Project title:	A review of the agronomic factors that influence postharvest pinking in lettuce
Project number:	Project FV 413
Project leader:	Dr Jim Monaghan
Report:	Final report, May 2013
Previous report:	None
Key staff:	Dr Laura Vickers
Location of project:	Harper Adams University, Shropshire, TF10 8NB
Industry Representative:	Dr Ed Moorhouse G's Fresh, Ely, Cambridgeshire, CB7 5TZ
Date project commenced:	1 March 2013
Date project completed	31 May 2013

DISCLAIMER

Authentication

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

[Name]
[Position]
[Organisation]

Signature .... Date ....
[Name]
[Position]
[Organisation]

Signature .... Date ....

Report authorised by:
[Name]
[Position]
[Organisation]

Signature .... Date ....
[Name]
[Position]
[Organisation]

Signature .... Date ....

Contents

GROWER SUMMARY

Following harvest some lettuce can produce pink colouring in the butt and ribs of the outer leaves. This is termed ‘pinking’ and, in spite of the development of new varieties with claims of reduced pinking, it continues to present substantial problems for producers of both UK and imported crops. Poor product on the shelf reduces sales and leads to complaints and consumer dissatisfaction. However, we do not have a good predictive system for this disorder; growers instead have to rely on gut-feelings and experience rather than having an informed best management strategy for this problem. There are suggestions in the literature that nutrition, high rainfall/over irrigation and temperature can have an influence on the expression of pinking along with physical damage at harvest. This study provides an overview of the underlying physiology of pinking; summarises the information available on the agronomic causes of pinking and recommends actions that growers could take to reduce pinking.

Key messages

- Growers should select cultivars with reduced pinking susceptibility.
- Care needs to be exercised when harvesting wholehead lettuce to minimise handling damage as wounding/bruising lettuce ribs can increase pinking.
- High levels of nitrogen (N) can increase pinking; growers need to optimise nitrogen application to prevent excessive levels of N being available to the crop. Particular care will be needed in crops grown on soils with high levels of residual N.
- Growers should consider whether phosphorus (P) nutrients have been optimised for lettuce as P nutrition may have a role in reducing pinking.
- Crop water status needs to be managed closely. Excess irrigation (and rainfall) can increase pinking.
- High temperatures 1-2 weeks before harvest can increase pinking and growers can monitor the climate of the growing crop to get an early warning of pinking potential. This may allow a temporary reduction in product-life to minimise wastage in the supply chain.

- There is no scientific evidence to support the commonly held belief that over mature heads having a greater pinking risk.

Key research needs

This review has identified a number of agronomic factors that have been reported as influencing pinking in lettuce. Some of these factors, such as temperature or rainfall, cannot be manipulated by growers but knowledge of their effects could be used to manage product shelf-life through the supply chain.

- **Work on predictive models that utilise local meteorological data is needed to allow growers to identify high risk crops.**

Wounding, nutrition and irrigation have been reported to be key factors in subsequent pinking.

- **In-field harvesting and handling systems that minimise damage to the ribs of leaves are needed to minimise pinking linked with wounding.**

High rates of nitrogen and irrigation have been associated with an increase in pinking but the interaction with other nutrients is not clear. It is not possible to identify an optimal nutrient regime or irrigation schedule from the research reviewed here.

- **Further research on optimal irrigation and nutrition regimes is needed to give growers confidence to adjust treatments.**

Reducing pinking in lettuce may require a mix of agronomy and genetic approaches. Identifying the agronomic conditions that reduce and conversely increase pinking will be of interest to researchers studying the genetic factors underlying cultivar responses.

- **Work linking environmental factors to genetic control of pinking (i.e. phenotyping in diverse environments) is needed for breeders to develop more resilient lettuce cultivars for the UK industry.**

SCIENCE SECTION

Introduction

Fresh produce are highly perishable and significant wastage can occur from producer through to retailer and consumer. With the cut salad industry growing year on year (Horticulture Research International Final Report, 2002) and worth an estimated £848 million in 2009 (Soininen, 2009), and with growth of 24% forecast between 2009-14 for the prepared salads market, there is an increased demand on maximising input efficiencies in the production of leafy vegetable crops, reducing wastage, increasing marketable yields and extending the shelf life of products.

Cut salads, in particular, suffer from a short and variable shelf-life, with processor wastage due to product discolouration or spoilage costing the UK industry in 2003 an estimated £2.5 million per annum (Wurr *et al.,* 2003). Lettuce heads and other leafy salads can often suffer non-infectious discolouration disorders, such as russet spotting, brown stain and pinking. Discolouration of lettuce butts and leaves can occur within only a few days after harvest, limiting product shelf life and contributing to retail consumer dissatisfaction. Losses due to pinking are hard to quantify but can account for substantial customer complaints at certain times of the year and batch rejections, particularly during the import season when products may take longer to reach the consumer from harvest.

This study provides an overview of the physiology of pinking with a focus on lettuce to summarise the available information in the literature, addressing the potential agronomic causes of pinking.

Methodology

Internet searches were conducted during March-April 2012 to retrieve articles related to pinking in lettuce. Literature was extracted from scientific journals, government websites, HDC reports, e-books, grower guides, e-thesis repositories and conference proceedings. Internet searches were conducted using the following search engines; Google, Google Scholar, Harper Adams University COPAC search engine and the University of Birmingham Library search engine. Boolean operators were used to link together keywords in searches. As pinking is also known as pink ribs in the literature a placeholder character (*) was used in searches after the word ‘pink’. Search terms used included; “pinking lettuce”, “pink rib lettuce”, “irrigation pink* lettuce”, “water stress pink* lettuce”, “fert* lettuce postharvest”, “N lettuce postharvest”, “temp postharvest lettuce”, “temp lettuce pink*”, “maturity lettuce pink*”, “light lettuce pink”, “UV lettuce pink”, “lettuce pink* cultivar”, “brown lettuce”, “lettuce PPO” and “lettuce PAL”. Articles retrieved were from a diverse range of sources and locations. The reference lists of selected articles were also reviewed in detail to find additional articles.

Defining pinking

Lettuces are subject to a number of pre- and postharvest disorders that are not caused by bacteria or fungi but can be easily confused as their symptoms are similar.

A common preharvest disorder is brown rib or rib blight, which occurs on the inner surface of outer head leaves, with discoloured areas turning a yellow/tan colour and then later turning brown/black (Lipton *et al.*, 1972). Tipburn or tip scorch is another common preharvest disorder caused by calcium deficiency, and characterised by the appearance of brown spots along the outer margins of leaves that eventually turn brown and die. Tip burn can occur internally in the lettuce head or externally, the disorder is usually prevalent in hot weather and when the plant is undergoing rapid growth (Swaider *et al.*, 1992). Other preharvest disorders include internal rib necrosis and rusty brown discolouration aka rusty rib (Lipton *et al.*, 1972).

