**FINAL REPORT**

Horticulture Australia Project Number VG04019

# Assessing nitrate and nitrite levels in Australian leafy vegetables


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## MEDIA SUMMARY

The concentration of nitrate and nitrite in a vegetable reflects its food safety, quality and even how efficiently fertiliser was used in the production of that vegetable. Vegetables provide the majority of nitrate found in the diet. Leafy vegetables tend to have a higher nitrate concentration than other vegetable types such as root or fruit vegetables. Further, leafy vegetables can accumulate nitrate beyond levels that are optimum for plant growth. Once eaten, nitrate can be reduced to nitrite in the body and can combine with amines to form carcinogenic nitrosamines that are associated with gastric cancer.

This project assessed the levels of nitrate and nitrite occurring in Australian produced leafy vegetables and the production factors that impact on these. A survey of leafy vegetables available on the market was conducted over a one-year period with vegetables obtained from different growing areas in New South Wales, Queensland and Victoria. Greenhouse experiments were then carried out at the NSW Department of Primary Industries Gosford Horticultural Institute investigating how light and nitrate fertiliser affect the concentration of nitrate in a range of leafy vegetables.

The survey clearly demonstrated that Australian leafy vegetables generally have a low concentration of nitrite but that 27% of samples had accumulated nitrate, leafy Asian vegetables in particular. It was demonstrated that nitrate supply is the key factor controlling the accumulation of nitrate in harvestable shoots. Light conditions in Australia, even in protected situations such as under shade, are not reduced enough to exacerbate nitrate accumulation. For Australia, management of nitrate accumulation in vegetables will be achieved through the efficient use of fertilisers in production. A

limitation to this is that growers currently do not have the tools to easily manage nitrate in Asian vegetable crops.


## TECHNICAL SUMMARY

The concentration of nitrate and nitrite in a vegetable reflects its food safety, quality and even how efficiently fertiliser was used in the production of that vegetable. Vegetables provide the majority of nitrate found in the diet. Leafy vegetables tend to have a higher nitrate concentration than other vegetable types such as root or fruit vegetables. Further, leafy vegetables can accumulate nitrate beyond levels that are optimum for plant growth. Once eaten, nitrate can be reduced to nitrite in the body and can combine with amines to form carcinogenic nitrosamines that are associated with gastric cancer. This has led some countries to set limits on nitrate and nitrite in vegetables.

This project assessed the levels of nitrate and nitrite occurring in Australian produced leafy vegetables and the production factors that impact on these. A survey of leafy vegetables available on the market was conducted over a one-year period with vegetables obtained from different growing areas in New South Wales, Queensland and Victoria. Greenhouse experiments were then carried out at the NSW Department of Primary Industries Gosford Horticultural Institute. The effect of the light level and supply of nitrate fertiliser on silverbeet responses, a known nitrate accumulator species was investigated. The response of 9 types of leafy vegetables to nitrate supply was investigated and experiments were carried out in both winter and spring to include a range of climatic conditions.

The survey clearly demonstrated that Australian leafy vegetables generally have a low concentration of nitrite. Nitrate-N concentrations ranged from 12 to 1400 mg/kg fresh weight. Vegetable samples with nitrate-N concentrations greater than 700 mg/kg were considered to have accumulated nitrate. In the survey 49% of leafy Asian vegetables had accumulated nitrate. It was demonstrated that nitrate supply is the key factor controlling the accumulation of nitrate in harvestable shoots. Light conditions in Australia, even in protected situations such as under shade, are not reduced enough to exacerbate nitrate accumulation. For Australia, management of nitrate accumulation in vegetables will be achieved through the efficient use of fertilisers in production. A limitation to this is that growers currently do not have the tools to easily manage nitrate in Asian vegetable crops.


## 1. INTRODUCTION

Public health concerns about high nitrate and nitrite in vegetables have led some countries to set limits on these compounds in vegetables. For example, China’s recommended tolerance level for nitrate-N in vegetables is below 700 mg/kg, and for nitrite-N is below 1.2 mg/kg fresh weight (Zhou *et al.* 2000). The European Commission (EC) has also set maximum safe eating limit standards for spinach and lettuce ranging from approximately 450 to 1000 mg/kg nitrate-N, depending on the season, vegetable-type and cropping situation (Table 1). Currently, Australia does not have standards for limits on nitrate and nitrite levels in vegetables.

Table 1. Maximum allowable nitrate-N levels in lettuce and spinach according to the European Commission Regulation no. 565/2002. Adapted from Santamaria (2006).

| *Product* | *Harvest period/ Production situation* | *Nitrate-N (mg/kg ) fresh weight* |
|---|---|---|
| Spinach | 1 November to 31 March | 700 |
| | 1 April to 31 October | 550 |
| | | |
| Open 'fancy' lettuce | 1 November to 31 March | |
| | Grown under cover | 1000 |
| | Grown in the open | 900 |
| | 1 April to 31 October | |
| | Grown under cover | 800 |
| | Grown in the open | 550 |
| | | |
| Head lettuce | Grown under cover | 550 |
| | Grown in the open | 450 |

A high level of nitrate in leafy vegetables is undesirable for reasons of public health and vegetable quality. Human dietary nitrate is largely obtained from vegetables and at high levels is considered harmful to human health (Santamaria 2006). Although nitrate is relatively non-toxic, in the body it is readily converted to nitrite and N-nitroso compounds that are toxic and have been associated with the potentially fatal methaemoglobinaemia, gastric cancer and bladder cancer (Abdel Mohsen *et al.* 1999, Mensinga *et al.* 2003). Vegetable quality is also compromised as increasing application rates of nitrogen-containing fertilisers can increase the incidence and severity of bacterial soft-rot in vegetables. This has been demonstrated in Chinese cabbage (Warner et al. 2004) and some susceptible broccoli cultivars (Canaday and Wyatt 1992). Further, the nutritional quality of lettuce is compromised by increased nitrate in terms of reduced vitamin C concentration (Poulsen *et al.* 1995).

There has been a lack of concern about nitrate accumulation in Australian produced vegetables and this is perhaps partly due to an assumption that high light conditions (irradiance) in Australia prevent any nitrate accumulation in vegetables. Increasing light can increase the activity of nitrate reductase that reduces nitrate, minimising its accumulation in plant tissues as reported by Chadjaa *et al.* (1999) and Gaudreau *et al.* (1995) for lettuce and spinach. However, in Australia it is possible that light may not be sufficient in the production of vegetables under shade or in greenhouses. The European maximum limits for nitrate in vegetables reflect the light-limited conditions of winter and protected cropping with higher limits set for these conditions compared with summer and open-air production (Table 1).

The reporting of nitrate values in Australia has been inconsistent (Bolger and Stevens 1999). In Australia, nitrate can be reported as either nitrate or nitrate-N but is only reported as nitrate (nitrate = nitrate-N x 4.43) in Europe. Mistaken comparisons of nitrate-N values obtained in Australia with nitrate values obtained elsewhere could lead to the impression that nitrate in Australian vegetables is generally low.

It is well established that the supply of nitrogen to a plant affects the concentration of nitrate in that plant. As excessive supply of fertilisers in vegetable production is a serious problem, fertiliser supply should not be ignored as potentially having an effect on nitrate accumulation in vegetables. High losses of nitrate have been recorded from market gardens in Australia (Pionke *et al.* 1990) and a nitrate leaching hazard index that has been developed for irrigated agriculture rates vegetables such as lettuce and broccoli as more likely to be associated with nitrate leaching than other tree and vine crops (Wu *et al.* 2005). This indicates that nitrate supply is likely to be high during the production of vegetables. Additionally, this situation is not confined to field production, as hydroponic nutrient solutions tend to supply nitrate in excess of need (Bugbee 2004).

Another important factor in nitrate accumulation is the vegetable species being cultivated. A number of leafy vegetables are known nitrate-accumulators. For example, spinach (*Spinacea oleracecea* L.) and silverbeet (*Beta vulgaris* L.) in the plant family Amaranthaceae are known to accumulate nitrate to very high concentrations. Other leafy vegetables classified as having a generally high concentration of nitrate include lettuce, rocket, celery, cress, radish, red beetroot, endive, fennel, parsley, leek, endive and Chinese cabbage (Santamaria 2006).

This project aimed to assess nitrate and nitrite occurring in Australian produced leafy vegetables and the production factors impacting on these. Initially, a survey of leafy vegetables available on the market was conducted. Greenhouse experiments were then carried out to evaluate the importance of light level and nitrate supply on plant nitrate and nitrite concentrations for a range of leafy vegetable types.

## 2. SURVEY OF NITRATE AND NITRITE IN AUSTRALIAN LEAFY VEGETABLES

Specific details of this work can be found in Appendix 1 in the paper *Nitrate and nitrite in Australian leafy vegetables* submitted to the Australian Journal of Agricultural Research in May 2007. The following highlights the key features of the survey.

### 2.1 Aims and objectives of the survey

The survey aimed to identify the range of nitrate and nitrite occurring in fresh leafy vegetables on the Australian market. To ensure that the survey was representative of the Australian market, leafy vegetables were sourced from distinctly different geographical regions including the vegetable growing areas of Queensland, New South Wales and Victoria. Vegetables were grown in a range of cropping situations. That is, in soil, in the field or in hydroponics, with some systems covered by hail netting. Leafy vegetables were also obtained over a one-year period, at different times, to take seasonality into account.

### 2.2 Main findings

Nitrite concentrations were generally low with 59% of samples being below the detection limit of (<0.003 mg/L). Most samples containing measurable amounts of nitrite-N were below 1 mg/kg.

The vegetable samples ranged widely in nitrate-N concentration from as low as 12 mg/kg fresh weight for silverbeet to as high as 1400 mg/kg for choy sum.

In the survey, the nitrate concentration of the Brassicaceae Asian vegetables buk choy, pak choy, mizuna, and choy sum (*Brassica rapa* L.), tatsoi (*Brassica rosularis* L.), and rocket (*Eruca sativa* Mill.) was generally higher than for the other leafy vegetable types.

Table 2 shows the number of vegetables for each type that exceeded 700 mg/kg nitrate-N and the number that exceeded 1.2 mg/kg nitrite-N, the maximum limits used in China. Overall, 27% of samples were considered high in nitrate-N. This highlights that Asian leafy vegetables feature strongly as having accumulated nitrate. When combined, 49% of the Asian leafy vegetable samples had nitrate-N concentrations greater than 700 mg/kg, whereas only 24% the lettuce samples.

Table 2. The number of survey vegetables with a high concentration of nitrate-N (>700 mg/kg)

| **Leafy vegetable** | **Number of samples** | **# samples >700 mg/kg Nitrate-N (% of samples)** | **# samples > 1.2 mg/kg Nitrite-N** |
|---|---|---|---|
| Baby leaf spinach | 23 | 1 (4%) | 2 |
| Buk choy | 21 | 8 (38%) | 0 |
| Choy sum | 3 | 3 | 0 |
| Lettuce, Butter | 3 | 0 | 0 |
| Lettuce, Coral green | 5 | 2 | 0 |
| Lettuce, Coral red | 22 | 1 (4.5%) | 0 |
| Lettuce, Cos | 9 | 0 | 0 |
| Lettuce, Iceberg | 11 | 0 (0%) | 0 |
| Lettuce, Oak green | 5 | 0 | 0 |
| Lettuce, Oak red | 8 | 1 | 0 |
| Mizuna | 10 | 7 (70%) | 0 |
| Pak choy | 2 | 0 | 1 |
| Rocket | 23 | 7 (30%) | 1 |
| Silver beet | 12 | 6 (50%) | 0 |
| Tat soi | 13 | 6 (46%) | 2 |

## 2.3 Conclusions from the survey

The survey clearly demonstrated that Australian leafy vegetables have the potential for accumulating nitrate with 27% of vegetables affected, with Asian leafy vegetables being particularly prone. However, nitrite concentrations considered high were detected in only a small number of vegetables. Conclusions could not be made about the causes of the nitrate and nitrite levels in vegetables from the survey such as the effect of different regions, systems or seasons that vegetables were grown in.

## 3. RESPONSE OF SILVERBEET TO NITRATE SUPPLY AND LIGHT LEVEL

Specific details of this work can be found in Appendix 1 in the paper *Nitrate and nitrite in Australian leafy vegetables* submitted to the Australian Journal of Agricultural Research in May 2007. The following highlights the key features of the silverbeet experiments.

### 3.1 Aims and objectives of the silverbeet experiments

Experiments were conducted with silverbeet, a known nitrate accumulator, to determine the effect of light level and nitrate supply on nitrate and nitrite concentrations in shoots. Three different light levels where created using shade (Fig. 1) and 5 rates of nitrate were supplied in nutrient solution fed to plants through drippers. The experiment was conducted in winter and in spring to provide a range of climatic conditions. Plant growth responses, nitrate accumulation and weight loss during storage were also measured.

Figure 1. A photo showing the set up of the silverbeet experiments. Light levels were the ambient light conditions of the greenhouse, light under bird netting (front, centre) and light under thermal shade cloth.

## 3.2 Main findings

The accumulation of nitrate in silverbeet was primarily influenced by increasing nitrate supply and not by light level (Table 3, Fig.s 2 & 3). Light was not reduced enough, even by the heaviest shade, to exacerbate nitrate accumulation. Growth was generally highest at a nitrate-N supply of 200 mg/L with growth depressed at the higher rate of 300 mg/L (Table 4). In spring, nitrate accumulation (>700 nitrate-N mg/kg) occurred in shoots at supplies of nitrate-N that were associated with the highest yields and in reduced yields associated with the highest nitrate-N supply. These results suggest that excessive nitrate-N concentrations in leafy vegetables are most likely to occur when high or excessive rates of nitrogen fertiliser are applied.

Treatment effects on nitrite-N in silverbeet could not be determined because nitrite was only detected in one third of plants. Only 62 samples out of the 180 measured in total for both experiments had detectable nitrite. The samples with nitrite had a mean nitrite-N concentration of 0.193 mg/kg (standard error 0.05) and a range 0.007-2.218 including four samples > 1.00 mg/kg.

In the spring experiment, the effect of storage for one week on postharvest fresh weight was investigated. Shoot fresh weight was reduced from a mean of 235g at harvest to 227g after storage but weight loss was not affected by nitrate supply or by shoot nitrate concentration.

Table 3. Predicted shoot nitrate concentrations for winter and spring experiments

| | Nitrate N supply (mg N/L) | Predicted shoot nitrate concentration winter | Standard error | Predicted shoot nitrate concentration spring | Standard error (s.e.) |
|---|---|---|---|---|---|
| Light 1 | 30 | 44.42 | 48.64 | 136.32 | 82.51 |
| | 50 | 114.03 | 37.39 | 256.06 | 63.43 |
| | 100 | 252.70 | 40.18 | 511.13 | 68.17 |
| | 200 | 378.50 | 50.18 | 831.49 | 85.13 |
| | 300 | 302.27 | 61.18 | 898.82 | 103.79 |
| Light 2 | 30 | 46.95 | 48.64 | 197.98 | 82.51 |
| | 50 | 112.26 | 37.39 | 317.70 | 63.43 |
| | 100 | 248.39 | 40.18 | 559.53 | 68.17 |
| | 200 | 404.28 | 50.18 | 796.92 | 85.13 |
| | 300 | 405.03 | 61.18 | 705.97 | 103.79 |
| Light 3 | 30 | 28.46 | 48.64 | 283.79 | 82.51 |
| | 50 | 74.97 | 37.39 | 332.26 | 63.43 |
| | 100 | 185.20 | 40.18 | 449.00 | 68.17 |
| | 200 | 379.75 | 50.18 | 663.51 | 85.13 |
| | 300 | 539.74 | 61.18 | 852.73 | 103.79 |
| s.e. of difference | | 66.59 | | 112.97 | |
| lsd | | 135.98 | | 230.68 | |

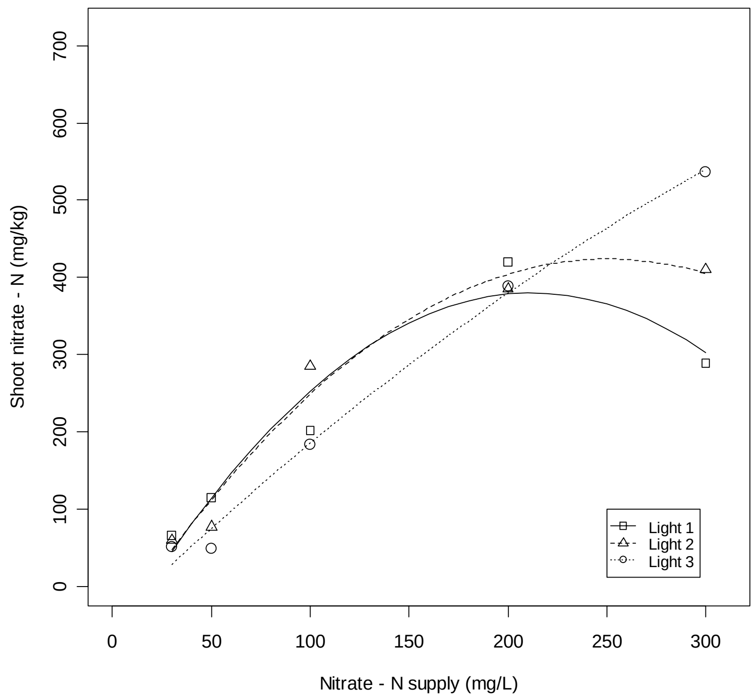


Figure 2. Silverbeet shoot nitrate-N response to nitrate-N supply in winter. At each nitrate-N supply the points are the average of 3 replicates.

