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Project number:	FV 413a
Project leader:	Jim Monaghan, Harper Adams University
Report:	Annual report, February 2015
Previous report:	None
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Location of project:	HAU
Industry Representative:	Ms Emma Garrod, G's Fresh
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The results and conclusions in this Annual Report are based on an investigation conducted over a one-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.

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GROWER SUMMARY

Headline

Postharvest pinking and other measures of postharvest quality varied over the season and between locations for both Iceberg and Cos lettuce. Iceberg lettuce had a greater range of pinking in the ribs; Cos lettuce had a greater range of pinking in the cut surface of the butt/stem.

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. 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 the expression of pinking.

This project is taking Iceberg and Cos lettuce samples from a number of UK commercial lettuce growing sites over two growing seasons. The heads are assessed over storage for the development of pinking and other quality measures. This information will be combined with data on the environmental conditions (agronomic and meteorological) that each crop has experienced. The availability of multiple lettuce crops from March - October means that a wide range of weather conditions can be incorporated into modelling over the two years. Guidelines for identifying high-risk crops will be developed based on local meteorological and crop input records enabling growers to manage crops through the supply chain to the benefit of the customer.

Summary

We have observed significant variation in Iceberg lettuce for rib cracking, rib pinking, butt browning, butt pinking and density. These values vary significantly over the season at each location. In addition, as expected post-harvest quality measures changed significantly during storage of Iceberg lettuce.

Significant variation was observed in Cos lettuce for rib cracking, rib pinking, butt browning and butt pinking but the range of response was less than that observed with Iceberg lettuce. These values varied significantly over the season at each location for most measures of qualitative post-harvest quality of Cos lettuce. Whilst rib pinking and cracking developed in Cos lettuce during storage, the more significant changes were observed for cut surfaces as observed in butt pinking and browning.

An overview of post-harvest data is presented in the Annual Report only with a particular focus on rib pinking. The meteorological data and agronomic inputs have been collected and formatted, and will be analysed and incorporated into the modelling of two years post-harvest data and this will be reported in full in the Final Report in 2016.

Financial Benefits

It is not possible to extrapolate financial benefits from this work until the model is completed in Year 2. 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

It is not possible to derive and propose Action Points from this work until the model is completed in Year 2.

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 pinking 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 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 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 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 tip burn. 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.

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We propose to utilise large sample sizes derived from multiple commercial sites 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 Practice to minimise risk and a HGCA mycotoxin risk assessment scheme where growers input agronomic factors and rainfall data to predict a low, moderate or high risk of exceeding legal limits of fusarium mycotoxins. This has led to growers clearly understanding the risk factors and modifying their agronomy accordingly.

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) from nine locations (Table 1 & 2, Figure A in Appendix 1). 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 sites: 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. Following discussion with the Industry Representative 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

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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.

Sample	G's Cambridge	TLC	PDM	Huntapac	Kettle	Intercrop	Jepco	LJ Betts
1	13/05	14/07	14/05	03/06	09/06	27/05	13/05	14/05
2	23/06	07/10	30/05	01/07	07/08	24/06	23/06	04/06
3	30/06		16/06	07/08	04/09	29/07	28/07	02/07
4	27/08		09/07	13/10	25/09	26/08	16/09	30/07
5	13/10		28/07			23/09	10/10	26/08
6			26/08					24/09
7			03/10					
Total	5	2	7	4	4	5	5	6

Table 1. Iceberg sample dates and locations, 2014

Table 2. Cos sample dates and locations, 2014

Sample	G's Cambridge	TLC	PDM	Huntapac	Kettle	Intercrop	Jepco	LJ Betts	G's Norfolk
1	13/05	07/10	14/05	03/06	09/06	27/05	13/05	14/05	25/06
2			30/05	01/07	07/08	24/06	23/06	04/06	03/07
3			16/06	07/08	04/09	29/07	28/07	02/07	21/08
4			08/07		25/09	26/08	16/09	30/07	06/10
5			28/07			23/09		26/08	
6			26/08					24/09	
7			03/10						
Total	1	1	7	3	4	5	4	6	4

