Optimising crop management and postharvest handling for baby leaf salad vegetables

Dr Gordon Rogers Applied Horticultural Research P/L

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Gordon Rogers et al

Applied Horticultural Research Pty Ltd

Horticulture Australia Project Number: VG05068

Project Leader

Dr. Gordon S. Rogers Applied Horticultural Research Po Box 3114 BUNDEENA NSW 2230

Key Personnel

Gordon Rogers – AHR Michael Titley – AHR Brad Giggins – AHR Bartley Bauers – AHR Jenny Jobling – AHR Tom McAuliffe – OneHarvest James Le Budd – OneHarvest

Statement of Purpose:

This project was a collaboration between OneHarvest, a commercial baby leaf manufacturer and Applied Horticultural Research. The research aimed to determine the optimum pre and post harvest management of baby leaf spinach, lettuce and rocket to ensure maximum shelf life and quality for the consumer.

Funding

This project is jointly funded by OneHarvest and Horticulture Australia Limited.

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Media Summary

The main industry partner in the project was OneHarvest, a major fresh food company and supplier of fresh cut baby leaf salads. Work was done with major growers and Applied Horticultural Research was the research provider. Funding was provided by Horticulture Australia and OneHarvest. The focus was on improving the quality and shelf life of processed baby leaf salads for Australian consumers.

Baby leaf salad crops such as lettuce and spinach are harvested and marketed in an immature stage of development compared to other vegetable crops. This means growing and handling practices which were developed for the mature selections of these crops are no longer valid. The baby leaf category also includes some new salad lines such as rocket, tatsoi and mizuna.

The field work was carried out across the main East coast growing regions of Australia in east Gippsland, Vic, Camden, NSW and SE Qld, with laboratory work conducted at the AHR facilities at the University of Sydney. The results were collected over 3 seasons and covered most aspects of agronomic production and postharvest handling.

Key project outcomes were that spinach grown in the optimum temperature range produced the best quality product. This can be achieved by managing planting to match the season and by using varieties that were identified as slower growing. A growing degree day (GDD) model was developed to assist farmers schedule their crops for the optimum conditions and also manage a regular harvest schedule.

Another key result was that agronomic management affects the shelf life of the product. For example; high nitrogen in the field reduced the shelf life, high density plantings increase yield but reduced shelf life and the new multi leaf varieties had the highest yield and longer shelf life.

Postharvest handling was also essential. The product must be vacuum cooled to 2°C within half an hour of harvest for maximum shelf life.

Future R&D should focus on the role of baby leaf salad vegetables as a valuable source of antioxidants, in particular agronomic factors affecting expression and postharvest factors affecting stability of these beneficial components.

Technical Summary

Baby leaf salads are an excellent convenience food. In Australia these products are marketed as either packaged fresh cut, minimally processed or fresh product and the baby leaf category is growing rapidly.

The term baby leaf describes a wide range of leafy vegetables harvested when only a few weeks old. However, this project focussed on the main components commercially blended, packaged and sold in major supermarkets and included lettuce (*Lactuca spp.*), spinach (*Spinacia oleracea*), wild rocket (*Diplotaxis tenuifolia*) and cultivated rocket (*Eruca sativa*),

Baby leaf salad crops such as lettuce and spinach are harvested and marketed in an immature stage of development compared to other vegetable crops. This means growing and handling practices which were developed for the mature selections of these crops are no longer valid. The baby leaf category also includes some new salad lines such as rocket, tatsoi and mizuna.

The main industry partner was OneHarvest, a major fresh food company and supplier of fresh cut baby leaf salads. Some of the major growers were also collaborators, and Applied Horticultural Research was the research provider. Funding was provided by Horticulture Australia and OneHarvest.

A key aim of the project was to attempt to understand the regional and climatic effects on productivity and "quality". Quality is an objective term. In this work a treatment "failed" (storage at 2°C) in terms of quality when 50% of the individual leaves in a replicate were rejected due to wilting, discoloration or physical damage. We agreed that consumers have a much lower tolerance to damaged leaves and the broader experimental tolerance was used to allow the treatment affects to be identified. It must be noted that even small incremental improvements in quality would have a commercial benefit.

The field work was carried out across the main East coast growing regions of Australia in east Gippsland, Vic, Camden, NSW and SE Qld, with laboratory work conducted at the AHR facilities at the University of Sydney. The results were collected over 3 seasons (2005 to 2008) and covered most aspects of agronomic production and postharvest handling.

The key project outcomes were:

- A crop life of at least 30 days, from seeding to harvest produced higher quality. This was achieved by using slower growing varieties under warmer conditions or by only growing other varieties when the season was cool enough for varieties to attain the 30 day minimum growth period.
- The growth rate of spinach was temperature dependant. The best quality was achieved when the average daily maximum temperature did not exceed 23° C and the average night temperature did not exceed 8-10°C.

- A growing degree day (GDD) model was developed with a base temperature of 0°C and high temperature cut off of 27°C. The requirement from seeding to harvest for slow, medium and fast growth rate spinach varieties was 443, 420 and 404 GDD respectively.
- In lettuce, nitrogen had a significant effect on shelf life. Concentrations of greater that 4% in the leaves at harvest resulted in a shorter shelf life.
- Rapid, vacuum cooling of the product after harvest maximised shelf life. Cooling within half an hour of harvest was best, any delay shortened the shelf life.
- The stability of Vitamin C in the harvested product was dependent on storage temperature. When held at 0°C, spinach retained 90% Vitamin C content for 7 days whereas at 4°–7°C, about 50% of the Vitamin C was lost.
- For European wild rocket, alternating higher day (24°C) and cooler night (<10°C) temperatures partially overcame low temperature inhibition of germination. Cultivated rocket germination was not sensitive to low soil temperature and germinated down to 5°C.
- The highest yields of spinach were obtained at a density of 1000 plants/m² but this reduced average leaf weight to 1g and reduced shelf life (stored at 2°C) from 23 days at 300 plants/m² to 16 days at 900 plants/m².
- Transplanted multi-leaf type lettuce were compared with more traditional; single leaf direct seeded lettuce types and found to yield better and displayed good quality attributes.
- Finally the agronomic modelling data was used to select a suitable new growing area. This resulted in a commercial pilot-to-prove farm being set up at Mt Gambier in South Australia and was shown to successfully produce market quality baby leaf products including spinach.

Future R&D should focus on the role of baby leaf salad vegetables as a valuable source of antioxidants, in particular agronomic factors affecting expression and postharvest factors affecting stability of these beneficial components.

Executive Summary

This project was a collaboration between OneHarvest, a commercial baby leaf manufacturer and Applied Horticultural Research. The research was carried out over a three year period between 2006 and 2008. The field research was done on commercial baby leaf farms that supplied product to Oneharvest. The trial sites were spread across the eastern seaboard of Australia and included Boisdale (Maffra), Victoria, Camden, New South Wales, Gympie, Queensland, Warwick, Queensland and Stanthorpe, Queensland.

The overall aim of the project was to determine the optimum pre and post harvest management of baby leaf spinach, lettuce and rocket to ensure maximum shelf life and quality for the consumer.

The most significant finding of the project was the effect of growth rate on quality. The results showed that for spinach to attain maximum quality, it needs to be grown for at least 30 days from seeding to harvest (Chapter 3). This 30-32 day minimum growth can be achieved by either selecting slow-growing varieties during warmer periods, or by growing the crop in a climate cool enough to give at least 30 days from seeding to harvest.

The effect of temperature on spinach growth rate was found to be a combination of maximum day temperature and minimum night temperature. High day temperatures can be offset to some extent by cooler nights, resulting in slower overall growth. The most rapid growth occurs when there is a combination of high day and high night temperatures (Chapter 3 and 16).

The crop growth rate of spinach for any given area and time of year can be predicted by using the crop growth model developed as part of the project (Chapter 8) utilising historical average temperature data. This model is based on accumulated growing degree days or heat units over the growing season with a base temperature of 0°C. The data shows that good quality spinach is grown in areas and seasons where the accumulated heat units are between 400 and 500 from emergence to harvest (Chapter 8).

Other agronomic factors such as variety evaluation (Chapter 13), germination temperature tolerance (Chapter 9), plant nutrition in particular nitrogen application (Chapter 6), spacing (Chapter 11) and weed control (Chapter 12) were all investigated. The overall results from these studies were that for maximum quality it is important to chose the right variety for the right time, don't over apply nitrogen or plant at a too high a density and be mindful of the herbicide you use as some are phytotoxic. Any agronomic factor that promoted rapid growth such as high density or high nitrogen resulted in "weaker" plants that did not have maximum shelf life. The overall message is that slow growth ensures maximum shelf life until more robust baby leaf cultivars become available.

The postharvest research carried out in this project validated the principles of best practice for leafy vegetables. The overall postharvest principle is that pre-harvest factors determine potential shelf life, while post-harvest handling determines to what extent this potential shelf life is realised.

For maintaining postharvest shelf life of baby leaf products harvested product must be vacuum must to 0°C within half an hour of harvest to achieve the longest shelf life and the longer vacuum cooling is delayed, the shorter the shelf life of the product. Storage temperature is also critical with storage at 0°C maintaining both visual and nutritional quality (Vitamin C) for a longer period than 4°C and 7°C. Unfortunately this is not commercial practice with products often stored between 4 and 7°C. Another principle that was validated was that it is important to pack dry product as free moisture encourages bacterial breakdown in the package.

Finally the agronomic and postharvest bench marks developed in the project were used to search for, select and establish a successful commercial pilot-to-prove farm at Mt Gambier in South Australia.

This project was conducted over three years and so further work is required to validate some of the results reported. This is particularly true for the data that is based on temperature and seasonal climate. Many of the results reported are novel and have not been previously reported in the scientific literature.

It is therefore recommended that more work is done to;

- 1. Validate the growing degree day model developed for spinach
- 2. Develop Growing Degree Day models for other baby leaf species
- 3. Validate the relationship between days to harvest and shelf life for baby leaf spinach and this model could be extended to other baby leaf species.
- 4. Investigate the level of nutritional bioactive compounds in baby leaf salad products and their stability during storage.

Some of the results from this project could be published in scientific journals such as the germination trials, development of the growing degree model and the relationship between preharvest factors on postharvest storage. An agronomic manual is also being prepared which will compile the key results in a format that is useful for growers.

1 Introduction

The overall aim of the project was to determine the agronomic factors that affected the shelf life of baby leaf spinach, lettuce and rocket.

Several factors or hypotheses were investigated and the results are summarised in the sections to follow. The first results section, Section 3 is an overview of the postharvest results from a series of trials. This Section has been compiled in an effort to give the reader an appreciation of the interacting agronomic factors that influence the postharvest quality of baby leaf spinach. An appreciation of the complexity of optimising spinach quality gives the reader an appreciation for the incremental agronomic changes that have to be made to managing baby leaf production in order to optimise yield and quality. As for many crops, the baby leaf production system must be managed to suit the location, species and growing season.

Other parts of the project focused on factors that affected crop yield and management efficiency. As a result the overall recommendations from this project relate to both consumer quality as well as yield and production efficiency for the grower.

The overall hypothesis of the project was that;

"Baby leaf spinach and rocket grown well, which includes an appropriate variety, grown without stress and with adequate nutrition will have the longest shelf life if the correct postharvest handling is used".

Researchers have proposed that a higher initial quality may help to extend the shelf life of fresh cut products and this is the concept identified in our overall hypothesis (Nicola *et al.*, 2006). This overall hypothesis may seem like common sense but it raises many questions for the relatively young baby leaf industry in Australia. This is particularly so in light of the fact that the industry produces product over a range of climatic zones across the country. Some of the key issues that this project aimed to address included identifying the climatic conditions that produced good quality baby leaf spinach and rocket; what varieties were most appropriate in what season; what was the optimum nutrition, spacing, irrigation and crop management schedule and what were the optimum cooling, packaging and storage conditions for baby leaf spinach and rocket.

From a physiological perspective this project addresses many aspects of plant growth and development. More specifically, research has shown that many preharvest factors affect the postharvest quality and shelf life of fresh produce (Lee and Kader, 2000). Reviews have reported the differences in the quality of baby leaf salad species resulting from cultivar differences (Johnson et al., 1989); harvest maturity (Bergquist, 2006, Lee and Kader, 2000) and climatic conditions (Lee and Kader (2000). These reviews all illustrate the significant impact that pre-harvest agronomic factors have on the physiology and quality of baby leaf products. This project aimed to quantify some of those effects for baby leaf spinach and rocket grown in Australia.

Seasonal Temperature affects plant growth

One of the main issues for the Australian baby spinach supply chain is year round supply. In order to achieve year round supply spinach is grown in different regions at different times of the year. The success of this scenario depends on a crop species or cultivars adaptability as well as yield stability across a range of geographical regions. Adaptability and yield stability are both influenced by seasonal climate. Adaptability refers to good performance over a wide geographic range under conditions of variable climatic and environmental influences (Stoskopf 1981) and stability of yield is defined as the ability of a genotype to avoid substantial fluctuations in yield over a range of environmental conditions (Heinrich *et al.* 1983).

In both case adaptability and yield stability relate to the relationship between photosynthesis and respiration of the plant. Within limits, the rate of photosynthesis and respiration both rise with increasing temperatures. As temperatures reach the upper growing limits for the crop, the rate of food used by respiration may exceed the rate at which food is manufactured by photosynthesis (Whiting *et al.* 2004) and at this point plant growth rates slow.

At very high temperatures cytological changes within the cell also occur and these include coagulation of the protoplasm, cytolysis, nuclear changes and altered mitosis, inhibition of protoplasmic streaming, increased protoplasmic viscosity, and loss of membrane semi permeability (Steponkus 1981). The temperature can therefore affect the plant on a cellular level disrupting normal metabolic functions, in turn causing stress to the plant. The physical and economic effect of this extreme exposure will result in a reduction of yield and hence profit.

For spinach the point at which optimum growth is obtained is between 15-18°C, therefore if temperatures exceed this range then the growth rate will decline but not cease unless temperature falls below the base temperature 2.2°C or exceeds the upper most limit of growth which is 36°C for spinach (Heidorn 2003). One of the aims of this project was to identify which varieties of spinach and rocket were best suited to different growing conditions in an effort to ensure yield stability in a region for as long as possible.

Crop Scheduling

Another important seasonal impact is the days from seedling emergence until harvest; this is commonly referred to as crop scheduling. For the spinach industry to maintain a continual supply to consumers there must be a reliable harvest volume. Therefore another aim of this project was to establish a crop scheduling model for baby leaf spinach.

Research has developed crop scheduling models for many field grown vegetables. These models are all based on accumulated heat units and some examples of crops where harvest models have been developed include peas, beans and sweet corn (Kafz,1952). The success of these heat unit models for these vegetables is the fact that optimum harvest can be measured objectively, using the maturometer index for peas and the percentage moisture of sweet corn kernels for freezing or canning corn. The difficulty for predicting the optimum harvest date for other vegetables is that there is not an objective measure of quality.

Work has been done to develop maturity indices for other field grown fresh market vegetables; asparagus, broccoli, brussels sprouts, cabbage, cauliflower and lettuce over the last two decades but there has been varying degrees of commercial adoption (Everaarts, 1999). Work done by Tan et al (2000) with export fresh market broccoli on the Eastern Darling Downs found the best prediction of harvest maturity was the time from emergence to 50% harvest using 0°C as the base temperature and 20°C as the optimum temperature.

Heat unit models are simplest if a clear harvest point can be determined. For baby leaf salad crops the criteria is usually leaf length (100 mm). Machado et al. (2004) states that the simplest method for predicting harvest date is based on the daily maximum temperature alone and this is as accurate as all the more complex methods in predicting the harvest date. Growing degree units are defined as the number of temperature degrees above a certain threshold base temperature, at which below no plant growth will result. The base temperature for spinach has already been established to be 2.2°C (Heidorn 2003). The result of which is that any temperature experienced above this base temperature will contribute to the growth of the plant and therefore bring the plant closer to harvest. One of the aims of this project was to develop a heat unit accumulation model for baby leaf spinach which was applicable across a range of growing regions in Australia. Such a model would help growers schedule their crops to ensure a reliable supply of product for processing.

Plant Varieties

Selecting the best variety for the growing conditions is also a very important decision for all growers. The baby leaf industry is relatively new as has been mentioned earlier and this means that there are few varieties that have been bred specifically for baby leaf production. For baby leaf spinach in particular traditional bunching spinach varieties have been planted at high densities and harvested at an earlier stage. There is however, one variety of spinach which has been bred for baby leaf production and the varieties commercial name is Crocodile (Rijk Zwaan Seeds). For lettuce more varieties are available for baby leaf production and these include many of the specialty head lettuce varieties such as red and green coral.

Spinach varieties have also been classified into one of three seasonal types; warm, transitional or cool, based on yield and days to harvest (Rijk Zwaan Seeds pers. Comm.). In this work a range of varieties of spinach, rocket and lettuce were trialled in different seasonal periods to determine which varieties should be planted when for optimum yield and quality.

Seasonal temperature affects leaf structure

Seasonal conditions have been shown to affect crop yield but what are the physiological factors that weather regulates that impact on the postharvest quality of baby leaf spinach and rocket? Research has shown that the structure of leaves changes in response to day length and temperature. For example research by van

Oorschot, (1960) focused on day length. This research found that spinach plants grown under long-day conditions (LD, 14 hours) had an increased total leaf area compared to plants grown with short days (SD, 7 hours). It was proposed that a long day-length promoted meristematic activity at the shoot apex, whilst a short day-length reduced the meristematic activity, leading to progressively smaller leaves with fewer cells and less dry weight (Adams & Langton, 2005). A similar response was reported in sugar beet, (Milford & Lenton, 1976) where a LD treatment reduced the leaf lamina thickness by 20% while increasing the leaf area by 92%.

Often a reduction in leaf thickness is seen as an increase in specific leaf area (SLA) (Milford & Lenton, 1976). The findings of Milford & Lenton (1976) suggest that long day grown plants increased leaf area, but only by reducing leaf thickness. The shelf life of baby leaf spinach has been reported to be correlated with both increased leaf thickness and leaf area (Zhang *et al.*, 2007). But what are the impacts of these changes in leaf dimensions on postharvest shelf life and quality?

Growing temperature is also known to affect plant physiology and structure. For example Boese & Huner (1990) reported that leaves grown at 5°C had a leaf area that was three times and dry weights that were five times greater than spinach leaves grown at 16°C. Boese & Huner (1990) also found that plants grown at 5°C increased leaf thickness twofold over spinach plants grown at 16°C. The difference in leaf thickness was 567 μ m and 290 μ m, respectively.

This increase in leaf thickness was attributed to a 30-40% increase in the mean lengths of both the palisade and the spongy mesophyll cells of the leaf. In addition, the thicker leaves were found to have more layers of palisade cells. The lower temperature treatment had two to three layers compared to one or two layers for the higher temperature treatment.

Other researchers have investigated the impact of leaf structure on shelf life but a different way to those previously discussed. Research by Clarkson *et al* (2003) and Pontinen *et al* (1992) have shown that mechanical stress applied to the plant through a brush, passing over the leaf during growth, strengthens the leaves by giving smaller leaves of lower weight and smaller epidermal cell size and this in turn increased the shelf life of the baby leaf products. This work indicates that leaf shape and structure does play a role in determining the shelf life of leafy products.

One of the hypotheses we investigated was that thicker leaves would be more robust and more able to resist physical damage and dehydration during postharvest handling than thinner leaves and that the thicker leaves would in turn have a longer shelf life.

Nitrogen fertiliser levels affects yield and quality

Another important preharvest factor in terms of baby leaf quality is plant nutrition. Plant nutrients represent only a tiny fraction of plant biomass but fulfil multiple roles as either catalytic or structural components of plant cells (Atwell et al 1999). Nutrient acquisition by the plant is a demanding process in terms of energy and carbon. Carbohydrates produced from photosynthesis provide the energy for the active uptake of nitrogen and other nutrients, across concentration gradients (Epstein and Bloom 2005). If nutrients are limiting then growth of the plant is compromised as more energy is used to acquire nutrients and in turn limiting growth. Our hypothesis was that inappropriate management of nutrition compromises baby leaf yield and quality.

Our work focused particularly on nitrogen uptake as this is the major determinant of leaf growth and leaf area and in terms of crop yield (Bacon 1995). There was also anecdotal evidence to suggest that baby leaf crops grown with high levels of applied nitrogen have a shorter shelf life. This project aimed to do a preliminary investigation into the relationship between nitrogen application rate and shelf life to clarify this issue for growers.

Germination, planting method and planting density

For vegetable growers there is often an important decision to make about whether a crop is direct seeded or transplanted. For many easy to germinate crops growers find that direct seeding is a cost effective method of plant establishment. Baby leaf rocket and spinach are commonly planted this way. For direct seeded crops seed germination is a critical step and it is widely acknowledged that multiple variables are important during the germination of seeds. Such things as the availability of moisture, seed depth, air and soil temperature, and seed desiccation (Akanda et al., 1996; Blackshaw, 1990, 1992; Blackshaw and Brandt, 2002; Horak and Sweat, 1994; Horak and Wax, 1991; Ray et al., 2005) all affect the germination rate. The ideal germination temperature has not been established for some of the new baby leaf varieties. This project work aimed to establish the temperature range for the germination of several rocket species. This work would demonstrate the importance of understanding the requirements of a specific species in order to optimise plant establishment if direct seeding was used.

For other crops such a lettuce transplanting is often the method used to establish the crop. Transplanting is a relative new practice in the vegetable industry. It was introduced in the USA about 30 years ago but took off around the world about 10 years ago. Many crops are transplanted. Examples include celery, seedless watermelon, bell peppers and fresh-market tomatoes, cauliflower, cabbage and more commonly lettuce. The driver for using transplants is often the cost of hybrid seed. When seed is expensive the risk of loss is reduced if germination is managed under controlled conditions in a glasshouse. Transplanting also shortens the season and provides uniformity (Katz, 2002).

Transplanting has higher initial expenses associated with greenhouse growing and placing the plugs into the field, there are significant savings in seed cost, weeding costs, fewer irrigations, and more likelihood of uniform plant populations and even stands. The relative benefit of transplanting versus direct seeding needed to be established for some of the baby leaf salad lines in Australia.

Another important aspect in relation to crop establishment is the planting density. Marketable yield per unit area has been shown to increase at a curve linear rate for most crops (Duthie et al., 1999; Cushman et al, 2004). The decreases at the high density plantings are the result of intraspecific competition. For all crops there is an optimum plant density where yield and quality are maximized. Maximum yield does not mean maximum quality (Cushman et al., 2004) and the optimum density for many baby leaf species had not been established. It may be that the driver for growers is maximum yield as they are paid per kilogram weight but this may be at the expensive of quality and shelf life for the consumer. This work aimed to establish the relationship between planting density and quality for baby leaf spinach.

Harvest time

Another area where an incremental improvement in product quality might have been achieved was thought to be the time of day for harvest. Recent research had shown that harvesting baby leaf products in the afternoon extended the shelf life of these products by 2 to 6 days (Forney and Austin, 1988; Clarkson et al, 2005). The hypothesis for this was that there was a greater level of accumulated carbohydrate at the end of the day compared to the beginning of the day which would provide the product with more stored energy for metabolism during postharvest handling. The difficulty with this hypothesis was that there are interactions between temperature, respiration rate and stored carbohydrate. This project did a small trial to determine if harvesting later in the day would extend the shelf life of baby leaf spinach.

Postharvest handling

Postharvest handling is a key part of the supply chain for any product but for baby leaf salads it is a limitation. Currently, the shelf life is around 5-7 days, whereas supermarkets require at least 10 days shelf life (Soliva-Fortuny & Martin-Belloso, 2003; Watada *et al.*, 1996). Consumers expect and demand consistent 'quality' and supply. This means that the shelf life of baby leaf spinach and rocket needs to exceed the minimum required distribution time from producer to the consumer, while allowing a reasonable period for home storage and use (Shewfelt, 1986).

The short shelf life of baby leaf spinach is the result of the rapid deterioration of the leaf components following tissue damage caused during processing, washing and cutting, as well as from microbial growth (Martin-Diana *et al.*, 2007). The shelf life of baby leaf spinach is has also been shown to be directly related to the visual quality, where shelf life is a measure of how long produce can stay at or above a predetermined level of quality under specified storage conditions (Shewfelt, 1986; Bergquist *et al.*, 2006).

Clarifying the terminology "quality" and "shelf life" is important. These words are used subjectively for consumers and consumers have a varying sense of what is an acceptable quality and shelf life that is often based on past experience. For most consumers baby leaf salads are unacceptable when there are any visual signs of leaves with damage (Oneharvest pers. Comm.).

Most of the field work for this project had a postharvest shelf life assessment so that we could determine which agronomic management factors impacted on shelf life and quality. This project also aimed to demonstrate the benefits of vacuum cooling for the baby leaf industry a technology that has been adopted overseas but the cost benefit has not been demonstrated in Australia.

Another postharvest aspect that was investigated was the change in Vitamin C content of baby leaf spinach over time at different storage temperatures. Research has shown that storage temperature and cultivar all impact on the level of Vitamin C in baby leaf spinach (Favell 1998; Kidmose et al., 2001; Pandjatan et al., 2005). This project carried out a preliminary study looking at Vitamin C changes for the cultivars currently grown in Australia and how the level of Vitamin C changes with time in storage. This nutrient content was seen as another aspect of product quality especially when consumers are becoming more knowledgeable about the health benefits of eating fresh fruit and vegetables.

In summary, the literature showed, pre-harvest factors significantly affected spinach growth and leaf structure. It was therefore likely that these same factors would impact on the shelf life of the product. This project aimed to determine the relative importance of cultivar, seasonal growing conditions, agronomic management and postharvest handling on the shelf life of baby leaf spinach. In so doing, it was intended that the project would provide vital new information on the growing and handling of baby leaf lines including spinach, rocket and lettuce in Australian growing areas, helping to provide a sound scientific basis on which the industry could build and grow.

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2 Materials and Methods

In general, the field work was carried out by AHR staff, with assistance from OneHarvest staff. Shelf life and quality assessments were done in OneHarvest factories (Brisbane and Bairnsdale) under the supervision of Dr Jobling.

The broad agronomic methods used for establishing and conducting trials as well as sampling, and assessing treatments across all trials are outlined here. However, more specific experimental design and location specific details for each trial are provided in each section.

2.1 Varieties

For the spinach trials the following varieties were used unless otherwise described in the individual Chapters.

Spinach *Spinacia oleracea* L. is the baby leaf salad vegetable and Crocodile, Parrot and Roadrunner are the commercial variety names. The characteristics of each variety are described below. RZ refers to Rijk Zwaan the Dutch based international vegetable seed company from where the seeds were sourced.

PARROT RZ is a new early variety with round, dark green, glossy leaves. The plant very is erect and vigorous. Parrot RZ is suited for machine harvesting. The variety is best suited for late autumn, winter and early spring harvest in cooler areas and winter harvest in warmer areas. This variety is suitable for baby leaf production only.

CROCODILE RZ is a dark green variety which is slow growing and suits the warm season summer period. It is very resistant to leaf burn during hot summer periods.

ROADRUNNER RZ has dark green thick leaves and this variety is best for shoulder period harvesting in the spring and autumn. It has an upright growth habit Reference:'Lettuce and outdoor vegetable crops' Rijk Zwaan Australia seed catalogue 2008-09 season



Figure 2.1 Photographs of Parrot (left), Crocodile (centre) and Road Runner (right) baby leaf spinach varieties

Lettuce *Lactuca sativa* (milk sap). All lettuce cultivars such as Cos, Leaf, Butterhead and Iceberg are regarded as being variants of *Lactuca sativa*. Lettuce has been transformed from an erect plant with bitter leaves to various cultivars including ones with distinctive heads of chlorophyll deficient leaves.

The trials in this report used red and green coral lettuce as well as Salanova lettuce which are the basis of many mesculan salad mixes.







Figure 2.2 Photographs of green coral (left), red coral (centre) and salanova (right) lettuce types.

Rocket. Two baby leaf rocket species were used; *Diplotaxis tenuifolia* (wild rocket) and *Eruca sativa* (cultivated rocket).

Wild rocket (*Diplotaxis tenuifolia*) is a species of the mustard family and is native to Europe and western Asia. The foliage is aromatic when crushed and is now sold commercially as wild rocket but in many countries it is a wild plant or weed.

Cultivated rocket (*Eruca sativa*) It is a species of the Eruca family and is native to the Mediterranean region. It is an annual plant growing from 20 – 100 cm tall.





Figure 2.3 Photographs of wild rocket (left) and cultivated rocket (right).

2.2 Geographic regions of trials

The trials were done in several growing regions across Australia and the trials were done on collaborating growers farms. The geographic locations included.

- **1. Boisdale (Maffra), Victoria** 37° 58'South 146° 59'East and 27 metres, about 228km east of Melbourne in Victoria.
- Camden, New South Wales 34° 3' South 150° 41' East and 70 metres is near the Nepean River 62 km and is south-west of Sydney South East Queensland
- **3.** Gympie, Queensland 26° 11' South 152° 40' East and 67 metres, about 180 km north of Brisbane

- **4. Warwick, Queensland** 28° 12' South 152° 6' East and 475 metres, about 180 km south west Brisbane
- 5. Stanthorpe, Queensland 28° 39' South 151° 56' East and 792 metres and is 260 km south west of Brisbane

2.3 Optimum growing and storage requirements for baby leaf salads

Growing conditions

The optimum growing conditions for the baby leaf salad vegetables studied are summarised below. In many cases in the summer these crops cannot be grown under ideal conditions.

Spinach

3
aximum
/)

Notes:

- 1. Soil temperature above 30°C limits germination
- 2. The range 14 24°C is best for growth and maximum leaf quality
- 3. If night temperatures > 15° C this reduces yield through higher plant respiration.

European wild rocket

	Germination (°C)	Growth (°C)		
Min	10 **	5-8		
Optimum	20-30	16-24		
range				
Max	35	30-32		

Notes:

- 1. 10 °C minimum soil temperature for germination is a major limitation when direct seeding
- 2. In Italy germination in greenhouses is controlled at 20 22°C

Lettuce		
	Germination (°C)	Growth (°C)
Min	2.2	7
Optimum	18-21	12-21
range		
Max	27 **	24

Notes:

- 1. high temperature dormancy above 27°C is a limitation
- 2. germination at $0^{\circ}C = 49$ days, and at $5^{\circ}C = 15$ days (see optimum range)

** = limiting factors

Postharvest handling of baby leaf salad products

Baby leaf products have higher rates of respiration than mature products of the same species. Young plants are actively growing and so use more energy in the active growth processes of cell division and cell expansion. This means that harvested baby leaf products use up stored carbohydrate much faster than mature plants.

Young plants are also more susceptible to bruising and physical damage as the leaves are not fully developed and so are not as robust as mature plants.

These baby leaf characteristics mean that a lot of damage can be done during harvesting, postharvest handling and processing. As a result a lot of care must be taken of these products if the optimum shelf life is to be achieved.

Ideally baby leaf salads should be stored as close to 0° C as possible for maximum shelf life. They need to be packaged in permeable plastic bags to prevent desiccation and physical damage resulting from washing and packaging should be minimised. There are both "wash-me, eat-me" types and "washed" types. The "wash-me, eat-me" types have a longer shelf life due to a lower level of physical damage but there is a higher microbiological risk for these products. The commercial reality is that the shelf life of these products is often compromised as they are usually stored between 4 and 7°C.

Key Factors for Maintaining Baby Leaf Product Quality*

- 1. Use the best quality raw product. Agronomic practices including crop nutrition, variety and season all affect product quality and shelf life.
- 2. Cool raw product before transport to factory particularly if field heat is high and transport distances or delays are long. Vacuum cooling is best for leafy vegetables.
- 3. Keep the product as close to 0°C as commercially possible. Leafy salad vegetables will freeze if exposed to temperatures of -0.15°C. Check temperature fluctuations around the set point of cool rooms and trucks to be sure the product is never exposed to damaging temperatures.
- 4. Any break in the cool chain or exposure of the product to temperatures above $1 2^{\circ}$ C will significantly reduce the shelf life of the product.
- 5. Use strict sanitation procedures. Most processors have a zero tolerance to microbial contamination. This means irrigation water, sheds, bins and knives must always be clean.
- 6. Don't underestimate the damage that processing can cause. The difference in shelf life between a "Wash me, Eat me" product and a washed product is usually the result of damage done during washing, drying and packaging.