Postharvest disorders develop once the lettuce has been harvested. Russet spotting is a disorder that can be induced by exposure to the hormone ethylene and is characterised by a reddish-tan colour and brown elongated pit-like spots on the midribs of Iceberg lettuce (Lipton & Ryder, 1989). Brown stain consists of lesions of 0.6-1.3cm that are slightly sunken and appear tan, brown or even black in colouration, and is associated with elevated carbon dioxide and low oxygen concentrations (Isenberg, 1979; Brecht *et al.*, 1973). These lesions can occur under the outer leaves and deeper in the head but don’t appear on the heart of the lettuce (Lipton *et al.*, 1972).

The focus of this study is on the postharvest disorder termed pinking, also known as pink rib in the USA (Image 1.1). Often developing during storage, pinking is characterised by a pink discolouration near the base of midribs on outer leaves. In severe cases, the discolouration can appear in all but the youngest head leaves and stem from the base of the midribs into the veins. The exact cause of pinking is not yet fully understood, although some enzymatic pathways have been indicated as being involved. In addition certain agronomic factors preharvest and storage conditions postharvest have been suggested as playing a role in the development of the disorder.

Image 1.1 Pinking in processed cut lettuce. Image courtesy of Taylor Farms Foodservice, 2012.

Chemistry behind pink discolouration in lettuce

Polyphenol-oxidase (PPO) is widely regarded as the enzyme involved in oxidative browning reactions in fresh produce (Image 1.2). It is also regarded as being the enzyme responsible for pinking in lettuce. PPO is found in young plants tightly bound to the thylakoid membranes of cell chloroplasts, and on chloroplast membrane disruption PPO is released (Chazarra *et al.*, 1996; Zaini *et al*., 2012), although it has been found in a soluble form in senescencing plants.

PPO is a copper containing enzyme which is involved in the hydroxylation of monophenols to diphenols and the oxidation of diphenols to quinones (Lopez-Galvez *et al*, 1996; Toivonen and Brummell, 2008); the products from these reactions then go through a series of non-enzymatic reactions to produce brown, pink or even black pigments (Karpeta, 2001). The major colour pigment produced by PPO in lettuce plants is pink (Lewis, 2001) although it has been suggested that pink pigments will gradually turn brown after prolonged storage (Van Vliet *et al*, 2009). The resulting pigment produced from PPO is dependent on the structure of the starting phenolic substrate (Toivonen and Brummell, 2008), with lettuce pinking being very susceptible to phenolic composition (Wurr *et al*, 2003; Toivonen and Brummell, 2008).

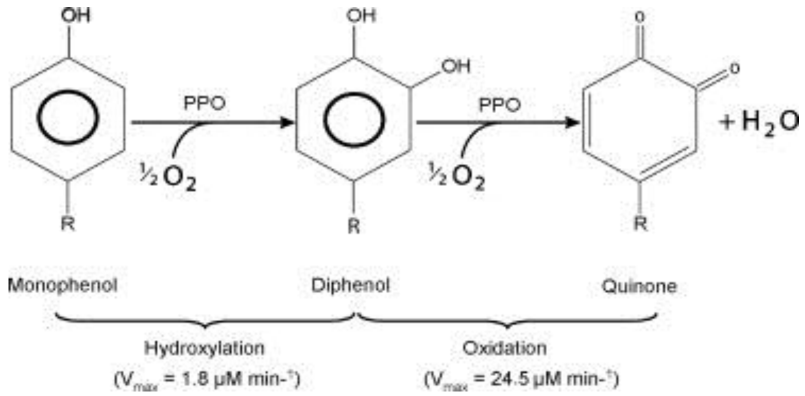

Image 1.2 Mechanism for polyphenol oxidase action on monophenols and diphenols, image taken from Toivonen and Brummell (2008)

The activity of PPO is a direct response to tissue injury (Lewis, 2001); however PPO levels in plant tissue do not change in response to wounding. Instead wounding shows increases in the level of polyphenols in plant tissue, the substrates of PPO. This increase in polyphenols is through the activity of another enzyme, phenylalanine ammonia-lyase (PAL). PAL is the first step in the production of plant phenolic compounds via the phenylpropanoid biosynthesis pathway (Image 1.3). PAL activity increases immediately after wounding and has been found to be closely related to the development of lettuce browning (Hyodo *et al.*, 1978). Following injury, PAL induction and PPO activation has been observed in six lettuce cultivars (Cantos *et al.*, 2002). This suggests that the regulation of polyphenol biosynthesis would be an effective avenue to consider for controlling discolouration in harvested lettuce (Hisaminato *et al.*, 2001). Therefore if we were able to fully understand how climatic conditions, agricultural practices, harvesting methods and storage affect polyphenol biosynthesis, and subsequent activation of PPO, then this information could be used to help inform a best management strategy for growers to manage the occurrence of pinking.

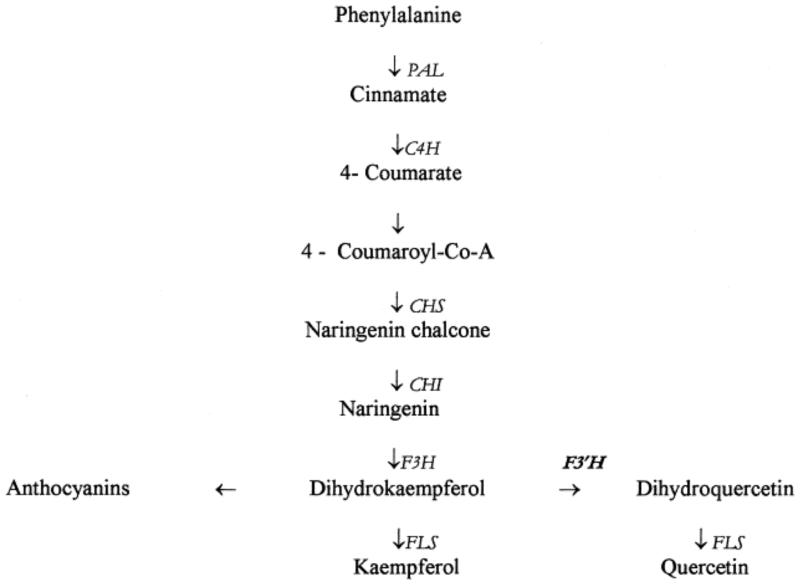

Image 1.3. Schematic diagram of part of the phenylpropanoid biosynthetic pathway taken from Ryan *et al*., (2002) Abbs: PAL, phenylalanine ammonia lyase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3 hydroxylase; F3′H, flavonoid 3′ hydroxylase; FLS, flavonol synthase.

