

Managing the risk of flesh browning for 'Cripps Pink' apples using a climate model

Dr Gordon Rogers
Applied Horticultural Research Pty Ltd

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Managing the risk of flesh browning for 'Cripps Pink' apples using a climate model

Gordon Rogers¹, John Golding².

¹. Applied Horticultural Research, NSW

². NSW Department of Primary Industries

Horticulture Australia
Project Number: AP08004

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Project Leader: Gordon Rogers
Applied Horticultural Research
Suite 352
1 Central Ave
Eveleigh NSW 2015

Key Personnel:
John Golding, NSW DPI
Lorraine Spohr, NSW DPI
Jenny Jobling, AHR

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This project has been funded by HAL using levy funds from the Australian apple and pear industry and matched funds from the Australian Government.

The broad objective of this project was to assess the use of using a growing degree day models (GDD) to predict the incidence of flesh browning disorder in Cripps Pink apples.

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Table of Contents

1	Media summary	4
2	Technical summary	5
3	Introduction	6
4	Material and Methods	9
4.1	Stakeholder Meeting	9
4.2	Temperature loggers	9
4.3	Harvest, storage and assessment	10
4.4	Fruit and temperature studies	11
4.4.1	Australia-wide studies.....	11
4.4.2	Regional Studies.....	12
4.4.3	Within orchard / block level studies	12
4.4.4	Within tree level study	13
4.5	Calculation of the growing day degrees	13
5	Results and Discussion	14
5.1	Australia-wide study	14
5.2	Regional study results	16
5.3	Batlow Block study - within orchard	20
5.4	Orange summary - within orchard	24
5.5	Within tree study	27
6	Conclusions	29
7	Recommendations	30
8	Communications	30
9	Acknowledgements	31
10	Appendices	32
10.1	Appendix 1: Report on the project planning meeting, Batlow December 2008. 32	
10.1.1	Australia-wide study	32
10.1.2	Regional Study.....	33
10.1.3	Within orchard / block level	33
10.1.4	Within tree level	34

1 Media summary

Flesh browning in Cripps Pink apples is a major issue for apple producers and consumers. Consignments with flesh browning can be rejected at the wholesale or retail level, representing a significant cost to Australian growers. Consumers purchasing internally browned apples will be dissatisfied and may be reluctant to buy the variety again, representing an additional loss for growers.

The previous HAL project AP04008 identified different potential causes of this disorder, including a correlation between cold growing conditions and increased internal browning. This project aimed to test and refine these predictions by recording the actual temperatures experienced by apple trees in different apple growing regions, within orchards and within trees. Fruit from each tree in the study was harvested, stored in air and then assessed for incidence of flesh browning after 8 months storage.

The overall conclusion from this study was that there is no clear relationship between radial flesh browning and growing degree days between 1173 and 1802.

It was highly likely there were factors which affect the relationship between temperature and the expression of radial flesh browning. These factors could include:

- Climatic factors such as humidity, temperature, solar radiation,
- Agronomic factors such as water stress, nutrient availability and soil type, and
- Other management factors, such as crop load.

Importantly, the results of the study do not invalidate the previous work of Jenny Jobling and Hannah James who studied the issue of flesh browning in Cripps Pink apples in detail. Their underlying work on the physiology of flesh browning and post harvest management practices to reduce expression of this disorder in storage remain valid.

The project recommends industry consider a meta analysis of other agronomic, climatic and management issues that could better explain the observed incidence of flesh browning over the regions and years studied.

2 Technical summary

Flesh browning is a postharvest disorder in Cripps Pink apples which is not evident at or before harvest, but only develops in storage. Flesh browning is a major issue for apple producers, especially in apples that are exported. Consignments with flesh browning can be rejected at the wholesale or retail level, representing a significant cost to Australian growers.

The project concept was to record the actual temperatures experienced by apple trees and relate this to the levels of flesh browning after storage. This was done across Australia, within different growing regions and orchards in NSW, and at the top and bottom of individual trees. Fruit from each tree in the study was harvested, stored in air and then assessed for incidence of flesh browning after 8 months storage.

The relationship between growing degree days (GDD, 10°C base temperature) and incidence of flesh browning was then evaluated to test the usefulness of GDD as a predictor of flesh browning.

The overall conclusion from this study was that there is no clear relationship between radial flesh browning and growing degree days between 1173 and 1802.

There was a general trend which showed that radial flesh browning was worse under cooler conditions. However, the high level of variability in the incidence of browning between sites, regions and individual trees was such that it was impossible to make any useful prediction on the likelihood of the harvested crop developing flesh browning in storage, based on GDD alone.

It was highly likely there were factors modifying any relationship between temperature and the expression of radial flesh browning in the range of GDD observed in this study. These factors could include:

- Climatic factors such as humidity, temperature, solar radiation,
- Agronomic factors such as water stress, nutrient availability and soil type, and
- Other management factors, such as crop load.

Importantly, the results of the study do not invalidate the previous work of Jenny Jobling and Hannah James who studied the issue of flesh browning in Cripps Pink apples in detail. Their underlying work on the physiology of flesh browning and post harvest management practices to reduce expression of this disorder in storage remain valid.

The data collected in this study was robust, as the development of the GDDs used the actual temperature data from the individual tree from which the fruit was harvested and stored, rather than from a local Bureau of Meteorology site.

The project recommends industry consider a meta analysis of other agronomic, climatic and management issues that could better explain the observed incidence of flesh browning over the regions and years studied.

3 Introduction

Flesh browning is a postharvest disorder in Cripps Pink apples which is not evident at or before harvest, but only develops in storage. Flesh browning is a major issue for apple producers, packers and marketers especially in apples that are exported. Consignments of apples with flesh browning can be rejected at the wholesale or retail level, representing a significant cost to Australian growers.

Project AP04008¹ investigated flesh browning in detail, and identified three separate types of flesh browning in Cripps Pink apples¹.

- *Diffuse Flesh Browning* (DFB)
- *Radial Flesh Browning* (RFB)
- *CO₂ injury*

Diffuse Flesh Browning is a chilling injury, and occurs mainly in districts or seasons where less than 1100 Growing Degree Days (GDD) above 10°C are accumulated between full bloom and harvest.

Radial Flesh Browning occurs in districts or seasons where 1100 to 1700 GDD above 10°C are accumulated between full bloom and harvest. Seasons or districts accumulating more than 1700 GDD are not at risk of developing either diffuse or radial flesh browning.

When the GDD during the season is between 1100 and 1400, Jobling and Morris¹ found that both diffuse and radial flesh browning symptoms could occur together.

CO₂ injury is not related to growing temperature, and results from storage at in atmospheres containing levels of CO₂ above 1%.

Images of the three types of flesh browning are shown in Figure 1.


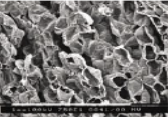

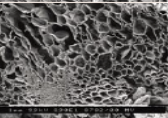

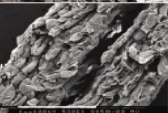
	Type	Damage observed	Physiological description	Climatic region where symptoms occur
Diffuse			Chilling Injury	Cool Regions
Radial			Senescent Breakdown	Warm Regions
CO ₂ injury			High CO ₂ Injury	All Cripps Pink apples susceptible

Figure 1 Types of flesh browning damage.

The growing degree day (GDD) thresholds referred to were developed as part of project AP04008, and the data suggested that a simple growing degree day, or heat unit model

¹ Jobling and Morris (2007) Continuation of the Flesh Browning of Cripps Pink Project to Validate Recommendations 2007. Horticulture Australia project AP04008 Final report.

could be developed and used to predict the likelihood that a given crop would be at risk of developing flesh browning in storage.

This information would be highly beneficial to growers because, if the risk of developing flesh browning can be identified as high, they can modify storage conditions and duration to prevent flesh browning developing². For example, apples which could potentially develop flesh browning could be marketed soon after harvest, before any symptoms develop.

The preliminary GDD model developed during project AP04008 showed promising trends for predicting the seasonal risk for flesh browning in Cripps Pink apples. In previous work (2003 to 2009) there was a significant relationship ($R^2 = 0.98$, $P < 0.001$) between accumulated growing degree-days in Batlow and the incidence of radial flesh browning (Figure 2). The model suggested that radial flesh browning occurs when the accumulated GDDs for the season are less than 1750.