- 7. Dry to remove excess water. Forced-air tunnels over the conveyor line are better for delicate products.
- 8. Package with an appropriate atmosphere. If a modified atmosphere package is to be used then it is essential that the cool chain is unbroken. Increases in temperature increase the respiration rate of the product and as a result can deplete the oxygen level inside a bag causing off flavours and aromas to develop.

*Adapted from Cantwell and Suslow, 2002

Microbial Aspects

Minimally processed products favour microbial growth more than whole unprocessed products. This is because the cut surfaces of processed products provide the nutrients and conditions that favour microbial growth. The extra handling of these products also means that there is greater opportunity for exposure to human pathogens such as *E.coli*, *Listeria* and *Salmonella* spp.

Another issue for fresh cut products is that they are consumed raw and so there is no critical kill step for the pathogens. As a result contamination is reduced by ensuring Good Agricultural Practice (GAP) is adopted in the field and Hazard Analysis and critical Control Point (HACCP) principles are used in the factory. Microbial growth is generally controlled with good sanitation and low temperature storage.

Optimising Postharvest Quality of Fresh Cut Baby Leaf Salads

- Pre-cool within half an hour of harvest, vacuum cooling is best.
- Ensure there is a reliable cool chain use data loggers to check
- Store product at low temperature as close to 0°C as commercially possible without freezing
- Only pack undamaged leaves
- Minimise physical damage and bruising during harvesting, packaging and processing
- Minimise free moisture in the bags
- Select the best product from the field best variety for the time of year

Reference

Cantwell M.I. and Suslow, T.V. (2002) Chapter 36. Postharvest handling systems: resh-cut fruit and vegetables. Kader A.A. Editor. Postharvest Technology of Horticultural Crops, University of California Agriculture and Natural Resources Publication 3311. USA.

2.4 Seeding procedure

All spinach and rocket trials were direct seeded and seed was sown using commercial equipment (eg. Seed Spider[®]) available on farms where trials were located. The procedure for all trials was to seed with the same seed batch number for varieties to reduce germination and seed size variation between trials. Some lettuce was direct seeded and some raised as seedlings and transplanted. The transplants were sourced from Withcott seedling, Queensland and the seedlings are commercially produced and are between 4 and 6 weeks old at the time of transplant depending on the season.

The differences in seeding density are explained in each section. The seeding method chosen was the commercially used method for baby leaf producers.

2.5 Irrigation and crop protection

Cooperating growers provided basic maintenance activities by incorporating the trial area into activities required by the adjacent or surrounding baby leaf crop. Irrigation was typically provided by solid-set or overhead sprinklers. Fungicides, insecticides and herbicides were applied with a tractor mounted boom-spray using either flat-fan or hollow-cone jets as was commercial best practice.

2.6 Sampling method for field trials

In all trials a metal quadrant 33cm × 33cm (= 0.1 m²) was used to take samples. In many direct seeded trials the numbers of plants per quadrant were not the same as direct seedling small seeded rocket and spinach is very difficult. As a result the area of the quadrant was the consistent unit. In all trials the number of plants per quadrant was counted and used as a cofactor in the statistical analysis to remove that source of variability. One quadrant was equal to one replicate and the details of the replication used for each trial are explained in detail in each Chapter. This method of sampling was used in all field trials for all species in all regions. The sampling method was different for the glasshouse and growth cabinet trials and the sampling method in those cases is described in that Chapter.

In most cases sub-plot sampling of individual treatments within respective blocks were taken randomly along the bed, excluding the first and last metre of treatment blocks to eliminate possible irregularities experienced with seeding. Sub-plot samples taken within individual treatments at respective sites improved the accuracy of treatment trial data obtained. The metal quadrant 33cm $\times 33$ cm (= 0.1 m²) was thrown randomly within a given treatment block; the area of baby leaf inside the quadrant was harvested by cutting all the above ground vegetative biomass with a pair of scissors and all the agronomic variables were measured on this sample.

2.7 Sampling method for growth cabinet trials.

Spinach plants were harvested when the fifth leaf reached commercial size (~100mm). Each tray was hand harvested by cutting all the above ground vegetative biomass using a scalpel. The number of plants and leaves counted and recorded. Any leaves under 2 cm long, including the plant meristem and cotyledons, were placed aside and counted as 'other' biomass. From each tray, 5 young and 5 old leaves were then randomly sampled and assessed for leaf thickness and chlorophyll content.

The leaves were then divided into two subgroups, one tested for leaf area, fresh weight and dry weight, and the other subgroup used for measuring the shelf life. The shelf life subgroup leaves were trimmed to commercial size (~100mm) and immediately placed in a refrigerator at 4°C inside perforated plastic bags. In most cases the samples were collected using a mixture of both young and old leaves unless otherwise stated.

The fresh weight of the leaves was weighed on a Mettler Toledo PB3002- S Topload Balance. The leaves were measured for their cumulative leaf area using a 'LiCor' Model 3100 area meter. The leaves were placed in labelled brown paper bags in a drying cabinet at 70°C for at least 72 hours or until constant weight (Cook et al., 2002), after which the dry weights of both leaves and 'other' biomass were measured and recorded.

Reference

Cock WRS, do Amaral AT, Bressan-Smith RE (2002) Biometrical analysis of phosphorus use efficiency in lettuce cultivars adapted to high temperatures. Euphytica 126(3): 299-308

2.8 Method for measuring leaf thickness

Leaf thickness was measured directly using a 'Pocket thickness gauge' size 0-9mm, made by Mitutoyo Corporation. The gauge is normally used for measuring the thickness of paper, leather, wire, plastic, sheet metal and similar materials in microns. Leaves were measured between the leaf midrib and veins in the centre of the leaf lamina.

2.9 Method for measuring chlorophyll content

Leaf chlorophyll content was measured using a Konica Minolta SPAD-502 chlorophyll meter, a handheld meter that measures chlorophyll content by measuring the absorbance of a leaf in the red and near-infrared regions. The meter uses these two transmittances to calculate a numerical value that is proportional to the amount of chlorophyll present in the leaf. The leaves were measured between the leaf midrib and veins in the centre of the leaf lamina.

2.10 Method for determining shelf life

The leaves were harvested in the field and sub samples of 30 leaves were placed in perforated plastic bags in an iced esky and then transported as quickly as possible to the University of Sydney for allocation into storage trials.

From each replicate/block in the field trial, 30 leaves were selected and placed in labelled perforated polyethylene resealable bags and stored at 4°C unless otherwise stated. To eliminate subjectivity from the scoring, a pictorial reference was used to illustrate leaves to be discarded. The discarded or 'failed' leaves were those that were desiccated, broken-down or unattractive/yellow. Each day any leaves determined to have 'failed' were discarded and the date and number of failed leaves noted. The shelf life was determined by the number of days until 50% of leaves in each bag 'failed'.

This meant that we could assess the quality of individual leaves and use a "days to fail" measure for shelf life. Shelf life was the number of days from harvest until the treatment replicate had half the leaves deemed to have "failed". When half the leaves "fail" is well passed the commercial specification for consumers but it allowed the treatment effects to be clearly demonstrated.



Figure 2.1. The scale reference used to determine the quality of the leaves.

In the OneHarvest factory samples are taken for each run and each of those bags are assessed daily for visual quality; crispiness; odour; taste; damage; consistency; and, freshness. For each category a number between 1 and 5 is assigned where 5 indicates excellent quality and 1 signifies an unacceptable level of quality. When a bag is given a score for any category less than 3 the bag fails.

3 Overview of the agronomic factors that affected postharvest shelf life of spinach

3.1 Introduction – Postharvest overview

It is a well know adage that postharvest handling can maintain quality but it cannot improve it (Wills et al., 1998). This has meant that more research is focussing on how preharvest factors affect postharvest quality. It is assumed that high quality at harvest will provide an inherently longer shelf life for fresh produce (Nicola *et al.*, 2006).

This project had an overall aim to understand how agronomic factors affected the postharvest shelf life of processed baby leaf salads. The results presented are for baby leaf spinach which was used a case study. This chapter summarises some of those factors. In particular the hypotheses tested included that;

- 1. The time of year of planting affects the shelf life of spinach
- 2. That seasonal temperatures affect the shelf life of spinach
- 3. That growing or storage conditions that promote high rates of respiration reduce the shelf life of baby leaf spinach
- 4. That different varieties of spinach suit different growing conditions

3.1.1 Postharvest overview - Materials and Methods

The results reported in this section are drawn from the Crop Scheduling experiments reported in Chapter 8 of this report. A more details description of the methods is reported in that Chapter. This work is a good overview of the interacting agronomic factors that impact on the shelf life of bay leaf spinach.

The sites used for this work included Boisdale in East Gippsland (Victoria) representative of cool temperate environment (cool in the winter and warm in the summer) and Camden, (NSW) south of Sydney in Hawkesbury/Nepean basin representative of a warm season temperate environment. The commercial grpwers where the trial were run were Edgar Grech (Grech Farms) at Camden and Bill Taylor (Taylor Farms) at Boisdale. All trials were planted at a density of 760 plants/m² using a specialised baby leaf seeder (Seed Spider[®], New Zealand).

The varieties planted were Crocodile (Rijk Zwaan), Roadrunner (Rijk Zwaan and Parrot (Rijk Zwaan) which are the current commercial varieties.

Samples of the three varieties were planted every month over a 12 month period in the 2 locations over 2006 and 2007, 11 plants in Camden, NSW and 12 plantings in Boisdale, Vic. This meant that samples were collected that represented all seasonal windows. The postharvest shelf life assessment was carried out on each sample at the University of Sydney as reported in Chapter 2 Materials ands Methods.

3.1.2 Postharvest overview - Results and Discussion

The effects of pre-harvest factors on the shelf life of baby leaf spinach.

A large amount of data was collected from the crop scheduling trial and so in the first instance correlation matrices were compiled to give a preliminary indication of the relationships between the parameters measured in the field with the shelf life of the harvested product stored at 2°C.

Table 3.1 shows that the factor with the highest linear correlation with shelf life was the days to harvest of the crop ($R^2 = 0.64$). This relationship illustrates the importance of a crop scheduling model for predicting harvest date and the need to determine how many growing days are required to give adequate shelf life for spinach.

There was also a weak correlation between shelf life and the thickness of the old and young leaves but subsequent analysis showed that this relationship was not statistically significant (P>0.05). The hypothesis that spinach harvested with thicker leaves was rejected for these trials. Although it would be interesting to look at this relationship in more detail in future work as more field replicates for shelf life would improve the strength of the statistical analysis and the trend our data indicates has previously been reported by Zhang et al (2007).

	Dry Weight	Days to harvest	Fresh weight per plant	Thickness of old leaves	Shelf life
Days to harvest	0.24		piciti		
Fresh weight per plant	0.57	-0.24			
Thickness of old leaves	0.19	0.54	-0.05		
Shelf life	-0.08	0.64	-0.13	0.49	
Thickness of young leaves	0.4	0.43	-0.06	0.74	0.32

Table 3.1: Correlation matrix comparing leaf growth and thickness with shelf life at 2°C.

The results indicate that seasonal growing temperature does have an impact on the postharvest shelf life.

The correlation matrix shown in Table 3.2 looks at the data in more detail with the traits of individual cultivars reported. This is important as the three cultivars have specific growth characteristics which are discussed in more detail in Chapter 8. The important differences between the cultivars are that: Crocodile is a slow growing variety that is most suited to warm summer conditions; Parrot is a fast growing variety and is suited to cooler winter conditions; and Road Runner, a transitional variety that has a growth rate somewhere between that of the slow growing Crocodile and fast growing Parrot.

In this correlation matrix the pairs that have numbers closest to 1 have a linear correlation. If the number is positive it means that as one goes up so does the other and if the number is negative then as one goes up the other goes down. Table 3.2 shows that the main characteristics associated with shelf life for these varieties of spinach were the days to harvest, the average minimum and the average maximum temperature during the growing season. These results indicate that seasonal growing temperature does have an impact postharvest shelf life.

	Crocodile shelf life	Days to harvest Crocodile	Days to harvest Parrot	Days to harvest Road Runner	Ave max temp	Ave min temp	Parrot shelf life (days)	Ave plant weight (g)	Road Runner Shelf life (days)	Ave shelf life for 3 varieties
Days to harvest Crocodile	0.45									
Days to harvest Parrot	0.51	0.96								
Days to harvest Road Runner	0.47	0.98	0.98							
Ave max temp	-0.49	-0.87	-0.85	-0.89						
Ave min temp	-0.56	-0.94	-0.88	-0.92	0.82					
Parrot shelf life (days)	0.87	0.79	0.78	0.78	-0.76	-0.79				
Ave plant weight (g)	0.07	-0.35	-0.29	-0.37	0.21	0.14	-0.25			
Road Runner Shelf life (days)	0.81	0.68	0.73	0.70	-0.63	-0.60	0.93	-0.46		
Ave shelf life for 3 varieties	1.00	0.45	0.51	0.47	-0.49	-0.56	0.87	0.07	0.81	
Total rainfall during growth (mm)	-0.32	-0.30	-0.20	-0.25	0.23	0.58	-0.23	-0.54	0.09	-0.32

Table 3.2: Correlation matrix comparing the shelf life at 2°C of baby leaf spinach varieties and pre-harvest parameters.

Shelf life and growing temperature

The strongest correlation (Table 3.2) with shelf life for the cultivar Crocodile was with the average minimum temperature during the growing season ($R^2 = -0.56$), for the cultivar Parrot the strongest correlation with shelf life was also average minimum temperature during the growing season ($R^2 = -0.79$) although the correlation with days to harvest was also strong ($R^2 = 0.78$). For the cultivar Road Runner the strongest correlation with shelf life was with days to harvest ($R^2 = 0.70$). This data shows that there is a difference between the cultivars. However the over arching message is that seasonal temperatures affect shelf life. When the data for all three cultivars was combined the strongest linear correlation with shelf life was with the average minimum temperature during the growing season.

When the data is presented as linear regression (Figure 3.1) the negative relationship between the average minimum temperature during the growing season and the shelf life of baby leaf spinach is clearly seen. This data shows that as the seasonal average minimum temperature increases, the shelf life of the spinach is reduced. This results supports the work of Boese & Huner (1990) who found that cooler growing conditions improved postharvest spinach quality. It is likely that the lower average minimum temperature is due to the lower night temperatures. During cooler nights the respiration rate of the leaves will be lower than if the night temperatures are warm and this means that there will be a greater accumulation of stored carbohydrate which can be used for growth (Cantwell and Suslow, 2002; Ueda et al., 1998). More carbohydrate means that the plant can grow well, without stress from limited metabolic energy reserves. Our hypothesis was that a plant grown well without stress would have a longer shelf life and this data supports that hypothesis.



Figure 3.1. Shelf life at 2°C of baby leaf spinach (three varieties combined) with the average minimum temperature during the growing season.
The data also showed that there was an important correlation between shelf life and the days to harvest (Table 3.2). When the data for the three cultivars was combined the regression analysis showed a significant (P<0.05) asymptotic relationship (Figure 3.2). The longest shelf life was achieved when spinach took more than 32 days from sowing to harvest and the shelf life was reduced by about half a day for every day reduction in growth rate below 32 days. Similar results have been reported previously for spinach by Boese & Huner (1990).



Figure 3.2. Shelf life at 2°C of baby leaf spinach (three varieties combined) with the days to harvest.

These two regression relationships show that spinach grown outside of its' climatic optimum does not have the same shelf life as when it is grown under optimum conditions. It will be important for growers across Australia to be mindful that hot weather during the growing season reduces the shelf life of baby leaf spinach. However it may be possible to reduce the impact of the seasonal weather by using a more heat tolerant variety. Crocodile for example, is the first heat tolerant cultivar bred especially for the baby leaf salad market but even Crocodile will have an optimum temperature range in terms of shelf life.

When the cultivars are looked at individually different relationships between shelf life and days to harvest become apparent as can be seen in Figure 3.3, Figure 3.4 and Figure 3.5. For the cultivar Parrot, which is a fast growing winter cultivar, there was a significant relationship between days to harvest and shelf life (Figure 3.3). This shows that seasonal weather conditions had a significant impact on the growth of this cultivar.



Figure 3.3. Shelf life at 2°C of the baby leaf spinach cultivar Parrot and the days to harvest.

A similar significant relationship was also apparent for the transitional variety, Road Runner where the shelf life was longer when there were more days between germination and harvest. The mathematical regression equations for Parrot and Road Runner are very similar and this shows that these 2 varieties behave in a similar way in response to the number of days from germination to harvest.



Figure 3.4. Shelf life at 2°C of the baby leaf spinach cultivar Road Runner and the days to harvest.

However, it is interesting to note that the relationship between days to harvest and shelf life was not significant for the cultivar Crocodile (Figure 3.5). Crocodile is an inherently slow growing cultivar bred to maintain a slow growth rate even under warm conditions. As a result the impacts of seasonal climate on the shelf life of this cultivar appeared to be less marked than for the cultivars Parrot and Road Runner. This result means that Crocodile can grow well over a wider temperature range than the other cultivars. For Crocodile it seems some other parameter is affecting shelf life more than the seasonal growing temperature.



Figure 3.5. Shelf life at 2°C of the baby leaf spinach cultivar Crocodile and the days to harvest.

The change in shelf life of the three cultivars over the harvest season for 2006/07 at Camden is shown in Figure 3.6. Crocodile had a more consistent shelf life each month than the cultivars Parrot and Road Runner. The warm weather over the summer in December to February had a much greater impact on the shelf life of Road Runner and Parrot than it had on Crocodile. This data helps to explain why there was not a significant correlation between days to harvest and shelf life for Crocodile but there was for Road Runner and Parrot.



Figure 3.6. Shelf life at 2°C of three baby leaf spinach cultivars the month of harvest at Camden, New South Wales.

The impact of seasonal weather conditions is seen more clearly if the data for the cultivar Road Runner is looked at on its own (Figure 3.7). Figure 3.7 shows the changes in fresh weight per plant and the differences in shelf life over the 8 plantings harvested from September 2006 until May 2007 at Camden, NSW. The data shows how the fresh weight or growth of the plants changed quite dramatically over the different planting dates and also that the shelf life for Road Runner was not the same for each harvest. The shelf life was longest when the crop was grown and harvested in the cooler months September, October and May but the plant fresh weight was greatest for plants harvested in the warmer months of November and December. The plants harvested in November and December would have been growing in the optimum weather conditions (day temperature 15 - 18°C).



Figure 3.7 Fresh weight, shelf life at 2°C and harvest date of the baby leaf cultivar, Road Runner at Camden 2006/07.

It is important to comment on the shelf life of the product harvested in March shown in Figure 3.7. Planting 10, harvested in March 2007, experienced 132mm of rainfall during the first 3 days after planting and this may have reduced the shelf life of the harvested product. Some possible explanations could be that the shorter shelf life is the result of poor establishment as see in the photographs below the Figure or because the base nitrogen application was leached from the soil.

The photographs below the figure show the difference in establishment of the plantings harvested in February, March and May with the March planting not being as dense or as well established as the plantings in February and May. This is seen by the fact that the plant stand is not as dense with soil being able to be seen at the edge of the bed, whereas in the other photographs the bed is densely covered with foliage. The impact of poor establishment on the shelf life of spinach was reported by Johnston et al. (1989) who showed that "high rainfall during the growing season reduced storage potential by 40%". This is another example of how significantly the pre-harvest seasonal conditions affect the postharvest shelf life of baby leaf spinach.

3.1.3 Postharvest overview - Conclusion

The results from the postharvest assessment of the crop scheduling trial show that the relationship between preharvest growing conditions and the postharvest shelf life of baby leaf spinach is complex.

Our results showed that seasonal weather during growth does impact on the postharvest shelf life of spinach. The shelf life was shorter when the average minimum and maximum temperature was high. More work is needed to establish what the temperature limits are but our results suggest that there is a linear relationship between shelf life and the minimum average temperature within the range of 6 to 18°C. Spinach grown when the minimum temperature was lower had a shelf life of about 26 days at 2°C compared to spinach grown with a minimum temperature of 18°C which had a shelf life of only 12 days. Growers must consider the idea that there is a seasonal window for the production of baby leaf spinach which has a long shelf life. As with other crops such as lettuce it is the transition times from one district to another when problems arise as this is when night and day temperatures begin to increase.

Another important result is that that was a strong correlation between the shelf life of spinach and the days from germination to harvest. Spinach that had a growing time of more than 32 days had a longer shelf life that spinach grown for a shorter time. The shelf life for a crop grown for more than 32 days was about 22 days whereas the shelf life of spinach grown for less than that time decreased in a linear way with decreasing growing time. For example, spinach that grew for 20 days before reaching marketable size had a shelf life of only 10 days compared to a 22 day shelf life for spinach that took 32 days to reach marketable size. This result again highlights the fact that there is a seasonal window for the production of spinach that as a long shelf life. However it is important to note that the impact of days to harvest on shelf life was greater for the faster growing cultivars Road Runner and Parrot but was not significant for Crocodile when the varieties were analysed separately. This

result suggests that selection and breeding are important when spinach is grown in warmer conditions.

Our original hypothesis was that seasonal weather influences the leaf morphology and this would be a factor in determining the shelf life of spinach. The hypothesis was that baby spinach with thicker leaves would be more robust than spinach with thinner leaves and therefore more able to withstand the damage resulting from processing. The results from the postharvest assessment of the crop scheduling trial showed that leaf thickness was not significantly correlated with shelf life. There was quite a lot of variability between the replicates used in these trials and this may have contributed to the insignificant results and so we believe that the relationship between shelf life and plant morphology warrants further investigation.

And finally some anecdotal evidence resulting from the comparison of plantings over the year suggested that rain at planting had a negative impact on shelf life of baby leaf spinach. This had has also been reported in the literature (Johnston et al.,1989). The reason for this result is not clear and so the impact of rain requires further investigation.

These results show that baby leaf spinach has a seasonal window where the maximum shelf life can be achieved. Growing baby leaf spinach when the weather conditions are favourable will have a positive effect on ensuring a long shelf life for the consumer.

3.1.4 Postharvest Overview - References

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3.2 Detailed study: Effect of growing temperature on the shelf life of baby leaf spinach under controlled conditions.

3.2.1 Introduction

In an effort to extend the postharvest shelf life of baby leaf salads a lot of research has focussed on improving cold chain logistics, modified atmosphere packaging, washing and cleaning. However, other researchers such as Weston & Barth, (1997) insist that pre-harvest factors have a profound influence on the post-harvest quality and nutritive value of vegetables. This concept has been confirmed for processed lettuce (Zhang et al., 2007; Newman et al., 2005). The postharvest aspect of the crop scheduling work discussed previously (Chapter 3.1) indicated that growing conditions did impact the shelf life of baby leaf spinach. The following study aimed to verify the impact of growing temperature on shelf life by growing spinach in growth cabinets where the day length as well as the night and day temperatures could be accurately controlled.

3.2.2 Materials and Methods

Two commonly grown commercial baby leaf spinach (*Spinacia oleracea L.*) varieties Crocodile and Parrot from Rijk Zwaan Pty. Ltd., were grown under two different temperature regimes:

- 1. 21°C day & 12°C night (Low or "Optimal") and
- 2. 27°C day & 18°C night (High)

A total of four trials were conducted under controlled conditions in a Thermoline Plant Growth Cabinet in the John Woolley building at the Sydney University, Camperdown campus. The growth cabinet, specifically designed for growing plants, contained five 1000W metal halide bulbs as well as eight 60W incandescent bulbs with an output of 764 μ mol/m²/s PAR.

The diurnal cycle was set to 16 hours of light (day) followed by 8 hours of dark (night). The plants were watered and fertilised to ensure these factors did not limit plant growth. The plants were monitored daily and watered accordingly. The plants were fertilised with a water-soluble fertiliser; Hortico Aquasol^a (N.P.K. 23:4:18) at a rate typically recommended for vegetables (1.6g/L) fortnightly.

3.2.3 Statistical analysis

The trial data was analysed using Microsoft[®] Excel and Genstat[®], using a two-way Analysis of Variance (ANOVA) and Non-linear (asymptotic curve fitted) regression analysis, where temperature, variety and blocks were factors. The parameters analysed included; shelf life, leaf thickness (young and old leaves), leaf chlorophyll content (young and old leaves), leaf area, leaf weight, and dry weight. The detail for how these measurements were taken is outlined in Chapter 2 Materials and

Methods. Differences were considered to be significant P < 0.05 and when the difference between means was greater than the LSD value.

3.2.4 Results

The arrangement of the plants within the growth cabinet is illustrated in Figure 3. The leaves of Parrot and Crocodile were glossy and dark green. The leaves of Parrot appeared slightly larger in leaf area, although were not significantly different to Crocodile (LSD=2.483). The leaves of Crocodile were slightly cupped at the edges, where the leaf margins are folded under the abaxial side of the leaf (Figure 3. and Figure 3.9).



Figure 3.8. Image of the baby leaf spinach plants growing at 21°C day/12°C night (low), where the variety is denoted by P (Parrot) and C (Crocodile).



Figure 3.8. Image of the baby leaf spinach plants growing at 27°C day/18°C night (high), where the variety is denoted by P (Parrot) and C (Crocodile).

In Figure 3.8, the spinach plants grown at 27°C day/18°C night (high) were stunted. The leaves of both Crocodile and Parrot showed signs of chlorosis at the leaf tips. The leaves were not fully expanded, and had short petioles causing the leaves to remain in a vertical position. A visual comparison of Figure 3.7 and Figure 3.8 showed an altered leaf phyllotaxy, where, spinach grown at 21°C day/12°C night had horizontally positioned leaves opposed to spinach grown at 27°C day/18°C night that had short and vertically positioned leaves.



Figure 3.9. Comparison of individual Crocodile baby leaf spinach plants grown at 21°C day/12°C night (left) and 27°C day/18°C night (right) respectively. Image taken on grid, where each grid square is equal to 1cm.

Figure 3.8 and Figure 3.9 also illustrate the cupping of the 'Crocodile' leaves and the chlorosis at the leaf tips in spinach grown under the high temperature environment.



Figure 3.10. Comparison of individual Parrot baby leaf spinach plants grown at 21°C day/12°C night (left) and 27°C day/18°C night (right) respectively. Images taken on grid, where each grid square is equal to 1cm.

Figure 3.10 shows the difference in leaf size between the two temperature treatments. Parrot, like Crocodile when grown at high temperature, showed signs of chlorosis at the leaf tips. Figure 3.8 and Figure 3.10 both show the leaf stunting and chlorosis at the leaf tips. Both varieties grown under the high temperature treatment produced a greater number of leaves per plant and these leaves were significantly smaller than the leaves of plants grown under the 'low' temperature treatment (Figure 3.15).



Figure 3.11. Shelf life of two varieties of baby spinach, grown at two different temperatures and stored at 4°C (P=0.041, LSD=6.197). Means followed by a different letter are significantly different.

In these experiments the "old" leaves or fully expanded leaves were separated from the young leaves as anecdotal evidence suggested that the young leaves are the ones that "fail" the shelf life test first. Figure 3.11 shows that temperature significantly affected the shelf life of the old leaves of Crocodile. The 'old' Crocodile leaves grown at 21°C day/ 12°C night had a 43% longer shelf life than leaves grown at 27°C day/ 18°C night. There was no significant difference between the shelf life of 'young' Crocodile leaves when grown at either 21°C day/ 12°C night or 27°C day/ 18°C night. Growing temperature had no significant affect on the shelf life of the leaves of the variety Parrot.



Figure 3.12. Leaf thickness and shelf life for old leaves of baby leaf spinach (P=0.019, fitted curve is shelf life= 15.22+0.00051^(leaf thickness), R-squared = 37.4).

As seen above in

Figure 3.12, Leaf thickness was significantly correlated to the shelf life of baby leaf spinach leaves, where, thicker leaves had a significantly longer shelf life, particularly when the thickness was greater than 0.9 mm.



Figure 3.13. Chlorophyll content of two varieties of baby spinach grown at two different temperatures (P=0.003, LSD=5.61).

Figure 3.11 shows that the chlorophyll content of the baby spinach leaves was significantly affected by temperature. The data shows that the chlorophyll content of the leaves of both varieties, Parrot and Crocodile was significantly lower in leaves grown at high temperature (27°C day/18°C night) than leaves grown at low temperature (21°C day/12°C night). There was no significant difference in chlorophyll content between varieties.





As seen in Figure 3.14, a significant correlation was found between chlorophyll content and the shelf life of baby leaf spinach, where, higher chlorophyll content resulted in a significantly longer shelf life. This supports the finding that plants grown under high temperature conditions had a shorter shelf life.



Figure 3.15. Leaf area of two varieties of baby spinach grown at two different temperatures (P<0.001, LSD=2.483)

Figure 3.15 shows that the leaf area in both varieties of spinach, Parrot and Crocodile were significantly smaller (P<0.001) for leaves grown at high temperature (27°C day/18°C night) compared to leaves grown at low temperature (21°C day/12°C night). There was no significant difference in leaf area between the varieties.



Figure 3.16. Fresh leaf weight of two varieties of baby spinach grown at two different temperatures (P<0.001, LSD= 0.266).

Figure 3.16 shows that the fresh leaf weight of baby spinach leaves was also significantly affected by temperature (P<0.001). The leaf weight of both Parrot and Crocodile was significantly lower in leaves grown at high temperature (27°C day/18°C night) compared to leaves grown at low temperature (21°C day/12°C night). No significant difference in leaf weight was observed between the varieties.

3.2.5 Discussion

The experiment investigated the relationship between growing temperature and the shelf life of two commonly grown baby leaf spinach varieties under controlled conditions. Temperature is widely acknowledged to dramatically affect the growth and development of plants (Lefsrud et al., 2005). The aim was to determine how growing temperature affected baby leaf spinach growth and morphology and whether any of these morphological changes were correlated with postharvest shelf life. The results of the experiment showed that growing temperature did significantly affect the shelf life of the spinach variety Crocodile not the variety Parrot.

The physical factors that were affected by temperature for the variety Crocodile were the shelf life response of the fully expanded "old" leaves, with leaves grown at 21°C day/12°C night having a 43% longer shelf life than leaves grown at 27°C day/18°C night (23 days and 16 days, respectively). The shelf life of the 'young' Crocodile leaves were not significantly impacted by shelf life, both had a shelf life of about 14 days. The leaves which had the longest shelf life were the older leaves grown in a cooler environment. This result supports that found in the scheduling experiments where spinach that was grown under cooler optimum conditions over a longer time frame had the longest shelf life.

The reason for this is still not clear however, growing temperature did significantly change the leaf area, leaf thickness, leaf weight and leaf chlorophyll content. Baby spinach leaves that had thicker leaves, higher chlorophyll content, a larger leaf area and greater leaf weight were positive attributes correlated with a longer shelf life (Figure 3.12 and Figure 3.14, respectively).

The increased leaf thickness, larger leaf area and greater leaf weight of baby spinach leaves could improve the shelf life due to larger amounts of stored carbohydrate. Plants grown at higher temperatures may be under a growth stress which impacts on the robustness of the leaves. This is because leaves grown under conditions with warm nights use up more carbohydrate during the warm nights than leaves grown under conditions with cooler nights, leaving less energy available for growth (Rokka et al. 2000). Our hypothesis is that stress during growth impacts negatively on the final shelf life of the product (Madakadze & Kwaramba, 2004).

Many studies have shown the significant effect of temperature on plant growth, leaf anatomy and morphology. Yamori et al. (2005) found that spinach plants grown at 30°C day/20°C night took six weeks for the seventh leaves to fully expand, while at 15°C day/10°C night the same stage of growth took about 2.5 times longer to

achieve (sixteen weeks). This result highlights the significant effect temperature has on plant growth, with high temperature promoting faster growth rates. The results from this experiment supported the work of Yamori et al. (2005), as plants grown at 27°C day/18°C night produced many more leaves than the plants grown at 21°C day/12°C night (Figure 3.9 and Figure 3.10). Wolfe (1990) also reported that the rate of leaf initiation generally decreased with decreasing temperature.