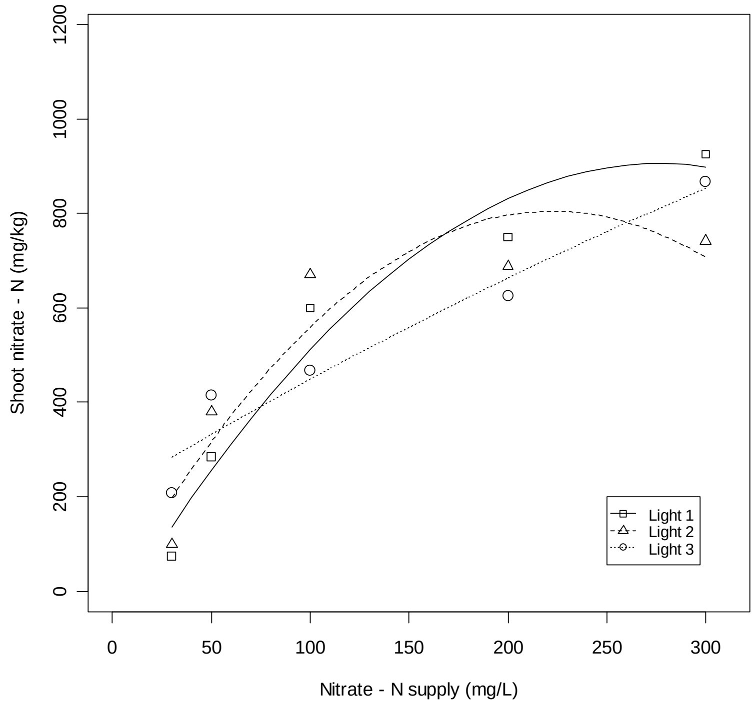


Figure 3. Silverbeet shoot nitrate-N response to nitrate supply in spring. At each nitrate-N supply the points are the average of 3 replicates.

Table 4. Predicted shoot fresh weight for winter and spring experiments

| | Nitrate N supply (mg N/L) | Predicted fresh weight winter (g) | Standard error | Predicted fresh weight spring (g) | Standard error (s.e.) |
|---|---|---|---|---|---|
| Light 1 | 30 | 56.14 | 22.45 | 83.24 | 29.18 |
| | 50 | 106.90 | 18.31 | 143.61 | 22.43 |
| | 100 | 210.54 | 19.32 | 268.80 | 24.11 |
| | 200 | 318.27 | 23.03 | 408.82 | 30.11 |
| | 300 | 293.24 | 27.27 | 401.74 | 36.71 |
| Light 2 | 30 | 54.98 | 22.45 | 119.29 | 29.18 |
| | 50 | 82.41 | 18.31 | 159.77 | 22.43 |
| | 100 | 145.36 | 19.32 | 241.55 | 24.11 |
| | 200 | 247.12 | 23.03 | 321.81 | 30.11 |
| | 300 | 316.69 | 27.27 | 291.02 | 36.71 |
| Light 3 | 30 | 67.07 | 22.45 | 122.91 | 29.18 |
| | 50 | 100.51 | 18.31 | 158.16 | 22.43 |
| | 100 | 167.97 | 19.32 | 226.53 | 24.11 |
| | 200 | 233.72 | 23.03 | 278.62 | 30.11 |
| | 300 | 207.24 | 27.27 | 217.83 | 36.71 |
| s.e. of difference | | 27.79 | | 39.95 | |
| lsd | | 56.68 | | 83.14 | |

### 3.3 Conclusions about nitrate supply and light conditions

The silverbeet experiments have clearly demonstrated that nitrate supply, rather than light conditions, is the most important factor affecting nitrate accumulation in a known nitrate-accumulator species. This highlights that light-limited production situations, such as inside a greenhouse or under shade are not likely to affect nitrate accumulation. Nitrate accumulation in silverbeet occurred at optimal to supra-optimal nitrate supplies. As nitrate supply is the major influence on the nitrate concentration in shoots, prevention of nitrate accumulation will be achieved in production by appropriate fertiliser management.

# 4. RESPONSE OF A RANGE OF LEAFY VEGETABLES TO NITRATE SUPPLY

## 4.1 Aims and objectives

Experiments were conducted to evaluate the range of nitrate levels likely to occur in a number of leafy vegetable types, in response to nitrate supply. This was important given that the silverbeet experiments highlighted the importance of nitrate supply in nitrate accumulation and that overuse of fertiliser is a recognised problem in Australia. The experiments were carried out in a greenhouse and determined the responses of 9 species of leafy vegetables grown at a low and a high supply rate of nitrate. The experiment was conducted twice, once in winter and once in spring to ensure the production period included contrasting climatic conditions.

## 4.2 Materials and Methods

### *4.2.1 Experimental design*

A greenhouse experiment was carried out at the Gosford Horticultural Institute, Narara, New South Wales, Australia (33$^{o}$22’S, 151$^{o}$20’E) in winter and was repeated in spring using nine leafy vegetable species, silverbeet (*Beta vulgaris* L.), baby spinach (*Spinacea oleracecea* L.), two varieties of *Brassica rapa* L. being buk choy (white stem) and pak choy (green stem), three varieties of lettuce (*Lactuca sativa* L.) being imperial head lettuce, cos lettuce and red coral lettuce, rocket (*Eruca sativa* Mill.) and endive (*Chichorium endiva* L.). Pak choy seedlings were unavailable for the spring experiment. Seedlings were planted in black plastic 200 mm pots (one per pot) containing a mixture of sand and perlite. Nutrient solution was supplied through drippers with one dripper supplying each pot. Nutrient solution was supplied in excess of requirement (at least twice daily) to maintain solution concentration around roots.

The experiment was a split plot design with 6 replications of tanks. Each individual N treatment tank supplied all species within a replicate. A similar design was used for both the winter and spring experiments. A photo of the experimental layout prior to harvest in the spring is shown in Fig. 4.

Figure 4. A photo taken of the spring experiment showing the set up within the greenhouse.

### 4.2.2 Control of greenhouse environment

Nitrogen was supplied at two rates: low 50 and high 300 mg N/L as nitrate, which is equivalent to 3.57 and 21.43 mmol N/L as nitrate. Other nutrients were (mmol/L) K 6.3, Ca , S 1.3 , Mg 1.3 , P 1.6 ; and (µmol/L) iron 145 , boron 18.5, manganese 7.1, copper 1.4, zinc 4.5, and molybdenum 0.5. At the low N supply the concentration of sodium was 3.4 µmol/L and of chlorine was 6.06 mmol/L. At the high N supply the concentration of sodium was 9.1 mmol/L and of chlorine was 3.4 µmol varied with N level.

Average minimum to maximum temperatures were 14-26$^{o}$C for winter, and 18-40$^{o}$C for spring. To characterise light conditions in the greenhouse, photosynthetically active radiation (PAR, 400-700 nm) was measured with a quantum sensor (Li-cor USA) every two hours between 8:00 and 16:00 on at least three clear days during the experiments (Fig. 5).

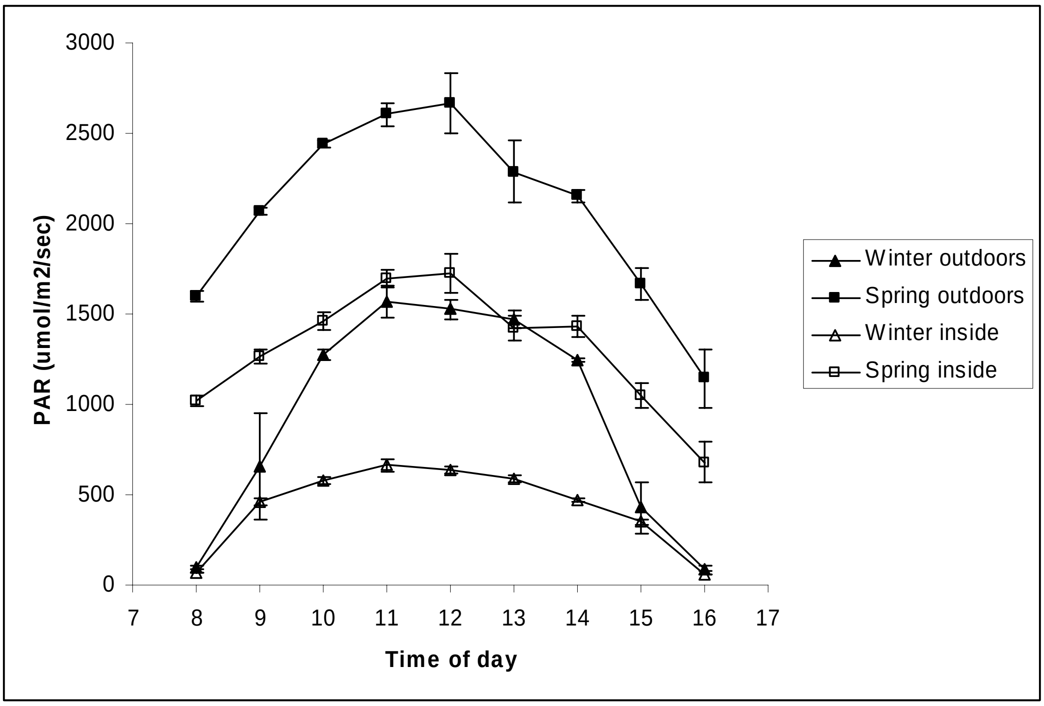


Figure 5. PAR conditions in the greenhouse and outdoors on clear days in winter and in spring (*n*=3, bars are standard error)

### *4.2.3 Plant measurements*

Growth responses were represented for all species by shoot fresh weight. Leaf number was also measured for silverbeet, pak choy, buk choy and baby spinach. Chlorophyll *a* and *b* was extracted from leaf disks taken from recently matured fully expanded leaves using the method of Inskeep and Bloom (1985).

### *4.2.4 Plant sampling and analyses*

Nitrite-N was measured for the spring experiment only. Nitrate-N analysis was determined on a fresh weight basis, in line with European procedures. The sample preparation protocol was developed using the method of Greenway (2001).

Plant samples were either refrigerated prior to processing immediately or frozen before being processed. Whole shoots were blended in a food processor. A sub sample of approximately 100 g was further blended in a plastic beaker, using a hand held blender, to fully homogenise the sub sample. The sub sample was then filtered through nylon gauze (0.23mm$^2$ mesh size) into a volumetric flask, usually 250ml, and made up to volume with distilled water. Supernatant was stored frozen before analysis of nitrate and nitrite. Samples were sent frozen from Gosford to NSW Department of Primary Industries diagnostic laboratories at Wollongbar for analysis.

Nitrate and nitrite were extracted from samples and analysed using flow injection analysis (Lachat QuikChem 8000 Automated Ion Analyzer, Zellweger Analytics Inc. Milwaukee, USA). The procedures for flow injection analysis are based on Method 4500 NO3-F Automated Cadmium Reduction (American Public Health Association 1999).

### *4.2.5 Statistical analyses*

Winter and spring experiments were analysed separately. Data was analysed using mixed linear regression analysis with ASReml (and asreml-r).

*Interpreting graphs* (Fig.s 6-13): for each vegetable type, half the data (fresh weight, leaf number, chlorophyll concentration and nitrate-N) for that vegetable lies within the box and the rest is found within the dashed lines, either side of the box. The median is represented by a solid dot and any outliers by an open circle outside of the box.

## 4.3 Winter results

### *4.3.1 Growth*

The fresh weight of the 9 leafy vegetable types was higher (*P*<0.001) at the high supply of nitrate compared with plants grown at the low supply of nitrate (Fig 6). There was also a significant interaction between nitrate supply and vegetable type (*P*<0.001) indicating that the effect of nitrate supply on fresh weight does depend on the vegetable type. Baby spinach plants had the smallest mean fresh weight of 60 g and head lettuce the largest mean fresh weight of 628 g.

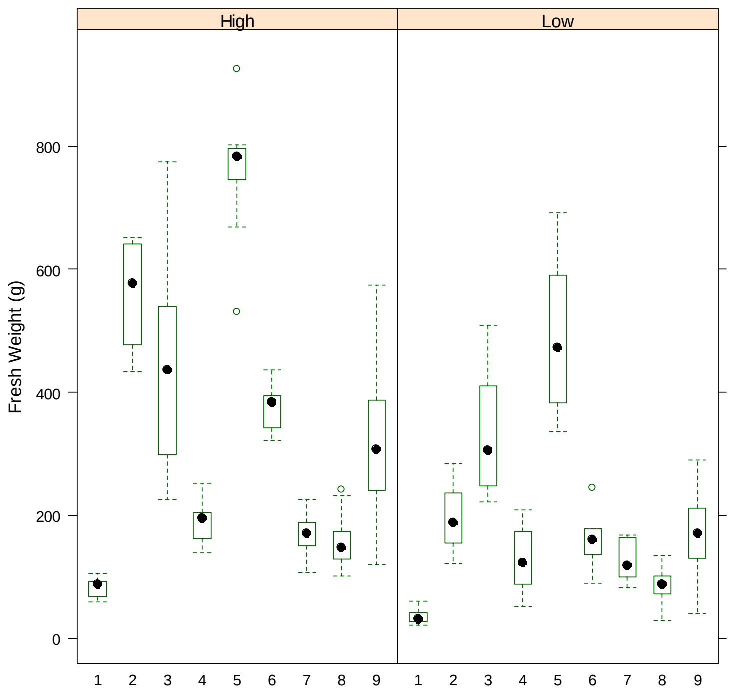


Figure 6. Winter fresh weights of leafy vegetables at high (left box) and low (right box) nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=pak choy, 7=red coral lettuce, 8=rocket, 9=silverbeet.

Leaf number was a useful measure of yield for baby spinach, buk choy and silverbeet. This measure was impractical for the other vegetable types due to a large number of leaves (endive) or where individual leaves are obscured by the rosette morphology of the vegetable or by wrapper leaves (lettuces). Leaf number was significantly (*P*<0.001) reduced by the lower supply of nitrate relative to the high supply (Fig. 7).

