Development of soft scald in ‘Scifresh’/Jazz™ apples

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Research Scientist                                                      Group Leader, Quality Systems

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EXECUTIVE SUMMARY

Development of soft scald in ‘Scifresh’/Jazz™ apples

Report to ENZA Limited

Brookfield PL, Johnston J, Farrell M, Bowen J.

October 2008

Soft scald is visible as a brown discoloration of the apple skin and can amount to substantial losses in affected lines of fruit. There is currently a lack of knowledge of the orchard factors that predispose the fruit to developing the disorder and of the biology involved in the expression of the disorder. The aim of this study was to investigate the possible role of oxidative stress in the development of soft scald in ‘Scifresh’ apples and to explore opportunities for developing predictive tools for identifying high risk lines of fruit at harvest.

The success of this study depended on obtaining and analysing lines of fruit with contrasting incidence of soft scald. To achieve this, ‘Scifresh’ apples were harvested at two stages of maturity from three commercial orchards, two of which were chosen as high risk and one as low risk, based on their history of soft scald incidence.

Key results:

• This study was successful in obtaining contrasting incidence of soft scald in different lines of fruit. One orchard had very high incidence (23-35%) of soft scald, while the other two orchards developed low to nil incidence (0-2%)
• Higher ethane emission at harvest was associated with higher incidence of soft scald in storage
• Lower antioxidant activity at harvest was associated with higher incidence of superficial scald after storage
• Total phenolic concentration showed no relationship with incidence of either disorder. Further research will be undertaken to identify the sub-classes of phenolic compounds for each orchard, as those important for disorder incidence may have been masked in the total phenolic assay.

Conclusions:

• There is correlative evidence that oxidative stress is associated with incidence of soft scald and superficial scald in ‘Scifresh’ apples. Studies with antioxidant applications (in the absence of apple waxing formulations) are required to strengthen this conclusion
• Ethane emission at harvest may have potential for predicting soft scald risk for ‘Scifresh’ apples.

Recommendations:

• Validate ethane emission as a predictor of soft scald based on a larger number of orchards. There is a possibility that this season’s result derived from three orchards could have occurred by chance
• Evaluate chlorophyll fluorescence as a non-destructive technology for detecting skin properties/defects not visible at harvest, which may subsequently progress into soft scald once in storage
• Preliminary studies show that 1-methylcyclopropene and controlled atmospheres can reduce expression of soft scald in ‘Scifresh’ apples. Some operators may opt to apply these technologies to ‘Scifresh’ apples as a standard practice but the commercial efficacy...
in disorder control are not known. Therefore, the response of ‘Scifresh’ apples to these technologies should be assessed, in order to develop appropriate commercial protocols.

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INTRODUCTION

‘Scifresh’ apples are susceptible to soft scald, a disorder that is visible as a brown discoloration of the apple skin and can amount to substantial losses in affected lines of fruit. Because susceptible fruit do not show the disorder until during storage, at risk fruit enter the distribution chain and may eventually erode the reputation of ‘Scifresh’ as a premium quality variety. Research has already identified some preliminary practices for minimising postharvest risk in susceptible lines, but there is a lack of understanding of the factors and processes involved in soft scald development that could enable identification of affected lines for appropriate treatment. The unpredictable nature of this disorder arises from a lack of knowledge of the orchard factors that predispose the fruit to developing the disorder, as well as of the biology involved in the expression of the disorder. The current practice for soft scald management involves keeping high risk lines of fruit on-shore for 6-8 weeks, to identify those that express the disorder. However, this approach is not sufficient for keeping affected fruit out of the distribution chain, because risk assessment is based on historical orchard block occurrence, which is not reliable for identifying all at-risk lines, and in some cases, expression of the disorder can still occur later in storage after fruit have been shipped off-shore.

Studies indicating the disorder is expressed in fruit at 0°C but not at 3°C provide evidence that the disorder is low temperature-related. Similarly, low temperature conditioning of fruit for 4 weeks at 3°C before transfer to 0°C has reduced the incidence of soft scald, although it is not known whether this involves an induced increase in cold-tolerance, an enhanced ripening response, a physical consequence of increased water loss, or combination of these and other factors.