In terms of leaf size Boese & Huner, (1990) found that leaves grown at 5°C had a final leaf area that was three times larger than spinach leaves grown at 16°C. Boese & Huner, (1990) also found that the leaves grown at 5°C were thicker and had a greater dry weight than spinach plants grown at 16°C. They reported leaf thicknesses of 567 μ m and 290 μ m, respectively. This increase in leaf thickness was attributed to a 30-40% increase in the mean lengths of both the palisade and the spongy mesophyll cells of the leaf. The difference in leaf thickness may be because Boese & Huner (1990) used a different spinach variety called Savoy that may have a different leaf morphology to the flat leaf cultivars of spinach used in this trial. Wolfe (1990) also grew spinach in growth chambers to compare warm (28°C/18°C day/night) and cooler (18°C/12°C day/night) temperature regimes. Wolfe (1990) also observed thicker leaves in spinach plants grown at the cooler temperature (18°C/12°C). This data suggests that growing temperature does have a direct impact on the growth and morphology of spinach leaves.

For many plants, different varieties are better suited to different periods of seasonal production. Rijk Zwaan Pty Ltd, the seed supplier for these experiments, currently recommends Parrot to be grown during the cooler autumn/spring and cold winter periods as plant development is much faster than the variety Crocodile at this time. In this experiment Parrot did not have significantly larger leaves than Crocodile (Figure 3.15), although, a varietal trend appeared where Parrot produced larger leaves (10.04cm²) than Crocodile (7.62cm²) at both temperatures (Figure 3.15). This trend in larger leaf area is attributed to Parrot's fast growth habit, which makes it an ideal transition, or winter baby leaf variety as it grows quickly in cooler conditions. The growth habit was also considerably different between varieties as Crocodile grows very flat and close to the soil surface while Parrot leaves are more erect.

During this experiment, it must be noted that the leaves harvested from the high temperature climate did not reach a commercially acceptable size and quality. When the days from planting (DAP) for both trials were equal, the leaves of the high temperature plants were too small and it was decided to give the plants another few days to expand. The leaves from the warm temperature regime never reached a marketable size and so the shelf life data must be interpreted with caution. This result highlights the relationship between high temperature tolerance and plant growth stress (Finch-Savage 1995). This is an important result for growers and processors as good quality baby leaf spinach with a long shelf life can only be sourced from regions with cooler growing temperatures.

Leaf development and morphology are fundamentally influenced by cell division, cell expansion and cell wall structure (Cosgrove et al., 2005). The cell wall is a thin, strong fibrillar network of cellulose microfibrils embedded in a hydrated matrix of complex polysaccharides and structural proteins (Cosgrove, 2005), and it provides

plant cells with mechanical strength and rigidity, while also protecting them from pathogenic attack and desiccation (Cosgrove, 2001). The cell wall loosens and reassembles (Cosgrove, 1993), and cells expand primarily due to increased turgor generated by increased internal osmotic pressure through water uptake (Zhang et al., 2007).

Recent studies have reported that cell expansion is highly responsive to environmental conditions (Zhang et al., 2007). Therefore, in this experiment it is hypothesized that growing temperature affected the rates of cell division and expansion within the baby leaf spinach. In the past, leaf growth has been seen to have two distinct and separate phases, with a cell division phase followed by a cell expansion phase (Saurer & Possingham, 1970). Both of these phases are regulated by temperature and so any disruption to these two fundamental processes will impact on the final leaf shape and structure.

Clarkson et al. (2003) and Zhang et al. (2007) suggested that the manipulation of leaf size and cell wall properties could significantly improve the shelf life of baby salad leaves. These researchers found shelf life of baby salad leaves could be significantly correlated with leaf size, leaf thickness, leaf weight, and leaf chlorophyll content. Plants with greater size, weight, chlorophyll content and leaf thickness had a longer shelf life. These characteristics are seen in plants grown under optimum temperature conditions. In these experiments baby leaf spinach leaves grown at high temperature compromised leaf morphology and in turn shelf life.

3.2.6 Conclusion

Growing temperature had a significant affect on the shelf life of baby leaf spinach. Leaf morphological characteristics such as increased leaf thickness, leaf area, leaf weight and chlorophyll content were positively correlated with increased shelf life of baby leaf spinach. The spinach variety Crocodile when grown at 21°C day/12°C night had a 43% longer shelf life, than leaves grown at 27°C day/18°C night due to having thicker, larger and heavier leaves with more chlorophyll.

Processors need to select produce from cool climate regions to obtain produce with a longer shelf life. In the future it may be possible to select new cultivars of baby leaf spinach based on leaf morphology. It would be helpful for the Australian industry if there was a new variety that was more heat tolerant than the ones currently available.

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4 The effect of the time of the day of harvest on shelf life

4.1 Introduction

Recent research has shown that harvesting baby leaf lettuce, roquette, arugula and lollo rosso lettuce in the afternoon can extend the shelf life of these products by 2 to 6 days (Forney and Austin, 1988; Clarkson et al, 2005). The research showed that the leaves of afternoon harvested crops had higher concentrations of sucrose, due to accumulation throughout the day via photosynthesis. As respiration uses up stored metabolites, leaves with a high sugar concentration at harvest had an increased shelf life but the extent depended on the species (Forney & Austin, 1988).

Baby spinach leaves have very high rates of respiration (Allende et al., 2004). Baby leaves in fact have a much higher respiration rate than mature full size leaves at any temperature (Cantwell et al., 1998). This experiment aimed to investigate if the shelf life of baby leaf spinach could be extended by harvesting them later in the day.

4.2 Materials and Methods

The experiments were conducted at the University of Sydney in a glasshouse with a day temperature of 21°C days and a night temperature of 12°C. The day length was set at a 16 hr day / 8 hr night. The plants were watered and fertilised to ensure that plant growth was not limited. The plants were monitored daily and watered accordingly. The plants were fertilised fortnightly with a water-soluble fertiliser; Hortico Aquasol[™] (N.P.K. 23:4:18) at the rate recommended for vegetables (1.6g/L).

The trial was set up as a completely randomised design with 6 replicates per treatment. Each replicate consisted of a seedling tray sown at a rate of approximately 50 plants per tray which related to a commercial planting density.

The plants were harvested when the fifth leaf reached commercial size (~100mm). Each tray was hand harvested using scissors, and all the above ground vegetative biomass was harvested.

Shelf life assessment

From each treatment replicate 30 leaves were selected and placed in labelled perforated polyethylene resealable bags and placed in controlled temperature cabinet set at 4°C and measured as described in Chapter 2.

Total carbohydrate content

The analysis was done using a Thermostable α -amylaze enzyme (Megazyme Total Starch Kit) Modified as reported in Hattey et al., (1994).

Each sample of leaves was finely milled in liquid nitrogen using a mortar and pestle. Then, 2-3 g of the finely milled sample was placed in two chilled and labelled 100 mL glass tubes. The exact weight of the samples was recorded and 0.2 g of insoluble polyvinylpyrolidine (PVP) added to each tube in order to eliminate any phenol interaction. After that, 1 mL of absolute ethanol was added to 'wet' the samples, followed by the addition of 17 mL of nanopure H₂O. The test tubes was mixed using a vortex and then placed in a boiling water bath for 10 min. The samples were mixed again and to one of the tubes 4 mL of α -amylase was added, with 4 mL of MOPS buffer added to the other tube to act as the blank. The two test tubes were then incubated at 80°C for 30 min in a thermostat controlled water bath. The tubes were mixed again after removal from the water bath, and allowed to cool.

After both tubes were allowed to cool, 5 mL of sodium acetate buffer was added to both tubes and mixed. To the same tube that received the α -amylase, 0.1 mL of amyloglucosidase (6100 units/mL) was added, 0.1mL of sodium acetate buffer was then added to the blank. Both tubes were mixed and then incubated at 50°C for 30 min. After removal from the water bath both tubes were centrifuged at 3000 rpm for 10 mins, then 0.1 mL aliquots of the samples were transferred into labelled duplicate 10 mL disposable glass test tubes (total of 4 tubes per sample). Two blanks using 0.1 mL of nanopure H₂O were also set up, including three glucose controls using 0.1 mL of glucose standard solution (1 mg/mL). To each tube 3 mL of the GOPOD reagent was added and mixed, these tubes were then incubated at 50°C for 20min. The absorbance at 510 nm of the tubes was recorded, with the glucose control read against the reagent blank. Total starch was then calculated.

4.3 Results and Discussion

Our results show that there was a significant difference in the quality of the spinach leaves harvested at different times during the day with the leaves harvested in the morning having better quality (Figure 4.1) and a longer shelf life (Figure 4.2).

It seems that the effect of harvesting at different times during the day is species specific and baby leaf spinach may not respond as dramatically as lettuce and roquette for example (Clarkson et al., 2005). Other research has shown species differences. For example, the postharvest quality of lettuce cultivars was affected by the time of harvest but the response varied between cultivars or "types" with the butterhead lettuce quality being highest when harvested at 6 pm at the end of the day and the Latin type of lettuce having better quality when harvested at 6 am in the morning (Moccia et al., 1998). The authors suggest that there is an interaction between genotype and the environment. Our results support this finding, although more research is needed.



Figure 4.1. The effect of harvest time during the day on the quality of baby leaf spinach leaves stored at 4° C. This data shows the significant main effect of harvest time, means followed by different letters are significantly different (P = 0.05).



Figure 4.2. Effect of harvest time during the day on the shelf life of baby leaf spinach stored at 4° C. (Least significant difference = 3.25, P = 0.05).



Figure 4.3. Starch content of baby leaf spinach leaves harvested at different times during the day. Means followed by different letters are significantly different (P= 0.05).

We also did a comparison between the starch content of the three main varieties of spinach and found that there was no significant difference between the cultivars (Figure 4.4).





Clarkson et al. (2005) propose that the differences in sugar metabolism between species may modulate the potential shelf life extension. In our research we measured total starch and not the individual sugars in the leaves. Our data shows a correlation between higher starch levels at 6 am and a longer shelf life but there was no significant difference between the starch content at 6 am and the content at 6 pm and so it is not clear from this data whether starch content is the main reason for the extension of the shelf life of baby leaf spinach (Figure 4.3).

It is important to note the results reported by Moccia et al (1998) where for both types of lettuce midday was the worst harvest time in terms of shelf life (Moccia et al., 1998). That trend was also in our data although not statistically significant and more work is needed to determine the best and worst time of day for harvesting baby leaf spinach. More research is also needed to understand the sugar metabolism of baby leaf spinach.

This preliminary investigation suggests that starch accumulation is not a major determinant of shelf life for baby leaf spinach.

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5 The effect of variety and storage temperature on the Vitamin C content of baby leaf spinach

5.1 Introduction

Vitamin C (ascorbic acid) is an important vitamin for human health. It is an antioxidant that participates in scavenging reactive oxygen species generated from metabolic activity in cells. Fruit and vegetables are the major sources of Vitamin C in the human diet.

Vitamin C is the most labile or unstable antioxidant in plants and it is prone to loss during storage and processing (Kalt 2005). Vitamin C is also highly water-soluble and is sensitive to heat, which makes it very sensitive to breakdown during cooking. From this point of view young spinach leaves consumed fresh have higher levels of Vitamin C than cooked spinach products (Gil et. al. 1999). This is one reason why baby leaf spinach is so popular for health conscious consumers.

In another study, Favell (1998) compared the Vitamin C content of fresh spinach stored at different temperatures. The results showed that spinach stored at ambient temperature (20°C) lost Vitamin C very rapidly with only 10 % remaining after 3 days. In comparison fresh spinach stored at 3-4°C had 20 % of the initial Vitamin C remaining after 7 days and this fell to zero after 14 days (Favell 1998).

Another factor that needs to be considered when assessing the Vitamin C content of baby leaf spinach is the cultivar as research has shown that plant genotype has an important influence on the concentration of total antioxidants, including Vitamin C in spinach leaves (Kidmose et al., 2001; Pandjatan et al., 2005).

This previous research shows that the selection of the cultivar and the postharvest handling of spinach are important considerations for managing the Vitamin C content of baby leaf spinach. Our work aimed to investigate the difference in Vitamin C content of three commonly grown baby leaf spinach varieties and the effect of storage temperature on the retention of Vitamin C in fresh baby leaf spinach leaves.

5.2 Materials and Methods

The experiments were conducted at the University of Sydney in a glasshouse with a day temperature of 21°C days and a 12°C night temperature. The day length was set at a 16 hr day / 8 hr night. The plants were watered and fertilised to ensure that plant growth was not limited. The plants were monitored daily and watered accordingly. The plants were fertilised fortnightly with a water-soluble fertiliser; Hortico Aquasol[™] (N.P.K. 23:4:18) at the rate recommended for vegetables (1.6g/L).

The trial was set up as a completely randomised design with 6 replicates per treatment. Each replicate consisted of a seedling tray sown at a rate of approximately 50 plants per tray which related to a commercial planting density.

The plants were harvested when the fifth leaf reached commercial size (~100mm). Each tray was hand harvested using scissors, and all the above ground vegetative biomass was harvested.

Shelf life

From each treatment replicate 30 leaves were selected and placed in labelled perforated polyethylene resealable bags and placed in controlled temperature cabinets set at 0, 4 and 7°C. The shelf life was determined based on the criteria describes in Chapter 2.

Vitamin C content

Vitamin C content was analysed using an HPLC system based on the method described by Carnevale (1980). A synopsis of the method is to take an accurately weighed portion of leaves (10-30 g) which was homogenised with an equal weight of stabilising solution (25 % w/v metaphosphoric acid) and then centrifuged. 1mL of supernatant was diluted with 4 mL water, syringe filtered through 0.45 μ m filter and injected into the HPLC or was frozen at – 80°C for analysis at a later time.

The HPLC analyses was performed using a C18 reverse phase column together with a guard column with a flow rate 1 mL/min detecting at 245 nm on a UV detector as a single peak eluting at around 4.2 minutes.

5.3 Results and Discussion

Baby leaf spinach has a high respiration rate, and it is about five times higher at 10°C than at 0°C (Bergquist *et al.*, 2006). Ko *et al.*, (1996) found respiration rates of spinach to be about 2 times greater at 5°C than at 0°C. This high rate of respiration is likely to contribute to the rapid depletion of Vitamin C at higher temperatures as the concentration of Vitamin C is directly linked to metabolic activity. At higher rates of metabolic activity Vitamin C is oxidised or "used up" more quickly.

Figure 5.1 and 5.3 shows that there were significant losses of Vitamin C in baby leaf spinach stored for 7 days at 4 and 7°C compared to leaves stored at 0°C. This data emphasises the importance of storing baby leaf spinach as close to 0°C as possible to maximise product quality.



Figure 5.1. Vitamin C content of baby leaf spinach, 0-6 days after harvest, stored in different temperatures (P = 0.05).



Figure 5.2. % Vitamin C loss of baby leaf spinach stored for 14 days at 3 different temperatures.

Figure 5.4 shows there was also a significant difference in the rate of Vitamin C loss between the two varieties Crocodile and Parrot with Crocodile maintaining a higher Vitamin C content than Parrot at both 0 and 7°C. Crocodile is a slow growing cultivar and the respiration rate of this variety may be lower and hence the rate of Vitamin C loss is also lower. More work is needed to quantify the differences in postharvest respiration rate of the two varieties.

Figure 5.5 shows the Vitamin C content of three varieties, Crocodile, Parrot and Road Runner at harvest and the results show that there was no significant difference between the varieties. There was quite a high variability between samples so although the mean values are lower for Parrot and Road Runner the difference is not significant. This means that the difference in Vitamin C retention between the varieties is likely to be the result of different rates of metabolism rather than being due to Crocodile having a higher initial concentration. More work is needed to repeat these experiments to clarify these findings.



Figure 5.3. Vitamin C content of two varieties of spinach, 0-15 days after harvest, stored at different temperatures (P = 0.05).



Figure 5.4. Vitamin C content at harvest of three varieties of baby leaf spinach (P = 0.05).

5.4 Conclusion

The results show that storage temperature and variety affect the postharvest rate of retention of Vitamin C in baby leaf spinach. It is very important to store baby leaf spinach as close to 0°C as possible if the maximum amount of Vitamin C is to be maintained during postharvest handling.

More research is needed to clarify the differences in Vitamin C retention for different varieties and to investigate the possibility that the rate of respiration of different varieties may be an indicator of the potential postharvest Vitamin C loss.

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6 The effect of nitrogen on the shelf life of baby leaf lettuce (nutrition and irrigation experiments)

6.1 Introduction

Baby leaf lettuce is a fast growing crop, and is harvested after a short growth period of 5 to 6 weeks. As a result, it requires a well-balanced nutrition to ensure the attributes of the harvested plant meet the markets demands. For leafy crops nitrogen uptake is the major determinant of leaf growth and leaf area and in turn crop yield (Bacon 1995) but there was anecdotal evidence to suggest that a large application of nitrogen to baby leaf spinach can cause a rapid flush of growth that resulted in a shorter postharvest shelf life.

These experiments aimed to investigate this suggestion under controlled conditions. The plants were grown in a glasshouse and the level of nitrogen applied was changed while all other nutrients remained the same. The additional nitrogen was balanced with calcium.

6.2 Materials and Methods (Overview)

For this trial the seedlings were planted in individual pots. Standard plant pots with a rim diameter of 10 cm were used, allowing a spacing of 36 seedlings /m², similar to commercial planting densities. The seedlings used were 48 Green Coral, 48 Red Coral and 48 Red Salanova lettuce.

Seedlings were planted into a potting mix of 50/50 Peat and Vermiculite, which was obtained from a local nursery and mixed by hand. This mixture was chosen as it is chemically inert, and nitrogen levels can be fully controlled within the experiment. Pots were filled up to 1cm below the rim. A layer of vermiculite was placed on the surface of each pot after planting to slow the evaporation of water from the potting media.

Three benches, or blocks, were used in this experiment (Figure 6. and Figure 6.2). Using a split-plot design, each bench had four rows, each row being one of the given nitrogen levels. Each bench had a row of each nitrogen level, and these rows of nitrogen levels were placed randomly on each bench. Within each row, varieties and replicates were randomised to ensure no bias between varieties.



Figure 6.1 Layout of Glasshouse bench space. Each bench had every nitrogen treatment, arranged in rows. Varieties and replicates were randomised among blocks at the beginning of the experiment.



Figure 6.2 Layout of plants in Glasshouse in Experiment Two immediately after planting, and prior to randomisation.

Plants were watered every 2 days, with two different soluble nutrient mixes. The first was a nutrient mix that contained all essential nutrients for growth excluding nitrogen, and was mixed especially for this experiment by R & D Aquaponics, Wetherill Park, NSW. The mix was 40L and contained:

- 31 kg/L Magnesium Sulphate (MgSO4)
- 8 kg/L Mono Potassium Phosphate (MKP)
- 7.5 kg/L Potassium Sulphate (K2SO4)
- 850 g/L Iron Chelates (Fe EDTA)

- 185 g/L Manganese Sulphate (MnSO4)
- 90 g/L Zinc Sulphate (ZnSO4)
- 20 g/L Copper Sulphate (CuSO4)
- 12 g/L Sodium Molybdenum (NaMo)

The nutrient mix was diluted in a ratio of 16mL nutrient mix to 34mL water so that 50mL was applied in a glass beaker to each plant every two days.

A separate nitrogen mix was created to give the required level of nitrogen for each treatment. Calcium chloride was also added to this nitrogen mixture to balance out the higher levels of calcium in the high nitrogen treatments. The nitrogen mix was placed in 20 L containers, with 4 x 20 L containers in total, 1 per nitrogen treatment. A tap was placed at the base of each 20 L container, and 50 mL of the relevant nitrogen mix was given to the relevant plants every two days via a 50 mL glass beaker. The mixture ingredients were calculated by determining the area of each pot in relation to the nitrogen level per hectare (Table 6.1)

N Level (kg/ha)	Density (plants/m²)	CaN Req. per 20 L (g)	CaCl Req. per 20 L (g)
0	36	0.00	11.94
50	36	17.06	7.96
100	36	34.13	3.97
150	36	51.19	0.00

Table 6.1 Calcium nitrate and calcium chloride required per 20L for nitrogen mixes.

Measurements were taken every four days throughout the experiment, using the same growth measurements as in Experiment One, such as number of leaves, average leaf length and leaf tip chlorophyll level.

After harvest, samples were measured for fresh weight in grams, then dried in an oven at 110°C for 1 week, and subsequently weighed. This measurement was then analysed as a percentage of fresh weight to determine the water content and structural percentage of the leaves. Fresh weight and dry weight was analysed as both a marketable weight and biomass. Marketable weight is the portion of the plant that would actually meet market specifications, whilst biomass was an entire lettuce plant, cut-off from soil height.

Shelf life was determined as described earlier in Chapter 2 – Materials and Methods.

6.3 Results

Leaf Thickness

Previous work described in Chapter 3 indicated that leaf thickness may be an objective measure of the potential shelf life of baby leaf salad leaves. Leaf thickness relative to nitrogen application concentration and shelf life was measured in this trial.

The leaf thickness was measured prior to harvest using a 'pocket thickness gauge' size (Mitutoyo Corporation). The results showed that 0N (0 kg N/ha) plants had significantly thicker leaves than 1.5N (150 kg N/ha) (P <0.001), whilst 0.5 & 1.0N (50 & 100 kg N/ha respectively) had similar leaf thicknesses, being thicker than 1.5N but thinner than 0N (**Error! Reference source not found.**).

When analysed by variety (Figure 6.3), Green Coral had significantly thicker leaves than both Red Coral and Salanova (P<0.001) whilst Salanova had the thinnest leaves of all varieties (P<0.001).



Figure 6.3. Leaf thickness measured prior to harvest

Marketable Fresh Weight

The marketable yield is a measure of the weight of product that can be packaged and sold from each plant. In the higher nitrogen treatments the leaves needed to be cut down to meet market specifications, with excess discarded as waste. This waste was not counted as part of the marketable fresh weight. Similarly, plants at lower nitrogen levels produced some leaves that were too small to meet specifications, and were also discarded as waste and were not counted as marketable weight. The marketable weight was significantly different between all nitrogen levels (P<0.001), with marketable yield increasing significantly as nitrogen level increased (Figure 6.4 and Figure 6.).

Both Green and Red Coral showed similar yields (Figure 6. and Figure 6.), and both were significantly higher than Salanova (P=0.013).



Figure 6.4 Marketable fresh weight. (LSD=5.73 across nitrogen levels)



Figure 6.5. The effect of nitrogen on yield. 150, 100, 50 & 0 kg N/ha from Left to Right.

Percentage Biomass

The percentage biomass was calculated from the fresh weight and dry weight measurements of the entire mass of harvested lettuce plants.

The results show that lettuce grown at 50 kg N/ha had a significantly lower water content and a higher percentage of structural cell components than lettuce grown at both 100 & 150 kg N/ha (P = 0.003) (Figure 6.).

When analysed by variety, Green & Red Coral had a similar percentage biomasses (Figure 6.), however Salanova had a significantly lower water content (P <0.001). These results indicate that there is a difference between the structural segregation of the leaves of the different varieties of lettuce.



Figure 6.6. Percentage dry weight to fresh weight (with standard error) - An indication of the ratio of structural components to water content of the lettuce (LSD=2.569 by nitrogen level, LSD=1.537 by variety).

Shelf Life

The shelf Life was measured as days until the end of shelf life at 0°C, and was determined based on the criteria described in Chapter 2.

When the analysis was done with all the varieties combined 0 & 100 kg N/ha had a significantly shorter shelf life than the 50 & 150 kg N/ha treatments (Figure 6.). However, Figure 6.7 also shows that Salanova had a significantly shorter shelf life that Green & Red Coral under all nitrogen levels except 150 kg N/ha.

The analysis also showed that Green & Red Coral had a significantly longer shelf life, over three days more on average than Salanova (P=0.034).

Shelf life was then re-analysed without Salanova to determine the effect of nitrogen on the shelf life of Green & Red Coral (Figure 6.8). When the results for Salanova were taken out of the analyse, nitrogen level was shown to have a significant effect on the shelf life of Green & Red Coral (Figure 6.8). The nitrogen rates of 0, 100 & 150 kg N/ha were shown to give the shortest shelf life, while those plants receiving 50 kg N/ha (half the industry standard) had a significantly longer shelf life (P = 0.006) up to four days longer compared to Coral lettuce at 0 kg/ha (Figure 6.8) This is up to six days longer than the average shelf life of Salanova lettuce.



Figure 6.7. Shelf life (with standard error) by nitrogen levels with all varieties combined.



Figure 6.8. Shelf life (with standard error) of Green & Red Coral against nitrogen levels with Salanova excluded.
6.4 Discussion

It was also hypothesised that an increase in leaf thickness and a lower percentage of water within the leaf may lead to an extended shelf life. In this experiment this hypothesis was true where Green Coral leaves were significantly thicker than Salanova (P<0.001) leaves and Green Coral had a significantly longer shelf life than Salanova in these experiments (P=0.034; Figure 6.).

This relationship was also noted with respect to nitrogen levels, with 50 & 100 kg N/ha treatments producing thicker leaves compared to plants grown with 150 kg N/ha. The 50 kg N/ha treatment had the longest shelf life for Green & Red Coral (P=0.006). The link between leaf thickness and shelf life is also supported by the fact that Salanova lettuce had significantly thinner leaves than all other varieties (P <0.001), and also had the shortest shelf life (P=0.034; Figure 6.). More work is needed to quantify this relationship further. It may be possible to select new baby leaf varieties based on leaf morphology with one important characteristic being leaf thickness.

In terms of leaf biomass lettuce grown at a rate of 50 kg N/ha had a significantly lower water content and higher percentage of leaf cellular structure than treatments of 100 & 150 kg N/ha nitrogen (P=0.003; Figure 6.) which visually related to the smaller leaves of plants in this treatment. A reduction in leaf water content correlated with the shelf life results when Green & Red Coral were analysed separately from Salanova. Green & Red Coral showed a significantly longer shelf life at 50 kg N/ha nitrogen compared to 100 & 150 kg N/ha (P=0.006). This suggests that the increased fresh weight to dry weight percentage and the increased leaf thickness has lead to an increased shelf life in Green & Red Coral. Unfortunately the 50 kg N/ha treatment also had a reduced biomass yield (Linker and Johnson-Rutzke 2005). These results indicate that there needs to be a compromise between yield achieved with high nitrogen application and a longer shelf life achieved with lower levels of applied nitrogen.

With Salanova, the cause of the lower shelf life is not as clear. When the results for Salanova lettuce were analysed separately to Green & Red Coral the shelf life response to nitrogen level applied was inconsistent. Other data showed that Green & Red Coral had a higher chlorophyll level than Salanova (P=0.030), indicating a great capacity for photosynthesis. In turn both Green & Red Coral had a longer shelf life that appeared to be correlated to an increased dry weight percentage and leaf thickness. The difference between the varieties may be due to a difference in photosynthetic capacity linked to structural growth but more work is needed to understand the differences more clearly.

The results from this experiment show that an increase in shelf life as a result of a lower application rate of nitrogen comes at a significant cost. Analysis of marketable yields from this experiment showed that by halving nitrogen rates, marketable yield is also halved. So while it may be desirable to increase the shelf life of the processed product, it appears that it will come at a commercial cost as yields will be halved.

6.5 Conclusion

This research has shown that it is feasible to increase the shelf life of baby leaf lettuce through the reduction in the rate of application of nitrogen levels applied to the plant during growth. However it appears that the commercially bred Salanova variety responds to nitrogen in a different way to the Coral types. For all treatments Salanova lettuce had a shorter shelf life.

In Green & Red Coral, the dry weight percentage appeared to give an indication of the potential shelf life. The data from this experiment did support the anecdotal evidence that plants grown with high nitrogen application had a reduced shelf life but whilst a reduction in the level of nitrogen applied has the potential to increase shelf life, it also decreases marketable yield. There is a commercial compromise between marketable yield and shelf life in relation to the level of nitrogen applied to baby leaf lettuce.

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7 The effect of moisture absorbing sachets and vacuum cooling on the shelf life of baby leaf spinach and lettuce

7.1 Introduction

The key factors that ensure an optimum postharvest shelf life of baby leaf spinach and lettuce include rapid cooling after harvest and the delivery of a cool product to the factory, a processing line that minimises the physical injury to the product during processing, good packaging and a consistent cold chain from the factory to the consumer.

The most important postharvest handling technique available is temperature management but it is also the technique most commonly misused. Many commercial operators are not aware of the impacts on quality and shelf life of a delay in cooling or of storage at a warmer incorrect temperature. As a result they do not use cooling to its maximum advantage.

The type of packaging used for the product is also an important area that is difficult to manage. In some situations the presence of condensation in the bag can have a negative effect on quality. Condensation causes solutes from the cut surface to leak out and this provides an ideal environment for bacteria growth. Bacteria contribute to the soft breakdown of the tissue in the package. Ideally excess moisture should be removed during drying to minimise this problem.

These experiments aimed to demonstrate the impact of delayed cooling and the presence of excess moisture in the bags on the quality of baby leaf spinach and lettuce. These are two important areas where improvements can be made in managing these baby leaf products and in turn, extend the shelf life.

7.2 The Effect of Moisture Reduction on the Quality of Baby Leaf Spinach and Lettuce

7.2.1 Materials and Methods

For these experiments the contents of each bag was weighed after manual drying with absorbent paper towel. The comparison of the initial weights allowed an estimate of leaf-surface moisture to be calculated. The average value of moisture per bag was found to be 11g for spinach and 14 g for baby leaf lettuce after normal commercial processing.

Sachets containing silica gel were placed into bags to absorb some of the moisture during storage. Each sachet could absorb approximately 11 - 15g of moisture and was similar in weight and dimensions as one of the condiment sachets that are normally included in the bag. The product shelf life was evaluated and compared to

that of control bags, containing material that was processed and packaged during the same production run.

All bags were stored at 7°C after processing as this was the commercial practice for quality evaluation.

The bags from the spinach trial were assessed for quality 9, 11, 12, 13, 15 and 16 days after processing and for the mescalin lettuce 6, 8 and 9 days after processing. The quality was assessed by turning out the contents of the bag and rating for the following characteristics on a 1-5 scale where 1 = worst and 5 = best: visual; crispiness; odour; taste; damage; consistency; and, freshness. These rating were then summed for each assessment to give a total score out of 35. Shelf life was defined as the number of days taken for the total score to fall below 13. ANOVAs were carried out in the summed score data, and for both spinach and mescalin lettuce each treatment was replicated 6 times (n=6).

The Mesculin salad mix was chosen to represent baby leaf lettuce as it contains velvet and coral lettuce leaf types.

Refer to Chapter 2 for more detailed methodology in terms of crop growth and management.

7.2.2 Results and Discussion

The results show that having an absorbent sachet in the bag increased the shelf life of the baby leaf spinach by about 1 day (Figure 7.1) and that there was no significant extension in the days to fail for baby leaf lettuce (Figure 7.2). These results may seem insignificant in terms of the improvement in total shelf life but the main factor is that there was an improvement in the quality of the drier product at each sampling time. This result was also achieved at a storage temperature of 7°C which is not the ideal temperature for storing baby leaf products and a much greater extension could be achieved with a cooler storage temperature. However 7°C is the temperature used by OneHarvest for quality evaluation as it is indicative of what happens in commercial practice.

Excess moisture in the bag reduces the shelf life of the product by encouraging the leakage of solutes from the cut surfaces into the water droplets on the surface. These water droplets, rich in nutrients become ideal sites for bacterial growth. The bacteria cause the soft breakdown of the tissue which makes the product unsaleable.



Figure 7.1: Quality of baby leaf spinach packaged with or without a moisture absorbent sachet.



Figure 7.2: Quality of baby leaf mescalin lettuce packaged with or without a moisture absorbent sachet.

7.2.3 Conclusion

The best solution to the problem of condensation in the bag would be to have a more effective drying system to minimise the water in the bag at the time of packing as adding sachets to the bags creates health and safety issues for processing.

