Project title:	Predicting high risk plantings to manage postharvest pinking in lettuce
Project number:	FV 413a
Project leader:	Jim Monaghan, Harper Adams University
Report:	Final report, March 2016
Previous report:	Annual report, February 2015
Key staff:	Ms Carol-Ann Woolley (HAU) Prof Simon Edwards (HAU) Prof Xiangming Xu (EMR)
Location of project:	HAU
Industry Representative:	Mrs Emma Garfield, G's Fresh
Date project commenced:	01 March 2014
Date project completed (or expected completion date):	31 March 2016

DISCLAIMER

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

AUTHENTICATION

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

Dr Jim Monaghan
Principal Lecturer
Harper Adams University

Signature .... Date ......30/03/2016.............................

Dr Andy Wilcox
Head of CES Department
Harper Adams University

Signature ....Date ....30/03/2016...............................

CONTENTS

Headline....................1

Background....................1

Summary....................2

Financial Benefits....................5

Action Points....................5

Introduction....................6

Materials and methods....................7

- Growing locations....................7
- Delivery to HAU....................7
- Plant assessments....................9
- Location information....................10
- Statistical analysis....................11

Modelling – Combined Year 1 and 2 data....................11

- Data set....................11
- Ordinal regression....................12
- Model interpretation....................13

Results....................14

- Year 2 Rib Pinking....................14

Combined model....................21

- Rib disorders – Iceberg lettuce....................22
- Rib disorders – Cos lettuce....................24
- Butt discolouration – Iceberg lettuce....................25
- Butt discolouration – Cos lettuce....................26

Discussion (Combined data)....................28

Conclusions....................33

Knowledge and Technology Transfer 33

References 34

Appendices..35

GROWER SUMMARY

Headlines

- Over both years Iceberg lettuces displayed moderate levels of rib pinking and Cos lettuces displayed low levels of rib pinking after cold storage for 2 weeks.
- Iceberg and Cos lettuces showed high levels of butt pinking after cold storage for 2 weeks.
- The main factors that influenced the risk of a crop developing pinking after harvest were SNS index > temperature > water inputs > fresh weight (maturity).
- Harvesting mature heads of both crops increased the probability of pinking.
- Soil type had greater effect on butt pinking than rib pinking in Iceberg lettuce but had similar effects in Cos lettuce
- Growing conditions during establishment had a significant effect on the risk of pinking developing after harvest, with both crops having a greater probability of rib and butt pinking if the first 20% of the crop cycle was warmer and wetter.
- The environmental conditions during the middle 20% of the crop growth cycle (3rd quintile) was important for both crops. This is the period when the leaves that will be found on the outside of the trimmed head are produced. The probability of rib pinking in Iceberg lettuce was greater with warmer and wetter conditions but the probability was reduced in Cos lettuce.
- The last 40% of the growth cycle (quintile 4 & 5) was important for rib pinking in Iceberg lettuce with cooler conditions associated with an increased probability of rib pinking. The conditions had no effect on Cos lettuce.

Background

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, continues to present substantial problems for producers in both UK and imported crops. Poor product on the shelf reduces sales and leads to more complaints and consumer dissatisfaction. A recent review of research into lettuce pinking (FV 413) identified that issues such as high rainfall/over irrigation have a direct influence on expression of pinking.

This project took Iceberg and Cos lettuce samples from a number of UK commercial lettuce growing locations over two growing seasons. The heads were assessed over storage for the development of pinking and other quality measures. This information was combined with data on the environmental conditions (agronomic and meteorological) that each crop had experienced. This data was separated into 5 equal periods (quintiles) of crop growth, Q1-5. The availability of multiple lettuce crops from March - October meant that a wide range of weather conditions were incorporated into the model over two years.

The aim of this work was to:

a) identify the environmental and crop factors that increase the risk of a crop developing rib and butt pinking after harvest;
b) develop guidelines identifying high-risk crops based on local meteorological and crop input records; and
c) enable growers to manage crops through the supply chain to the benefit of the customer.

Summary

The crop growth duration ranged from 41 days from planting (Cos and Iceberg) to 78 days (Cos) and 87 days (Iceberg) over the season. The crop cycle was separated into five periods (quintiles). Hence, each quintile represents a period of between 1 and 2 weeks depending on the crop duration. It was assumed that the developmental stages (i.e. hearting) would occur at a consistent stage in crop growth.

Variation in Soil Nitrogen Supply (SNS) Index was very strongly associated with a changed risk of pinking developing. Fertilizer records were inconsistent between locations and nutrient input from fertilizer was not included in the model. The use of SNS represents the soil type and an indication of residual nitrogen (and wider soil nutrient and organic matter properties). The risk of rib pinking in iceberg lettuce was greatest in crops grown in soil of Index 1, but it was greatest in Index 3 soils for Cos lettuce. Cos lettuce also had the lowest risk of rib pinking when grown in Peat soils. The pattern with soil index and butt pinking was confusing, with the risk of butt pinking in Iceberg lettuce greatest in soils of Index 1 and 2 and peat soils. In contrast the risk of pinking in Cos lettuce was least in Index 1 and 2 soils.

Temperature was associated with a number of significant responses in discolouration (Table A). In Iceberg lettuce, higher temperatures in Q1 and Q3 were associated with a significant increase in the risk of rib pinking but a significant reduction in risk if the temperatures was higher in Q5. A similar response was observed in Q1 and Q5 for butt pinking in Iceberg lettuce.

Cos lettuce had a different pattern of response, higher temperatures in Q1 increased the probability of butt and rib pinking, this response changed in Q2 with a reduction in risk of rib pinking but an increase in risk for butt pinking. The reduction in risk of rib pinking was an

even stronger response to higher temperatures in Q3, with no response in butt pinking. By the end of the growing cycle higher temperatures increased the risk of butt pinking only.

The effect of **water inputs** was generally less than that of temperature (Table A). This can be explained by the fact that low rainfall can be mitigated by irrigation meaning that commercial crops should not be short of water during growth, although heavy rain would lead to excess water availability. Rib pinking in Iceberg lettuce was sensitive to water inputs in Q3 and Q5 with greater volume of water input associated with a small increase in probability in Q3 and a small decrease in probability in Q5. Butt pinking responded to early availability of water with a small increase in risk in Q1 and a small decrease in risk in Q2. In Cos lettuce the risk of both rib and butt pinking increased with higher volumes of water in Q1 and decreased with higher volumes in Q2. The risk of butt pinking was also more strongly reduced when Q5 was wetter.

Table A. Effect of water availability and temperature over 5 periods during the crop cycle on the probability of rib pinking increasing from score 1 – score 2 in Iceberg and Cos lettuce.

Iceberg Lettuce

	Q1	Q2	Q3	Q4	Q5
↑Water			↑		↓
↑Temp	↑↑		↑↑	↓↓	↓↓

Cos Lettuce

	Q1	Q2	Q3	Q4	Q5
↑Water	↑↑	↓			
↑Temp	↑↑↑↑	↓↓	↓↓↓↓		

Harvesting more **mature crops** has been suggested as a factor that increases the risk of pinking (HDC FV 413) in whole heads. More mature crops are generally heavier and larger, which would lead to higher fresh weight and greater head circumference being associated with maturity. In contrast more dense (less mature) heads would have higher weight but smaller circumference. In this study greater fresh weight was associated with increased risk of rib and butt pinking in both Iceberg and Cos lettuce suggesting that maturity of the head at harvest is a key factor in subsequent pinking. Increased density could also explain the increased risk of pinking in Cos ribs.

Early growth

This study suggests that both Iceberg and Cos lettuce are influenced by the growing conditions in the first 1-2 weeks following transplanting with higher temperatures and water availability being associated with a significant increase in the probability of pinking in the butt and ribs following harvest (Table A). This was a surprising finding. The leaves produced during this time are not part of the harvested head, so any response to temperature and water status at this time must be due to changes in long term factors within the plant, maybe linked to the biochemistry of the ribs and butts and/or a consequence of growth form i.e. cell differentiation in the meristem leading to larger or smaller cell size in subsequent ribs and leaves.

Mid growth

Cos and Iceberg responded differently to conditions during mid-growth (Table A). In Iceberg lettuce, the probability of rib pinking increased when Q3 was warmer and wetter, in contrast, with Cos lettuce, the probability decreased strongly when Q3 was warmer. Butt pinking was not influenced by Q3 conditions in either crop. The explanation of this response is not obvious. In Iceberg lettuce, it suggests that leaves that develop and expand under warmer conditions have more fragile ribs, as seen by the increased rib cracking, whereas the same conditions in Cos lettuce produce more resilient ribs. The morphology of leaf ribs differs between the two crops with Cos lettuce having more pronounced ribs, often with a hollow core. Understanding this response may be of use to breeders in developing crops that are more resilient to post harvest handling.

