Hort Innovation

Final Report

Improved management of pumpkin brown etch

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Delivery partner:

Applied Horticultural Research

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Improved management of pumpkin brown etch VG15064

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Content

Summary

"Brown etch", or ''rust mark" is a major issue for pumpkin growers. The problem affects butternut pumpkins (*Cucurbita moschata*) and, occasionally, related hybrids such as Kent. Brown etch regularly results in major losses; up to 50% of a crop may be affected. Paddocks may be abandoned due to the large percentage of affected fruit. Even where fruit appears clean at harvest, etch can develop during storage and transport. Etched fruit arriving at wholesale markets may be rejected outright or resorted and downgraded, further increasing costs to growers.

Brown etch has commonly been thought to be caused by disease. Initial laboratory work isolated a number of different pathogens from affected pumpkins. These included *Stagonosporopsis cucurbitacearum* (gummy stem blight/black rot) as well as *Fusarium* species, *Colletotrichum* and *Rhizoctonia* spp.. A high percentage of immature pumpkins scratch inoculated with *S. cucurbitacearum* developed etch-like symptoms. The pathogen could then be re-isolated from sections of etched skin, suggesting the Koch's postulates had been satisfied. However, many of these pumpkins also developed symptoms of black rot, whereas this did not occur in naturally etched fruit. Moreover, many attempts to isolate this pathogen from etched fruit were unsuccessful. It was concluded that while *S. cucurbitacearum* can trigger etch it is not the sole cause of this disorder.

This was supported by RNA analysis of etched pumpkins. A number of genes involved in lignin synthesis were strongly upregulated in etched compared to non-etched sections of tissue, with borderline areas intermediate. Lignin is produced as a defence against stress – microbial or physical. Observation of this tissue with light and scanning electron microscopes revealed cellular disruption, including massive lignification of the cell walls. However, no fungal hyphae were found in these areas.

A large number of field trials were conducted with both commercial and experimental pumpkin crops. Treatments included foliar nutrients (potassium, silicon, calcium + boron), elicitors of plant resistance (*Bacillus subtilis*, chitosan), inoculation with disease (*S. cucurbitacearum, Fusarium* spp), fungicide programs, plastic mulch and different varieties. Temperature and relative humidity (RH) were recorded along with development of etch in the field.

None of the treatments consistently reduced or increased etch. However, a strong correlation was observed between wet conditions in the 14 days before harvest and high RH and etch development. Modelling suggests that 50 to 150 hours at >90%RH, with fruit wet 15 to 30% of the time, will result in 5 to 10% of the crop developing etch. Longer periods will further increase the number of etched fruit. For example, if fruit is wet >50% of the time, at least one in four pumpkins is likely to develop etch. A second model suggests that in the month before harvest, a continuous period of wetness for more than 24hrs is likely to result in >10% of the crop developing etch.

Postharvest trials demonstrated that etch continues to expand after harvest and can appear on initially clean fruit. The amount of etch occurring postharvest is strongly related to etch in the field: non-etched crops will not develop etch postharvest. In contrast, if etch is observed in the field then even clean fruit may develop symptoms, especially during the first week after harvest. High RH during storage increases etch, whereas low RH and cold temperatures can greatly reduce appearance of the disorder.

Brown etch is a superficial disorder and does not reduce the eating quality of the pumpkins. A retail study examined consumer willingness to purchase etched / non etched pumpkins displayed as cut, overwrapped halves. Discounting etched fruit by 50c/kg slightly increased sales compared to clean fruit. When the discount was reduced to 20c/kg or zero, sales were generally similar. This suggests that consumers are actually willing to purchase cut fruit when they can see the quality of the flesh, and may not even notice that the skin is discoloured.

Keywords

Pumpkin, brown etch, rust mark, disorder, gummy stem blight, fusarium, black rot

Introduction

"Brown etch", or ''rust mark" is a major issue for pumpkin growers. The problem affects butternut pumpkins (*Cucurbita moschata*) and, occasionally, related hybrids such as Kent. Brown etch regularly results in major losses; up to 50% of a crop may be affected, and in some cases paddocks may be abandoned due to the large percentage of affected fruit. Even where fruit appears clean at harvest, etch can develop during storage and transport. Etched fruit arriving at wholesale markets may be rejected outright or resorted and downgraded, further increasing costs to growers.

Brown etch appears as a light tan to dark brown spreading stain across the surface of the fruit. Symptoms may develop as a series of concentric rings, as a marbled brown area, or as a simple blotch spreading over the skin. With time, the etched tissue dries out, developing a whitish appearance reminiscent of petrified wood. Fungal pycnidia may appear in the centres of these dead areas.

Despite variability in appearance, symptoms of brown etch are almost always only superficial; the underlying flesh is not affected. Unfortunately, although the eating quality of the pumpkin is unaffected, etched pumpkins are largely unsalable. Major retailers will tolerate only a few fruit with etched areas no larger than a 50c coin. This relates to the assumption that etch is a disease that will progress into the flesh, as well as the poor appearance of the fruit itself.

Pathologists investigating brown etch have isolated a range of different organisms from affected areas. These include various species of *Fusarium*, as well as gummy stem blight (*Stagonosporopsis cucurbitacearum*). However, proof of causation (via Kochs postulates) has proven elusive. Some have posited that etch is not caused by a disease but is a physiological reaction to internal and/or external stresses.

This project aimed to determine the cause / causes of brown etch and develop management solutions.

Methodology

The causes and management of brown etch have been investigated using a range of different methodologies. These include:

- A comprehensive **literature review** of research relating to brown etch worldwide.
- **Pathology** testing to isolate potential causative pathogens and re-inoculate into clean fruit (Koch's postulates). These studies have included:
    - Attempted isolation of various pathogens from affected and unaffected fruit tissue
    - Re-inoculation of pathogens onto plant leaves and fruit in the field using a range of different techniques (scratch inoculation, spray inoculation, wounding)
    - Inoculation into harvested mature green, cream and fully mature fruit using a range of different techniques (scratch inoculation, addition of surfactant, placement of soaked tissue)
    - Field studies focused on gummy stem blight (GSB)
- **RNA analysis and microscopy** to examine changes in etched tissue (conducted as part of a University of Sydney Masters Thesis by Firdause Al Haj Hasson)
    - Global RNA sequencing and analysis of affected, unaffected and "edge" tissue to determine upregulated / downregulated genes
    - Light microscope examination of cellular changes associated with development of etch
    - Observation of presence / absence of fungal hyphae within affected cells
    - Scanning electron microscope images of affected, unaffected and "edge" cells
- **Monitoring of commercial crops** to examine environmental factors that may be linked to development of etch. Based on both the literature review and reports from growers and wholesalers, there appeared to be a good correlation between occurrence of brown etch and damp climatic conditions. Numerous growers reported that previously clean crops developed etch after rain and/or a major cold change.

  Climatic conditions were measured using remotely monitored portable weather stations, and the crops periodically scouted for etched pumpkins. Scouting was conducted by walking a transect through each paddock, examining at least 100 pumpkins for etch. Regions monitored included:
    - Griffith, NSW
    - Ayr, Qld
    - Lockyer Valley, Qld
    - Donnybrook, WA
    - Mareeba, Qld
- **Field trials** with treatments designed to potentially increase / decrease incidence of brown etch. Field trials and treatments included:
    - Somersby 2016 – 2017
        - Increase / decrease RH around fruit using hessian sacking / plastic supports
        - Field inoculation with *Fusarium* spp. and GSB on fruit and leaves
        - Differences between butternut cultivars Sunset QHI and Jacqueline
    - Somersby 2017 – 2018
        - Foliar applications of calcium + boron (Sett-Enhance®), silicic acid (AgriSiL®), potassium (K_2SO_4) or chitosan
        - Soil drench with *Bacillus subtilis* (Serenade®)

        - Differences between butternut cultivars Sunset QHI and Jacqueline
        - Inoculation of leaves with spore suspension of *Fusarium* spp.
    - Cowra 2017 – 2018
        - Plastic covers vs open (to increase RH around fruit)
        - Foliar applications of calcium + boron (Sett-Enhance®), potassium silicate and Envy® (an anti-transpirant)
    - Bowen 2018
        - Differences between six varieties (Sunset QHI, Tiana, Hannah, Matilda, Jacqueline, E308)
        - Effect of plastic mulch vs bare soil
        - Foliar applications of chitosan
    - Richmond 2018 – 2019
        - Inoculation with GSB +/- a rigorous fungicide program using systemic and non-systemic products
        - Foliar application of chitosan
        - Differences between four varieties (Hannah, Havana, Matilda, Tiana)
- **Supply chain** studies examining transport conditions and a range of treatments:
    - Monitoring temperature and relative humidity (RH) in cardboard vs vented plastic bins
    - Forced air curing before transport
    - Postharvest treatment with 1-MCP fumigant (SmartFresh®), chitosan or fungicide (Graduate A+)
- **Postharvest storage** trials examining the continued expansion of etched areas postharvest as well as the development of etch on previously clean fruit:
    - Development of etch during storage with high (95%), medium (70%) and low (20%) RH over two seasons
    - Effects of pre-harvest treatment of calcium + boron (Sett-Enhance®), silicic acid (AgriSiL®), potassium (K_2SO_4) or chitosan on postharvest development of etch
    - Cold vs ambient storage effects on expansion of etched areas
- **Retail studies** examining acceptability to consumers of etched fruit when presented as a cut product:
    - Effect of price differential on purchase
    - Inclusion of explanatory signage

Full methodology for each of these activities is included in the appendices to this report.

Outputs

A number of outputs have been produced as a result of the project. These include articles in WA Grower and Vegetables Australia magazines, presentations at field days and a Fact sheet;

- Literature Review (Appendix 1)
- WA Growers article "Have you seen this etch?" Summer 2016 https://issuu.com/vegetableswa/docs/wagrower_summer_16_lr
- Vegetables Australia article "Attention pumpkin growers, Have you seen this etch?" March-April 2017 https://ausveg.com.au/app/data/publications/VA/VA-MarApr2017.pdf
- Lockyer Valley Growers article "The mysterious case of pumpkin etch" February 2018
- Presentation at Gatton field day, March 2018 "Managing brown etch of pumpkins"
- Vegetables Australia article "Project update: Managing brown etch in pumpkins" May-June 2018 https://ausveg.com.au/app/uploads/publications/Vegetables-Australia_May-June-2018_Web.pdf
- Vegetables Australia article "Managing brown etch of pumpkins" submitted for publication in the Vegetables Australia March-April 2020
- Fact sheet "Understanding brown etch of pumpkins"

The magazine articles and Factsheet are included as Appendix 7 to this report.

Note that fewer outputs have been created than originally envisaged due to the lack of conclusive results.

Outcomes

Literature review (Appendix 1)

Brown etch specifically affects varieties of *Cucurbita moschata*, particularly butternut pumpkins.

A number of fungal pathogens have been named as the cause of symptoms resembling brown etch. Some prominent USA pathologists have attributed etch symptoms to gummy stem blight / black rot of fruit caused by *Stagonosporopsis cucurbitacearum* (Stone and Selman 2014, Keinath 2011, Zitter 1992). However, others have suggested various species of *Fusarium* including *F. solani,* and *F. acuminatum* are responsible for these symptoms (Koike et al. 2006, Elmer 1996). Australian researchers have also noted possible involvement of *Fusarium* spp, with *F. avenaceum*, *F. oxysporum* and *F. roseum* Equiseti all being reported as inducing etch in butternut pumpkins (Chambers 1975, Johnson 1976).

It has also been suggested that cold conditions during later fruit development can also induce etch (Letham 1981).