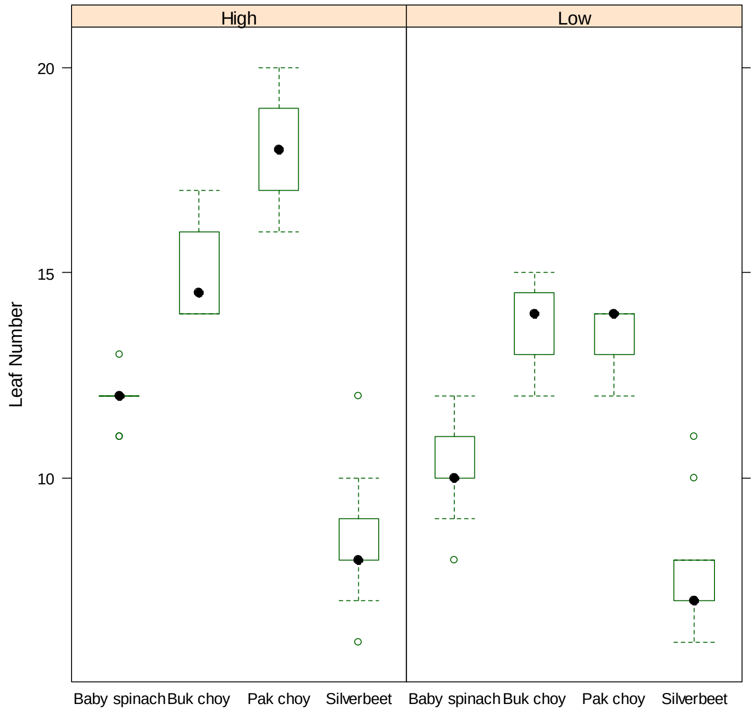


Figure 7. Winter leaf number of baby spinach, buk choy, pak choy and silverbeet at high and low nitrate supply.

### *4.3.2 Nitrate-N in shoots*

In winter, the nitrate-N concentration of the vegetables was higher ($P<0.001$) for plants supplied with a high nitrate supply compared with a low nitrate supply, with the exception of red coral lettuce (Fig. 8). Red coral had a higher mean nitrate-N concentration at the low nitrate supply (195 mg/kg) than at the high nitrate supply (175 mg/kg), although this difference was not significant. This occurrence does not appear to be related to fresh weight as the red coral lettuces were significantly heavier for the high nitrate supply compared with the low nitrate treatment. Nitrate-N concentration depended on vegetable type ($P<0.001$) with cos lettuce having the lowest mean nitrate-N concentration of 174 mg/kg fresh weight compared with 432 mg/kg for buk choy. There was also a significant interaction between nitrate supply and vegetable types for nitrate-N concentration ($P<0.001$).

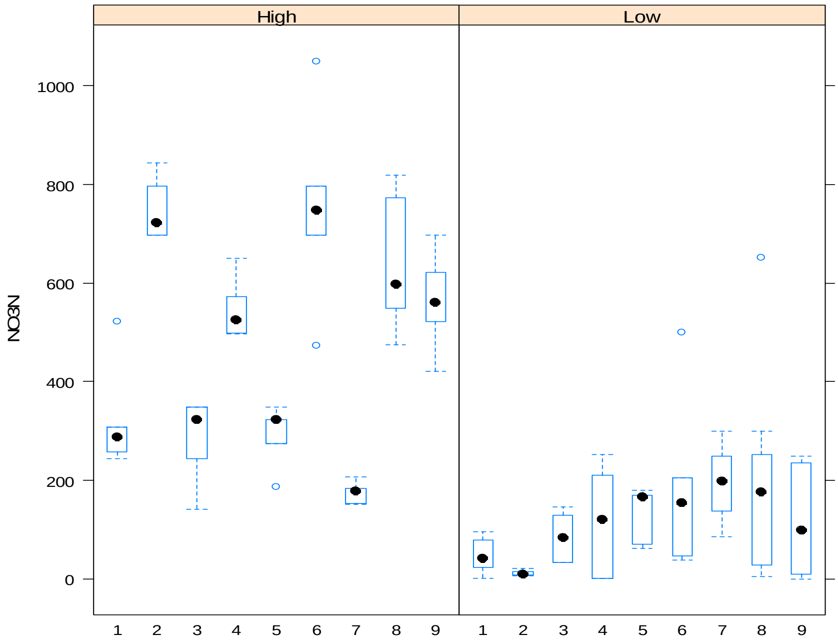


Figure 8. Winter nitrate-N concentrations (mg/kg) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=pak choy, 7=red coral lettuce, 8=rocket, 9=silverbeet.

### *4.3.3 Chlorophyll in shoots*

A high nitrate supply significantly increased the chlorophyll concentration in leaves compared with plants grown at the low nitrate supply (Fig. 9). As expected there were significant differences among the vegetable types with baby spinach having the highest density of chlorophyll (0.54 µg/mm$^2$) and red coral lettuce having the lowest density of chlorophyll (0.07 µg/mm$^2$) in leaves. The interaction between nitrate supply and vegetable type was not significant for chlorophyll concentration suggesting that the nitrate supply effect on chlorophyll concentration was consistent regardless of vegetable type.

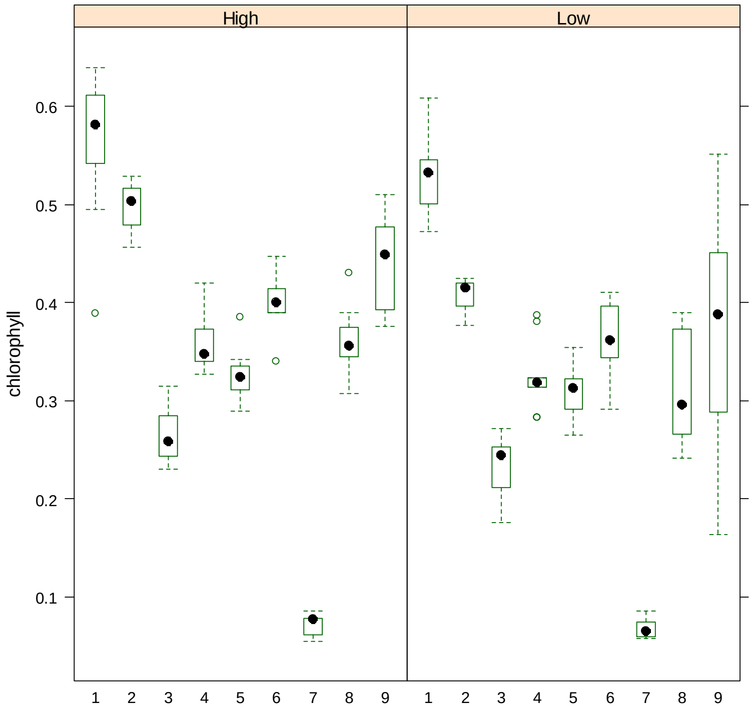


Figure 9. Winter chlorophyll *a+b* concentration (µg/mm$^2$) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=pak choy, 7=red coral lettuce, 8=rocket, 9=silverbeet.

## 4.4 Spring results

### *4.4.1 Growth*

In spring, pak choy was not available for the experiment so 8 leafy vegetable types were grown instead of 9 types. The fresh weight of the 8 leafy vegetable types was high ($P$<0.001) at the high nitrate supply compared with the low nitrate supply (Fig 10). There was also a significant interaction ($P$<0.01) between nitrate supply and vegetable type indicating that the effect of nitrate supply on fresh weight does depend on the vegetable type. Red coral plants had the smallest mean fresh weight of 139 g and head lettuce the largest mean fresh weight of 388 g. In contrast to the winter experiment, the fresh weight of red coral in the spring experiment did not significantly differ between the low and high nitrate supply treatment.

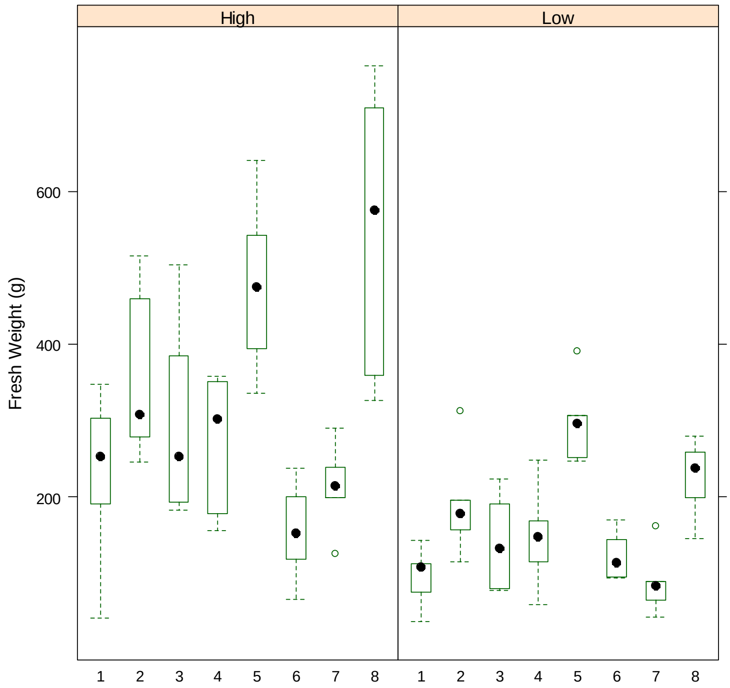


Figure 10. Spring fresh weights of leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=red coral lettuce, 7=rocket, 8=silverbeet.

### *4.4.2 Nitrate-N and nitrite-N in shoots*

In spring the nitrate-N concentration of the vegetables was higher ($P<0.001$) for plants with a high nitrate supply compared with a low nitrate supply, including red coral lettuce, in contrast to the winter experiment (Fig. 11). There were significant differences in nitrate-N concentration among the vegetable types ($P<0.001$) with cos lettuce having the lowest mean of 89 mg/kg fresh weight compared with 420 mg/kg for buk choy. There was also significant interaction between nitrate supply and vegetable types for nitrate-N concentration ($P<0.001$). Only 37% of plants tested for nitrate-N had detectable levels of nitrite-N. Of those plants with nitrite-N concentration ranged between 0.008-0.105 mg/kg.

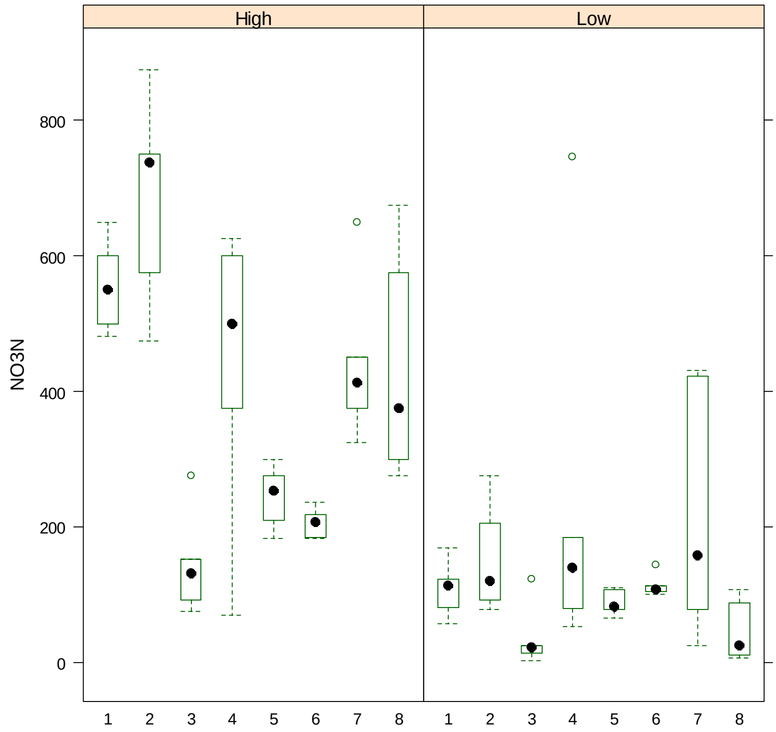


Figure 11. Spring nitrate-N concentrations (mg/kg) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=red coral lettuce, 7=rocket, 8=silverbeet.

### *4.4.3 Chlorophyll in shoots*

As for winter, in spring a high nitrate supply significantly increased the chlorophyll concentration in leaves compared with plants grown at the low nitrate supply (Fig. 12). There were significant differences among the vegetable types with baby spinach having the highest density of chlorophyll (0.41 $\mu g/mm^2$) and red coral lettuce having the lowest density of chlorophyll (0.08 $\mu g/mm^2$) in leaves, similar to the winter results. The interaction between nitrate supply and vegetable type for chlorophyll concentration was not significant suggesting that the nitrate supply effect on chlorophyll concentration was consistent regardless of vegetable type.

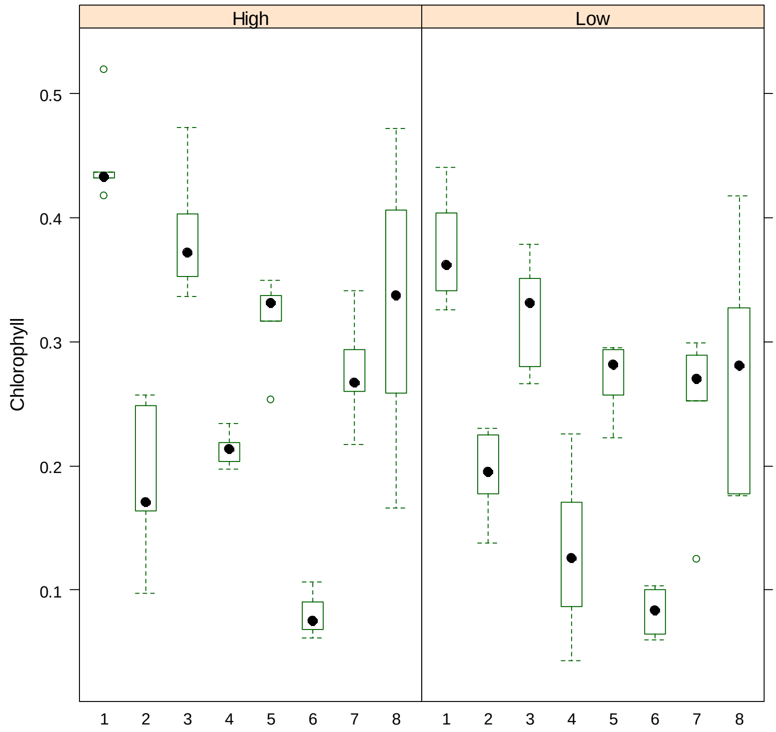


Figure 12. Spring chlorophyll *a+b* concentration (μg/mm$^2$) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=red coral lettuce, 7=rocket, 8=silverbeet.

## 4.5 Distribution of nitrate in shoots as affected by nitrate supply

The winter experiment was used to evaluate the distribution of nitrate-N in the shoots of head lettuce grown at the low and high nitrate supply. Lettuces were separated into halves, hearts and outer leaves. Mixed linear regression statistical analysis with ASReml showed that nitrate supply ($P$=0.001) and plant part ($P$<0.001), and the interaction of nitrate supply and plant part ($P$=0.001), significantly affected the distribution of nitrate-N in head lettuce. For lettuce hearts, the difference in nitrate-N for low (88 mg/kg) and high (144 mg/kg) nitrate supply was not significant (Fig. 13). However, nitrate-N in the outer leaves produced in plants fed the low nitrate supply (169 mg/kg) was significantly lower than those fed the high nitrate supply (511 mg/kg).

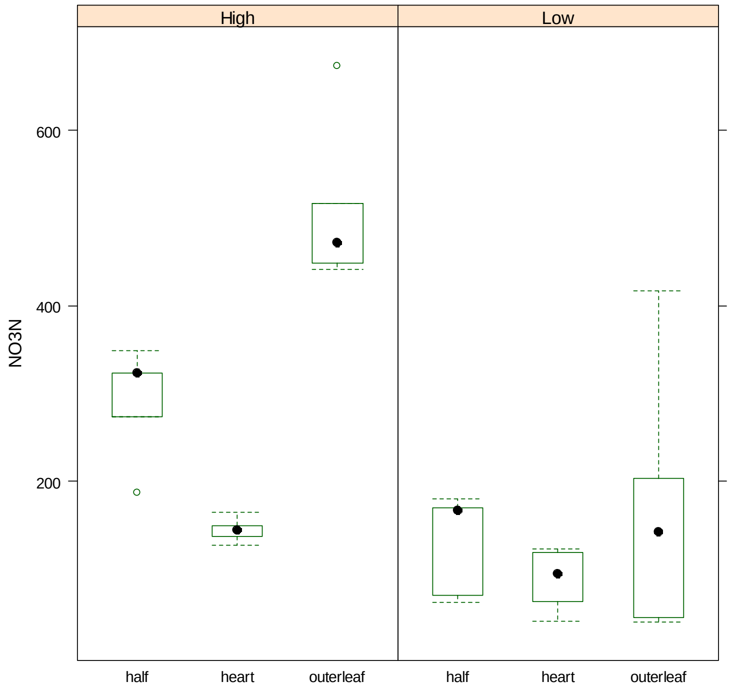


Figure 13. Distribution of nitrate-N (mg/kg) in fresh head lettuce at low and high nitrate supply for the winter experiment.