Late growth

The stage of growth before harvesting was important for iceberg lettuce but less so for Cos. Wetter and warmer conditions reduced the risk of rib and butt pinking in Iceberg lettuce i.e. cooler and dryer conditions would increase the risk. In Cos there was no effect on rib pinking and dryer, warmer conditions increased the risk of butt pinking. The leaves produced in Q5 would be within the head so any response would be due to general head water status and the turgidity of ribs and leaves at harvest. It may be that cooler conditions prior to harvest slow down growth and lead to higher turgidity at harvest in Iceberg lettuce. This could lead to greater rib cracking when the plants were handled. However, rib cracking and pinking were a lower risk when Q5 had greater water availability. In Cos lettuce the robust ribs may be less sensitive to plant water status. More work is needed to understand this response, which was counter to grower expectations that wetter environments close to harvest are associated with pinking.

Financial Benefits

Pinking losses are hard to quantify, but can account for substantial customer complaints at certain times of the year and batch rejections. The importance of the work to the industry can be gauged from the willingness of seven businesses to provide crop samples for the study.

Action Points

In order to reduce the probability of pinking of wholehead lettuces, growers should:

1. Avoid harvesting over mature heads
2. Monitor temperature during crop growth and trend values against long-term averages.
3. Identify crops planted at higher than average temperatures as higher risk crops
4. Monitor growing environment during the middle of the crop cycle when the outer leaves of the trimmed head are being formed. Iceberg lettuce produce more delicate ribs and Cos lettuces more resilient ribs when this middle 20% of the crop cycle is warmer.
5. Identify Iceberg crops that experience a cooler end to the season as being at a greater risk of pinking.

SCIENCE SECTION

Introduction

Leafy salads often suffer from discolouration on the butt and leaf ribs within a few days after harvest, limiting their shelf life. Enzymatic and non-enzymatic oxidative processes cause 'browning' and 'pinking' which results in the emergence of coloured pigments (brown and pink/red respectively) are produced via the phenylpropanoid (PPO) pathway (Toivonen and Brummell 2008). Pinking continues to present substantial problems for producers with both UK and imported crops. Poor product on the shelf reduces sales and leads to more complaints and consumer dissatisfaction. It is understood that issues such as high rainfall/over irrigation have a direct influence on expression of pinking, but we do not have a good predictive system for this disorder and growers rely on fairly unscientific "gut-feel".

Workers have reported that high temperatures are associated with pinking in lettuce. The crop stage most sensitive to temperature is not clear. Positive correlations have been identified between discolouration and the temperature a lettuce experiences in the 7 days before harvest for wholehead lettuce (Sharples, 1965), 14 days prior to harvest in fresh cut lettuce (Wurr *et al*., 2003) and 2 weeks after heading in wholehead lettuce (Jenni, 2005). The temperature range associated with discolouration is also unclear. Research suggests that temperatures of 35 °C during the day and 15-25 °C during the night are associated with increased pinking expression (Jenni, 2005; Sharples, 1965). Whether the day or night temperature is more important in influencing discolouration is still unknown, furthermore whether lettuce are sensitive to accumulated high temperature exposure or single instances of high temperature exposure has not yet been established.

Studies also report that increased irrigation can decrease storability with higher subsequent pinking expression postharvest (Wurr *et al.,* 2003; Monaghan *et al*., 2007; Luna *et al.,* 2012). There is no work studying the effect of rainfall but it can be assumed that the response to heavy rain would be similar. 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. However, the level of irrigation/rainfall that would lead to increased pinking has not been reported.

Limitations of previous studies into pinking in lettuce include the scale of the sample size and the use of extreme experimental treatments (to generate consistent responses) but Pinking is a sporadic physiological disorder seen to some extent throughout the season. We propose to utilise large sample sizes derived from multiple commercial locations experiencing a range of environmental conditions over the season. This approach has been used to successfully identify cereal crops at high risk of exceeding mycotoxin levels (Edwards, 2007). The statistical modelling approach (see methodology) utilised in the FSA/HGCA work will be applied to lettuce pinking. There are some similarities between the two projects, like lettuce pinking the level of mycotoxins (derived from *Fusarium* spp. found in the ear of cereal crops at harvest) is influenced by rainfall prior to harvesting. However, the availability of multiple lettuce crops from March - October means that a wide range of conditions can be incorporated into the model over two years, in contrast to the 5 years needed for the FSA/HGCA work conducted on wheat which has a single harvest each year.

The mycotoxin research has been successfully implemented by the cereal industry with the generation of Guidelines and Codes of Practices to minimise risk and a HGCA mycotoxin risk assessment scheme where growers input agronomic factors and rainfall data to predict a low, moderate of high risk of exceeding legal limits of *fusarium* mycotoxins. This has led to growers clearly understanding the risk factors and modifying their agronomy accordingly.

The aim of this work was to a) identify the environmental and crop factors that increase the risk of a crop developing rib and butt pinking after harvest; b) develop guidelines identifying high-risk crops based on local meteorological and crop input records; and c) enable growers to manage crops through the supply chain to the benefit of the customer.

Materials and methods

Growing locations

Commercially grown Iceberg and Cos/Romaine lettuce were sampled routinely through the growing season from week 20-41 (week commencing 12/05/2014 – week commencing 6/10/2014) in 2014 and week 20-44 (week commencing 11/05/2015 - week commencing 27/10/2015) from seven locations in 2015 (Table 1 & 2). The crop sampling schedule was agreed with the growers involved in the study at the start of the trial to fit in with availability and supply period.

Delivery to HAU

The heads were harvested and overwrapped by the commercial crews and vacuum cooled at the grower pack house in the morning/early afternoon. Forty heads were sampled from the crop, boxed and a pre-arranged courier collected them late afternoon (usually between 3 and 5 pm). The heads were delivered to HAU before 9 am on the following day. This schedule differed for three locations: Jepco held the heads overnight in a refrigerated store before collection by the courier using the above timings as they were routinely harvested in the afternoon rather than the morning. Huntapac heads were harvested, vacuum cooled and delivered to HAU on the same day by the business and samples from PDM were delivered to HAU on the day of harvesting after cooling. During the second year, a subsample of Huntapac and PDM samples were kept at 15°C overnight to ensure that there was no difference between those that were delivered on the same day and only out of the cold store for a few hours compared to those that were kept out overnight; no difference was found. Following discussion with the Grower Coordinator at the start of the trial an unrefrigerated courier service was used. Samples were collected towards the end of the day, and either transported or held in a distribution centre overnight and delivered before 9 am at HAU. This avoided the samples being exposed to transport during the full heat of the day.

Table 1. Iceberg sample dates and locations, 2015. The location numbers are common between 2014 and 2015.

Sample	Location 1	Location 2	Location 3	Location 5	Location 6	Location 9
1	09.06.15	26.05.15	13.05.15	28.05.15	03.06.15	20.07.15
2	06.07.15	30.06.15	25.05.15	11.06.15	01.07.15	01.09.15
3	03.08.15	28.07.15	09.06.15	25.06.15	05.08.15	30.09.15
4	02.09.15	25.08.15	23.06.15	13.07.15	09.09.15	
5	05.10.15	29.09.15	07.07.15	27.07.15		
6		20.10.15	21.07.15	10.08.15		
7			04.08.15	25.08.15		
8			18.08.15	07.09.15		
9			02.09.15	21.09.15		
10			15.09.15	05.10.15		
11			29.09.15	19.10.15		
12			12.10.15	27.10.15		
13			27.10.15			
Total	5	6	13	12	4	3

Table 2. Cos sample dates and locations, 2015. The location numbers are common between 2014 and 2015.

Sample	Location 1	Location 2	Location 4	Location 5	Location 6	Location 9
1	09.06.15	26.05.15	18.05.15	28.05.15	03.06.15	20.07.15
2	06.07.15	30.06.15	25.05.15	11.06.15	01.07.15	01.09.15
3	03.08.15	28.07.15	09.06.15	25.06.15	05.08.15	
4	02.09.15	25.08.15	23.06.15	13.07.15	09.09.15	
5	05.10.15	29.09.15	06.07.15	27.07.15		
6		20.10.15	21.07.15	10.08.15		
7			04.08.15	25.08.15		
8			02.09.15	07.09.15		
9			15.09.15	21.09.15		
10			29.09.15	05.10.15		
11			12.10.15	19.10.15		
12			27.10.15	27.10.15		
Total	5	6	12	12	4	2

Plant assessments

On arrival, all of the heads were re-trimmed – chopping the butts off and removing 2-3 of the outer leaves and removing any pinking or butt discolouration which may have occurred prior to arriving and before the assessments began. Each head was labelled (location, date of harvest, date of arrival, variety etc.), weighed fresh and the circumference measured before it was placed in a new plastic bag (supplied by PDM Ltd, Newport, UK), and sealed (twisted and taped). The samples were placed in trays in a lit cold store at around 4˚C.

The following post-harvest destructive assessments were made from randomly selected heads from across the batch:

Harvest +1d (10 heads per lettuce type and location):

Heads were scored qualitatively for external and internal appearance (1 = perfect, 2 = mild symptoms, 3 = moderate symptoms, 4 = severe symptoms) using a commercial visual scoring chart (G's Fresh Ltd, Barway, UK) for:

- Rib Pinking
- Rib Cracking
- Butt Pinking
- Butt Browning

As well as qualitative scoring the following quantitative measurements were made destructively:

Dry weight - The chopped heads and trimmed leaves were the placed in individual oven bags and dried at 80°C until constant weight, usually after 48 hours and dry weight was recorded.