If sachets were the only option available then more work is needed to optimise this system. The sachet used in this trial had a small surface area and ideally this would be increased and the absorbent could also be improved by using a different salt that dried the air to the equilibrium relative humidity of the processed product to prevent dehydration.

7.3 The effect of delayed vacuum cooling on the quality of baby leaf spinach compared to forced air cooling.

Fresh-cut products generally have a higher respiration rate than the corresponding intact products. Higher respiration rates indicate a more active metabolism and usually a faster deterioration rate. The most important aspect of postharvest handling of fresh cut products is refrigeration at as close to 0°C as possible.

Storage temperature affects the respiration rate and the rate of deterioration of the product with products stored at temperatures close to 0°C having the longest shelf life. Any period of temperature abuse will shorten the shelf life of the product.

One option for cooling baby leaf spinach is vacuum cooling. Vacuum cooling works by evaporating water from the product at very low atmospheric pressures. Products that easily release water cool in 20 to 30 minutes.

Vegetables that have a high surface to mass ratio and that release water easily, such as leafy vegetables are best suited to this method of cooling. Vacuum cooling causes about 1% product weight loss for each 6°C of cooling. Some coolers can spray water on the surface of the product before or during cooling to reduce the level of weight loss. The water must be disinfected and in some cases the rapid release of the vacuum can cause some vegetables to have a water-soaked appearance.

7.3.1 Materials and Methods

Freshly harvested baby leaf spinach was used at Cox farms, Bairnsdale, and the leaves treated according to Table 7.1. While harvested material was waiting for vacuum cooling, it was held in the shed which had an ambient temperature of 21°C. Once the treatments were applied, the cooled product was stored at 2°C in the cool room on the farm and then transported in a refrigerated truck to the Vegco (OneHarvest) factory at Bairnsdale for shelf life assessment. Data loggers were placed in all treatments.

The product processing and assessments were carried out by Vegco and AHR staff at the Bairnsdale factory. On receival at the factory, spinach was packed on Line 5 at a length of 310mm into standard WW Spinach film (42EL90), see Table 7.2 for details.

Treatment No.	Ingredient	Treatment Method
1	Baby Spinach	Hydro-vac 1/2 hour after harvest
2	Baby Spinach	Hydro-vac 2 hours after harvest (initially store in shed under ambient conditions)
3	Baby Spinach	Hydro-vac 4 hours after harvest (initially store in shed under ambient conditions)
4	Baby Spinach	Hydro-vac 6 hours after harvest (initially store in shed under ambient conditions)
5	Baby Spinach	Therma-fresh forced air cool as current standard practice (no vacuum).

Table 7.1: Cooling Treatments

The machine settings were set to pack standard spinach bags at a length of 310mm. About 100 bags were packed off (10 cartons) of each trial to gain relevant processing data and for shelf life assessments. All relevant data was noted down.

All packed products were kept at 7°C for shelf life purposes. All trials were sent for microbiological testing at the end of shelf life. There were no problems with the samples in terms of the microbiological results for all samples in this trial. Samples were washed through the Line 3 "Tsunami Bath" and spun in the NON Dryer (using the existing baby leaf setting – Spinach No 6). The product was caught into Blue crates at the end of Line 3 and was labelled and kept separately to be packed later.

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Bag Size	480 x 310mm				
Gas Flush (O2)	NA	NA	NA	NA	NA
Film Type	42 EL 90				
Pack weight (g)	150g	150g	150g	150g	150g
Order Multiple	10	10	10	10	10
Carton Type used	NO1	NO1	NO1	NO1	NO1

Table 7.2: Packing Details

7.3.2 Results and Discussion

The report from the trimming room on the samples processed through the factory is outlines in Table 7.3. These values are within normal ranges for the factory including minor bruising.

Description	Hydro Vac cooled within 0.5 hour	Hydro Vac cooled within 2 hours	Hydro Vac cooled within 4 hours	Hydro Vac cooled within 6 hours	Forced air cooling within 0.5 hour
No. of people involved in the trial	2	2	2	2	2
Time taken from start to finish	6min	6min	6min	6min	6min
Raw material quality Baby Spinach	Minor bruising on spinach	Minor bruising on spinach	Minor bruising on spinach	Minor bruising on spinach	Minor bruising on spinach
Raw material issued Baby Spinach	~30kg	~30kg	~30kg	~30kg	~30kg

Table 7.3: Trim Room Results

Figure 7.3 shows the effect of delays in cooling on the shelf life of processed spinach and Figure 7.4 shows the damage score of the same material. Spinach that was vacuum cooled within half an hour of harvest had the best shelf life. The product that was forced air cooled within half an hour of harvest performed as well as the product that had a 2 hour delay in vacuum cooling.



Figure 7.3 Effect of Vacuum cooling treatments on the shelf life of baby leaf spinach.



Figure 7.4 The effect of delayed hydro vacuum cooling on the 5 defects of baby leaf spinach compared to forced air cooling.

Vacuum cooling 6 hours after harvest resulted in the poorest quality and the shortest shelf life. The results demonstrate the benefits of vacuum cooling as quickly as possible, ideally within half an hour of harvest to ensure maximum shelf life.

7.3.3 Conclusions

Reducing moisture in the bags

The results show that having an absorbent sachet in the bag improved the quality of baby leaf spinach and lettuce. This is an important result as it demonstrates the fact that the current processing can be improved.

Postharvest temperature management

Baby leaf spinach must be vacuum cooled with in half an hour of harvest to ensure maximum shelf life.

Baby leaf spinach that was vacuum cooled 6 hours after harvest had the highest level of defects and in turn the shortest shelf life. Delays in cooling have a significant impact on the quality of baby leaf products.

Vacuum cooling within half an hour of harvest improved product quality compared to forced air cooling half an hour after harvest.

8 Crop scheduling in baby leaf spinach

8.1 Introduction

The baby leaf sector in Australia has developed from grower processors to contract growers supplying processors, who both require accurate prediction of harvest date and specific quantities. The accurate prediction of time of harvest is critically important for baby leaf production where the optimum size of leaf occurs inside a one day window and the harvested leaf has a relatively short shelf life (Everaarts, 1999).

8.2 Materials and Methods

Five distinct climatic regions were selected along the eastern coast of Australia: Boisdale in East Gippsland (Victoria) representative of cool temperate environment (cool in the winter and warm in the summer); Camden, (NSW) south of Sydney in Hawkesbury/Nepean basin representative of a warm season temperate environment; Gympie in Southeast Queensland representative of a sub tropical environment for optimum winter production; Stanthorpe in south east Queensland representative of a sub tropical environment for midsummer, high altitude production.

Three contract commercial growers were used; Ryan McLeod (Australian Fresh Salads) at Gympie and Stanthorpe, Edgar Grech (Grech Farms) at Camden and Bill Taylor (Taylor Farms) at Boisdale. All trials were planted at a density of 760 plants/m² using a specialised baby leaf seeder (Seed Spider[®], New Zealand).

The varieties planted were:

- Crocodile (Rijk Zwaan); a dark green slow growing variety for warm season growing conditions with semi savoyed thick leaves, very strong against leaf burn in hot periods and high yielding.
- Roadrunner (Rijk Zwaan): dark green with thick leaves, for the transition (shoulder) period growing season (autumn and spring), upright growth habit, uniform sized leaves and very high yielding.
- Parrot (Rijk Zwaan), vigorous growing, erect spinach for cooler cold growing conditions with round dark green glossy uniform leaves being the industry standard for cold season production.

Site:	Camden (NSW)	Boisdale (Vic)	Gympie (QLD)	Stanthorpe QLD)
Design:	Randomised Complete Block Design	Randomized Complete Block Design	Randomized Complete Block Design	Randomized Complete Block Design
Number of Trials	11	12	4	Two (2) 1 hailed out
Replication:	Six (6) blocks x three treatments per block	Four (4) blocks x 3 treatments per block	Four (4) blocks x 3 treatments per block	3 random samples per treatment (36 in total
Plot Size	1m x 9m x 6 rows x 75 mm	1m x 5m x 8 rows x 125 mm apart	1m x 10 m long plots	1m x 10 m long plots
Samples	2 random samples per treatment (36 in total)	3 random samples per treatment (36 in total	3 random samples per treatment (36 in total	3 random samples per treatment (36 in total
Seeder:	Seed Spider	Seed Spider	Seed Spider	Seed Spider
Spinach Variety	Crocodile	Crocodile	Crocodile	Crocodile
Spinach Variety	Parrot	Parrot	Parrot	Parrot
Spinach Variety	Roadrunner	Roadrunner	Roadrunner	Roadrunner

Table 8.1. Experimental design for the crops at the different locations

The methods used for assessment of leaf thickness and yield are outlined in Chapter 2 - Materials & Methods.

The three varieties were selected after consultation with the three grower's stakeholders and representatives from vegetable seed companies who breed spinach and distribute it around Australia. They all agreed that currently Rijk Zwaan dominate the varieties used commercially in Australia and supply approximately 70% of the domestic seed requirements. For developing the crop scheduling model the use of slow growing warm weather types, a shoulder type and a vigorous growing winter type provided sufficient genetic diversity to allow 'new' varieties in the future to be placed into one of the three groups. Graded high germination seed of the three varieties was acquired for all of the four sites and 29 seeding dates.

8.3 Results and Discussion

8.3.1 Boisdale

Days from emergence to harvest

The data collected from the 12 plantings at Boisdale summarised in Table 8.1, Table 8.2 and Table 8.3 for the varieties Crocodile, Roadrunner and Parrot respectively.

Days from emergence to harvest for the three varieties follow a similar shaped curve, taking 55-60 days from an early winter seeding to as quick as 21-22 days during mid summer and increasing out to 30 plus days in late autumn/early winter.

Location Boisdale	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	17/05/06	26/05/06	22/07/06	88	154	9	57	482	490	6.7	17.9	3.2	13.8	13.5	N/A	N/A
2	15/06/06	28/06/06	20/08/06	117	183	13	53	486	492	3.0	14.8	3.2	15.1	16.9	1.08	0.64
3	5/07/06	17/07/06	4/09/06	137	198	12	49	483	486	3.8	15.5	3.9	15.9	16.6	0.91	0.57
4	1/08/06	11/08/06	16/09/06	164	210	10	36	384	385	4.1	16.5	4.3	16.9	19	0.72	0.43
5	5/09/06	14/09/06	20/10/06	199	244	9	36	480	474	5.3	20.2	5.4	21.2	16.1	1	0.61
6	11/10/06	18/10/06	22/11/06	235	277	7	35	496	487	7.0	21.8	7.3	21.3	10.4	0.92	0.5
7	16/11/06	22/11/06	19/12/06	271	304	6	27	440	428	8.2	24.6	8.5	24.2	19.2	0.6	0.4
8	19/12/06	24/12/06	13/01/07	304	329	5	20	392	374	13.0	25.8	12.9	26.0	19.4	0.59	0.39
9	31/01/07	5/02/07	28/02/07	347	375	5	23	480	451	14.7	28.1	15.4	28.5	9.6	0.67	0.43
10	27/02/07	4/03/07	27/03/07	374	402	5	23	394	383	11.6	24.2	10.9	23.7	19.9	0.68	0.38
11	4/04/07	10/04/07	7/05/07	410	443	6	27	401	401	8.7	21.0	8.8	21.0	12.8	0.66	0.37
12	26/04/07	3/05/07	11/06/07	432	478	7	39	465	465	6.7	17.9	6.3	17.7	12.1	0.86	0.6

Location Boisdale	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	17/5/06	26/5/06	21/7/06	88	153	9	56	473	481	6.7	17.9	3.3	13.7	16.7	N/A	N/A
2	15/6/06	28/6/06	16/8/06	117	179	13	49	448	453	3.0	14.8	3.3	14.9	19.1	0.8	0.48
3	5/7/06	17/7/06	31/8/06	137	194	12	45	430	431	3.8	15.5	3.5	15.7	19.9	0.72	0.4
4	1/8/06	11/8/06	15/9/06	164	209	10	35	371	372	4.1	16.5	4.2	17.0	19.2	0.57	0.34
5	5/9/06	14/9/06	19/10/06	199	243	9	35	467	461	5.3	20.2	5.5	21.2	15.2	0.75	0.44
6	11/10/06	18/10/06	20/11/06	235	275	7	33	453	449	7.0	21.8	6.9	20.8	11.4	0.76	0.39
7	16/11/06	22/11/06	19/12/06	271	304	6	27	440	428	8.2	24.6	8.5	24.2	14.3	0.73	0.35
8	19/12/06	24/12/06	12/1/07	304	328	5	19	356	338	13.0	25.8	13.1	26.1	18.6	0.48	0.32
9	31/1/07	5/2/07	27/2/07	347	374	5	22	459	430	14.7	28.1	15.4	28.6	9.8	0.54	0.33
10	27/2/07	4/3/07	27/3/07	374	402	5	23	394	383	11.6	24.2	10.8	23.7	19.4	0.52	0.28
11	4/4/07	10/4/07	5/5/07	410	441	6	25	372	372	8.7	21.0	8.8	21.1	12.5	0.5	0.3
12	26/4/07	3/5/07	8/6/07	432	475	7	36	435	436	6.7	17.9	6.5	17.9	13.1	0.67	0.44

Table 8.2 Roadrunner (Boisdale, Vic)

Table	8.3	Parrot	(Boisdale.	Vic)
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Location Boisdale	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	17/05/06	26/05/06	20/07/06	88	152	9	55	467	474	6.7	17.9	3.3	13.7	20.2	N/A	N/A
2	15/06/06	28/06/06	16/08/06	117	179	13	49	448	453	3.0	14.8	3.3	14.9	22.7	0.7	0.39
3	5/07/06	17/07/06	28/08/06	137	191	12	42	396	398	3.8	15.5	3.5	15.5	24.3	0.62	0.32
4	1/08/06	11/08/06	11/09/06	164	205	10	31	325	326	4.1	16.5	4.3	16.5	23	0.49	0.28
5	5/09/06	14/09/06	15/10/06	199	239	9	31	416	411	5.3	20.2	5.1	21.0	18	0.6	0.36
6	11/10/06	18/10/06	19/11/06	235	274	7	32	435	431	7.0	21.8	6.8	20.6	12.4	0.67	0.36
7	16/11/06	22/11/06	18/12/06	271	303	6	26	423	411	8.2	24.6	8.4	24.4	15.3	0.56	0.34
8	19/12/06	24/12/06	10/01/07	304	326	5	17	334	320	13.0	25.8	13.2	25.7	21.1	0.44	0.26
9	31/01/07	5/02/07	28/02/07	347	375	5	23	480	451	14.7	28.1	15.4	28.5	8.5	0.49	0.3
10	27/02/07	4/03/07	26/03/07	374	401	5	22	380	368	11.6	24.2	10.9	23.8	18.4	0.49	0.25
11	4/04/07	10/04/07	6/05/07	410	442	6	26	387	387	8.7	21.0	8.9	21.0	12.6	0.45	0.3
12	26/04/07	3/05/07	5/06/07	432	472	7	33	411	412	6.7	17.9	6.9	18.3	15.5	0.65	0.44



Figure 8.1 Days to emergence – Boisdale, Vic

A quadratic model accounted for 90-92% of the variability in the duration from emergence to harvest maturity for each variety. For Crocodile, Roadrunner and Parrot (Figure 8.1) seeded in May 2006 through to April 2007 these quadratic models, for Emergence to Harvest Maturity (EHM) are based on chronological time and can be used by growers to plan a spinach sowing schedule for all year round production.

Degree day model for Boisdale

A degree day model was developed using 0°C as the base temperature as well as the minimum cut-off temperature and 27°C as the maximum cut-off temperature and a similar model was developed without a high temperature cut-off. The results are shown in Table 8.1, Table 8.2 and Table 8.3. The aim is to develop a robust model that can be used from year to year by collecting seasonal temperature data.

The mean degree days calculated from the mode (0°C BT/27°C cut-off) was 443, 420 and 404 for Crocodile, Roadrunner and Parrot respectively from emergence to harvest (Figure 8.2).



Figure 8.2 Degree day model – Boisdale, Vic

8.3.2 Camden

Days from emergence to harvest

In Camden the data was collected from 11 plantings and the results are summarised in Table 8.4, Table 8.5 and Table 8.6 respectively for the spinach varieties Crocodile, Roadrunner and Parrot.

Table 8.4	Crocodile	(Camden,	NSW)

Location Camden	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	8/05/06	16/05/06	25/06/06	79	127	8	40	465	452	4.8	18.4	4.6	18.0	25.8	N/A	N/A
2	10/06/06	20/06/06	5/08/06	112	168	10	46	466	474	3.1	17.4	3.3	17.5	21.2	1.25	0.73
3	3/07/06	14/07/06	19/08/06	135	182	11	36	415	417	3.4	18.5	4.3	18.7	25.6	1.02	0.58
4	29/07/06	7/08/06	9/09/06	161	203	9	33	425	426	4.5	20.5	4.7	21.0	25.1	0.99	0.58
5	18/09/06	25/09/06	19/10/06	212	243	7	24	461	413	8.9	26.9	9.1	26.4	7.9	0.95	0.44
6	25/10/06	30/10/06	24/11/06	249	279	5	25	515	473	11.4	27.4	11.4	27.8	19.9	0.85	0.48
7	25/11/06	29/11/06	21/12/06	280	306	4	22	467	437	14.8	28.5	14.6	28.0	23.8	0.89	0.38
8	28/12/06	1/01/07	18/01/07	313	334	4	17	398	365	16.3	29.7	16.4	30.2	25.4	0.62	0.35
9	31/01/07	4/02/07	26/02/07	347	373	4	22	510	482	17.7	28.7	17.7	28.8	16.7	0.67	0.45
10	5/03/07	9/03/07	2/04/07	380	408	4	24	483	472	15.0	25.7	14.6	25.6	12.6	0.77	0.49
11	2/04/07	7/04/07	3/05/07	408	439	5	26	437	436	10.9	23.5	10.8	23.2	21.4	0.83	0.52

Location Camden	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0C BT E-H	Accum- ulated DD 27 oC Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	8/05/06	16/05/06	24/06/06	79	126	8	39	457	444	4.8	18.4	4.7	18.2	27.2	N/A	N/A
2	10/06/06	20/06/06	4/08/06	112	167	10	45	464	472	3.1	17.4	3.2	17.5	24.8	1	0.53
3	3/07/06	14/07/06	17/08/06	135	180	11	34	391	393	3.4	18.5	4.3	18.5	26.7	0.79	0.42
4	29/07/06	7/08/06	7/09/06	161	201	9	31	397	399	4.5	20.5	4.4	21.3	25.7	0.7	0.38
5	18/09/06	24/09/06	18/10/06	212	242	6	24	442	395	8.9	26.9	9.0	26.3	10.8	0.69	0.38
6	25/10/06	30/10/06	24/11/06	249	279	5	25	491	452	11.4	27.4	11.3	27.6	17.65	0.68	0.46
7	25/11/06	29/11/06	20/12/06	280	305	4	21	444	415	14.8	28.5	14.5	28.0	25.6	0.6	0.3
8	28/12/06	1/01/07	17/01/07	313	333	4	16	373	342	16.3	29.7	16.2	30.1	23.6	0.58	0.34
9	31/01/07	4/02/07	26/02/07	347	373	4	22	510	482	17.7	28.7	17.7	28.8	14.8	0.52	0.32
10	5/03/07	9/03/07	30/03/07	380	405	4	21	432	421	15.0	25.7	15.4	25.8	13.4	0.65	0.45
11	2/04/07	7/04/07	2/05/07	408	438	5	25	422	421	10.9	23.5	10.8	23.2	19.6	0.62	0.4

Table 8.5 Roadrunner (Camden, NSW)

Location Camden	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	8/05/06	16/05/06	22/06/06	79	124	8	37	435	422	4.8	18.4	4.5	18.3	36.7	N/A	N/A
2	10/06/06	20/06/06	1/08/06	112	164	10	42	435	443	3.1	17.4	3.3	17.5	32	0.9	0.51
3	3/07/06	14/07/06	15/08/06	135	178	11	32	364	367	3.4	18.5	4.4	18.2	34.6	0.8	0.37
4	29/07/06	7/08/06	5/09/06	161	199	9	29	372	374	4.5	20.5	4.2	21.5	30.5	0.59	0.31
5	18/09/06	24/09/06	16/10/06	212	240	6	22	404	359	8.9	26.9	8.8	26.4	17.1	0.69	0.31
6	25/10/06	30/10/06	24/11/06	249	279	5	25	491	452	11.4	27.4	11.3	27.6	17.6	0.6	0.36
7	25/11/06	29/11/06	20/12/06	280	305	4	21	444	415	14.8	28.5	14.5	28.0	25	0.64	0.28
8	28/12/06	1/01/07	18/01/07	313	334	4	17	398	365	16.3	29.7	16.4	30.2	24.3	0.51	0.32
9	31/01/07	4/02/07	25/02/07	347	372	4	21	489	460	17.7	28.7	17.7	29.0	15	0.56	0.3
10	5/03/07	9/03/07	29/03/07	380	404	4	20	414	403	15.0	25.7	15.5	25.9	15.7	0.57	0.38
11	2/04/07	7/04/07	1/05/07	408	437	5	24	407	406	10.9	23.5	10.8	23.2	21.9	0.58	0.33

The days from emergence to harvest for the three cultivars follow a similar shaped curve taking a maximum of 46 days during the cooler months and being as quick as 16-17 days in mid summer, increasing out to 26-30 days in the spring and autumn (Figure 8.3).



Figure 8.3 Days from emergence to harvest – Camden, NSW

A quadratic model accounted for 86-87% the variability in the duration from emergence to harvest.

Degree day model for Camden

A degree day model was developed for Camden using 0°C as the base temperature as well as the minimum cut-off temperature and 27°C as the maximum cut-off temperature and a similar model was developed without a high temperature cut-off. The results are shown in (Table 8.4, Table 8.5 and Table 8.6).

The mean degree days for Camden (0°C BT/27°C high temperature cut-off) was 441, 421 and 406 respectively from emergence to harvest (Figure 8.4)



Figure 8.4 Degree day model – Camden, NSW

8.3.3 Gympie

Days from emergence to harvest

Data generated from four (4) plantings (AFS ran out of irrigation from Widgee Creek restricting the number of seeding dates in September- November from the number originally planned). The data is summarised in Table 8.7, Table 8.8 and Table 8.9 for Crocodile, Roadrunner and Parrot respectively.

Table 8.7 Crocodile (Gympie, SE QId)

Location Gympie	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27 °C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	19/04/06	23/04/06	15/05/06	60	86	4	22	398	390	9.8	27.4	9.3	27.0	14.6		
2	16/05/06	21/05/06	17/06/06	87	119	5	27	380	381	6.8	23.7	5.9	23.5	9.4		
3	14/06/06	20/06/06	18/07/06	116	150	6	28	428	428	7.9	22.6	8.0	22.6	20.4		
4	26/07/06	1/08/06	28/08/06	158	191	6	27	410	404	5.8	25.1	5.3	25.1	23.3		

Table 8.8 Roadrunner (Gympie, SE Qld)

Location Gympie	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	19/04/06	23/04/06	15/05/06	60	86	4	22	398	390	9.8	27.4	9.3	27.0	13.5		
2	16/05/06	21/05/06	15/06/06	87	117	5	25	366	367	6.8	23.7	6.0	23.5	9.3		
3	14/06/06	20/06/06	18/07/06	116	150	6	28	428	428	7.9	22.6	8.0	22.6	21.2		
4	26/07/06	1/08/06	28/08/06	158	191	6	27	410	404	5.8	25.1	5.3	25.1	19.8		

Location Gympie	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg- ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha	Oldest Leaf thick- ness mm	Youngest Leaf thickness mm
1	19/04/06	23/04/06	14/05/06	60	85	4	21	381	373	9.8	27.4	9.4	27.1	17.5		
2	16/05/06	21/05/06	13/06/06	87	115	5	23	336	337	6.8	23.7	5.9	23.6	12.2		
3	14/06/06	20/06/06	15/07/06	116	147	6	25	380	380	7.9	22.6	7.7	22.7	20.5		
4	26/07/06	1/08/06	26/08/06	158	189	6	25	369	364	5.8	25.1	4.7	25.0	23.9		

The days from emergence to harvest followed a similar curve for the three varieties (moon shaped) taking 21-22 days in late summer to 28 days in winter, coming back to 26 days in early spring (Figure 8.5.)



Figure 8.5 Days from emergence to harvest – Gympie, SE Qld

A quadratic model accounted for 98-99% of the variability in the duration from emergence to harvest for Crocodile, Roadrunner and Parrot respectively.

Degree day model for winter production at Gympie

A degree day model was developed using 0°C as the base temperature as well as the minimum cut-off temperature and 27°C as the maximum cut-off temperature and a similar model was developed without a high temperature cut-off. The results are shown in (Table 8.7, Table 8.8 and Table 8.9).

The mean degree days for winter production in Gympie (0°C BT/27°C high temperature cut-off) were 401, 397 and 364 for Crocodile, Roadrunner and Parrot respectively, from emergence to harvest (Figure 8.6).



Figure 8.6. Degree day model – Gympie, SE Qld

8.3.4 Stanthorpe

Days from emergence to harvest

The data generated from two plantings at Stanthorpe during the summer (2006-2007) is summarised in Table 8.10, Table 8.11 and Table 8.12 for Crocodile, Roadrunner and Parrot respectively. The harvest date for planting seeded 20th December 2006 was inspected late on 13th January 2007 by Brad Giggins (AHR) and judged to be ready for harvest the next morning. Late on 13th January 2007 a severe hail storm shredded the trial. The optimum harvest dates are as recorded however no yields were obtained.

Table 8.10 Crocodile (Stanthorpe, SE Qld)

Location Stanthorpe	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg-ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha
1	20/12/06	24/12/06	14/01/07	305	330	4	21	416	401	14.0	25.8	13.9	25.8	n/a
2	31/01/07	4/02/07	1/03/07	347	376	4	25	529	521	15.9	26.8	15.7	26.6	15.7

Table 8.11 Roadrunner (Stanthorpe, SE Qld)

Location Stanthorpe	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg-ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha
1	20/12/06	24/12/06	14/01/07	305	330	4	21	416	401	14.0	25.8	13.9	25.8	n/a
2	31/01/07	4/02/07	1/03/07	347	376	4	25	529	521	15.9	26.8	15.7	26.6	14.4

Table 8.12	Parrot (St	anthorpe	, SE QId)											
Location Stanthorpe	Planting date	Emerg -ence date	Harvest date	Planting Julian day	Harvest Julian day	Days from planting to emerg-ence	Days from E-H	Accum- ulated DD 0°C BT E-H	Accum- ulated DD 27°C Cut off E-H	Growing Season Mean (Min)	Growing Season Mean (Max)	Growing Season Mean E-H (Min)	Growing Season Mean E-H (Max)	Yield t/ha
1	20/12/06	24/12/06	13/01/07	305	329	4	20	393	378	14.0	25.8	13.7	25.7	n/a
2	31/01/07	4/02/07	28/02/07	347	375	4	24	506	498	15.9	26.8	15.6	26.6	15.6

Days from emergence to harvest for Crocodile and Roadrunner range from 21-25 days while Parrot matured earlier in 20-24 days.

Degree day model for summer production in Stanthorpe

The mean degree day (0°C BT/27°C high temperature cut-off) for Crocodile, Roadrunner and Parrot from the 31 January 2007 planting was significantly higher than late summer data from Camden and Boisdale; 521 GDD for Crocodile and Roadrunner and 498 GDD for Parrot. A possible explanation is that the 25mm of rain that fell on 11 February 2007 in a violent storm severely compacting the granite soil and also caused nutrient leaching that may have delayed maturity. In addition the minimum average temperature was >15°C which could have resulted in increased respiration.

8.3.5 Commercial Data – Boisdale 2006-2007

While collecting the comprehensive trial data from Taylor Farms at Boisdale, data was collected from commercial plantings on the same configuration as the trials. Harvesting however was undertaken on a commercial demand basis rather than at an optimum leaf size for the scheduling data. This is commercially realistic but not as precise as the trial data used to develop the model.

The commercial varieties used were Crocodile for the summer months, Parrot for the late autumn, winter and early spring and Roadrunner as a transition variety for autumn and spring harvesting.

All the commercial data generated from 2006-07 is summarised in Table 8.13, Table 8.14 and

Table 8.15 for Crocodile, Roadrunner and Parrot respectively. The harvest date is the actual harvest date if only one harvest occurred, or the mid-harvest date if multiple harvests occurred especially in the cooler growing periods.



Figure 8.7 Days from emergence to harvest - spinach Crocodile (Boisdale)

The actual commercial schedule was not an accurate fit due to severe shortages of baby leaf spinach in the warm summer months with the Vegco field officers requesting the grower harvest at a smaller leaf specification. The shape of the curve is however very similar. It is just that the leaves were harvested as young as 15 days after emergence.



Figure 8.8 Days from emergence to harvest – spinach Roadrunner (Boisdale).

The actual commercial harvest schedule for Roadrunner was the closest fit to the predicted model (R^2 =0.84) illustrating the predictable and versatile nature of this transitional variety of spinach.



Figure 8.9 Days from emergence to harvest – spinach Parrot (Boisdale).

The actual commercial harvest schedule for Parrot was quite accurate (R^2 =0.77) which was surprising as Bill Taylor scheduled Parrot in the late autumn and into the winter of 2007. The actual commercial data took longer than predicted, in part due to a cold snap in June and July 2007.

Location (Boisdale)	Planting date	Emergence date	Harvest date	Planting Jday	Harvest Jday	Days from planting to emergence	Days from emergence to harvest
1	29/11/2006	5/12/2006	27/12/2006	284	312	6	22
2	7/12/2006	12/12/2006	2/01/2007	292	318	5	21
3	15/12/2006	20/12/2006	8/01/2007	300	324	5	19
4	21/12/2006	26/12/2006	15/01/2007	306	331	5	20
5	29/12/2006	3/01/2007	20/01/2007	314	336	5	17
6	4/01/2007	9/01/2007	24/01/2007	320	340	5	15
7	12/01/2007	17/01/2007	8/02/2007	328	355	5	22

Table 8.13 Commercial Crocodile 2006-2007 (Boisdale, Vic)

Location (Boisdale)	Planting date	Emergence date	Harvest date	Planting Jday	Harvest Jday	Days from planting to emergence	Days from emergence to harvest
1	23/08/2006	2/09/2006	6/10/2006	186	230	10	34
2	10/09/2006	19/09/2006	19/10/2006	204	243	9	30
3	12/09/2006	21/09/2006	19/10/2006	206	243	9	28
4	20/09/2006	29/09/2006	24/10/2006	214	248	9	25
5	4/04/2007	11/04/2007	10/05/2007	410	446	7	29
6	10/04/2007	17/04/2007	15/05/2007	416	451	7	28
7	18/04/2007	25/04/2007	28/05/2007	424	464	7	33
8	27/04/2007	4/05/2007	5/06/2007	433	472	7	32

Table 8.14 Commercial Roadrunner 2006-2007 (Boisdale, Vic)

Table 8.15 Commercial Parrot 2006-2007 (Boisdale, Vic)

Location	Planting	Emergence	Harvest date	Planting	Harvest	Days from	Days from
(Doisdale)	uale	uale		Judy	Judy	emergence	to harvest
1	7/06/2006	20/06/2006	2/08/2006	109	165	13	43
2	14/06/2006	27/06/2006	14/08/2006	116	177	13	48
3	21/06/2006	4/07/2006	15/08/2006	123	178	13	42
4	28/06/2006	11/07/2006	25/08/2006	130	188	13	45
5	5/07/2006	17/07/2006	29/08/2006	137	192	12	43
6	14/07/2006	26/07/2006	7/09/2006	146	201	12	43
7	1/08/2006	11/08/2006	12/09/2006	164	206	10	32
8	8/08/2006	18/08/2006	22/09/2006	171	216	10	35
9	17/08/2006	27/08/2006	1/10/2006	180	225	10	35
10	1/09/2006	10/09/2006	13/10/2006	195	237	9	33
11	8/09/2006	17/09/2006	18/10/2006	202	242	9	31
12	2/05/2007	3/05/2007	14/06/2007	438	481	1	42
13	9/05/2007	18/05/2007	26/06/2007	445	493	9	39
14	15/05/2007	24/05/2007	14/07/2007	451	511	9	51
15	22/05/2007	31/05/2007	25/07/2007	458	522	9	55
16	29/05/2007	7/06/2007	7/08/2007	465	535	9	61

8.4 Conclusions

The formula for calculating Growing Degree Days (GDD) is

Daily GDD = $((T_{max} + T_{min}) \div 2) - T_{base}$

where:

Tmax = the daily maximum air temperature °C Tmin = the daily minimum air temperature °C Tbase = the GDD base temperature below which no significant growth occurs.