### 4.6 Conclusions about the response of leafy vegetables to nitrate supply

For some vegetable types, nitrate accumulation occurred in both the winter and spring experiments, associated with the high nitrate supply. These were samples of pak choy, buk choy and rocket. Nitrate-N in shoots exceeded 700 mg/kg for these vegetables. These experiments demonstrated that under controlled conditions shoot nitrate concentration can vary widely among vegetable types. This highlights that plant nitrate requirements depend on the species, or even variety, of vegetable. To determine the optimal nitrate requirements for the species used in these experiments, excluding that completed for silverbeet in this project and some lettuce varieties in other work, further experimentation is necessary. The optimal nitrate requirements determined for a species allow an agronomist or grower to assess the nitrate status of a crop. This provides an important tool in crop fertiliser management.

## 5. GENERAL DISCUSSION

This project was initiated to provide the vegetable industry with information about nitrate and nitrite in Australian produced leafy vegetables and how this impacts on food safety. This project has successfully determined the range of nitrate and nitrite in some Australian of leafy vegetables and some of the production factors that affect nitrate accumulation in vegetables. This issue will not be problematic if nitrate is well managed in leafy vegetable production systems.

### 5.1 Light conditions and the supply of nitrate

The investigation in this project of the effects of nitrate supply and light conditions on the model nitrate-accumulator species silverbeet has demonstrated that nitrate supply, and not light, is the key factor controlling the accumulation of nitrate in harvestable shoots in Australian conditions. The use of shade was sufficient to reduce yield but not to exacerbate nitrate accumulation. The lowest irradiance level used in the silverbeet experiment was 348 *u*mol/m$^2$/sec and during winter, exceeded the critical level required to increase leaf nitrate.

### 5.2 Vegetable type and the supply of nitrate

The performance of buk choy and pak choy in experiments, and the results of the survey, highlight that Asian vegetables are particularly susceptible to nitrate accumulation, compared with other leafy vegetables, when supplied with excessive amounts of nitrate. This would apply generally to leafy vegetables of the Brassicaecae family, to which most Asian leafy vegetables belong. Rocket, the other Brassicaceae member investigated in the project, also had very high nitrate concentrations, particularly at the high supply of nitrate.

As Asian vegetables are ‘new crops’ in Australia, and are increasing in popularity, it is vital that nitrate is managed in these crops in order to avoid an increasing problem of nitrate accumulation. An impediment to this is the lack of production information in Australia about the range of Asian vegetables now being produced. Information on agronomic practices and the nutritional requirements of individual Asian vegetable species needs to be translated from overseas sources and/or developed for Australian conditions. Other strategies include using cultivars that have been identified as possessing low nitrate accumulation properties. For example, JinKui *et al.* (2006) demonstrated that the Shanghaiqing pak choy cultivar did not have a nitrate accumulation problem because it had a high nitrate reductase activity when compared with other varieties.

## 5.3 Managing nitrate accumulation in the production of leafy vegetables

The silverbeet experiments demonstrated that nitrate accumulation can occur at optimal to supra-optimal nitrate supply. To exceed the optimum supply of nitrate to a crop is likely to be detrimental. For example, the maximum recorded nitrate-N concentration of hydroponic head lettuce recorded by Huett and Dettmann (1991) was 2300 mg $kg^{-1}$ grown at 36 mmol $L^{-1}$ nitrate (N). However the highest fresh weight was obtained at a lower nitrate supply of 11 mmol $L^{-1}$. It is entirely possible that the lettuces with accumulated nitrate in the survey were supplied with an excessive amount of nitrate as lettuces supplied with 21 mmol $L^{-1}$ in the experiments were of a marketable size and did not exceed 348 mg/kg nitrate-N.

There are good management tools available to growers that prevent excessive levels of nitrate in soil. Like soil, a hydroponics system also has the potential to deliver an over supply of nitrate to plants. However, for some crops such as lettuce, it is often desirable to have a low concentration of nutrients in the nutrient solution to prevent disorders such as tip burn and so nitrate accumulation is less likely to occur in these situations. This may not be the case for new crops such as Asian vegetables being grown in hydroponics, particularly if growers find that they respond well to a highly concentrated nutrient solution.

For hydroponics, alternative nutrient solutions, such as the partial replacement of nitrate with ammonium, achieve a lower nitrate concentration in lettuce as demonstrated by Savvas *et al.* (2006) in a closed system. However this can compromise growth and quality depending on the cultivar (Al-Redhaiman 2001). Another strategy is to reduce the nitrate concentration in the solution just days before harvesting. Santamaria and Gonnella (2001) reduced the nitrate concentration of rocket by 70% by reducing total nitrogen in the solution by one quarter five days before harvesting. This did not affect yield. Being a relatively recent area of research there is likely to be more scope for nitrate management strategies in hydroponics.

Measuring nitrate in the plant is an excellent tool in determining the nitrate status of the crop. Huett and White (1992) showed that petiole sap nitrate concentrations, measured using Merck test strips clearly differentiated between inadequate and adequate application rates of nitrogen to lettuce. This approach could also be applied to Asian vegetables for use on-farm by growers and agronomists. Critical nitrate concentrations for petiole sap have already been developed for the Brassica species canola (Hocking *et al.* 1997).

## 5.4 Potential for Australian nitrate and nitrite limits in leafy vegetables

Currently there are no limits set for Australia. Publication in the near future of the results of the 22nd Australian Total Diet Study (Food Standards Australia & New Zealand) will indicate if the typical Australian diet exceeds the acceptable daily intake (ADI) of nitrate and nitrite. The establishment of nitrate and nitrite limits in Australian vegetables may depend on this survey.

A recent New Zealand dietary survey (Thomson *et al.* 2007) showed that the mean adult daily intake of nitrate and nitrite from food and water should not pose a health risk to the average consumer. However, when the conversion of nitrate to nitrite in the body is taken into account, about 10% of people with an average conversion rate will be at risk of exceeding the ADI.

Successful export of Australian vegetables in the future may also depend on there being evidence that the product does not exceed maximum limits in nitrate and nitrite established in the target country or region.

## 6. CONCLUSIONS AND RECOMMENDATIONS

The outcomes and results of this project have determined that nitrate accumulation does occur for a range of leafy vegetables produced in Australia. These findings have been reported to the Australian vegetable industry, at two industry conferences and through five media articles, and in the scientific literature. Nitrate accumulation is associated with an excessive supply of nitrate fed to crops and could be avoided with better fertiliser management practices. Vegetables of the Brassicaceae family, in particular many Asian leafy vegetables, are prone to nitrate accumulation. However, currently there is little Australian information on the nutritional management of Asian vegetables. In order for growers of Asian vegetables to easily manage nitrate in leafy vegetables the following developments are recommended:

- The nutritional requirements of leafy Asian vegetable species need to be translated from overseas sources and/or determined experimentally for Australian production systems, including hydroponic systems.
- Protocols need to be developed, with commercial laboratories as partners, for commercial laboratory nutrient analysis of Asian vegetables
- Protocols need to be developed for growers on the use of cheap nitrate test strips for monitoring Asian vegetable crop nitrate status in the field
- Training of agronomists in Asian vegetable nutritional requirements
- Training of growers in the nutrition management of Asian vegetable crops

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## 8. APPENDICES

### 8.1 Appendix 1 Scientific paper manuscript

**The following manuscript was submitted to the Australian Journal of Agricultural Research 10/05/07. The manuscript incorporates changes in response to referee comments received 23/07/07.**

**Nitrate and nitrite in Australian leafy vegetables**

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**Abstract**
A market survey of Australian leafy vegetables and a winter and spring experiment with Swiss chard were conducted to examine nitrate and nitrite levels in leaves. The relationship between growth response to nitrogen (N) supply and light level and accumulation of N in leaves was of particular interest. The survey that included 7 types of lettuce and endive (Asteraceae), 6 leafy Asian vegetables (Brassicaceae) and Swiss chard and spinach (Amaranthaceae) showed that fresh leafy vegetables available on the Australian market can range in nitrate-N from 12-1400 mg/kg fresh weight and nitrite-N from 0 - 37.5 mg/kg. Some samples exceeded the limits for nitrate and nitrite based on international food safety standards. The response of Swiss chard to N supply and light was investigated. The accumulation of nitrate in Swiss chard was primarily influenced by increasing N supply and not by light level. Light conditions for all treatments in both the winter and spring experiments exceeded the critical level (approximately 200 µmol/m$^2$/s) required to increase leaf nitrate. Growth and leaf nitrate concentration were higher for spring, associated with average minimum to maximum temperatures of 18-39$^o$C, compared with 14-28$^o$C for winter. Treatment effects on nitrite-N in Swiss chard could not be determined because nitrite was only detected in one third of plants. The importance of N supply in affecting nitrate accumulation in vegetables is highlighted by the Swiss chard experiments. It confirmed that nitrate accumulation occurs at optimal to supra optimal nitrate supply, emphasising the undesirable effect of excessive fertiliser use to growers.

Additional key words: nitrates, shading, *Beta vulgaris*, lettuce, Asian vegetables

**Introduction**
Vegetable crops have high growth rates and consequently, are very responsive to nutrient applications. Nitrogen (N) is the dominant nutrient for most horticultural crops and maximum uptake rates were estimated at (kg/ha/wk) 23 for lettuce, 66 for tomato and 65 for zucchini squash (Huett 1996) compared with 1.3 for a high density

peach orchard (Stassen *et al.* 1981). Fertiliser management is driven by the need to maximise production and as a consequence, optimal and occasionally supra-optimal rates are used (Goulding 2000, Thompson *et al.* 2007). Nitrate is very prone to leaching and in Europe, monitoring networks indicate that agriculture accounts for 50 to 80% of nitrate entering water where over 20% of groundwater and 30 to 40% of lakes and rivers show excessive concentrations (European Commission 2005).

Most plants utilise nitrate as their main source of nitrogen. Large applications of nitrogenous fertiliser are associated with high levels of nitrate in leafy vegetables such as lettuce (Demsar *et al.* 2004), rocket (Santamaria *et al.* 2001) and Swiss chard (Santamaria *et al.* 1999a). Nitrate is translocated to the leaves where it is reduced and incorporated into proteins, and has an essential role in photosynthesis as a component of chlorophyll.

A high level of nitrate in leafy vegetables is undesirable for reasons of public health and vegetable quality. Human dietary nitrate is largely obtained from vegetables and at high levels is considered harmful to human health (Santamaria 2006). Although nitrate is relatively non-toxic, it is readily converted to nitrite and N-nitroso compounds that are toxic and have been associated with the potentially fatal methaemoglobinaemia, gastric cancer and bladder cancer (Abdel Mohsen *et al.* 1999; Mensinga *et al.* 2003). Vegetable quality is also compromised as increasing application rates of nitrogen-containing fertilisers can increase the incidence and severity of bacterial soft-rot in vegetables. This has been demonstrated in Chinese cabbage (Warner *et al.* 2004) and some susceptible broccoli cultivars (Canaday and Wyatt 1992). Further, the nutritional quality of lettuce is compromised by increased nitrate in terms of reduced vitamin C concentration (Poulsen *et al.* 1995). In Europe, widespread evidence of nitrate accumulation in leafy vegetables led to the European Community developing limits on fresh nitrate concentrations (Santamaria 2006).

Both nitrate supply and light levels have been reported as important controls for nitrate assimilation. Gaudreau *et al.* (1995) and Chadjaa *et al.* (1999) reported that light levels affected nitrate reductase activity and therefore nitrate accumulation in lettuce and spinach. Wallace and Steer (1983) demonstrated that nitrate supply was the primary controller of nitrate reductase activity. Crawford *et al.* (1992) indicated that nitrate supply was the primary signal for nitrate reductase activity and light, because it increased photosynthetic activity and carbohydrate production, promoted nitrate assimilation and hence was a secondary stimulus for nitrate uptake.

In Europe, the incidence of excessive nitrate accumulation is highest during winter when European Commission maximum limits are highest (Santamaria 2006) and similarly in Denmark (Peterson and Stoltze 1999). This response is consistent with the very low light levels that are required to increase nitrate accumulation with a high nitrate supply (Cantliffe 1972a,b).

Australia is fortunate that its climate ranges from temperate to tropical, allowing commercial vegetable production under near optimal environmental conditions. As a consequence, there has been little focus on nitrate levels in vegetables. Only one study has been conducted where Lyons *et al.* (1994) reported some evidence of high nitrate in Australian vegetables. They also reported that hydroponically grown head lettuce contained twice the nitrate concentration of field grown lettuce lettuce. As in Europe, excessive application of nitrogen fertilisers to horticultural crops is a problem and in Australia, high losses of nitrate have been recorded from market gardens (Pionke *et al.* 1990).

Given that high nitrate supply is the primary control over excess nitrate accumulation it is important to establish at what point on the Nitrogen yield response curve this occurs. Many studies report effects of high nitrogen supply on nitrate accumulation without establishing whether excessive nitrate accumulation requires optimal or supra-optimal rates (Dapoigny *et al.* 2000, Proietti *et al.* 2004). Only one study has been conducted to establish this response and it was by Chen *et al.* (2004) in Central China. However, light responses were not examined. No data are available on the nitrate accumulation by leafy vegetables to nitrate supply and light under Australian conditions and the limited study by Lyons *et al.* (1994) have not raised any health concerns.

The objectives of this study were to identify the range of nitrate and nitrite levels occurring in leafy vegetables on the Australian market and to investigate the response of a common leafy vegetable, Swiss chard (*Beta vulgaris* L. var. *cicla*) to nitrate supply and light during typical Australian growing conditions. Swiss chard is a known nitrate accumulator and also a source of high dietary nitrate (Santamaria *et al.* 1999b). Plant growth responses and nitrate and nitrite accumulation in shoots were measured.

## Materials and Methods

*Survey*

The survey included 7 varieties of lettuce (*Lactuca sativa* L.) and endive (*Cicihorium endivia* L.) of the Asteraceae family, 4 leafy Asian vegetable varieties of *Brassica rapa* L., tatsoi (*Brassica rosularis* L.) and rocket (*Eruca sativa* Mill.) of the Brassicaceae, and, baby leaf spinach (*Spinacea oleracecea* L.) and Swiss chard (*Beta vulgaris* L.) of the Amaranthaceae. A total of 165 fresh samples were collected between June 2005 and January 2006. Samples originated from the major vegetable growing areas in three states; Queensland, Victoria and New South Wales and were sourced directly from growers or the central markets in Sydney.

Plant samples were either refrigerated prior to processing immediately or frozen before being processed. Whole shoots were blended in a food processor and then a sub sample (approximately 100 g) was filtered through nylon gauze into a volumetric flask and made up to 250 ml with deionised and distilled water at room temperature. The supernatant was stored frozen before analysis of nitrate and nitrite.

Nitrate and nitrite were extracted from samples and analysed using flow injection analysis (Lachat QuikChem 8000 Automated Ion Analyzer, Zellweger Analytics Inc. Milwaukee, USA). The procedures for flow injection analysis are based on Method 4500 NO3-F Automated Cadmium Reduction (American Public Health Association 1999). The concentration of the standards was within the range 1-10 mg N/L. Values are reported here for nitrate nitrogen and nitrite nitrogen.

Since the survey design was not statistically strong, results presented for the survey are a summary of the data only and no statistical inferences are made.

*Swiss chard experiments*

A greenhouse experiment was carried out at the Gosford Horticultural Institute, Narara, New South Wales, Australia (33$^{o}$22'S, 151$^{o}$20'E) in winter and was repeated in spring using Swiss chard, *B. vulgaris* cv. Silverstar. Seedlings were planted at the 2 leaves stage in black plastic 200 mm pots (one per pot) containing sand. The winter experiment was planted on the 12$^{th}$ July and harvested on the 23$^{rd}$ August, 2005. The spring experiment was planted on the 26$^{th}$ October and harvested on the 30$^{th}$ November, 2005. Nutrient solution was supplied through drippers with one dripper supplying each pot. Nutrient solution was supplied in excess of requirements (at least twice daily) to maintain solution concentration around roots.