Moisture content – the moisture content percentage of the whole lettuce head was calculated as ((1-(DW/FW)) x 100).

Pinking validation grid score – After the first year it became apparent that the rib and butt colour differences were not being picked up by the Minolta colorimeter which lead to the development of the grid scoring validation. A 10 x 2cm rectangle was drawn onto a transparent sheet of acetate marking out 5 identical squares. The base of the rectangle was placed at the butt of the lettuce and positioned travelling up the rib. For each square, the level of pinking was recorded on a scale of 0 – 3 (0 – absent, 1 – faint, 2 – moderate, 3 – dark) and it was noted whether the rib was cracked or not. This was repeated for the first three exposed ribs on each head. Additionally, a 4cm by 4cm square (made up of 4, 2 x 2cm squares) was used to record the level of pinking using the same scale on the butt of each head.

Harvest +8d (10 heads per lettuce type and location):

Ten heads were randomly selected removed from the bag and weighed to give fresh weight. The same assessments were then made as described for Harvest +1d.

Harvest +15d (10 heads per lettuce type and location):

Ten heads were randomly selected removed from the bag and weighed to give fresh weight. The same assessments were then made as described for Harvest +1d.

Harvest +22d (10 heads per lettuce type and location):

The remaining 10 heads were removed from the bag and weighed to give fresh weight. The same assessments were then made as described for Harvest +1d.

Location information

The following information has been collected where available from each location for each crop sample.

- Soil type
- Previous cropping
- Nutrient application and timing
- Irrigation timing and quantity
- Cultivar
- Transplanting date

At the start of the trial it was established the met data that each grower (and growing location) could provide. In addition to grower data, data from the nearest Met Office synoptic and climate stations to the growing locations was accessed. HAU provided thermocrons to each location in Year 1 but most of these were lost during commercial field working and this approach was stopped.

Statistical analysis

Data were analysed for significance using two way ANOVA for each location with day of analysis and week of harvest as main effects using Genstat 16th Edition.

Modelling – Combined Year 1 and 2 data

Data set

The following variables describing various aspects of lettuce head quality were used as response variables: butt pinking, butt browning, rib pinking and rib cracking. All these variables were recorded on a scale of 1 (perfect) to 4 (unmarketable) and hence cannot be analysed using usual statistical methods.

The independent variables consist of climatic variables, soil SNS index and lettuce head attributes. For each location, we recorded daily average temperature and water input (i.e. daily irrigation and rainfall). The whole lettuce growing season for each harvest [from sowing to harvest] was divided equally into five periods [Q1-Q5]; if the total length could not be divided exactly by five, one additional day was added to a number of later periods. For instance, if the total length is 42 days, the first three periods [from sowing] have eight days each, and the last two periods [Q4, Q5] have nine days each. Then average daily temperature and total daily water input (mm) were calculated for each period. Several lettuce head traits were also used as independent variables: fresh weight (g) at harvest prior to storage, dry weight (g), head circumference (cm) and moisture content (%).

Ordinal regression

A cumulative logit model was used to model a given head quality trait (Y_i), which was recorded in four categories (j = 4). Y_i follows a multinomial distribution with a parameter π; π_{ij} denotes the probability that ith head falls in the score j. The cumulative probability is then defined as

$$\gamma_{ij} = P(Y_i \leq j) = \pi_{i1} + \cdots + \pi_{ij} \qquad (1).$$

A cumulative logit is defined as:

$$logit(\gamma_{ij}) = logit(P(Y_i \leq j) = ln\left\{\frac{P(Y_i \leq j)}{1-P(Y_i \leq j)}\right\} = ln\left\{\frac{P(Y_i \leq j)}{P(Y_i > j)}\right\} \qquad (2).$$

By definition, a cumulative logit does not exist for the last category of the response variable. A cumulative logit model is a regression model for a cumulative logit (3):

$$logit(\gamma_{ij}) = ln\left\{\frac{P(Y_i \leq j)}{P(Y_i > j)}\right\} = \alpha_j - \sum \beta_k x_{ik} \qquad (3),$$

where x_k represents the kth explanatory variable, β_k the effect of x_k, and α_j the intercept for each cumulative logit. The regression part $\sum \beta_k x_{ik}$ is independent of j. The larger the value of $\sum \beta_k x_{ik}$, the higher the probability of Y_i falling in a category at the upper end of the category scale (i.e., worse quality in the present case). Figure 1 shows an example cumulative logit model with five categories. For small and large $\sum \beta_k x_{ik}$ values, the response is likely to fall in the first (j = 1) and the last (j = 5) category. α_j is the intercept for each cumulative logit and determines the horizontal displacements of the curves (Fig. A). In the present study, there were maximum four categories for each quality score (1, 2, 3, and 4).

It is also possible that the effect of x_k on the response variable also depends on the category, namely β_k varies with the value of j.

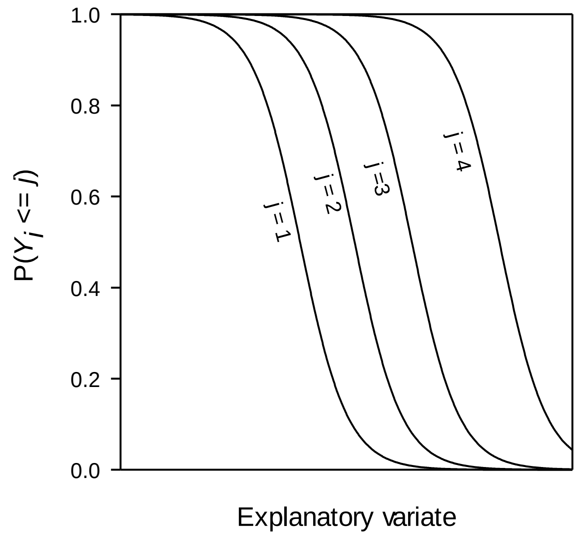

Figure A. Example curves illustrating cumulative logit models. This example describes a cumulative logit model with five categories for the response variable; the logit of the cumulative probability of the response variable linearly related to a set of explanatory variates. The four curves represent the cumulative probability of P($Y_i \leq j$ = 1, 2 ,3 and 4) and the probability of the response variable in the category of j = 5 equals to 1 - P($Y_i \leq j$ = 4).

Model interpretation

Interpretation of ordinal logit models is difficult if only α_j and β_k estimates are presented. However, these models are much easier to understand if model parameter estimates are presented in terms of odds ratio of the response variable being below or above a particular category.

Equation 3 describes a linear relationship of the logit of the odds ratio of the response being below/above a particular category with explanatory variables. It is the extension of the common logistic model with only two possible outcomes (for example, diseased or healthy). Thus, cumulative logit models are a class of generalized linear models, assuming that the errors follow a binomial distribution. This model satisfies

$$ln\left\{\frac{Pr(Y_i \leq j\ |x_1)/Pr(Y_i > j\ |x_1)}{Pr(Y_i \leq j\ |x_2)/Pr(Y_i > j\ |x_2)}\right\} = \beta(x_2 - x_1) \qquad (4)$$

for all j (i.e., proportional odds property, hence it is also called proportional odds model). Thus**, β estimates the change in the cumulative odds ratio (on the ln scale) for one unit increase in the explanatory variable x [absolute increase, not relative to any other values].**

Continuous independent variables

Thus exp(β) measures the proportional odds change for one unit increase [absolute increase] in the variable ***x***: **< 1 indicates that increasing the variable leads to smaller scores (greater probability being ≤ j), = 1 indicates that variable has no effect on the score i.e. there is a 50/50 chance that the event will occur with a small change in the independent variable, and > 1 indicates that increasing the variable leads to increased probability of scores being > j.**

SNS ordinal categories

There are five categories of soil SNS indices for cos lettuce: 0, 1, 2, 3 and 5; six categories for iceberg lettuce: 0, 1, 2, 3, 4.5 and 5. Soil index was included as a factor in the analysis with the category SNS index 0 as the base. Thus the effects of all other soil types are interpreted in terms of its relative effect to the SNS index 0. Hence exp(β) in the tables measures the proportional odds change for the soil type concerned relative to the SNS index 0: **< 1 indicates that quality with the SNS index under consideration is better than that grown under soil of SNS index 0, and vice versa**

Results

An overview of post-harvest data is reported here with a particular focus on rib pinking in Year 2. The data for Year 1 was reported in the previous Annual Report. The model and interpretation for both Years is then described.

Year 2 Rib Pinking

Iceberg Lettuce

Main effect of harvest date on post-harvest quality

The average value differed significantly between different harvest dates for quantitative measures of head fresh and dry weight, circumference and moisture content for all locations except rib cracking and pinking at Location 9 (Table 3). A similar response was observed for the qualitative assessments of rib cracking, rib pinking, butt browning and butt pinking (Table 3).

Table 3. Significance of main effect of week of harvest on average mean score of post-harvest quality parameters across all days of assessment for Iceberg lettuce, 2015.