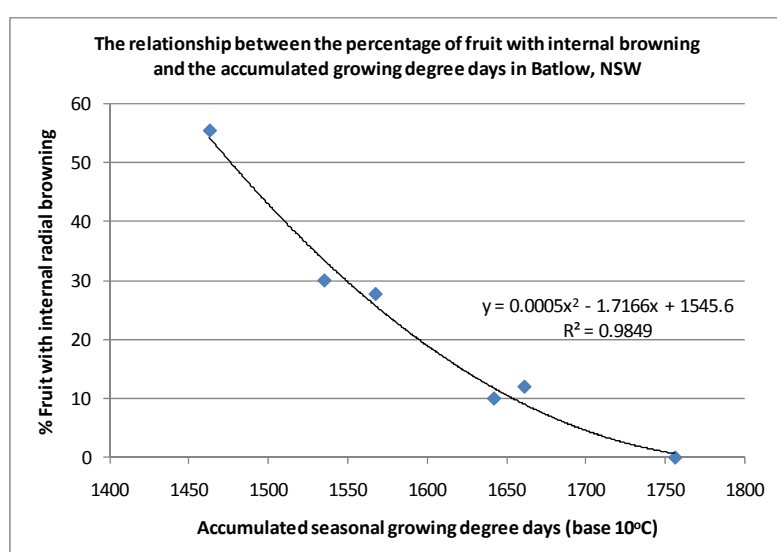


Figure 2 The relationship between the percentage of fruit with internal radial flesh browning and the accumulated growing degree-days in Batlow, NSW (2003-2009)³.

This model was based on three seasons data using base temperature of 10°C, and suggested that the incidence of flesh browning in Cripps Pink apples is strongly related to the temperature developing fruit experience during the growing season, especially from flowering to harvest. More data was required to test the theory, and to evaluate the robustness of the model over a wider range of temperature conditions during the growth cycle of Cripps Pink apples. For example, the last three seasons of project AP04008 were dry and warm, and the natural incidence of flesh browning was low.

Apple postharvest expert, Colin Little⁴ suggested weather data from a number of districts locations be collected to form a data matrix which could then be used to identify the degree

² Jobling, J. (2008) Managing the Flesh Browning Disorder of Cripps Pink Apples. A summary of Australian research investigating the causes and management of the problem. Applied Horticultural Research.

³ James, H (2007) Understanding the flesh browning disorder of 'Cripps Pink' apples (electronic resource, University of Sydney).Thesis (Ph. D.) University of Sydney, 2007.

of influence that weather conditions over one season or the cumulative effect of adverse weather conditions over two or more successive seasons might have on the development of flesh browning in Cripps Pink apples⁵. Little suggested a minimal term for such a climatological study should be seven years. Studies overseas have shown that year-to-year seasonal differences can have a significant influence on apple storability. In this project the initial duration was four years, which is less than ideal. The project was therefore extended, without additional funding in 2013 for another year. This allowed five years of data to be collected.

The project concept was to record the actual temperatures in apple trees and relate this to the levels of flesh browning after storage. This was done across Australia and within different growing regions and orchards in NSW. Fruit from each tree in the study was harvested, stored in air and then assessed for incidence of flesh browning after 8 months storage.

In this trial, we selected fruit with the greatest risk of flesh browning development. Fruit were harvested from second pick (starch plate 4), stored at low temperatures (0°C) for seven months. These conditions were the most severe to induce this disorder⁶.

The relationship between GDD (10°C base temperature) and incidence of flesh browning was then evaluated to test the usefulness of GDD as a predictor of flesh browning.

⁴ Colin Little pers comm.

⁵ Storage technology for apples and pears: a guide to production, postharvest treatment and storage of pome fruit in Australia (2000) Little, C. R. ; Holmes, R. J. 528 pp. Department of Natural Resources and Environment; East Melbourne; Australia.

⁶ Jobling, J. (2008) Managing the Flesh Browning Disorder of Cripps Pink Apples. A summary of Australian research investigating the causes and management of the problem. Applied Horticultural Research.

4 Material and Methods

4.1 Stakeholder Meeting

The first activity for the project was to hold a stakeholder meeting to plan the project. The meeting was held at the Batlow Co-op on 16 December 2008 and involved: Jenny Jobling, John Golding, Jeremy Bright and Ron Gordon to discuss and plan the project. The detailed outcome of this meeting is included as Appendix 1.

From this meeting and other discussions a detailed project plan was developed and this is described below.

The project plan was to measure the fruit growing temperatures from full bloom to harvest and related these to the levels of internal flesh browning of Cripps Pink apples after long term cold storage over five seasons (from 2008/9 through to 2012/13).

The following standard methods were used for each season.

4.2 Temperature loggers

Temperature loggers (Tiny Tag Transit, TG-0050) were encased in a double plastic open-ended sleeve to prevent direct solar radiation onto the logger Figure 3. The loggers were placed on the lowest hanging branch (about 1 – 1.5m from the ground) at full bloom and programmed to record the air temperature at 45-minute intervals until fruit harvest.

All loggers were calibrated against an ISO standard reference thermometer before each measurement season. The loggers were collected at harvest and sent to NSW Department of Primary Industries for data collection.



Figure 3 Temperature logger in plastic casing in apple tree.

4.3 Harvest, storage and assessment

One box of fruit (around 100 apples) from the tree closest to the logger was harvested at commercial maturity (starch plate = post plate 4) on the second or third pick (Figure 4). This ensured the growing degree calculation used data as close to the tree where the fruit were harvested as possible. As soon as possible after harvest, fruit were transferred to NSW Department of Primary Industries at Gosford, or Ourimbah for cold storage in a common storage facility.



Figure 4 Harvesting Cripps Pink apples from around the temperature logger before storage.



Figure 5 Storage of apple fruit in common storage facility at NSW Department of Primary Industries.

All fruit were stored in air at 0°C for eight months at NSW Department of Primary Industries (Figure 5). After long term cold storage and one week simulated shelf life at 20°C, each fruit was cut in half equatorially (Figure 6) and scored for browning using a 1 to 5 scale (1 = no browning and 5 = severe browning (Figure 7).



Figure 6 Cutting each fruit in half to assess the level of internal flesh browning.

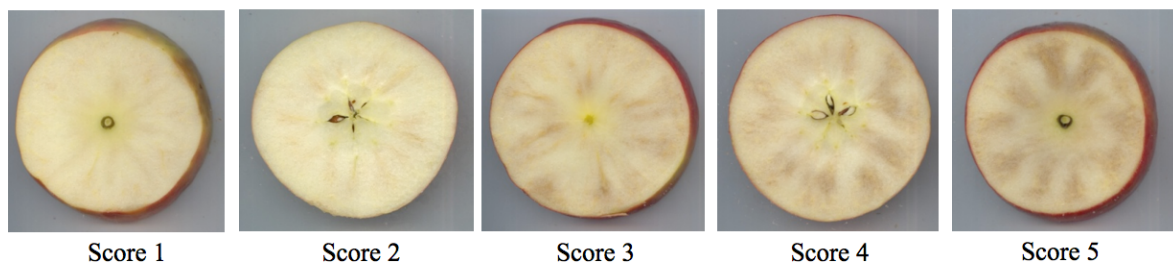


Figure 7 Internal flesh browning rating scale of Cripps Pink apples after storage.

4.4 Fruit and temperature studies

Four studies were conducted to match fruit growing temperatures to internal browning following cold storage:

1. Australia-wide
2. Regional
3. Within orchard
4. Within tree

4.4.1 Australia-wide studies

Different orchards around Australia were used to study climate and internal flesh browning after storage. The use of the particular orchards varied each year, but orchards from at least three different states were compared each season:

- New South Wales – Batlow and Orange
- Queensland – Stanthorpe
- South Australia – Lenswood
- Tasmania – Grove and Legana
- Victoria – Narre Warren and Harcourt

(Western Australia was not included due to the quarantine restrictions for transferring fruit from WA to the Eastern states as a result of Mediterranean fruit fly restrictions).

In year one, temperature loggers were not placed into the orchard until December 2008. This meant that for the period from full bloom to December 2008 in year one only, we used local Bureau of Meteorology data on temperature.

4.4.2 Regional Studies

Within an apple-growing region, different orchard blocks with known susceptibility and tolerance to internal flesh browning were compared. Four trees per orchard, each with their own temperature logger were compared. Fruit growing temperatures were measured and the fruit were harvested, stored and assessed for internal flesh browning, as described in section 4.3.