Nitrogen was supplied at five rates: 30, 50, 100, 200, 300 mg N/L as nitrate, which is equivalent to 2.14, 3.57, 7.14, 14.29 and 21.43 mmol N/L as nitrate. Other nutrients were (mmol/L) K 6.3, Ca 3.8, S 1.3, Mg 1.3, P 1.6; and (µmol/L) Fe 145, B 18.5, Mn 7.1, Cu 1.4, Zn 4.5, and Mo 0.5. The concentrations of Na (3.4 µmol - 9.1 mmol/L) and Cl (3.4 µmol - 7.5 mmol/L) varied with N level.

Light treatments were the ambient light levels in the greenhouse (Light 1, approximately 57% of light outdoors), light reduced by 20 mm black polypropylene bird netting mounted on frames and suspended over plants (Light 2, approximately 46% of light outdoors) and light reduced by aluminium and polyester screen (AB Ludvig Svensson, Kinna, Sweden) suspended over plants (Light 3, approximately 27% of light outdoors). To characterise light conditions for each treatment, photosynthetically active radiation (PAR, 400-700 nm) was measured with a quantum sensor (LI-COR Lincoln NE, USA) under each light treatment every two hours between 8:00 and 16:00 on at least three clear days during the experiments (Fig. 1 a,b).

Total solar radiation was recorded daily on an unshaded solarimeter located next to the greenhouse at the highest point of the roof (Fig. 2).

Mean daily temperatures inside the greenhouse ranged from 13.8-27.8$^{o}$C in winter and 17.5-38.7$^{o}$C in spring.

The combinations of the three light treatments and five nitrate treatments formed a 3 x 5 factorial treatment structure with treatments arranged in 3 replicates of split plot design. Three rows in the greenhouse were used, with each replicate comprising a complete row. Within each replicate row, the 3 light treatments were allocated randomly to the 3 main plots. The 5 nitrate treatments were then randomised within each main plot to the subplots so that each light treatment contained a complete set of the 5 nitrate treatments. An experimental unit comprised of 3 pots, with one plant per pot. Each replicate row had 5 different tanks supplying the 5 levels of nitrate treatment to all experimental units within the 3 light main plots. Within a replicate row, at the subplot level, the nitrate treatments were not independently supplied, as each individual nitrate treatment tank supplied all three shade treatments. This lack of independence is ignored in the statistical analysis. Within a replicate, the nutrient solution for each nitrate treatment came from a common source leading to a lack of independence between experimental units. This will be ignored in the statistical analysis. A similar design was used for both the winter and spring experiments.

Plant measurements

Plant growth was represented by shoot fresh weight. Shoot nitrate and nitrite concentrations were measured as described for the survey samples.

Statistical analyses

Averages of the 3 plants per experimental unit were calculated prior to analysis for the growth responses. Single plants were used to measure the concentration of shoot nitrate and nitrite present at harvest. Linear mixed models allowing variation in the observations to be separated into fixed and random effects were fitted to the shoot fresh weight and nitrate concentration for each experiment using the ASReml program (Gilmour *et al.* 2006). Since nitrate concentration is a treatment with 5 quantitative levels, its linear and quadratic effects and their interactions with the light treatment were included in the model. A model accommodating the experimental design elements such as treatments, rows and main plots within rows was fitted and predicted treatment effects and error components were calculated accordingly. The significance of the fixed effects was assessed using a Wald statistic (WS). Predicted treatment means were compared using the least significant difference (LSD) technique. Maximum values of the shoot nitrate concentrations were calculated together with the corresponding nitrate supply values. Winter and spring experiments were analysed separately.

## Results

*Survey*

The fresh leafy vegetable samples in the survey ranged widely in nitrate concentration (Table 1). Nitrate concentrations were as low as 12 mg/kg for Swiss chard and as high as 1400 mg/kg for the Asian vegetable Choy Sum. China's tentative tolerance level for nitrate-N in vegetables is 700 mg/kg (Zhou *et al.* 2000). The detection limit for nitrate in sample extracts was 0.02 mg/L which approximates to 0.05 mg/kg on a fresh weight basis. Reliable comparisons between different vegetable types are difficult as all types were not available on each sampling occasion from each state. Nonetheless, it is worth noting that the three samples highest in nitrate concentration were Asian vegetables: choy sum, tat soi and buk choy.

Of the 165 vegetable samples, 59% of samples were below the detection limit (<0.003 mg/L) for nitrite nitrogen. Of the 68 samples containing measurable amounts of nitrite nitrogen, most were below 1 mg/kg fresh weight. The exceptions were six samples with nitrite levels well above 1 mg/kg. These were two samples of baby leaf spinach (33.5 and 37.5mg $kg^{-1}$), one sample of pak choy (33.5 mg $kg^{-1}$), two samples of tatsoi (11.6 and 5.3 mg $kg^{-1}$) and one sample of rocket (6.5 mg $kg^{-1}$).

*Swiss Chard Experiments*

The response of fresh weight of Swiss chard shoots to increasing supply of nitrate followed a quadratic form ($P<0.001$) in both winter and spring, although this response was dependent upon light level (Figure 3 and 4). In winter the shoot fresh weight response to nitrate supply under Light 2 responded differently to the other light treatments. In spring, plants in the Light 1 treatments generally exhibited a significantly different response to plants in the other 2 light treatments (Figure 4).

The plants grown in spring (mean=229.6g, standard error=17.0) were generally heavier than those grown in winter (mean=173.9g, standard error=15.2).

In both winter and spring, light treatment did not affect the concentration of nitrate in Swiss chard shoots and its response to increasing nitrate N levels followed a quadratic form in both winter and spring (Fig. 5). In winter, the model predicted a maximum shoot nitrate concentration of 420.9 ± 27.8 mg/kg occurring at 271.1 mg/L of nitrate. The predicted maximum shoot nitrate for spring (824.4 ± 47.1 mg/kg) occurred at 277.4 mg/L of nitrate. The overall range of shoot nitrate levels concentrations in spring (3-1175 mg/kg) was greater than in winter (3-720 mg/kg).

The number of Swiss chard samples with detectable nitrite was insufficient to investigate the effects of light, N supply or storage on nitrite in fresh tissue. Only 62 samples out of the 180 measured in total for both experiments had detectable nitrite. The samples with nitrite had a mean nitrite-N concentration of 0.193 mg/kg (standard error=0.05) and a range 0.007-2.218 including four samples > 1.00 mg/kg.

## Discussion

The survey revealed that nitrate-N levels in Australian leafy vegetables can exceed the European Commission (EC) maximum safe eating limit standards (Santamaria 2006). Samples of green coral lettuce, red coral lettuce and green oak lettuce exceeded the EC limits for leafy lettuce grown outdoors in summer (560 mg/kg nitrate-N). Members of the Brassicaceae family, known to contain a number of nitrate-accumulating species (Santamaria 2006), were included in the survey and had nitrate-N concentrations as high as 1400 mg/kg (6160 mg NO3/kg). Particularly high levels of nitrate-N (>900 mg/kg) were obtained for samples of rocket, and the Asian vegetables buk choy, choy sum, tat soi and mizuna, exceeding China's tentative tolerance level for nitrate-N in vegetables of 700 mg/kg (Zhou *et al.* 2000). In contrast, the maximum nitrate-N level obtained for an iceberg lettuce sample was half the European Commission Regulation maximum limit of 450 mg/kg (European Commission 2005).

High nitrite-N levels (>6.5 mg/kg), well above the tolerance limit of 1.2 mg/kg proposed for the Chinese National Standard, were detected for several samples in the survey. The storage temperature and time period in storage of these samples prior to purchase was not known but were possibly unfavourable. High storage temperature and long storage periods have been shown to increase nitrite in Chinese cabbage and spinach (Aworh *et al.* 1978, 1980 and Chung *et al.* 2004). Although the number of samples high in nitrite was few, the importance of appropriate storage of leafy vegetables in Australia should not be ignored. Furthermore, as nitrite is a known carcinogen where, under favourable conditions, nitrate is converted to nitrite in the body, concentrations of both ions should be monitored.

In the Swiss chard study, the shape of the growth response curve was similar to that recorded for lettuce grown in sand culture over the same range of nitrate concentrations (Huett and Dettmann 1991). The maximum shoot nitrate concentrations in Swiss chard occurred at optimal to supra-optimal nitrate supply for growth in both the winter and spring experiments, consistent with response studies on rape, Chinese cabbage and spinach by Chen *et al.* (2004). Shoot nitrate concentrations in Swiss chard were higher in spring than in winter and were not affected by irradiance or photoperiod. These results appear to contrast with the lettuce greenhouse studies by Dapoigny *et al.* (2000) and Pavlou *et al.* (2007) where the authors presumed that limiting light conditions towards the end of the cropping cycle increased nitrate accumulation.

Cantliffe (1972a) reported that nitrogen supply increased spinach leaf nitrate concentrations with little effect of light over irradiance levels of around 450 to 900 µmol/m$^2$/s, consistent with the Swiss chard study. At the lowest irradiance level of around 160 µmol/m$^2$/s, Cantliffe (1972b) reported an increase in leaf nitrate concentration of 46 to 48% compared with higher irradiance levels at the two highest nitrogen rates. Similarly, Proietti *et al.* (2004) showed that spinach grown at a light intensity of 200 µmol/m$^2$/s had a higher nitrate concentration than those plants grown at 800 µmol/m$^2$/s. The lowest irradiance level used in the Swiss chard experiment was 348 µmol/m$^2$/s and during winter, exceeding the critical level required to increase leaf nitrate. The lowest irradiance level used by Cantliffe (1972b) and Proietti *et al.* (2004) equates to dull, heavily overcast conditions, common during European winters that are associated with high nitrate concentrations in leafy vegetables, but uncommon in commercial vegetable growing regions of Australia. A reduction in photoperiod has also been reported to increase nitrate accumulation (Cantliffe 1972b) and would compound the European high nitrate problem during winter.

Temperature may affect nitrate accumulation in leafy vegetables. The range of temperatures in the spring and autumn experiments conducted by Pavlou *et al.* (2007) were similar, ranging from an average minimum to maximum of 13-33$^o$C for spring and 12-30$^o$C for autumn. In contrast, the average minimum to maximum temperatures in the current Swiss chard winter experiment were 14-28$^o$C and 18-39$^o$C in spring. A reduction in root zone temperature from 8$^o$C to 22$^o$C reduced nitrate reductase activity in leaves of peas, potentially leading to nitrate accumulation (Vasilieva *et al.* 1999). An increase in nitrate uptake will accompany a growth response as temperatures increase towards an optimum without increasing leaf nitrate concentration, as was the case for lettuce (Frota and Tucker 1972). Cantliffe (1972c) demonstrated an increase in both uptake and leaf concentration of spinach to temperature and nitrate supply. When the temperature was further increased to 30$^{o}$C spinach growth and shoot nitrate concentration were reduced. Lo Piero *et al.* (2000) showed in heat-stressed sweet pepper, nitrate uptake was enhanced, associated with increased nitrate reductase activity, and this did not affect growth. The higher growth and leaf nitrate concentrations recorded in the spring than the winter experiments in the current study are consistent with the responses reported by Lo Piero *et al.* (2000) and Cantliffe (1972a,b,c)

The results from our study reveal that nitrate accumulation can occur in Australian leafy vegetables. The European problem of low winter light levels and short photoperiod causing higher nitrate concentrations is unlikely to be a problem in Australia. However, the importance of N supply in affecting nitrate accumulation in vegetables is highlighted by this work. The critical issue is that excessive nitrate accumulation occurs at optimal to supra optimal nitrate supply, emphasising to growers the undesirable effect of excessive fertiliser use. There is a tendency worldwide in horticulture to adopt management practices that maximise yield at the expense of the environment (Goulding 2000, Thompson *et al.* 2007) because fertiliser is a small part of the total production cost.

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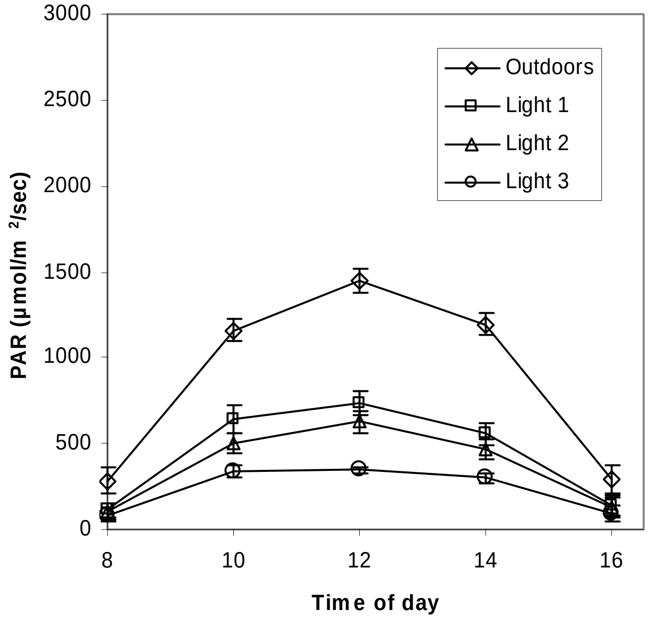


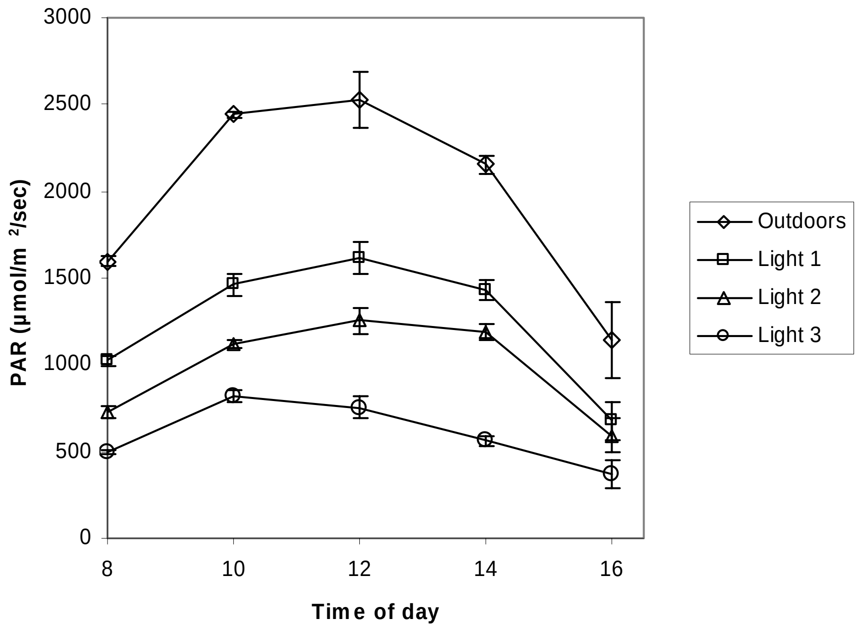


Figure 1. PAR conditions under shade treatments on clear days in winter (a) and in spring (b) (*n*=3, bars are standard errors).

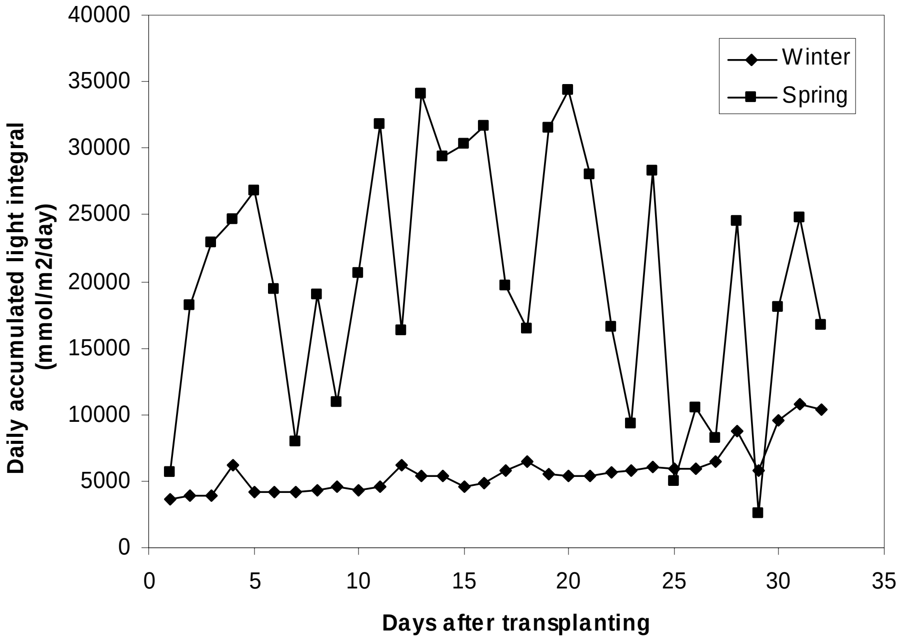


Figure 2. Daily total solar radiation for the winter and spring experiment.