Oxidative stress is considered to have an important role in the cold sensitivity of plant tissues (Mittler 2002; Suzuki & Mittler 2006). Oxidative stress can result in damage to lipids (through lipid peroxidation), DNA and proteins, and can ultimately lead to cell death (Mittler 2002). Anecdotal evidence suggests soft scald could also arise through oxidative stress, since expression of the disorder is reduced by the application of commercial apple waxes containing wax-stabilising antioxidants (Brookfield et al. 2006). The supplementation of these waxes with antioxidant-rich apple extracts further reduced incidence, suggesting that susceptible fruit may have insufficient antioxidants in the skin to prevent scald development during postharvest low temperature storage. However, this concept needs verification using antioxidant applications in the absence of antioxidant-containing commercial waxing formulations. Storage in controlled atmospheres and 1-methylcyclopropene application has also been shown to suppress expression of soft scald (Brookfield et al. 2006). The mechanisms involved are not known; however, any treatment that lowers the respiratory activity of the fruit could reduce the rate of free radical production. Free radical production occurs during respiratory metabolism and has an important role in cell signalling (Mittler 2002); oxidative stress occurs when this production (often in bursts) exceeds the antioxidant capacity of the tissue. If oxidative stress is associated with expression of soft scald, it would be expected that susceptible orchards have fruit with a high respiratory activity and/or a low antioxidant activity.

The aim of this study was to investigate the possible role of oxidative stress in the development of soft scald in ‘Scifresh’ apples and to explore opportunities for developing predictive tools for identifying high-risk lines of fruit at harvest. Although there are numerous techniques available to evaluate oxidative stress in plant tissues, many are time-consuming and require complex equipment. Those used in this study are considered to be the most rapid and simple of the options available, although they measure holistic changes rather than
changes in individual compounds. This relative simplicity was considered to be an important component of this study, in that any positive outcomes could be more readily and rapidly applied commercially to predict the risk of soft scald incidence for different lines of fruit.
MATERIAL AND METHODS

FRUIT SOURCE AND TREATMENTS

Two ‘Scifresh’ orchards with a previous history of soft scald incidence and one without were used in an attempt to provide two plantings of high risk and one of low risk for soft scald, respectively. All orchards were located in Hawke’s Bay. One high risk orchard, located near Highway 50 to the south west of Hastings, was six years old and planted on ‘M.9’ (dwarfing) rootstock (Orchard 1). The other was located in Twyford Rd to the west of Hastings and was three years old from grafting onto a ‘MM.106’(semi-dwarfing)/‘M.9’ interstem rootstock that had previously been worked to ‘Scired’ as the scion (Orchard 2). The low risk block, located in Lawn Rd to the north east of Hastings, was seven years old and planted on ‘M.9’ rootstock (Orchard 3).

At each orchard, five rows of trees were allocated as five replicates for fruit sampling. Pre-harvest fruit development was monitored by assessment of harvest maturity characteristics two weeks (21 February) and one week (28 February 2008) before anticipated harvest. At each orchard, fruit for the study were harvested evenly by proportion from each replicate on 5 and 17 March, corresponding to low and high harvest maturity based on harvest starch pattern index (SPI), being ~ 2.5 (low maturity) and ~ 4 (high maturity), respectively. At each harvest, the fruit from each orchard were used as follows:
1. Twenty fruit (4 fruit per replicate) were assessed for harvest maturity characteristics at harvest
2. Three hundred fruit (60 fruit per replicate) were assessed for incidence of disorders after 7 days at 20°C following 16 weeks of cool storage at 0°C
3. One hundred and sixty fruit (32 fruit per replicate) were assessed for incidence of soft scald after 1 day at 20°C following cool storage at 0°C and at 3°C for 12 weeks
4. Twenty fruit (4 fruit per replicate) were assessed for skin ethane and ethylene production at harvest and after 2, 4, 6 and 12 weeks of cool storage at 0°C and at 3°C
5. Twenty fruit (4 fruit per replicate) were assessed for skin total phenolic concentration and total antioxidant activity at harvest and after 4 and 12 weeks of cool storage at 0°C and at 3°C.