Location	1	2	3	5	6	9
Rib Cracking	***	***	***	***	***	NS
Rib Pinking	***	***	***	***	***	NS
Butt Browning	***	***	***	***	***	***
Butt Pinking	***	***	***	***	***	***
Density	*	***	***	***	***	***
Dry Weight	***	***	***	***	***	***
Fresh weight	***	***	***	***	***	**
Head Circumference	***	***	***	***	***	***
Moisture Content	***	***	***	***	***	***

p<0.05 *; p<0.01 **; p<0.001 ***

Comparison of average rib pinking for separate locations and harvest dates.

The average rib pinking score ranged from 1.1 to 2.8 with the highest average score observed with samples harvested from one location in week 40 (Figure 1). However, the pattern of scores varied between locations.

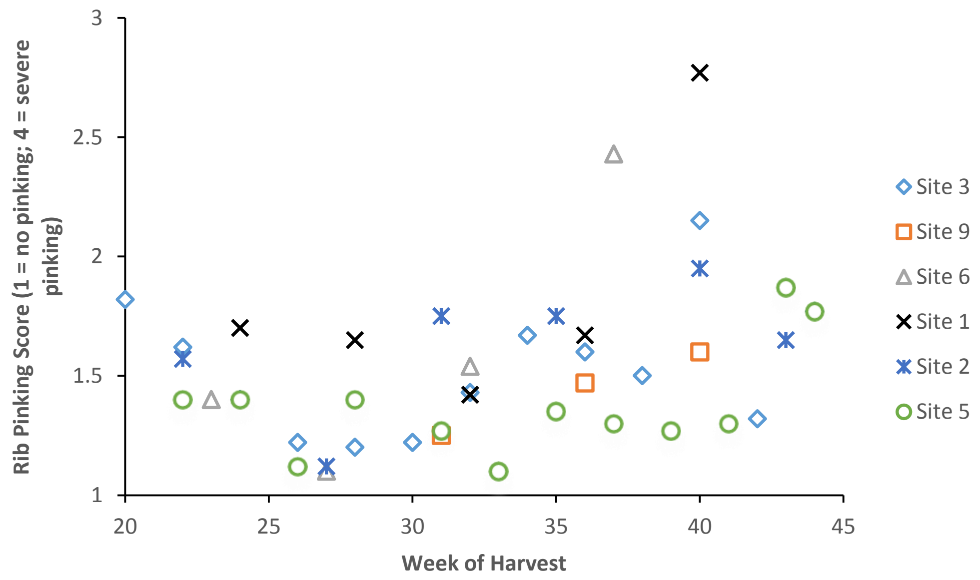

Figure 1. Average Iceberg lettuce rib pinking score for all locations over the harvest season 2015.

Main effect of storage duration on post-harvest quality

When the data was averaged over all harvests the quantitative measures showed some significant change during storage at HAU (Table 4) with samples from five locations showing one or more variates exhibiting significant change during storage. However, all the qualitative measures showed significant changes during storage (Table 4).

Table 4. Significance of main effect of day of assessment after harvest on average mean score of post-harvest quality parameters across all harvest weeks for Iceberg lettuce, 2015.

Location	1	2	3	5	6	9
Rib Cracking	***	***	***	***	*	**
Rib Pinking	***	***	***	***	***	**
Butt Browning	***	***	***	***	***	***
Butt Pinking	***	***	***	***	***	***
Density	**	NS	NS	**	NS	**
Dry Weight	NS	*	NS	NS	***	***
Fresh weight	**	NS	NS	*	NS	*
Head Circumference	NS	*	NS	NS	NS	NS
Moisture Content	***	NS	NS	***	***	***

$p<0.05$ *; $p<0.01$ **; $p<0.001$ ***

Comparison of average rib pinking during storage for separate locations.

Rib pinking increased during post-harvest storage and this effect was consistent across all harvest locations (Figure 2). When averaged over locations and harvest dates a low level of pinking (1.1) was observed on Day 1 after harvest. The level of pinking then increased significantly to 1.7 by Day 8, remained similar, at 1.7, by Day 15 and 22 (Table 5).

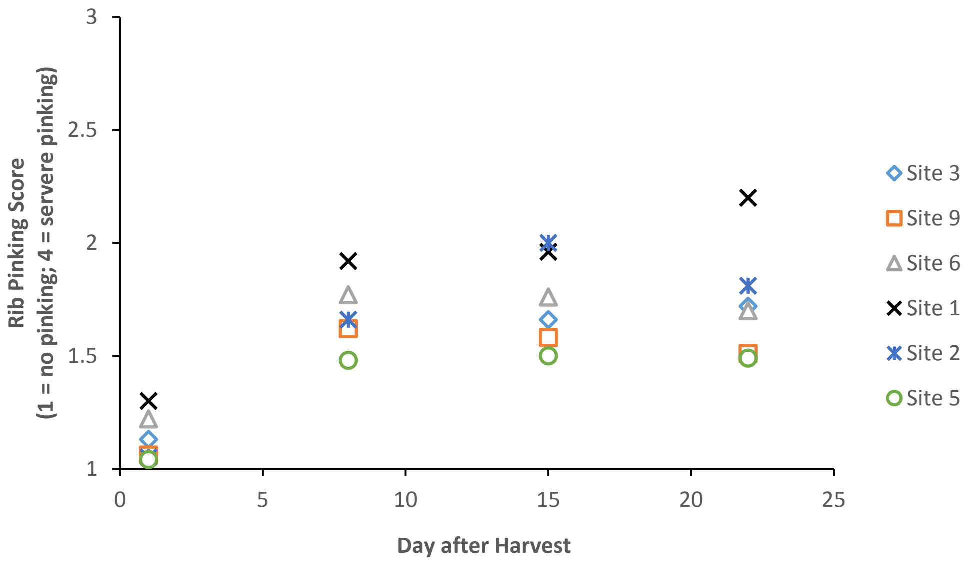

Figure 2. Average Iceberg lettuce rib pinking score during storage for all harvests during 2015.

Table 5. Iceberg lettuce rib and butt pinking score during storage, averaged for all locations, 2015. Different letters, within columns, indicate that values are significantly different (P<0.05).

Day	Rib pinking Score (1-4)	Butt Pinking Score (1-4)
1	1.1 a	1.0 a
8	1.7 b	2.9 b
15	1.7 b	3.3 c
22	1.7 b	3.5 c
Mean	1.6	2.7
SED	0.1	0.1

Cos Lettuce

Main effect of harvest date on post-harvest quality

The average value between different harvest dates for quantitative measures of head fresh and dry weight, circumference and moisture content differed significantly for all locations (Table 6). In contrast to Iceberg lettuce, less significant difference was observed for the qualitative assessments with three locations showing no significant difference between harvests for rib cracking or rib pinking (Table 6). Butt browning displayed no significant difference between harvests for two locations and butt pinking for one location (Table 6).

Table 6. Significance of main effect of week of harvest on average mean score of post-harvest quality parameters across all days of assessment for Cos lettuce, 2015.

Location	1	2	4	5	6	9
Rib Cracking	***	**	***	NS	NS	NS
Rib Pinking	*	***	***	*	*	NS
Butt Browning	NS	**	***	***	NS	**
Butt Pinking	***	***	***	***	NS	***
Dry Weight	***	***	***	***	***	**
Fresh weight	***	***	***	***	***	***
Head Circumference	***	***	***	***	***	***
Moisture Content	***	***	***	***	***	***

p<0.05 *; p<0.01 **; p<0.001 ***

Comparison of average rib pinking for separate locations and harvest dates.

The average rib pinking score ranged from 1.0 to 1.4 with the highest average score observed with samples harvested at Location 2 in week 35 and 43 (Figure 3). The range of scores was less than observed with Iceberg lettuce but varied between locations.

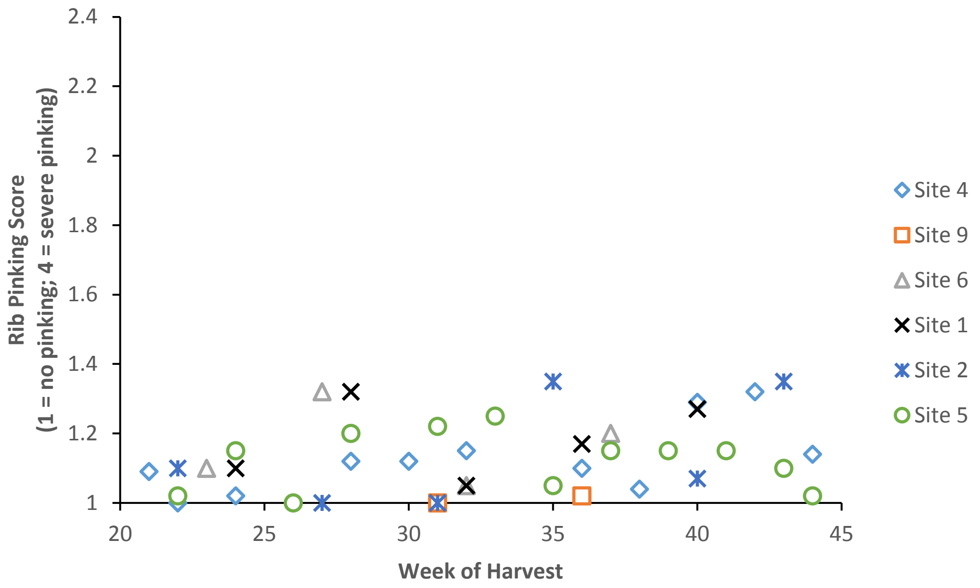

Figure 3. Average Cos lettuce rib pinking score for all locations over the harvest season 2015.