Table 1. Nitrate-N in fresh Australian leafy vegetables.

| Leafy vegetable (n)[a] | Mean nitrate-N (s.e.) (mg/kg) | Range (mg/kg) |
|---|---|---|
| Baby leaf spinach (23) | 310 (51) | 14 - 727 |
| Buk choy (21) | 648 (51) | 336 - 1162 |
| Choy sum (3) | 1017 (200) | 725 - 1400 |
| Endive (1) | 466 | 466 |
| Lettuce, Butter (3) | 128 (3) | 123 - 130 |
| Lettuce, Coral green (5) | 505 (162) | 176 - 995 |
| Lettuce, Coral red (22) | 396 (33) | 129 - 816 |
| Lettuce, Cos (9) | 254 (29) | 108 - 350 |
| Lettuce, Iceberg (11) | 138 (18) | 73 - 239 |
| Lettuce, Oak green (4) | 291 (38) | 220 - 399 |
| Lettuce, Oak red (8) | 493 (66) | 193 - 815 |
| Mizuna (10) | 755 (85) | 214 - 1014 |
| Pak choy (2) | 374 (39) | 335 - 413 |
| Rocket (23) | 522 (46) | 235 - 1059 |
| Swiss chard (10) | 678 (91) | 12 - 1078 |
| Tat soi (10) | 678 (114) | 105 - 1197 |

[(a)] number of samples

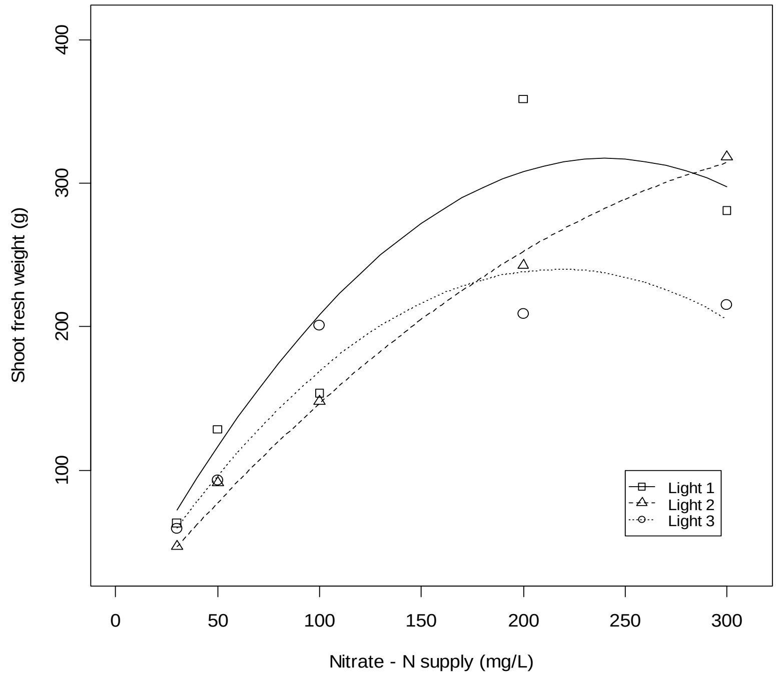


Figure 3. Predicted shoot fresh weight response of Swiss chard to nitrate-N supply at light 1 (☐), light 2 (△) and light 3 (○) levels in winter. Values are averages of 9 pots.

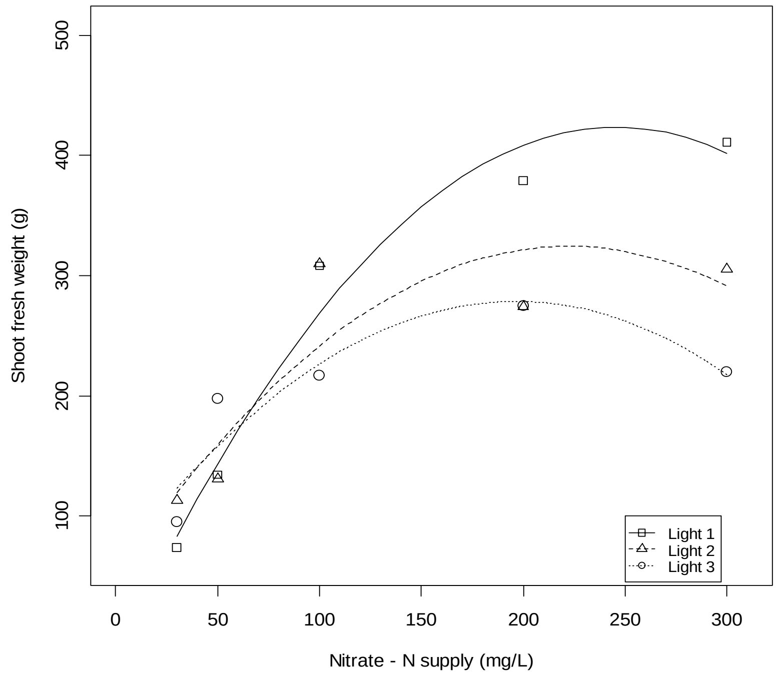


Figure 4. Predicted shoot fresh weight response of Swiss chard to nitrate-N supply at light 1 (☐), light 2 (△) and light 3 (○) levels in spring. Values are averages of 9 pots.

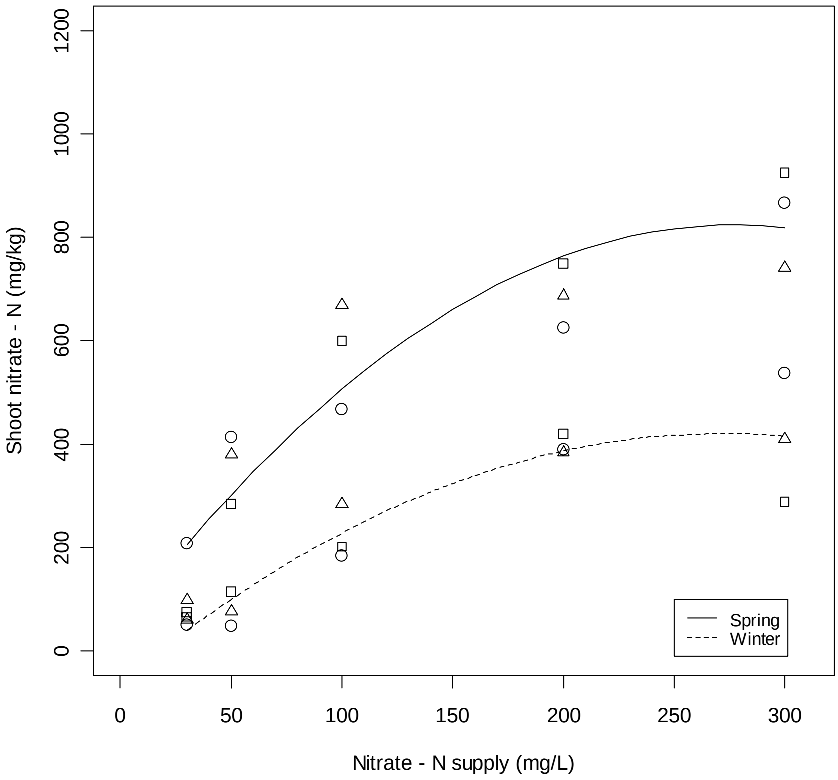


Figure 5. Response of nitrate-N in fresh shoots of Swiss chard to nitrate-N supply in winter and spring. At each nitrate-N supply the points are the average of 3 replicates. light 1 (□), light 2 (△) and light 3 (○)

Winter: y= - 60.6 ± 46.2 + 3.55 ± 0.78 x -0.00652 ± 0.00234 $x^2$

Spring: y= 46.9 ± 75.3 + 5.61 ± 1.27 x -0.01011 ± 0.00381 $x^2$

## 8.2 Appendix 2 Conference proceedings paper

**Proceedings, Australian Hydroponic and Greenhouse Industry National Conference 2007 June 24-27, Launceston Tasmania.**

**This article was also submitted to *Practical Hydroponics and Greenhouses* 17/08/07**

NITRATE AND NITRITE IN VEGETABLES: CONSEQUENCES FOR HORTICULTURE

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Abstract
A survey of leafy vegetables grown in Australia has shown that 27% of samples had an elevated concentration of nitrate and/or nitrite. High levels of nitrate or nitrite are a concern for reasons of food safety, product quality and sustainability in production. Experimental work showed that increasing the supply of nitrate, through fertiliser inputs, increases the likelihood of nitrate accumulating in leafy vegetables. By avoiding the excessive use of nitrogen fertilisers, growers can deliver high quality vegetables without accumulated nitrate. Additionally, fertiliser costs are reduced and the impact of production on the environment is minimised.

INTRODUCTION
It is important to measure nitrate and nitrite in vegetables for reasons of food safety, product quality and sustainability of production. Vegetables provide the majority of nitrate found in the diet. Leafy vegetables tend to have a higher nitrate concentration than other vegetable types such as root or fruit vegetables. Once eaten, nitrate can be reduced to nitrite in the body and can combine with amines to form carcinogenic nitrosamines that are associated with gastric cancer (Mensinga et al. 2003). Therefore, it is important to avoid having a high concentration of both nitrate and nitrite in vegetables.

Poor quality can be a problem if vegetables have a high nitrate concentration. Increasing application rates of nitrogen-containing fertilisers can increase the incidence and severity of bacterial soft-rot in vegetables. This has been demonstrated in Chinese cabbage (Warner et al. 2004) and some susceptible broccoli cultivars (Canaday and Wyatt 1992). High nitrate concentrations in vegetables also reflect unnecessarily high fertiliser inputs. This is not cost effective and can lead to environmental pollution such as the contamination of ground water (Pionke et al. 1990).

A number of countries have set maximum allowable limits for nitrate and nitrite in some leafy vegetables. The European Community has developed limits on nitrate concentrations of fresh spinach and lettuce (Santamaria 2006). For example, leafy lettuce grown outdoors in summer has a maximum allowable limit of 560 mg/kg nitrate-N. China's tentative tolerance level for nitrate-N in vegetables is 700 mg/kg fresh weight and for nitrite-N it is 1.2 mg/kg fresh weight (Zhou et al. 2000).

Currently there are no limits set for Australia. Publication in the near future of the results of the 21st Australian Total Diet Study (Food Standards Australia & New Zealand) will indicate if the typical Australian diet exceeds the acceptable daily intake of nitrate and nitrite. The establishment of nitrate and nitrite limits in Australian vegetables may depend on this survey. Successful export of Australian vegetables in the future may also depend on there being evidence that the product does not exceed maximum limits in nitrate and nitrite established in the target country or region.

NITRATE AND NITRITE LEVELS IN AUSTRALIAN LEAFY VEGETABLES

A survey conducted of leafy vegetables available in the marketplace showed that 27% of samples were above 700 mg nitrate-N /kg fresh weight and/or above 1.2 mg nitrite-N /kg fresh weight (Parks *et al.*, 2007). Leafy vegetables included 7 varieties of lettuce (butter, green coral, red coral, cos, iceberg, green oak, red oak), endive, 4 leafy Asian vegetable varieties (buk choy, mizuna, pak choy, tat soi), baby leaf spinach and silverbeet. Samples were collected over a one year period and were sourced from the major vegetable growing areas in three states; Queensland, Victoria and New South Wales. Only 4% of samples in the survey had high levels of nitrite. The samples above the 700 mg nitrate-N /kg fresh weight appeared to be more frequent for mizuna, rocket, tat soi, silverbeet, and buk choy but were certainly not confined to these types.

THE CAUSES OF NITRATE ACCUMULATION IN LEAFY VEGETABLES

An over supply of nitrate fed to plants is the primary reason for nitrate accumulation in leafy vegetables. Parks *et al.* (2007) showed that the nitrate concentration of silverbeet increased with an increase in nitrate supply in both winter and in spring. In contrast, nitrite levels in silverbeet were not affected by fertiliser supply and were detected in only a few samples.

Another important factor in nitrate accumulation is the vegetable species being cultivated. A number of leafy vegetables are known nitrate-accumulators. For example, spinach (*Spinacea oleracecea* L.) and Swiss chard (*Beta vulgaris* L.) in the plant family Amaranthaceae are known to accumulate nitrate to very high concentrations. Other leafy vegetables classified as having a generally high concentration of nitrate include lettuce, rocket, celery, cress, radish, red beetroot, endive, fennel, parsley, leek, endive and Chinese cabbage (Santamaria, 2006). Evidently vegetables from the plant family Brassicaceae are also susceptible. In the survey (Parks *et al.*, 2007) the Brassicaceae vegetables were buk choy, pak choy, mizuna, and choy sum (*Brassica rapa* L.), tatsoi (*Brassica rosularis* L.) and rocket (*Eruca sativa* Mill.). The nitrate concentration of these was generally high, possibly reflecting a combination of choice of species and a high nitrogen fertiliser supply during cultivation.

HYDROPONICS & NITRATE ACCUMULATION IN LEAFY VEGETABLES

A number of studies have investigated nitrate accumulation in leafy vegetables grown using different cultivation systems. These studies show that nitrate accumulation can occur, in lettuce for example, in a hydroponic system (Guadagnin et al. 2005). This is not surprising given that hydroponic nutrient solution typically delivers an over supply of nitrate to roots.

Alternative nutrient solutions, such as the partial replacement of nitrate with ammonium, achieve a lower nitrate concentration in lettuce as demonstrated by Savvas et al (2006) in a closed system. However this can compromise growth and quality depending on the lettuce cultivar (Al-Redhaiman, 2001). Another strategy is to reduce the nitrate concentration in the solution just days before harvesting. Santamaria and Gonnella (2001) reduced the nitrate concentration of rocket by 70% by reducing total nitrogen in the solution by one quarter five days before harvesting. This did not affect yield.

## CONCLUSION

Australian produced leafy vegetables can have a high concentration of nitrate and occasionally of nitrite. The nitrate concentration in leafy vegetables is largely controlled by the supply of nitrate fed to the plant. Vegetable producers will be able to avoid nitrate accumulation in their produce by managing the nutrition of the crop. For hydroponic producers clear strategies to avoid nitrate accumulation will need to be developed for this type of production system.

## Acknowledgements

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## 8.3 Appendix 3 Media articles

### *8.3.1 Soilless*

**Article submitted to Soilless 21/05/07**

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INTRODUCTION

It is important to measure nitrate and nitrite in vegetables for reasons of food safety, product quality and sustainability of production. Vegetables provide the majority of nitrate found in the diet. Leafy vegetables tend to have a higher nitrate concentration than other vegetable types such as root or fruit vegetables. Further, leafy vegetables can accumulate nitrate beyond levels that are optimum for plant growth. Once eaten, nitrate can be reduced to nitrite in the body and can combine with amines to form carcinogenic nitrosamines that are associated with gastric cancer.

Poor quality can be a problem if vegetables have a high nitrate concentration. Increasing application rates of nitrogen-containing fertilisers can increase the incidence and severity of bacterial soft-rot in vegetables such as Chinese cabbage and broccoli. High nitrate concentrations in vegetables also reflect unnecessarily high fertiliser inputs during production. Excessive fertiliser use is the primary cause of nitrate accumulation in vegetables. This is inefficient, costly and can lead to environmental pollution such as the contamination of ground water.

A number of countries have set maximum allowable limits for nitrate and nitrite in some leafy vegetables. The European Community has developed limits on nitrate concentrations of fresh spinach and lettuce. For example, leafy lettuce grown outdoors in summer has a maximum allowable limit of 560 mg/kg nitrate-N. China's tentative tolerance level for nitrate-N in vegetables is 700 mg/kg fresh weight and for nitrite-N it is 1.2 mg/kg fresh weight.