For our model, the base temperature = 0° C and if the measured T_{max} ever exceeded 27°C, then 27°C was used in place of the actual maximum for calculating degree days.

In order to produce a usable set of GDDs, an estimate of the GDD requirement from seeding to emergence was predicted to be110 GDD. This estimated was made using the data from these trials.

In summary, the growing degree day requirements for the three spinach types used in the project grown in either location are given in Table 8.16 and Table 8.17.

 Table 8.16 Spinach Growing Degree day requirement for four regions from emergence to harvest

Emergence to harvest	E. Gippsland GDD	Camden GDD	Gympie GDD	Stanthorpe GDD	T _{base} (°C)	T _{max} (°C)
Crocodile	443	441	401	521	0	27
Roadrunner	420	421	397	-	0	27
Parrot	404	406	364	498	0	27

Table 8.17 Spinach Growing Degree day requirement for four regions from seeding to harvest

Seeding to harvest	E. Gippsland GDD	Camden GDD	Gympie GDD	Stanthorpe GDD	T _{base} (°C)	T _{max} (°C)
Crocodile	553	551	511	631	0	27
Roadrunner	530	531	507		0	27
Parrot	514	516	474	608	0	27

These GDD estimates can be used by growers to manage planting and harvest dates over the year and they will provide a more accurate method of crop scheduling than planting and harvesting on a calendar basis.

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9 A germination comparison between wild rocket (Diplotaxis tenuifolia) & cultivated rocket (Eruca sativa).

9.1 Introduction

It is widely acknowledged that multiple variables are important during the germination of seeds. These variables include; available moisture, seed depth, air and soil temperature, and seed desiccation (Akanda et al., 1996; Blackshaw, 1990, 1992; Blackshaw and Brandt, 2002; Horak and Sweat, 1994; Horak and Wax, 1991; Ray et al., 2005). Knowledge of the conditions which optimise seed germination is crucial for the commercial production of field grown rocket.

Few papers have compared the differences in germination success between wild rocket (*Diplotaxis tenuifolia*) and cultivated rocket (*Eruca sativa*) at either constant or diurnal temperatures. The aim of this study was to identify the optimum temperature for germination of *Diplotaxis tenuifola* and *Eruca sativa* and to determine if natural diurnal temperature fluctuations have an effect on germination levels.

9.2 Methods and Materials

Two germination experiments used three baby leaf rocket species; *Diplotaxis tenuifolia* (wild rocket), *Hesperis matronalis* (sweet rocket), and *Eruca sativa* (cultivated rocket). Although taxonomically sweet rocket and cultivated rocket are different, commercially they are considered the same and are collectively referred to as cultivated rocket. For this reason both of these species have been combined into the same germination experiments and will be referred to throughout this report simply as cultivated rocket.

Experiment One evaluated germination counts of individual species/varieties over a period of ten days, under a range of different constant temperatures, ranging from 5-35°C in increments of 5°C.

Experiment Two also evaluated the respective species/varieties over a period of ten days. Treatments were alternatively exposed to twelve hour diurnal or variable temperature regimes. The variable temperature exposures were chosen to closely replicate actual seasonal day/night ambient temperature fluctuations. This experiment had three diurnal temperature exposures, each of which represent major seasons of plant development, being summer (16-28°C), winter (5-18°C) and an intermediate season representing spring and autumn (10-24°C).

9.2.1 Experiment One - constant temperatures

Experiment one had seven different temperature regimes of 5, 10, 15, 20, 25, 30, and 35°C. The selection of these temperatures was based on previous rocket germination studies conducted by Ray et al. (2005). Within each temperature regime, there were four replicates of 25 seeds per species/variety. A total of seven rocket species/varieties were assessed with wild rocket making up three of these, sweet rocket accounting for one treatment, and cultivated rocket the remaining three treatments (Table 9.1). The seeds were placed on two damp grade 1803 Filtech[®] filter paper sheets and put into glass Petri dishes with a diameter of 100 mm. The Petri dishes were then sealed using PM-992 Para film to prevent moister evaporation during the experiment. A total of four replicates per species/variety per temperature regime were assessed for germination over a period of ten days. The experiment was repeated three times which is consistent with other rocket germination work conducted by Ray et al. (2005), to ensure that sufficient and reliable data was collected.

Species/Variety No.	Species	Seed supplier
1	Diplotaxis tenuifolia	Lefroy valley Pty Ltd
2	Diplotaxis tenuifolia	Fairbanks Seed Co.
3	Diplotaxis tenuifolia	Seminis Ltd.
4	Hesperis matronalis	South Pacific Seeds Pty Ltd
5	Eruca sativa	Fairbanks Seed Co.
6	Eruca sativa	Fairbanks Seed Co.
7	Eruca sativa	Fairbanks Seed Co.

 Table 9.1 Treatment list for both constant and diurnal germination experiments.

9.2.2 Experiment Two - diurnal temperatures

Experiment Two had three different alternating or diurnal temperature regimes per treatment. The temperature regimes were designed to closely replicate actual day/night temperature fluctuations experienced during seasonal field germination at Ellis Lane, New South Wales latitude 33° and longitude 150°. The following temperature regimes imposed a 12 hour exposure of each combination of 16-28°C (summer), 5-18°C (winter) and 10-24°C (intermediate, combined spring/autumn). A total of seven species/varieties were used and these covered the three commonly grown Rocket species (Table 1). Individual temperature regimes had four replicates of 25 seeds per seed treatment. The seeds were placed on two damp grade 1803 Filtech[®] filter paper sheets and put into glass Petri dishes with a diameter of 100mm. The Petri dishes were then sealed using PM-992 Para film to prevent moister evaporation during the experiment. A total of four replicates per species/variety per temperature regime were assessed for germination over a period of ten days. The experiment was repeated a total of three times (Ray et al., 2005).
9.2.3 Data collection

Individual experiments ran for ten days with germination data being recorded every day during this period. This enabled the measurement of germination vigour and total germination for each given species/variety. The point at which germination was considered to have been reached was when the first visual appearance of the radical protruding from the epidermis was greater than 2mm.

9.2.4 Statistical analysis

The data was stacked and analysed using the general analysis of variance, with temperature and species/variety number being converted to factors. The analysis of individual species/varieties, individual days, and total germination percentages were conducted using this function (GenStat[®] (9th edition)). Differences between treatments and species/variety were considered to be significant when P<0.05 and when the difference between treatment means were greater than that of the least significant difference (LSD) 5%.

9.3 Results

9.3.1 Germination of rocket species at constant temperatures

There were significant differences between the total germination counts of wild and cultivated rocket at all temperatures except for 25°C. At 25°C some of the different species where not statistically different from each other, in particular species/variety 2 (93.33%), species/variety 6 (92.33%) and species/variety 7 (95.33%) did not show significantly different germination rates (Figure 9.1)



Figure 9.1 Mean germination percentage of wild rocket (*Diplotaxis tenuifolia*) circles 1-3, and Cultivated rocket (*Eruca sativa | Hesperis matronalis*) squares 4-7, after 10 days exposure to various constant temperatures.



Figure 9.2. Comparison of germination between wild rocket (*Diplotaxis tenuifolia*) left column, and cultivated rocket (*Eruca sativa | Hesperis matronalis*) right column, over one (i & iv), two (ii & v) and three (iii & vi) days of seed exposure to constant temperatures.

Significant differences were identified from 25-35°C between all wild rocket varieties on day 1, with variety 2 recording the highest germination percentage over this temperature range, Figure 9.2 (i). Significant differences were also identified at 20, 30 and 35°C between all wild rocket varieties on day 2, with variety 2 recording the highest germination at 20°C and 35°C, and variety 1 at 30°C, Figure 9.2 (ii).

At 25°C on day 2, variety 3 was significantly different to both varieties 1 and 2. On day 3 all wild rocket varieties were significantly different at 15°C, with variety 1 recording the highest germination percentage at this temperature, Figure 9.2 (iii). At 20, 25, and 30°C on day 3, variety 3 was significantly different to both varieties 1 and 2, and recorded the highest germination over this range. At 35°C on day three, variety 2 was significantly different to both varieties 1 and 3, recording the lowest germination on this day.

Significant differences were identified at 15°C for all species/varieties on day 1 for cultivated rocket, with species/variety 6 recording the highest germination at this temperature, Figure 9.2 (iv). Significant differences were also identified on day 2 for all species/varieties at 10 and 15°C, with species/varieties 4 and 5 respectively recording the highest germination, Figure 9.2 (v). On day 3 all species/varieties were again determined to be significantly different from each other at 10°C with species/variety 5 achieving the highest germination, Figure 9.2 (v).

Alternating Temperature Regimes Germination % 0-24 16-28 -18 Species Number

9.3.2 Germination of rocket species at alternating diurnal temperatures

Mean Germination Percentage of Two Rocket Species at

Figure 9.3. Mean total germination percentage of wild rocket (*Diplotaxis tenuifolia*) 1-3, and cultivated rocket (*Eruca sativa / Hesperis matronalis*) 4-7, after ten days exposure to various diurnal temperature regimes.

Significant differences in germination percentage were found between species/varieties of rocket (P<0.001; LSD=2.293). Species/variety 3 was found to be significantly different to all others across all diurnal temperature regimes (10-24, 53 seeds; 16-28, 61 seeds; 5-18, 18 seeds).



Figure 9.4. Comparison of germination vigour between wild rocket (*Diplotaxis tenuifolia*) left column, and cultivated rocket (*Eruca sativa / Hesperis matronalis*) right column, over one (i & iv), two (ii & v) and three (iii & vi) days of seed exposure to constant temperatures.

Figure 9.4 shows the difference in germination between the different species of wild and cultivated rocket. There are no clear trends although the wild rocket had the highest germination percentage on day 2 at the $16 - 28^{\circ}$ C treatment and the cultivated rocket had the highest germination percentage on day 1 at the $10 - 24^{\circ}$ C and the $16 - 28^{\circ}$ c treatments.

9.4 Discussion

The three rocket species examined; *Diplotaxis tenuifolia* (wild rocket), *Hesperis matronalis* (sweet rocket), and *Eruca sativa* (cultivated rocket) showed significantly different germination patterns when subjected to both constant and varied diurnal temperature regimes. Individual species/varieties also exhibited significantly different germination patterns within the same temperature

treatments, how individual seed species/varieties response to temperature was not consistent.

This observed difference within species/varieties is most likely due to the large genetic diversity and original seed collection techniques. The environmental conditions in which seed development and formation occurs has been shown to remarkably influence seed germination and dormancy (Gutterman, 1992a, 1992b, 1994; Wulff, 1995; Perez-Garcia, 1997; Villamil et al., 2002).

At constant temperatures there was a significant difference in the germination percentage of individual seed samples at temperatures above and including 25°C. At temperatures below this, no significant differences were observed between species/variety samples. The results indicated that species/variety number 3 had comparatively poor germination at temperatures between 25°C and 30°C, and species/variety 1 had poor germination at temperatures > 35°C. The response curve indicates that the optimal germination temperature for *Diplotaxis sp* (wild rocket) was between 20-30°C (Figure 9.1), with a 62% to 93% total germination rate inside this range.

The variation within individual species did not have any clear relationship with temperature or time. The comparison between species on the same day, however, was significant, indicating that there were different seed vigour responses to temperature. *Eruca sp.* (European rocket) was shown to germinate faster than *Diplotaxis sp.* (wild rocket) which tended to have a delayed germination response. For example, *Eruca sp.* showed 85% germination after 1 days exposure to summer diurnal temperature, whereas *Diplotaxis sp.* showed only 65% germination after 3 days.

The results from the alternating diurnal temperature treatments indicate the importance of this physical factor on the rate and speed of germination, particularly for *Diplotaxis sp.* (wild rocket) (Figure 9.3). Variety number 3 from the *Diplotaxis sp.* was significantly different from both other varieties in this species. The individual relationship within species and across species under diurnal temperature conditions was however not clear.

From these results it can be concluded that the optimal temperature for germination of *Eruca sativa* (European rocket) is 10-30°C while a narrower range is required by *Diplotaxis tenuifolia* (wild rocket) which is best germinated at 20-30°C.

This study has identified the ideal temperature ranges for germinating the two commonly grown rocket species, *Diplotaxis tenuifolia* and *Eruca sativa*. There were no significant differences in total germination when the seeds were exposed to varied diurnal temperature regimes. Both species with the exception of one of the *Diplotaxis sp*. (#3), achieved >90% germination across the three diurnal temperature regimes after 10 days.

In a commercial context where diurnal temperature differences apply, the results show that *Eruca sativa* (European rocket) germinates more quickly than *Diplotaxis tenuifolia* (wild rocket) but the delay is not commercially significant 10 days after seedling.

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10 The effect of direct seeding versus transplanting on baby leaf plant establishment

10.1 Introduction

In the Baby leaf production little research has been conducted to evaluate the advantages and disadvantages of direct seeding versus transplanting crops.

If we look at traditional iceberg lettuce production for example, we see that the vast majority of lettuce produced is established from seedlings. Both forms of establishment have inherent advantages and disadvantages ranging from cost and shelf life to thermo-dormancy and evenness of plant stand. The following data was generated from a series of three summer trials conducted at Stanthorpe, in south-east Queensland I an effort to provide scientific data to demonstrate the advantages of both methods of plant establishment.

10.2 Methods and Materials

All varieties for each of the respective trials were either direct seeded or transplanted on the same day using commercial equipment. Direct seeded treatments were planted using a Seed Spider[®], transplanted treatments were transplanted using a standard "peg planter". Growth and performance data was collected throughout the life of each trial. Each variety was harvested and assessed as they reached "commercial" maturity.

Transplanted varieties were planted at a density of 36 plants per square metre which is considered standard for Salanova type lettuce. The Direct seeded "standard" type red and green coral were planted at 400-500 plants per square metre. Each variety was replicated 3 times in the trial in a 20 metre section of bed. Standard fertiliser, watering and pesticide treatments were applied to all plots. On the day of harvest a 0.31 m x 0.31 m (0.1 m²) wire quadrant was used to randomly sample a subsection of each plot. All material inside this quadrant was harvested. It was from this material that all assessments were made. Characteristics measured and calculated included fresh weight yield, weight of 30 standard leaves, leaf thickness, percentage dry matter and shelf life. A total of 3 sub-samples were taken from each of the 3 replicates.

10.3 Results and Discussion

In the warmer summer months establishing lettuce from seed under field conditions can be very difficult. High temperature during germination can lead to thermodormancy in lettuce and this can greatly reduce plant stand establishment. Several methods to circumvent this dormancy have been proposed including using resistant germplasm, adjusting the seed production environment, the use of growth hormones, and seed priming (Nascimento, 1999). Many of the practices mentioned by Nascimento (1999) are now common practice when direct seeding lettuce in Australia.

Early plant stand counts were made during each of the trials. For direct seeding a typical establishment target is somewhere around 650-800 plants per square metre.

As can be seen from Table 10.1, early plant stand establishment varied greatly. This can have obvious effects on final yield and also plant habit and time to harvest.

Trial	Establishment Counts (Green coral)	Establishment Counts (Red coral)
Planting 1	371	227
(January)*	552	611
(February)*	552	011
Planting 3 (March)*	477	481

Table 10.1: 1 Average plant stand establishment* This months refers to harvest date not planting date.

All three trials showed an improvement in shelf life (Figure 10.1, Figure 10.2 and Figure 10.3) of between 2 and 3 days when transplanted lettuce was used rather than seeded lettuce. This represented a 17-25% improvement in shelf life. Conservatively, it seems reasonable to expect at least a 2 day improvement in shelf life in the S.E. Queensland summer from using transplanted lettuce instead of direct seeding.

There was no significant relationship between the days to harvest and the shelf life for these trials. However, with only 6 days difference between the longest and shortest crops, the difference in days to harvest was probably too small to impact on shelf life.



Figure 10.1. January Planting - Shelf life of direct seeded and transplanted baby leaf lettuce (Average of all varieties) - Stanthorpe 2008



Figure 10.2. February Planting - Shelf life of direct seeded and transplanted baby leaf lettuce (Average of all varieties) - Stanthorpe 2008



Figure 10.3. March Planting - Shelf life of direct seeded and transplanted baby leaf lettuce (Average of all varieties) - Stanthorpe 2008

The results also showed that higher yields (Figure 10.4 and Figure 10.5) were achieved using transplants compared to direct seeding. On average the yields were about 0.55 kg/m² (23%) lower than the direct seeded lettuce.



Figure 10.4. Yield of direct seeded and transplanted red and green coral baby leaf lettuce varieties - Stanthorpe - combined summer (Jan-Mar 08) average.



Figure 10.5. Yield of direct seeded and transplanted red and green coral baby leaf lettuce varieties – Planting 1 (Jan), Planting 2 (Feb) & Planting 3 (Mar) - Stanthorpe 2008.

The difference in plant stand can be seen very clearly in Figure 10.6 where there are lots of gaps between the plants in the direct seeded photograph (right) compared to the transplanted plants (left).



Figure 10.6 Transplanted Salanova (left) and direct seeded (right) "red coral" types at 27 days post transplanting and seeding.

10.4 Conclusions

The results show that transplanting rather than direct seeding lettuce in south east Queensland has many advantages. In particular transplanted red and green coral varieties have a longer shelf life and a higher yield compared to direct seeded plant stands.

One of the reasons for this difference in shelf life and yield may be that transplanted lettuce is planted at a lower density. Direct seeded stands of baby leaf lettuce have many more plants/ m² than transplants, resulting in a stand of thinner, more upright leaves. Through competition for light, these dense plants may be forced to grow more rapidly, laying down less structural cell material and as shown previously (Chapter 3 and 6) this relates to a shorter shelf life and yield.

The commercial recommendation from these trials would be that growers should use transplanted seedlings rather than direct seedling for the establishment of baby leaf lettuce.

10.5 References

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11 The effects of plant density on harvest quality in baby leaf spinach (Spinacia oleracea)

11.1 Introduction

Plant density is an important agronomic consideration as it affects the plants light use and dry matter portioning (De Camacaro et al., 2004.) At high density the plant habit is more up right than at low density as the plants try and intercept more light. Leskovar and Stein (2000) found that spinach grown at a high spacing had a higher leaf area, leaf number, leaf dry matter and root dry weight. Their work was done on semi-savoy spinach rather than baby leaf spinach but it is likely that the same principles will apply to baby leaf production.

This work aimed to identify the optimum spacing for baby leaf production. The optimum spacing needs to deliver a commercial yield for the grower as well as a long shelf life for the consumer. As seen in previous work on establishment of lettuce in Chapter 10 there is a compromise between maximum yield and shelf life.

11.2 Methods and Materials

During the winter of 2006, five density trials were established in southeast Queensland. The planting dates in Gympie were 19 April, 14 June, and the 26 July. Another Gympie trial was planned for mid May however weather conditions and a lack of suitable country prevented this trial from being planted. An alternative trial was planted at Wheatvale on 16 Oct 2006 and the last at Stanthorpe on 31Jan 2007.

For these trials the spinach variety Whale (RZ) was used in all plantings. A Seed Spider[®] commercial planter was calibrated to deliver the targeted seeding density for each treatment. However, the final plant population sometimes varied from the targeted population. All treatments were replicated in a randomized, complete block design.

The treatments were agronomically managed using commercial practice. Harvest timing was determined by the average leaf length cut under commercial conditions. The specification used was that no harvested material was to exceed 120mm-140mm in length from the cut petiole to the end of the leaf blade. Fresh yield (g/m²), average leaf weight (mg), leaf thickness (mm) and shelf life (days)

were measured for all varieties to determine the varieties that performed best under commercial conditions.

11.3 Results and Discussion

Gympie Trials

The target and actual plant populations varied between trials (Figure 11.1, Figure 11.2 and Figure 11.3). However, in all three trials, plant density and fresh yield were well correlated (R^2 =0.759-0.952), with higher densities consistently resulting in higher fresh yields. This suggests that an optimum planting density could be established for main stream varieties and these optimums used to maximize yield. In reality however planting at higher densities comes with its own set of challenges. Firstly, many of the lines of hybrid spinach seed are expensive, so that the additional cost of seed would have to be offset by a sufficient yield increase to justify the added expense.



Figure 11.1 Gympie Trial 1 – Planted 19 April 2006 – Fresh yield and plant population.



Figure 11.2 Gympie Trial 2 – Planted 14 June 2006 – Fresh yield and plant population.



Figure 11.3 Gympie Trial 3 – Planted 26 July 2006 – Fresh yield and plant population.

The average leaf weight and density were negatively correlated ((Figure 11.4, R^2 =0.990), with higher densities yielding lower average leaf weights. The average leaf weight was calculated from a random sub-sample of 30 leaves collected from the original sample. As density increases, competition for light, nutrient and space also increase resulting in smaller leaves (Figure 11.4) (De Camacaro, 2004).



Figure 11.4 Gympie Trial 2 – Planted 14 June 2006 - Harvested leaves at different densities



Figure 11.5 Gympie Trial 3 – Planted 26 July 2006 - Plant population and average leaf weight

It is important to nots some other quality observations. In particular, Sclerotinia leaf spot appeared to be slightly more prevalent in some of the higher density treatments in all three trials. The higher densities, above 600 plants/m² also showed more erect cotyledons on young plants. This could lead to a higher percentage of cotyledons cut at harvest in higher density plantings. The

cotyledons at harvest are often yellow and senescent and are undesirable harvest contaminants in the harvested product and subsequent salad mixes.

Wheatvale trials

In the Wheatvale density trial, similar results relating yield and density were recorded (data not shown). In the Wheatvale trial a detailed study of the relationship between plant density and leaf thickness was carried out. Previous work described in Chapter 3 described a potential link between leaf thickness and shelf life, with thicker leaves having a longer shelf life.

The results in Figure 11.6 show a strong link between "old leaf thickness" and plant density, where lower densities resulted in thicker leaves. The trend for "young leaf thickness" was similar but not as strong. The thickness of the old leaves refers to the average thickness of the second true leaf, while "young leaf thickness" refers to the thickness of the youngest fully expanded leaf at harvest, usually true leaf number six to eight.



Figure 11.6 Wheatvale – Planted 16 Oct 2006 – Density and average leaf width

Stanthorpe Trials

The previous trials focussed on the effect of plant density on crop yield. In Stanthorpe the trial investigated the relationship between planting density and shelf life. The hypothesis was that as planting density increased average leaf size, weight and thickness were reduced and this resulted in a shorter shelf life. There was some anecdotal evidence from the industry to support this hypothesis. Figure 11.7 shows that there was a significant negative correlation (R^2 =0.993) between planting density and shelf life, as density increased the shelf life decreased.



Figure 11.7 Stanthorpe – Planted 31 Jan 2007 - Density and shelf life.

11.4 Conclusions

The results from these trials provide the industry with some important agronomic information by highlighting the compromise that must be made between planting density, yield and shelf life. Increasing planting density increases fresh weight yield, it also resulted in smaller leaves and a shorter shelf life. The shelf life is reduced at densities of greater than 750-900 plants/linear metre.

These results also validate previous research such as the impact of seasonal climate (Chapter 3) and plant establishment method (Chapter 10) where leaf thickness can be used as an objective measure of the potential shelf life of baby leaf spinach and where planting density, direct seeding versus transplants affects shelf life. However in many situations the optimum production system for baby leaf spinach must be compromised to match the commercial market demand for a continuous supply of baby leaf product into the factory.

These trials also highlighted the fact that growers must adjust their seeding equipment to account for seed size (75,000-147,000 seeds/kg) and germination viability (87-99%) of spinach seed batches in order to achieve the appropriate seeding density. The trials also suggested that higher plant densities appear to be correlated with a higher incidence of Sclerotinia leaf spot a damaging disease of baby leaf spinach.

11.5 References

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12.1 Phenmedipham (Betanal[®]) Herbicide Tolerance Trial – Spinach (Spinacia oleracea)

12.2 Introduction

Weeds are defined as any plant that crowds out cultivated plants and they tend to thrive at the expense of the more refined edible or ornamental crops. Weeds compete with the commercial crop for space, nutrients, water and light, although how seriously they will affect a crop depends on a number of factors. Some crops have greater resistance to the negative impacts of weed than others but slower growing seedlings are more likely to be overwhelmed than those that are larger and more vigorous (Wikipedea, 2008).

Spinach is a small seeded crop that is negatively impacted by weeds in terms of yield and quality. In terms of quality, there is a zero tolerance for weeds in the processed product. Despite the importance of weed control in baby leaf spinach there are few herbicides that are registered for use on baby leaf salad crops. This is due to the fact that it is a relatively new industry. The aim of this trial was to determine the effectiveness of the commercially available herbicide Betanal[®] (active ingredient, phenmedipham; Bayer CropScience Pty Ltd) for weed control in baby leaf spinach.

12.3 Methods and Materials

During Mar 2006, a number of small strips were sprayed in a 4 true leaf stage crop of spinach cv. "Whale" RZ, using a conventional boom spray with standard flat-fan jets, 300 kPa pressure and 200 L total water volume/ha producing a medium-fine droplet spectrum. Each strip (plot) was 20 metres long and a single bed in width. Four treatments were applied – 0, 1.5, 3.0 & 5.0 L/ha of Betanal[®] (157 g phenmedipham/L; Bayer CropScience Pty Ltd) and each treatment was replicated twice. Treatments were applied 9 Mar 06 (Time: 3:30-4:30 pm; Temp: 26°C; Relative humidity ~65%; winds light and variable up to 5 km/h; sky: approx. 20% cloud-cover). The crop was visually assessed for weeds 6 days after treatment (DAT) on 15 Mar 06 and then again immediately prior to harvest.

12.3.1 Results

In this trial severe phototoxicity was observes 6 days after treatment for all herbicide rates1 (Figures 12.1 to 12.4). Unfortunately, the high level of damage was sustained through to harvest. In order to prevent phytotoxicity it is recommended that rates below 1.5 L/ha be used. However at rates of 1.5-3.0

L/ha wild radish (*Raphanus raphanistrum*) the main weed of spinach was reduced but control was only achieved at rates of 5.0 L/ha.



Figure 12.1 4 leaf Crop & 3-4 leaf wild radish at spraying (9 Mar 06)



Figure 12.2 Betanal® 1.5 L/ha 6 DAT (15 Mar 06) Note – Most severe damage to wild radish but crop damage still unacceptable.



Figure 12.3 Betanal® 3.0 L/ha 6 DAT (15 Mar 06)



Figure 12.4 Betanal® 5.0 L/ha 6 DAT (15 Mar 06)

12.3.2 Conclusion

The results showed that the herbicide Betanal® (a.i Phenmedipham) was phytotoxic to baby leaf spinach grown in SE Queensland at rates of 236 g a.i./ha (1.5 L Betanal/ha). The registered label rate 5.5 - 8.5 L/ha for other crops. At the low rate required to prevent phytotoxicity on spinach the weed control is compromised with only small weeds being controlled.

This results from this field trial showed that a review needed to be done to identify other potential herbicides that could be used for weed control in baby leaf spinach crops.

12.3.3 References

Wikipedia (2008) http://en.wikipedia.org/wiki/Weed_control (Viewed Dec 2008).

12.4 Review of registered and prospective herbicide options for weed control in Australian spinach (Spinacia oleraceae) production

12.4.1 Overview

Herbicide options for weed control in spinach (*Spinacia oleraceae*) can be broadly described as intermediate to poor in comparison to other vegetables. Currently, no selective herbicides are registered for use in spinach in Australia, although a number of herbicides may well be made available under the APVMA (Australian Pesticides and Veterinary Medicines Authority) minor use permit system.

The primary reason for there being few herbicide options available either locally or overseas is that spinach is a minor crop, only representing a small proportion of arable land use. Most global investment by the chemical industry in herbicides is focussed on the major crops (grown over the largest area) which obviously represent a much better return on any research and development investment.

The second major reason for few herbicide options for spinach is related to a lack of metabolic selectivity for this crop. As a member of the family Chenopodiaceae, spinach is highly susceptible to many of the selective herbicides developed for use in other crops that are used to control weeds belonging to the same family, which in Australia include fat hen (*Chenopodium album*), clammy goosefoot (*Chenopodium pumilio*) and Tumbleweed (*Salsola kali*). There are 302 species belonging to the family in Australia, many of which are regarded as weeds (Various authors, Flora of Australia, 1984). The following review describes some of the weed control options that could be available for baby leaf spinach production.

12.4.2 Soil Fumigants

Metham

The main fumigant still available to growers is metham sodium. As with the nonselective herbicides, metham has generalised label instructions not specifically written for spinach. It's use in Eastern Australia is relatively limited, although it has been more extensively used in Western Australia. It is broadly effective as a pre-emergent control on a range of weeds but is normally used only when pests, especially nematodes and diseases need to be controlled prior to planting. It also requires specialised application equipment to seal the metham in the ground; this involves using large quantities of product and water and it is a relatively expensive treatment. Plant-back times for cropping after treatment can be several weeks, especially in heavy soils and it is usually necessary to plant some indicator seedlings (such as lettuce) to see whether the metham has sufficiently dissipated so as not to cause crop injury. Fumigation is generally not a favoured method for weed control as it destroys beneficial soil organisms as well as undesirable ones.

12.4.3 Non-selective ("knock-down") Herbicides

As with many vegetable crops having few or no selective herbicide options available, growers rely heavily on reducing weed burdens by controlling emerged weeds outside of the crop growing period. This is achieved by a combination of mechanical (cultivating) and chemical methods. The major herbicides used for this purpose are glyphosate as well as the bipyridyls - paraquat and/or diquat in various combinations (Gramoxone[®], Spray.Seed[®] and Reglone[®]). After application, these herbicides are generally tightly bound to foliage, organic matter and soil (the clay fraction in particular). This extremely tight adsorption prevents these herbicides from being taken up by plants subsequently sown (whether seeds or seedlings) in the treated area. Label recommendations for the use of these herbicide are of a general (not crop specific) nature.

As glyphosate is extremely systemic, it is capable of moving from foliage to roots and therefore killing a broad range of both annual and perennial weeds even if they are well established. However, diquat and paraquat are contact herbicides and their activity damages cell membranes, causing cell leakage before any significant translocation can occur. As a result the bipyridyls are mostly effective only on smaller weeds with poorly established root systems. Nonetheless, the extremely rapid activity of bipyridyls means weeds can be effectively sprayed and crops seeded immediately afterwards (hence the tradename Spray.Seed).

While glyphosate takes considerably longer to kill weeds, it is rapidly absorbed and translocated. This means that it is possible to spray and then plant within several hours. However, despite it being generally bound up quickly by soil and organic matter, there is some evidence that glyphosate may have some limited residual capacity to cause plant damage in very sandy soils or where sprayed weeds (Blowes, 1987 & Campbell, 1974) may come into contact with emerging or planted seedlings (Lynch & Penn, 1980). It is therefore recommended that heavy weed infestations show complete plant death or desiccation (no green material) before new crop planting occurs (Monsanto Roundup CT label, 2008).

Some bipyridyl registrations in Australia, eg. Gramoxone and Spray.Seed also permit use post-sowing but pre-emergence of the crop (Syngenta label handbook, 2008-09) and post-emergence of weeds (colloquially referred to sometimes as the "hair-cut" method of control). However, extreme caution needs to be exercised with this technique, because the longer the application is delayed after sowing, the higher the risk of the crop emerging and being damaged or killed.