Main effect of storage duration on post-harvest quality

When the data was averaged over all harvests the quantitative measures showed some significant change during storage at HAU (Table 7).

Of the qualitative measures, butt pinking and browning displayed significant differences during storage for heads from all locations. Rib pinking and cracking showed significant change over storage for all locations but Location 9 (Table 7).

Table 7. Significance of main effect of day of assessment after harvest on average mean score of post-harvest quality parameters across all harvest weeks for Cos lettuce, 2015.

Location	1	2	4	5	6	9
Rib Cracking	*	*	***	*	*	NS
Rib Pinking	*	*	***	***	***	NS
Butt Browning	***	***	***	***	***	***
Butt Pinking	***	***	***	***	***	***
Dry Weight	*	***	***	***	NS	***
Fresh weight	*	***	***	*	NS	NS
Head Circumference	***	***	***	*	*	NS
Moisture Content	*	***	NS	***	NS	***

p<0.05 *; p<0.01 **; p<0.001 ***

Comparison of average rib pinking during storage for separate locations.

Rib pinking increased during post-harvest storage although this effect was not consistent across all harvest locations (Figure 4). When averaged over locations and harvest dates no rib pinking (1.0) was observed on Day 1 after harvest. The level of pinking increased at a consistent rate during storage to 1.1 by Day 8, 1.2 by Day 15 and 22 (Table 8). This increase in rib pinking over two weeks storage was significant (p<0.05) with the level of pinking observed after 15 days being significantly greater than that observed at the start of storage (Table 8).

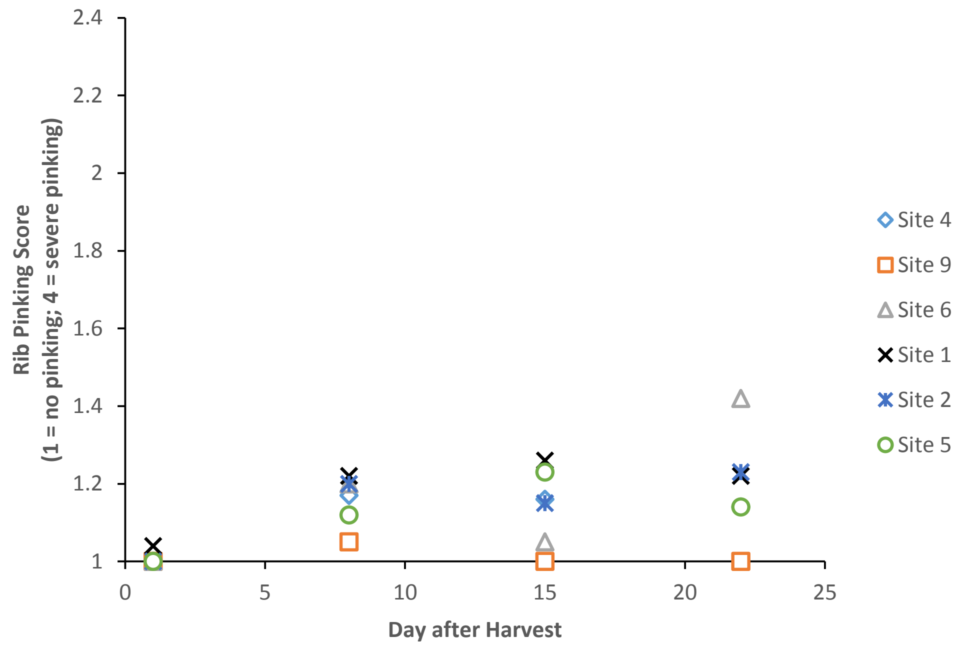

Figure 4. Average Cos lettuce rib pinking score during storage for all harvests during 2015.

Table 8. Cos lettuce rib and butt pinking score during storage, averaged for all locations, 2015. Different letters, within columns, indicate that values are significantly different (P<0.05).

Day	Rib pinking Score (1-4)	Butt pinking Score (1-4)
1	1.0 a	1.0 a
8	1.1 ab	2.2 b
15	1.2 b	2.8 c
22	1.2 b	2.9 c
Mean	1.1	2.2
SED	0.05	0.13

Combined model

The main focus of the research was to study the effect of growing environment on rib pinking. However, the collection of a wider range of observations has allowed additional analysis of rib cracking, butt pinking and butt browning. The raw model outputs are shown in Appendix 1, Tables A1-8.

For this report the output has been summarised graphically to show the change in likelihood of the variable changing from a score of 1 (perfect) to 2 (mild symptoms) and has been derived from the consolidated data for week 1 and week 2 shelf life scores. Week 3 has not been included as the data is often erratic, reflecting the greater breakdown after three weeks in some crops. Additionally, it is not standard practice to hold lettuce heads for 21 days in the UK. The greatest value observed for either week 1 or week 2 data is used. The key to the graphical summary is shown in Table 9.

Table 9. Key to summary tables for the model output.

Exp(β) = 1 or more	NS	1<1.1	1.1<1.2	1.2<1.3	>1.3
		↑	↑↑	↑↑↑	↑↑↑↑
Exp(β) = 1 or less	NS	1>0.9	0.9>0.8	0.8>0.7	<0.7
		↓	↓↓	↓↓↓	↓↓↓↓

Crop duration ranged from 41 days from planting (Cos and Iceberg) to 78 days (Cos) and 87 days (Iceberg) over the season. Hence, a quintile (Q1-5) would represent a period of between 1 and 2 weeks depending on the crop duration. It can be assumed that the developmental stages (i.e. hearting) would occur at a consistent stage in crop growth.

Soil Nitrogen Supply (SNS) index was calculated for each harvest following the Field Assessment Method (Defra, 2011) using soil type, rainfall and previous cropping. Fertilizer records were inconsistent between locations and nutrient input from fertilizer was not included in the model and the use of SNS represents the soil type and an indication of residual nitrogen (and wider soil nutrient and organic matter properties). The SNS for organic and peat soils span index 3-6 and 4-6 respectively (Defra, 2011). The number of harvest from soils of different SNS index are shown in Table 10. The greatest number of crops were grown in Light Sand soil (SNS index 0) and the model takes SNS 0 crops as the baseline when comparing the response to SNS. It would be anticipated that the soil moisture holding capacity of a Peat soil would differ to that of a Light Sand, as such SNS index also represents wider soil properties by default. The SNS category does not take account of additional nutrients added to the soil before planting or during the crop growth.

Table 10. Number of harvests at each SNS index derived from RB209 Field Assessment Method (Defra, 2011). N.B. the index does not account for nutrient input into the crop.

SNS index	Soil type	2014	2015	Combined
0	Light sand	25	33	58
1	Light sand	9	7	16
	Medium	0	2	2
	Deep silty	12	4	16
2	Deep silty	12	7	19
3	Deep silty	2	4	6
Organic*	Organic	2	8	10
Peat**	Peat	11	19	30

* SNS index 3-6
** SNS index 4-6

Rib disorders – Iceberg lettuce

Does growing environment increase or reduce the probability of rib disorders?

There was one strong pattern and two weaker patterns of response to growing environment. The strongest response was seen with higher temperatures in Q3 (i.e. halfway through crop cycle) which increased the probability of cracking and pinking after harvest (Table 11). Higher temperatures in Q1 also increased the risk of cracking and pinking after harvest. The conditions at the end of the crop cycle had an opposite effect. Higher temperatures and water inputs in Q5 reduced the risk of cracking and pinking after harvest i.e. cooler and dryer growth conditions in Q5 increased the risk of cracking and pinking after harvest.

Table 11. Effect of water availability and temperature over 5 periods during the crop cycle on the probability of rib disorder parameters increasing from score 1 – score 2 in Iceberg lettuce.

Rib cracking

	Q1	Q2	Q3	Q4	Q5
↑Water	↑				↓
↑Temp	↑↑		↑↑↑		↓↓

Rib Pinking

	Q1	Q2	Q3	Q4	Q5
↑Water			↑		↓
↑Temp	↑↑		↑↑	↓↓	↓↓

Does maturity/head fresh weight increase or reduce the probability of rib disorders?

There was a small increase in the risk of rib cracking and pinking with more mature heads, as measured by fresh weight at harvest (Table 12). The scale of response was small compared to the effect of growing environment.

Table 12. Effect of Fresh weight and Head circumference at harvest on probability of rib disorder parameters increasing from score 1 – score 2 in Iceberg lettuce.

	Rib cracking	Rib pinking
↑ Fresh weight	↑	↑
↑ Head circumference		

Does SNS index increase or reduce the probability of rib disorders?

Compared to SNS Index 0 soil rib cracking and rib pinking were sensitive to SNS and soil type with a strongly reduced risk of rib cracking in SNS Index 1 soils but strongly increased risk of rib pinking (Table 13). There was a small reduction in the probability of rib cracking where crops were grown in Organic soils.

Table 13. Effect of SNS before planting on probability of rib disorder parameters increasing from score 1 – score 2 in Iceberg lettuce.