Queensland researchers have attempted to test these theories, with limited success. Projects funded by HRDC, and later HAL, failed to reliably induce etch or confirm the cause of this issue (Loader et al 1996). However, while the authors of the latter report assume that etch was caused by *S. cucurbitacearum* (gummy stem blight) the butternut pumpkin variety that was developed from the work (Sunset QHI) is sold as resistant to "etch caused by *Fusarium* strain" (Herrington et al 2000).

In summary, there is only slender evidence that etch is the result of infection by a specific pathogen. However, three key conclusions may be drawn from this review:

- Etch may be triggered by the hosts reaction to a fungal infection, but the timing of this infection is likely to be critical
- Wet conditions are associated with increased etch
- Resistance to etch is not simple and may require multiple genetic changes

Pathology testing (Appendix 2)

Initial laboratory work, CropDoc, 2015-2018

Senior plant pathologist Dr Len Tesoriero attempted to isolate fungal pathogens from etched pumpkins on a number of occasions between 2015 to 2018. Few of these plates yielded fungi, no growth was observed for at least 86% of the tissue samples that were plated.

Fungi that were found included black rot (*Phoma cucurbitacearum*, the asexual form of *S. cucurbitacearum*) as well as *Fusarium* sp., *Colletotrichum* sp., and *Rhizoctonia* sp.. In 2017 black rot and *Fusarium* were each re-inoculated into 30 clean pumpkins. In total, 23 pumpkins inoculated with Fusarium developed symptoms similar to etch, compared to 11 and 12 for black rot and water only respectively.

Dr Tesoriero states that "*The lack of consistent recovery of potential pathogens from fruit with typical brown etch symptoms is consistent with earlier pathology studies conducted in this project suggesting that fungi are not the primary cause of this disease*."

AHR Laboratory work, 2019

When Dr Natalie Elias joined the project team in 2018, she consulted extensively with USA pathologists regarding this issue. Prof. Thomas Zitter (Cornell University), Prof. Anthony Keinath (Clemson University), Prof. Mary Ruth McDonald (University of Guelph) and Prof. Natalie Goldberg (New Mexico State university) all considered the cause of brown etch (or "alligator skin") to be *S. cucurbitacearum*. Prof. McDonald also stated she had completed Koch's postulates, proving causation. However, Prof. Zitter advised that it was difficult to infect plants in the field with this pathogen and suggested that a lab based trial would be more likely to succeed.

A fungal isolate from an etched pumpkin was purified and identified as *S. cucurbitacearum* by the Royal Botanic Gardens Disease Diagnostics Service. This was bulked and incubated to induce sporulation. The spore suspension was inoculated onto immature pumpkins picked that same day. Controls were either left intact or wounded only. This methodology was consistent with that used by Dr Greg Johnson in 1976. Twelve of the 15 pumpkins which

were wounded and inoculated with the spore suspension developed etch-like symptoms. None of the untreated controls or wounded-only controls developed etch.

Sections of etched skin were re-isolated, yielding a new *S. cucurbitacearum* fungal culture. Dr Elias concluded that Koch's postulates had been satisfied and "a major of cause of pumpkin etch is the pathogen *S. cucurbitacearum*."

Final laboratory work, CropDoc 2019.

Following the successful eliciting of brown etch at the AHR laboratory, Dr Tesoriero repeated this methodology at NSW DPI Ourimbah. The work was conducted during autumn-winter, so pumpkins were supplied from a farm in North Queensland. As a result, pumpkins were several days old before treatments were applied. Two isolates of *S. cucurbitacearum* and one of *Fusarium* spp. were applied using different methods to green and semi-mature pumpkins.

Inoculation with either isolate of *S. cucurbitacearum* increased the incidence of brown etch. The effect was more pronounced in green fruit than in those approaching maturity. It is noted that up to 50% of inoculated fruit also developed the soft rot symptoms typical of black fruit rot.

Attempts to re-isolate the pathogen from affected tissue were partially successful, only two samples yielding a new fungal culture from etched tissue. In contrast, the pathogen could be readily re-isolated from all samples with black fruit rot.

These results suggest that etch can occur as a response to infection by certain pathogens. It may be triggered by metabolites released by the invading mycelium as it attempts to kill plant cells, releasing the nutrients it needs. This explanation appears consistent with the results of Dr Elias, as well as those of Dr Greg Johnson, who described a similar effect with detached green fruit and *Fusarium* sp.p.

In summary, brown etch can be triggered by infection by *S. cucurbitacearum*. However, this appears to be due to a hypersensitive response by the pumpkin rather than a direct disruption of the epidermal cells by the invading fungus.

RNA analysis and microscopy (Appendix 3)

Masters candidate at the University of Sydney Firdause Al Haj Hasson extracted and sequenced RNA from etched, unaffected and transitioning areas of pumpkins. Ten genes associated with phenylpropanoid biosynthesis were significantly up-regulated in etched compared to non-etched tissue. Transitioning areas were intermediate between the two.

Phenylpropanoids are involved in formation of compounds such as lignin as well as flavonoids, stilbenes and other compounds involved in defending plant tissue against biotic and abiotic stresses.

Increased lignification of the cell walls was confirmed using light microscopy and scanning electron microscopy (SEM). The light microscopy showed that etch affected cells were deformed and highly disrupted, possibly due to increased internal turgor pressure. The SEM images clearly show a massive increase in the thickness of cell walls, as well as highly compressed and disordered cell contents.

Interestingly, the number of genes identified in all three tissue types was similar. In addition, no fungal hyphae were observed invading the epidermal cells. This suggests that no fungal pathogen was present in either the etched or unaffected tissue samples examined.

The results are consistent with the physical symptoms of etch. Excessive formation of lignin leads to the appearance of the rich, reddish brown stain typical of brown etch. As the disrupted cells die, the whitened skeletons of the empty cell walls remain. This accounts for the "petrified wood" appearance that develops inside old areas of etch.

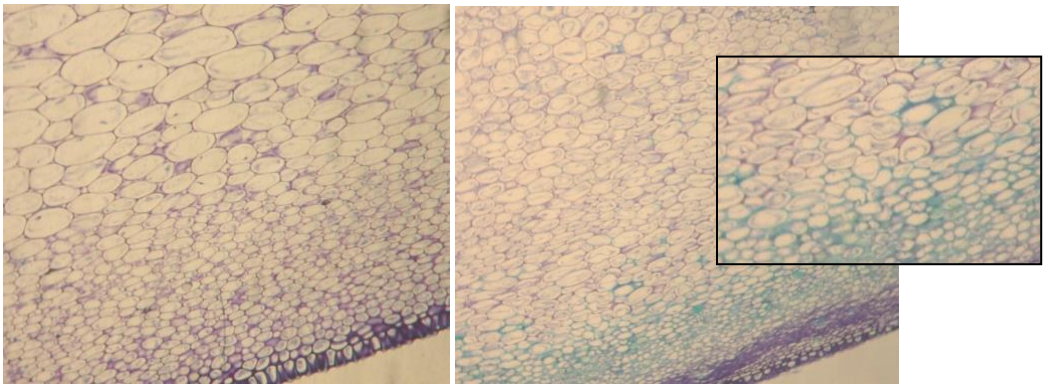

Figure 1. Light microscope images of unaffected (left) and etched tissue (right). Inset shows closeup of deformed and disrupted cells with thickened cell walls.

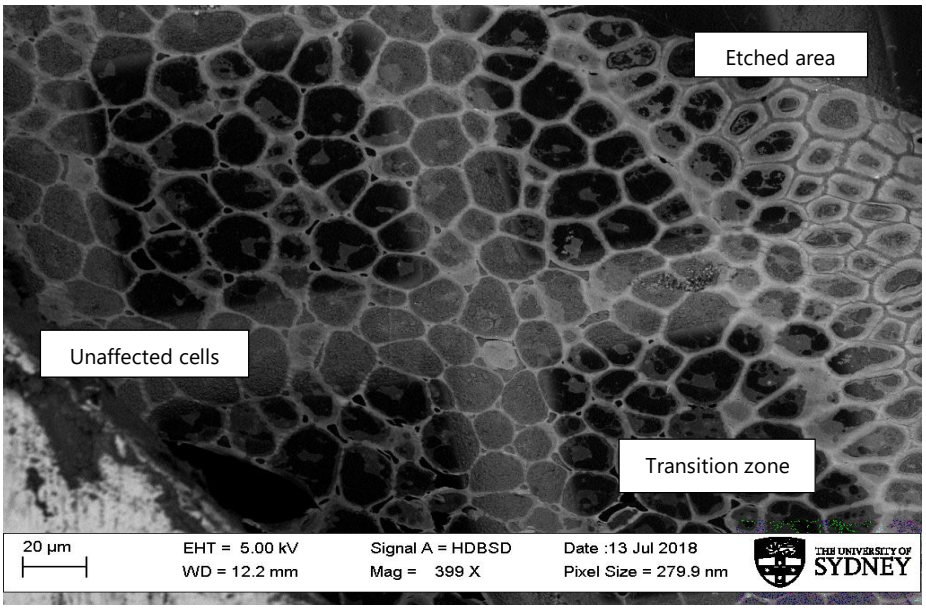

Figure 2. Scanning electron microscope image of the transition zone between etched and unaffected tissue. There is a clear increase in cell wall thickness in the etched area to the right of the image, the cell contents here being compressed and disrupted..

Field trials and monitoring of commercial crops (Appendix 4)

Weather data was collected from all field sites, along with data on incidence of etch.

One of the factors hampering this project has been the dry conditions in many pumpkin production areas. While there was significant rain during the first season of the project (2016 to 2017), pumpkin production areas have since been unusually dry. The current severe drought in NSW, Qld and WA has virtually eliminated etch, with few reports of the issue by growers or wholesalers.

Outcomes from specific sites includes:

Griffith, NSW

No significant etch developed during the summer of 2016 to 2017, either pre or postharvest.

Ayr, Qld

During 2017 etch incidence peaked at around 10% of fruit affected. The majority developed in the three weeks leading up to harvest. In almost all cases etch developed at contact points with soil, plant material or other fruit.

Lockyer Valley, Qld

Despite heavy rain earlier in the year, levels of etch were low in the 2018 autumn crop. Up to 5% of fruit had moderate or severe etch, rendering it unmarketable. When bins of harvested fruit were inspected, it was noted that rates of etch were higher if fruit were muddy, suggesting they had grown in a damp area of the paddock.

Donnybrook, WA

The onset of etch in an autumn crop was associated with a steep increase in RH. Etch affected approximately 9% of the crop, however this appeared to have occurred in at least two separate events, with some etch already at the "petrified wood" stage while other fruit displayed new, brown etch.

Mareeba, Qld

Both the 2017 and 2018 crops were virtually free of etch. In 2018 a number of developing fruit were sealed inside plastic bags to determine if this would increase etch. Not only did it not trigger etch, but RH inside the bags was lower than in the surrounding crop, possibly due to increased temperatures.

Field trials non-commercial crops (Appendix 5)

A series of experimental trials were conducted attempting to both induce etch and find ways to manage this issue.

Somersby 2016

Butternut pumpkins cv. Jacqueline and Sunset QHI were grown as these were nominally susceptible and resistant varieties. Pumpkins were scratch inoculated with *S. cucurbitacearum* or *Fusarium* spp., wrapped in hessian or elevated on stands. Conditions were very damp over the growing season with frequent rain and high RH. Rates of etch were extremely high, affecting approximately 28% and 50% of fruit at the first and second harvests respectively. There were no differences between the two varieties. Etch nearly always initiated from the point of contact with the soil or vegetation. All treatments (including inoculation) appeared to *reduce* rates of etch.

Somersby 2017

A mixed crop of Jacqueline and Sunset QHI was grown as previously. The area was divided into treatment plots with foliar applications of silicon (AgriSil), calcium + boron (SettEnhance) or potassium (K_2SO_4) or a soil drench with *Bacillus subtilis* (Serenade). Only 5% of pumpkins treated with SettEnhance had etch compared to approximately 12% of fruit from the other treatments. However, results were affected by excessive weed growth and the trial was cut short.

