FINAL REPORT

Horticulture Australia Project Number VG04019

Assessing nitrate and nitrite levels in Australian leafy vegetables

Organisation: NSW Department of Primary Industries

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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.

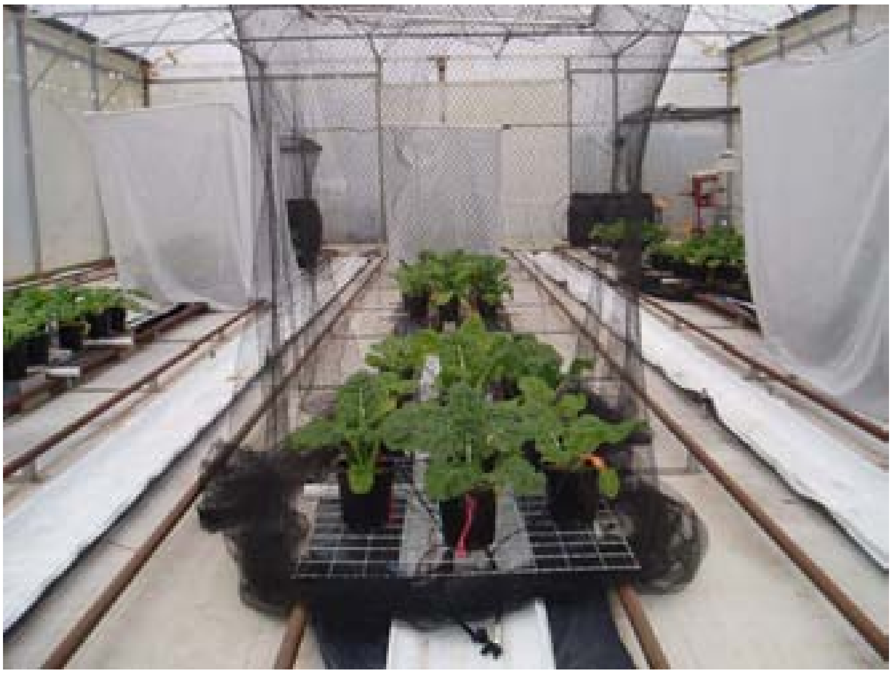

Figure 1. A photo showing the set up of the silverbeet experiments. Light levels were the ambient light conditions of the greenhouse, light under bird netting (front, centre) and light under thermal shade cloth.

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	

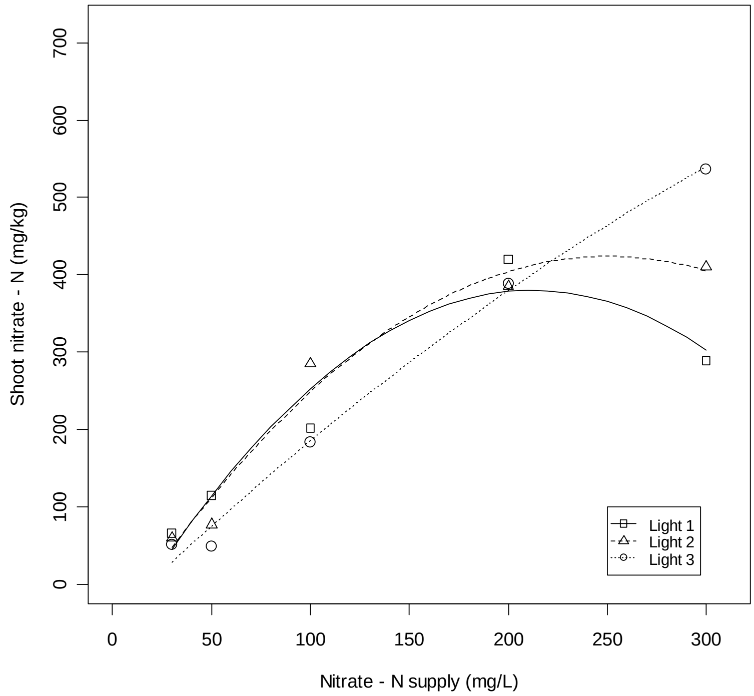

Figure 2. Silverbeet shoot nitrate-N response to nitrate-N supply in winter. At each nitrate-N supply the points are the average of 3 replicates.