4.4.3 Within orchard / block level studies

Batlow study: The effect of increasing elevation on internal flesh browning was assessed at Batlow (NSW). Previous observations had shown the browning incidence in trees at the top the ridge was less than browning incidence in trees at the bottom of the gully. Loggers were placed in individual trees at four different locations within four adjacent rows up the slope of the hill:

- a. Bottom gully
- b. Top gully
- c. Middle ridge
- d. Top ridge

Fruit growing temperatures were measured and the fruit were harvested, stored and assessed for internal flesh browning, as described in Section 4.3. Approximately 100 fruit per tree were studied. The experimental unit was a single tree, although each individual apple from each tree was assessed for internal browning. This study was repeated over five years (2008-2009 until 2012-2013).

Orange study: Previous observations showed the browning incidence in two locations in the same orchard in Orange (NSW) were different. Trees in the back block had been observed to have less browning incidence after storage than fruit from trees near the dam. The back block was higher in elevation than the dam.

In the back block, 3 or 4 trees and approximately 100 fruit per tree were studied. In the dam location, between 7 and 12 trees were studied. The experimental unit was a single tree, although each individual apple from each tree was assessed for internal browning. This was repeated over five years (2009-2013).

GenStat (VSN, 2013) was used for all statistical analyses⁷.

⁷ VSN International (2013). GenStat for Windows 16th Edition. Hemel Hempstead, UK. VSN International

4.4.4 Within tree level study

It is well known there is natural variation within a tree in terms of fruit maturity. It was thought that this might affect individual fruit susceptibility to browning. For example, fruit at the top and external parts of the tree may experience more/less heat units and therefore have different susceptibilities to internal browning. Alternatively, earlier flowering at the bottom of the tree may mean these fruit are more mature at harvest and therefore more prone to browning. A small study was conducted to determine the variation in growing degree-days within a tree. For this study, temperature loggers were placed in two positions (top and bottom of the tree) in four trees in Batlow, NSW.

4.5 Calculation of the growing day degrees

The equation to calculate growing day degrees (GDD) was as follows:

In this trial, air temperatures were recorded every 45 minutes giving a total of 32 readings per day.

$$\text{Daily GDD} = \frac{\sum_{i=1}^{32} (\text{Temperature } i - 10^{\circ}\text{C})}{32}$$

* If Temperature i is less than or equal to 10°C , the $(\text{Temperature } i - 10^{\circ}\text{C})$ equals zero.

The seasonal GDD = \sum of all daily GDD from full bloom to harvest

5 Results and Discussion

All the flesh browning observed in this study was radial flesh browning. No diffuse flesh browning was observed. This was consistent with the findings of project AP04008⁸ which concluded that diffuse flesh browning only occurs at GDDs of less than 1100. In this study, individual tree GDDs ranged from 1173 to 1802.

5.1 Australia-wide study

The Australia-wide study measured the incidence of flesh browning from a total of 193 trees over 5 years and in 5 separate regions (Table 1 and Table 2). Approximately 100 fruit were harvested from each tree, stored and then assessed for the incidence of (radial) flesh browning. In total 17, 544 fruit were assessed and approximately 993,000 temperature readings were recorded.

There was a wide range in the observed incidence of radial flesh browning over the entire study, with the levels of flesh browning ranging from 1% to 98% (Table 1). There were also wide ranges in average GDDs, from 1242 (Orange 2012) to 1711 (Batlow, 2010) (Table 3).

There were some seasons where radial flesh browning was a significant issue, and other seasons where it was not. For example, 2009, 2011 and 2013 were bad years for flesh browning in Orange with 72%, 62% and 59% (respectively) of fruit affected (Table 1). In Batlow, 2012 was also a bad year for flesh browning with 46% of the fruit affected (Table 1).

There were some weak, localised correlations between GDD and the incidence of flesh browning, but they were the exception rather than the rule.

For example, 2012 and 2013 were both cold years in Batlow (1381 and 1324 GDD respectively). The incidence of flesh browning was high in these years (29% and 46%) which is consistent with the idea that browning is worse in cold years. However, 2010 was a very warm year (GDD = 1711) and yet there was still 22% flesh browning (Table 1 and Table 3).

In contrast, 2012 was a cold year in Orange (GDD = 1242) and yet the incidence of flesh browning in that year was only 15%. In 2013 flesh browning in Orange was severe (59%) even though this was a warm year (GDD = 1579). (Table 1 and Table 3).

In summary, the results suggest that while there were large seasonal and regional differences in the expression of radial flesh browning in Cripps Pink apples, this expression does not appear to be closely related to GDD in the range experienced by the trees in this survey.

This lack of correlation between GDD and radial flesh browning can be readily seen when mean GDD and flesh browning incidence across regions and seasons are plotted together. Figure 8 shows this relationship over all sites and years (2009-2013) and clearly there is no consistent trend in the data.

⁸ Jobling and Morris (2007) Continuation of the Flesh Browning of Cripps Pink Project to Validate Recommendations 2007. Horticulture Australia project AP04008 Final report

Table 1. Percentage of apples with internal browning across all regions over 5 years.

Year	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
District					
Batlow	11.9 ± 5.5	21.5 ± 8.4	29.4 ± 9.5	46.0 ± 10.2	16.2 ± 7.7
Orange	72.4 ± 11.2	-	62.0 ± 14.6	15.2 ± 11.4	59.0 ± 14.8
Qld	-	86.52	2.7	98.48	21.12
SA	-	1.03	79.17	62.5	30.77
Tas	53.2 ± 35.3	-	-	-	-
Vic	37.2 ± 34.2	19.33	27.27	1.56	67.5

The values are means ± standard error.

Table 2. Number of trees assessed.

Year	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
District					
Batlow	35	24	23	24	23
Orange	16	0	11	10	11
Qld	0	1	1	1	1
SA	0	1	1	1	1
Tas	2	0	0	0	0
Vic	2	1	1	1	1

Table 3. Growing degree days across all regions over 5 years.

Year	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
District					
Batlow	1632 ± 14	1711 ± 14	1381 ± 14	1324 ± 16	1446 ± 19
Orange	-	-	1339 ± 8	1242 ± 16	1579 ± 20
Qld	-	1691	1575	1472	1548
SA	-	1684	1251	1550	1489
Tas	-	-	-	-	-
Vic	-	1641	1355	1433	1570

The values are means ± standard error.

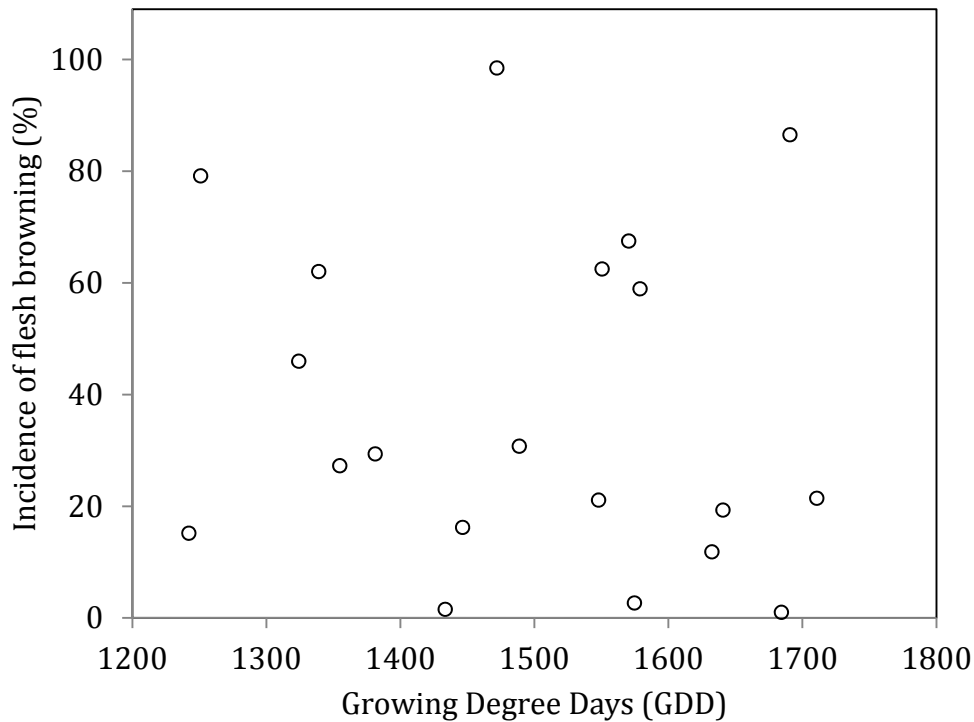


Figure 8. Relationship between the incidence of flesh browning and growing degree days over all sites and years (2009-2013).