Somersby 2018

Pumpkins cv. Jacqueline were planted into black plastic mulched beds. Plots were given foliar applications of SettEnhance or chitosan or inoculated with a solution of *Fusarium* spp.. The percentage of etched pumpkins (18% overall) was not reduced by SettEnhance or increased by inoculation with *Fusarium*.. Chitosan slightly reduced etch. There appeared to be a greater effect of position in the field than treatment, with plots on the lower side having consistently more etch than those in the upper part of the crop.

Cowra 2018

A section of a commercial butternut pumpkin crop was purchased to use for the trial. As Cowra has a dry climate, plastic tunnels were built over two rows to increase RH in the period leading up to harvest. Foliar applications were made of SettEnhance, potassium silicate and Envy (an anti-transpirant). One of the tunnels appeared to increase RH and rates of etch. However the plastic covering the other tunnels dislodged several times. Both RH and etch were no different to the field controls. There were no significant differences between the treatments.

Richmond 2018 to 2019

Three crops were grown. Crop 1, planted November 2018, cv. Jacqueline were inoculated with *S. cucurbitacearum* then left untreated, subjected to an intensive fungicide program, or treated with chitosan sprays. Results were compared to a non-inoculated control. Crop 2, planted January 2019, repeated these treatments with the exception of the chitosan application. Crop 3, also planted in January, consisted of four different cultivars: Matilda,

Hannah, Havana and Tiana. All were inoculated with *S. cucurbitacearum.*

An initial harvest of crop 1 found low levels of etch across all treatments, with a (non-significant) trend to reduced rates of etch in the intensive fungicide treatment. All other treatments were similar. At the second and final harvest in April 2019 high rates of etch (approx. 46%) were found in both crops 1 and 2. Again there was a slight trend to fewer etched pumpkins where the plants had been treated with fungicide. There was no difference between inoculated / non-inoculated plants. Etch was far less in the variety trial, averaging only 7% with no differences between varieties.

The reasons for the extreme difference between crops 1 and 2 and crop 3 are unclear. The canopy coverage for crops 1 and 2 was denser, the plants growing in double rows, whereas for crop 3 empty buffer rows were left between the plots, reducing RH. It is also possible that the cv. Jacqueline pumpkins were infected by a seed borne pathogen; molecular testing of the seed revealed very low levels of an organisms resembling *S. cucurbitacearum*.

Field trial conclusions

The results suggest that the causes of brown etch are complex. It is unlikely there is a simple solution. While it appears that the gummy stem blight pathogen can trigger etch, it also seems likely that this is not the sole cause of the disorder.

There is a clear influence of climate on brown etch with wet or humid conditions increasing incidence of the disorder. Comparing RH to rates of etch appears to give a good correlation. The results are best if using the data from the two weeks prior to harvest. As shown in Figure 3, If RH is over 90% for more than 50 hours during the two weeks before harvest, or 15% of the time, then there is a risk that more than 10% of fruit will be affected by etch. However, if RH exceeds 90% for extended periods levels of etch may be much higher. It is interesting to note the outlier point from the April harvest in Richmond, where 46% of the pumpkins cv. Jacqueline were etched, even though RH was relatively moderate.

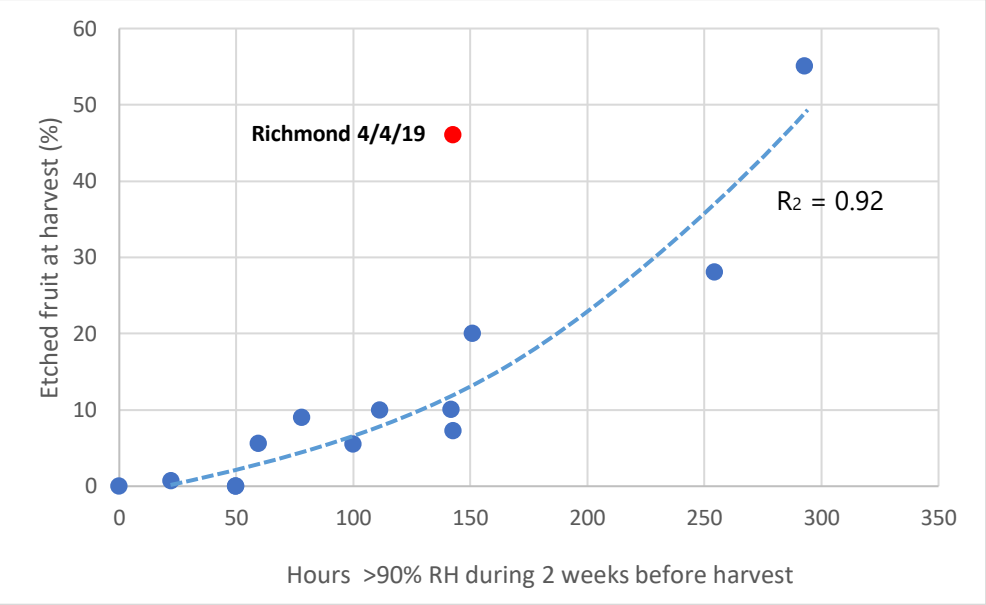

Figure 3. Correlation between RH during the two weeks before harvest and the incidence of etched fruit. Each point represents the mean values from a field trial.

On further examining the weather relating to the "outlier" value from Richmond, it is apparent that very wet conditions occurred for approximately one week, 3-4 weeks before harvest. During this period, pumpkins were likely to have been continuously wet for approximately 3.5 days.

A slightly better correlation can be achieved by estimating the maximum continuous period that pumpkins could potentially be wet in the 30 days before harvest (Figure 4). That is, air temperature is within 1°C of dewpoint. This model suggests that the risk of etch increases if fruit stay continuously wet for more than 24 hours in the 30 days leading up to harvest.

This is likely to explain why etch usually starts from a contact point with the soil, a stem or another fruit. This is the

area which is likely to stay wet longest.

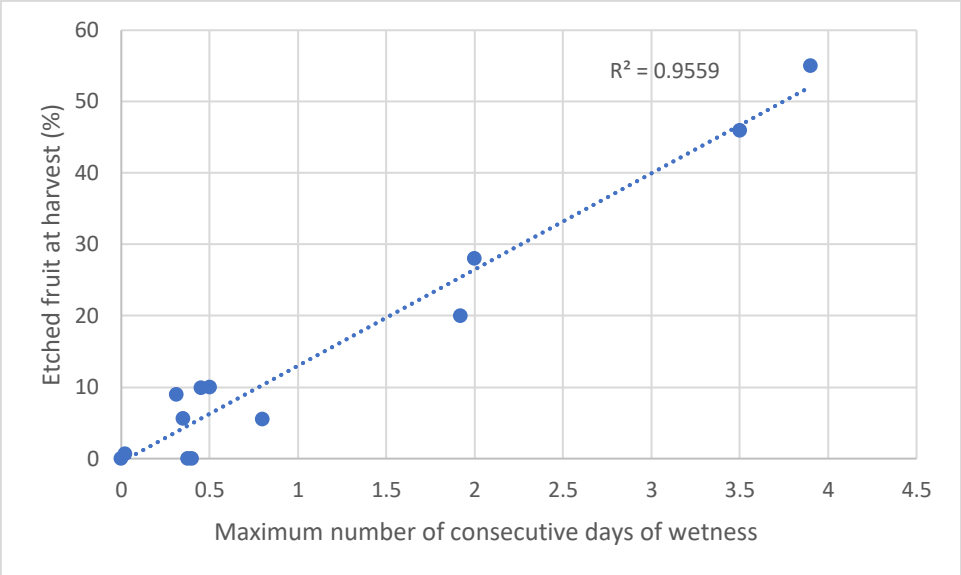

Figure 4. Correlation between the maximum consecutive time of wetness (calculated by comparing dewpoint with actual temperature) during the 30 days before harvest and the incidence of etched fruit. Each point represents the mean values from a field trial.

Postharvest trials (Appendix 6)

Griffith supply chain

Temperature and RH were monitored inside bins of clean pumpkins during transport to Sydney. A number of pumpkins were placed in moistened plastic bags to increase RH around those fruit. Humidity inside the bins averaged 70-80%. The pumpkins were inspected on arrival in Sydney and no etch was found. The results support the observation that if no etch is found in the field, etch does not develop postharvest.

Ayr supply chain

Pumpkins from a crop with a high rate of etch in the field were packed into unvented cardboard “hat bins” or vented, foldable plastic bins provided by CHEP. Some etched pumpkins were included, with the etched area outlined in pen. The fruit were examined on arrival at Sydney markets.

Surprisingly, RH was initially higher in the plastic than the cardboard bins, suggesting cardboard absorbs moisture from the pumpkins. Approximately 20% of the fruit that were clean when packed developed etch during two days transport. After 24 hours this increased to 23% of fruit. Etch continued to expand on 50% of fruit which had etch marked at packing. Differences between the bin types were not significant. The results demonstrate that if etch is observed in the field it can develop and expand rapidly during transport, even if fruit appeared clean at packing.

A second supply chain study was conducted from Ayr, this time when etch was less of an issue. Pumpkins in plastic bins were forced air cured overnight before transport. In this study etch rates were low regardless of treatment, averaging only 3-4%. This prevented any conclusions as to the effectiveness of curing.

Postharvest storage of fruit from Somersby trials

Trials examined the effects of pre-harvest treatments on postharvest development of etch, the effects of low compared to high RH during storage, and whether low temperature storage could slow progression of etch.

- Postharvest etch development was unaffected by pre-harvest treatment with SettEnhance, foliar potassium, silicon or Serenade, with affected fruit increasing from 5-15% to 28-50% after 10 days at ambient. Rates of etch did not increase significantly over a further 7 days storage.
- Virtually all etch recorded at harvest continued to spread during ambient storage. The rate of new etch development was greatest between 2 to 7 days after harvest. While new etch could occur for up to 2

weeks, after 10 days likelihood was greatly reduced.

- Many of the pumpkins that were clean at harvest developed etch during storage. However, the rates of new etch development were significantly lower for these fruit.
- Etch development was significantly increased at 95% RH compared to 15% RH for both etched and clean fruit, with 70% RH intermediate between these values. These results were confirmed over two seasons.
- Only 13% and 10% of etched and clean pumpkins developed new etched areas after one week at 5°C. This compares to 90% and 45% of etched and clean pumpkins under ambient conditions,. While 5°C is too cold for butternut pumpkins, the results suggest that cooling to a moderate temperature (e.g. 10°C) could substantially reduce development of etch during transport and storage. This strategy could be used for high risk crops. That is, where moderate to high rates of etch have been observed in the field.

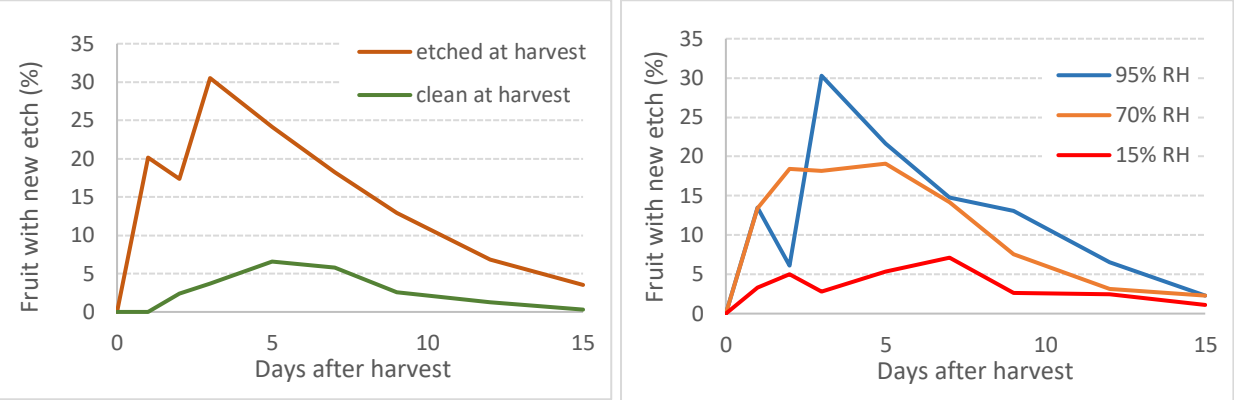

Figure 5. Effect of etch at harvest and RH during ambient storage on postharvest development or expansion of etch

Effect of etch on retail sales

Butternut pumpkins are mainly sold cut in half and overwrapped. Slightly more etched than clean pumpkins were sold when offered with a 50c/kg discount. When the discount was reduced to 20c/kg or zero, sales of etched / clean fruit were generally similar. It seems likely that consumers often do not look at the pumpkin skin, especially if they can see that the flesh is good quality.