Plant assessments

On arrival, all of the heads were re-trimmed – chopping the butts off and removing a few of the outer leaves which are likely to have been damaged in transit and removing any pinking or butt discolouration which may have occurred prior to arriving and before the assessments began. Each head was labelled (site, date of harvest, date of arrival, variety etc.), weighed fresh and the circumference measured before it was placed in a new plastic bag, 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 using a commercial visual scoring chart for:

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

In addition, the following factors were scored following standard commercial scoring scales:

- Chill Damage
- Dehydration
- Downy Mildew
- Internal and external breakdown
- Misshapen Head
- Pest Damage
- Ribbiness
- Soiling
- Tip burn
- Twisting
- Viral Infection

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

Leaf and rib colour - The outer leaf was removed from the head and laid on a white background before the rib colour was quantified for each head using a Minolta colorimeter at 1 cm up the rib (from the base which was attached to the butt) and 5 cm up the rib. These were marked out on the background to ensure that the same measurement was used for every leaf. The butt readings were taken from the centre of the intact butt. Readings were taken in the L, a and b dimensions.

Membrane integrity - The leaf tissue was then used for assessment of membrane integrity and 2 discs were cut from the leaf along the rib. Solute leakage into 100 ml deionised water over 3 hours was measured as change in EC. The same tissue was then frozen for 48 hours to break the cells open, and the same process to quantify solute leakage was followed giving a maximum leakage value. Solute leakage was calculated as the percentage of maximum leakage.

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

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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.

Site information

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

- Soil type
- Soil nutrient index/residual analysis
- Applied nutrients(1)
- Presence and timing of crop covers
- Irrigation system (overhead, drip tape or sub irrigation)
- Irrigation timing and quantity
- Cultivar
- Transplanting date

HAU provided thermocrons to each site but most of these were lost during commercial field working. However, those recovered will allow comparison between the recorded conditions and the reported meteorological data for that location.

At the start of the trial it was established the met data that each grower (and growing site) could provide. In addition to grower data, data from the nearest Met Office synoptic and climate stations to the growing locations was accessed.

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 ONLY

Statistical analysis to determine the impact of environmental and agronomic factors on the level of pinking will be performed using a stepwise selection ANOVA. Models of level of

pinking and other quality assessments will be validated using residual plots and the predictive ability of the models will be assessed by observing the stability of the parameter estimates for each year and by calculating the Prediction Error Sum of Squares (PRESS). Other forms of model development (e.g. all subset regression) and validation (e.g. cross year/ location validation) will be explored.

Results

An overview of post-harvest data is reported here with a particular focus on rib pinking. The met data and agronomic inputs have been collected and formatted to be analysed and incorporated into the modelling of two years post-harvest data. The model and interpretation will be reported in full in the Final Report in 2016.

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 head circumference at one location (Table 3). A similar response was observed for the qualitative assessments of rib cracking, rib pinking, butt browning, butt pinking and density with only butt browning showing no significance between harvests from two locations (Table 3). In contrast, there were no significant differences observed with the colorimeter values and only half of the locations showed significant differences between harvest dates for membrane leakage.

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

The average level of pinking observed in the samples increased significantly (p<0.001) over the season and is described by the model:

Pinking score = 0.025 week number + 0.810 (R² = 0.27)

The average rib pinking score ranged from 1.0 to 2.3 with the highest average score observed with samples harvested from one location in week 42 (Figure 1). However, the pattern of scores varied between sites. For illustration, the lettuce from Location 5 had high pinking scores at week 20 which then declined before increasing again towards the end of the season but the lettuce from Location 6 showed an inverse response with the mid-season samples having the highest average pinking scores. The spread of response is useful for the modelling at the end of Year 2.

Main effect of storage duration on post-harvest quality

When the data was averaged over all harvests the quantitative measures showed little significant change during storage at HAU (Table 4) with only samples from four locations showing any variates exhibiting significant change during storage. However, the qualitative measures, with the exception of density at all sites but one location and rib cracking in samples from one location, showed significant changes during storage (Table 4).

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 1.6 by Day 8, remained similar, at 1.7, by Day 15 but then increased significantly again to 2.0 by Day 22 (Table 5).