Whole head vs fresh cut lettuce

Lettuce is predominantly consumed as a raw product in salads. Lettuce can be purchased from retailers in mainly two forms, as wrapped whole heads or as fresh cut lettuce in pre-packaged salad bags. Pinking is a postharvest issue that is more likely to affect fresh cut lettuce than whole head lettuce. The process of cutting or shredding lettuce leaves can disrupt the chloroplast membrane, releasing PPO, which acts on polyphenol substrates to produce a pink pigment (Wurr *et al*, 2003; Toivonen and Brummell, 2008).

Despite fresh cut lettuce being more likely to suffer pinking it can also occur in harvested whole head lettuce. As pinking is a physiological disorder and not a fungal disease, it is possible to learn lessons from research into both whole head and fresh cut lettuce. Therefore studies into the effects of agronomic factors on pinking expression in fresh cut and whole head lettuce are discussed.

What are agronomic factors? Can they affect pinking?

Agronomic factors are principally physical and biological factors that can affect crop production. Factors include irrigation, fertilisation (NPK, macro and micronutrients), pests and pesticide application (insect, weed, bacterial and fungal control), sowing date, climate (temperature, rainfall, light and wind speed), soil properties (soil pH and salinity), soil management practices (crop rotation, tillage and aeration), irrigation water quality and cultivar use. These different factors all have the capacity to influence crop yield and quality. In the literature, nutrient availability, irrigation, temperature, cultivar use, bacterial presence and sowing/harvest dates have all been suggested as having an effect on the subsequent quality and storability of harvested lettuce. These factors shall each be reviewed in turn with respect to their influence on pinking incidence in lettuce.

Wounding

Typically harvested lettuce is sold as intact whole heads or as processed cut leaves in salad bags. Lettuce is quite a fragile crop which can be prone to handling damage especially when processed. Damage and wounding from cuts or punctures sustained during crop development, harvesting or storage can affect the postharvest storability of lettuce. Lettuce can sustain wounding in a variety of ways; mechanical wounding during or postharvest, freeze injury when sown early or late in the season, or cell disruption as a consequence of fast or abnormal growth (Sharples, 1965).

The Vegetable Inspection Manual produced by the Canadian Food Inspection Agency (2011) listed bruising and the crushing of lettuce midribs as common condition defects that can result from packaging practise. The guide suggested that these defects could lead to cellular disruption and the formation of air spaces which could make lettuce more susceptible to pinking.

Studies into the effects of wounding on lettuce have shown that within 4 hours of wounding Iceberg lettuce increases PAL and peroxidise activity, as well as the concentrations of several phenolic compounds (Ke and Saltveit, 1989). Whilst cutting lettuce leaves rapidly enhances phenolic content after 24 hours, even when stored at 4 °C (Wurr *et al*., 2003). From the literature we can conclude therefore that wounding incurred during the processing of fresh-cut lettuce can induce the synthesis of PAL protein, increase PAL activity and stimulate the accumulation of phenolic compounds that contribute to tissue discolouration (Ke and Saltveit, 1989; Tomás-Barberán *et al*., 1997; Campos-Vargas *et al*., 2004; Saltveit, 2004).

The deterioration of product quality as a result of wounding can accelerate with increasing storage temperature (Wurr *et al*., 2003). Wounding not only stimulates the phenylpropanoid pathway but can also stimulate respiration in injured plant tissue (Devlin, 1975), and increasing respiration in storage can lead to an increase in the rate of product deterioration (Morad, 2003).

- ***Wounding or processing that results in lettuce damage needs to be minimised as this can increase the expression or incidence of pinking***

Nutrients

Nutrients have a major effect on the growth of plants. Nutrients such as nitrogen influence cell size and number in leaves, as well as the proportion of cell wall material volume to total tissue volume. It might be expected therefore that the amount of nitrogen available to the lettuce crop through its growth period could influence the postharvest quality and shelf life of the product (Steenhuizen and Boon, 1985).

Fresh cut salad shelf life can be maximised simply by applying balanced NPK fertiliser, however if nitrogen is increased whilst the other nutrients are kept constant then the shelf life of Iceberg lettuce can reduce by three days (Rogers *et al.*, 2006). Adding nitrogen to whole head lettuce (Monaghan *et al.*, 2007) can reduce whole head and processed leaf shelf life, with significantly more physical damage occurring in processed leaves where nitrogen was added. These findings support earlier studies that decreasing nitrogen application can lead to an improvement in shelf life at 5 °C and 20 °C (Poulsen *et al.*, 1995).

However, in other studies (Hilton *et al.*, 2009) the degree of pinking was not affected by nitrogen application. With no response reported in external quality traits in whole head lettuce where nitrogen was added (D'Antuono & Neri, 2001). Hartz and Breschini (2001) reported no significant effect on postharvest quality under different nitrogen managements systems in both Iceberg and Romaine lettuce.

This contradiction in the literature may be attributed to the different amounts of nitrogen applied in each of the studies; furthermore the amount of available nitrogen in the soil could differ significantly. The soil properties, cropping system and irrigation scheduling used in each field trial could affect the amount of nitrogen left in the soil after fertilisation. Jackson *et al*., (1994) reported that a double-cropped lettuce field can lose as much as 150 kg ha-1 of nitrogen from nitrate leaching even with conservative fertilisation.

Generally the current research in this area indicates that high nitrogen application leads to an increase in pinking. However, from the current work it is not possible to identify an optimal nitrogen application rate that incorporates the level of available nitrogen already within the soil. The research in this area to date is insufficient to inform a strategy for nitrogen management that would benefit growers without further work being required.

Regarding other nutrients, the application of phosphorus has been indicated as being able to improve shelf life (Yano and Hayami, 1978). Monaghan *et al.* (2007) reported phosphorus application was shown to increase the quality of lettuce postharvest with reduced pinking scores in plants with additional phosphorus, whereas calcium application showed no effect on whole head or processed leaf shelf life. Hilton *et al*. (2009) showed that calcium application reduced postharvest discolouration at low potassium: nitrogen (K:N) ratios, whereas varying K:N ratios without calcium application produced inconsistent results. The mechanism in which calcium is able to reduce discolouration may be in reducing the total concentration of plant phenolics in plant tissue that can be oxidised by PPO. Wurr *et al*. (2003) demonstrated that calcium application reduced the resulting total amount of caffeates (a plant phenolic) when crops where grown at low K:N ratios, but the effect of calcium on crops is dependent on the environmental conditions, as calcium uptake can be influenced by temperature, crop growth rate and water availability. Calcium application was also found to increase leaf strength and stiffness in lettuce (Wurr *et al*., 2003), which could help the crop resist damage when harvested whole, or by strengthening membrane integrity in fresh cut lettuce.