These results suggest that etch need not impact on retail sales if pumpkins are sold as cut halves. So long as consumers can see that the inside is unaffected, skin colour may be less of an issue than thought. This information has been provided to Coles with the objective of increasing tolerance of etched fruit.

Figure 6. Effect of discounting on sales of etched pumpkins compared to clean fruit, and header cards used on the display.

Monitoring and evaluation

The original project plan had a large extension component. This was based on determining the key causes of brown etch in year 1, developing management options and then communicating these options to growers as a key activity in year 3. The main annual project activities were planned to be (in summary);

Year 1 – Clarify the causative agents of brown etch, identify conducive conditions and assess varietal susceptibility

Year 2 – Develop and assess management options and evaluate cost:benefits of control

Year 3 – Extension and communication, including fact sheets, farm walks and field demonstrations

However, it has proven far more difficult to determine the causes of brown etch than expected. Even now, we still do not fully understand the causes of this condition. Results from different times were frequently inconclusive and sometimes contradictory. Moreover, the lack of etch over the last two seasons has significantly hampered trials examining management options. For example, a major trial was conducted in Bowen testing a number of different varieties as well as plastic mulch vs growing direct on soil and application of foliar chitosan. Dry conditions meant that no etch was experienced. Similar results were obtained for a number of supply chain studies, not all of which are reported here due to the lack of meaningful results.

As a result, the activities proposed for year 1 and year 2 were not completed as planned. This made it impossible to conduct the planned extension and communication activities.

Only now, when all data collection is complete, can we formulate useful guidelines regarding:

- What is occurring in the fruit skin where etch develops
- Environmental conditions that increase the risk of etch
- Pathogen/s that can trigger development of etch
- Management options that (may) reduce risk of etch in the field
- Postharvest conditions that reduce the risk of etch developing during transport and storage
- Consumer acceptance of etched fruit

These guidelines are included in the final Fact Sheet prepared as an outcome from the project.

Recommendations

Reducing humidity in pumpkin crops

Many of the trials conducted testing ways to reduce etch were unsuccessful due to the dry conditions experienced on the east coast during the last two years. This includes the trials testing different varieties, foliar applications and plastic mulch vs soil. The modelling suggests that RH and wetness are key to development of etch. Reducing RH in the crop canopy could therefore be an effective way to reduce the risk of etch if wet or humid weather is expected. Trials could examine:

- Plant spacing and row width
- Irrigation method (overhead vs drip)
- Mulch type (plastic vs organic) or non-mulched
- Management of nutrition, so as to reduce canopy density

Seed treatment

S. cucurbitacearum can be introduced to the field via infected seed (demonstrated previously in other cucurbit species). Very low levels of an organisms resembling *S. cucurbitacearum* were identified in this project. It would therefore be useful to determine how widespread *S. cucurbitacearum* is in butternut pumpkin seed used in Australia, and consider methods of seed treatment for control of the pathogen, such as heat.

Forced air curing of pumpkins

Two forced air curing trials were conducted, both of which were inconclusive due to low levels of etch in the harvested crop. However, the early results from postharvest storage trials demonstrated that development of new etch peaks within one week of harvest. Shortening this risk period by forced air curing could potentially reduce etch development during transport. It would be useful to repeat this trial with pumpkins at higher risk of etch development.

Effect of temperature on postharvest development of brown etch

The results from the final postharvest trial, where etched and non-etched pumpkins were refrigerated, are extremely promising. Simply cooling the fruit reduced development and expansion of etch more than any of the other treatments tested. In this case normal refrigeration temperatures (5^{o}C) were used, which can cause chilling damage to butternut pumpkins. This trial should therefore be repeated on a larger scale using appropriate storage temperatures (10-12^{o}C).

Retail studies

Even though etched fruit are considered unacceptable by retailers, this is a superficial disorder that does not affect eating quality. Moreover, most butternut pumpkins are sold as cut halves, so the flesh is clearly visible. A small retail study conducted within this project found that there was little consumer resistance to purchasing etched fruit. Confirming this result on a larger scale would improve understanding of what, if any, discount would be appropriate. Presenting this information to retailers has the potential to greatly reduce rejections due to etch. This would enable growers to sell affected pumpkins for a reasonable price instead of wasting what is perfectly edible food.

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Zitter TA. 1992. Fruit rots of squash and pumpkins. Cornell University Fact Sheet 732.10, accessed online 27/9/2016 at vegetablemdonline.ppath.cornell.edu/factsheets/Cucurbit_FrtRots.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

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Appendices

Appendix 1 – Literature Review

Appendix 2 – Laboratory studies of brown etch

Appendix 3 – RNA analysis and microscopy

Appendix 4 – Field trials and monitoring of commercial crops

Appendix 5 – Field trials with experimental crops

Appendix 6 – Supply chain and postharvest studies

Appendix 7 – Extension materials

Appendix 1. Literature review

Brown etch, or 'Rust mark' is a major issue for Australian pumpkin growers, particularly producers of butternut varieties. It regularly results in significant losses on farm, product quality downgrades or rejections in the market. Losses of 50% are not unknown, and in some cases crops may be abandoned as not worth harvesting, due to the large percentage of affected fruit.

As a cause of major economic loss, it could be expected that brown etch would have been the subject of significant research effort in Australia and in other countries (eg the USA) that grow susceptible pumpkin varieties. The following review attempts to collate what has been published on this issue.

Description of symptoms

Brown etch usually appears as a light tan to dark brown spreading stain across the surface of the pumpkin. It is most common on butternut (*Cucurbita moschata*) but can appear on other varieties as well. The symptoms may appear as a series of concentric rings, as a somewhat marbled browned area, or as a simple, brown blotch spreading across the fruit skin (Figure 1).

Figure 1. Symptoms of brown etch on Australian butternut variety pumpkins.

Brown etch rarely extends into the flesh, but is usually a purely superficial skin discolouration. The affected skin may be slightly depressed compared to the healthy skin, but this is not always the case.

According to many growers and packers, what appear to be slight symptoms at harvest can grow and darken in the few days after packing and during transport. So, fruit that looked relatively undamaged when packed can be unacceptable after several days haulage to central markets. It is interesting to note that in most cases the area does not continue to develop or expand after this initial transformation, and remains unchanged over days or even weeks of storage (Figure 2).

Eventually the affected areas dry out, becoming whitish, and the skin surface may crack. This allows easy entry for the opportunistic bacteria and fungi that cause soft rots and fruit decay.

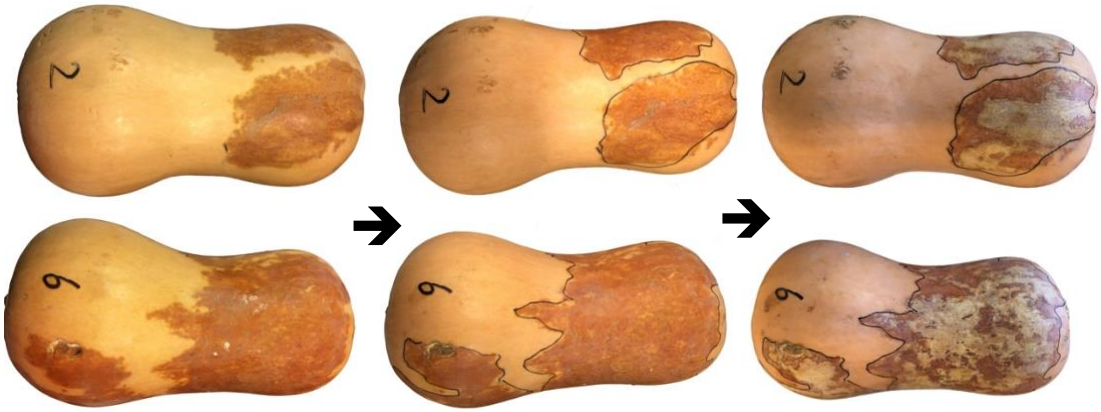

Figure 2. Brown etch symptoms on pumpkins on arrival in Sydney from Mareeba (left), after one week at 22°C (centre) and after a further 2 months at 22°C (right).

The symptoms of brown etch are not limited to the area in contact with the ground, but may extend right around the fruit. Symptoms can occur in fruit grown on plastic mulch as well as that grown directly on the soil, and have been reported from every major growing region within Australia. There are currently no control strategies in place for brown etch, although it is noted that fungicide sprays may be applied for other cucurbit diseases, particularly powdery mildew and gummy stem blight.

International research and reports

The United States

Resources identifying fungal disorders of winter squash or pumpkin include a number of references and photographs of butternut pumpkin with similar symptoms to those seen in Australia. Symptoms are usually attributed to different pathogen families;

1. *Didymella bryoniae*, now known as *Stagonosporopsis cucurbitacearum*. This is described as causing black rot in fruit and gummy stem blight in plants more generally. The fungus also has an asexual stage called *Phoma cucurbitacearum*[1].Two further species have recently been described as causing gummy stem blight: *S.*

[1] Stone A, Selman L. 2014. Winter squash storage rots and their management. Oregon State University Fact Sheet, accessed online 27/9/2016 at horticulture.oregonstate.edu/content/winter-squash-storage-rots-and-their-management.

citrulli and *S. caricae*[2]. All three of these species have also been found in Australia infecting cucurbits[3].

2. *Fusarium solani* f. sp. *cucurbitae*, as well as several other *Fusarium* species[1].

Gummy stem blight has been described as "*resulting in widespread financial disaster wherever it gains foothold*[4]". The symptoms primarily occur in foliage, with the fungus growing rapidly in all parts of the plant. Lesion expansion rates of 1cm/day have been reported (Keinath, unpublished data). The spores can be carried in wind or water, survive for extended periods in the soil, or be transmitted on seed coats[5]. Crop losses of 25-80% have been reported from Brazil, the Netherlands and Florida and South Carolina in the USA[6].

When gummy stem blight occurs in fruit, it may be referred to as black rot. Symptoms can vary widely between different types of cucurbits, ranging from black to brown, reddish or white[7], and occur as sunken, watersoaked lesions or dry rots[Error! Bookmark not defined.]. On butternut pumpkin they are described as "*superficial, bronzed irregular patches that may show raised corklike areas*"[8]. Sherf and Macnab[6] also note that "unique, superficial" symptoms of black rot appear on butternut squash, described as "Large, irregular areas of the fruit become bronzed with distinct concentric rings". Again, the photograph supplied bears superficial similarity to brown etch.

T.A Zitter, of Cornell University, has published a number of photographs described as the symptoms of infection by black rot (*D. bryoniae*)[8]. Many – although not all of these pictures – appear similar to the brown etch symptoms observed in Australia. Figure 3 shows a number of these images. Images a, b and c closely resemble symptoms of brown etch observed in Australia. The images in d and e show a spotty effect – perhaps more similar to the symptoms occasionally observed on Kent pumpkins. The final image, f, shows a soft rot, which is a very different symptom to the dry rots otherwise observed. This is the 'classic' expression of black rot as observed on other cucurbits, being a soft, spreading rot penetrating the flesh.