HARVEST INDICES

The following measurements were made at each harvest on 20 fruit per orchard:
1. Fruit weight (g)
2. Background colour, using a HortResearch colour chart, where 1 = green and 10 = yellow
3. Red colour coverage (%)
4. Internal ethylene concentrations (IEC) (µL/L), measured from a 1-mL sample of gas drawn from the cortical cavity of each fruit using a Hewlett Packard 6890 Series gas chromatograph fitted with a flame ionisation detector
5. Flesh firmness (kgf), using a GÜSS fruit texture analyser with an 11.1-mm diameter probe
6. Soluble solids (%), using an Atago Smart-1 automatic refractometer
7. Starch pattern index (SPI, 0-6 where 0 = no starch hydrolysed and 6 = all starch hydrolysed) (using the ENZAFRUIT Starch Pattern Index Chart for apples)
8. Titratable acidity (% malic acid, with tissue from each fruit bulked across replicates) using a Metrohm autotitrator.
HEADSPACE ANALYSIS FOR ETHANE AND ETHYLENE

Detection of ethylene is not difficult given the abundance in ripening fruit, whereas ethane production tends to be substantially lower. For this reason, two methods of headspace analysis were used to extract ethane, the first being a microwave method, the second being a vacuum method. Gas samples from both extraction techniques were analysed using a Hewlett Packard 6890 Series gas chromatograph equipped with an activated alumina grade F-1 80/100 column at 130°C, nitrogen as the carrier gas (flow-rate of 35 mL/min), and a flame ionisation detector (flow rate for H$_2$ = 35 mL/min and air 350 mL/min; temperature = 150°C). Ethylene and ethane concentrations were determined using a Hewlett Packard model HP3395 integrator calibrated with an external standard (1 µL/L of each in nitrogen, β-standard, BOC gases Ltd).

The microwave method was developed to improve resolution of ethane detection by increasing the liberation of ethane from tissues (Degousee et al. 1995), and has successfully been used for oxidative stress studies on pears (Larrigaudiere et al. 2001). However, this method can be problematic in that it can accelerate or invoke new oxidative reactions, and requires precise control of the heating process to ensure samples are heated to the same temperature for the same length of time. This method involved placing 10 g of diced skin peel removed from the circumference of the fruit inside the barrel of a 60-mL syringe with the plunger set at 15-mL; the end was then capped with a rubber septum and the syringe heated in a microwave for 10 seconds at 700 W (Black & Decker Model No. BMO700). Two minutes after heating, a 1-mL gas sample was removed through the rubber septum for analysis by gas chromatography. Syringes were rinsed in water and dried between samples. Control syringes containing no tissue were also heated to check for ethane or ethylene background contamination from the septum and syringe.

For the vacuum method, 10 g of diced skin peel removed from the circumference of the fruit were also placed in a 60-mL syringe with the plunger set at 15-mL, the end capped with a rubber septum and the syringe handle pulled to 60-mL to produce a vacuum inside the syringe barrel for 2 minutes. The vacuum was then released and a 1-mL gas sample removed through the rubber septum for analysis by gas chromatography. Syringes were rinsed in water and dried between samples. Control syringes containing no tissue was also used to check for ethane or ethylene background contamination from the septum and syringe.

Following an initial comparison of the two ethane extraction methods, it was found that ethane levels were approximately four-fold higher following microwaving, but the results tended to be more variable. The vacuum method was subsequently chosen as the method of choice for this study, although concentrations were close to the minimum detection threshold for ethane when using a flame ionisation detector (e.g. mean value 0.037 µL/L).

PHENOLIC AND ANTIOXIDANT ASSAYS

Skin peel samples (1 mm thick by 15-20 mm wide) were removed from the entire circumference of the fruit equator, snap-frozen in liquid nitrogen, and stored at -80°C until analysis. Care was taken to avoid sampling damaged or dead tissue. Tissue samples were then ground to a coarse powder using a mortar and pestle pre-cooled with liquid nitrogen. The tissues were then extracted for phenolic and antioxidant activity by adding 100 mg of frozen tissue to 1 mL of 95% cold methanol (4°C), weighing to determine actual tissue amount in the tube, and incubating on ice for 30 min with samples vortex-mixed at 10-minute intervals. Samples were then centrifuged at 13000 g for 10 minutes at 4°C and the supernatant transferred to clean tubes on ice. An aliquot of extract was then diluted 10-fold in 95%
methanol to avoid saturating the assay reagents. All chemicals were sourced from Sigma-Aldrich.