- ***High levels of nitrogen can increase pinking. Phosphorus may have a role in reducing pinking but calcium results are variable. Optimal nitrogen application rates are not able to be determined from present studies.***

Irrigation

Water content in lettuce is related to saleable weight with lettuce being roughly 95% water. A loss of 5% body weight can make a product look shrivelled and reduce saleability (Atkinson, 2012). Generally the consensus is that lettuce should be maintained by providing water right up to harvest for maximum yields (Rogers *et al.*, 2006). Irrigation can have a marked impact on plants, with water being important for transpiration, photosynthesis, the vascular transport of minerals, cell expansion and turgor. Decreases in photosynthesis due to limited water availability could reduce plant yields in crops. In a drying soil it may not be possible to generate the pressures and tension using transpiration needed to absorb water and nutrients from the soil, decreasing ion uptake and transport to shoots (Kuslu *et al*., 2008). Furthermore, in response to water stress plants can accumulate a wide range of antioxidants to quench the reactive oxidant species induced by water stress (Sofo *et al*., 2005).

Studies in lettuce have shown that reducing irrigation reduces marketable yield (Gallardo *et al*., 1996; Karam *et al*., 2002), however, interestingly drier treatments (maintained at levels of 30-50mm water deficit) had a longer shelf life than their counterpart wet treatments (of 10-

20mm water deficits) in Monaghan *et al*. (2007). Whilst in Wurr *et al*. (2003) minor water stress which is relieved by excess water is thought to trigger increased discolouration of lettuce heads, this discolouration being amplified under increased temperatures.

Excess irrigation was found to increase total caffeates (a plant phenolic) in Wurr *et al*. (2003), whilst in Luna *et al.* (2012) well irrigated treatments led to a 17-fold increase in PAL activity and an increase in PPO activity with an associated rise in leaf browning. In contrast, other studies have shown that mild water stress can increase the total phenolic concentration and antioxidant capacity in lettuce (Oh *et al*., 2010), potentially increasing product nutritional quality. However, this observed increase in phenolic concentration was mainly in chicoric acid, whereas pinking has been reported as associated with plant phenols like chlorogenic acid, with no evidence to suggest chicoric acid is a phenol involved in pinking so far in the literature (Sharples, 1965).

The studies mentioned above have only touched on the effect irrigation could have on lettuce storability. Irrigation could also have a large effect on the mechanical properties of the lettuce leaf by influencing cell expansion and tissue turgor pressure. In transgenic lines of lettuce the effect of reducing membrane permeability was a reduction in the amount of bacteria found within leaves as well as an extended shelf life when compared to controls (Wagstaff *et al*., 2010). These findings indicate that internal water relations could have large implications on the quality of lettuce postharvest, and at the moment we have some way to go to fully understanding the effect water has on lettuce storability and quality.

Overall current studies suggest that increased irrigation can decrease storability with higher subsequent pinking expression postharvest. Higher water contents in lettuce heads could affect tissue turgor pressure and cell expansion. Changes in turgor pressure could result in the lettuce leaf being more susceptible to rupture, resulting in the induction of PPO activity. Increased irrigation could impact on growth, with rapid growth in lettuce contributing to the occurrence of tipburn (Swaider *et al.*, 1992). From these studies it is not possible to identify an optimal irrigation treatment that would maximise yield and lettuce storability with particular emphasis on reducing pinking expression. Further work in this area is required to sufficiently form an irrigation strategy that would be of benefit to growers.

Regarding irrigation techniques, although Wurr *et al*. (2003) found no effect of irrigation method (drip *vs* overhead) on postharvest discolouration in fresh cut lettuce, in Rogers *et al*. (2006) trickle irrigation was suggested as improving shelf life in fresh cut Cos lettuce. This was despite higher yields being obtained in both Iceberg and Cos using overhead irrigation techniques. However, these conclusions were reached by running two trial sites over a three year period, with one site using overhead and the other using trickle irrigation. Therefore it is questionable whether the results are influenced by site location or related to the growers scheduling of irrigation. For the results to be more conclusive the trial should have compared both irrigation systems within the same trial at a single site. Further work is therefore required to reveal any potential postharvest benefits with trickle irrigation. Trickle irrigation could be an attractive option for growers as it has the potential to reduce leaf water contact, improve water use efficiency, target irrigation around the root zone and provide a means for improving scheduling of irrigation.

- ***Excess irrigation can increase yield but also result in pinking***

Temperature

Temperature can affect the productivity and growth of a crop. Increasing temperature can increase the rate of respiration, transpiration and photosynthesis. Generally, plants produce maximum growth when exposed to a day temperature that is about 5.5 to 8 °C higher than the night temperature, allowing photosynthesis and respiration to occur during an optimum daytime temperature and respiration to be curtailed during the night (Evans, 2013). For good growth to occur the rate of photosynthesis must be greater than the rate of respiration. High temperatures can sometimes increase respiration above the rate of photosynthesis and when this occur the products of photosynthesis are used up more rapidly than they are being produced.

For cool season vegetables such as lettuce this can have a variety of effects. High temperatures and fast growth have been associated with tipburn, bolting and other physiological disorders. Positive correlations have been identified between pinking and the temperature a lettuce experiences 14 days prior to harvest in fresh cut lettuce, suggesting pinking increases with increasing temperature (Wurr *et al*., 2003). These results were supported in a study by Jenni (2005), who found whole head lettuce was most sensitive to rib discolouration when exposed to heat stress 2 weeks after heading, whereas stressing plants at earlier or later stages resulted in significantly less incidences of rib discolouration. In another study the percentage of lettuce heads with rib discolouration at harvest was correlated with the minimum mean temperature 7 days prior to harvest, suggesting that it is night temperatures over day temperature that may be having an effect on rib discolouration (Sharples, 1965). This concurs with findings in other crops (namely tomatoes and potatoes), whereby predominantly night temperatures are important in the control of processes such as fruit and tuber formation (Went, 1953). However, additional work in this area has established that lettuce night temperature or the duration of temperature stress did not have an effect on the incidence or severity of rib discolouration in lettuce (Jenni, 2005).

Overall, the current research in this area suggests that high temperatures of 35 °C during the day and 15-25 °C during the night 1-2 weeks before harvest can increase pinking expression (Jenni, 2005; Sharples, 1965). Whether the day or night temperature is more important in influencing pinking is still unclear, furthermore whether lettuce are sensitive to accumulative high temperature exposure or single instances of high temperature exposure has not yet been established. Further work in this area in required before any recommendations can be made to growers on the optimal growing temperature requirements for lettuce to maximise shelf life.