In-crop use of bipyridyl products such as Gramoxone are also permitted for postemergent inter-row spraying where shielded sprayers can be effectively used without any risk of spray drift touching the crop (Syngenta label handbook, 200809). However, this is not likely to be practically possible in any situation where spinach is currently grown.

Application rates vary widely according to the target weed spectrum and are beyond the scope of this review. Suffice to say manufacturers labels should be referred to for selecting rates appropriate for species and growth stage.

Another non-selective chemical that has a technical fit for baby leaf spinach production, although there is no current registration or permit allowing its use in Australia, is glufosinate-ammonium (Basta[®]). Like glyphosate, it is considered to have a much higher degree of user safety than the bipyridyl herbicides. It is a contact herbicide like the bipyridyls, though its speed of activity is slower, taking several days rather than several hours to be bound in the plant or soil. Other than these technical distinctions glufosinate does not offer any particular advantages for controlling weeds compared with glyphosate or the bipyridyls.

Inhibitors of protoporphyrinogen oxidase (PPOs) (CropLife Australia, 2008) -Oxyfluorfen (Goal[®]) and carfentrazone (Hammer[®]) are contact herbicides most commonly used as additives or "spikes" with the other non-selective herbicides already mentioned for improving the control and speed of kill on certain weeds they are particularly active on. However, as with glufosinate, there are no registrations or permits in place for use in spinach in Australia. They also have some soil persistence which may result in plant-back problems for spinach if it is especially sensitive to them.

12.4.4 Selective Herbicides –Pre-emergent (and) Early Postemergent

s-metolachlor

The most extensively researched and widely used pre-emergent herbicide in spinach both locally and globally is metolachlor and its more recently resolved isomeric form s-metolachlor (Dual[®] Gold). While s-metolachlor has never been registered for use in spinach in Australia, permits for its use under the APVMA minor use permit system have allowed the herbicide to be used by growers commercially in the past.

The minor use permit system was introduced and continues to evolve to help smaller agricultural industries have access to agricultural chemicals to address particular crop protection problems these industries face in the absence of complete domestic registration packages for the crops in question. The main requirements for such a permit is that the proposed product has an existing Australian registered use in one or more food crops and that sufficient residue studies in the prospective crop are available using appropriate rates and application timings and that these studies have been submitted for evaluation by the APVMA to determine whether a use pattern is likely to result in acceptable residues or not. Permits are granted for a specified period, typically up to several years at a time before an applicant must then apply to renew the permit or request a full registration package is submitted by a manufacturer.

Permit PER 5729 was issued in Mar 2003 and was in force until Mar 2008 (NRA, 2003). It allowed growers to apply s-metolachlor at 1440-1920 g a.i./ha (1.5-2.0 L Dual Gold/ha) immediately before or after sowing, in both spinach and silverbeet (*Beta vulgaris*). These rates of application are among the higher rates recommended on the Australian Dual Gold label, suggesting spinach is not known to be particularly sensitive to s-metolachlor. However, the permit did not allow growers to apply post-emergence of the crop, most likely because of crop safety concerns based on field experience or inadequate data to support this use pattern. It is active mainly on weed seeds immediately after germination, being taken up through the coleoptile. It is generally ineffective on weeds and crops once they have emerged but can still be sufficiently taken up in sensitive plants to cause phytotoxicity, mainly seen as stunting and often accompanied by chlorosis and purpling at the leaf margins. While no permit is in place at the time of this report (02 Sep 2009), there is a reasonable expectation that this will be renewed (Dal Santo, pers comm, 2008) within the next few months.

Globally, s-metolachlor is used in corn, cotton, soybeans and sorghum and is particularly effective on annual grass weeds but controls a range of broadleaf weeds as well (Syngenta label handbook, 2008-09). As with most pre-emergence herbicides, appropriate use rates are likely to vary with soil texture. Generally higher rates of metolachlor are required for similar herbicidal activity on heavier soils than on sandy soils and crop tolerance on heavier soils is correspondingly higher. This is due to the herbicide binding to the clay and organic fraction of the soil. However, a study in the USA showed spinach was generally tolerant to preemergent applications of s-metolachlor at rates up to 1060 g a.i./ha on all soil types (Fennimore et al, 2001). However, at 1570 g a.i./ha s-metolachlor reduced shoot dry weight by 10%. While s-metolachlor does not have a general registration for use in spinach in the USA, a Section 24(c) Special Local Need Label permits use in spinach in specific states, eg. California (US EPA), allows a single application of 360-1060 g a.i./ha applied pre-emergence of weeds and crop (spinach).

Propachlor

Research in the USA has shown propachlor (Ramrod[®]), another chloroacetamide, is well tolerated by spinach when applied at 6 lb/acre (6736 g/ha) as a pre-plant incorporated treatment, although it appears to have no current registrations for commercial use in any crop in the USA. Propachlor is sold for use in a range of vegetable crops in the UK (Monsanto UK Ramrod product label 2008) at rates up 6240 g a.i./ha, from pre-planting through to early crop development, but not in spinach and like s-metolachlor it is used to control a range of annual grass and broadleaf weeds (Monsanto UK product label 2008). In Australia, propachlor is still registered for use in other crops but not currently for use in spinach. While a lower industry priority, it has been identified as worthy of investigation for the issuing of a permit (Dal Santo, pers comm, 2008) in spinach at a later date.

Dimethenamid-P

Dimethenamid-P (Outlook[®]) has been registered in the USA and elsewhere overseas for some years mainly in the corn and soybean market. It has not yet been registered in Australia, although intent to register the product (Frontier-P[®]), appeared in the APVMA Gazette 8, 2 August 2007. Dimethenamid-P, like metolachlor is a chloroacetamide and would be classified in Australia as a Group K herbicide. In the same study (Wallace et al, 2007) mentioned in the ethofumesate section, dimethenamid-P at 560 g a.i./ha produced only minor crop injury while providing excellent control of London rocket (*Sisymbrium irio*) in trials conducted over a two year period. No other global references to trials in spinach were found and spinach does not appear on the crop list for which registration is sought in the APVMA Gazette (above). Given the similarity to s-metolachlor and the proven safety of s-metolachlor, seeking a use pattern for dimethenamid-P in spinach is not likely to be a high priority.

Ethofumesate

Ethofumesate (Tramat[®]), a resistance Group J (Australia) herbicide in the benzofuran class of chemicals, is an inhibitor of fatty acid synthesis (not ACCase) (CropLife Australia, 2008). It is registered for use in Australia in several beet crops, oilseed poppy, onions, ryegrass, several turf and ryegrass crops and non-crop areas (Bayer Australia, Tramat product label, 2008). Ethofumesate in not currently registered in spinach, though residue data is being compiled for the lodgement of an application for a minor use permit in Australia (Dal Santo, pers comm, 2008).

Crop selectivity appears highly varied, being used for controlling wintergrass in turf and ryegrass crops, while controlling barleygrass and a range of broadleaf weeds in the above broadleaf crops. Even more notable is the label claim for controlling fat hen in various beet crops, where weeds and crop are both Chenopodiaceae. Recommended rates for application vary from 300-3000 g a.i./ha according to crop and situation. Application windows range from preplanting through post sowing, pre-emergence to post emergence. It appears that ethofumesate can be applied earlier in more tolerant crops but must be delayed until crops are more developed and used at lower rates in more sensitive crops. The beet group appear to be the most tolerant to the herbicide, allowing 200-3000 g a.i./ha to be applied pre-emergence (crop and weed). This might suggest selectivity in spinach is also likely to be good but confidence is diminished by the fat hen control claim as already noted.

The most reliable indication on the performance of this herbicide in spinach, comes from a recent two year study (Wallace et al, 2007) conducted in processing spinach in the USA at a number of sites located on a range of soil types. Ethofumesate applied early post emergence caused no injury in year 1, although in year 2 injury was higher in all plots where it was applied twice, regardless of rate (90-180 g a.i./ha). When applied pre-emergence, ethofumesate produced minor crop injury at 840-1120 g a.i./ha and high levels of injury at 2240 g a.i./ha. The reference treatment of s-metolachlor at 730 g a.i./ha showed no

signs of crop injury. Nonetheless, the authors concluded ethofumesate should still be considered a candidate for expanding weed control options in spinach.

Chloridazon

Chloridazon (Pyramin[®]), a pyridazinone class herbicide, is registered for use in Australia in several beet (Chenopodiaceae) crops only. However, a permit application has been lodged for use in spinach (Dal Santo, pers comm, 2008), though it has yet to be approved for this use. Chloridazon is classified in Australia as a Group C herbicide (Inhibitors of photosynthesis at photosystem II). Use rates for beets vary from 1430-5200 g a.i./ha depending on use situation and soil texture. It is applied either pre-planting or post-sowing but prior to crop emergence, to control a broad range of broadleaf weeds and wintergrass. It has a similar range of registrations and use rates in the UK (Sipcam UK, Better Flowable product label, 2008). Chloridazon is no longer for sale in the USA. No global product labels were found showing a registration for use in spinach. However, some evidence exists to show chloridazon has either been investigated for use in spinach or actually granted permits for this use (Hort. Dev. Co., 2004).

Cycloate

Cycloate (Ro-Neet[®]) is not available in Australia. However, it is considered an important spinach herbicide in the USA and is available in a number of specified states, under the Special Local Needs (SLN) registration system (Helm Agro USA, Ro-Neet product label, 2008). It is used to control a range of annual grasses and broadleaf weeds (including Chenopodiaceae weeds). Maximum recommended application rates are 3368 or 4491 g a.i./ha, depending on soil type and the state it is used in (the higher rate permitted in California only). While it is primarily effective on germinating seeds, it is also effective on two perennial sedge (*Cyperus spp.*) species. Like ethofumesate and EPTC, cycloate would be assigned to the Group J mode of action grouping, an inhibitor of fatty acid synthesis (not ACCase) (CropLife Australia, 2008), were it registered in Australia, although chemically it is a thiocarbamate.

The most outstanding feature of cycloate appears to be its very high margin of crop safety. In the same study (Wallace et al, 2007) mentioned in the ethofumesate section, cycloate was used as a reference standard for crop safety and applied at 2520 g a.i./ha, showed no phytotoxicity in any of the trials over a two year period.

Of all the herbicides not registered (for any use) in Australia, cycloate appears to show the most potential for effective and selective broad based weed control in spinach.

EPTC

EPTC (Eptam[®]) is registered in Australia at 2016-5040 g a.i./ha for use in a range of field crops but not in spinach. No global spinach registrations could be found for EPTC in spinach. Like ethofumesate and cycloate, EPTC is in the Group J mode of action grouping, an inhibitor of fatty acid synthesis (not ACCase) (CropLife Australia, 2008), and in the thiocarbamate chemical group. It appears

to be weak on cruciferous weeds and therefore relatively safe in cruciferous crops – It is registered in dryland oilseed rape and has also previously been granted permits in canola and mustard. However, it controls fat hen (Chenopodiaceae) and in the same USA study (Wallace et al, 2007) mentioned in the ethofumesate section, caused inconsistent light to moderate crop damage at 2940-3400 g a.i./ha. Based on the above, EPTC does not seem like a priority candidate for further evaluation in spinach.

12.4.5 Selective Herbicides – Post-emergent (grasses and broadleaf weeds)

Phenmedipham

Phenmedipham (Betanal[®]), a phenylcarbamate class herbicide, is registered for use in Australia in several beet crops and non-fruiting strawberries only. Like chloridazon, it is classified as a mode of action Group C herbicide. While no Australian use pattern in spinach has been registered, residue data is being compiled for the lodgement of an application for use in spinach (Dal Santo, pers comm, 2008), although it has yet to be approved for this use. Unlike chloridazon, phenmedipham is a selective contact herbicide that is only effective when applied post-emergence of the weeds. Like other contact herbicides the symptoms develop relatively rapidly and with phenmedipham can be seen within 2-3 days of application. Weed death then takes about 7-10 days. Weeds are generally targeted at the 2-4 leaf stage using rates of 864-1335 g a.i./ha (using higher rates for larger weeds). It is effective against a range of broadleaf weeds but has no grass weed activity except for wintergrass. As a contact herbicide, targeting smaller weeds is particularly important. Recommendations often involve combinations with ethofumesate to improve the weed control spectrum. especially grasses. Tank-mixing a pre-emergent, also adds residual weed control.

While phenmedipham appears to be widely used around the world in sugar beet, research data in spinach is difficult to locate. Our preliminary data suggests that this herbicide may not be that useful in baby leaf spinach.

12.4.6 Selective Herbicides – Post-emergent (grasses only)

In Australia, the Group A herbicides or "ACC'ase Inhibitors" (Acetyl Co-enzyme A carboxylase inhibitors) inhibit the synthesis of fatty acids. There are three chemical sub-groupings, the "fops" (aryloxyfenoxypropionates), the "dims" (cyclohexanediones) and more recently the first "den" (phenylpyrazoles). These herbicides are so selective that some are used for controlling grass weeds in grass crops (cereals). In fact this is the major use of these chemicals in Australia and throughout the world. All have their particular target strengths and weaknesses. Some are more effective on annual ryegrass for example, some are selective in wheat but not barley and so on. One common feature of all these herbicides, however, is their complete lack of activity on dicotyledon crops and weeds. While there may be other isolated instances in the world, the author is

only aware of one peculiar exception to this – In Australia, haloxyfop has been found to control Erodium spp. and this claim appears on the Verdict[®] 520 label. Because of their importance in the broadacre market, there are a lot of different active ingredients from this mode of action grouping that are registered in Australia.

These herbicides are generally very effective on annual grasses but tend to translocate slowly and so are not always effective on larger, more established perennial species. They are also extremely susceptible to being rendered ineffective due to resistance, most notoriously in annual ryegrass. Care therefore needs to be taken not to overuse these herbicides if they are to maintain efficacy when they are used for ryegrass control. One species these herbicides are not effective against is wintergrass (*Poa annua*). Fortunately many of the pre-emergent herbicides already described are effective against this important weed species.

A number of these herbicides such as sethoxydim and clethodim are used in spinach elsewhere in the world. However, within this group, only fluazifop-p (Fusilade[®] Forte) and to a lesser extent sethoxydim (Sertin[®] 186) have broad registrations in fruit and vegetable crops in Australia. While neither of these are registered in spinach, fortunately there is a current permit (APVMA PER8489, 2005) in force until October 2010 allowing the use of clethodim (Select[®]) at 36-90 g a.i./ha. These are typical label rates and should control most annual grass species well, though the 28 day withholding period may prove difficult to work with in baby leaf spinach.

12.4.7 Selective Herbicides – Post-emergent (broadleaf weeds only)

Clopyralid

Normally, Group I chemicals (Australia) are kept well away from horticultural production areas as they include herbicides such as 2,4-D, MCPA, triclopyr or dicamba which can be any of; very volatile, persistent or broadly active against dicotyledonous plants. They can consequently cause a great deal of damage to broadleaf crops. However, clopyralid (Lontrel[®]), a pyridine carboxylic acid group herbicide, is unusually selective compared with other members of the group. It is for example relatively weak on cruciferous weeds and therefore registered in canola where it is one of the very few post-emergence options for controlling broadleaf weeds in that crop. It is however, highly active against legumes and weeds from the family Asteraceae.

Clopyralid is widely used in broadacre agriculture in Australia around 30-90 g a.i/ha. While the author is unaware of any registrations or use under permits (globally) in spinach, one greenhouse study (Wallace and Petty, 2007) in the USA showed that while clopyralid applied at the relatively high rate of 140 g a.i./ha caused slight to moderate leaf buckling, yields were not significantly different to untreated 35 days after application. Based on this information, and

the knowledge that clopyralid has some short term soil persistence, seeking to establish a legitimised use pattern in Australian grown spinach is probably not a high industry priority. However, there might well be improved tolerance at more commonly used local rates and might be worth looking at if aster or leguminous weeds become especially difficult to control with other herbicides.

12.5 Conclusions

Weed control in spinach in Australia heavily reliant on mechanical and nonselective herbicides to reduce seed-banks outside of the cropping period. There are currently no selective herbicides (pre or post emergence, broadleaf or grass) registered in Australia for weed control in spinach. In the current market this situation is unlikely to change.

The APVMA's minor crop permit system is an essential mechanism for allowing growers access to appropriate selective herbicides (registered in crops other than spinach) for in-crop control of grass and broadleaf weeds.

The only selective herbicide permit in spinach that is active at 02 Sep 08 (PER8489) is for clethodim, a post-emergent herbicide for annual grass weed control. This is considered extremely safe for use in spinach and very effective for this purpose.

The most extensively evaluated, broad-spectrum, pre-emergent herbicide, previously granted a permit in Australia (PER5729) is s-metolachlor. An application for renewal of this permit was submitted in February 2008 and is likely to be issued in the coming months, although no permit is in place at 02 Sep 08. S-metolachlor has been shown to be well tolerated in spinach when used as directed and is likely to be the most effective, readily available herbicide in this application window in the near future. It is used in spinach under permit in certain states in the USA. The Australian label (Dual Gold) shows it is effective against a range of annual grass and several important broadleaf weeds.

A permit application for the use of the pre-emergent herbicide chloridazon, has been submitted to the APVMA and the outcome of this is expected in the coming months. It is effective on broadleaf weeds and winter grass. Chloridazon is not known to be registered or used under permit in spinach elsewhere in the world.

A permit application for the use of the selective post-emergent contact herbicide, phenmedipham (Betanal[®]) is likely to be lodged with the APVMA by the end of 2008. It is effective against broadleaf weeds and winter grass. Phenmedipham is not known to be registered or used under permit in spinach elsewhere in the world.

A permit application for the use of the selective pre and early post emergent herbicide, ethofumesate is likely to be lodged with the APVMA by the end of 2008. It is effective against broadleaf weeds, barley grass and winter grass. Ethofumesate is not known to be registered or used under permit in spinach elsewhere in the world. It is not always to be well tolerated by spinach.

Of all the herbicides not registered (for any use) in Australia, cycloate appears to show the most potential for effective and selective broad based weed control in spinach. It is considered an important spinach herbicide in the USA and is available in a number of specified states, under permit. It is used to control a range of annual grasses and broadleaf weeds and is extremely well tolerated by spinach.

12.6 References

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13 Spinach variety trials

13.1 Introduction

The advent of harvesting spinach at an early stage of growth is relatively new. Until recently, the majority of varieties used for spinach production were bred for traditional bunching spinach production. Recently varieties have been bred specifically for baby leaf production. In this experiment, we compare two varieties which were bred for bunching spinach and have been adapted to baby leaf production by planting them at a high densities and harvesting the plants when young, to a variety which has been bred specifically for warm season baby leaf production.

While the essential difference between these three spinach types is growth rate, this trial also provides an opportunity to investigate the influence of growth rate on a number of plant characteristics such as leaf thickness, shelf life and yield.

13.2 Materials and Methods (Boisdale and Camden Trials)

New varieties were classified into one of three seasonal types; warm, transitional or cool, based on yield, days to harvest and shelf life management throughout the year.

In this study, 3 spinach varieties from Rijk Zwaan Seeds were selected which covered the range of currently available growth rates. These were:

- Parrot : Fast growing type suited to cool conditions
- Roadrunner: Intermediate growth rate and suited to transitional times between warmest and coolest seasons
- Crocodile: Slow growing type suited to warm growing conditions and specifically bred for baby leaf production



Figure 13.1 Cool Season Parrot (left), Transition Road Runner (centre) and Warm Season Crocodile (right)

The effect of spinach variety type was tested in replicated spinach variety trials at Boisdale (East Gippsland, Vic) & Camden (Sydney Basin, NSW). The experimental design for the spinach variety trials over the two sides are outlined in Table 13.1.
Site:	Camden (NSW)	Boisdale (Vic)
Design:	Randomised Complete Block Design	Randomized Complete Block Design
Number of Trials	12	12
Replication:	Six (6) blocks x three treatments per block	Four (4) blocks x 3 treatments per block
Plot Size	1 m x 9 m x 6 rows x 75 mm	1m x 5m x 8 rows x 125mm
Samples	2 random samples per treatment (36 in total)	3 random samples per treatment (36 in total
Seeder:	Seed Spider	Seed Spider
Spinach Varieties	Crocodile	Crocodile
	Parrot	Parrot
	Roadrunner	Roadrunner
		Plus the following trial varieties:
		Planting 1: Nagano
		Plantings 2 & 3: Turtle Planting 6 & 7: Whale

Table 13.1 Trial Design and Set up Camden and Boisdale

The varieties were assessed at harvest for yield, leaf thickness and shelf life as describes in Chapter 2 – Materials and Methods.

13.1 Results and Discussion (Boisdale and Camden Trials)

Yield

For growers, yield is the single-most important characteristic as they are paid per tonne produced. Figure 13. shows the relative differences between the yields of the three cultivars grown at Camden, NSW. The results show that Road Runner, the relatively fast growing transitional variety, yielded more that the other cultivars. Crocodile, the slowest growing cultivar had the lowest yield. This data shows that there is a practical compromise between yield and shelf life for baby leaf spinach grown under commercial conditions. This result has also been reported in Chapter 10, Establishment Methods and Chapter 11, Planting Density. There is a commercial need to work out how best to provide growers with greater incentive to supply superior quality rather than simply higher yields to processors.



Figure 13.1. Yield of baby leaf spinach cultivars at Camden NSW, 2006/07.



Figure 13.2. Fresh weight of plants, average minimum temperature and harvest date for baby leaf spinach cv. Road Runner at Camden NSW, 2006/07.

The impact of seasonal climate on the growth rate and potential yield was clearly demonstrated in the data for Road Runner at Camden (Figure 13.). There was a very clear link between the average minimum temperature during the growing season and the fresh weight of the plant. When the minimum temperature, was above 15°C, plant weight declined. Plant weight was lowest when the night

temperature was highest (in February) a similar result was reported in Chapter 3 where the change was also linked to postharvest shelf life.

These differences in yield are very likely related to the rate of respiration of the crop. When the night temperatures are high the respiration rate at night is also high and so there is a low net accumulation of carbohydrate for growth. Boese & Huner (1990) reported a similar result with spinach leaves grown at 5°C having dry weights that were five times greater than spinach leaves grown at 16°C.

Interestingly there was also a significant linear relationship between the days to harvest and the average minimum temperature during the growing season (Figure 13.3).



Figure 13.3. Days to harvest and average minimum temperature during the growing season of baby leaf spinach at Camden NSW, 2006/07.

Figure 13.3 showed that as the night temperature decreased, the days to harvest increased. It seems that the night temperature was a key factor in determining baby leaf shelf life (Chapter 3), yield and also in predicting harvest date (Chapter 8).

13.2 Leaf Thickness

The results from the variety assessment at Camden also showed a significant difference between the thicknesses of the leaves of the three cultivars. Crocodile, the slowest growing variety, had consistently thicker leaves than the other faster growing varieties (Figure 13.2 and Figure 13.3).

A correlation between leaf thickness and growth rate was reported by Boese & Huner (1990) who found that plants grown at 5°C increased leaf thickness twofold over spinach plants grown at 16°C, resulting in leaf thicknesses of 567 μ m and 290 μ m respectively. The plants grown in the 5°C treatment would have grown more slowly than those grown at 16°C. It seems that differences in leaf thickness are related to the growth rate of the plant. The difference in growth rate may be the result of temperature or may be a genetic trait of the variety. The leaves of the spinach plants grown by Boese and Huner (1990) were thinner than reported in this.



Figure 13.2. Leaf thickness of baby leaf spinach cultivars at Camden NSW, 2006/07.







Figure 13.4. Leaf thickness of baby leaf spinach cultivars by harvest date at Camden NSW, 2006/07.

Figure 13.4 shows the changes in leaf thickness of the three cultivars harvested at different times of the year at Camden. The data shows that Crocodile was consistently thicker than the other two cultivars and that the thickness of Crocodile decreased as the growing conditions became warmer. A similar trend was seen for the other two varieties but those varieties did not have the same spread of thickness values over the season that Crocodile had.

It is important to note that the thickness of baby leaf spinach leaves alone does not determine the robustness of the leaves or the leaves ability to withstand the rigours of processing. Clarkson et al (2003) demonstrated that shelf life of processed lettuce was related to the biophysical properties of the cell wall, including plasticity and epidermal cell size. Clarkson et al. (2003) found that mechanical stress applied at a daily dose of "100 paper strokes" or brushing the leaves daily increased the shelf life of baby leaf lettuce by 33%. The mechanical stress reduced the plasticity of the cells and also reduced the size of the epidermal cells. Increased plasticity means that the leaves were able to withstand a greater force during processing before they sheared or incurred damage. This work suggests that there is potential to target cell wall attributes when breeding cultivars for the processed salad market.

13.3 Shelf life

Data from the Camden and Boisdale variety trials both show that Crocodile had the longest shelf life followed by Road Runner and Parrot when all the data was pooled (Figure 13.7 and Figure 13.8). This concurs with earlier findings on the relationship between growth rate and shelf life as Crocodile was the slowest

growing cultivar. The intermediate growth rate variety, Roadrunner had the next longest shelf life, and the fast growing winter variety Parrot had the shortest shelf life. These findings were also consistent with observations by Johnson et al. (1989) which showed that the cultivar of baby leaf spinach had a very significant impact on shelf life and quality.



Figure 13.7. Shelf life at 2°C of baby leaf spinach at Camden, NSW 2006/07.



Figure 13.8. Shelf life at 2°C of baby leaf spinach at Boisdale, Victoria 2006/07.

It is interesting to observe that the average shelf life of the three cultivars was consistently higher for the Camden data set compared to the Boisdale data. The difference was likely to be the result of the extra transport time required to get the samples from Victoria to the University of Sydney where the post-harvest storage assessments were done. This is a practical example of how an inconsistent cool chain can impact on baby leaf quality despite the fact that the samples were transported from Victoria to Sydney in an insulated cooler within 12 hours of harvest. The samples from Camden were able to be transported to the Sydney campus in a much shorter time and the impact on shelf life was evident when comparing these two data sets.

13.4 Conclusion (Boisdale and Camden)

The results from these variety trials show that there was a significant difference in shelf life of the three cultivars with the variety Crocodile consistently having the longest shelf life. There was also a significant difference in the thickness of the leaves of the three cultivars with Crocodile the thickest leaves followed by Road Runner and Parrot.

Leaf thickness was influenced by cultivar and temperature with the leaves being thinner for all cultivars during warmer growing conditions.

From a commercial perspective the faster growing cultivars Road Runner and Parrot had higher yields than the slower growing cultivar Crocodile. For the fast growing cultivar Road Runner, average minimum night temperature impacted on plant weight. When temperatures were maintained above 15°C, the average plant weight decreased with the lowest leaf weight being recorded during the hottest growing period.

As was reported earlier in the Chapter 8 - Crop Scheduling the average minimum temperature was significantly correlated with days to harvest.

The results show that the variety Crocodile which has been bred especially for baby leaf production had the longest shelf life of the other two varieties. However this came at a commercial cost with the two faster growing cultivars having a consistently higher yield. As with many production systems there is a commercial compromise between yield and quality.

13.5 Methods and Materials (Gympie, Wheatvale and Stanthorpe)

During 2006-2007 six variety trials where planted and assessed in southeast Queensland. Four cool season trials were planted at Gympie in South east Queensland, on the 19 April, 16 May, 14 June, and the 26 July for trials 1-4 respectively. One transitional season trial was planted at Wheatvale on 16 Oct 2006 and a summer trial was planted at Stanthorpe on 31 Jan 2007. A "Seed Spider" commercial planter was calibrated to deliver the targeted seeding density. An average of 760 plants to the square was established across the 4

trials. All treatments were replicated in a randomized, complete block design (Table 13.2).

Site:	Wheatvale (QLD)	Gympie (QLD)	Stanthorpe (QLD)
Design:	Randomized Complete Block Design	Randomized Complete Block Design	Randomized Complete Block Design
Number of Trials	1	4	1
Replication:	Four (4) blocks x 3 treatments per block	Four (4) blocks x 3 treatments per block	3 random samples per treatment (36 in total
Plot Size	1m x 5m x 8 rows x 125 mm apart	1m x 10 m long plots	1m x 10 m long plots
Samples	3 random samples per treatment (36 in total	3 random samples per treatment (36 in total	3 random samples per treatment (36 in total
Seeder:	Seed Spider	Seed Spider	Seed Spider
Spinach Varieties	Crocodile, Parrot, Road Runner, Monza, Neo, Trump, Typhoon, Erecto, Whale	Crocodile, Parrot, Road Runner, Monza, Neo, Trump, Typhoon, Veno, Whale	Crocodile, Parrot, Road Runner, Mendora, Sardinia

Table 13.2 Trial Design and Set up Gympie, Wheatvale and Stanthorpe

Treatments were managed in the same manner as the commercial planting. The harvest timing was determined by the average length leaf cut under commercial conditions. The specification used was that no harvested material exceeded 120mm-140mm in length from the cut petiole to the end of the leaf blade. After harvest the fresh yield (g/m^2) and shelf life (days) were determined as described in Chapter 2 – Materials and Methods.

13.6 Results and Discussion

Gympie – Cool Season Trials

Figure 13.5 shows the first variety trial harvested in May. In this trial the average yield across all varieties was around 1200 g/m². Parrot yielded significantly higher than all other varieties; about 16% higher than Whale and Crocodile, about 22% higher than Road Runner and about 44% higher than the poorest yielding variety Monza.

For the trial harvested in June, Trial 2 (Figure 13.7), the average trial yield across all varieties was around 1800 g/m² more than in the previous trial. Parrot again yielded significantly better than all other varieties; about 15% higher than Crocodile, about 20% higher than Sardinia, about 24 higher than Road Runner, Tsunami and Rotunda and about 39% higher than the poorest yielding variety LV495.



Figure 13.5 Trial 1 (Gympie) – Cool season – Planted 19 Apr 06, harvested 15 May 06 (26 Days To Harvest) - Yield



Figure 13.6 Trial 2 (Gympie) – Cool season – Planted 16 May 06, harvested 12 Jun 06 (27 Days To Harvest) – Yield



Figure 13.7 Trial 3 (Gympie) – Cool season – Planted 14 Jun 06, harvested 18 Jul 06 (34 Days To Harvest) - Yield

The variety trial harvested in July, Trial 3 (Figure 13.7), had an average trial yield across all varieties of around 2700 g/m² significantly higher than the other trials. Blizzard yielded significantly better than all other varieties; about 8% higher than Road Runner and Whale, about 10% higher than Crocodile, Parrot and Night Hawk, and about 26% higher than the poorest yielding variety San Antonio.



Figure 13.8 Trial 4 (Gympie) – Cool season – Planted 26 Jul 06, harvested 28 Aug 06 (33 Days To Harvest) - Yield

Trial 4 (Figure 13.8) was harvested in August, and the average trial yield across all varieties was around 3000 g/m². The variety LV385 had the highest yield although not significantly higher than Parrot, Night Hawk or Whale, which all cut about 5% less than LV385. The poorest yielding variety was San Antonio which cut 25% less than LV385.



Figure 13.9 All 4 Gympie Cool, season trials - Yield comparison over four harvest periods (May, June, July and August) of cultivars Crocodile, Parrot and Road Runner.

Only the varieties Crocodile, Parrot and Roadrunner were sown in all four winter trials. Figure 3.14 shows the change in yield of these 3 varieties over the winter period with the lowest yield in May and June.

Over the May through to August harvest period, the trial yields steadily increased from about 1200 to around 3000 g/m². The trial showed that the leaves attain market size sooner in warmer periods and this was at the expense of yield. A longer period to maturity was also associated with reduced differences in yields between varieties.

Parrot proved to be the standout performer for the earlier May-June harvest period. Blizzard gave the best yield in the July period and there was no clear best performing variety in August, with LV385, Whale, Parrot and Crocodile all performing similarly.

Wheatvale – Transitional Season Trial

As the trials moved into the summer periods days to harvest shortened and yields in general dropped. Some low levels of soil surface crusting were also seen in Wheatvale and Stanthorpe. The crusting caused non-uniform germination across all of the varieties in the transition and summer trials. While no one particular variety was disadvantaged more than any others it may have contributed to the generally lower yields recorded.