5.2 Regional study results

This aspect of the study investigated possible relationships between GDD and flesh browning by season and within growing region.

Figure 9 shows the GDD and incidence of flesh browning present by year, with the different sites identified by the shape of the data points. The differences in GDD can be clearly seen, and are due to season temperatures being either warmer or cooler. In some cases, years are consistently warm over all regions (e.g. 2010) and in other cases regional differences are more important, and so the GDDs are more spread out (e.g. 2013).

Across regions the 2009 and 2010 seasons were generally warm, 2011 and 2012 were cold, 2013 was somewhere between. In each of the seasons, the mean flesh browning incidence ranged from close to 0% to between 80-100%. More importantly, no strong relationship between GDD and flesh browning could be identified (Figure 9). There are some weaker trends e.g., 2009 was a warm year and the incidence of browning was low, but this trend was not consistent with other years (Figure 10).

If the data from individual sites are pooled, so that a single point represents one region for each season, then some trends start to emerge (Figure 11). The broad trend in the data supports the idea that the incidence of flesh browning is higher in cooler weather (between GDDs of 1242 and 1711). However, the very high level of variability in expression of flesh browning at the tree level means that calculations of GDD cannot be reliably used to guide management of the harvested crop.

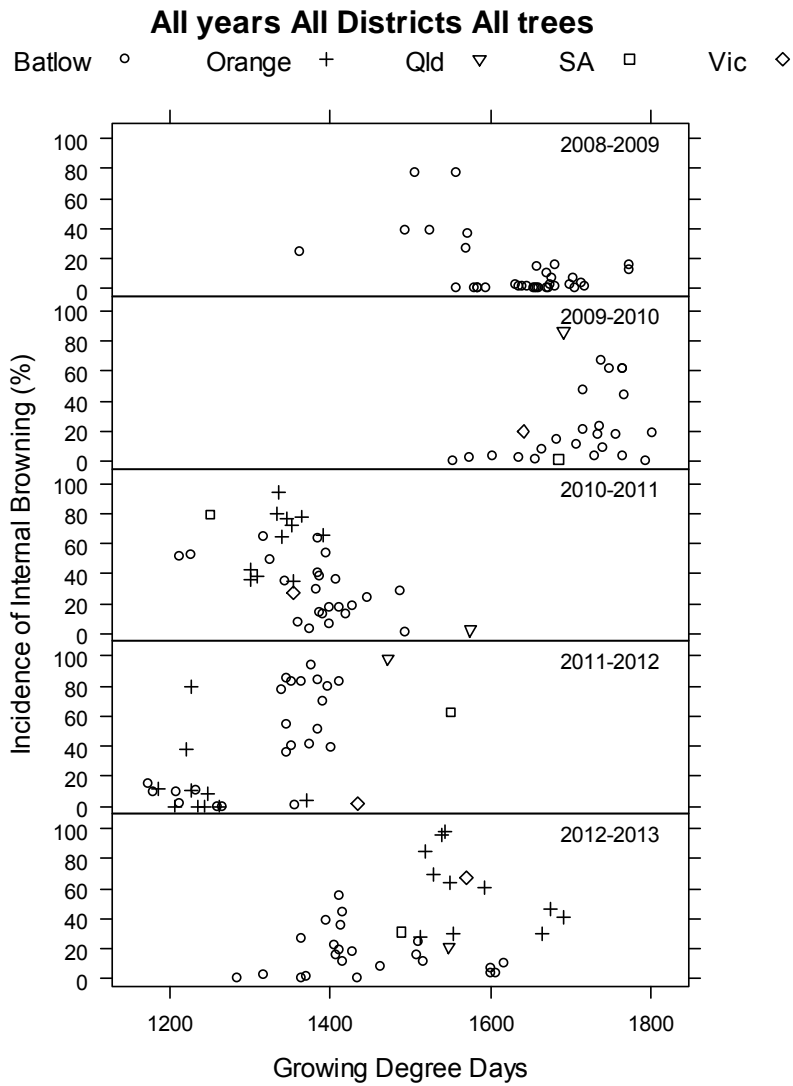


Figure 9. Relationship between the incidence of flesh browning and Growing Degree Day (GDD) and 2008-9 to 2012-13 across different regions in Australia.

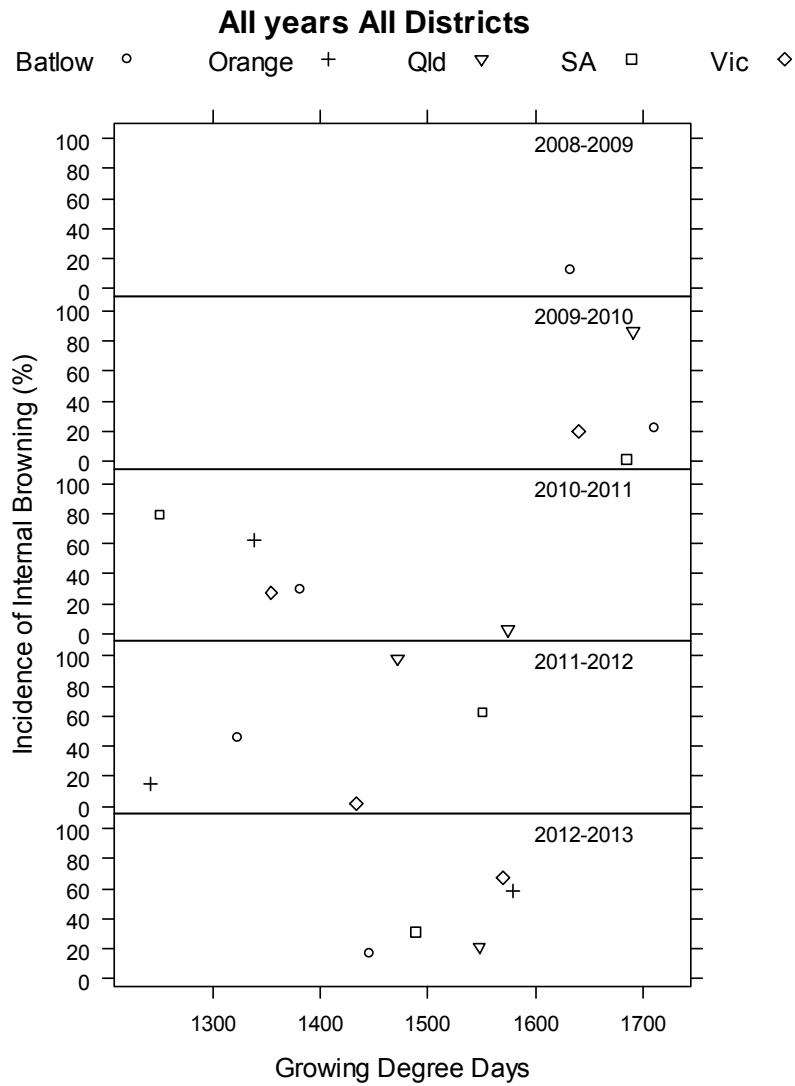


Figure 10. Relationship between the incidence of flesh browning and Growing Degree Day (GDD) and 2008-9 to 2012-13 for each district.