- ***The role of temperature is unclear, but high temperature 1-2 weeks before harvest may increase pinking***

Maturity

It has been shown that lettuce can accumulate 70% of the final fresh weight during the 21 days prior to harvest (Zink and Yamaguchi, 1962), suggesting that lettuce maturity at harvest might be an important factor in maximising yield. Rogers *et al*. (2006) showed that a later harvest offered the potential for a substantial increase in yield in Cos and Iceberg lettuce. The study also showed that when Iceberg was grown for an extra seven days relative to current commercial practise, yield was significantly increased but there was no impact on shelf life for fresh cut lettuce. These results were supported by Wurr *et al*. (2003) which found there was little difference in absolute phenolic content in immature, mature and over mature lettuce heads. Wurr *et al*. (2003) also found that the time of day of harvest had no significant effect on postharvest discolouration of cut lettuce leaves.

Interestingly, these findings go against most of the current consensus in the grey literature which indicates that over mature lettuce heads have a greater occurrence of pinking. However, despite Kasmire and Cantell (1992) relating storage life to lettuce maturity (with small immature lettuces exhibiting the lowest storage life, whilst mature firm heads exhibit the maximum storage life), there has been no comparable published information on lettuce head maturity at harvest in relation to storage life (Gray, 1997). Therefore although it is considered that a delay in the harvest of mature heads increases the risk of pinking, there is no conclusive study in the literature to confirm this.

- ***Contrary to many comments in the grey literature there is no scientific evidence of over mature heads having greater pinking***

Bacteria

Although pinking is considered physiological disorder in lettuce, it has also been associated in some studies with the bacterium *Pseudomonas marginalis* (Marlett and Stewart, 1956; Hall *et al*., 1971). *P. marginalis* is a widespread organism and is thought to enter lettuce through breaks in tissue and crushed leaves or cut stems. Marlett and Stewart (1956) first showed that *P. marginalis* was associated with the disorder of pinking by inoculating lettuce with the bacterium and observing the development of pinking. Another study a few years later showed that injecting *P. marginalis* into the ribs of disease-free lettuce followed by storage at 1.6-8.6 °C resulted in the development of pinking, whereas storage at 15.5-22.2 °C resulted in browning (Hall *et al*., 1971). However, in Wurr *et al*. (2003) discoloured regions in postharvest fresh cut lettuce where not found preferentially associated with specific types of bacteria, but were associated with greater bacterial numbers.

Lettuce injury and wounding can therefore not only stimulate the phenylpropanoid pathway and increase respiration, but also increase the opportunity for *P. marginalis* to infect lettuce tissue, leading to pinking. It is important then to minimise any practices that could potentially cause tissue damage or injury.

Cultivar

Fertilisation, irrigation and wounding have been shown in some studies to have an effect of postharvest shelf life and quality in lettuce (Monaghan *et al.*, 2007; Poulsen *et al.*, 1995; Luna *et al.*, 2012; Ke and Saltveit, 1989; Tomás-Barberán *et al*., 1997; Campos-Vargas *et al*., 2004; Saltveit, 2004). Additionally, it has been observed that there are varietal effects on lettuce postharvest quality; with some lettuce varieties being more susceptible to the disorder of pinking (Ryder and McCreight, 1996). Varietal differences have been suggested to be due to differences in tissue strength or stiffness, but no relationship was found between tissue strength or stiffness and harvest discolouration in Wurr *et al*. (2003).

More recently, rates of discolouration have been identified as being specific to lettuce type, with certain accessions significantly more susceptible to pinking (Atkinson *et al*., 2013). This indicates that there is a genetic basis for phenotype variation and that this natural allelic variation can be exploited to develop cultivars that are resistant to pinking (Wurr *et al*., 2003; Atkinson *et al*., 2013).

Preharvest Factors Summary

Discussed above are a variety of different preharvest factors that can influence pinking incidence and lettuce quality when in storage. To summarise the conclusions of these studies a summary table is featured below (Table 1.1). Where the arrow faces upwards, it indicates the studies in the right column have found the influence of that particular agronomic factor is positive in increasing storage life, whereas when the arrow facing downwards indicates it may reduce storage life whilst an equals sign means that no effect was found.

Table 1.1 Summary table of the current literature that discusses the effect of preharvest agronomic factors on shelf life in lettuce

Agronomic Factor	***Effect***	***Shelf Life*** **↑↓ =**	***References***
Nitrogen	Higher levels of nitrogen increase pinking and decrease shelf life	↓	Monaghan *et al*., 2007; Poulsen *et al*., 1995; Rogers *et al*., 2006
	No effect of nitrogen on pinking	=	Hilton *et al*., 2009; Hartz and Breschini, 2001; D'Antuono and Neri, 2001
Phosphorus	Adding phosphorus increases shelf life	↑	Yano and Hayami, 1978; Monaghan *et al*., 2007
Calcium	Addition of calcium increases shelf life	↑	Hilton *et al*., 2009; Wurr *et al*., 2003
	No effect of calcium on shelf life	=	Monaghan *et al*., 2007
Irrigation	High levels of irrigation can increase pinking and reduce shelf life	↓	Wurr *et al*., 2003; Monaghan *et al*., 2007; Luna *et al*., 2012
Irrigation system	Trickle irrigation increases shelf life versus overhead systems	↑	Rogers *et al*., 2006
	No effect of different irrigation systems	=	Wurr *et al*., 2003
Temperature	High temperatures 1-2 weeks before harvest can increase pinking and decrease shelf life	↓	Wurr *et al*., 2003; Jenni, 2005
Maturity at harvest	Harvesting mature/overmature heads can increase pinking	↓	Grey literature no primary resources
	Harvesting lettuce when mature increases yield and shelf life	↑	Kasmire and Cantwell, 1992
	No effect of maturity at harvest on shelf life or pinking	=	Rogers *et al*., 2006; Wurr *et al*., 2003
Wounding	Wounding/ damage to tissue can increase pinking and decrease shelf life	↓	Canadian Food Inspection Agency, 2011; Ke and Saltveit, 1989; Wurr *et al*., 2003; Tomás-Barberán *et al*., 1997; Campos-Vargas *et al*., 2004
Bacterial infection	Inoculation/infection with *P. marginalis* can increase pinking and reduce shelf life	↓	Marlett and Stewart, 1956; Hall *et al*., 1971
Cultivar	Susceptible cultivars can reduce shelf life and increase pinking	↓	Atkinson *et al*., 2013

Postharvest

The focus of this review has been on agronomic factors preharvest that can affect the postharvest quality of lettuce with specific regard to pinking. However, there are also many postharvest factors that can influence the expression of pinking and pinking incidence. Although it is not the aim of this study to review all of the literature in the area of postharvest technology, some agronomic factors preharvest can make lettuce predisposed to pinking postharvest, and so this review will summarise briefly some of the recent research in this area.