Total phenolics were determined using the Folin-Ciocalteu reagent. This consisted of adding 500 μL of reagent and 400 μL of 0.7 M sodium carbonate to 100 μL of sample. Samples were incubated for 90 minutes at 21°C before reading the absorbance at 760 nm using a microplate reader (SpectraMax 384 Plus). Assays for standards (0 to 12.5 μg/μL catechin) and samples were performed in triplicate.

Total antioxidant activity was determined using the Ferric Reducing Antioxidant Power assay (FRAP). The assay involved adding 198 μL of FRAP reagent (2 mM FeCl₃ / 1 mM 2,4,6-tris(2-pyridyl)-s-triazine / 300 mM acetate buffer, pH 3.6) to 10 μL of sample in a microplate. Samples were then incubated for 20 minutes at 21°C before reading the absorbance at 593 nm using a microplate reader (SpectraMax 384 Plus). Assays for standards (0 to 62.5 μg/μL Trolox – a water soluble α-tocopherol analogue) and samples were performed in triplicate.
RESULTS AND DISCUSSION

MATURITY AT HARVEST

To identify potential predictors of soft scald it was critical for this study to obtain and analyse lines of fruit with contrasting incidences of soft scald. To achieve this, orchard blocks with a history of high or low incidence of soft scald were used. Fruit were also picked at two stages of maturity based on previous studies which have shown a higher harvest maturity can be associated with an increased incidence of soft scald (Brookfield et al. 2005a; 2005b). Fruit from the first pick were harvested at a mean SPI of 2.0-2.7, while those from the second pick were harvested at a mean SPI of 3.5-4.1 (Table 1). Fruit from Orchard 1 had less intense red colour development and were softer at harvest than fruit from the other two orchards (Table 1, Figure 1). Fruit from Orchard 2 tended to have a higher fruit IEC at harvest than the other two orchards, while fruit from Orchard 3 had the highest red colour coverage and intensity. These results support earlier studies with ‘Scifresh’ apples (Brookfield et al. 2005b), which demonstrate for any given SPI the other harvest indices, and particularly IEC, can vary substantially across orchard blocks.

Table 1. Harvest maturity characteristics of ‘Scifresh’ apples picked from three Hawke’s Bay orchards on two harvest dates, 2008. Means (n = 20 fruit) and standard errors of the mean are shown.

<table>
<thead>
<tr>
<th>Orchard 1</th>
<th>Orchard 1</th>
<th>Orchard 2</th>
<th>Orchard 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest 1</td>
<td>Harvest 2</td>
<td>Harvest 1</td>
<td>Harvest 2</td>
</tr>
<tr>
<td>Harvest date</td>
<td>5-Mar-08</td>
<td>17-Mar-08</td>
<td>5-Mar-08</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>183 ± 3</td>
<td>206 ± 4</td>
<td>183 ± 3</td>
</tr>
<tr>
<td>Red Colour coverage (%)</td>
<td>53 ± 2</td>
<td>54 ± 4</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>Skin background colour (1-9)</td>
<td>4.0 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Internal ethylene (µL/L)</td>
<td>1.3 ± 0.7</td>
<td>3.8 ± 2.0</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>Firmness (kgf)</td>
<td>7.8 ± 0.1</td>
<td>8.0 ± 0.1</td>
<td>8.7 ± 0.1</td>
</tr>
<tr>
<td>Soluble solids (%)</td>
<td>11.8 ± 0.2</td>
<td>12.6 ± 0.1</td>
<td>12.8 ± 0.2</td>
</tr>
<tr>
<td>Starch pattern index (0-6)</td>
<td>2.7 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>0.52</td>
<td>0.52</td>
<td>0.5</td>
</tr>
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</table>

1Titratable acidity samples were bulked across replicates, therefore no standard errors are shown.
Figure 1. External appearance of ‘Scifresh’ apples harvested from three Hawke’s Bay orchards, 2008. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1).
**DISORDER INCIDENCE**

Soft scald and superficial scald were the two main disorders found after 16 weeks at 0°C and 7 days at 20°C (Figure 2, Table 2). Orchard 2 had the highest incidence of soft scald (23-35%), while the two other orchards had low incidence (0-2%). Orchard 3 had the highest incidence of superficial scald (38%), while the two other orchards had moderate incidence (11-15%). Harvest maturity was a key factor determining the incidence of superficial scald, with incidence being 11-38% for low maturity fruit and 0-2% for more mature fruit. The impact of maturity on soft scald incidence was less pronounced, although for the most affected orchard, fruit picked at the higher maturity had higher incidence, as found in previous studies (Brookfield et al. 2005a; 2005b). Storage temperature had a major influence on the expression of soft scald after 12 weeks of storage, with the disorder occurring at 0°C, but not at 3°C (Table 3).