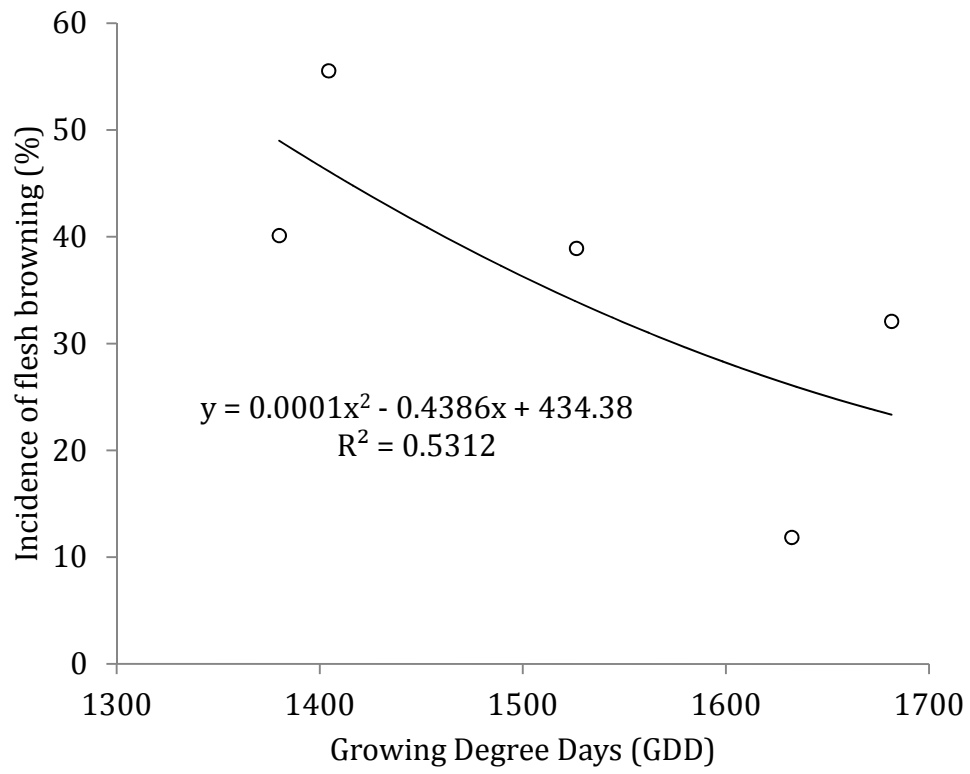


Figure 11. Relationship between seasonal mean incidence of flesh browning and seasonal mean GDD.

5.3 Batlow Block study – within orchard

The purpose of this within orchard study was to compare different locations within a single orchard at Batlow, NSW, measure GDD and the incidence of flesh browning at each location, then look for any relationships. Preliminary observations suggested there were significant differences in the incidence of flesh browning within a single orchard block by year. The four locations were chosen based on elevation, the idea being these locations would provide a range of temperatures and expected incidence of flesh browning. The locations chosen were:

- **Bottom gully**
- **Top gully**
- **Middle ridge**
- **Top ridge**

There were significant differences in the incidence of flesh browning, but only in 2012, and then only between the top of the ridge in the middle of the ridge (Table 4). In 2012 there were no significant differences in GDD between any of the locations (Table 5). There were some minor significant differences in GDD at different locations in 2009 and 2013, but in those years, there were no differences in flesh browning.

The major differences in GDD and flesh browning were between **years**, not between **locations** within the orchard. The 2009 and 2010 seasons at Batlow were warm and the other years were cold. The 2009 season did have a low incidence of flesh browning but in 2010 (a warm year), 31% of fruit were affected by flesh browning (Table 4).

The level of browning in 2011 was low (19%) and in 2012 it was high (65%), yet the GDD for both of these years at Batlow were similar.

Figure 12 shows the relationship between GDD and browning over the 5 years of the study at Batlow, and the whole data set is shown together in Figure 13. Both figures clearly show there is no relationship between GDD and radial flesh browning.

It is very likely there were significant modifying factors, which were responsible for the differences observed in flesh browning between sites and seasons. Possible factors include water stress, soil type, nutrient availability or a climatic factor other than temperature.

Statistically, there was no evidence that internal browning was affected by elevation in 2009, 2010 or 2013 (Table 4). There was weak evidence to suggest an effect in 2011 ($P=0.072$) and stronger evidence in 2011-2012 ($P=0.026$).

For each year, the large variation (residual mean deviance) in the proportion of apples with browning between trees at the same elevation resulted in very high extra-binomial variation (Table 4). Under the binomial distribution we expect a residual mean deviance close to 1. For each year, analysis of variance was used to test whether GDD differed between the 4 elevations (Table 5).

Statistical approach: For each year, a generalised linear model with quasi-binomial family and logit link function was used to test the effect of elevation on the proportion of apples with internal flesh browning. Use of the quasi-binomial family, as opposed to the binomial distribution, allowed for greater variation in browning incidence between trees (over-dispersion) than one would expect under a binomial distribution. Comparisons between proportions of apples with internal flesh browning at the four locations were made on the logit scale at the 5% significance level and back transformed to percentages for presentation.

Table 4. Effect of location within an orchard on internal browning Batlow, NSW.

Year	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
Bottom gully	4.53	36.92	23.46	65.75 ab	10.25
Top gully	4.02	13.65	29.89	66.12 ab	33.34
Middle ridge	1.26	41.36	9.55	46.11 b	25.40
Top ridge	0.33	35.79	13.64	85.51 a	19.34
Approximate F probability	0.393	0.293	0.072	0.026	0.245
Residual mean deviance	6.306	15.53	6.672	8.293	15.25

Back transformed predicted means with different letters within columns are significantly different ($P < 0.05$).

Table 5. Effect of location within an orchard on growing degree days (GDD), Batlow, NSW.

Year	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
Bottom gully	1684 b	1737	1424	1366	1429 bc
Top gully	1664 ab	1730	1397	1368	1412 ab
Middle ridge	1649 a	1749	1378	1378	1365 a
Top ridge	1686 b	1756	1440	1371	1482 c
F probability	0.048	0.651	0.075	0.915	0.014

Means with different letters within columns are significantly different ($P < 0.05$).

Batlow Block Survey

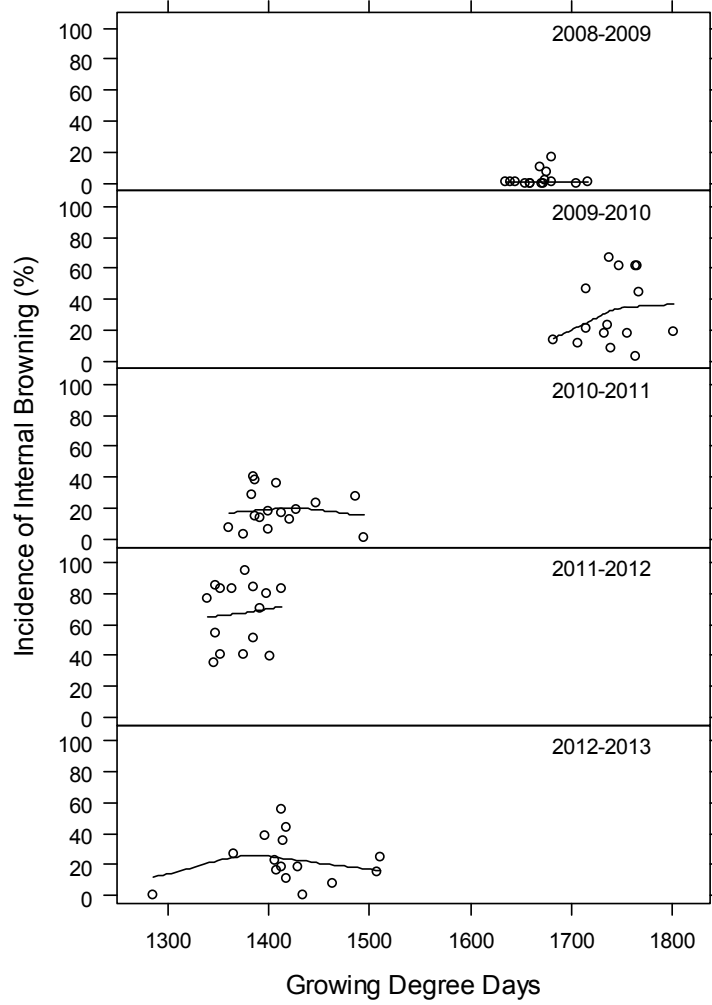


Figure 12. Relationship between the incidence of flesh browning and Growing Degree Day (GDD) and 2008-9 to 2012-13 in Batlow, NSW.