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NITRATE AND NITRITE LEVELS IN AUSTRALIAN LEAFY VEGETABLES
A recent survey conducted by the author on leafy vegetables available in the marketplace has shown that 27% of samples were above 700 mg nitrate-N /kg fresh weight and/or above 1.2 mg nitrite-N /kg fresh weight. The leafy vegetables tested were 7 varieties of lettuce (butter, green coral, red coral, cos, iceberg, green oak, red oak), endive, 4 leafy Asian vegetable varieties (buk choy, mizuna, pak choy, tat soi), baby leaf spinach and silverbeet. Samples were collected over a one year period and were sourced from the major vegetable growing areas in three states; Queensland, Victoria and New South Wales. Only 4% of samples in the survey had high levels of nitrite. The samples above the 700 mg nitrate-N /kg fresh weight appeared to be more frequent for mizuna, rocket, tat soi, silverbeet, and buk choy but were certainly not confined to these types.

THE CAUSES OF NITRATE ACCUMULATION IN LEAFY VEGETABLES
An over supply of nitrate fed to plants is the primary reason for nitrate accumulation in leafy vegetables. In contrast, nitrite levels are not affected by fertiliser supply and are detected infrequently in vegetables. Another important factor in nitrate accumulation is the vegetable species being cultivated.

A number of leafy vegetables are known nitrate-accumulators. For example, spinach (*Spinacea oleracecea* L.) and Swiss chard (*Beta vulgaris* L.) in the plant family Amaranthaceae are known to accumulate nitrate to very high concentrations. Other leafy vegetables classified as having a generally high concentration of nitrate include lettuce, rocket, celery, cress, radish, red beetroot, endive, fennel, parsley, leek, endive and Chinese cabbage. Evidently vegetables from the plant family Brassicaceae are also susceptible. In the survey the Brassicaceae vegetables were buk choy, pak choy, mizuna, and choy sum (*Brassica rapa* L.), tatsoi (*Brassica rosularis* L.) and rocket (*Eruca sativa* Mill.). The nitrate concentration of these was generally high, possibly reflecting a combination of choice of species and a high nitrogen fertiliser supply during cultivation.

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Alternative nutrient solutions, such as the partial replacement of nitrate with ammonium, can achieve a lower nitrate concentration in lettuce. However this can compromise growth and quality depending on the lettuce cultivar. Another strategy is to reduce the nitrate concentration in the hydroponic solution in the days before harvesting. One study has shown that the nitrate concentration of rocket can be reduced by 70% when total nitrogen in the solution is reduced by one quarter, five days before harvesting. Importantly, this did not affect yield.

## CONCLUSION

Australian produced leafy vegetables can have a high concentration of nitrate and occasionally of nitrite. The nitrate concentration in leafy vegetables is largely controlled by the supply of nitrate fed to the plant. Vegetable producers will be able to avoid nitrate accumulation in their produce by managing the nutrition of the crop. For hydroponic producers clear strategies to avoid nitrate accumulation will need to be developed for this type of production system.

### 8.3.2 Vegetables Australia





# Shedding new light on nitrate levels

**High nitrate levels in leafy vegetables have the potential to threaten Australian exports. Graham Gosper speaks to NSW DPI researcher Sophie Parks to find out why.**

A survey of leafy vegetables from Australia's leading growing areas has revealed high nitrate levels in some samples and identified a need for a reassessment of on-farm fertiliser inputs.

The survey is part of a research project investigating nitrate and nitrite accumulation in Australian produced leafy vegetables. The three-year project, due for completion early next year, is also examining likely causes of high nitrate levels in leafy vegetables and best practices for managing such levels.

Sophie Parks, a NSW Department of Primary Industries (DPI) research horticulturist based at the Gosford Horticultural Institute on the NSW Central Coast, is the project leader. She said excessive nitrate concentrations in leafy vegetables are recognised as undesirable for human health reasons and because of their affect on the quality of the produce. "Vegetables with a high level of nitrate, for example, are more likely to develop soft rots compared with those with lower nitrate levels," she said.

Despite such evidence there has been little industry focus on nitrate levels in Australia. Sophie said one reason for this is that excessive nitrate levels in vegetables have been commonly associated with poor growing conditions involving low light levels. "Research in Europe has shown that nitrates are high in lettuce and spinach grown in the short days and low light levels of winter," she said. "The high light intensities enjoyed year round by growing areas in Australia have led to a widespread belief that the industry here is free of problems associated with excessive nitrate levels in vegetables."

*Sophie has found no evidence in her study to support the theory that poor storage of vegetables can exacerbate nitrite levels.*

However the results of the survey and experiments conducted as part of the leafy vegetables project suggest otherwise. Sophie conducted the survey over 12 months using leafy vegetables sourced from Queensland, Victoria and NSW. "We found high concentrations of nitrate in some samples and very high concentrations in some Asian vegetables such as choy sum, tat soi and buk choy," she said. "No leafy type had exclusively high levels and some lettuce types tended to have lower concentrations, especially head lettuce."

Sophie said a series of experiments revealed that the high nitrate levels found in some samples were most likely to due to the supply of nitrogen fertiliser fed to the plants, and not due to poor light conditions.

The survey also identified some vegetable samples with high nitrite levels, though Sophie said there were probably too few for this to be a concern for the industry. "Nitrite levels were not affected by fertiliser supply or light," she said. "However, nitrite may still be worth monitoring alongside nitrate in the future." Sophie has found no evidence in her study to support the theory that poor storage of vegetables can exacerbate nitrite levels.

Sophie said one clear message from the leafy vegetables study was that effective on-farm fertiliser management programs hold the key to controlling the nitrate concentration in vegetables in Australia.

She said with high nitrate levels almost impossible to detect without expensive testing, growers face a difficult task in getting fertiliser applications just right. With that in mind Sophie is investigating the possible use of an instrument, the ion selective electrode, which directly measures nitrate in solution, as a simple and relatively cheap way of measuring nitrate in fresh vegetables. "The application would allow larger vegetable industry conference 2007

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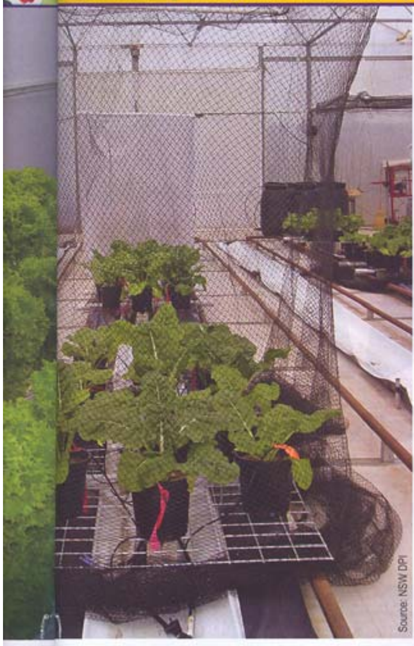


growers to regularly monitor nitrate in their crop and soil in order to fine-tune their fertiliser management programs," she said.

Sophie will also seek further funding to enable her to develop simple (and cheap) test protocols that growers could use to monitor plant nitrate levels in their crops. "Ultimately by using these techniques they will improve the quality of the crop and save in fertiliser costs," she said.

Sophie believes the industry cannot afford to brush aside the threat posed by excessive nitrate levels in vegetables. "As well as being important to the quality of the crop effective management of nitrate levels is becoming increasingly important in terms of vegetable exports," she said. "Europe has developed guidelines for nitrate concentration in some leafy vegetables and China is also developing guidelines. Ensuring that nitrate levels in Australian produced vegetables are not excessive will keep our options open for export markets."

**The bottom line:**

- High levels of nitrates in leafy vegetables are found to have adverse effects on vegetable quality.
- Nitrogen fertilisers may contribute to excessive nitrate levels.
- Export markets may be affected by excessive nitrate levels, and the guidelines for the Australian industry may be useful.

For more information: Visit
Visit www.ausveg.com.au/levy-payers
Project number: **VG04019**
Keywords: **Leafy vegetables, nitrate**

## 8.3.3 *Agriculture Today*

**AGRICULTURE TODAY** ■ Thursday, February 23, 2006 **11**

**Vegetables**

**Primary Industries**

# Project assesses nitrate in vegetables

IT IS desirable to prevent nitrate accumulation in vegetables, according to NSW DPI research horticulturist, Sophie Parks.

"From a human health perspective, vegetables with high levels of nitrate are of a poorer quality, for example containing reduced amounts of vitamin C, compared with vegetables having lower levels of nitrate," she said.

"In terms of vegetable production it is also cost effective and environmentally responsible for growers to avoid excessive use of fertilisers supplying nitrate to growing plants. This reduces the risk of nitrate accumulation in plants," she said.

Dr Parks said the aim of a Horticulture Australia project 'Nitrates in vegetables on the Australian market' which commenced in June last year was to assess the potential health benefits of Australian vegetables, in particular leafy vegetables, in terms of nitrate content.

"The effect of location, season, type of production system and post-harvest conditions on vegetable nitrate concentration is being investigated with the work expected to be completed by January 2008," she said.

Dr Parks said the research included a regular survey of leafy vegetables sourced from Queensland, Victoria and NSW and a series of experiments investigating nitrate fertiliser supply, shading level and post-harvest conditions on plant nitrate concentration.

"Through this project determination of nitrate levels in Australian vegetables will reflect the quality of Australian vegetables and will identify any issues that can be addressed in production or post-harvest to further increase quality," she said.

Dr Parks said nitrate could accumulate in vegetables for several reasons.

"Some plants are more likely than others to accumulate nitrate, such as the leafy vegetables lettuce and species from the Chenopodiaceae and Brassicaceae plant families, for example spinach and some Asian vegetables," she said.

"Growing conditions are also important. High fertiliser inputs and low light levels encourage nitrate accumulation in plants."

For example, Dr Parks said it has been shown in Europe that nitrates are high in lettuce and spinach grown in the short days and low light levels of winter.

"In Australia, we have high light intensities and so have the potential to produce high quality vegetables year-round using good agricultural practices," she said.

■ **Contact Sophie Parks, Gosford, (02) 4348 1914.**

NSW DPI's Sophie Parks samples nutrient solution for analysis.

## *8.3.4 Asian Foods Newsletter*

# ASIAN FOODS NEWSLETTER


### Asian Vegetables at the Australian Vegetables Industry Conference

Asian vegetables were an important part of the Vegetables Industry Conference this year. RIRDC conducted its annual researchers meeting at the Conference and also presented at the 'Innovation Showcase'. These presentations included an overview of RIRDC's Asian Foods Program, Angela Sparrow's research on Wasabi production, Len Tesoriero's research on Integrated Pest Management and Andrew Drummond's presentation on the demand for Asian vegetables. Of note, Dr Sophie Parks presented a paper on nitrate and nitrite in leafy vegetables and this was found to be of particular relevance to Asian vegetables including buk choy, pak choy, mizuna, and choy sum. A summary of Sophie's paper is included in this newsletter.

***Barry Lee***
***Editor***

### This Month at RIRDC

At the Australian Vegetables Industry Conference, RIRDC conducted its annual meeting for researchers of Asian vegetables. The major theme for the meeting was communication of research with the Asian vegetables industry. During the meeting, Mr David Chung of the NSW Chinese Vegetable Growers Association and Mr Andrew Drummond of Barden Fresh Produce presented their industry perspectives and RIRDC would like to acknowledge their contribution and support. We shall provide further updates from the Conference and meeting in future newsletters.

***John Oakeshott***
***RIRDC***

### Vegetable Industry Awards Winners

AUSVEG would like to acknowledge and congratulate the following as the winners of the 2007 AUSVEG Vegetable Industry Awards:

- NAB Agribusiness Grower of the Year: Dino Musolino, South Australia.
- Young Grower of the Year: Danny Trandos, Western Australia.
- Researcher of the Year: Dennis Phillips, Department of Agriculture, Western Australia.
- Brisbane Produce Market Innovative Marketing Award: John Said, Victoria.
- AUSVEG Chairman's Award: Ian Young, Tasmania.

***John Roach, Chief Executive Officer***
***AUSVEG***

### Nitrate and Nitrite in Australian Leafy Vegetables

**Introduction**

It is important to measure nitrate and nitrite in vegetables for reasons of food safety, product quality and production sustainability. Vegetables provide the majority of nitrate found in diets. Leafy vegetables tend to have a higher nitrate concentration than other vegetable types such as root or fruit vegetables. Further, leafy vegetables can accumulate nitrate beyond levels that are optimum for plant growth. Once eaten, nitrate can be reduced to nitrite in the body and can combine with amines to form carcinogenic nitrosamines that are associated with gastric cancer.

Poor quality can be a problem if vegetables have a high nitrate concentration. Increasing application rates of nitrogen-containing fertilisers can increase the incidence and severity of bacterial soft-rot in vegetables such as Chinese cabbage and broccoli. High nitrate concentrations in vegetables also reflect unnecessarily high fertiliser inputs during production. Excessive fertiliser use is the primary cause of nitrate accumulation in vegetables. This is inefficient, costly and can lead to environmental pollution such as the contamination of ground water.

A number of countries have set maximum allowable limits for nitrate and nitrite in some leafy vegetables. The European Community has developed limits on nitrate concentrations of fresh spinach and lettuce. For example, leafy lettuce grown outdoors in summer has a maximum allowable limit of 560 mg/kg nitrate-N. China's tentative tolerance level for nitrate-N in vegetables is 700 mg/kg fresh weight and for nitrite-N it is 1.2 mg/kg fresh weight.

Currently there are no limits set for Australia. Publication in the near future of the results of the 21st Australian Total Diet Study (Food Standards Australia & New Zealand) will indicate if the typical Australian diet exceeds the acceptable daily intake of nitrate and nitrite. The establishment of nitrate and nitrite limits in Australian vegetables may depend on this survey. Successful export of Australian vegetables in the future may also depend on there being evidence that the product does not exceed maximum limits in nitrate and nitrite established in the target country or region.

## Nitrate and Nitrite Levels

A recent survey conducted by the author on leafy vegetables available in the marketplace has shown that 27% of samples were above 700 mg nitrate-N /kg fresh weight and/or above 1.2 mg nitrite-N /kg fresh weight. The leafy vegetables tested were 7 varieties of lettuce (butter, green coral, red coral, cos, iceberg, green oak, red oak), endive, 4 leafy Asian vegetable varieties (buk choy, mizuna, pak choy, tat soi), baby leaf spinach and silverbeet. Samples were collected over a one year period and were sourced from the major vegetable growing areas in three states; Queensland, Victoria and New South Wales. Only 4% of samples in the survey had high levels of nitrite. The samples above the 700 mg nitrate-N /kg fresh weight appeared to be more frequent for mizuna, rocket, tat soi, silverbeet, and buk choy but were certainly not confined to these types.


**Effects of Differing Nitrogen Supply for Buk Choy**

## The Causes of Nitrate Accumulation

An over supply of nitrate fed to plants is the primary reason for nitrate accumulation in leafy vegetables. In contrast, nitrite levels are not affected by fertiliser supply and are detected infrequently in vegetables. Another important factor in nitrate accumulation is the vegetable species being cultivated.

A number of leafy vegetables are known nitrate-accumulators. For example, spinach (*Spinacea oleracecea* L.) and Swiss chard (*Beta vulgaris* L.) in the plant family Amaranthaceae are known to accumulate nitrate to very high concentrations. Other leafy vegetables classified as having a generally high concentration of nitrate include lettuce, rocket, celery, cress, radish, red beetroot, endive, fennel, parsley, leek, endive and Chinese cabbage. Evidently vegetables from the plant family Brassicaceae are also susceptible. In the survey the Brassicaceae vegetables were buk choy, pak choy, mizuna, and choy sum (*Brassica rapa* L.), tatsoi (*Brassica rosularis* L.) and rocket (*Eruca sativa* Mill.). The nitrate concentration of these was generally high, possibly reflecting a combination of choice of species and a high nitrogen fertiliser supply during cultivation.

## Hydroponics & Nitrate Accumulation

A number of studies have investigated nitrate accumulation in leafy vegetables grown using different cultivation systems. These studies show that nitrate accumulation can occur, in lettuce for example, in a hydroponic system. This is not surprising given that hydroponic nutrient solution typically delivers an over supply of nitrate to roots.