![Figure 2. Soft scald (left and middle) and superficial scald (right) on ‘Scifresh’ apples.](image-url)
Table 2. Incidence of storage disorders in ‘Scifresh’ apples after 16 weeks of storage at 0°C and 7 days at 20°C. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1) from three Hawke’s Bay orchards, 2008. Data are means derived from 300 fruit assessed for each orchard and harvest.

<table>
<thead>
<tr>
<th></th>
<th>Orchard 1</th>
<th></th>
<th>Orchard 2</th>
<th></th>
<th>Orchard 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low maturity</td>
<td></td>
<td>High maturity</td>
<td></td>
<td>Low maturity</td>
<td></td>
</tr>
<tr>
<td>Soft scald (%)</td>
<td>0</td>
<td></td>
<td>23</td>
<td></td>
<td>0</td>
<td></td>
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<tr>
<td>Superficial scald (%)</td>
<td>15</td>
<td></td>
<td>11</td>
<td></td>
<td>38</td>
<td></td>
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<tr>
<td>Lenticel blotch (%)</td>
<td>0</td>
<td></td>
<td>1</td>
<td></td>
<td>0</td>
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<tr>
<td>Bitter pit (%)</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Defect free (%)</td>
<td>85</td>
<td></td>
<td>66</td>
<td></td>
<td>62</td>
<td></td>
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</table>

Table 3. Incidence (%) of soft scald in ‘Scifresh’ apples after 12 weeks of storage at 0°C or 3°C, plus one day at 20°C. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1) from three Hawke’s Bay orchards, 2008. Data are means derived from 160 fruit assessed for each orchard and harvest.

<table>
<thead>
<tr>
<th>Storage temperature (°C)</th>
<th>Maturity</th>
<th>Orchard 1</th>
<th></th>
<th>Orchard 2</th>
<th></th>
<th>Orchard 3</th>
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<tr>
<td></td>
<td>Low</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>37</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>High</td>
<td>0</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Low</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>High</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

**ANTIOXIDANTS AND MARKERS OF OXIDATIVE STRESS**

**Antioxidant activity**

Total antioxidant activity relates to the capacity of the tissue for quenching free radicals. Tissues with high antioxidant activity should be better able to prevent oxidative stress when exposed to more frequent bursts of free radical production which can occur under stress conditions such as low storage temperature. The antioxidant activity in the skin of ‘Scifresh’ apples differed between orchards and changed with storage time, but was not affected by storage temperature (Figure 3). Low maturity fruit from Orchard 3 had 60-75% lower antioxidant activity at harvest than the other orchards, but after 4 weeks of storage all orchards had similar activity. The low maturity fruit from Orchard 3 then decreased markedly between 4 and 12 weeks of storage, while activity in the other orchards was maintained or decreased slightly. When comparing the high maturity fruit, all three orchards had a similar response to storage, although fruit from Orchard 3 tended to have lower activity at harvest and during storage.
Figure 3. Total antioxidant activity in the skin of ‘Scifresh’ apples during storage at 0 and 3°C. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1) from three Hawke’s Bay orchards, 2008. Skin peel samples were removed from the fruit equator. Means (n = 5 replicates, 4 fruit per replicate) and standard errors of the mean are shown.

The rapid increase in antioxidant activity in the skin of low maturity fruit from Orchard 3 may be indicative of a storage stress response related to development of superficial scald. Low maturity fruit from Orchard 1 and 2 had a similar early storage response, although this response was smaller in magnitude and these orchards had lower incidence of superficial scald than fruit from Orchard 3. The early storage response was minimal or not apparent for fruit picked later at a higher maturity, and which did not develop superficial scald. These
results suggest that fruit with low antioxidant activity at harvest, followed by a rapid increase during the first four weeks of storage, may have a higher risk of developing superficial scald. This assay appears to have no predictive capacity for soft scald risk, as the antioxidant values and storage responses were similar for fruit from Orchards 1 and 2, despite these having contrasting incidence of soft scald.