After lettuce is harvested, respiration and transpiration can still continue with shorter product longevity being associated with higher respiration rates. Respiration and transpiration are processes that are affected by temperature. Storing lettuce at a low temperature immediately after harvest is a way to maximise the longevity of the product. Temperatures of <10 °C decrease the activity of the PPO enzyme which has an optimal temperature of 25-30 °C and can thereby reduce the rate at which discolouration develops. Lower temperatures can also inhibit the growth of decay organisms and inhibit water loss (Hardenburg *et al*., 1986). The greatest shelf life for lettuce is therefore achieved when stored just above freezing at the lower limit for metabolism (Wills *et al*., 1989).

In addition to work looking into the storage of lettuce at lower temperatures there has also been research in the area of heat shocking lettuce prior to storage, particularly of lightly cut processed lettuce. Heat shock involves exposing the product to a drastic temperature shift upwards to 39-42 °C for a short period of time. This induces heat shock proteins (HSPs) that are involved in establishing an organism's thermotolerance. When lettuce is wounded and then exposed to a heat shock, the induction of HSPs diverts protein synthesis away from the wound induced PAL pathway which is involved in discolouration (Saltveit, 2000). In theory at very high temperatures (70-90 °C) PPO activity could be deactivated (Jacxsens *et al*., 2004). Martin-Diana *et al*. (2005) showed that when minimally processed fresh cut lettuce was washed at 50 °C there was a lower PPO activity then when washed at 4 or 25 °C, interestingly the 50 °C treatment also repressed synthesis of PPO during subsequent storage.

Heat shocking prior to storage at low temperatures can therefore have a positive impact on product storage by reducing PPO activity. Other postharvest techniques used to prolong product longevity are dipping in chemicals and additives (McEvily *et al*., 1992). Dipping into ascorbic acid, calcium chloride and citric acid can reduce darkening in potatoes (Sapers *et al*., 1990). Pinking in lettuce was inhibited when freshly cut tissue was exposed to 0.1M ascorbic acid and then steamed for 2 minutes (Sharples, 1965). Whilst exposing lettuce to cinnamaldehyde and PAL inhibitors reduced discolouration as they inhibited PAL and subsequently phenolic formation (Peiser *et al*., 1998; Tanaka *et al*., 2011).

Calcium has also been suggested as a potential additive to dip lettuce in as it plays an integral role in membrane and cell wall structural integrity (Poovaiah, 1986). However, care needs to be exercised as some treatments may negate against the effects of other treatments when used in conjunction together. For example a heat shock applied before cutting lettuce with the addition of calcium reduced PAL activity by up to four fold, but if ascorbic acid was added to the thermal bath after the tissue was heat shocked then an increase in PAL activity was observed (Roura *et al*., 2008).

Other methods that have investigated in terms of their effect on storage of processed lettuce include high pressures, irradiation, ultrasound, edible coating, moderate vacuum packaging (MVP), and modified atmosphere packaging (MAP). However not all of these methods have been successful. Recent research into the effect of gamma ray irradiation of lettuce showed that it increased discolouration disorders as it promoted respiration (Fan *et al*., 2012). Currently the most effective and commonly used method of preserving processed lettuce is modified atmosphere packaging (Sandhya, 2010; Brecht *et al*., 2003), as it reduces the rate of respiration and water loss in the enclosed product packet, slowing down tissue metabolic rate and reducing discolouration (Hilton *et al*., 2009). The most commonly used gases in modified atmosphere packaging are oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2). Low oxygen levels can provoke pinking inhibition as oxygen is needed for the phenylpropanoid pathway, alternatively high oxygen levels (>70%) can cause substrate inhibition but can also raise the rate of respiration (Jacxsens *et al*., 2004). Stewart *et al*. (1970) found that elevated carbon dioxide concentrations could control pinking but induced brown stain. Therefore for lettuce the gas composition is typically recommended at 1-3% oxygen, 0% carbon dioxide and 97-99% nitrogen gas. However the effectiveness of MAP is dependent on storage temperature and the integrity of the packaging, as biological reactions can increase three fold for every 10 °C rise in temperature (Sandhya, 2010) and once the packaged is opened oxygen enters and the modified atmosphere is lost.

Conclusions and recommendations

This review has identified a number of agronomic factors that have been reported as influencing pinking in lettuce. Some of these factors, such as temperature or rainfall, cannot be manipulated by growers but knowledge of their effects could be used to manage product shelf-life through the supply chain and work on predictive models could be useful in this area. Other factors can be controlled by growers. Wounding, nutrition and irrigation have been shown to be key factors in subsequent pinking. Handling systems that minimise damage to the ribs of leaves will need to be developed to minimise pinking linked with wounding. In general, high rates of nitrogen and irrigation have been associated with an increase in pinking but the interaction with other nutrients is not clear. It is not possible to identify an optimal nutrient regime or irrigation schedule from the research reviewed here. Further research will be needed in this area before guidelines can be produced for growers.

The following conclusions have arisen from this review:

- Growers should select cultivars with reduced pinking susceptibility
- Care needs to be exercised when harvesting wholehead lettuce to minimise handling damage as wounding/bruising lettuce ribs can increase pinking.
- High levels of nitrogen can increase pinking; growers need to optimise nitrogen application to prevent excessive levels of N being available to the crop. Particular care will be needed in crops grown on soils with high levels of residual N.
- Growers should consider whether phosphorus nutrients have been optimised for lettuce as P nutrition may have a role in reducing pinking.
- Crop water status needs to be managed closely. Excess irrigation (and rainfall) can increase pinking.
- High temperatures 1-2 weeks before harvest can increase pinking and growers can monitor the climate of the growing crop to get an early warning of pinking potential. This may allow a temporary reduction in product-life to minimise wastage in the supply chain.
- There is no scientific evidence to support the commonly held belief that over mature heads having a greater pinking risk.
- Further work is needed to develop nutrition and irrigation recommendations that minimise pinking whilst maintaining marketable yield.