The first harvest in Wheatvale was in October, Trial 5 (Figure 13.10), the average trial yield across all varieties was around 2300 g/m². At about 2900 g/m² Whale was numerically the highest yielding variety, but there was no significant separation between Whale, Erecto, Parrot, Road Runner, Veno or Crocodile. The poorest yielding variety was Monza which cut 41% less than Whale.





Figure 13.10 Trial 5 (Wheatvale) – Transition season –Planted 16 Oct 06, harvested Oct 06 (Days To Harvest) - Yield



Figure 13.11 shows the shelf life of the varieties grown in Trial 5. There were few statistically significant difference within this trial. The main differences were that Parrot, while achieving a shelf life of around 24 days (the longest in the trial) was only significantly better than the poorest shelf life variety, Crocodile at around 18.5 days. Numerically though the four varieties that stored the longest were Parrot, Road Runner, Typhoon and Whale and the two poorest Crocodile and Monza.

While there was no significant relationship between yield and shelf life, there was a trend that better yielding varieties tended to store for longer. More work is needed to investigate this hypothesis in more detail.

Stanthorpe – Warm Season Trial

Stanthorpe, Qld was the site for the warm season variety trials the first trial at this site was harvested in March 07, Trial 6 (Figure 13.12). The average trial yield across all varieties was around 1350 g/m². At about 1410 g/m² Menorca was numerically the highest yielding variety, but there were no significant differences between any variety. The poorest yielding variety was Sardinia which cut about 17% less than Menorca.



Figure 13.12 Trial 6 (Stanthorpe) – Warm season – Planted 31 Jan 07, harvested Mar 07 (Days To Harvest) – Yield



Figure 13.13 Trial 6 (Stanthorpe) – Warm season – Planted 31 Jan 07, harvested Mar 07 (Days To Harvest) – Shelf life

Figure 13.13 shows the different shelf lives of the varieties grown in the warm season trial at Stanthorpe. There was a high variability between the samples and so there are no statistical differences between the varieties. However the trend was for Crocodile to have the longest shelf life in this trial at 20.5 compared to the poorest performing variety in terms of shelf life, Road Runner at around 16.5 days.

Again, while there was no statistical relationship between yield and shelf life, the trend was for higher yielding varieties to have a longer shelf life. More work is required to segregate varieties in terms of shelf life, but in these trials, Parrot performed best.

13.7 Conclusions (Gympie, Wheatvale and Stanthorpe)

Rijk Zwaan (70%) and Seminis (30%) are the current suppliers of baby leaf spinach variety seed. In most trials the Rijk Zwaan varieties had higher yields than the Seminis varieties but the differences are relatively small and do vary depending on the seasonal conditions. The trials showed that the seasonal temperature affected yield much more than the selection of variety of spinach.

In summary the shelf life was highest in the cooler and transitional times during periods of slower plant growth and the yields were highest in the transitional period, when the conditions were warm but not hot.

The yield and shelf life were lowest during the summer period regardless of variety and this was dur to the high temperatures, humidity and rainfall during the production period.

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14 An agronomic comparison between wild rocket (Diplotaxis tenuifolia) and cultivated rocket (Eruca sativa) cultivars

14.1 Introduction

Lettuce, chard, rocket, and spinach, are the main components of pre-packaged baby leaf salad mixes. One of the major contributing factors leading to an increased demand for rocket and other baby leaf products, is that they are perceived, and in fact deliver compounds beneficial to human health such as; high concentrations of bioactive phytochemicals, such as vitamin C, polyphenols, and glucosinolates (Bennett et al., 2006; Martinez-Sanchez et al., 2007; Martinez-Sanchez et al., 2008).

The widespread study of rocket has only taken place within the last 5 years. To date very few studies have examined the commercial characteristics of either wild or cultivated rocket under Australian conditions. The majority of studies have thus far focused on factors affecting germination and seed formation (Kleemann et al., 2007; Ray et al., 2005; Villamil et al., 2002). A study by Kleemann et al., (2007) focused on understanding the germination behaviour in an attempt to control the plant, as it is considered a weed of both pastures and brad acre crops in western and southern Australia.

Wild rocket belongs to the Brassicaceae family and is often found in regions of low to moderate rainfall, and in neutral to high pH sandy soils (Moerkerk 2006). The seeds are relatively small and a formed during autumn, the crop has a tendency to bolt to seed during summer cultivation, subsequently reducing its commercial value. Wild rocket is a perennial species which seems to be well adapted to poor soils, which are characteristic of its region of origin in and around the eastern Mediterranean (Pignone, 1997). The collection of commercial Diplotaxis sp. seems to have occurred around the coastal regions of Apulia and Basilicata, in Italy (Pignone, 1997). The leaves are extensively used as a specialty food and a delicacy, both in Europe and around the world (Pignone, 1997). The commercial production of rocket in Australia takes place via direct sowing throughout the year, although a few growers also transplant wild rocket during winter to overcome reduction in germination during this period. The germination vigour of rocket has been determined to be around 85%, with a reduction of 15-20% if seeds are collected during September to October (Pimpini & Enzo, 1997). The crop is multi-harvested and can be cut between 3-5 times before re-seeding is required.

Cultivated rocket is also a member of the Brassicaceae family and is an erect annual, naturally growing to around 100cm, with lobed dark green leaves. If favourable climatic conditions permit, *Eruca sativa* can be grown in almost any type of soil, in comparison with wild rocket which prefers calcareous soils

(Pimpini & Enzo, 1997). The domestic production of cultivated rocket is less than that of wild rocket. The commercial cultivation of *Eruca sativa* is almost exclusively restricted to winter when wild rocket germination and growth is impaired by the lower ambient temperatures.

The harvesting of leaves can start anywhere from 20-60 days after emergence depending on species, season and market location (Pimpini & Enzo, 1997).

The following study aimed to improve the current understanding of both *Diplotaxis tenuifolia* and *Eruca sativa* growth characteristics, under Australian summer conditions.

14.2 Methods and Materials

Field experiments – Overview

Two field experiments were conducted to gather agronomic data on three commonly grown baby leaf rocket species; *Diplotaxis tenuifolia* (wild rocket), *Hesperis matronalis* (sweet rocket), and *Eruca sativa* (cultivated rocket). Although taxonomically sweet rocket and cultivated rocket are different, commercially they are considered the same and are collectively referred to as cultivated rocket, for this reason both species have been combined into the same field experiment. As the majority of domestically produced cultivated rocket is from the *Eruca sp.*, there are more representatives within the cultivate rocket field experiment than there are from the *Hesperis sp.*, as it is of greater commercial importance.

<u>NB</u>: For the purpose of these trials, the word "treatment" will be used to mean seed from a particular batch belonging to a particular species as designated by the treatment number in Tables 14.1 & 14.2.

Field experimental location

Field experiments were conducted on a commercial enterprise located approximately 70km south west of Sydney, at Ellis Lane. The reason for conducting experiments on a commercial farm was to closely replicate actual crop management and commercial production of baby leaf rocket under typical summer production conditions.

Treatments (Species/varieties) for wild rocket and cultivated rocket

The following Tables summarise the varieties used in the field trials.

Treatment	Species	Seed batch number	Estimated germination %
1	Diplotaxis tenuifolia	ST06-2040B V59	93
2 3	Diplotaxis tenuifolia Diplotaxis tenuifolia	BMP7206262 B1330863	87 82

Table 14.1. Wild rocket seed details

Treatment	Species	Seed batch number	Estimated germination %
1	Hesperis matronalis	4461C	92
2	Eruca sativa	BRG0807056	90
3	Eruca sativa	BRN0807058	90
4	Eruca sativa	BRJ0807094	90

Table 14.2. Cultivated (& "sweet") rocket seed details

Experimental design of wild rocket field experiments

A randomised complete block design was used for the wild rocket field experiments, consisting of four replicates or blocks, with three treatments randomly allocated across the growing bed (Figure 14.1). Individual treatments were randomly allocated within individual blocks; an example of the spatial layout of wild rocket experiments is depicted below in Table 14.3 and Figure 14.1.

1.5 Metre growing bed				
Block	Left side of the bed	Centre of the bed	Right side of the bed	
1	1	3	2	
2	2	1	3	
3	1	2	3	
4	2	3	1	

 Table 14.3. Example of the spatial layout for wild rocket field experiments, complete randomised block design. Each number represents a different treatment.



Figure 14.1. This figure illustrates the growing bed for the wild rocket experiment and the region of the bed individual treatments occupy.

As the seeder has a total of 18 tines with three seeding boxes, 6 rows per treatment or one third of the growing bed is occupied by individual treatments.

Experimental design of cultivated rocket field experiments

An incomplete randomised block design was used for cultivated rocket field experiments, consisting of four replicates or blocks, and four treatments. Treatments were randomly allocated across the growing bed (Figure 14.2). This experimental designed was used as four treatments were being assessed but only three physical locations per block were available. Individual treatments were randomly allocated within individual blocks; an example of the spatial layout of cultivated rocket experiments is depicted below in Table 14.4 and Figure 14.2.

1.5 Me	etre growing bed		
Block	Left side of the bed	Centre of the bed	Right side of the bed
1	2	1	3
2	4	1	2
3	4	2	3
4	1	3	4

 Table 14.4. Example of the spatial layout for cultivated rocket field experiments,

 incomplete randomised block design. Each number represents a different treatment.



Figure 14.2. This figure illustrates the growing bed for the cultivated rocket experiment and the region of the bed individual treatments occupy.

Sampling Methods

The sampling procedures and individual variables measured were the same for both the wild and cultivated rocket experiments. Unless otherwise stated the following information refers to both wild and cultivated rocket field experiments. Emergence counts were conducted at 5, 10, 15, 20, and 25 days after seeding. Three sub-samples were taken per treatment block using a quadrant measuring 33 cm \times 33cm (1.0 m²). The quadrant was randomly placed within the treatments with emergence counts recorded within the sampling area. The point at which emergence was considered was when both cotyledons had emerged from the soil.

Rocket is commercially harvested more than once as the crop grows back well after the first few cuts, and growing the crop from seed for only one cut would not be financially viable. For this reason field experiments were sampled twice for harvest data first and second cut harvests were sampled for individual experiments.

Within both first and second cut sampling events, three sub-plot samples or pseudo replicates were taken with individual treatment blocks. A quadrant was randomly placed within each plot after which all vegetative material within the quadrant was removed for agronomic measurement of the following; plants per quadrant, volume, fresh weight, dry weight, weight of 30 leaves and leaf thickness were measured.

Statistical analysis

All data was analysed using GenStat[®] (Release 9.2). All sub-samples or pseudoreplicates were pooled for analysis within individual variables. Differences between treatments were considered to be significant when P<0.05, and between individual treatment means when the least significant difference (LSD) was greater than the difference between respective means. The variable plants per quadrant were used as a covariant for the analysis of agronomic data which was influenced by plant density.

14.3Results

Wild rocket experiment - summer: first cut

For the first cut of wild rocket statistically significant differences were observed between individual treatments at all of the measured time intervals after seeding (5, 10, 15, 20 and 25 days) (Figure 14.3). On day 25 the different cultivars had different numbers of plants per quadrant despite being seeded at the same rate. This trend between varieties was consistent across all sampling times. Treatment 1 (48 plants) had more plants per quadrant after 25 days than 2 (35 plants) and Treatment 3 (21 plants).



Mean Establishments Counts for Grown Summer Wild Rocket

Figure 14.3. Wild rocket summer establishment count relationship over a period of 5, 10, 15, 20 and 25 days after seeding. Refer to Table 14.1 for the meaning of the treatment numbers.

Figure 14.4 shows the average volume of the leaves collected from a quadrant. A higher volume is regarded as better as this means that the leaves fill out a bag to make it more attractive for consumers.



Mean Volume for First Cut Summer Grown Wild Rocket

Figure 14.4. Mean volume for first cut wild rocket grown during summer plant developmental conditions. Refer to Table 14.1 for the meaning of the treatment numbers.

The results show that Treatment 3 had a significantly lower volume (3342mL) than the other treatments, Treatment 1 (4591mL) and 2 (4867mL). There was also a similar pattern for the leaf thickness of the three varieties with Treatment 3 having significantly thinner leaves (0.49mm) than Treatments 1 (0.45mm) and 2 (0.41mm).

No other significant differences were identified between wild rocket treatments and any other agronomic variables measured for first cut of summer grown wild rocket.

Wild rocket experiment - summer: second cut

For the second cut of wild rocket the plant counts between Treatments were again significantly different with Treatment 3 (22 plants) having less plants than Treatments 1 (40 plants) and 2 (45 plants). No other significant differences were identified between wild rocket treatments and any other agronomic variables measured for second cut summer grown wild rocket.

Cultivated rocket experiment - summer: first cut

For the cultivated rocket 4 varieties were compared. As with the wild rocket there were differences in the numbers of plants per quadrant for the different varieties over time but by day 25 there were no significant difference between Treatments.

No other significant differences were identified between cultivated rocket treatments and any other agronomic variables measured for first cut summer grown cultivated rocket.

Cultivated rocket experiment – summer: second cut

No significant differences were identified between cultivated rocket treatments and any of the agronomic variables measured for the second cut of summer grown cultivated rocket.

Wild & cultivated rocket experimental comparisons – summer: first cut

Figure 14.5 compares the average plants per quadrant for all the wild and cultivated rocket varieties tested. The results showed that Treatment 3 (22 plants) had the lowest number of plants compared to the Treatments, 1 (51 plants), 2 (45 plants), 5 (57 plants) and 7 (46 plants). No other significant differences were identified between or within the varieties examined.



Figure 14.5. Mean plants per quadrant for first cut wild & cultivated rocket grown during summer plant developmental conditions. Treatments 1-3 are wild rocket, and 4-7 cultivated rocket. Refer to Table 14.1 for the meaning of the treatment numbers.

When the volume of the leaves were compared Treatment 3 (3220 mL) had a significantly lower volume compared to all the other treatments (Figure 14.6).



Figure 14.6. Mean volume for first cut wild & cultivated rocket grown during summer plant developmental conditions. Treatments 1-3 are wild rocket, and 4-7 cultivated rocket. Refer to Table 14.1 for the meaning of the treatment numbers.

Figure 14.7 shows the difference in fresh weight of the different wild and cultivated rocket varieties. The fresh weight was significantly higher for the wild

rocket varieties; 1 (284.5g) and 3 (215.2g) compared to the cultivated rocket varieties (4, 396.2g; 5, 431.7g; 6, 439.6g; 7, 405.9g). In general, cultivated rocket had more plants per quadrant, a higher volume and in turn a higher fresh weight than the wild rocket species.



Figure 14.7. Mean fresh weight for first cut wild & cultivated rocket grown during summer plant developmental conditions. Treatments 1-3 are wild rocket, and 4-7 cultivated rocket. Refer to Table 14.1 for the meaning of the treatment numbers.

There were also differences in leaf thickness but the differences were variety specific and not consistent across species (data not shown).

Similar trends to those discussed were shown for the second cut of wild and cultivated rocket (data not shown).

14.4 Discussion

The results showed that there were differences between the plant establishment counts of different varieties of rocket within a species.

The results showed for wild rocket that Treatment 1 (European wild rocket – Lefroy Valley Pty Ltd) was superior to both treatments 2 (Apollo – Fairbanks Seed CO.) and 3 (Nature – Seminis Ltd.) with respect to summer stand establishment. The poor performance of treatment 3 may be the result of poor seed collection techniques with seeds collected in summer performing better than those collected in cooler months (Esiyok, 1997; Pimpini & Enzo, 1997; Yaniv, 1997; Villamil et al., 2002). The analysis of the difference in volume of the wild rocket varieties showed that Treatment 3 had a significantly lower volume than both treatments 1 and 2. This result is consistent with a lower plant density but the volume data was analysed using density as a covariate and so the variability due to plant numbers was taken into account during the analysis. Differences in volume is usually due to differences in leaf shape.

The results also showed that Treatment 3 had the thickest leaves and this may correlate with a lower plant density as a similar result was reported for baby leaf spinach in Chapter 11. In summary the results show that the European wild rocket supplied by Lefroy Valley Pty Ltd(Treatment 1) was superior to both the variety Apollo (Fairbanks Seed CO.) and Nature (Seminis Ltd.) in terms of yield and volume.

For the cultivated rocket the seed supplied by Fairbanks Seed Co.(Treatment 5) had the highest plant establishment counts early on but interestingly no significant differences between varieties were observed 25 days after seeding. In fact there were no significant differences for any of the parameters measured for the cultivated rocket species.

In terms of a comparison between the types, cultivated rocket had a higher fresh weight and volume compared to wild rocket and this is most likely due to the fact that cultivated rocket leaves are wider than Wild rocket leaves and occupy more area and produce more weight per leaf.

14.5 Conclusion

In summary wild rocket (*Diplotaxis tenuifolia*) and cultivated rocket (*Eruca sativa*) produce different yields and volumes when grown over the summer period. From a commercial perspective cultivated rocket is better suited to summer growth than wild rocket, as it consistently produces a larger volume and fresh weight at this time.

The results of these trials highlight the importance of growers selecting the best seed from suppliers, as there is a large variation in performance between varieties of the same species, particularly for wild rocket.

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15 Lettuce variety trials

15.1 Introduction

Lettuce is susceptible to downy mildew and lettuce leaf aphid (*Nasanovia*). These two pest and disease problems can be overcome to some degree by planting resistant varieties. These trials aimed to evaluate the new downy mildew and *Nasanovia* resistant baby leaf lettuce types, red and green lettuce, the Coral, Batavia and Oak leaf types which were sourced from the main international breeding companies, Rijk Zwaan, Enza Zaden, Seminis, Nunhems and Nickerson Zwaan.

15.2 Methods and Materials.

Replicated lettuce variety trials conducted at Warwick (QLD), Stanthorpe (QLD) and Gympie (QLD). The yield and shelf life of the varieties were assessed as described in Chapter 2 – Materials and Methods.

Experimental Design for Lettuce Variety Trials

Site	Gympie (QLD)	Wheatvale	Stanthorpe
Design	Small plot observational trial with 3 plots across the bed	Randomised Complete Block Design	Randomised Complete Block Design
Replications	Unreplicated due to weather and	Three (3)	Three (3)
	time of planting.		Trial run 3 times
Plot Size	Small Plot	1m x 10m long plots	1m x 20m long plots
Samples	3	3	3

Varieties used in the Gympie Trials

Initial observation trials were planted at Gympie in winter 2006. The purpose of this trial was to initially screen 33 varieties and types, in an attempt to visually identify varieties with commercially desirable characteristics, to be more thoroughly evaluated in later trials (Table 15.1).

Baby leaf Type – category	Variety
Dark Green " Tango type"	BONITO
Dark Green Cos	MARLIN
Dark Green Green Oak	SLAMMER SPINNER
Dark Red "Frillice Type"	PRO 1226 PIRANA SNOOK
Dark Red Leaf type Dark Velvet	BARRACUDA FARADIA RZ
Frilled Green	PAMPANO PRO 1022
Green Mutlileaf	SNAPPER CARPEDIA RZ SPS 697-5 SPS 698 5
	KYLIE
Green Oak	PRO 1048
Light green Cos	PRO 1053
Red Corai	
Red Frillice	GROUPER
Red Italian Oak	FIONA
Triple Red	PRO 1051
	PRO 1052
	PRO 1064
Dark Green " Tango type"	BONITO
	TRIGGER
Dark Green Cos	MARLIN
	PRO 1420
Dark Green Green Oak	SLAMMER

 Table 15.1: Varieties initially screened at Gympie, winter 2006.

Varieties used in the Wheatvale Trials

The 14 best varieties from Gympie were more thoroughly evaluated at Wheatvale later in 2006, specifically comparing yield and shelf life. The varieties were 698-5, 697-5, Faradia, Pirana, PRO1052, Xenon, Snapper, Pampano, PRO1053, Marlin, PRO1048, PRO1022, PRO1051 and Granita.

Varieties used in the Stanthorpe Trials

Three further trials of new material were made in Stanthorpe in the summer of 2007. These trials looked at a range of varieties of both standard baby leaf lettuce and the newer multi-leaf or Salanova[®] types, comparing yield and shelf life. The varieties included Obregon, Saigon, Victoire, Faradia, Carpedia, Gaugin, Sartre, 79-63, Xsara, Catamaran and 79-42.

15.3 Results and Discussion

In the Gympie trials the 14 highest yielding varieties were selected and trialled again at Wheatvale, Qld.

In the Wheatvale trials the highest yielding variety was PRO1051 at 2650 g/m², followed by PRO1048 and 698-5. The lowest yielding variety was Pirana at around 1400 g/m² or 47% less than PRO1051 (Figure 15.1 and 15.2).

No shelf life data was determined from the Wheatvale trial as a result of logistical difficulties experienced by the cooperator (OneHarvest).



Figure 15.1 Average yield - 14 best varieties - Trial 1 - Wheatvale, 2006.



Figure 15.2 14 Best varieties - Trial 1 - Wheatvale, 2006



Figure 15.3 cont. 14 Best varieties - Trial 1 - Wheatvale, 2006

In the Stanthorpe trials the highest yielding variety was Sartre at 3500 g/m^2 , followed by Victoire and Catamaran. The lowest yielding variety was Faradia at around 1550 g/m² or 56% less than Sartre. The varieties Xsara and 79-42 also yielded poorly (Figure 15.3).



Figure 15.4. Average yield - Trial 1 - Stanthorpe, 2007.



Figure 15.5. Average shelf life (days to fail) - Trial 1 - Stanthorpe, 2007.

Figure 15.4 shows that the three best storing lettuce varieties were Victoire (around 14.1 days), Obregon and Saigon. Carpedia deteriorated the most rapidly (8.5 days or 40% more rapidly than Victoire). Catamaran, Faradia and Gaugin also stored poorly.



Figure 15.6. Average yield - Trial 2 - Stanthorpe, 2007.



Figure 15.7. Average shelf life (days to fail) - Trial 2 - Stanthorpe, 2007.

In the second trial at Stanthorpe the highest yielding variety was again Sartre at 2550 g/m², statistically better than all other varieties, followed by Carpedia, Victoire and Obregon. The lowest yielding variety was Xsara at around 1300 g/m² or 49% less than Sartre. The varieties Catamaran and Faradia also yielded poorly.

The three best storing varieties were Gaugin (around 16.0 days), 79-63 and Sartre. Again, Carpedia deteriorated the most rapidly (9.5 days or 41% more rapidly than Gaugin). Faradia, Saigon and Obregon also stored poorly (Figure 15.7).



Figure 15.8. Average yield - Trial 3 - Stanthorpe, 2007.



Figure 15.9 Average shelf life (days to fail) - Trial 3 - Stanthorpe, 2007.

In the third trial at Stanthorpe the highest yielding variety was again Sartre at 3400 g/m², statistically better than all other varieties, followed by 79-63 and Gaugin. The lowest yielding variety was Faradia at around 1350 g/m2 or 60% less than Sartre. Saigon also yielded poorly (Figure 15.7).

The three best storing varieties were Saigon (around 15.5 days), and Obregon. Again, Carpedia deteriorated the most rapidly (8.5 days or 45% more rapidly than Saigon). Sartre also stored poorly (Figure 15.8).

15.4 Conclusions

The results show that there are many lettuce varieties that perform well. Although the "green" leaf types tend to have a shorter shelf life than the "red" types. The variety Sartre performed consistently well followed by 79-63 and Gaugin. The lowest yielding varieties were Faradia and Saigon.

It is important to note that the coral leaf types have been losing market share in recent years compared to the highly productive 'Salanova/multi-leaf types that are established by transplants.
15.5 Multi-leaf lettuce variety trial and comparison to traditional fancy lettuce types

15.5.1 Introduction

There is a new style of lettuce being used by the baby leaf sector known as multileaf. One major seed company has really pioneered this category, and their multi-leaf lettuce varieties are indicated by the registered name Salanova[®]. The main advantage of multi-leaf lettuce is that when the lettuce is cut near the base of the plant and the leaves separate naturally into individual leaves (leaves are attached low down on then plant rather than up a central core as in more traditional plants).

The alternative to the multi-leaf lettuce are the more traditional varieties such as Red Oak, Green Oak, Red Coral and Green Coral. While the multi-leaf types are generally established by transplant as they are larger plants, more traditional lettuce types used for baby leaf production are normally established by direct seeding.

These trials used factorial experiments to evaluate different varieties and methods of establishment.

15.5.2 Methods and Materials

Three randomized complete block designed trials were conducted during summer in South East Queensland as described in earlier in Chapter 15.2. These trials compared a range of multi-leaf lettuce with standard type direct seeded baby leaf red and green coral varieties (Table 15.2). All varieties for each of the respective trials were either direct seeded or transplanted on the same day. Growth and performance data was collected through the life of each trial. Each variety was then subsequently harvested and assessed as they reached commercial maturity.

Three planting times were evaluated (December, January and February) the multi-leaf varieties were compared to traditional varieties. The variables measured included yield and shelf life as described in Chapter 2 – Materials and Methods.

The varieties established by transplants were planted at a density of 36 plants per square metre which is an industry standard for multi-leaf type lettuce. The direct seeded "standard" type red and green coral were planted at 700 plants per square metre.

Each variety was replicated 3 times in the trial in a 20 metre section of bed. Standard fertiliser, watering and pesticide treatments were applied to all plots. On the day of harvest, a $0.31 \text{ m} \times 0.31 \text{ m}$ wire quadrant was used to randomly select

a subsection of variety. All material inside this quadrant was harvested. It was from this material that all assessments were made. Characteristics measured and calculated included; fresh weight, yield, weight of 30 standard leaves, leaf thickness, percentage dry matter and shelf life. A total of 3 subsamples were taken from each of the 3 replicates.

Variety	Lettuce Type	Establishment
Faradia Carpedia Xsara Gaugin Saigon Obregon 79-63 Victoire	traditional traditional multi-leaf multi-leaf multi-leaf multi-leaf multi-leaf multi-leaf	direct seeded direct seeded transplanted transplanted transplanted transplanted transplanted transplanted
Sartre	multi-leaf	transplanted

Table 15.2. Varieties planted in the trial

15.5.3 Results and Discussion

Yield

The varieties listed in Table 15.2 were trialled in Stanthorpe and harvested in January, February and March 2008. Figure 15.10, Figure 15.11 and Figure 15.12 show the yields of varieties for each of the trials. Table 15.3 shows the yield results numerically. In general trials the yields in trial one and three were consistent and the yields in a trial two were lower than the other two.

The best performing varieties were Sartre, Victoire, 79-63 and Obregon which were all multi-leaf types. Of the traditional leaf types, only Carpedia performed acceptably. The other three multi-leaf types and Faradia performed below standard. This trial showed that the multi-leaf types were able to significantly out yield traditional types despite the much lower populations used in establishment.



Figure 15.10. Hand harvest yield comparison of multi-leaf and directed seeded varieties; Stanthorpe, January, 2008.



Figure 15.11. Hand harvest yield comparison of multi-leaf and directed seeded varieties; Stanthorpe, February, 2008.



Figure 15.12. Hand harvest yield comparison multi-leaf and directed seeded varieties; Stanthorpe, March, 2008.

Variety	January	February	March
	Harvest	Harvest	Harvest
Faradia	1.6	1.4	1.3
Xsara	1.6	1.3	1.9
Gaugin	2.2	1.5	2.5
Saigon	2.5	1.7	1.7
Carpedia	2.6	2.0	2.5
Obregon	2.8	1.9	2.4
79-63	3.0	1.8	2.6
Victoire	3.2	2.0	2.4
Sartre	3.5	2.6	3.6
Average	2.6	1.8	2.3

Table 15.3. Variety yields (kg per square metre); Stanthorpe, 2008.

Table 15.3 shows that there were some strong yield trends. Several varieties performed relatively poorly across all three trials and others performed consistently well above the trial average in each of the trials. The shaded area in the Table represents the varieties that performed below the trial average in each of the three trials. From a yield perspective it is important to remember that these yields represent total above ground material produced. As these were hand harvested, the yields in general are much higher than what would be expected in a comparable commercial situation where losses at harvest would occur.

Shelf Life

The shelf life results from the three trials harvested in January, February and March 2008 are shown in Figure 15.13, Figure 15.14 and Figure 15.15 and show some trends across varieties. On average the new multi-leaf varieties had a longer shelf life than the traditional varieties. Table 15.4 is an attempt to show those trends. Saigon, Victoire and Obregon performed best, with an average shelf life of 13.3.- 13.6 days. Carpedia performed the worst of all varieties. This variety was ranked last in all three trials with the days from harvest to fail being 9, 9.5 and 8 for each of the three trials.



Figure 15.13. Shelf life comparison of multi-leaf and directed seeded varieties; Stanthorpe, January, 2008.



Figure 15.14. Shelf life comparison of multi-leaf and directed seeded varieties; Stanthorpe, February, 2008.



Figure 15.15. Shelf life comparisons multi-leaf and directed seeded varieties; Stanthorpe, March, 2008.

Variety	She	If Life Ran	k	Average Shelf life (days)
Obregon	1st	2nd	7th	13.2
Victoire	= 1st	3rd	= 5th	13.6
Saigon	1st	1st	8th	13.6
Carpedia	11 th (11)*	10 th (10)*	9th(9)*	9.3
Faradia	9th (11)*	8th(10)*	5th(9)*	11.3

Table 15.4. Shelf life pooled over three trials.

* This number represents the total entries for each trial



Figure 15.16. Carpedia – 21 days post seeding, one of the two "standard" type varieties and included in the trial as controls



Figure 15.17. Saigon – 21 days post transplant.

15.5.4 Conclusion

The trials conducted in Stanthorpe in January, February and March 2007 reinforced the potential benefits in developing the multi-leaf "Salanova" type varieties. Within these multi-leaf types there is a great deal of variation in shelf life and yield. Varieties such as Obregon, Victoire and Saigon gave consistently longer shelf life. Carpedia on the other hand performed consistently poorly in this area.

The individual varieties yielded reasonably consistently over the three trials. Faradia, Xsara and Saigon yielded below the trial average in all three trials. Carpedia, Obregon, 79-63, Victoire and Sartre consistently yielded above trial averages. It must be remembered that Carpedia's performance, while good in relation to yield, was the poorest in relation to shelf life, having the shortest shelf life in all three trials.

16 Pilot to Prove Trials

16.1 Introduction

The concept behind this part of the project was to take the key learning's achieved in the research phase and apply these to commercial baby leaf production.

The focus was on spinach since this is the main commercial baby leaf crop grown in Australia. Key project findings which were used in this commercial pilot to prove exercise were:

- The effect of crop growth rate on quality, specifically shelf life of the unprocessed product.
- Scheduling planting using the heat unit model developed.
- Determination of an optimal temperature range for growing spinach.
- Selection of a production area as climatically ideal as possible for spinach.
- Selection of the correct variety types for year round production.

16.1.1 Crop Growth Rate

A major outcome of the 12 month sequential spinach planting trials at Camden and Boisdale was the confirmation of the need to achieve a minimum period of 30 days from sowing to harvest to produce high quality spinach. Crops grown faster than this did not achieve satisfactory shelf life.

This 30 day minimum growth period was achieved either by choice of variety or growing location. In this study, 3 spinach varieties from Rijk Zwaan Seeds were selected which covered the range of growth rates.

Variety Parrot	Description Fast growing type suited to Winter conditions
Roadrunner	Intermediate growth rate, suited to Autumn or Spring production
Crocodile	Slow growing type suited to Summer growing conditions and specifically bred for baby leaf production in warmer locations and periods

Table 16.1Spinach varieties from Rijk Zwann Seeds

The 30 day minimum growth period was shown to reliably produce product with superior shelf life, whether the growing period was varied by manipulating

planting date (season) or variety type, and provided the cut, unprocessed baby spinach was stored at 2°C, (Figure 13.7). A similar trend was observed for spinach grown in East Gippsland, Victoria (data not shown).



Figure 16.1 The effect days from sowing to harvest on shelf life of unprocessed baby spinach stored at 2°C. The data is from plants grown at Camden, NSW planted monthly over the year.

16.2 Crop Scheduling

The heat unit model developed in this project accurately predicted the number of days from planting to maturity for the three spinach variety types which were studied in this project. The model predicts the growth rate of spinach based on the number degree days that accumulate above 0°C and below 27°C.