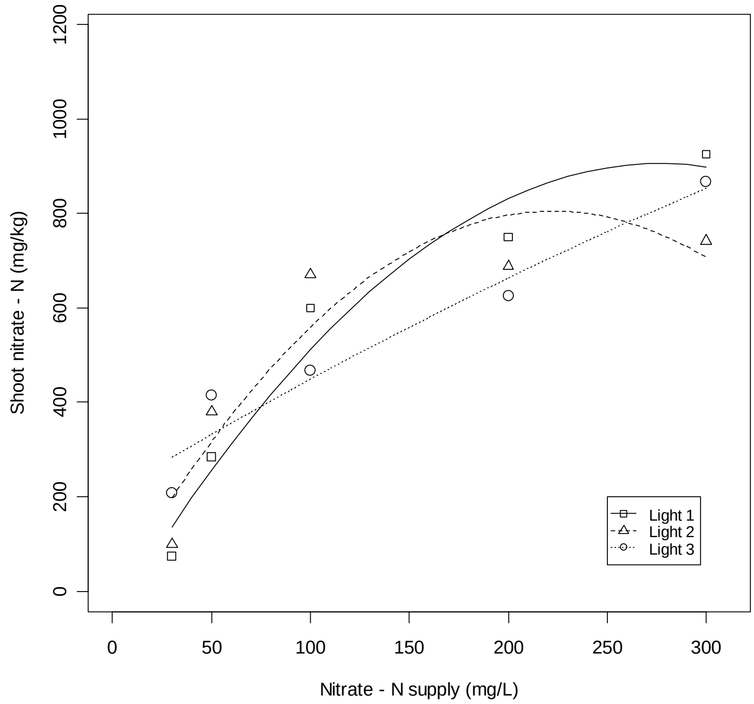

Figure 3. Silverbeet shoot nitrate-N response to nitrate supply in spring. At each nitrate-N supply the points are the average of 3 replicates.

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°22'S, 151°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).

