available to the following crop by 100 kg N/ha and where N was applied to the second crop following the previous fallow or cropped plots this increased SMN at harvest in all cases. An overspread of fertiliser N occurred between the two crops causing the SMN pre second crop to increase higher than expected.

Crop yield and SPAD reading both appeared to have a decreasing trend with increasing N applied, although these effects were not statistically significant. TNC and N offtake decreased significantly with increasing level of applied N. Tissue nitrate concentration in all treatments exceeded the limits set in the regulations. These data show a similar pattern to site 11/5 in Study 1, where increasing N applied also reduced yield, and effectively killed the crop at very high N levels. Residual SMN at harvest increased significantly with increasing N rate.

Crop rotation as a factor had no significant effect on yield, SPAD, TNC or N offtake. This was because the SMN was high over the whole trial and so crop growth (as reflected in the declining yield response to increasing levels of applied N) was adversely affected in both treatments. Residual SMN at harvest was higher in the previously cropped treatment, but this also had very high levels of N applied due to being overspread.

SMN pre first crop	111 kg N/ha	SMN pre seco SMN pre seco	ond crop (r ond crop (r	mean fallow p mean cropped	lots) 279 kg N/ I plots) 376 kg	'ha' N/ha <sup>†</sup>
Factor	Treatment	Yield (fresh weight t/ha)	SPAD	TNC (mg/kg)	N offtake (kg N/ha)	SMN (kg N/ha)
Crop rotation						
	No crop (fallow)	18.0	43.58	7,289	106.3	440
	Crop	8.4	42.61	7,516	74.0	641
N applied treatment						
(kg N/ha)	1 (0)	13.4	44.16	8,070	149.8	196
	2 (50)	14.4	42.05	7,972	128.3	346
	3 (100)	10.6	42.80	7,473	77.9	589
	4 (150)	14.4	43.22	6,873	62.0	729
	5 (200)	13.0	43.25	6,623	32.6	843
Crop rotation x N ap	plied treatment					
(kg N/ha) No crop	1 (0)	16.2	45.07	7,365	172.5	59
	2 (50)	22.2	44.52	7,754	124.9	220
	3 (100)	14.6	43.22	7,639	92.6	507
	4 (150)	15.2	42.74	7.050	93.9	600
	5 (200)	21.8	42.36	6,635	47.6	814
Crop	1 (0)	10.6	43.26	8,776	127.2	332
	2 (50)	6.5	39.58	8.189	131.7	473
	3 (100)	6.6	42.39	7.308	63.2	671
	4 (150)	13.7	43.70	6.697	30.2	858
	5 (200)	4.3	44.13	6,611	17.6	872
LSD Crop (3 df)		NS	NS	NS	NS	29.0**
LSD N applied (23 df)		NS	NS	291.7***	33.19***	49.2***
LSD Crop x N applied (23 df)		NS	NS	461.6*	NS	NS

**Table 9.** Effect of nitrogen applied on yield, greenness, TNC and SMN at final harvest forthe rotational experiment (harvested 16 August 2011)

<sup>†</sup>plots affected by overspread from nearby beds increasing SMN between first and second crops.

# Discussion

The experiments described in this report satisfied the objectives defined at the outset of the project by providing detail on the responses to N fertiliser on seven growers' sites on representative soil types across the 2011 season for the wild rocket crop. The results defined the optimum N needed to produce a marketable crop while remaining below the EC limit of 6,000 mg NO<sub>3</sub>/kg fresh weight in these situations. More importantly, the results allowed us to define the crop N requirement based on typical N offtake, a factor which was unknown at the outset of the project. Rather than relying solely on empirical observations from N response experiments, the data therefore allow us to match crop N requirement to the supply of N by soil plus fertiliser.

A second objective was to determine whether residues from incorporated previous crops affect subsequent N response, and to what extent this can be predicted from SMN measurements. A specific experiment designed to investigate this (Study 2) was inconclusive because of high SMN levels across all treatments. However data from the other six sites (Study 1) also gave us important indicators that crop residues and/or applications of N to preceding crops must be taken into account in determining optimum N rate. These results are discussed in more detail below.

#### **Yield responses**

The data collected in Study 1 indicate that the average wild rocket crop N requirement is around 110 kg N/ha, based on the maximum offtake measured. This provides a useful basis to understand the N responses. The values vary between sites (range 37-169 kg N/ha) depending on the yield level, with the highest yield site 11/1 (fresh weight yield 30 t/ha) showing the strongest yield response to N.

In broad terms these results suggest that if the soil supplies about 65 kg N/ha as SMN (which was the case at the two sites 11/1 and 11/6 which showed significant yield responses), then the crop requirement for additional N is likely to be around 55 kg/ha. Since some mineralisation of N is likely to occur between sowing and harvest, particularly as these crops are growing in warm soils and often irrigated, which might reduce applied N requirement by say 10 kg/ha, this explains why there was only a yield response seen with 40 kg N/ha applied.