Location	1	2	3	4	5	6	7	8
Rib Cracking	***	***	***	***	***	***	***	***
Rib Pinking	***	***	***	***	***	***	***	***
Butt Browning	**	*	***	NS	***	***	***	NS
Butt Pinking	***	***	***	***	***	***	***	*
Density	***	***	***	***	***	***	***	***
Colorimeter	NS							
Membrane leakage	***	NS	NS	***	NS	*	*	NS
Dry Weight	***	***	***	***	***	***	***	**
Fresh weight	***	***	***	***	***	***	***	***
Head Circumference	***	***	***	***	***	***	***	NS
Moisture Content	***	***	***	***	***	***	***	*

Table 3. Significance of main effect of week of harvest on average mean score of postharvest quality parameters across all days of assessment for Iceberg lettuce, 2014.

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

Table 4. Significance of main effect of day of assessment after harvest on average	score of
post-harvest quality parameters across all harvest weeks for Iceberg lettuce, 2014.	

Location	1	2	3	4	5	6	7	8
Rib Cracking	*	***	**	*	***	**	NS	***
Rib Pinking	***	***	***	***	***	***	***	***
Butt Browning	***	***	***	***	***	***	***	***
Butt Pinking	***	***	***	***	***	***	***	***
Density	NS	NS	NS	NS	NS	NS	**	NS
Colorimeter	NS							
Membrane leakage	NS							
Dry Weight	NS	NS	NS	NS	***	NS	***	NS
Fresh weight	NS	NS	NS	NS	*	NS	NS	NS
Head Circumference	NS							
Moisture Content	NS	NS	NS	*	**	**	**	NS

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



Figure 1. Average Iceberg lettuce rib pinking score for all sites over the harvest season 2014.



Figure 2. Average Iceberg lettuce rib pinking score during storage for all harvests during 2014.

Table 5. Iceberg lettuce rib pinking score during storage, averaged for all sites and locations, 2014. Different letters, within columns, indicate that values are significantly different (P<0.05).

Dav	Rib Pinking
Day	Score (1-4)
1	1.1 a
8	1.6 b
15	1.7 b
22	2.0 c
Mean	1.6
SED	0.11

Cos Lettuce

Main effect of harvest date on post-harvest quality

Two locations supplied only one Cos harvest sample and are excluded from this analysis (Table 6). 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 one location but butt pinking showed a significant response to harvest date for all the locations (Table 6). No significant difference was observed for colorimeter readings and membrane leakage exhibited significant differences at three locations only.

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

The average level of pinking observed in the Cos samples increased significantly (p<0.01) over the season and is described by the model:

Pinking score = 0.013 week number + 0.766 (R² = 0.19)

The average rib pinking score ranged from 1.0 to 2.1 with the highest average score observed with samples harvested at Location 5 in week 39 although this was an unusually high score with the next highest score being 1.4 observed with Cos harvested from Location 4 and Location 7 on week 31 and 39, respectively (Figure 3). The range of scores was less than observed with Iceberg lettuce but varied between sites.

Main effect of storage duration on post-harvest quality

When the data was averaged over all harvests the quantitative measures showed little significant change during storage at HAU (Table 7) the exception were the samples from one location where significant differences were observed over storage in dry and fresh weight, head circumference and moisture content (Table 7).

Of the qualitative measures, butt pinking displayed significant differences during storage for heads from all locations and butt browning for all but one location. In contrast, rib pinking only changed significantly during storage with heads from four locations. The heads from the other locations did not show significant change over storage (Table 7).

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 1.3 by Day 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).

Location	1	2	3	4	5	6	7	8	9
Rib Cracking	**	NS	***	NS	***	***	NS	-	-
Rib Pinking	NS	NS	NS	***	***	***	**	-	-
Butt Browning	***	*	NS	***	***	***	***	-	-
Butt Pinking	***	***	***	**	***	***	***	-	-
Colorimeter	NS	-	-						
Membrane Leakage	***	NS	NS	*	NS	***	NS	-	-
Dry Weight	***	***	***	***	***	***	***	-	-
Fresh weight	***	***	***	**	***	***	***	-	-
Head Circumference	***	***	***	***	***	***	***	-	-
Moisture Content	***	***	***	***	***	***	***	-	-

Table 6. Significance of main effect of week of harvest on average score of post-harvestquality parameters across all days of assessment for Cos lettuce, 2014.

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

Table 7.	Significance	of main	effect of	day of	assessment	after	harvest	on	average	mean
score of	post-harvest c	juality pa	arameter	s acros	s all harvest v	veeks	s for Cos	lett	uce, 2014	4.