**Total phenolic concentration**

Phenolics are an abundant and diverse group of secondary metabolites present in all plant tissues, which on reaction with other cellular components cause black/brown discolouration. There was a general trend across all orchards for the phenolic concentration to increase between 0 and 4 weeks of storage and then stay the same or decrease between 4 and 12 weeks (Figure 4), the exceptions being in low maturity fruit from Orchard 1 where the phenolic concentration continued to increase through to 12 weeks of storage at 3°C, and in high maturity fruit from the same orchard, where the concentration continually decreased during storage at 3°C. Fruit from Orchard 3 generally had the highest concentration of the three orchards, more so when picked later at a higher maturity. The phenolic concentrations were similar at 0°C and 3°C storage temperatures.
Figure 4. Total phenolic concentration in the skin of ‘Scifresh’ apples during storage at 0 and 3°C. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1) from three Hawke’s Bay orchards, 2008. Skin peel samples were removed from the fruit equator. Means (n = 5 replicates, 4 fruit per replicate) and standard errors of the mean are shown.

In relation to storage disorder incidence, the total phenolic concentration did not predict orchard or maturity differences in incidence of soft scald or superficial scald, suggesting the total concentration of these compounds with tissue browning potential are not important. Instead, biological processes that could be preventing phenolic-mediated oxidation of cellular components may dominate. Phenolic acids can also have pro-oxidative roles (can produce potentially damaging oxygen byproducts of metabolism) in plant cells (Sakihama et al. 2002)
and the measurement used in this study encompasses many different types of phenolic compounds, each with different roles in plant cells. Further studies are being undertaken in a parallel FRST programme (Pipfruit - A Juicy Future; C06X0705) using more complex methodology to quantify the different sub-classes of phenolics in each of these orchard blocks.

**Ethane emission**

Ethane is used in medical and plant studies as a marker of oxidative stress on the basis that this volatile is an end-product of membrane breakdown (primarily breakdown from the fatty acid linolenic acid in the membrane) (Degousee et al. 1995; Frankel 1982; Kimmerer & Kozlowski 1982). A similar pattern of skin ethane emission occurred for fruit from all orchards during storage (Figure 5). Ethane emission was highest at harvest, followed by a decrease in emission within the first two weeks of storage, and either did not change for the remainder of storage (high maturity samples) or had a slight increase in emission, which peaked after 4-6 weeks and decreased thereafter (low maturity samples). The initial decrease in ethane emission during the first two weeks of storage is probably a temperature response, as the measurements at harvest were made on fruit at 20°C, while the storage measurements were made on fruit at 0 or 3°C. Fruit from Orchard 2 generally had the highest and most variable ethane emission at harvest and during storage, while fruit from the other two orchards had similar emissions at harvest. Calculation of cumulative ethane emission during 12 weeks of storage showed that fruit from Orchard 2 had the highest overall accumulation, with fruit from the other two orchards being similar (Figure 6). This analysis also showed that ethane emission was similar for the two storage temperatures and tended to be higher for fruit picked earlier at a lower maturity.
Figure 5. Ethane emission from the skin of ‘Scifresh’ apples after different storage times at 0 and 3°C. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1) from three Hawke’s Bay orchards, 2008. Skin peel samples were removed from the fruit equator. Means (n = 5 replicates, 4 fruit per replicate) and standard errors of the mean are shown.
Cumulative ethane emission during storage (µL/kg)

Figure 6. Cumulative ethane emission from the skin of ‘Scifresh’ apples during 12 weeks of storage at 0 and 3°C. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1) from three Hawke’s Bay orchards, 2008. Skin peel samples were removed from the fruit equator. Means (n = 5 replicates, 4 fruit per replicate) and standard errors of the mean are shown.