This understanding of crop N requirement also explains why no significant yield response was seen at the other four sites, where the initial SMN was in the range 82 to 205 kg N/ha.

In these cases it is very likely the initial SMN plus the N which mineralises during the growing season supplies sufficient N to match crop demand. Where SMN was high (>150 kg N/ha) no significant yield responses were gained from additional applications of N but nitrate continues to build up in the soil and the plant tissues. At two sites (11/5 in Study 1 and in the rotational experiment in Study 2) yields were seen to decline in response to applied N because the initial SMN levels before drilling the crop were very high (>200 kg N/ha) and further applied N probably became toxic to plant growth.

#### **Tissue nitrate concentration**

It is clear that a proportion of wild rocket crops are still at significant risk of exceeding the EU limit of 6,000 mg NO<sub>3</sub>/kg. In the 2010 study it was found that the typical amount of N applied to rocket crops was 120 kg/ha. The data here suggest that at 120 kg N/ha applied, the median value for TNC in individual crops samples was 6,000 mg NO<sub>3</sub>/kg across all six sites in Study 1. Moreover in crops where responses might be anticipated at 80 kg N/ha applied, approximately 25% of samples would have exceeded the limits. Therefore a decision rule to only apply approximately 40 kg N/ha when initial SMN is <65 kg N/ha and none when SMN is >65 kg N/ha on the basis of expected yield response would also be correct in terms of minimising the risks from exceeding the limit for TNC.

#### Issues regarding measurement of SMN

Soil mineral N measurements are notoriously variable. A low average SMN value identified here at ca. 65 kg N/ha (site 11/6) if sampled again, could easily be measured as anywhere between 50 and 100 kg N/ha simply due to spatial variability. The amount of N which will be made available to the crop by mineralisation during the season is also unpredictable, although for rocket crops which are located in the south of the country and generally irrigated (hence warm and wet soils) this source of N is likely to be significant.

Any field that remains uncropped between April and June, prior to sowing, is likely to mineralise appreciable quantities of SMN. This must be taken into account when deciding on N applications to the first drilled crop. Further work is required to quantify this amount, but such mineralisation could easily contribute 50 kg N/ha to soil N supply in the spring. To illustrate this point, the manager at Site 11/4 measured SMN on 3<sup>rd</sup> March 2011 with a result of 11 kg N/ha and again on the 5<sup>th</sup> May with a result of 56 kg N/ha, showing N to be mineralising up to this point. By the time the rocket crop was sown in early August, further mineralisation of N would have occurred. Even if N offtake of the lettuce crop that preceded it was equal to the N inputs, another 30 kg N/ha could easily have mineralised giving a measured SMN before drilling the rocket crop of 84 kg N/ha (Table 6). It should be noted

that the tables given in RB209 for soil N supply based on the field assessment method only (soil type, over-winter rainfall and previous cropping) only give an estimate of SMN after winter (i.e. February-March). The tables do not adequately deal with N subsequently mineralised from soil organic matter (OM) between April and June.

Two sites in study 1 had initial low SMN in the range 0-65 kg N/ha which based on the earlier discussion would be expected to respond to fertiliser N (sites 11/1 and 11/6). Conversely the four sites with SMN in the range 65 - 205 kg N/ha showed no response to fertiliser N. Current advice in arable crops is that in low soil N index situations (N indices 0-2 in the spring as defined in RB209), carrying out SMN analysis does not confer any benefits to the grower and simply adds cost. In part this is because of the imprecision in SMN measurements. At low SMN sites, by using the soil assessment method (soil type, annual rainfall, previous crop and N applied to it), soil N supply can be adequately predicted for most crops when sown early in the spring.

However it is also clear that late-season rocket crops which have had previous crop residues incorporated and a previous application of N within the same season will have large amounts of readily available N in the soil for rapid crop uptake. These residues must also be taken into account, and it is these situations which would benefit from SMN testing.

The important point however is to be able to identify those crops which are like to have a soil N supply greater than 110 kg N/ha and therefore be likely to satisfy crop N demand without having to apply fertiliser N. For instance site 11/2 had a previous grass and clover crop with 100 kg N/ha applied in 2010 on a medium soil, and might be expected to have had a soil N index somewhere between 1 and 2 (i.e. up to 100 kg N/ha according to RB 209) in the spring of 2011. The field then had two rocket crops grown, each with 100 kg N/ha applied in the spring. We do not know the yields of these rocket crops, but if for any reason N offtake was less than 110 kg/ha, appreciable residues might be left for the third rocket crop on which the N response experiment was carried out. Given mineralisation of N from incorporated crop residues from the first two rocket crops and continued mineralisation of N from the previous season's OM until drilling on the 26th May, these observations might explain the high SMN measured (180 kg N/ha to 30 cm depth) at site 11/2. A similar accounting exercise could be carried out for the other crops in this study, although lack of detail on previous cropping means this was not always possible.