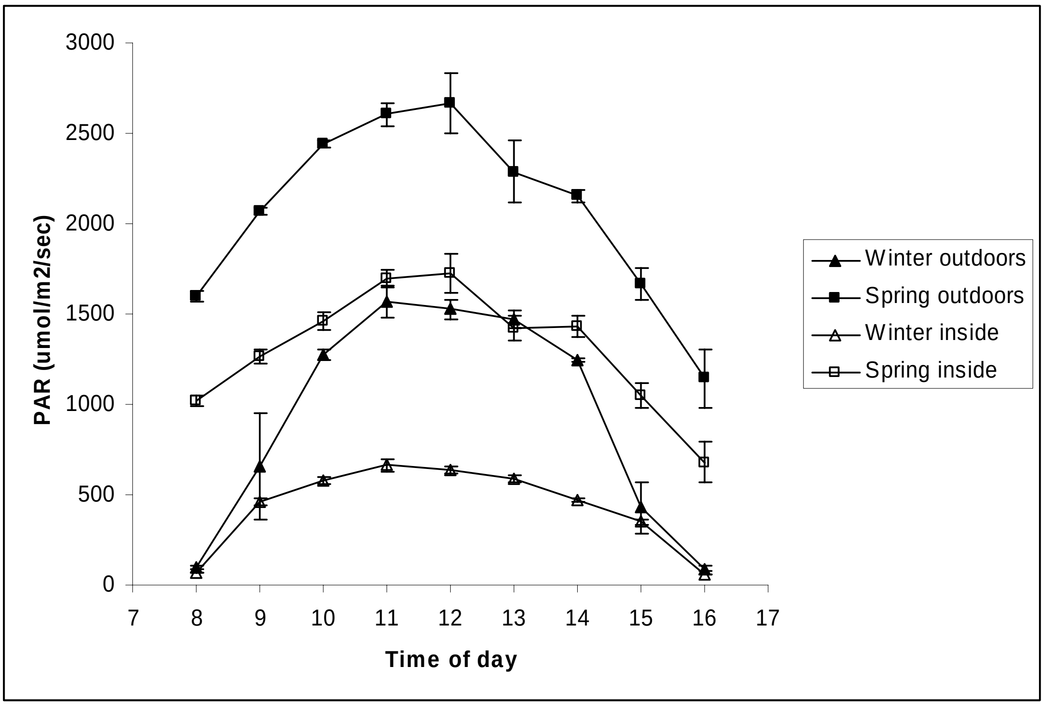

Figure 5. PAR conditions in the greenhouse and outdoors on clear days in winter and in spring (*n*=3, bars are standard error)

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.

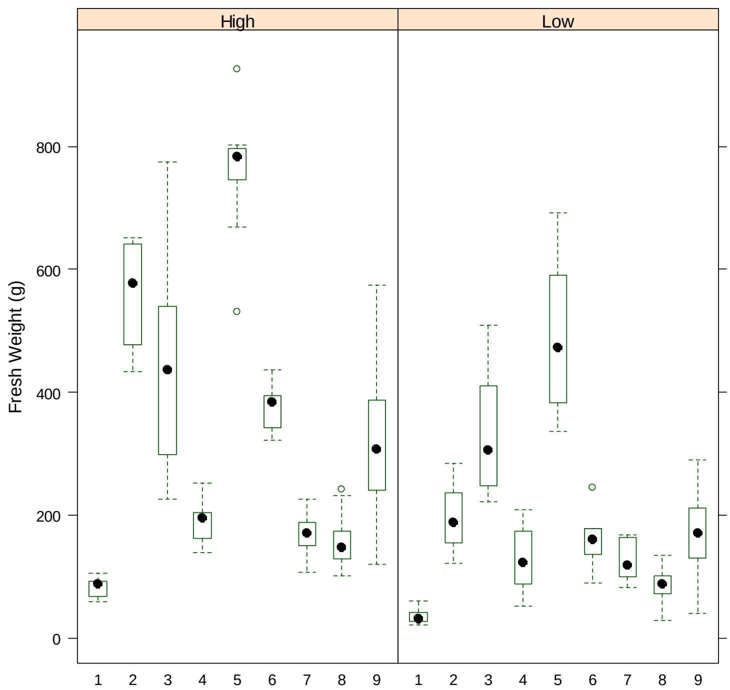

Figure 6. Winter fresh weights of leafy vegetables at high (left box) and low (right box) nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=pak choy, 7=red coral lettuce, 8=rocket, 9=silverbeet.

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).

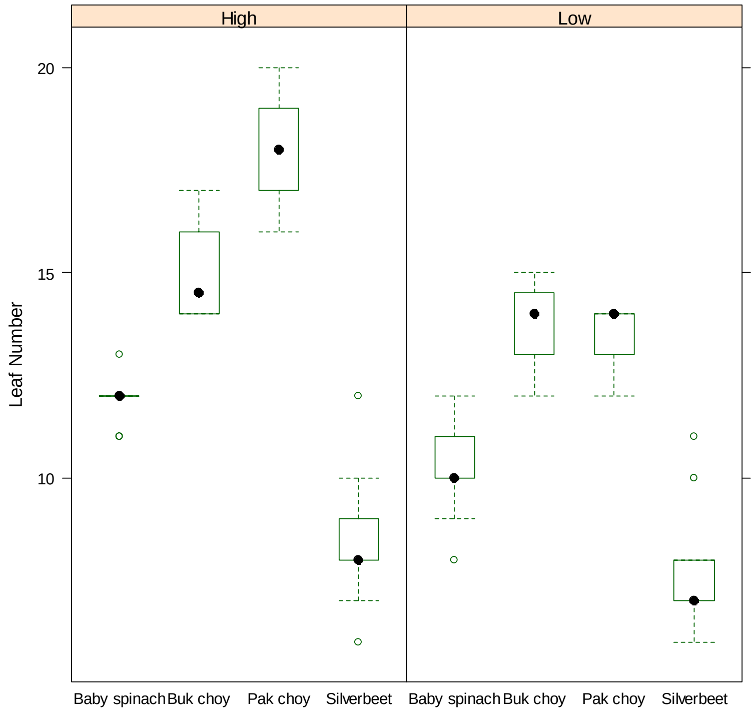

Figure 7. Winter leaf number of baby spinach, buk choy, pak choy and silverbeet at high and low nitrate supply.