Alternative nutrient solutions, such as the partial replacement of nitrate with ammonium, can achieve a lower nitrate concentration in lettuce. However this can compromise growth and quality depending on the lettuce cultivar. Another strategy is to reduce the nitrate concentration in the hydroponic solution in the days before harvesting. One study has shown that the nitrate concentration of rocket can be reduced by 70% when total nitrogen in the solution is reduced by one quarter, five days before harvesting. Importantly, this did not affect yield.

## Conclusion

Australian produced leafy vegetables can have a high concentration of nitrate and occasionally of nitrite. The nitrate concentration in leafy vegetables is largely controlled by the supply of nitrate fed to the plant. Vegetable producers will be able to avoid nitrate accumulation in their produce by managing the nutrition of the crop. For hydroponic producers clear strategies to avoid nitrate accumulation will need to be developed for this type of production system.

***For further information: Dr Sophie Parks***
***NSW Department of Primary Industries*** sophie.parks@dpi.nsw.gov.au

| | |
|---|---|
| Editor: | Barry Lee, Connectica International |
| Email: | barrywlee@ozemail.com.au |
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| Email: | John.Oakeshott@rirdc.gov.au |
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| ISSN | 1329-9174 |
| Mailing list: | Please direct mailing list inquiries to<br>June Murphy, RIRDC Asian Foods Program |
| Email: | june.murphy@rirdc.gov.au |



The Asian Foods Program in funded from RIRDC core funds which are provided by Commonwealth, with contributions from Horticulture Australia who manages the vegetable levy.

Australian Government
Rural Industries Research and Development Corporation

## 8.4 Appendix 4 Further requests for funding

### *8.4.1 RIRDC preliminary research proposal 2006*

# Rural Industries Research and Development Corporation

| **Research Organisation: NSW Department of Primary Industries** |
|---|
| **Organisation type:**<br>☐ Australian Government ☑ State Government ☐ CSIRO ☐ Tertiary ☐ Consultant ☐ Private ☐ Overseas ☐ Other |

| **PRINCIPAL INVESTIGATOR/S' CONTACT:** | | |
|---|---|---|
| Title: Dr | First Name: Sophie | Surname: Parks |
| Mailing Address: Locked Bag 26<br>Gosford, NSW 2250 | | |
| Phone Number:<br>(02) 4348 1914 | Fax Number:<br>(02) 4348 1910 | Email:<br>sophie.parks@dpi.nsw.gov.au |
| **ADMINISTRATIVE CONTACT:** | | |
| Title: Mr | First Name: Graham | Surname: Denney |
| Mailing Address: Locked Bag 21<br>Orange, NSW 2800 | | |
| Phone Number:<br>(02) 6391 3219 | Fax Number:<br>(02) 6391 3336 | Email:<br>graham.denney@dpi.nsw.gov.au |

| **RIRDC Portfolio:** ☑ New Industries ☐ Established Industries ☐ National Rural Issues<br><br>**RIRDC Program: Asian foods** |
|---|

| **Project Title (10 words maximum):**<br>Production of high quality Asian vegetables |
|---|
| **KEY R&D ISSUE ADDRESSED:** (See relevant program five year plan) |
| Improve crop productivity, sustainability and produce quality |
| **R&D OBJECTIVE/S:** |
| The objective of this project is to reduce the nitrate concentration of leafy Asian vegetables at harvest to ensure food safety and quality of produce and environmental sustainability. This objective will be met by equipping the grower with techniques to manage the nitrate level in his/her Asian leafy vegetable crop. Experimentation, development of production protocols and extension activities are required. Work includes the determination of leaf nitrate levels associated with high quality produce, the development of a protocol for cheap and rapid field testing of nitrates in leaves, and a protocol for growing practices that minimise nitrate levels in produce, without compromising yields. In particular, as hydroponics allows for fine control of nutrient delivery, guidelines will be developed and published for growing Asian vegetables in this specific production system. Protocols for field testing of nitrates will be integrated into an existing soil and fertiliser training workshop, aimed at non-English speaking background (NESB) growers. The protocol for growing practices of these crops in hydroponic systems will be disseminated through workshops and publications. These will be delivered nationally. |
| **BACKGROUND AND EXPECTED OUTCOMES:** (Clearly identify economic, environmental and social benefits) |
| High levels of nitrate in vegetables are undesirable impacting on food safety, product quality and the environment. Dietary nitrate is largely obtained from vegetables. At excessive levels dietary nitrate is associated with gastric cancers and blue baby syndrome. A survey of leafy vegetables in the Australian marketplace being carried out by the principal investigator has identified that leafy Asian vegetables can have extremely high |

nitrate levels compared with other leafy types. For example, some samples contained levels three times the limit set for lettuce in the European Union. Additionally, high nitrate levels in leafy vegetables are associated with poor storage quality and postharvest rots, and reflect unnecessarily high fertiliser inputs in production. These issues can be addressed with new information on leafy Asian vegetable crop nutrition, relevant tools for farm management of nitrate (including guidelines for production in hydroponics), and grower training in developed protocols. This project is also designed to coordinate with the proposed project 'Adding value to Asian vegetables' (Dr Jenny Ekman, NSW DPI), that aims to evaluate high quality product and identify marketing options for Asian vegetables.

Economic benefits: For the grower immediate cost savings result from reduced fertiliser use. Additionally, better management of crop nitrate will improve produce quality, reducing crop losses and increasing profits, and the demand for products. For the industry, production of high quality and safe Asian vegetables will increase domestic and export opportunities.
Social benefits: Food safety of Asian leafy vegetables will be assured, availability of high quality vegetables will increase, and provision of crop production information will assist growers of traditional commodities to diversify maintaining vibrant rural communities.

Environmental benefits: Greater control of Asian vegetable crop nutrition will reduce fertiliser inputs, reducing the environmental impact of farm run off. Additionally, reduced nitrogen fertiliser inputs will reduce the risk of acid soil problems in field systems. The protocol for growing these crops in hydroponic systems will greatly assist the transition from conventional practices to the more environmentally sustainable hydroponic production system.

## PROPOSED R&D ADOPTION/COMMERCIAL PATHWAYS:

Information on managing nitrate in Asian vegetable production will be extended from this project to growers through an existing process to ensure adoption. For NESB market gardeners soil and fertiliser workshops are currently being conducted and developed by NSW DPI and funded by the NSW Department of Education and Training. Group training is delivered on-farm in modules and includes resources such as fact sheets and DVDs in a number of languages. In addition, practices for growing these crops in hydroponics will be disseminated to growers through workshops and the growing guidelines. Training will be delivered on a national scale.

## PROJECT DESIGN AND METHOD:

The project will consist of experimental work, the development of production protocols and extension activities. Experimental work will be conducted at the Gosford Horticultural Institute. Experiments conducted in hydroponic systems will determine critical values for leaf nitrate concentration and strategies to minimise nitrate levels in produce at harvest in a range of Asian leafy vegetables. On-farm methods for rapid testing of leaf nitrate will be developed for Asian vegetables using readily available and cheap tools such as hand blenders and commercially available colorimetric test strips for nitrate. Grower co-operators will assist in the testing and fine tuning of draft protocols before these are developed into training materials. Mr Jeremy Badgery-Parker, NSW DPI extension officer (Greenhouse and Hydroponics) will liaise with growers and manage the dissemination of information developed from the project to each state.

## RESEARCH CAPABILITY AND EXPERIENCE: (including previous RIRDC projects)

Dr Parks has proven experience in plant nutrition and vegetable research and in obtaining research grants. She was an author of the 'Guide to Nutritional Requirements of Some Proteaceae' produced from the RIRDC project UWS-1A (Nutrition of Proteaceae used in cutflower production, Researcher A. Haigh) and this work contributed towards her PhD project on Proteaceae nutrition (University of Western Sydney, 2001). Since, Dr Parks has successfully lead a NSW DPI Research and Development Initiatives project 'Nitrate accumulation in hydroponic lettuce' (2004) and the HAL project 'Pesticide residues in hydroponic lettuce systems' (2005). Dr Parks is currently leading 'Nitrates and nitrites in Australian leafy vegetables' (HAL). Other team members on this and the proposed project, Dr David Huett (NSW DPI) and Dr Lindsay Campbell (U Syd), have a strong and demonstrated experience in plant nutrition.

## APPLICATIONS TO OTHER SOURCES OF FUNDING: (for this & related proposals plus existing projects/applications)

Industry levies (AusVeg) will be sought for similar work in an application to HAL for commencement in 2007/2008. This would broaden the focus of the research to other leafy vegetables not considered in this project such as lettuce, silver beet and rocket. The current HAL project 'Nitrates and nitrites in Australian leafy vegetables' due for completion in late 2007 has determined, to date, that nitrate can occur at high levels in a number of leafy vegetable types, and that fertiliser inputs are a major factor in determining these levels.

## PRELIMINARY BUDGET (exclusive of GST):

| Funding Per Year | 2007–08 | 2008–09 | 2009–10 | 2010–11 | 2011–12 | TOTAL $ |
|---|---|---|---|---|---|---|
| RIRDC Contribution | 100447 | 111627 | 118918 | | | 330992 |
| Research Organisation – Cash | | | | | | |
| Industry Contribution – Cash | | | | | | |
| Other Funding - Cash | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Total Cash Funding** | 100447 | 111627 | 118918 | | | 330992 |
| Research Organisation – In-kind | 123254 | 123254 | 123254 | | | 369762 |
| Industry Contribution – In-kind | | | | | | |
| Other Funding – In-kind | | | | | | |
| **Total In-kind Funding** | 123254 | 123254 | 123254 | | | 369762 |
| **TOTAL OF ALL FUNDING SOURCES** (CASH & INKIND) | **223701** | **234881** | **242172** | | | **700754** |

**Commonwealth Government (DITR) Time Box Initiative:**

| | | |
|---|---|---|
| If the Research Organisation has less than 20 employees please provide an estimate of the time taken to complete this research proposal: | Hrs: | Mins: |

### *8.4.2 General concept development proposal 2007*

Sent to Horticulture Australia Ltd and RIRDC

**Improving the quality and safety of leafy Asian vegetables through management of crop nutrition**

Project proposal for commencement in 2008

Introduction

The project *Nitrate and nitrite in Australian vegetables* (HAL VG04019) has identified that approximately half of the leafy Asian vegetables purchased in the market place have an undesirably high nitrate concentration. This is likely to be due to the naturally high uptake of nitrate by Brassica vegetables, to which most Asian leafy vegetables belong, combined with excessive use of fertilisers in the production of these vegetables. High nitrate in these vegetables is undesirable from a food safety perspective and it reduces their postharvest quality.

Good fertiliser management of leafy Asian vegetable crops would avoid this problem and increase profit margins. Currently, growers do not have the means to achieve good fertiliser management in Asian vegetables. There is little information about the nutritional requirements of these vegetables and therefore there are no standard guidelines available. To develop guidelines, the nutritional requirements of leafy Asian vegetables need to be determined experimentally. This information can be used to develop plant nitrate test standards and protocols for use by commercial laboratories, agronomists and growers. The availability of plant analysis tools for Asian vegetables, in combination with existing soil testing tools provides the best method of managing the nutrition of these crops.

The aim of this proposed project is to determine the nitrate requirements of Asian leafy vegetables so that routine plant analysis tools can be developed for these specific crops. An important part of the project would be the extension of the information developed to commercial plant analysis laboratories, agronomists and growers to facilitate the improvement of fertiliser management in Asian leafy vegetable production.

Activities include:

- Experimentally determining optimal nutrition standards (critical nitrate concentrations) for Asian vegetables
- Developing protocols for commercial laboratory nutrient analysis of Asian vegetables
- Developing protocols for growers on the use of cheap nitrate test strips for monitoring Asian vegetable crop nitrate status in the field
- Training of agronomists in Asian vegetable nutrition standards
- Training of growers in the nutrition management of Asian vegetable crops

Funding
An opportunity has been identified for this project to combine resources with an ACIAR project (Enhancing the role of women in the safe produce, promotion and utilisation of indigenous vegetables) and would represent a significant cost saving of approximately $115,000 to vegetable levy payers. The approximate cost to vegetable levy payers would be $215,000 over 3 years.

### *8.4.3 Ausveg expression of interest 2007*

**VEGETABLE R&D PROJECTS 2008/09**
**Expression of Interest (EOI)**

**Application Process**
R&D Service Providers are invited to submit an EOI based on the 2008/09 R&D priorities identified by the Vegetable Industry Advisory Committee (IAC) and its advisory groups. The submission of an EOI is voluntary and will provide the opportunity for the Vegetable IAC to give feedback on outline project proposals.

The submission of an EOI is a process independent to the annual HAL Industry Call, but may assist service providers shape their proposals when responding to HAL's Industry Call.

**Timing of Application**
The period for submitting an EOI is 30 July - 7 September 2007. Early submission of EOIs is encouraged. The vegetable IAC will provide feedback by 5 October 2007. HAL's Industry Call for 2008/09 is 7 October - 20 November 2007.

**Completing the EOI**

The Expression of Interest form is not to exceed these 2 pages. Applications must be submitted in electronic format, and sent to the following email address idm@ausveg.com.au.

## A: About You

| **Organisation(s) Conducting the Research** |
|---|
| NSW Department of Primary Industries (Sophie Parks)<br>Central Queensland University (David Midmore) |

| **Contact Details:** | | | |
|---|---|---|---|
| Name | Sophie Parks | Position | Research Horticulturist |
| Phone (w) | (02) 4348 1914 | Email | sophie.parks@dpi.nsw.gov.au |

## B: About the Project

**Project Title** (Maximum of fifteen words)

**Nutrition management to save costs, raise quality, and reduce water pollution in leafy vegetable production**

**VegVision 2020 Strategy being addressed** (eg 4.1 Refining industry data and information collection)

**Innovation and breakthrough technologies**

**Establishing an effective whole-of-supply chain R&D system**

**This EOI is in response to Industry Priority Reference No P9 3.5 on AUSVEG website**

**Environment: determine extent farming has a direct impact on the quality of water downstream etc**

**Description and Background of proposed research**

The project *Nitrate and nitrite in Australian vegetables* (HAL VG04019) has identified that 27% leafy vegetables, and 50% of Asian vegetables, have undesirably high nitrate levels due to excessive nitrogen (N) fertiliser use. Significant cost savings could be made through efficient management of fertilisers allowing for higher quality vegetables, whilst reducing the impact of nutrient pollution leaching from production areas to waterways. Both PIs have had practical research experience with N nutrition in vegetable species.

**Objective/s and Outcomes of Project**

This proposed project will determine the efficiency of N fertiliser use for leafy vegetable production in three systems (conventional field, hydroponic, organic). Research will be undertaken to improve upon the efficiency with which N is utilized, taking a more integrated management approach. Further, the nitrate requirements of Asian leafy vegetables will be determined so that routine plant analysis tools can be developed for these specific crops. An important part of the project will be the extension of the information developed to commercial plant analysis laboratories, agronomists and growers to facilitate the improvement of fertiliser management in Asian leafy vegetable production. This will not only have benefit for the consumer, it will also optimise input use, with possible consequent reduction in costs and in off-site pollution.

**Benefits to Vegetable industry**
**Higher quality leafy vegetables, increased profit margins for producers, ability to manage runoff pollution from production areas**

**Location of research work**
Gosford, NSW and Rockhampton, QLD

**Brief project design and methods**

- Determining the efficiency of N use (NUE) in the commercial production of a range of leafy vegetables in different production systems (conventional field, hydroponic, organic).
- Research new options to improve upon NUE in each production system
- Experimentally determining optimal nutrition standards (critical nitrate concentrations) for Asian vegetables in organic, conventional and hydroponic production systems across a range of environments.
- Developing protocols for commercial laboratory nutrient analysis of Asian vegetables
- Developing protocols for growers on the use of cheap nitrate test strips for monitoring Asian vegetable crop nitrate status in the field
- Training of agronomists in Asian vegetable nutrition standards
- Training of growers in the nutrition management of Asian vegetable crops

**How we will disseminate project outcomes/results:**
**Guidelines, publications and workshops**

**Indication of resources required:**
A. Project duration**- 3 years**
B. Total funds required (in the order of..) $200,000/yr for three years = total $600,000