Location	1	2	3	4	5	6	7	8	9
Rib Cracking	NS	NS	***	NS	NS	*	NS	NS	NS
Rib Pinking	*	NS	NS	***	***	NS	*	NS	NS
Butt Browning	***	***	***	***	***	***	***	***	NS
Butt Pinking	***	***	***	***	***	***	***	***	***
Colorimeter	NS	NS-							
Membrane Leakage	***	NS							
Dry Weight	NS	**	NS	*	NS	***	*	NS	NS
Fresh weight	NS	NS	NS	*	NS	**	NS	NS	NS
Head Circumference	NS	NS	*	NS	NS	**	NS	*	NS
Moisture Content	NS	NS	NS	***	**	***	***	*	NS

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



Figure 3. Average Cos lettuce rib pinking score for all sites over the harvest season 2014.



Figure 4. Average Cos lettuce rib pinking score during storage for all harvests during 2014.

Table 8. Cos lettuce rib pinking score during storage, averaged for all sites and locations, 2014. Different letters, within columns, indicate that values are significantly different (P<0.05).

Day	Rib pinking Score (1-4)				
1	1.0 a				
8	1.1 ab				
15	1.2 bc				
22	1.3 c				
Mean	1.1				
SED	0.05				

Discussion

This report summarises the extent of usable variation in the data set from Year 1. It is not possible to discuss the underlying physiology of the observed patterns of response this year.

Does pinking vary between locations and harvest dates?

Significant main effects of harvest on rib pinking, butt pinking, butt browning and rib cracking were observed for Iceberg lettuce as required by the modelling phase in Year 2. There was a different response for Cos with rib pinking values lower than expected and only four of seven locations showing significant main effects of harvest date. However butt pinking and browning appeared to be more affected by harvest date at most locations.

This suggests that the growing environment has less effect on rib pinking in Cos than it does on Iceberg. If this response is consistent over two years it may lead to greater understanding of the physiological basis of the pinking phenomenon. In addition, this also suggests that the discolouration of cut surface is more sensitive in Cos; of potential interest to the fresh-cut sector.

Does pinking change during storage?

As expected, there were significant increases in rib pinking during storage for both Cos and Iceberg lettuce. This response was more marked in Iceberg. It may be that the response in Cos lettuce to be modelled in Year 2 is focused on butt discolouration rather than the ribs. This will become clearer when the data from Year 2 is analysed.

Can we use objective measures of pinking in the model?

There was no significant overall variation observed between different harvest dates or during storage for either Iceberg lettuce or Cos lettuce for the base/butt, 1 cm and 5 cm along the rib (detailed data not presented). Membrane leakage showed more potential identifying significant variation between harvests for four locations growing iceberg lettuce and three locations growing Cos lettuce. However, there was no consistent change in membrane leakage during storage for either Cos or Iceberg. As both measures were hoped to give more clarity to the colour changes of tissues during storage, this has been a disappointment. The approaches are both time consuming and the lack of sensitivity suggests that these measures should be removed from Year 2 analysis.

Conclusions

- Significant variation was observed in Iceberg lettuce for rib cracking, rib pinking, butt browning, butt pinking and density.
- Overall, there was a main effect of harvest date at each location for rib cracking, rib pinking, butt browning, butt pinking and density for Iceberg lettuce.
- The qualitative post-harvest quality measures changed significantly during storage of Iceberg lettuce
- Significant variation was observed in Cos lettuce for rib cracking, rib pinking, butt browning and butt pinking but the range of response was less than that observed with Iceberg lettuce.
- There was a main effect of harvest date at each location for most measures of qualitative post-harvest quality of Cos lettuce.
- Whilst rib pinking and cracking developed in Cos lettuce during storage the main effect of storage duration was only significant for the minority of locations.
- Main effect of storage duration was significant for either butt pinking or browning in Cos lettuce was significant for all locations.
- Quantitative measures of tissue colour and membrane integrity lacked sensitivity and will be removed from the assessments in Year 2.
- The Year 1 data set will be combined with the Year 2 data set before being modelled.

Knowledge and Technology Transfer

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

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Appendices



Figure A. Location of grower's field sites, 2014.