Batlow Block Survey

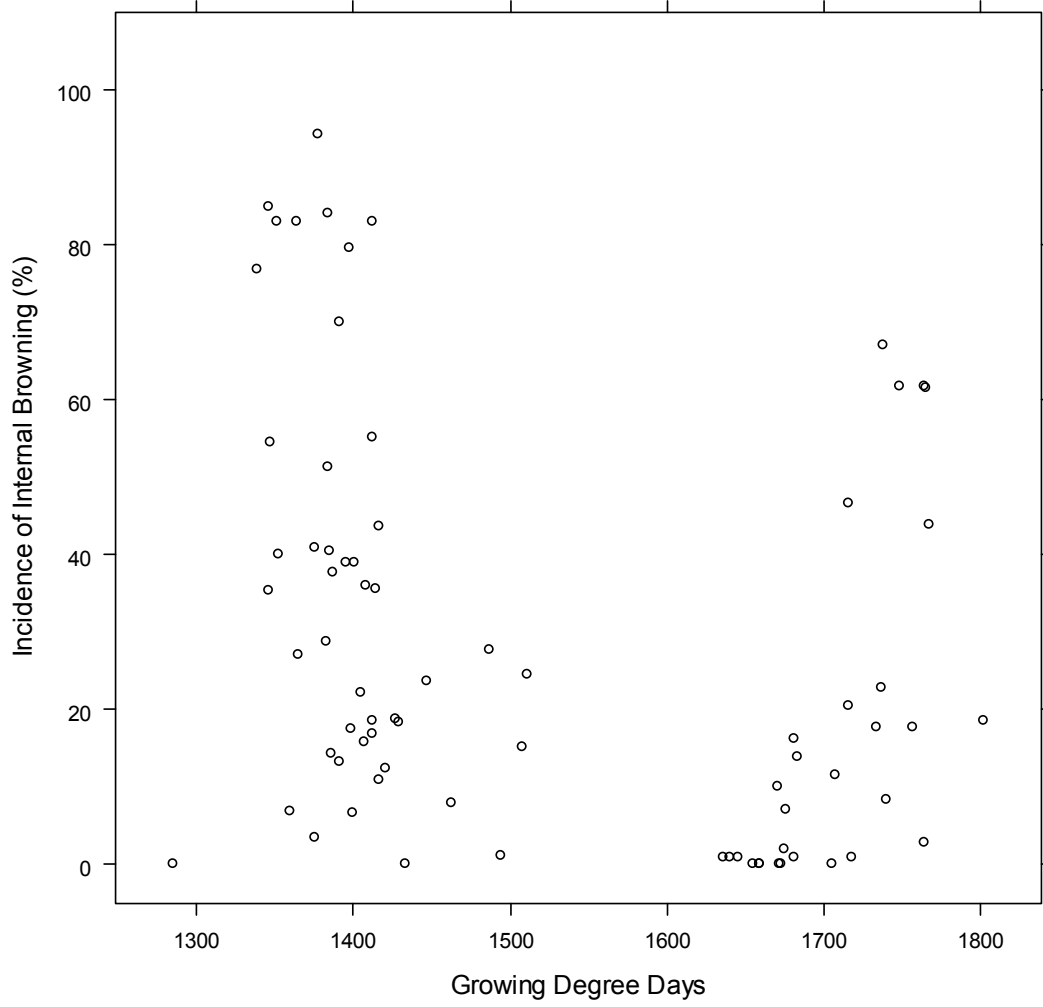


Figure 13. Relationship between the incidence of flesh browning and Growing Degree Day (GDD) from 2008 to 2013 in Batlow, NSW.

5.4 Orange summary – within orchard

Previous observations have shown that two locations within a single orchard block in Orange (NSW) had different predispositions to develop flesh browning in storage, where fruit from the back block generally had low / no flesh browning, while fruit from the dam block had higher levels of flesh browning after storage.

There was a significant difference in GDD in 2013 between these two locations. The dam was cooler than the back block (Table 7). However there were no corresponding differences in the incidence of flesh browning (Table 6).

There were significant differences in flesh browning in 2009 ($P < 0.001$) and 2011 ($P = 0.026$). For each year, the large variation (residual mean deviance) in the proportion of apples with browning between trees at the same location resulted in very high extra-binomial variation (Table 7). Under the binomial distribution we expect a residual mean deviance close to 1.

In both cases fruit from the dam had a significantly higher level of flesh browning than fruit from the back block (Table 6). These differences were not related to GDD, again suggesting other agronomic, climatic or environmental factors may be responsible for these observed differences in flesh browning.

Seasonal differences in GDD were clear (Figure 14) but as for the other studies, there was no significant correlation between these differences in GDD and flesh browning (Figure 14 and Figure 15).

Statistical approach: A preliminary analysis suggested a significant interaction existed between treatment and year. For each year, a generalised linear model with quasi-binomial family and logit link function was used to test the effect of location on the proportion of apples with internal flesh browning. Use of the quasi-binomial family, as opposed to the binomial distribution, allowed for greater variation in browning incidence between trees (over-dispersion) than one would expect under a binomial distribution. Comparisons of proportion of apples with internal flesh browning between locations were made on the logit scale at the 5% significance level and back transformed to percentages for presentation. For each year when data was available, analysis of variance was used to test whether GDD differed between the two locations.

Table 6. Effect of location within an orchard on internal browning Orange, NSW

Year	2008-2009	2009-2010	2010-2011	2011-2012[#]	2012-2013
Location					
Back block	26.55 a	-	44.60 a	0	39.14
Dam	87.70 b	-	71.97 b	21.70	66.38
Approximate F probability	<0.001	-	0.026	-	0.243
Residual mean deviance					
	11.44	-	8.691	-	29.9

Back transformed predicted means with different letters within columns are significantly different ($P<0.05$).

[#] Data was not complete and therefore not analysed.

Table 7. Effect of location within an orchard on growing degree days (GDD) Orange, NSW.

Year	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
Location					
Back block	-	-	1337	1246	1677 b
Dam	-	-	1340	1240	1544 a
F probability	-	-	0.880	0.872	<0.001

Means with different letters within columns are significantly different ($P<0.05$).

Orange Survey

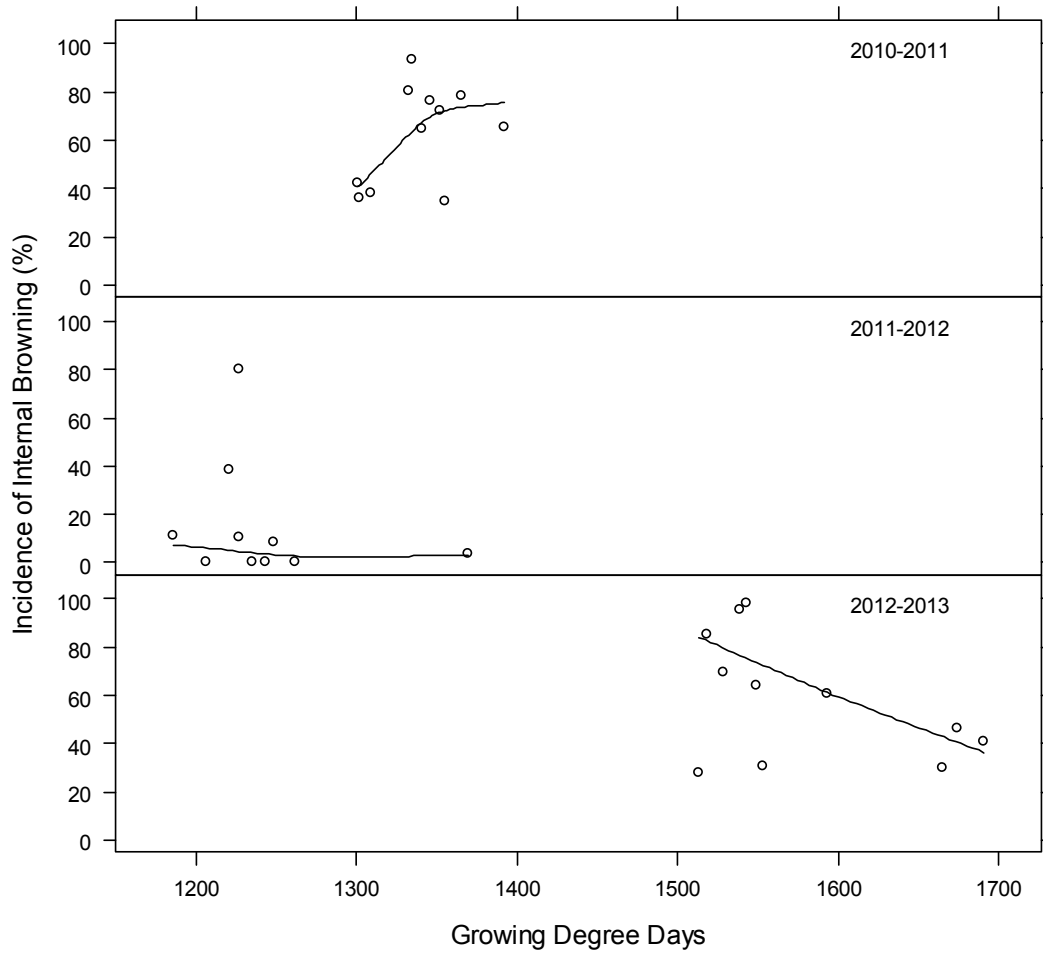


Figure 14. Relationship between the incidence of flesh browning and Growing Degree Day (GDD) from 2010-11 to 2012-13 in Orange, NSW.