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[2] Stewart JE, Turner AN, Brewer MT. 2015. Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. Fungal biol. 119:370-382.

[3] Shivas R. pers. com. Preliminary information data sheet; *Stagonosporopsis citrulli*.

[4] Chester FD. 1891. Notes on three new or noteworthy diseases of plants. Bull. Torrey Bot. Club. 18:371-374.

[5] Keinath AP. 2011. From native plants in central Europe to cultivated crops worldwide: The emergence of *Didymella bryoniae* as a cucurbit pathogen.

[6] Sherf AF, Macnab AA. 1986. Vegetable Diseases and their Control. Second Edition. Wiley Interscience. 736pp.

[7] Grube M et al. 2011. Emerging multi-pathogen disease caused by Didymella bryoniae and pathogenic bacteria on Styrian oil pumpkin. Eur J Plant Path. 131:539.

[8] Zitter TA. 1992. Fruit rots of squash and pumpkins. Cornell University Fact Sheet 732.10, accessed online 27/9/2016 at vegetablemdonline.ppath.cornell.edu/factsheets/Cucurbit_FrtRots.

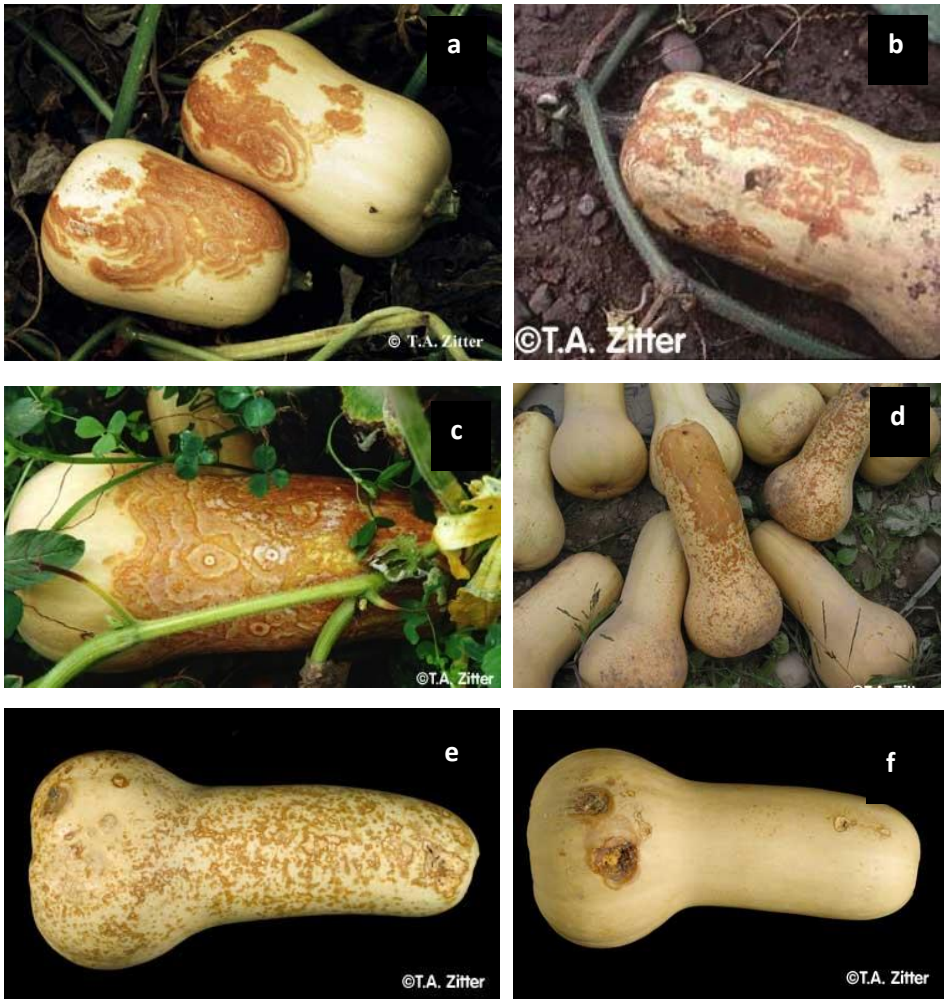

Figure 3. Symptoms of black rot on butternut pumpkin. Photos by TA Zitter, Cornell University

Gerald Holmes (California Polytechnic State University) has also published photographs of symptoms that appear similar to brown etch (Figure 4). These are shown on butternut squash and an unknown variety of light skinned pumpkin. In this case the causal organism is named as *Stagonosporopsis cucurbitacearum*.

Figure 4. Symptoms of *Stagonosporopsis cucurbitacearum* on butternut winter squash and pumpkin (Photos by G. Holmes, Calif. Polytech, Bugwood.org).

In California, researchers and extension staff have noted the impact of *Fusarium solani* f. sp. *cucurbitae* on pumpkin and winter squash crops. Crown and root infections appear as water

soaked lesions near the soil line. However, surfaces of fruit that are in contact with the soil are noted by Koike *et al*. as "*developing circular to oblong, tan to brown, firm, dry, sunken lesions (which) may have concentric rings within them. These spots remain firm unless secondary decay organisms enter the infected area and cause soft, wet rots"*[9].

Figure 5. Fruit lesions on spaghetti squash caused by *Fusarium solani* (Photo in Koike et al, 2006) and "Discolouration of summer squash" (Photos by M. Cantwell, UC Davis).

The effects of *Fusarium* infection on pumpkin (cv. Howden) were extensively studied by Elmer[10]. He classified lesions according to whether they remained hard and dry (type 1) or resulted in a soft rot (type 2). Type 1 lesions were most commonly observed in immature green fruit. These penetrated up to 0.5cm into the rind, but did not expand as the fruit ripened. The *Fusarium* spp. isolated from these lesions, in decreasing order of occurrence, were *F. acuminatum*, *F. graminearum*, *F. equiseti* and *F. avenaceum*. When these fungi were re-inoculated into mature pumpkins, only *F. acuminatum* produced new, type 1 lesions. Type 1 lesions never developed into soft rots. While this suggests that there may be two distinct symptom types, it is suggested that time of infection and environmental conditions strongly influence symptom expression.

Elmer also conducted testing against *F. acuminatum* in-vitro. Most fungicides were found to be relatively ineffective, although mancozeb (Manzate 200) and chlorothalonil (Ridomil Bravo 81W) significantly inhibited spore germination and benomyl (Benlate 50DF) slowed growth. Despite this, the author suggests that chemical suppression is unlikely to be an effective strategy for this disease[10].

New Zealand

Black rot (then named *Mycosphaerella melonis*) was reported on butternut pumpkins in 1967[11]. The symptoms reported were a dry brown rot of the skin, similar to brown etch. However, a later investigation of diseases affecting cucurbits found that *Didymella bryoniae*

[9] Koike ST, Gladders P, Paulus AO. 2006. Vegetable Diseases: A colour handbook. CC Press 320pp.

[10] Elmer WH. 1996. Fusarium fruit rot of pumpkin in Connecticut. Plant Dis.:80:131-135.

[11] Procter CH, Young BR. 1967. A fungous disease of butternut squash and pumpkin. NZ Commercial Grower. 23:35-37.

infection of butternut squash resulted in water-soaked lesions with a watery ooze, commonly filled with fruiting bodies of the fungus. In this study, six species of *Fusarium* were also isolated, some of which were associated with restricted, brown superficial spots with a distinct and non-expanding margin. Unfortunately, the pathogenicity of these fungi was not tested on butternut, only on other pumpkin varieties. In most cases infection resulted in large, rotting lesions with visible mycelium on the surface, rather than dry rots.[12].

In New Zealand, *Fusarium* spp. and *Didymella bryoniae* can affect 10-90% of stored pumpkins. Hawthorne[13] found that the main factors that increased rots were damage at harvest and leaving harvest late. Fungicides had little effect on the development of disease in stored pumpkins. Unfortunately neither the causes, nor the specific symptoms of disease, are described in this study. However, it seems likely that the symptoms observed were primarily soft rots, rather than the dry skin discoloration that is typical of brown etch.

Australian research and reports

The first known report of a syndrome similar to brown etch was in 1974[14]. Butternut pumpkins growing in a commercial crop in Victoria were observed to have a series of reddish-brown concentric rings on the skin that was in contact with the soil. A survey revealed that this symptom was quite common both in the field and during storage for butternut pumpkins. However, it was not found on any other pumpkin variety.

Disease or disorder?

Brown etched pumpkins were collected by Chambers in an attempt to find the responsible pathogen. He was able to consistently isolate *Fusarium avenaceum* from different samples of the diseased tissue. To test pathogenicity, discs of fungal mycelium were inserted into healthy butternut pumpkins. These subsequently developed a dry rot, during storage at 15-20°C, suggesting this pathogen was indeed the cause of the observed symptoms[14].

However, only a year later these findings were questioned by Johnson[15]. Uniquely, this study was designed specifically to examine "*brown etch, or zonate ring spot*" of butternut pumpkin, which is stated to be "*a common disorder in Queensland*". Indeed, symptoms were observed on 50%, 20% and 20% of fruit during three sequential experimental crops. It was particularly observed that flushes of the disease occurred 7-10 days after wet weather.

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[12] Hawthorne BT. 1988. Fungi causing storage rots on fruit of *Cucurbita* spp. NZ J. Exp. Agric. 16:151-157.

[13] Hawthorne BT. 1989. Effects of cultural practices on the incidence of storage rots in Cucurbita spp. NZ J. Crop Hort. Sci. 17:49-54.

[14] Chambers SC. 1975. *Fusarium avenaceum* on butternut pumpkin. Australian

[15] Johnson GI. 1976. Brown etch or zonate ring spot of butternut gramma.

Initial symptoms were orange-brown spots, which appeared on fully grown yet immature fruit. These enlarged, sometimes covering nearly the entire fruit. However, the brown areas stopped expanding once the fruit matured. A number of different fungi were isolated from the diseased areas; *Fusarium oxysporum, Fusarium roseum* 'Equiseti', *Fusarium roseum* 'Acuminatum'*, Ascochyta cucumis.*

These fungi were then inoculated into half grown, fully grown but immature, and fully mature pumpkins. The effects after 5 days of storage are summarized in Table 1.

Table 1. Result of inoculation of fungus into different maturity stages of butternut pumpkin.

	Butternut pumpkin development stage		
	Half grown	*Full grown immature*	*Fully mature*
Fusarium oxysporum	Sunken lesion	**Typical brown etch**	Small, superficial lesion
Fusarium roseum 'Equiseti'			
Fusarium roseum 'Acuminatum'	None	Small, discoloured area	None
Ascochyta cucumis			

The author concluded that;

- Brown etch symptoms are caused by a host reaction to fungal infection
- Symptom expression is affected by weather at the time of infection
- Fruit are most susceptible when fully grown, but still immature

However, a third potential cause was proposed by DB Letham, a senior plant pathologist at the prestigious Biological and Chemical Research Institute. His publication on Diseases of Cucurbits describes symptoms of "reddish brown surface markings on the skin, commonly seen late in the season". This is illustrated by a photograph of a butternut pumpkin with clear symptoms of brown etch. In this case, the cause is attributed to cold conditions during late fruit growth[16] – not a fungal disease.

It was therefore still unclear whether the cause of brown etch was primarily physiological, or fungal. Trials in 1988 at the Gatton field station in Qld (an area where brown etch is common) attempted to test both of these possibilities. Butternut pumpkins were grown on reflective mulch with trickle irrigation and compared to normal production methods. Unfortunately, both trials failed to produce any pumpkins with symptoms of brown etch. While this suggested that keeping pumpkins dry prevented brown etch, results were inconclusive[17].