	SNS 1	SNS 2	SNS 3	Organic	Peat
Rib cracking	↓↓↓↓			↓	
Rib pinking	↑↑↑↑				

Rib disorders – Cos lettuce

Does growing environment increase or reduce the probability of rib disorders?

There were two consistent patterns of response over the five quintiles. At the start of the crop cycle (Q1) a higher temperature was associated with a higher risk of rib cracking and rib pinking (Table 14). This response was particularly strong compared to that of Iceberg lettuce (Table 11). Another strong response was observed in mid growth (Q3) where higher temperature was associated with a reduced probability of rib cracking and pinking. The risk of rib cracking and pinking was not affected by the growing environment in the last two quintiles of the crop cycle (Table 14).

Table 14. Effect of water availability and temperature over 5 periods during the crop cycle on the probability of rib disorder parameters increasing from score 1 – score 2 in Cos lettuce.

Rib Cracking

	Q1	Q2	Q3	Q4	Q5
↑Water	↑		↑		
↑Temp	↑↑↑↑		↓↓↓↓		

Rib pinking

	Q1	Q2	Q3	Q4	Q5
↑Water	↑↑	↓			
↑Temp	↑↑↑↑	↓↓	↓↓↓↓		

Does maturity/head fresh weight increase or reduce the probability of rib disorders?

Denser heads, as suggested by a greater fresh weight and reduced head circumference, increased the risk of pinking, suggesting that more mature Cos heads were more prone to rib pinking (Table 15).

Table 15. Effect of Fresh weight and Head circumference at harvest on probability of rib disorder parameters increasing from score 1 – score 2 in Cos lettuce.

	Rib cracking	Rib pinking
↑ Fresh weight		↑
↑ Head circumference		↓↓

Does SNS index increase or reduce the probability of rib disorders?

No clear pattern was apparent in the response to soil SNS index although the effects were very strong. Compared to those grown in SNS Index 0, crops grown in peat soils were at a lower risk of developing rib cracking or pinking but crops grown in SNS Index 3 had at a greater probability of rib cracking and pinking (Table 16).

Table 16. Effect of SNS before planting on probability of rib disorder parameters increasing from score 1 – score 2 in Cos lettuce.

	SNS 1	SNS 2	SNS 3	Organic	Peat
Rib cracking	↑↑↑↑		↑↑↑↑		↓↓↓↓
Rib pinking			↑↑↑↑		↓↓↓↓

Butt discolouration – Iceberg lettuce

Does growing environment increase or reduce the probability of butt discolouration?

Higher temperatures at the start of the crop cycle (Q1) were associated with an increased risk of butt pinking but there was no effect on butt browning. The risk of both butt pinking and browning were increased with higher inputs of water in Q1 but the response differed after then. Butt pinking risk reduced with higher volumes of water input in Q2, but the risk of butt browning increased and did so for Q3 also. At the end of the crop cycle, the pattern was reversed between butt pinking and browning. Increased temperature in Q5 was associated with a greater risk of butt browning but a lower risk of butt pinking.

Table 17. Effect of water availability and temperature over 5 periods during the crop cycle on the probability of butt discolouration increasing from score 1 – score 2 in Iceberg lettuce.

Butt Pinking

	Q1	Q2	Q3	Q4	Q5
↑Water	↑	↓			
↑Temp	↑↑↑				↓↓↓

Butt Browning

	Q1	Q2	Q3	Q4	Q5
↑Water	↑	↑	↑		↓
↑Temp					↑↑↑

Does maturity/head fresh weight increase or reduce the probability of butt discolouration?

Denser, heavier heads were at a greater risk of butt pinking but a lower risk of butt browning (Table 18). Head circumference had a stronger relationship than fresh weight.

Table 18. Effect of Fresh weight and Head circumference at harvest on probability of rib disorder parameters increasing from score 1 – score 2 in Iceberg lettuce.

	Butt pinking	Butt browning
↑ Fresh weight	↑	↓
↑ Head circumference	↓↓	↑↑

Does SNS index increase or reduce the probability of butt discolouration?

There were very strong effects of SNS index on butt discolouration (Table 19). There was an inverse relationship between SNS index and pinking or browning. Compared to SNS 0 crops, iceberg lettuce grown in SNS 1, 2 and peat soils had a greater probability of butt pinking. The opposite was observed for butt browning in SNS 1 and 2 soils. Crops grown in organic soils were at a relatively greater risk of butt browning and lower risk of butt pinking than those grown in SNS 0 soils.

Table 19. Effect of SNS before planting on probability of rib disorder parameters increasing from score 1 – score 2 in Iceberg lettuce.

	SNS 1	SNS 2	SNS 3	Organic	Peat
Butt pinking	↑↑↑↑	↑↑↑↑		↓↓↓↓	↑↑↑↑
Butt browning	↓↓↓↓	↓↓↓↓	↓↓↓↓	↑↑↑↑	

Butt discolouration – Cos lettuce

Does growing environment increase or reduce the probability of butt discolouration?

Butt pinking in Cos lettuce was more sensitive to environmental conditions at the start and end of the growth cycle, whereas butt browning was more sensitive to the conditions in the middle of the crop cycle (Table 20). Warmer temperatures in Q1, 2 and 5 increased the probability of subsequent butt pinking. Increased volumes of water had opposite effects on butt pinking in Q1 and 2 but strongly reduced butt pinking where they occurred at the end of the crop cycle (Q5). In contrast the probability of butt browning was increased where the mid stage of crop growth (Q3) was warmer.

Table 20. Effect of water availability and temperature over 5 periods during the crop cycle on the probability of butt discolouration increasing from score 1 – score 2 in Cos lettuce.

Butt Pinking

	Q1	Q2	Q3	Q4	Q5
↑Water	↑	↓			↓↓↓
↑Temp	↑↑	↑↑			↑↑↑

Butt Browning

	Q1	Q2	Q3	Q4	Q5
↑Water		↑		↑	↓
↑Temp		↓↓	↑↑↑↑		

Does maturity/head fresh weight increase or reduce the probability of butt discolouration?

Cos lettuce with a greater fresh weight were generally associated with a higher probability of both butt browning and pinking (Table 21). The probability of butt pinking was also greater with heads of reduced circumference, again suggesting that more mature, dense heads were at greater risk of butt pinking.

Table 21. Effect of Fresh weight and Head circumference at harvest on probability of rib disorder parameters increasing from score 1 – score 2 in Cos lettuce.

	Butt pinking	**Butt browning**
↑ Fresh weight	↑↑	↑
↑ Head circumference	↓	

Does SNS index increase or reduce the probability of butt discolouration?

Compared to Iceberg lettuce, the SNS index had less effect on the probability of butt discolouration in Cos lettuce (Table 22). Compared to SNS index 0 soils, butt pinking was less likely in crops grown in SNS index 1 and 2 soils. In contrast, butt browning was a greater risk in those lettuce grown in peat soils.

Table 22. Effect of SNS before planting on probability of rib disorder parameters increasing from score 1 – score 2 in Cos lettuce.

	SNS 1	**SNS 2**	**SNS 3**	**Organic**	**Peat**
Butt pinking	↓↓↓↓	↓↓↓↓			
Butt browning					↑↑↑↑

Discussion (Combined data)

Over both years significant levels of discolouration were observed in ribs and butts of lettuce. The level of discolouration differed between locations and over time. Iceberg lettuce consistently displayed greater symptoms of rib pinking than Cos lettuce. Iceberg lettuce had an average rib pinking score of 1.6 in both 2014 and 2015, which compares to an average score of 1.1 in both years for Cos lettuce. The maximum rib pinking score was also higher in Iceberg lettuce for both years. Similar levels of butt pinking were seen in Cos and Iceberg lettuces.

Which environmental and crop factors increase the risk of a crop developing rib and butt pinking after harvest?

The main factors that influenced the risk of a crop developing pinking after harvest were: SNS index, temperature, water inputs and fresh weight.

Variation in SNS Index was very strongly associated with a changed risk of pinking developing. The risk of rib pinking in iceberg lettuce was greatest in crops grown in soil of Index 1, but it was greatest in Index 3 soils for Cos lettuce. Cos lettuce also had the lowest risk of rib pinking when grown in Peat soils. The pattern with soil index and butt pinking was confusing, with the risk of butt pinking in iceberg lettuce greatest in soils of Index 1 and 2 and peat soils. In contrast the risk of pinking in Cos lettuce was least in Index 1 and 2 soils.

The SNS index was derived from soil type, previous cropping and rainfall level. It did not take account of additional fertiliser inputs that would have an effect on nutrient availability. SNS Index was also a measure of soil type which was different between locations and soil type and texture would have an effect on nutrient holding capacity and availability as well as water holding capacity and availability. It was not possible in this analysis to identify which of these factors led to the marked response to SNS Index and further detailed work would be needed to establish the individual underlying soil properties of importance to pinking.

Temperature was associated with a number of significant responses in discolouration. In Iceberg lettuce, higher temperatures in Q1 and Q3 were associated with a significant increase in the risk of rib pinking but a significant reduction in risk if the temperatures was higher in Q5. A similar response was observed in Q1 and Q5 for butt pinking in Iceberg lettuce.