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).

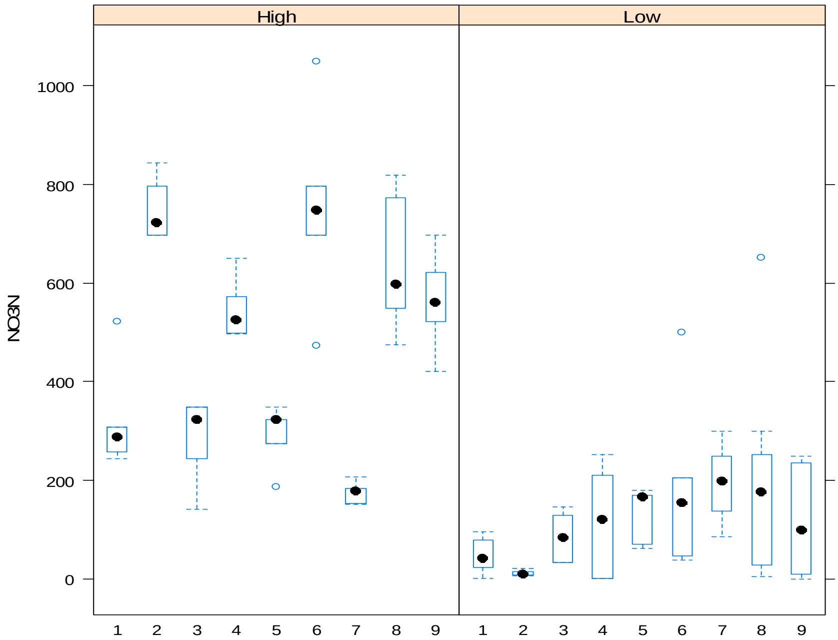

Figure 8. Winter nitrate-N concentrations (mg/kg) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=pak choy, 7=red coral lettuce, 8=rocket, 9=silverbeet.

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.

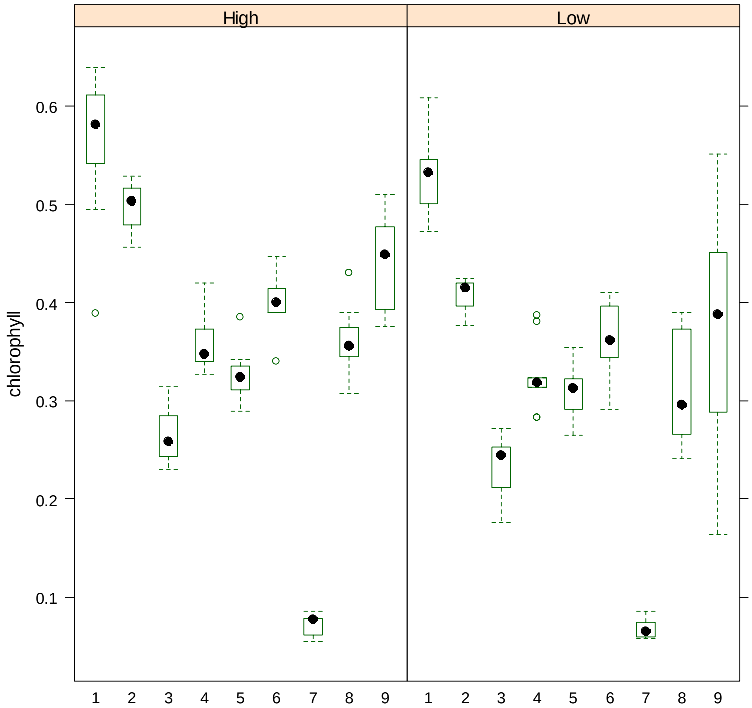

Figure 9. Winter chlorophyll *a+b* concentration (μg/mm^2) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=pak choy, 7=red coral lettuce, 8=rocket, 9=silverbeet.