It was developed using crop growth and temperature data from Boisdale, near Sale in East Gippsland, Victoria, and also from Camden in NSW. It was validated using separate commercial data from the Victorian farm, and also sites in SE Queensland.

The formula for calculating Growing Degree Days (GDD) is:

Daily GDD = $((T_{max} + T_{min}) \div 2) - T_{base}$

where:

 $\begin{array}{l} T_{max} = \mbox{the daily maximum air temperature °C} \\ T_{min} = \mbox{the daily minimum air temperature °C} \\ T_{base} = \mbox{the GDD base temperature below which no significant growth occurs.} \end{array}$

For our model, the base temperature = 0° C and if the measured T_{max} ever exceeds 27°C, then 27°C should be used in place of the actual maximum for calculating degree days.

The growing degree day requirements for the three spinach types used in the project grown in either location are given in Table 16.2.

Spinach Variety	Growing degree days (seeding to harvest)	Base temperature (°C)	High temperature cut off (°C)
Crocodile	552	0	27
Roadrunner	530	0	27
Parrot	515	0	27

Table 16.2 Spinach Growing Degree Day (GDD) requirement

16.3 Determination of the temperature limits for spinach

The growing temperature limits for spinach were determined on the basis of two ideas. First, for maximum quality, spinach should be grown under conditions which result in the plants reaching harvest maturity in 30 days or more from seedling (Figure 16.1), and second that temperature can limit the germination of seeds.

16.3.1 Maximum Temperature

In Figure 16.2, a line was drawn horizontally at 30 days on the model of days to maturity for intermediate growth rate spinach based on historical average temperature data for Sale, near Boisdale in East Gippsland, Victoria. Spinach grown for 30 days or longer is the best quality, so the point where the 30 day line intersects the days to harvest line should define the optimum growing period for Sale.

The long-term daily maximum and minimum temperatures were then plotted underneath the crop development data (Figure 16.2).

Vertical lines were drawn corresponding to the optimal growth period. Where these lines intersect the average daily maximum, this defines the maximum average temperature at which spinach quality (shelf life) was ideal. This is 23°C (Figure 16.2).





It is possible to grow spinach above 23°C and still produce a crop, but the quality will be reduced. The practical upper limit for spinach is 28°C, above which germination becomes severely limited.

The estimation of 28°C as a maximum temperature for baby leaf spinach is consistent with the 27°C high temperature, determined by the model.

16.3.2 Minimum Temperature

For determination of the lower temperature limit, it is reasonable to look to the heat unit model that was developed. In this model, the best fit to the growth data was obtained with a minimum temperature of 0°C. Given the temperatures are long-term averages, and the risk of severe frost damage of setting the minimum temperature too low, a minimum temperature of 2°C is more reasonable.

Temperature	Germination (0°C)	Growth (0°C)	Mapping Limits
Min	2	5	Min. = 2
Optimum Range	7-24	Maximum 23°C for	23°C for
		maximum quality	Maximum quality
Max.	2	28	Max. = 28

Table 16.3 Temperature requirements of baby leaf spinach

16.3.3 Temperature requirements for other baby leaf species

It was not within the scope of the current project to determine upper and lower temperature limits for the other key baby leaf species; rocket and lettuce using the same method as that used for spinach. It was however, possible to determine upper and lower temperature limits from previous projects in conjunction with the experience of project collaborators.

The estimated limits for rocket and lettuce are given in Table 16.4 and Table 16.5.

Temperature	Germination (0°C)	Growth (0°C)	Mapping Limits
Min	10**	5-8	Min. = 10
Optimum Range	20-30	16-24	Max. night = 18
Max.	35	30-32	Max. = 32

 Table 16.4 Temperature requirements for European wild rocket

 Notes:

10°C minimum soil temperature for germination is a major limitation In Italy, germination in greenhouses is controlled at 20-22°C.

Temperature	Germination (0°C)	Growth (0°C)	Mapping Limits
Min	2.2	7	Min. = 7
Optimum Range	18-21	12-21	Max. night = 15
Max.	27**	24	Max. = 24

 Table 16.5 Temperature requirements for baby leaf lettuce

<u>Notes</u>: Soil temperature above 28°C is a limiting factor for germination. 23°C average daily maximum for best quality.

Notes:

High temperature dormancy above 27° C is a limitation Germination at 0° C = 49 days, and at 5° C = 15 days (see optimum range)

16.3.4 Selection of an appropriate area to grow baby spinach

Average historical daily maximum and minimum temperature, and rainfall data was obtained from the Bureau of Meteorology website for 16 potential growing locations in Queensland, 30 in New South Wales, 14 in Victoria, 2 in South Australia, 3 in Tasmania and 5 in Western Australia. In addition, climate data for two major baby leaf production areas in the United States of America; Yuma, Arizona and Salinas, California were included to check the prediction is robust.

The potential growing regions screened all had available horticultural land, and water. The available infrastructure varied considerably.



Germination temperature (day/night °C)



The mapping limit data in Table 16.3, Table 16.4 and Table 16.5 were used to draw upper and lower limit lines on the plotted daily average maximum and minimum temperature data. Figure 16.4 is an example of this approach used for spinach, rocket and lettuce in Cranbourne, Vic.



Figure 16.4 Example of mapping data plotted for Cranbourne, Vic

Figure 16.5, Figure 16.6 and Figure 16.7 illustrate a review of the weather data of a range of sites. The six potential sites identified were Mt Gambier, Hobart, Stanthorpe, Bairnsdale, Cranbourne and Gympie. Districts that were green for 12 months represented potential production areas, the lighter green represented borderline production temperature.

Spinach	lan	Fab	Mor	Anr	May	lun	Lubz	Aug	Cont	Oct	Nov	Dee
QLD Maroochydore	Jan	reb	war	Apr	way	Jun	July	Aug	Sept	Oct	NOV	Dec
Rockhampton												
Mackay												
Millaroo												
Emerald												
Pittsworth/Brookstead												
Inglewood												
Gympie												
Bundaberg												
Bowen												
Atherton												
Gatton (Lawes)												
Toowoomba												
Beechworth												
NSW	lan	Feb	Mar	Anr	May	lun	luly	Aug	Sent	Oct	Nov	Dec
Bellingen	Jan	1.60	wich		May	oun	oury	Aug	oept	001	1407	Dec
Kempsev												
Southwest rocks (Stuarts Point)												
Coffs harbour												
Waughope												
Taree												
Cessnock												
Nowra												
Robertson (Mittagong)												
Murwillumbah												
Lismore												
Bega												
Sunny Corner, NSW					_							
Milthorpe, NSW												
Albury												_
Hillstone												
Oberon												
Camden												
Guyra												
Condobolin												
Canowindra												
Gayndah												
Hav												
Orange												
Bathurst												
Cooma												
Tumbarumba NSW												
Victoria	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Orbost												
Cranbourne												
Rochester												_
Boort												
Echuca												
Mildura												
Colac												
Bairnsdale												
Robinvale (Wemen)												
Warrpambool												
Maffra												
Werribee (Laverton)												
South Australia	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Murray Bridge, SA												
Mt Gambier												
Tasmania	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Forth												
Hobart Swansea (Tas)												
Northern Territory	Jan	Feb	Mar	Apr	Mav	Jun	Julv	Aua	Sept	Oct	Nov	Dec
Katherine		1		<u> </u>			<u> </u>			<u> </u>		
Ti Tree (Territory Grapes)	1	1	1									
Ti Tree (Barrow Creek)												
Middle Point (Humpty Doo												
Western Australia	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Wanneroo												
Albally												
Maniimup												
Kununurra												
USA	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Yuma, AZ												
Salinas, CA												

Figure 16.5 Potential spinach growing times x regions in Australia

European Wild Rocket (not including Aragula)

QLD	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Maroochydore					,	• ••••		g	00p.			
Rockhampton												
Maakay												_
Milleree												
Transla												
Eilleralu Dittewerth/Dreeksteed												
Pillsworth/Brooksleau												
Inglewood												
Gympie												
Warwick												
Bundaberg												
Bowen												
Atherton												
Gatton (Lawes)												
Toowoomba												
Beechworth												
Stanthorpe												
NSW	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Bellingen												
Kempsey												
Southwest rocks (Stuarts Point)												
Coffs harbour												
Waughope												
Taree							I					
Cessnock												
East Maitland (Tocal)												
Nowra												
Robertson (Mittagong)							I					
Munwillumbab												
LISINOTE							I					
Bega										-		
Sunny Corner, NSW							L					
Milthorpe, NSW						ļ	I			ļ		
Albury												
Griffith												
Hillstone												
Oberon												
Camden												
Guyra												
Condobolin												
Canowindra												
Gavndah												
Tumut												
Hav												
Orange												
Bathurst												
Cooma												
Tumbarumba NSW												
Victoria	lan	Eab	Mar	Apr	May	lun	luby	Aug	Sont	Oct	Nov	Dec
Victoria Orte a st	Jan	гер	Wal	Api	way	Jun	July	Aug	Sept	UCI	NOV	Dec
Orbost												_
Cranbourne												
Sale												
Rochester												
Boort												
Echuca												
Mildura												
Colac												
Bairnsdale												
Robinvale (Wemen)												
Gundagai												
Warrnambool												
Maffra												
Werribee (Laverton)												
South Australia	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Murray Bridge, SA							· ·					
Mt Gambier												
Tasmania	Jan	Feb	Mar	Apr	Mav	Jun	Julv	Aug	Sept	Oct	Nov	Dec
Forth							<u> </u>	Ť			-	· ·
Hobart					1		l					
Swansea (Tas)							<u> </u>					
Northern Territory	Jan	Feb	Mar	Anr	Мау	Jun	July	Auc	Sent	Oct	Nov	Dec
Katharina	Jan		mai	- ide	may	Juil	July	nug	Jepi	501		500
Ti Tree (Territer: Oracia)			_					_				
Ti Tree (Territory Grapes)		 										
IIIree (Barrow Creek)	L	L								l	l	
Midale Point (Humpty Doo	I						.					
western Australia	Jan	Feb	Mar	Apr	Мау	Jun	July	Aug	Sept	Oct	Nov	Dec
Wanneroo												
Albany												
Denmark												
Manjimup												
Kununurra												
USA	Jan	Feb	Mar	Apr	Мау	Jun	July	Aug	Sept	Oct	Nov	Dec
Yuma, AZ	Ì			-			<u> </u>					
Salinas CA	1		1									

Figure 16.6 Potential rocket growing locations x regions in Australia

Lettuce												
QLD	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Maroochydore								, j				
Rockhampton												
Mackay												
Millaroo												
Emerald												
Pittsworth/Brookstead												
Gympie												
Warwick												
Bundaberg												
Bowen												
Atherton												
Gatton (Lawes)												
Toowoomba												
Beechworth												
Stanthorpe	Law.	F .1				1	laste a		0	0.1	Num	
NSW	Jan	Feb	war	Apr	way	Jun	July	Aug	Sept	UCT	NOV	Dec
Kempsov												
Southwest rocks (Stuarts Point)												
Coffs harbour												
Waughope	1		1									
Taree	1		i									
Cessnock												
East Maitland (Tocal)												
Nowra												
Robertson (Mittagong)												
Murwillumbah	<u> </u>		<u> </u>									⊢
LISINOFE	<u> </u>											┝──┤
Suppy Corper NSW							I				-	
Milthorpe NSW												
Albury												
Griffith												
Hillstone												
Oberon												
Camden												
Guyra												
Condobolin												
Canowindra												
Gayndah												
lumut												
Orango												
Bathurst												
Cooma												
Tumbarumba NSW												
Victoria	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
Orbost												
Cranbourne												
Sale												
Rochester												
Boort												
Echuca												
Mildura												
Baimadala								-				
Robinvale (Wemen)												
Gundagai												
Warrnambool												
Maffra												
Werribee (Laverton)												
South Australia	Jan	Feb	Mar	Apr	Мау	Jun	July	Aug	Sept	Oct	Nov	Dec
Murray Bridge, SA												
Mt Gambier												
Tasmania	Jan	Feb	Mar	Apr	Мау	Jun	July	Aug	Sept	Oct	Nov	Dec
Forth						<u> </u>	<u> </u>					
Hopart							I					
Swansea (Tas)	lan	Fab	Mar	Anr	May	lue	lubr	Au.~	Sant	Oct	Nov	Dec
Northern Territory	Jan	reo	war	Apr	way	Jun	July	Aug	Sept	UCT	VOV	Dec
Ti Tree (Territon/Grapos)												
Ti Tree (Barrow Creek)	-		<u> </u>									┝──┤
Middle Point (Humpty Doo	1		1	<u> </u>						l —	<u> </u>	
Western Australia	Jan	Feb	Mar	Apr	Mav	Jun	Julv	Aug	Sept	Oct	Nov	Dec
Wanneroo	1		i					-9			<u> </u>	<u> </u>
Albany												
Denmark												
Manjimup												
Kununurra												
USA	Jan	Feb	Mar	Apr	Мау	Jun	July	Aug	Sept	Oct	Nov	Dec
Yuma, AZ												
Salinas, CA		1										-

Figure 16.7. Potential lettuce growing locations x regions in Australia

The screening data and sun shine hours were also investigated for each of the potential growing areas selected.

16.4 Report on the suitability of alternative sites for winter baby leaf production in Australia.

16.4.1 Summary

In Mt Gambier the analysis shows that the temperatures are generally suitable for winter production of lettuce (only slightly below the desired minimum).

The number of cloudy days during winter is very high (18-19 per month) and the average sunlight hours during this time is only about 4 hours per day. This could be a limitation, and is likely to slow growth. Hobart by comparison has a similar number of daylight hours per day over winter, but less cloudy days (14 per month).

Stanthorpe is a bit cold for winter lettuce, but the higher day temperature may compensate. It has a lot less cloudy days in winter than Mt Gambier (7-8 cloudy days per month).

16.4.2 Recommendation

It is recommended that OneHarvest trial winter lettuce and spinach production at Mt Gambier, but expect slow growth rates. The rainy and overcast weather may cause significant issues with foliar diseases such as downy mildew and *Sclerotinia*. Operations such as planting, harvesting, land preparation and spraying may also be affected by the wet weather.

Mt Gambier - climatic details

The winter temperature for winter production is just marginally too cold for lettuce. It would be fine for spinach, but too cold for European wild rocket. There is significant cloud cover and limited sunshine hours in winter. There are 18-19 cloudy days per month between May – October and only 4 h per day (on average) of sunlight for May, June and July. It starts to increase in September.



Cloudy days and sunshine hours



Spinach



European Wild Rocket



Hobart – Climatic details

The winter temperatures are slightly colder than Mt Gambier.

Please note, the much cooler summer temperature which will result in better quality lettuce in mid summer.

There are about 14 cloudy days per month for most of the year, except for summer where the number drops slightly. There are still only 4 sunshine hours on average in May, June and July, which is the same as Mt Gambier.





Stanthorpe – Climatic details

The winter minimum temperatures are below those recommended for lettuce, but the relatively high day temperatures may compensate for this.

Winter temperatures are suitable for spinach and too cold for European wild rocket. There are about 7-8 cloudy days per month during winter, which is much higher than for Mt Gambier.

No data was available on average sunlight hours.







Spinach







Bairnsdale – Climatic details

The winter temperatures are marginally too cold for lettuce.

Winter temperatures are suitable for spinach and too cold for European wild rocket.

There are 14 cloudy days during winter which is less than Mt Gambier.



Cloudy days and sunshine hours



Spinach



European Wild Rocket



Cranbourne – Climatic details

The winter temperatures are suitable for lettuce.

Winter temperatures are suitable for spinach and too cold for European wild rocket.



There are 12-14 cloudy days during winter which is less than Mt Gambier.

Cloudy days and sunshine hours



European Wild Rocket





Gympie – Climatic details

Gympie is suitable for winter lettuce production but really only from May to August. Gympie is also suitable for spinach, and slightly too cold for rocket during mid winter.

There are only 5-6 cloudy days per month during winter which is the lowest of all the growing regions.



Cloudy days and sunshine hours











16.5 Assessment of Mt Gambier Site

This report was an assessment that Gordon Rogers carried out for OneHarvest on a property that Oneharvest acquired at Mt Gambier with a view to growing baby leaf spinach and lettuce as a commercial pilot-to-prove operation.

16.5.1 Climate

Latitude: 37.75 °S





From a temperature perspective, the site should be suitable for all year round production. The summer maximums are not excessive, although the winters are slightly too cold on average. There may be high temperature spikes in summer which may cause quality problems and frosts in winter may also result in some crop losses.

There is some significant winter rainfall which may cause some foliar disease problems, but perhaps more importantly, cloudy weather combined with low temperature may result in a very long crop growth cycle in winter. This will need to be taken into account for scheduling purposes.



Figure 16.8 Mt Gambier site before development.

16.5.2 Comments on Soil Test Report

The soil appears to be a sandy loam (from photos) with a reasonably high nutrient holding capacity (CEC=17 meq/100g; although units not shown on soil test report).

The soil organic matter level is good and it is not saline or sodic. The pH is slightly acidic but within the normal range and will not limit nutrient uptake.

The background nitrogen levels reported in the soil test are likely to be in the form of soil organic matter and probably not available for immediate plant uptake. Nitrate or ammonium N levels were not shown. Applying full crop nutrient requirement is recommended.

Phosphorus levels are only moderate for a lettuce crop and will benefit from an additional basal application of up to 80 kg/ha P.

The level of Potassium is low. The ideal level is at least 5% of cations present. Crop removal for a 30 t/ha crop is about 75 kg of potassium/ha. I suggest applying in excess of 100 kg/ha of potassium per crop.

Calcium and Magnesium: Calcium is adequate but magnesium is slightly low. Crop Mg levels should be monitored using leaf analysis.

Micronutrients: All plant micronutrients except Mo are low and may need to be applied to the crop as a basal application or through foliar sprays. The key micronutrients to apply are boron, manganese, copper and zinc.

16.5.3 Water

The water analysis is normal and should be suitable for growing lettuce. Iron should not be a problem in trickle irrigation tubes.

Lettuce will require about 3.5 ML/ha per crop if using sprinkler irrigation and about 1.5 ML/ha for trickle irrigation, which includes an initial irrigation with sprinklers for establishment.

The soil appears sandy and therefore the lateral spread of water from a trickle irrigation tube may be limited and insufficient to successfully establish a crop without supplementary sprinkler irrigation. This is a key issue and may require setting up both sprinkler and trickle irrigation system infrastructure if trickle is selected as the irrigation source.

16.5.4 Other Issues

These responses were provided by Colin Britton to questions asked by Gordon Rogers.

Land area suitable for cultivation: The initial area available for the Pilot is 45 ha. The potential future area available in the district is thousands of ha. The Pilot property has about 300 ha available.

<u>Water Supply</u>: All water is Bore water. Flow rates of 25,000-150,000 gallons/hour (112,500-675,000 litres/hour). The bore next to the pilot block is running a 50,000 gallon per hour pivot for up to 24 hours a day. Water is all pumped from 7 - 20 m depth. The Pilot has a water allocation of 150 ha, i.e. (approximately 1,000 ML).

Existing infrastructure on the farm, e.g. sheds, power, irrigation sub mains, bores, pumps: Machinery shed available, also block opposite pilot site with area for amenities / lunch block with bore and 240 volt power available if reconnected. Also bay 2 has a shed on it. Furthermore cold rooms, loading facilities, office space, workshop, and processing area all have been acquired at a location about 8km up the road.

Transport availability for getting the lettuce to Bairnsdale/Wacol for processing: Liz Hall has been involved with logistics; Mt Gambier is the home base for a number of large freight companies including Scott's, K & S, and SWF ECT. Sheaths transport (Scott's) will deliver a minimum of 4-6 pallets in the short term to Melbourne. Multiple companies cater for full pallet loads direct to Bairnsdale. Freight costs are around \$50-60 a pallet.

Levels of local support available include insect scouting, chemical and fertilizer stores etc: Mt Gambier is a major centre of more than 20,000 people and consequently has local branches of all of the major produce companies such as Landmark and Elders. It has a good base of Agronomists and support services.

Machinery requirements: All machinery will be acquired and transported to the pilot.

Other Issues to consider:

- Is there a risk if flooding?
- Has a supply of seedlings been secured?

16.6 Preliminary Conclusions

Providing transport, support services and other inputs such as water supply are as indicated by Colin (and within the constraints included in this report), the farm appears to be suitable for commercial lettuce production.

16.7 Establishment of Commercial Pilot to Prove Trials

In 2007, OneHarvest set up a pilot to prove trial on the farm at Mt Gambier. There were seven plantings from 15th November to 12th December 2007. Species planted included spinach, tatsoi, fine mizuna, chard and european wild rocket.

Areas planted over that time were: spinach, 1.78 ha of cv. Crocodile and 0.4 ha of cv. Greyhound which produced a total yield of 19.3 tonnes over that area. Also planted was 0.63 ha of rocket which produced a yield of 3.2t.

The area was proved capable of successfully producing summer baby leaf spinach and rocket, and is now being used commercially by OneHarvest.



Figure 16.9 Spinach cv. Crocodile established at Mt Gambier and ready for harvest.



Figure 16.10 Young spinach growing at Mt Gambier showing excellent establishment of by direct seeding.

17 PROJECT CONCLUSIONS & RECOMMENDATIONS

17.1 Pre-harvest effects on quality

Post-harvest decline in quality was significantly affected not just by post-harvest handling and storage but by pre-harvest growing conditions. The key indicator of this was the shelf life of the unprocessed product.

The most significant finding of the study was the effect of growth rate on quality. For spinach to attain maximum quality, it needs to be grown for at least 30 days from seeding to harvest (Chapter 3). This 30-32 day minimum growth can be achieved by either selecting slow-growing varieties during warmer periods, or by growing the crop in a climate cool enough to give at least 30 days from seeding to harvest.

The effect of temperature on spinach growth rate is a combination of maximum day temperature and minimum night temperature. High day temperatures can be offset to some extent by cooler nights, resulting in slower overall growth. The most rapid growth occurs when there is a combination of high day and high night temperature.

Again for spinach, the best quality was achieved where the average daily maximum temperature did not exceed 23°C (Chapter 16). The best quality was achieved when the average night temperature did not exceed about 8-10°C. Poorest quality occurred when the night temperature did not fall below 17-18°C (Chapter 3). The crop growth rate of spinach for any given area and time of year can be predicted by using the crop growth model developed as part of the project (Chapter 8) and using historical average temperature data.

The effects of growing temperature on quality of spinach was confirmed in a controlled growth cabinet study where a 21°C Day/12°C night produced 43% longer shelf life compared to the same heat tolerant variety (Crocodile) grown at 27°C Day/18°C night (Chapter 3).

Seasonal weather conditions were also significant in determining spinach quality, e.g. in Camden in March 2007, a rainfall event of 132mm within 3 days after seeding, significantly reduced yield and quality compared to crops planted either side of this event.

17.2 Harvest Time of Day

The time of day that spinach is harvested at was found to have a significant effect on the shelf life of the product. The shelf life of spinach harvested at 6.00am was about 3 days longer than product harvested at 9.00am or 12 noon, and this trend followed the amount of starch remaining in the leaves: more starch = longer shelf life (Chapter **Error! Reference source not found.**).

In lettuce, nitrogen has a significant effect on shelf life. Where leaf nitrogen exceeded 4% at harvest, shelf life was reduced (Chapter 6). The same is likely to be true for spinach. However, it is also likely that if higher rates of N are "balanced" by other nutrients such as potassium, phosphorus and calcium that the negative affect of excessive nitrogen on shelf life could be minimized.

17.3 Post-harvest handling

Once the crop is harvested, quality immediately begins to decline. Two key aspects of post-harvest handling that were investigated in the project were temperature management and moisture in the bag of processed lettuce.

Harvested lettuce was vacuum cooled at various times after harvest and the best shelf life was achieved if the product was cooled within half an hour of harvest. This is the best option but may not be practical in many cases. Nonetheless, the longer vacuum cooling is delayed, the shorter the shelf life of the product, and forced air cooling is not a viable alternative to vacuum cooling (Chapter 7).

A useful concept is that pre-harvest factors determine potential shelf life, while postharvest handling determines to what extent this potential shelf life is realised.

17.4 Vitamin C Content

Some work was done on the stability of the common antioxidant, vitamin C (ascorbic acid) which is present at high levels in spinach. Vitamin C is known to be highly thermolabile and this was reflected in this study, with vitamin C persisting longer in harvested product stored at lower storage temperatures. When held at 0°C, spinach retains 90% of the vitamin C content for 7 days whereas at the higher temperatures of either 4°C or 7°C, about 50% of the vitamin C is lost in the same time. However, after 14 days only about 30% of the vitamin C remains regardless of storage temperature (Chapter 5).

17.5 Production

An outstanding achievement of the project was the development of a reliable heat unit growth model which predicts the time from seeding to harvest for the major growing regions in Australia. The GDD requirement for Camden and East Gippsland are virtually identical and slightly different to Gympie and Stanthorpe.

Daily GDD =
$$((T_{max} + T_{min}) \div 2) - T_{base}$$

where:

 T_{max} = the daily maximum air temperature °C

T_{min} = the daily minimum air temperature °C

 T_{base} = the GDD base temperature below which no significant growth occurs.

For our model, the base temperature = 0°C and if the measured T_{max} exceeds 27°C, then 27°C should be used in place of the actual maximum for calculating degree days.

Emergence to harvest	E. Gippsland GDD	Camden GDD	Gympie GDD	Stanthorpe GDD	T _{base} (°C)	T _{max} (°C)
Crocodile	443	441	401	521	0	27
Roadrunner	420	421	397	-	0	27
Parrot	404	406	364	498	0	27

Table 17.1 Spinach Growing Degree day requirement for four regions from emergence to harvest

Table 17.2 Spinach Growing Degree day requirement for four regions from seeding to harvest

Seeding to harvest	E. Gippsland GDD	Camden GDD	Gympie GDD	Stanthorpe GDD	T _{base} (°C)	T _{max} (°C)
Crocodile	553	551	511	631	0	27
Roadrunner	530	531	507		0	27
Parrot	514	516	474	608	0	27

The optimum days to harvest for each of the main spinach varieties in the districts used in this trial are shown in Tables 17.1 and 17.2. These heat unit models can be used by commercial growers to schedule crops for a continuous supply of spinach to the processor. A heat unit model is more reliable than planting using a calendar schedule.

The germination sensitivity of rocket seed to temperature is a practical limitation to field production, particularly in European wild rocket where germination drops sharply below 20°C. In this study, we have found that alternating day/night temperature partly overcomes this steady temperature effect. European wild rocket will germinate effectively when night temperatures drop down as low as 10°C provided day temperatures reaches 24°C. Cultivated rocket by comparison is not sensitive to low soil temperatures and can germinate down to as low as 5°C (Chapter 9).

Planting density has a significant effect on spinach yield and leaf size. The highest yields were obtained at a density of 1,000 plants/m² but this high density reduced average leaf weight to 1 g and reduced shelf life from 23 days at 300 plants/m² to 16 days at 900 plants/m2, measured at 2°C on unprocessed leaves (Chapter 0).

Weeds are also a significant issue and some data is presented on current and anticipated permits and registrations as well as some preliminary work with phenmedipham (Betanal[®]) (Chapter 12).

17.6 Varieties

The spinach variety work was mainly focused on identifying key attributes of seasonal types. The Rijk Zwaan varieties Crocodile, Roadrunner and Parrot were chosen as examples of slow, intermediate and fast growing types respectively. Of these, Crocodile was the only slow growing variety bred specifically for baby leaf production and as a result had the best shelf life and the thickest leaves. There was some screening work done on a wider selection of spinach varieties on the Queensland sites (Chapter **Error! Reference source not found.**).

European wild rocket varieties and cultivated/Arugula type varieties were compared at Camden. The summer results are presented and work is continuing for other times of the year. Yield, shelf life and other quality data are presented and some of the cultivated types have performed better that expected but do not have the distinctive rocket taste which comes from the glucosinolate content of european wild rocket (Chapter 14).

Lettuce varieties were also studied; newer, multi-leaf types were compared with more traditional, single leaf, direct seeded lettuce types. Multi-leaf types were found to yield more and have good quality attributes (Chapter 15).

17.7 Pilot to Prove

Finally the agronomic modelling data developed in the project were used to search for, select and establish a commercial pilot-to-prove farm at Mt Gambier in South Australia. This successfully produced quality baby leaf spinach and other baby leaf species.

18 Recommendations for Future Work

This project was conducted over three years and so further work is required to validate some of the results reported. This is particularly true for the data that is based on temperature and seasonal climate. Many of the results reported are novel and had not been previously reported in the scientific literature.

It is therefore recommended that more work is done to;

- 5. Validate the growing degree day model developed for spinach
- 6. Develop Growing Degree Day models for other baby leaf species
- 7. Validate the relationship between days to harvest and shelf life for baby leaf spinach and this model could be extended to other baby leaf species.
- Investigate in more detail the relationship between leaf thickness and shelf life for baby leaf spinach and this could be extended to include a more detailed study of the structure of the leaves relative to their capacity to withstand processing.
- 9. Investigate the level of nutritional bioactive compounds in baby leaf salad products and their stability during storage.

It is likely that several research papers will be prepared from this report. A paper on the germination characteristics of rocket has already been submitted to the Journal of Weed Science. It is likely that the development of the growing degree day model could be published as well as a paper linking preharvest growing conditions to the postharvest shelf life of baby leaf spinach.

An agronomic guide for the production of baby leaf spinach is currently being prepared which will summarise the results in a way that will be useful for growers.

19 Technology Transfer

Review Meetings:

- 23rd March, 2006 Management group meeting Wacol Factory Brisbane-PowerPoint presentation attached (01)
- 26th July, 2006 Management group meeting Wacol Factory Brisbane
- 7-8th September 2006 Management group meeting Bairnsdale , Vic PowerPoint presentation attached (02)
- 20th February 2007. Management group meeting Wacol office Brisbane. PowerPoint presentation appended (03)
- 26th of September 2007. Management committee meeting Wacol Qld PowerPoint presentation attached (04)

Grower Meetings

- March 2006. Baby Leaf Update OneHarvest
- 08 September 2006. Baby Leaf Update, OneHarvest Growers, Bairnsdale, Vic.
- 15 September 2006. Baby Leaf Update, OneHarvest Growers, Stanthorpe, Vic.
- March 2007 Baby Leaf Varieties
- 13 November 2007 Baby Leaf update to OneHarvest growers, Stanthorpe, Qld – PowerPoint presentation attached (06)
- 15th November, 2007 Baby Leaf update to OneHarvest growers, Bairnsdale Vic – PowerPoint presentation attached (07)
- 17th January 2007. Jan den Braber (Rijk Zwaan world wide spinach breeder) & Harry Turna (RZ) Australia inspected trials at Bill Taylor's Boisdale with Tim Kimpton.
- 15th February 2008. Mike Titley met with following technical staff from Rijk Zwaan at Melbourne airport to discuss genetic material from Rijk Zwaan for the balance of the project and to review spinach growth and post-harvest results to date. Links established with RZ spinach breeder in Holland.
 - Arie Baelde CEO of Rijk Zwaan seeds in Australia and Harry Turna (Australia)
 - Pieter Egelmeers Head of Plant Breeding for all Terra Crops (Salad baby leaf, spinach etc.) for Rijk Zwaan International based in Holland
 - Frans Carree Chard and Rocket breeder based in Holland
- 13th March 2008 Mike Titley attended annual Rijk Zwaan Field Day baby leaf trials at Daylesford observing specifically the new generation Salanova types.
- Jan-Mar 2008. The sub committee of the multi-leaf lettuce variety and establishment trials met at Colin Britton's trial site at each of the three harvests during first three months of 2008.
Workshops

OneHarvest field and technical staff - Bairnsdale, Vic - 19 October 2006 – PowerPoint presentation attached (05). Programme:

- Planting Density
- What is a good indicator of shelf life?
- Effect of nitrogen on growth and shelf life on red and green coral lettuce
- Scheduling Spinach
- Post-harvest handling of leafy processed vegetables

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