Orange Survey

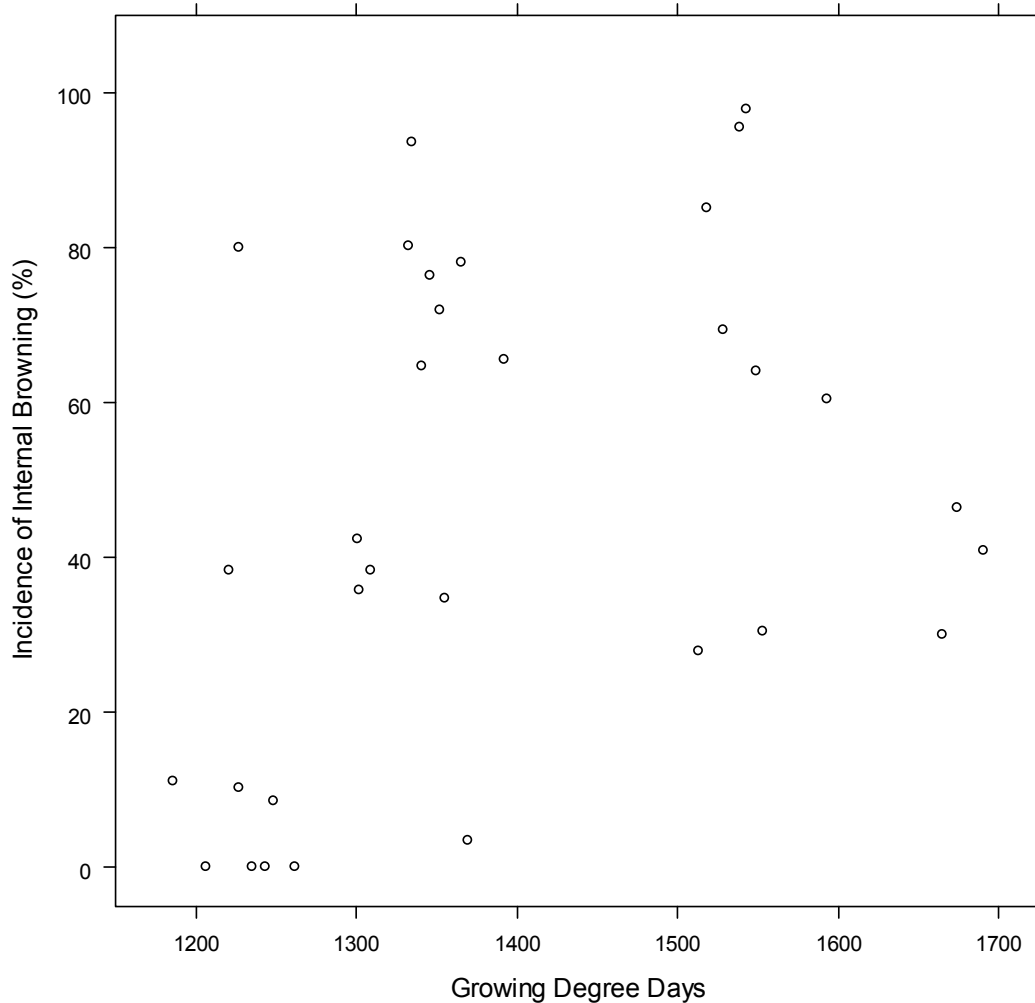


Figure 15. Relationships between the incidence of flesh browning and Growing Degree Day (GDD) and from 2010-11 to 2012-13 in Orange, NSW.

5.5 Within tree study

The within tree study showed the average temperature during the growing period was less at the base of the tree compared to the top of tree, i.e. the GDD at the bottom was 1662 compared to 1740 at the top (Table 8). The incidence of flesh browning was slightly lower at the bottom of the tree, inconsistent with the idea that radial flesh browning is more likely to occur below 1700 GDD (Table 8). In addition, Figure 16 does not show a clear correlation between GDD and flesh browning within the tree.

Given the lack of supporting evidence on the relationship between GDD flesh browning in the orchard and regional level studies, it is difficult to be confident there is a link between GDD and the small difference in flesh browning within trees in this study.

Table 8. Preliminary data on the incidence of radial flesh browning relative to position in a tree in Batlow, NSW (2008-9). Different letters denote a significant difference between positions.

Position in the Tree	GDD	% Fruit with internal browning
Batlow tree top	1740	8.95a
Batlow tree bottom	1662	5.44b

Means with different letters within columns are significantly different ($P < 0.05$).

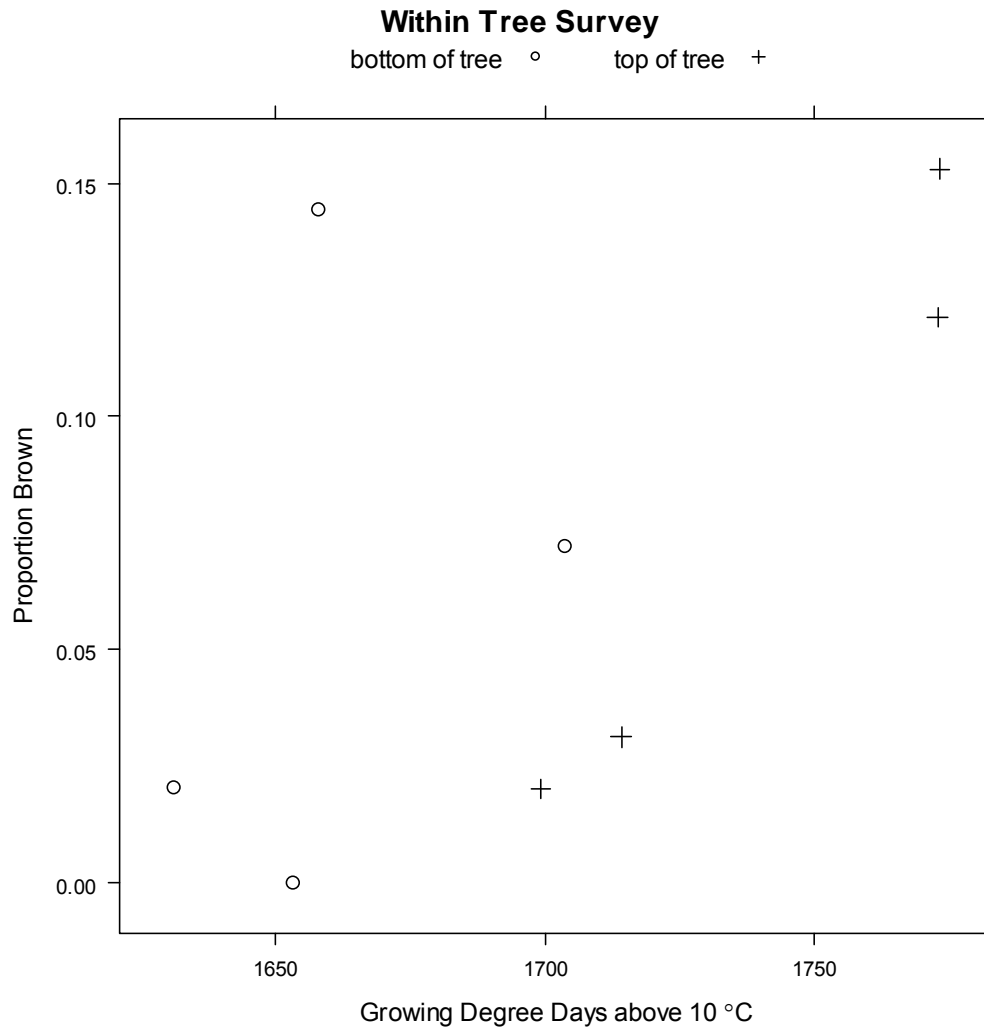


Figure 16 Relationship between the incidence of flesh browning and Growing Degree Day (GDD) for the within tree study undertaken in 2009 at Batlow, NSW.

6 Conclusions

The data collected in this study was robust, as the development of the GDDs used the actual temperature data from the individual tree from which the fruit was harvested and stored, rather than from a local Bureau of Meteorology site.