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.

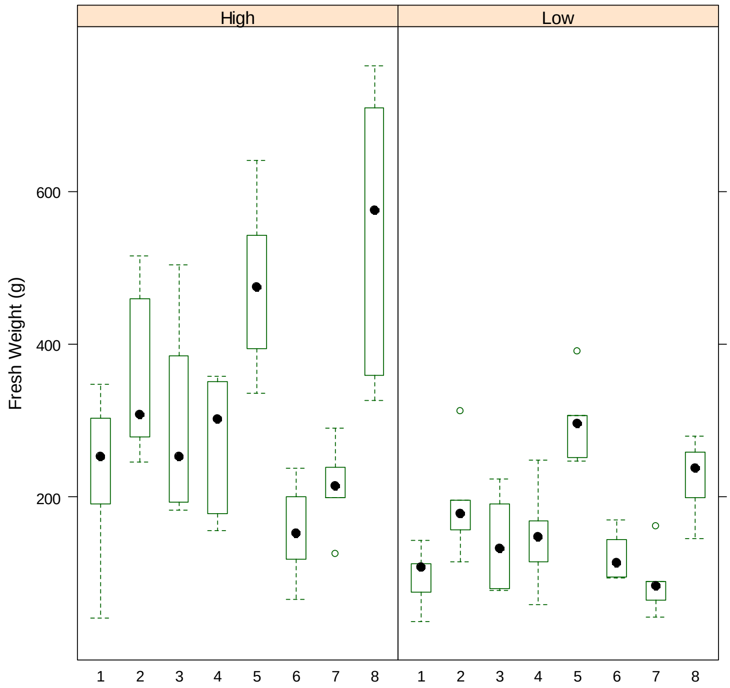

Figure 10. Spring fresh weights of leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=red coral lettuce, 7=rocket, 8=silverbeet.

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.

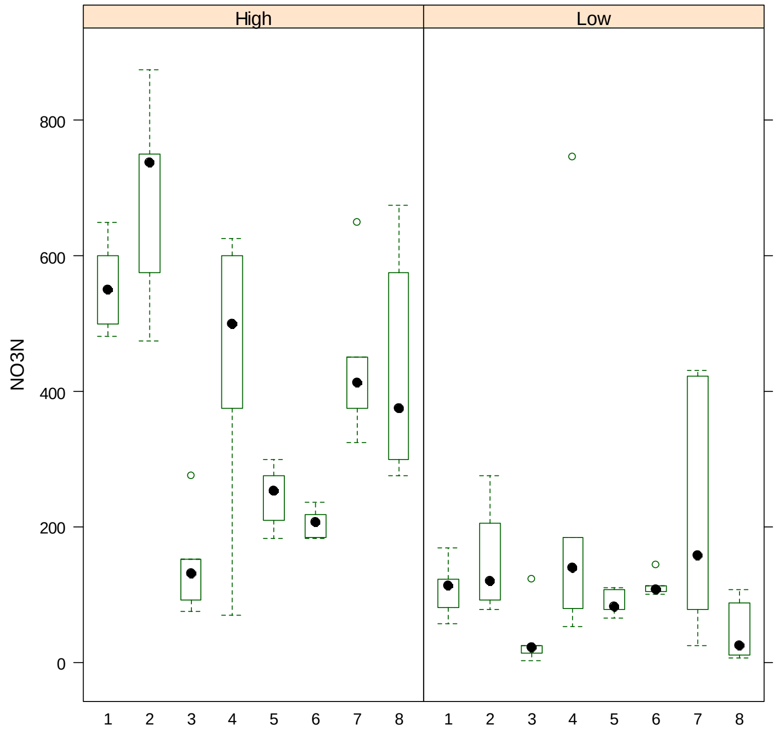

Figure 11. Spring nitrate-N concentrations (mg/kg) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=red coral lettuce, 7=rocket, 8=silverbeet.

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.