[16] Letham DB. 1981. Diseases of cucurbits. Agfact H8.AB.24, eighth edition. Biological and Chemical Research Institute Rydalmere

[17] Jackson KJ, Harper TW, Schrodter GN, Duff AA. 1989. Marketing aspects of heavy vegetable research in Queensland. Acta Hort. 247:137-142.

Despite this, subsequent texts have asserted that the cause of brown etch is primarily fungal. Brown, or surface etch, is included in 'Diseases of vegetable crops in Australia'[18]. The cause is given as *Fusarium* spp. and *Didymella bryoniae*. It is further stated that these fungi "*are soil inhabitants and invade the fruit where ground contact has occurred. The disease is favoured by warm, wet weather*".

Breeding for brown etch resistance

The one fact clear from previous research was that – regardless of the cause – there are wide differences in brown etch susceptibility between different cucurbit varieties. A project was therefore funded by HRDC and the Queensland vegetable growers to develop butternut varieties resistant to brown etch.

Initial fieldwork identified a number of butternut breeding lines that appeared to have strong resistance to brown etch. Trials in Mareeba and Gatton in 1995 compared ten hybrid selections with a commercial variety (Yates butternut large). At Mareeba, 74% of the Yates butternuts had symptoms of brown etch compared to 0 – 46% of the trial selections. Rates of brown etch were generally lower in Gatton, with 18% affected fruit in the Yates butternuts. In this trial five of the hybrid selections had no etch symptoms at all, four selections had less than 5% affected fruit, and one had 13% affected fruit.

While there were differences between the two sites in terms of the best performing variety, a number of the selections (12.05, 26.12, 2.1) had significantly lower levels of etch than the Yates butternuts at both sites. Not only were the number of etched fruit significantly lower in the selected varieties, but the severity of infection (% of surface area affected) was reduced from 24% to 3% [19].

This promising research was continued in a subsequent project – VG96010[20]. This project on pumpkin varietal improvement aimed to develop a butternut pumpkin with good agronomic qualities as well as resistance to both virus infection and brown etch.

Developing brown etch resistance proved particularly problematic, as occurrence of brown etch within different 'families' and crosses was highly variable, and did not fit well with any simple genetic model. It is suggested that resistance to brown etch depends on the presence of more than one gene, and that there may be both dominance and additive genetic effects. So, for example, to produce an F1 hybrid with good resistance to brown etch, both parents must carry that trait.

The report also notes the strong effect of environmental conditions on the appearance of brown etch.

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[18] Persley D, Cooke T, House S. 2010. Diseases of Vegetable Crops in Australia. CSIRO Publishing. Collingwood, Victoria.

[19] Loader L, Herrington M, Jackson K, Reid D, Trevorrow P. 1996. Etch resistance development in butternut pumpkin. HAL Final Report VG316.

[20] Herrington M *et al*. 2000. Pumpkin varietal improvement. HAL Final Report VG96010.

In this work, it is assumed that brown etch symptoms are the result of black rot (*Didymella bryoniae*) infection. Certainly, this pathogen could be consistently isolated from brown etch affected pumpkins in Mareeba.

Given the complexity of breeding programs, and the relatively long time required from planting to fruit maturity, the authors attempted to develop a rapid screening method for black rot resistance. This involved inoculating the pathogen into or onto glasshouse grown pumpkin plants and observing how disease symptoms developed.

Unfortunately, this method did not prove successful. A number of the lines selected for resistance to brown etch were more strongly affected by disease than the Yates butternut control plants. Results were also extremely variable, regardless of the method used to inoculate the young plant. For example, for 20 plants of line 3272 (selected for brown etch resistance), leaf death ranged from 30 to 100%, and petiole death ranged from 0 to 100%.

While there would be clear advantages to a rapid screening, glasshouse based method, it was concluded that field trials were the only way to confidently assess brown etch resistance in different breeding lines of butternut pumpkins.

The outcome of the project was development of "Sunset QHI', a butternut pumpkin variety stated to have high resistance to brown etch, high virus resistance, good fruit quality and high productivity. Field trials in Mareeba comparing Sunset QHI to Yates Butternut Large resulted in etch ratings of 0.12 and 1.75 respectively, where the percentage of fruit surface affected was rated from 0% (grade=0) to >30% (grade=4). Fruit size and total yield were the same for the two varieties, however it was clear that marketable yield was higher for the new variety[21].

Sunset QHI has been commercialised, and is available from South Pacific Seeds (SPS). It is claimed to have intermediate resistance to a number of viruses as well as partial resistance to brown etch. According to the SPS information sheet[22], it is "*resistant to surface browning or etch (caused by Fusarium strain), with significantly less etch under extreme conditions than standard Butternut Large*".

[21] Herrington M. 2007. Success and limitations of some cucurbit releases in Queensland. Acta Hort. 731:505-511.

[22] South Pacific Seeds. 2015. Sunset QHI data sheet. spssales.com.au/seeds/pumpkin/all/. Accessed online 29/9/16.

Conclusions

Brown etch is a major issue in Australia, and is reported as affecting up to 50% of butternut pumpkins. Even though it is superficial, and does not usually affect the flesh, heavily marked pumpkins are unsalable. The result is major losses every year. While brown etch mainly affects butternut pumpkins, superficial brown spotting has also been reported on Kent varieties.

Butternut pumpkin is a variety of *Cucurbita moschata*. Also known as 'tropical pumpkins', *C. moschata* are more tolerant of hot, humid weather than cultivars of *C. maxima* (traditional, hard skinned pumpkin) or *C. pepo* (summer squash such as zucchini and patty pans). Varieties such as "Ken's special' or 'Kent' are often listed as *C. moschata* (butternut types), but may in fact be *C. maxima* x *C. moschata* hybrids. This may explain why symptoms still sometimes appear on these varieties, but are less severe.

Varieties of *C. moschata* are grown extensively around the world. They are believed to have originated in the Americas, and are grown in large quantities in southern USA and Mexico. They are also an important crop in Africa and parts of Europe. In addition to butternuts, varieties include cheese squash, crookneck squash and a wide range of hybrid pumpkins (Figure 6).

Figure 6. Some varieties of C. moschata include Long Island cheese squash, crookneck squash and many others, such as this 'Bliss' kabocha type hybrid (right).

The lack of research on brown etch, both in Australia and internationally, therefore seems extraordinary. While brown etch symptoms do occur in the USA, the extent of losses and severity of symptoms is unclear. It could be useful to know whether butternut pumpkin growers in other parts of the world suffer the same losses to brown etch as we do in Australia and if not, why not.

The majority of reports suggest that brown etch is caused by *Fusarium* sp. and / or black rot (*Didymella bryoniae*). Such theories are generally proven using Koch's postulates; isolating the pathogen, re-inoculating into a healthy plant or fruit, observing to see if the same symptoms re-occur, then re-isolating the same pathogen from the diseased tissue.

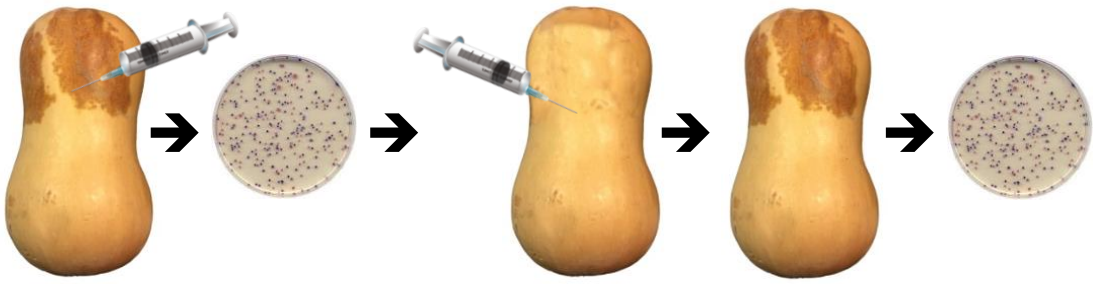


Figure 7. Kochs postulates. The pathogen needs to be isolated from diseased tissue, inoculated into a healthy specimen, observed to see if the same symptoms develop, then re-isolated again from the diseased tissue.

In this case, there is only slender evidence supporting these fungi as having a causal role. They are common, soil dwelling organisms, which can infect damaged tissue. This review has found only three publications that specifically tested whether the organisms isolated from brown etched pumpkins could produce the same or similar symptoms in inoculated fruit. Interestingly, no two of these studies agree on the organisms responsible;

- Elmer[10] re-inoculated *Fusarium acuminatum* isolated from diseased fruit into healthy pumpkins, which subsequently developed a dry rot. Other *Fusarium* sp. caused sunken, wet rots in inoculated fruit.
- Chambers[14] inoculated discs of *F. avenaceum* mycelium into butternut pumpkin, which then developed a dry rot.
- Johnson[15] produced symptoms of brown etch by inoculating fruit with *F. oxysporum* and *F. roseum* 'Equiseti'. However, this was only successful when the pumpkins were fully grown but still immature.

It seems possible that brown etch is at least in part a physiological, rather than a pathological disorder. There seems to be an association between wetness and the onset of symptoms, although perhaps with a delay between[15]. However, the assertion by Letham[16] that brown etch is due to exposure to cold weather does not seem probable given the prevalence of the disorder in Far North Queensland.

Regardless of the cause of brown etch, considerable effort has been put into breeding resistant varieties. The result is 'Sunset QHI', developed by QDAF and commercialised by South Pacific Seeds. It is unclear how widely this variety has been adopted, or whether it is suitable for all growing regions and seasons.

One other outcome from the breeding program was the observation that resistance to brown etch did not appear to be a simple mechanism, but may require more than one genetic change. However, susceptibility to brown etch appears to be specific to *C. moschata* varieties and hybrids. With faster and cheaper DNA profiling than ever before, it may yet be possible to identify the genetic changes that increase susceptibility to brown etch, as well as those that confer resistance.

Appendix 2. Laboratory studies of brown etch

2017 – Dr Len Tesoriero, CropDoc

Background

This report details studies that provide further data to determine if fungal plant pathogen is the cause pumpkin brown etch. It follows after previous attempts in 2016 and 2017 failed to consistently isolate suspect pathogens from affected fruit. However there was sporadic recovery of various fungal isolates of which some were consistent with pathogens described in the literature. In 2017 a pathogenicity study was conducted in field pumpkins where fruit were inoculated with candidate fungal isolates of *Fusarium solani* and *Didymella bryoniae*. Inoculated fruit did not consistently develop brown etch symptoms. In fact, a higher proportion of non-inoculated control fruit had developed brown etch symptoms at harvest. Those results were inconsistent with several overseas references that identify these fungi as causing brown etch-like symptoms in butternut pumpkins[1, 2.] Furthermore an earlier study in Australia determined that *Fusarium* species could give rise to brown etch symptoms[3]. Personal communication with the author of that study (Dr Greg Johnson) suggested only immature fruit could be successfully infected with the pathogens. Therefore pathogenicity testing was replicated in this study taking into account that precondition.

Methods

1. Isolations from brown etch affected tissue

Five mature fruit with typical brown etch symptoms were collected from a field (Somersby, NSW) and tested for potential fungal pathogens. Fruit surfaces from affected and healthy areas were swabbed with 70% ethanol aseptically cut from the fruit (Figure 1) and sub-epidermal and cuticle tissue was plated to agar media (1/4-strength potato dextrose agar amended with 100ppm novobiocin [1/4PDA+N). Plates were incubated at 25°C and regularly inspected for fungal growth. Fungi were sub-cultured and identified using morphological taxonomy. Isolates of *Fusarium* sp. were sub-cultured and kept for pathogenicity studies detailed below.