Cos lettuce had a different pattern of response, higher temperatures in Q1 increased butt and rib pinking, this response changed in Q2 with a reduction in risk of rib pinking but an increase in risk for butt pinking. The reduction in risk of rib pinking was an even stronger response to higher temperatures in Q3, with no response in butt pinking. By the end of the growing cycle higher temperatures increased the risk of butt pinking only.

Rib discolouration, with browning lesions as contrasted to pink ribs, has been previously described as a response to high temperatures in the 7 days before harvest (Sharples, 1965), and ~2 weeks before harvest (Jenni, 2005) in wholehead lettuce. These would equate to Q5 and Q4 in their growth cycles. The temperature range associated with pinking is also unclear. Research suggests that temperatures of 35 °C during the day and 15-25 °C during the night are associated with increased rib discolouration (Jenni, 2005; Sharples, 1965). However, the crops in this study did not experience unusually hot summers. The UK mean temperature for summer 2014 was 14.8 °C which was 0.5 °C above the 1981-2010 average. June and July were both warmer than average, but it was the coolest August since 1993 (Met Office, 2016a). In summer 2015 mean temperatures were below average for all three months. Temperatures were generally near normal across eastern England (Met Office, 2016b).

Temperature also influences rate of growth and development in a crop and periods of moderately higher temperature will increase the rate of leaf expansion, as long as nutrients and water are not limiting. This may lead to leaves with larger cells, potentially more prone to damage or breakdown during subsequent growth or harvest handling.

The effect of **water inputs** was generally less than that of temperature. This can be explained by the fact that low rainfall can be mitigated by irrigation, meaning that commercial crops should not be short of water during growth, although heavy rain would lead to excess water availability. Rib pinking in Iceberg lettuce was sensitive to water inputs in Q3 and Q5 with greater volume of water input associated with a small increase in probability in Q3 and a small decrease in Q5. Butt pinking responded to early availability of water with a small increase in risk in Q1 and a small decrease in risk in Q2. In Cos lettuce the risk of both rib and butt pinking increased with higher volumes of water in Q1 and decreased with higher volumes in Q2. The risk of butt pinking was also more strongly reduced when Q5 was wetter.

Increased irrigation can decrease storability with higher subsequent pinking expression postharvest (Wurr *et al.,* 2003; Monaghan *et al*., 2007; Luna *et al.,* 2012). There is no work studying the effect of rainfall but it can be assumed that the response to heavy rain would be similar. 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 and hence pinking. Increased irrigation could also impact on growth. However, the UK had close to average summer rainfall in 2014. June and July were generally dry months, although heavy summer downpours resulted in above average rainfall for parts of East Anglia in July. Rainfall in August was generally above average (Met Office, 2016a). June 2015 was drier than average for most of the UK and it was especially dry in East Anglia. Most of the country was then wetter than average in both

July and August, with approaching double the normal rainfall amount for parts of Scotland and East Anglia in July, and southern coastal counties in August (Met Office, 2016b).

Timing of responses – in general there were three main timings of response: Q1, Q3 and Q5. The crop cycle ranged from 41 days from planting (Cos and Iceberg) to 78 days (Cos) and 87 days (Iceberg) over the season, with the shortest durations during mid-summer. This suggests that a quintile would represent a period of between 1 and 2 weeks depending on the crop duration. Previous work at HAU has counted leaves on Iceberg lettuce plants growing in soil in a polytunnel. The lettuce took 50 days from transplanting to harvest and had produced 45 leaves by harvest. The trimmed head contained 25 leaves, indicating that the outer leaves of the head were produced during Q3 (Figure 5).

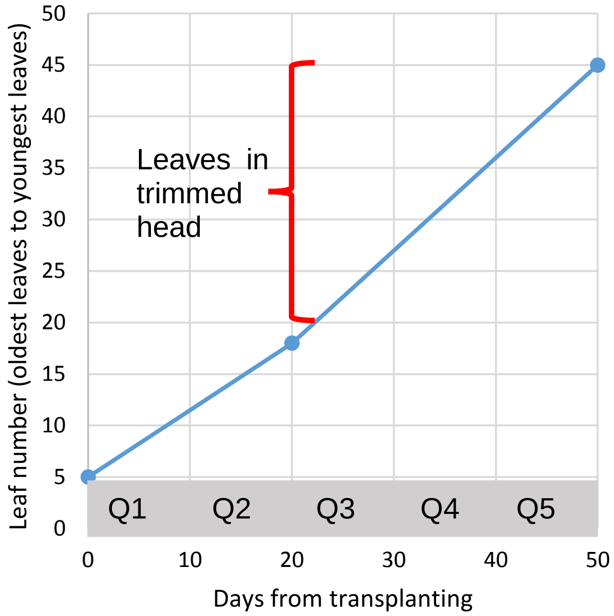

Figure 5. Production of leaves - polytunnel grown Iceberg lettuce. HAU, 2014.

Similar development in a Cos lettuce can be derived from data reported by Nothmann (1976) where pot grown plants were raised from seed. Assuming that a transplant would have 4-5 leaves a transplant to harvest crop cycle would approximate to 48 days. Heading/hearting started at about leaf 17, on day 22 after transplanting and a further 35 leaves were produced before the heads started to bolt. If a trimmed head contained 30 leaves, the leaves at the outside of the trimmed head would also have developed during Q3.

Assuming that the developmental stages (i.e. hearting/heading) occurred at a consistent stage in crop growth: Leaves developed in Q1 & 2 will establish the 'framework' of the plant but will be left as waste following trimming at harvest; leaves developed in Q3 will be at the outside of the trimmed head; leaves developed in Q4 & 5 will make up the internal leaves of the head and influence density and fresh weight of the harvested head.

Early growth

This study suggests that both Iceberg and Cos lettuce are influenced by the growing conditions in the first quintile following transplanting with higher temperatures and water availability being associated with a significant increase in the probability of pinking in the butt and ribs following harvest. This was a surprising finding. The leaves produced during this time are not part of the harvested head, so any response to temperature and water status at this time must be due to changes in long term factors within the plant, maybe linked to the biochemistry of the ribs and butts and/or a consequence of growth form i.e. cell differentiation in the meristem leading to larger or smaller cell size in subsequent ribs and leaves.

Mid growth

Cos and Iceberg responded differently to conditions during Q3. In Iceberg lettuce, the probability of rib pinking increased when Q3 was warmer and wetter, in contrast, with Cos lettuce, the probability decreased strongly when Q3 was warmer. Butt pinking was not influenced by Q3 conditions in either crop. The explanation of this response is not obvious. In Iceberg lettuce, it suggests that leaves that develop and expand under warmer conditions have more fragile ribs, as seen by the increased rib cracking, whereas the same conditions in Cos lettuce produce more resilient ribs. The morphology of leaf ribs differs between the two crops with Cos lettuce having more pronounced ribs, often with a hollow core. Understanding this response may be of use to breeders in developing crops that are more resilient to post harvest handling.

Late growth

The stage of growth before harvesting was important for iceberg lettuce but less so for Cos. Wetter and warmer conditions reduced the risk of rib and butt pinking in Iceberg lettuce i.e. cooler and dryer conditions would increase the risk. In Cos there was no effect on rib pinking and dryer, warmer conditions increased the risk of butt pinking. The leaves produced in Q5 would be within the head so any response would be due to general head water status and the turgidity of ribs and leaves at harvest. It may be that cooler conditions prior to harvest slow down growth and lead to higher turgidity at harvest in Iceberg lettuce. This could lead to greater rib cracking when the plants were handled. However, rib cracking and pinking were a lower risk when Q5 had greater water availability. In Cos lettuce the robust ribs may be less sensitive to plant water status. More work is needed to understand this response, which was counter to grower expectations that wetter environments close to harvest are associated with pinking.

Harvesting more **mature crops** has been suggested as a factor that increases the risk of pinking (HDC FV413) in whole heads. More mature crops are generally heavier and larger, which would lead to higher fresh weight and greater head circumference being associated with maturity. In contrast more dense heads would have higher weight but smaller circumference.

In this study greater fresh weight was associated with increased risk of rib and butt pinking in both Iceberg and Cos lettuce suggesting that maturity of the head at harvest is a key factor in subsequent pinking. Increased density could also explain the increased risk of pinking in Cos ribs.

Can we identify high-risk crops based on local meteorological and crop input records?

Iceberg and Cos lettuce behaved differently to both temperature and water input during growth. In addition, there were relatively low levels of pinking in Cos. Rib and butt pinking also responded differently and are clearly not the same response with the butt being a cut surface where rib pinking is closely linked to rib cracking. Nevertheless, some general observations could be made:

The **greatest risk of rib pinking in Iceberg Lettuce** would follow a warm and wet start, warm mid growth and cool and dryer end to the crop cycle. This would be expected to give rapid initial growth, rapid growth at hearting when the outer leaves of the head are developing and slower growth prior to harvest. Risk was increased slightly by heavier heads at harvest (i.e. more mature). No clear pattern of response to SNS was observed. There was a suggestion that SNS 1 soils were associated with an increased risk of pinking only.