#### **Environmental discussion**

Low levels of solar radiation can have cause an increase in TNC as shown in the previous project (Roques and Weightman 2010). To a lesser extent this effect was also seen in the present project (Figure 12) but the data overall show that N supply had the largest influence on TNC at harvest, in the 2011 season.



Figure 12. Influence of solar radiation in last 5 days before harvest on TNC at harvest

If N fertiliser is applied in excess of crop requirement, while there will be a measurable increase in TNC, the majority of the N will not be taken up by the crop. Importantly, N in excess of rocket's requirements will remain in the soil as residual SMN and will significantly increase the risk of leaching over winter. The data show that at 200 kg N/ha total available N (soil SMN plus fertiliser N), there could be 100-200 kg N/ha left in the soil at harvest. Moreover, it should be noted that SMN in these experiments was only measured to 30 cm depth. For arable crop sampling and for deeply rooted crops, SMN sampling to 90 cm depth is advised, which means the values in this report are probably underestimates of the true leaching risk. If sampling were carried out to 90 cm depth, it is likely that the residual SMN in a comparable situation would be 140-280 kg N/ha, giving a significant environmental risk.

For illustrative purposes the traditional 'economic' optimum N was calculated using site 11/1 which had the lowest background SMN of 52.4 kg N/ha and a typical N response curve to give a breakeven ratio of 0.4:1 of applied N at 170 kg N/ha. This break-even ratio was calculated using the current price of CAN (27.5% N) at £285/tonne and an ex farm price for wild rocket of £2.50/kg. Figure 13 shows that although superficially there are no economic losses likely from applying extra nitrogen, this ignores the environmental risk from high residual SMN, as well as the risk of high TNC.



Initial SMN 52.4 kg N/ha

**Figure 12.** Schematic diagram showing likely economic and environmental optima at the site with the lowest initial soil N status (site 11/1).

# Conclusions

#### Nitrogen requirement and yield response

- The average crop requirement for N in wild rocket was estimated at 110 kg N/ha in the present studies, although this could be appreciably greater where yields are high (>20 t/ha fresh weight);
- A large range of actual N offtakes was observed (37-169 kg N/ha), and individual growers are advised to record typical yields and N offtakes, in order to provide an evidence base for future N applications on their premises;

- Significant responses to applied N fertiliser were only seen at two out of seven sites, and these were sites at which initial SMN was less than 65 kg N/ha;
- These recommendations for fertiliser N are appreciably less than the typical 120 kg N/ha applied commercially in 2010;
- At the two sites where a significant response to applied N was seen, applications of no more than 40 kg N/ha could be justified;
- Increasing N applied above this level had no effect on yield or leaf greenness and only increased the risk of exceeding the 6,000 mg NO<sub>3</sub>/kg limit set for TNC;

# Tissue Nitrate Concentration (TNC)

- Examining the individual measurements of TNC recorded samples harvested from each replicate, 25% of the samples exceeded the 6,000 mg NO<sub>3</sub>/kg limit with only 80 kg N/ha applied;
- While light levels prior to harvest did have an effect on TNC, it was smaller than seen in 2010, with available N having the dominant effect on TNC in the present studies;

#### Site variations

- As in the previous study in 2010, some sites had appreciable levels of SMN prior to drilling (>150 kg N/ha) which must be taken into account when deciding on N fertiliser rates;
- At two sites, increasing levels of fertiliser N reduced yields, which in conjunction with the high levels of SMN recorded, implied that excess N can be toxic to plant growth;

#### Risk of nitrate leaching

 As well as increasing the risk of exceeding the limits set for TNC, providing the crop with an N supply greater than 200 kg N/ha (total of SMN at drilling + fertiliser N) significantly increased residual N at harvest, hence increasing the risk of nitrate leaching;

### Further work

- A better understanding of the amount of N mineralised from soil OM in the spring, between March and June (when many rocket crops begin to be sown) is required;
- Further work is required to quantify the supply of N from preceding salad and rocket crops within the season, based on actual N applied, N offtakes and mineralisation of crop residues.

# Knowledge and Technology Transfer

No knowledge transfer activities were undertaken during the course of the project. An article for HDC News will be prepared in spring 2012.

# Glossary

CAN	Calcium ammonium nitrate
OM	Organic matter
RB209	DEFRA Fertiliser manual
SMN	Soil Mineral Nitrogen (sum of ammonium and nitrate-N, kg/ha)
SPAD	Arbitrary units relating to Chorophyll Concentration Index
TNC	Tissue nitrate concentration

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