Microscopic examination of further fruit with brown etch symptoms on which a white scaly cuticle (Figure 2) had formed revealed it was impregnated with small black fungal fruiting bodies (pycnidia) consistent with a *Phoma* species which is the asexual state of the fungus causing Gummy Stem Blight (GSB) and Black Rot (BR) of cucurbits[2, 4]. Spores were released into a water suspension and streaked onto 1/4PDA+N. Single spore isolated were sub-cultured to the same medium and kept for pathogenicity studies detailed below.

[1] Sherf AF & Macnab AA. 1986. Vegetable diseases and their control. 2nd Ed Wiley Interscience, 736pp.

[2] Zitter TA. 1996. Black Rot. In: *Compendium of Cucurbit Diseases*, APS Press (Eds. Zitter, Hopkins & Thomas) p48.

[3] Johnson GR. 1976. Brown etch or zonate ring spot of butternut gramma. Australasian Plant Pathology, 5:48.

[4] Stewart JE, Turner AN & Brewer MT. 2015. Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. Fungal Biology, 119:370-382.

2. Pathogenicity testing of fungi isolated from brown etch affected fruit

Spore suspensions from isolates of *Didymella brioniae* and *Fusarium* sp.(originally isolated from pumpkins with brown etch symptoms) were inoculated onto the upper (surface facing the sky) and lower surface (surface sitting on the ground) of 30 immature fruit. On each fruit surface a 5x5cm square was drawn with a marker pen onto which the spore suspension (approximately 10^7 spores) was applied with a paint brush. A set of fruit was treated in the same way with sterile water, serving as controls. Treated fruit were marked with coloured flag tape and left to mature when they were harvested and assessed for brown etch symptoms.

Results

There was sporadic fungal development from brown etch-affected and unaffected tissue samples with no distinct correlation between consistent recovery and symptoms. A number of fungi commonly associated with cucurbits were identified including: *Fusarium* sp.; *Colletotrichum* sp.; *Plectosphaerella* sp; and *Rhizoctonia* sp. (Figure 3).

In the pathogenicity study there were no fruit that had been inoculated with the *Phoma* isolate developed typical BR symptoms with a scaly white cuticle studded with fungal pycnidia. However several fruit that had been inoculated with *Fusarium* did develop these symptoms: on approximately 10% of the upper surface of a single fruit; and in association with varying areas on 18 of 23 fruit with etch symptoms on their lower surfaces. There appeared to be no significant difference in the proportion of fruit that developed brown etch between those inoculated with the *Phoma* isolate and the water controls, but there were about twice as many fruit affected with BE when fruit were inoculated with *Fusarium* (Table 1).

Table 1. Proportion of pumpkin fruit lower surfaces displaying brown etch symptoms

Water control	*Phoma* sp.	*Fusarium* sp.
12/30	11/30	23/30

Conclusions

It is difficult to make firm conclusions from these experiments. The lack of consistent recovery of potential pathogens from fruit with typical brown etch symptoms is consistent with earlier pathology studies conducted in this project suggesting that fungi are not the primary cause of this disease. The field pathogenicity study is also difficult to interpret, particularly given there was clearly a background of the *Phoma* sp. at the field site which appears to have confounded the effect of inoculum treatments. This was particularly evident where the *Phoma* sp. was recovered from scaly white surfaces on fruit that also had brown etch symptoms following inoculation with *Fusarium* sp. The relatively dry environmental conditions during the trial period may have affected these results. Such conditions would not be conducive to successful infection of the upper surfaces of fruit. Another approach which could be applied to confirm if the *Fusarium* and *Phoma* spp. are causal of brown etch is to conduct a pathogenicity experiment with excised fruit – similar to how Johnson conducted the work in his published work.

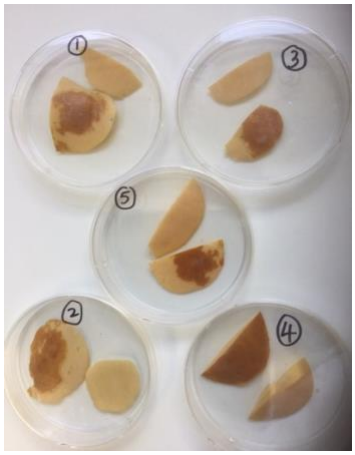


Figure 1. Segments of pumpkins used for isolations

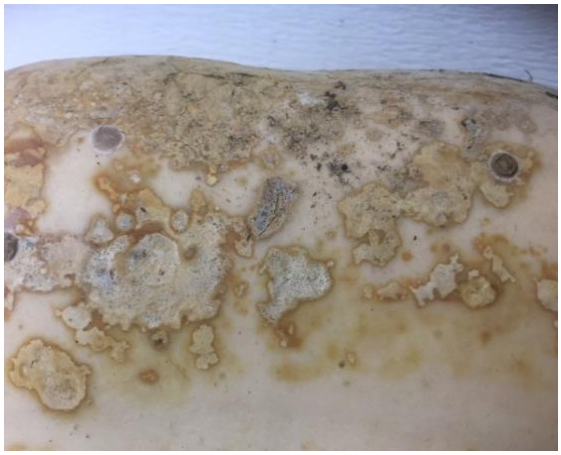


Figure 2. Pumpkin fruit embedded with black fungal pycnidia in scaly cuticle

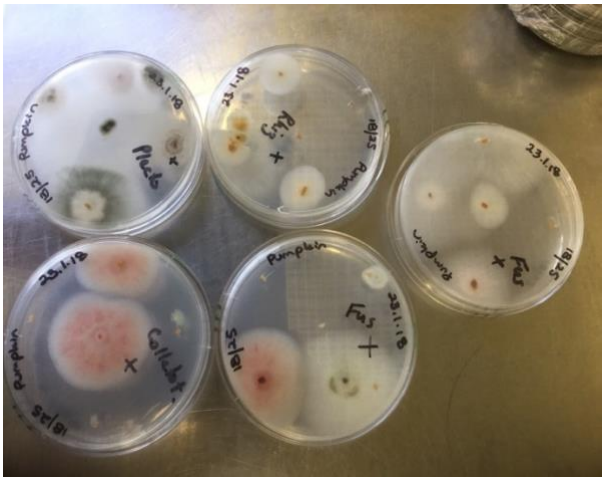


Figure 3. Fungal agar cultures from pumpkin fruit

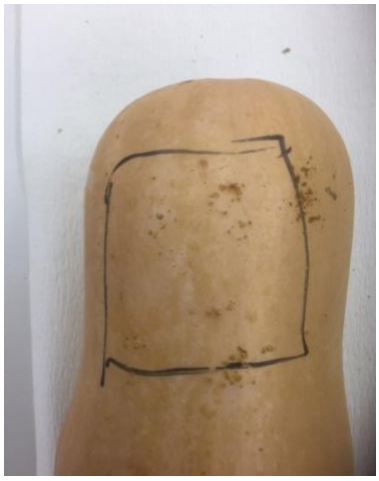


Figure 4. Upper surface of pumpkin inoculated with *Fusarium* sp. isolate

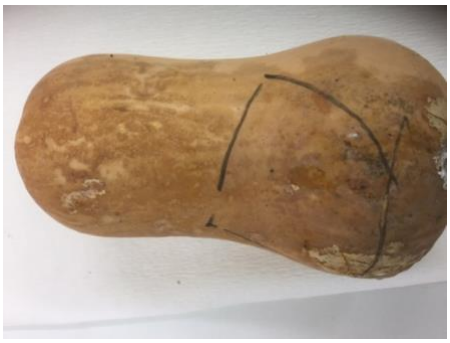


Figure 5. Lower surface of water control pumpkin

2019 – Dr Natalie Elias, AHR

Introduction

Brown etch, or 'rust mark' is a major issue for Australian butternut pumpkin growers. It can result in significant losses, product quality downgrades or rejections in the market. It may develop in the field or post-harvest. Some growers have experienced losses of up to 50% , and in some cases, crops may be abandoned due to the large percentage of affected fruit.

Stagonosporopsis cucurbitacearum (previously *Didymella bryoniae*) is known to cause gummy stem blight (GSB) (vegetative matter infection) or black rot (fruit infection) in cucurbits. Description and images of black rot in butternut pumpkins from North America are identical to the symptoms of brown etch in Australia. Previous research conducted in Australia have also implicated *S. cucurbitacearum* and *Fusarium* sp. as the cause of etch symptoms on butternut fruit (Johnson, 1976). An alternate theory proposed is that these symptoms are not related to a fungal pathogen and are a physiological response to environmental conditions.

Further research is required to determine if *S. cucurbitacearum* or other fungal pathogens are the cause of etch symptoms of butternut pumpkins.

A part of Koch's postulates is the inoculation of healthy fruit with a purified isolate to determine if the same disease symptoms can be replicated.

Aim

To determine if inoculation of healthy butternut pumpkins with an isolate of *Stagonosporopsis cucurbitacearum* induces symptoms similar to brown etch.

Method

A fungal isolate obtained from an etched pumpkin was purified and identified using ITS-region/BLAST analysis techniques, as being *S. cucurbitacearum* (analysis conducted by Royal Botanic Gardens Disease Diagnostics Service).

This isolate was bulked up on half-strength potato dextrose agar (PDA) and placed under UV-A light with a 12-hour photoperiod for 10 days to induce sporulation. Plates were blended with tap water to create a spore suspension.

On the 13th February 2019 Healthy green immature pumpkins were harvested from a field site in Richmond, NSW with no known history of cucurbit production. The pumpkins were brought back into the laboratory and treated on the same day.

The pumpkins were split into the following three treatment groups, with 15 pumpkins in each group:

1. No wounding, no inoculation
2. Wounding , no inoculation
3. Wounding, inoculation with spore suspension of *S. cucurbitacearum*

All pumpkins were surface sterilised with 75% ethanol. For pumpkins being wounded, superficial wounding was done with a scouring pad. Each pumpkin had its own scourer pad to avoid cross-contamination (see figure 1a). For those being inoculated, up to 2ml of the spore suspension was placed over the wound (figure 1b). All

pumpkins were placed in an unsealed zip-lock bag and into a sealed plastic tub with warm water in the bottom (figure 1c). Pumpkin were elevated so that they were not in direct contact with the water and left for 72 hours.

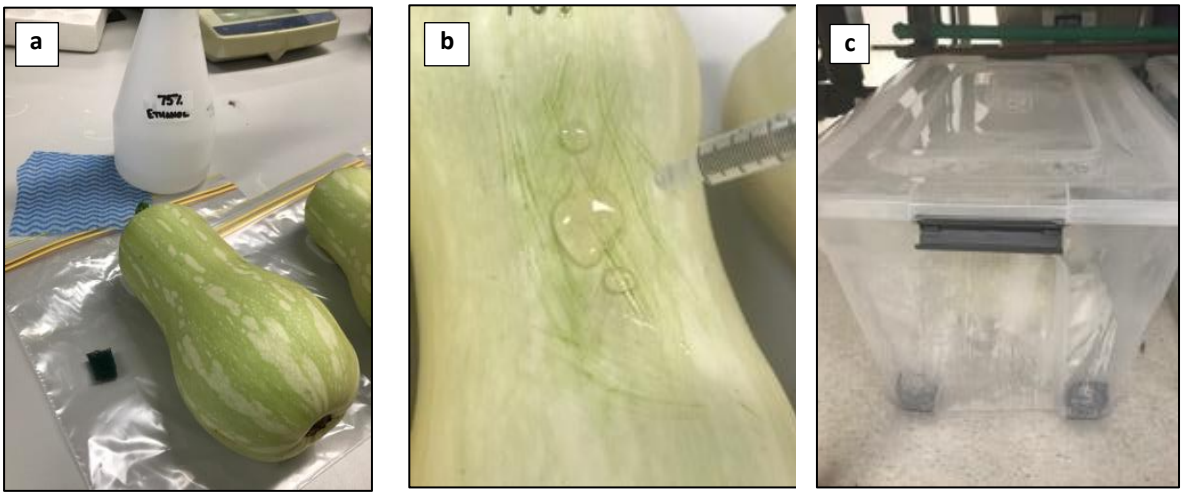


Figure 1. a) Green pumpkin with individual scourer pad for wounding b) wounded pumpkin being inoculated with *S. cucurbitacearum* spore suspension c) humid chamber that pumpkins were incubated in for 72 hours.