References

Atkinson, L. (2012). Genetic Characterisation of Post Harvest Spoilage in Lettuce. Chapter 1: Introduction. PhD thesis, University of Warwick

Atkinson, L., Hilton, H. W. and Pink, D. A. C. (2013). A study of variation in the tendency for postharvest discolouration in a lettuce (*Lactuca sativa*) diversity set. International Journal of Food Science and Technology 48: 801-807

Brecht, J. K., Chau, K. V., Fonseca, S. C., Oliviera, F. A. R., Silva, F. M., Nunes, M. C. N. and Bender, R. J. (2003). Maintaining optimal atmosphere conditions for fruits and vegetables throughout the postharvest handling chain. Postharvest Biology and Technology 27: 87-111

Brecht, P., Morris, L. L., Chesey, C., Janecke, D. (1973). Brown stain susceptibility of selected lettuce cultivars under controlled atmospheres and temperatures. Journal of the American Society for Horticultural Science 98: 261-264

Campos-Vargas, R., Nonogaki, H., Suslow, T. and Saltveit, M. E. (2004). Isolation and characterization of a wound inducible phenylalanine ammonia-lyase gene (LsPAL1) from Romaine lettuce leaves. Physiologia Plantarum 121: 429-438

Canadian Food Inspection Agency (2011). Lettuce. Vegetable Inspection Manual

Cantos, J. A., Tudela, M. I. and Espín, J. C. (2002). Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. Journal of Agricultural Food Chemistry 50: 3015-3023

Chazarra, S., Cabanes, J., Escribano, J. and Garcia-Carmona, F. (1996). Partial purification and characterization of latent polyphenol oxidase in iceberg lettuce. Journal of Agricultural Food Chemistry 44: 984-988

Devlin, R. M. (1975). Plant Physiology, 3rd Edition. Litton Educational Publishing Inc, New York

D'Antuono, L. F. and Neri, R. (2001). The evaluation of nitrogen effect on lettuce quality by means of descriptive sensory profiling. Acta Horticulturae, 563: 217–222

Evans, E. (2013). Temperature effect on plants. NC State University, North Carolina Cooperative Extension Service web article:

www.ces.ncsu.edudepts/hort/consumer/weather/tempeffect-plants.html

Fan, X., Sokorai, K. J. and Niemira, B. A. (2012). Quality of Gamma Ray-irradiated Iceberg Lettuce and Treatments to Minimize Irradiation-induced disorders. HortScience 47 (8): 1108-1112

Gallardo, M., Jackson, L. E., Schulbach, K. F., Snyder, R. L., Thompson, R. B. and Wyland, L. J. (1996). Production and water use in lettuces under variable water supply. Irrigation Science 16: 125–137

Gray, D. (1997). Iceberg Lettuce: Improving post-harvest quality. Horticultural Development Company final report for project FV198

Hall, C. B., Stall, R. E. and Burdine, H. W. (1971). Association of *Pseudomonas marginalis* with pink rib of lettuce. Florida Agriculture Experiment Stations Journal Series 4132: 163-165

Hardenburg, R. E., Watada, A. E. and Wang, C. Y. (1986). The commercial storage of fruits, vegetables and nursery stocks. US Department of Agriculture, Agricultural Research Service, Agricultural Handbook, 66

Hartz, T. K. and Breschini, S. J. (2001). Pre-sidedress soil nitrate testing (PSNT) improves N management in lettuce production. Western Nutrient Management Conference, 20-26

Hilton, H. W., Clifford, S. C., Wurr, D.C. E. and Burton, K. S. (2009). The influence of agronomic factors on the visual quality of fieldgrown, minimally-processed lettuce. Journal of Horticultural Science & Biotechnology 84 (2): 193–198

Hisaminato, H., Murata, M. and Homma, S. (2001). Relationship between enzymatic browning and phenylalanine ammonia-lyase activity of cut lettuce, and the prevention of browning by inhibitors of polyphenol biosynthesis. Bioscience, Biotechnology and Biochemistry 65 (5): 1016-1021

Horticulture Research International (2002). Establishing the basis for marker-assisted selection of salad genotypes with different discolouration. Department for Food and Rural Affairs, final report for project number HH3707SFV

Hyodo, H., Kuroda, H. and Yang, S. F. (1978). Induction of phenylalalnine ammonia-lyase and increase in phenolics in lettuce leaves in relation to their development of russet spotting caused by ethylene. Plant Physiology 62: 31-35

Isenberg, F. M. R. (1979). Controlled atmosphere storage of vegetables. Horticultural Reviews 1: 337-394

Jackson, L. E., Stivers, L. J., Warden, B. T. and Tanji, K. K. (1994). Crop nitrogen utilization and soil nitrate loss in a lettuce field. *Fertiliser Research* 37 (2): 93-105

Jacxsens, L., Devlieghere, F. and Debevere, J. (2004). Quality of Equilibrium Modified Atmosphere Packaged (EMAP) Fresh-Cut Vegetables. Production Practices and Quality Assessment of Food Crops, Vol. 3, Quality Handling and Evaluation. Eds Dris, R. and Jain, S. M. Kluwer Academic Publishers 473-523

Jenni, S. (2005). Rib discolouration: A physiological disorder induced by heat stress in crisphead lettuce. *HortScience* 40 (7): 2031-2035

Karam, F., Mounzer, O., Sarkis, F. and Lahoud, R. (2002). Yield and nitrogen recovery of lettuce under different irrigation regimes. Journal of Applied Horticulture 4 (2): 70-76

Karpeta, A. (2001). Control of the pinking of lettuce inhibition of polyphenol oxidase processing. MSc Thesis. Proctor Department of Food Science, University of Leeds

Kasmire, R. F. and Cantwell, M. (1992).. Post-harvest handling systems: flower, leafy and stem vegetables. In Postharvest Technology of Horticultural Crops. Kader, A. A. (Editor) Publication Number 3311 University of California Agriculture

Ke, D. and Saltveit, M. E. (1989). Wound induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. Physiologia Plantarum 76 (3): 412-418

Kuslu, Y., Dursun, A., Sahin, U., Kiziloglu, F. M. and Turan, M. (2008). Short communication. Effect of deficit irrigation on curly lettuce grown under semiarid conditions. Spanish Journal of Agricultural Research 6 (4): 714-719

Lewis, D. (2001). The development procedure for the extraction and assay of polyphenol oxidase in Iceberg lettuce. MSc thesis. Proctor Department of Food Science, University of Leeds

Lipton, W.J. and Ryder, E.J. (1989). Lettuce. In Quality and preservation of vegetables, edited by N.A. Erskin, CRC Press, 217–244

Lipton, W. J., Stewart, J. K. and Whitaker, T. W. (1972). An illustrated guide to the identification of some market disorders of head lettuce. US Department of Agriculture, Agricultural Research Service, Market Research Report No 950

López-Galvez, G., Saltveit, M. and Cantwell, M. (1996). Wound-induced phenylalanine ammonia-lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuces. Postharvest Biology and Technology 9: 223-233

Luna, M. C., Tudela, J. A., Martínez-Sánchez, A., Allende, A. and Gil, M. I (2012). Long-term deficit and excess of irrigation influences quality and browning related enzymes and phenolic metabolism of fresh-cut iceberg lettuce (*Lactuca sativa* L.) Postharvest Biology and Technology 73: 37-45

Marlett, R. B. and Stewart, J. K. (1956). Pink rib of head lettuce. Plant disease Reporter 40: 742-743

Martin-Diana, A. B., Rico, D., Barry-Ryan, C., Mulcahy, J., Frias J. and Henehan, T. M. (2005). Effect of heat shock on browning-related enzymes in minimally processed iceberg lettuce and crude extracts. Bioscience, Biotechnology and Biochemistry 9: 1677-85