The orchard with the highest ethane emission from fruit also had the highest incidence of soft scald. Fruit from Orchard 2, which developed high incidence of soft scald, emitted 2-3 times more ethane at harvest than fruit from the two other orchards, which developed nil or low soft scald. Given that ethane is a marker of stress, these results also suggest that the skin from Orchard 2 fruit was showing symptoms of stress at the time of harvest. The higher fruit skin ethane emissions from Orchard 2 may have been a response to picking on this particular orchard, or they may have arisen from particular conditions of stress that developed in the orchard and were present at the time of picking. A further component of the ethane response was that peak emission during storage occurred after 4-6 weeks, which coincides with the time when soft scald begins to express in storage (Brookfield et al. 2005a). However, an exception to this was for low maturity fruit from Orchard 1, which showed a peak in ethane emission after 4-6 weeks, but didn’t develop high levels of soft scald. This suggests that if ethane is to be used as a predictor of soft scald, it may have value to determine high and low risk lines of fruit at harvest, but would have minimal value for monitoring the progression of fruit in storage.

Ethylene production

While ethylene production is useful for monitoring the ethylene climacteric and progression of ripening in apples, it can also be used as a marker of membrane integrity. Under conditions of severe stress, membrane function can be compromised in a way that results in decreased ethylene production (Benson & Withers 1987; Kimmerer & Kozlowski 1982). In contrast, tissues under mild stress can increase ethylene production as a stress response (Benson & Withers 1987; Kimmerer & Kozlowski 1982). For ‘Scifresh’ apples, ethylene production in the skin followed a climacteric type pattern during storage. Ethylene production started low and then increased to peak after 4-6 weeks of storage (Figure 7).
Storage temperature had no effect on ethylene production for all orchards, the exception being higher ethylene production during 4-6 weeks of storage at 0°C than at 3°C for low maturity fruit from Orchard 2. Fruit from the three orchards had similar skin ethylene production, although production from fruit from Orchard 2 tended to decline more rapidly following the peak in production. There was no evidence of any orchard differences in skin ethylene production that related to orchard differences in incidence of soft scald or superficial scald.
Figure 7. Ethylene emission from the skin of ‘Scifresh’ apples after different storage times at 0 and 3°C. Fruit were harvested at two stages of maturity (low = orchard mean starch pattern index of 2.0-2.7; high = orchard mean starch pattern index of 3.5-4.1) from three Hawke’s Bay orchards, 2008. Skin peel samples were removed from the fruit equator. Means (n = 5 replicates, 4 fruit per replicate) and standard errors of the mean are shown.
CONCLUSIONS

- This study provides correlative evidence that oxidative stress may be associated with the incidence of soft scald and superficial scald in ‘Scifresh’ apples. High ethane emission at harvest was associated with high incidence of soft scald, while low antioxidant activity at harvest was associated with high incidence of superficial scald. Antioxidant application studies in the absence of apple waxing formulations with wax stabilising antioxidants are required to further strengthen the concept that oxidative stress has an important role in the development of soft scald.

- Total phenolic concentration showed no relationship with incidence of either disorder, although further research will be undertaken to identify the different sub-classes of phenolics for each orchard. It is possible that the particular sub-classes of phenolic compounds important for disorder incidence may have been masked in the total phenolic assay.

- Following validation in another season, ethane emission and antioxidant activity may be developed as at-harvest predictors of soft scald and superficial scald, respectively, for ‘Scifresh’ apples.
RECOMMENDATIONS

- Ethane emission was shown to segregate the three orchard blocks for incidence of soft scald. This result was only based on three orchards, and therefore it is recommended that this result be validated using a larger number of orchards, as there is a possibility that this season’s result could have occurred by chance.
- Chlorophyll fluorescence has been successfully used to monitor low oxygen stress responses in apples, providing the basis for dynamic controlled atmosphere storage. There is also scope to evaluate this technology for detecting skin properties/defects responses not visible at harvest, which may then progress into soft scald once in storage. This technology also has the advantages of being non-destructive and portable.
- Preliminary studies have shown that 1-methylcyclopropene and controlled atmospheres can suppress the expression of soft scald in ‘Scifresh’ apples. There has been considerable interest by growers in using these postharvest technologies for controlling soft scald, but the commercial efficacy of either technology in relation to their application to ‘Scifresh’ apples is not known. There is also the likelihood that some storage providers may opt to use these technologies on ‘Scifresh’ apples as a standard practice, the quality consequences of which are not known. Accordingly, it is recommended that the response of ‘Scifresh’ apples to these technologies be assessed, in order to develop appropriate commercial protocols.
REFERENCES


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