Butt pinking and browning in iceberg lettuce generally showed opposite responses. A warm end of the crop cycle reduced the probability of butt pinking. The risk of butt pinking was increased by heavier heads with a smaller circumference at harvest (i.e. denser). Soil index had a marked effect on the probability of butt discolouration but with a greater SNS index generally increasing the probability of butt pinking, the exception was organic soils which reversed this response.

Greatest risk of rib pinking in Cos Lettuce would follow a warm and wet start and a cool period mid growth in the crop cycle. This would be expected to give rapid initial growth then slower growth at hearting when the outer leaves of heads are developing. As with Iceberg lettuce, the risk was increased slightly by more mature heads at harvest. No clear pattern of response to SNS was observed. SNS 3 soils were associated with an increased risk of pinking and cracking but peat soils were associated with a reduced risk of pinking and cracking.

Butt pinking and browning in Cos lettuce generally showed opposite responses. There was an increased risk of butt pinking in Cos lettuce when there was a warmer start and a warmer, dryer end of the crop growth cycle. More mature, dense heads were at greater risk of butt pinking. The probability of butt pinking increased in SNS 1 and 2 soils.

Conclusions

- Over both years Iceberg lettuces displayed moderate levels of rib pinking and Cos lettuces displayed low levels of rib pinking after cold storage for 2 weeks.
- Iceberg and Cos lettuces showed high levels of butt pinking after cold storage for 2 weeks.
- The main factors that influenced the risk of a crop developing pinking after harvest were SNS index > temperature > water inputs > fresh weight (maturity).
- Harvesting mature heads of both crops increased the probability of pinking.
- Cos and Iceberg lettuces differed in the extent and timing of sensitivity to soil and environmental factors.
- Soil type had greater effect on butt pinking than rib pinking in Iceberg lettuce but had similar effects in Cos lettuce
- The first 20% of the crop growth cycle (1st quintile) had a significant effect on the risk of pinking developing after harvest, with both crops having a greater probability of rib and butt pinking if the early growth stage was warmer and wetter.
- The environmental conditions during the middle 20% of the crop growth cycle (3rd quintile) was important for both crops. This is the period when the leaves that will be found on the outside of the trimmed head are produced. The probability of rib pinking in Iceberg lettuce was greater with warmer and wetter conditions but the probability was lower in Cos lettuce.
- The last 40% of the growth cycle (quintile 4 & 5) was important for rib pinking in Iceberg lettuce with cooler conditions associated with an increased probability of rib pinking. The conditions had no effect on Cos lettuce.

Knowledge and Technology Transfer

The work was presented and discussed at the HDC Salad day at Huntapac 6 Nov 2014.

Preliminary data was reported at the Lettuce Research Meeting at HAU 24 Feb, 2016.

An article is planned for AHDB grower in 2016.

References

Defra (2011) RB209 Fertiliser manual. Available on-line at: http://www.ahdb.org.uk/documents/rb209-fertiliser-manual-110412.pdf. Accessed 5/03/2016

Edwards, S.G. (2007) Investigation of Fusarium mycotoxins in UK wheat production. Final report FSA CO4022/HGCA 2452

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

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

Met Office (2016a) Summer 2014. Available on-line at: http://www.metoffice.gov.uk/climate/uk/summaries/2014/summer. Accessed 20/03/2016

Met Office (2016b) Summer 2015. Available on-line at: http://www.metoffice.gov.uk/climate/uk/summaries/2015/summer. Accessed 20/03/2016

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

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

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

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.

Appendices

Table A1: exp(β) for **Butt Pinking of Cos lettuce** after 1, 2 and 3 storage; there were four classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j. For each storage time, if there is only one value, it means that β does not depend on *j* [see M & M]

	Week 1			Week 2			Week 3		
	> 1	> 2	> 3	> 1	> 2	> 3	> 1	> 2	> 3
SNS 1				0.419			0.347		
SNS 2	0.522			0.198			0.267		
Fresh weight	0.908	NS	NS	0.903	0.973	0.980	0.919	0.945	NS
Dry Weight							1.230	1.077	NS
HC	1.123	NS	NS				1.075		
RainQ1	NS	0.988	0.990	1.008			1.013		
TempQ1	0.881	0.846	NS	1.128					
RainQ2				0.989					
TempQ2				1.107					
RainQ4	NS	NS	1.050						
TempQ4				0.731					
TempQ5				1.268			1.113		

Table A2: exp(β) for **Butt Pinking of Iceberg lettuce** after 1, 2 and 3 storage; there were four classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j. For each storage time, if there is only one value, it means that β does not depend on *j* [see M & M]

	Week 1			Week 2			Week 3		
	> 1	> 2	> 3	> 1	> 2	> 3	> 1	> 2	> 3
SNS 1	2.046			1.995					
SNS 2	1.643			1.619					
SNS 4.5				0.631					
SNS 5	1.637			2.006					
Fresh weight	1.037			1.062			1.029		
Dry Weight				0.926					
HC	0.935			0.897			0.938		
RainQ1				1.024			1.017		
TempQ1	1.293			1.248			1.198		
RainQ2				0.991			0.990		
RainQ4	NS	1.011	NS						
RainQ5							0.992		
TempQ5	0.800			0.843			0.921		

Table A3: exp(β) for **Rib Cracking of Cos lettuce** after 1, 2 and 3 storage; there were only two classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j

	Week 1	Week 2	Week 3
	> 1	> 1	> 1
SNS 1	3.917		
SNS 3	15.66		
SNS 5		0.348	0.403
HC			0.930
RainQ1	1.016		1.018
TempQ1	1.328	1.223	1.166
RainQ2			0.974
RainQ3	1.017		
TempQ3	0.666	0.777	
TempQ5			0.807

Table A4: exp(β) for **Rib cracking of Iceberg** after 1, 2 and 3 storage; there were four classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j

	Week 1	Week 2	Week 3
	> 1	> 1	> 1
SNS 1	0.661		1.545
SNS 2			1.983
SNS 3			3.062
SNS 4.5		0.954	0.579
SNS 5			2.129
Fresh weight	1.019	1.031	1.061
Dry Weight			0.890
RainQ1		1.011	1.012
TempQ1	1.191		1.093
RainQ2			1.009
TempQ3		1.201	
RainQ5		0.991	
TempQ5		0.884	0.856

Table A5: exp(β) for **Rib pinking of Cos lettuce** after 1, 2 and 3 storage; there were three classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j

	Week 1		Week 2		Week 3	
	> 1	> 2	> 1	> 2	> 1	> 2
SNS 3	6.754					
SNS 5			0.453			
Fresh weight	1.081		1.059		1.059	
Dry Weight	0.872					
HC	0.920		0.873		0.84	
RainQ1	1.112				1.006	
TempQ1	1.343		1.299		1.122	
RainQ2	0.982					
TempQ2			0.837			
TempQ3	0.683					

Table A6: exp(β) for **Rib Pinking of Iceberg lettuce** after 1, 2 and 3 storage; there were three classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j

	Week 1		Week 2		Week 3	
	> 1	> 2	> 1	> 2	> 1	> 2
SNS 1	0.580		1.556			
SNS 4.5					0.397	
SNS 5					1.661	
Fresh weight	1.027		1.038		1.030	
RainQ1					1.008	
TempQ1			1.135		1.196	
RainQ2					1.008	
TempQ2					0.870	
RainQ2	1.009					
TempQ3	1.119					
TempQ4	0.840					
RainQ5	0.993					
TempQ5			0.885			

Table A7: exp(β) for **Butt Browning of Cos lettuce** after 1, 2 and 3 storage; there were three classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j. For each storage time, if there is only one value, it means that β does not depend on *j* [see M & M]

	Week 1		Week 2		Week 3	
	> 1	> 2	> 1	> 2	> 1	> 2
SNS 5			2.047			
Fresh weight	1.035					
Dry Weight	0.946					
RainQ2			1.011			
TempQ2			0.804			
TempQ3	1.085		1.400		1.152	
RainQ4	1.012					
RainQ5	0.982		0.980		0.975	
TempQ5					0.904	

Table A8: exp(β) for **Butt Browning of Iceberg lettuce** after 1, 2 and 3 storage; there were three classes of scores. The estimates with green colour indicates the specific variable has no effect on the particular score transition for this quality trait. Values < 1 and > 1 indicates that increasing the variable by one unit (temperature – one degree, fresh weight – 10 g, dry weight - 1 g, HC – 1 cm, rain – 1 mm) will lead to proportional decrease (i.e. better quality) or increase (worse quality) in the odds of the quality score being above or below the score j. For each storage time, if there is only one value, it means that β does not depend on *j* [see M & M]

	Week 1		Week 2		Week 3	
	> 1	> 2	> 1	> 2	> 1	> 2
SNS 1	0.576		0.569		1.506	
SNS 2	0.309					
SNS 3	0.295		0.445			
SNS 4.5	1.687		3.180		2.629	
Fresh weight	0.961	NS				
Dry Weight	1.093	NS				
HC			1.114			
RainQ1			1.018	1.033	NS	1.014
TempQ1					0.870	
RainQ2			1.015		NS	0.998
TempQ2					0.712	NS
RainQ3			1.010	NS	0.990	
TempQ3					1.421	NS
TempQ5	1.210		1.211		0.840	NS
RainQ5	0.990					