The overall conclusion from this study is that there is no clear relationship between radial flesh browning and growing degree days in the range 1173 to 1802 GDD.

There was an overall trend which showed that radial flesh browning was generally worse under cooler conditions. However, the high level of variability in the incidence of browning between sites, regions and individual trees was such that it was impossible to make any useful prediction on the likelihood of the harvested crop developing flesh browning in storage, based on GDD alone.

It was highly likely there were other factors modifying any relationship between temperature and the expression of radial flesh browning in the range of GDD observed in this study.

These factors could include:

- Climatic factors such as humidity, temperature, solar radiation
- Agronomic factors such as water stress, nutrient availability and soil type
- Other management factors, such as crop load

A review of the literature on the topic has revealed one of the paper by South African group mainly focused on diffuse flesh browning⁹. They raised the possibility of other factors being responsible for the expression of flesh browning.

Importantly, the results of the study do not invalidate the previous work of Jenny Jobling and Hannah James who studied the issue of flesh browning in Cripps Pink apples in detail. Their underlying work on the physiology of flesh browning and post harvest management practices to reduce expression of this disorder in storage remain valid.

Jobling and Golding suggested that GDD could possibly be used to predict radial flesh browning in Australian apples. The purpose of this study was therefore to collect sufficient data to say whether GDD was a realistic tool for the prediction of flesh browning in warmer regions in Australia. The overall conclusion therefore is that GDD alone is not a sufficiently sophisticated tool to predict the likely expression of flesh browning in Cripps Pink apples.

If the industry wishes to further investigate this issue, additional work could be done on looking for correlations between the incidence of internal flesh browning and other climatic soil agronomic and other factors. This additional work was beyond the scope of this project.

⁹ Bergman, H. et al. (2012) Update on the possible causes and management strategies of flesh browning disorders in 'Cripps Pink' apples. SA Fruit Journal, Feb/March, pp 56-59.

7 Recommendations

Consider a meta analysis of other agronomic, climatic and management issues that could better explain the observed incidence of flesh browning over the regions and years studied.

8 Communications

1. A presentation entitled *Managing the risk of flesh browning for 'Cripps Pink' apples using a climate model* was presented at the Research Review, speed updating workshop held at the Gold Coast, 17th July, 2013 by Gordon Rogers and John Golding.
2. A two page summary of the 2008/09 seasonal risk relative to 20 year average growing degree days has been prepared and this has been sent to Andrew Dick and Neil Offner to post on the 'Pink Lady Australia' website for all Pink Lady growers to access. (Attached as appendix).
3. Stephen Tancred prepared a seasonal weather prediction data set for growers as well using our model when he did a series of talks for the 2012 Orchard walk series.
4. A summary of the 2009/10 seasonal risk relative to 20 year average growing degree days has been prepared (see document below) and this was been sent to Andrew Dick and Neil Offner to put in the 'Pink Lady Australia' newsletter for all Pink Lady growers to access.
5. Internal Browning Update for APAL Newsletter March 2010 – Dr Jenny Jobling and Dr John Golding. AP08004 – Flesh Browning Risk for Pink Lady apples 2010.
6. The project was discussed with researchers at the 7th International Postharvest Conference in Malaysia (June 2012). Detailed discussions were held with Dr. Angelo Zanella (Laimburg Research Centre for Agriculture and Forestry in Italy) and Dr. Juan Pablo Zoffoli (Pontificia Universidad Católica de Chile).
7. Annual project summaries have been written for the HAL industry annual report.

9 Acknowledgements

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- Matt McMahon and Ron Gordon, Batlow Fruit Co-operative, Batlow New South Wales
- The Pearce family, Orange New South Wales
- Steve Tancred, Orchard Services, Stanthorpe Queensland
- Don and Robyn Nightingale, Nightingale Bros. Wandiligong Victoria
- John Plummer, Plummers Border Valley Orchards, Lenswood South Australia
- Andrew Smith, Grove Tasmania
- Chris Peters, Narre Warren North Victoria

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NSW Department of Primary Industries provided on-going support and significantly contributed to the outcomes of this project

10 Appendices

10.1 Appendix 1: Report on the project planning meeting, Batlow December 2008.

Outline and Project plan: *Managing the risk of flesh browning for 'Cripps Pink' apples using a climate model (HAL AP08004)*

Jenny Jobling, John Golding, Jeremy Bright + Ron Gordon met at the Batlow Co-op on 16 December 2008 to discuss and plan this project.

This project aimed to develop the preliminary climatic model to account for the seasonal weather conditions which may have a large role in determining the seasonal incidence of flesh browning in Cripps Pink apples.

There are four levels of investigation in this project. The possible link between climate and internal browning at;

- (1) the national level (across states and growing regions in Australia) [Australia],
- (2) within a single growing region [regional],
- (3) within a single orchard / block [block], and
- (4) within a single tree [within tree].

10.1.1 Australia-wide study

Five sites (orchards) around Australia will be used to study climate and internal browning after storage:

1. Tasmania
2. Victoria
3. Victoria
4. Batlow, New South Wales
5. South Australia

Data loggers will be placed in trees at the lowest hanging branch (about 1 – 1.5m from the ground). The 'Tiny Tag Transit' loggers are protected with two plumbing pipes and programmed to record the temperature at half hourly intervals until up to fruit harvest. But as we are starting measuring temperatures half way through growth, we will have to use BOM data to get the first part of growth for this season (pre-December).

We want to collect fruit from the same tree to ensure the exact DDG experienced by the fruit. This fruit will be sent to Gosford for storage under similar storage conditions (0°C in air). After 7 months storage, cut open and assess for internal browning. We will have 2 complimentary scoring systems for internal browning (Binary data = yes or no browning, and 1-5 scale of browning and type of browning). We will only collect fruit from the second (or third pick).

At each of the five sites:

Harvest 100 fruit (1 box) at harvest stage post plate 4 and send to Gosford (NSW DPI).

10.1.2 Regional Study

Within two apple-growing regions (Batlow and Orange), look at different blocks with known susceptibility and resistance to internal browning.

x 1 harvest time (all fruit will be second pick fruit (post plate 4)

x 2 growing regions (Batlow and Orange)

x 2 internal browning susceptibilities (no browning and prone to browning)

x 4 loggers / tree

TOTAL 16 loggers / tree

Loggers were placed in trees in Batlow on 16 December 2008. Four loggers were placed in individual trees in adjacent rows at two grower properties (Casey = no history of browning, and Sheen = lots of previous internal browning).

Jeremy Bright to do the same at two grower properties in the Orange district. (Farmer A = no internal browning and Leonie = lots of internal browning)

At harvest, collect 100 fruit from each of the logger tagged trees. This fruit will not have any postharvest treatments (ie no SmartFresh or DPA) and will be centralised and stored under commercial CA conditions at the Batlow Co-op with no SmartFresh or DPA. The fruit will be gathered into one storage lot then all stored in the same room, with step-wise cooling and regular commercial CA.

10.1.3 Within orchard / block level

Jenny Jobling observed a block on the side of the hill in a block of James Oag's Pink Lady apples that had a different propensity for internal browning. Loggers were placed in individual trees at four different locations within four adjacent rows up the slope of the hill:

- a. Top of the hill (tree 3 from the road)
- b. Top – mid of the hill (tree 18 from the road)
- c. Bottom – mid of the hill (tree 33 from the road)
- d. Bottom of the hill (tree 48 from the road)

x 4 orchard positions

x 4 rows

TOTAL 16 treatment units / temperature loggers

At harvest, 100 fruit from each tree (x 16) with the logger will be stored (no SF or DPA) in CA at Batlow Co-op. The fruit will be stored in plastic orange crates to be supplied by NSW DPI. The fruit will be later assessed for internal browning.

10.1.4 Within tree level

What variation in GDD exists within a single tree? Do fruit at the top and external parts of the tree experience more heat units and therefore have different susceptibilities to internal browning. Fruit at the bottom of the tree flower earlier and are more mature.

x 2 tree positions (top and bottom of the tree)

x 4 trees

Loggers were placed at the top and bottom of four adjacent James Oag's Pink lady trees (2 trees in 2 adjacent rows) on 16 December 2008.

Other fruit / orchard assessments on trees with loggers

We should measure / estimate and record:

- crop load (number of fruit per tree)
- Tree vigour
- Water use (neutron probe in 3 locations in those trees in Row 5 at Oag's Pink Lady block)