McEvily, A. J., Iyengar, R. and Otwell, W. S. (1992). Inhibition of enzymatic browning in foods and beverages. Critical Reviews in Food Science and Nutrition 32: 253-273

Monaghan, J., Hilton, H., Akehurst, J., Fellows, J., Wood, M., Simmonds, L. and Rahn, C. (2007). Improved efficiency of nutrient and water use for high quality field vegetable production using dynamic fertigation. Hortlink Final Report for project HL0165

Morad, R. (2003). The effect of different postharvest treatments on the longevity and russet spotting of iceberg lettuce. MSc thesis, Rand Afikaans Unviersity

Oh, M., Carey, E. E. and Rajashekar, C. B. (2010). Regulated water deficits improve phytochemical concentration in lettuce. Journal of American Horticultural Science 135 (3): 223-229

Peiser, G., López-Gálvez, G., Cantwell, M. and Saltveit, M. E. (1998). Phenylalanine ammonia lyase inhibitors control browning of cut lettuce. Postharvest Biology and Technology 14: 171-177

Poovaiah, B. W. (1986). Role of calcium in prolonging storage life of fruits and vegetables. Food Technology 40: 86-89

Poulsen, N., Johansen, A. S. and Sorensen, J. N. (1995). Influence of growth conditions on the value of crisphead lettuce. 4. Quality changes during storage. Plant Foods for Human Nutrition 47: 157–162

Rogers, G. S., Titley, M., Giggins, B., Bauer, B., Poynton, R., Kocks, A., McAuliffe, T. and Le Budd, J. (2006). Post harvest improvement in Iceberg and Cos lettuce to extend shelf life for fresh cut salads. Horticulture Australia, project number VX03092

Roura, S. I., Pereyra, L. and del Valle, C. E. (2008). Phenylalanine ammonia lyase activity in fresh cut lettuce subjected to the combined action of heat shocks and chemical additives. LWT Food Science and Technology 41: 919-924

Ryan, K. G., Swinnya, E. E., Markhama, K. R. and Winefield, C. (2002). Flavonoid gene expression and UV photoprotection in transgenic and mutant *Petunia* leaves. Phytochemistry 59: 23–32

Ryder, E. J. and McCreight, J. D., edited by (1996). Vegetable cultivar descriptions for North America. Lettuce (M-Z), Lists 1-26 combined. U.S. Agricultural Research Station, CA

Saltveit, M. E. (2000). Wound induced changes in phenolic metabolism and tissue browning are altered by heat-shock. Postharvest Biology and Technology 21: 61-69

Saltveit, M. E. (2004). Effect of 1-methylcyclopropene on phenylpropanoid metabolism, the accumulation of phenolic compounds, and browning of whole and fresh-cut 'iceberg' lettuce. Postharvest Biology and Technology 34: 75-80

Sandhya (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. LWT Food Science and Technology 43: 381–392

Sapers, G. M., Garzarella, L. and Pilizota, V. (1990). Application of browning inhibitors to cut apple and potato by vacuum ad pressure infiltration. Journal of Food Science 55: 1049-1053

Sharples, G. C. (1965). Biochemical Studies of Rib Discoloration and Pink Rib of Lettuce. Vegetable Report, University of Arizona Repository, 15-19

Sofo, A., Dichio, B., Xiloyannis, C. and Masia, A. (2005). Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Functional Plant Biology 32: 45–53

Soininen, K. (2009). Salads and Salad Dressings - UK - August 2009. Mintel Oxygen Report

Steenhuizen, J. W. and Boon, J. van der (1985). The nitrate content of lettuce in nutrient solution. 2. Nitrogen level and ammonium:nitrate ratio; replacement before harvest by a solution with a higher proportion of ammonium. Rapport, Instituut voor Bodemvruchtbaarheid, Netherlands, 1-85

Stewart, J. K., Ceponis, M. J. and Beraha, L. (1970), Modified atmosphere effects on the market quality of lettuce shipped by rail. US Department of Agriculture Marketing Research Report No. 863

Swaider, J. M., Ware, G. W. and McCollum, J. P. (1992). Producing vegetable crops, 4th ed. Interstate Publishers, Danville, IL

Tanaka, E., Okumura, S., Takamiya, R., Hosaka, H., Shimamura, Y. and Murata, M. (2011). Cinnameldehyde inhibits enzymatic browning of cut lettuce by repressing the induction of phenylalanine ammonia-lyase without promotion of microbial growth. Journal of Agricultural and Food Chemistry 59 (12): 6705-6709

Toivonen, P. M. A. and Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biology and Technology 48 (1): 1-14

Tomás-Barberán, F. A., Loaiza-Velarde, J., Bonfanti, A., Saltveit, M. E. (1997). Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. Journal of the American Society for Horicultural Science 122: 399-404

Van Vliet, W., Van Dun, C. M. P. and Schut, T. W. (2009). Method for identifying plant material that shows a reduced discolouration, plants thus identified and use of the identified plants for the production of commercial crop plants showing a reduced discolouration. EU patent EP2011389

Wagstaff, C., Clarkson G. J. J., Zhang, F., Rothwell, S. D., Fry, S. C., Taylor, G. and Dixon, M. S. (2010). Modification of cell wall properties in lettuce improves shelf life. Journal of Experimental Botany 61 (4): 1239-1248

Went, F. W. (1953). The effect of temperature on plant growth. Annual Review of Plant Physiology 4: 347-362

Wills, R. H. H., McGlasson, W. B., Graham, D., Lee, T. H. and Hall, E. G. (1989). Postharvest, An Introduction to Postharvest Handling and Physiology of Fruits and Vegetables, 3rd Edition. New South Wales, University Press, Australia

Wurr, D., Parr, A., Feuerhelm, S., Kennedy, S., Pennings, H., Oost, E., Cornai, I., Harriman, M., Sawday, J. and Tucker, A. (2003) Improving the quality and shelf-life of cut salad products. Department for Environment, Food and Rural Affairs final report for project number HLO142

Yano, M. and Hayami, A. (1978). A Studies on the improvement of storage ability in head vegetables. I. The relationship between cultivar, stage of maturity and fertilizers and the storage ability of lettuce and cabbage. Bulletin of the Vegetable and Ornamental Crops Research Station 4: 77-88

Zaini, N. A. M., Osman, A., Hamid, A. A., Ebrahimpour, A. and Saari, N. (2012). Purification and characterization of membrane-bound polyphenoloxidase (mPPO) from Snake fruit [*Salacca zalacca* (Gaertn.) Voss]. Food Chemistry 136: 407-414

Zink, F. W. and Yamaguchi, M. (1962). Studies on the Growth Rate and Nutrient Absorption of Lettuce. Higardia 32 (11): 471-500