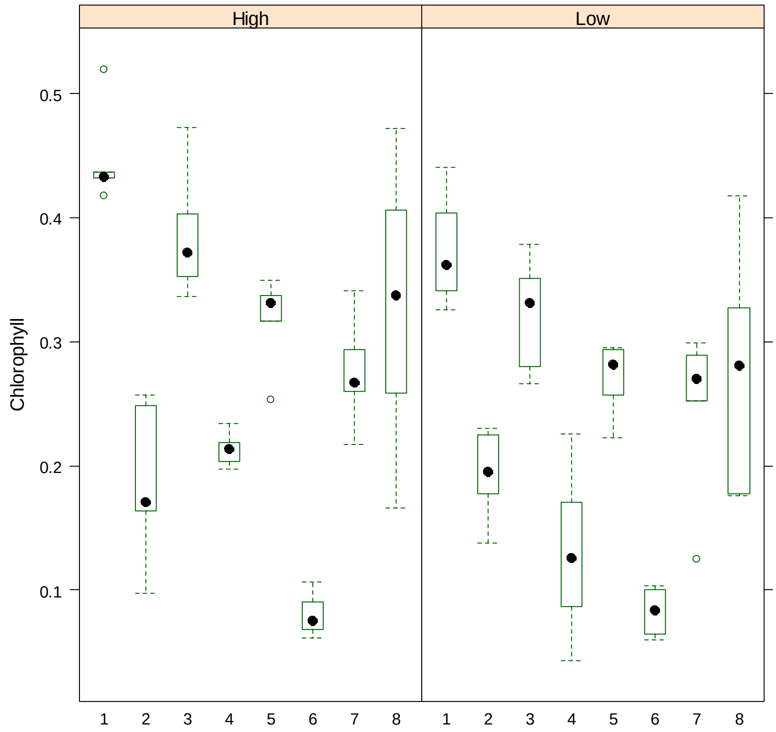

Figure 12. Spring chlorophyll *a+b* concentration (µg/mm^2) of fresh leafy vegetables at high and low nitrate supply. 1= baby spinach, 2=buk choy, 3=cos lettuce, 4=endive, 5=head lettuce, 6=red coral lettuce, 7=rocket, 8=silverbeet.

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).

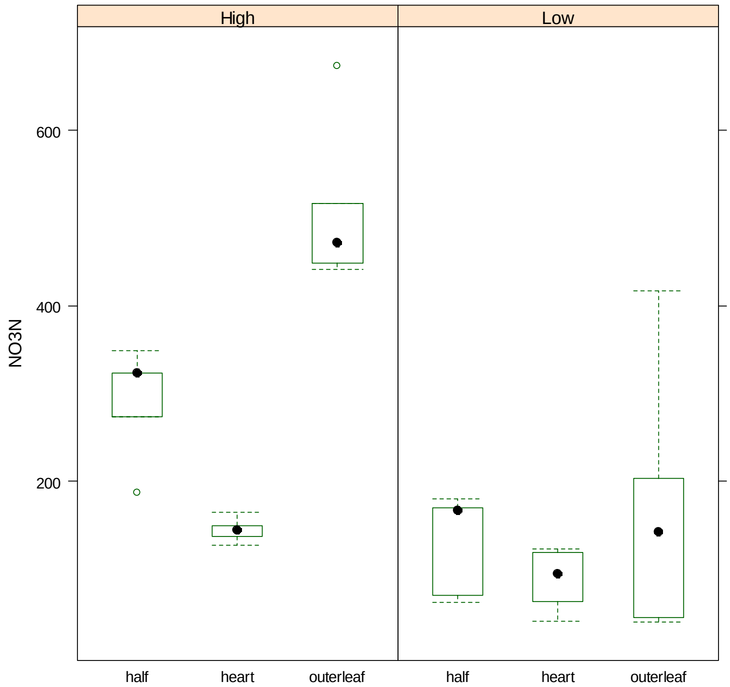

Figure 13. Distribution of nitrate-N (mg/kg) in fresh head lettuce at low and high nitrate supply for the winter experiment.

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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