Results

After 72 hours pumpkins were removed from the humid chamber and assessed for symptoms of etch. They were continually assessed for 30 days post-inoculation for symptom development. All of the unwounded, uninoculated pumpkins and wounded, uninoculated pumpkins remained free of etch-like symptoms (see figure 2a and 2b). All 15 of the wounded and inoculated pumpkins formed lesions only around the site of wounding (see figure 2c).

Figure 1. Pumpkins that were a) unwounded, uninoculated; b) wounded, uninoculated; c) wounded, inoculated.

Lesion formation as also evaluated based on whether it was typical or atypical in its likeness to etch seen in the field. Atypical formed light brown lesion that look more superficial compared to darker typical etch-like lesions (see figure 3a and 3b). Of the 15 pumpkins which responded with lesion formation, 12 displayed typical etch and 3 with atypical etch.

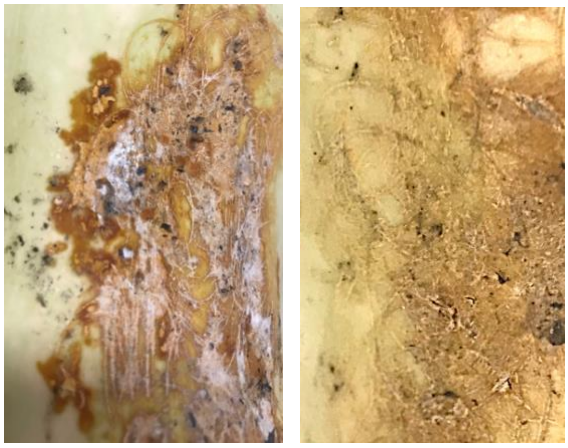


Figure 2. Examples of typical (left) and atypical (right) lesions seen on immature fruit inoculated with *S. cucurbitacearum*

Etched fruit were surface sterilised and re-isolation conducted from etched and non-etched areas on the fruit. Non-etched regions did not produce any growth and etched regions grew a fungal isolate with morphology similar to the *S. cucurbitacearum* isolate that was identified using the ITS region. It was concluded based on the morphology that this was the same pathogen.

Discussion

The recreation of etch like symptoms in healthy pumpkins with the introduction of *S. cucurbitacearum* and the re-isolation of this pathogen, suggests that it is a major cause of pumpkin etch. The difference in responses, i.e. typical vs atypical may be due to slight difference in maturity which have previously been shown to be a critical factor in expression of this disease[5].

It should also be noted that with lab inoculations the pathogen is being artificially introduced at high spore concentrations via a wound in the skin, whereas often in the field the infection is systemic and therefore expression of symptoms is likely to differ.

It should be noted that in obtaining the original pure isolated other pathogens were found in etched pumpkin, including *Fusarium equiseti* and *Colletotrichum* spp. It is possible that these pathogens are working in a complex with S. cucurbitacearum and further research should be conducted to elucidate their role in pumpkin etch.

Conclusion

A major of cause of pumpkin etch is the pathogen *S. cucurbitacearum*. For lab inoculations, symptom expression is highly dependent on pumpkin maturity at time of infection.

5 Johnson, G. I. (1976). Brown etch or zonate ring spot of butternut gramma. Australasian Plant Pathology, 5(4), 48-48.

2019 – Dr Len Tesoriero, CropDoc

Introduction

A key question for this project is to determine if brown etch (*BE*) of butternut pumpkin is an environmental disorder/physiological condition, or a disease caused by a plant pathogen. Certain overseas reports suggest *BE* is due to an infection by the fungus *Didymella bryoniae* which causes Gummy Stem Blight (*GSB*) and Black Fruit Rot (*BFR*) on a range of cucurbits[2,5,6]. However, none of these reports clearly demonstrate the causal nature of this fungus as they are extension publications. Meanwhile similar extension publications include images and descriptions of *BFR* only[1,7].

A previous Australian study[3] implicated both *D. bryoniae* and three *Fusarium* species with *BE* symptoms. Sunken or superficial lesions typical of *BE* developed in 5 days after inoculation of fully-sized but immature fruit. The author suggested that the symptoms were a 'host reaction following infection' and postulated that symptom development was strongly influenced by fruit maturity and environmental conditions. Alternatively, other reports from the USA and Australia suggested *BE* was an environmental disorder linking it with cold injury ('cold pox')[8,9].

Previous attempts in the course of this project failed to consistently isolate suspect pathogens from *BE*-affected fruit that had been collected from commercial farms. Most tissue pieces taken from across *BE* affected fruit and cultured on media produced no fungal colonies although there was sporadic and low recovery of various fungi from affected tissue that included: *D. bryoniae* (now known as *Stagonosporopsis cucurbitacearum*[4]; the former name is used for simplicity in this report), *Fusarium solani* (also known as *Nectria haematococca* and shown in the literature to cause a cucurbit fruit rot[1,5]) and *Colletotrichum orbiculare* (the cause of anthracnose disease on cucurbits which includes fruit spotting symptoms[1,5]). It could be interpreted that these fungi were secondary invaders and not the primary cause of *BE* symptoms.

A field experiment in 2017 attempted to demonstrate pathogenicity of fungal isolates of *Fusarium solani* and *D. bryoniae*. Fruit on growing vines that had been inoculated with fungal spore suspensions did not consistently develop brown etch symptoms. In fact, a higher proportion of non-inoculated control fruit had developed brown etch symptoms when assessed at harvest. Again, these results were inconsistent with several references cited above.

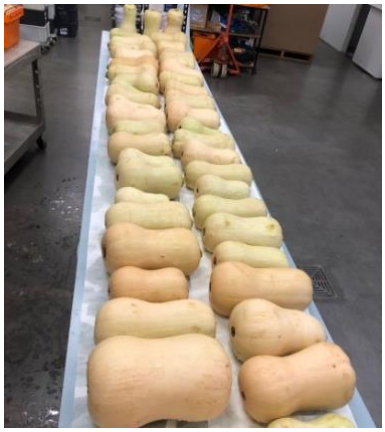
This experiment was designed to test pathogenicity of *F. solani* and *D. bryoniae* isolates on excised fruit that were incubated under defined environmental conditions using methods similar to that described by Johnson (1976) in the previous Australian study.

Methods & Results

Fruit were supplied by a commercial farm in Mareeba, North Queensland. They were in transit and storage for approximately 10 days prior to commencement of this experiment. Fruit were washed and graded into immature fruit (which had green or white skin) and mature fruit (pale butterscotch-coloured skin) (figure 3). Two fruit with *BE* symptoms were rejected from the experiment and put aside for diagnostic analysis. Treatments were applied (Table 1) to batches of six fruit in each of 20 plastic containers (figure 3):

Table 1. Treatments applied to immature and mature pumpkins

1	Nil control
2	*D. bryoniae* isolate #1 – mycelial fragments (10^6 cfu/mL) applied with a brush
3	*D. bryoniae* isolate #2 – mycelial fragments (10^6 cfu/mL) applied with a brush
4	*F. solani* – spore suspension (10^6 cfu/mL) applied with a brush
5	*D. bryoniae* culture filtrate – blended mycelial extracts from both isolates that were passed through 0.2micron filter to remove live fungus



Washed fruit ready for maturity grading

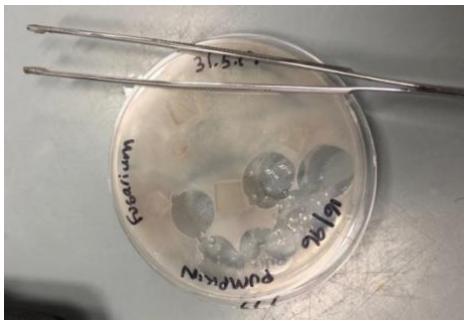
Fruit were marked with a 55mm circle and the area within scoured lightly to facilitate infection

Filter paper was moistened and placed over inoculated area on fruit to maintain conditions conducive for infection

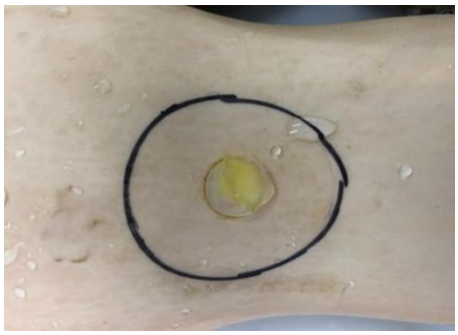
Boxes with 6 fruit each were covered in a sealable plastic bag and stored in the temperature-controlled room at 22°C.

Figure 3. Initial application of inoculum

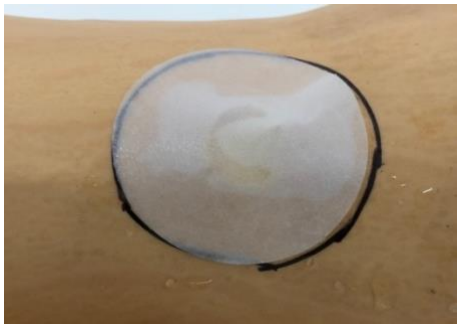
Fruit were checked regularly to ensure high relative humidity was maintained inside plastic bags. No evidence of infection was recorded after 15 days incubation. At this time fruit were re-inoculated using 12mm plugs of fungal agar culture which were placed at the centre of the previous infection area on fruit (Figure 2B). Fresh moistened filter papers were used to re-cover plugs (Figure 2C). The culture filtrate treated fruit were not re-inoculated while the negative control fruit received a sterile agar plug as per the fungal inoculations.



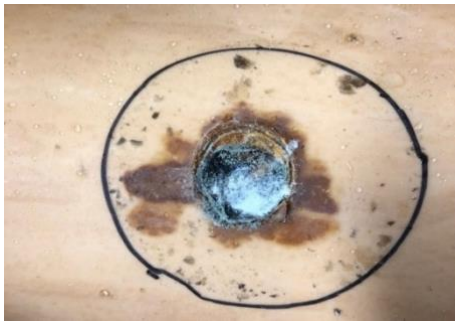
A. Plugs of fungal agar cultures were removed with a sterilised cork borer



B. The same cork borer was used to excise a plug of skin from pumpkins onto which the agar plug was placed

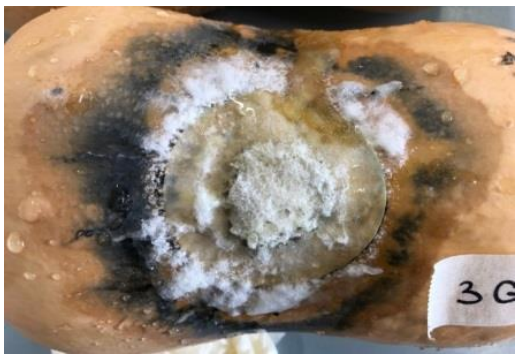


C. Fruit were covered with an agar plug with and re-covered with a moistened filter paper



D. BE symptoms extending from the D. bryoniae #1 inoculated area on a fruit after incubation for14 days.

E. BFR symptoms developing on all fruit inoculated with isolate 2 of D. bryoniae. Note one fruit has BE symptoms.



F. Close-up view of BFR symptoms at 21 days incubation. Note that there is a brown area at the margin of the BFR zone on fruit that is similar in colour to BE.

Figure 4. Re-inoculation of butternut pumpkins with agar plugs and development of fruit rot symptoms

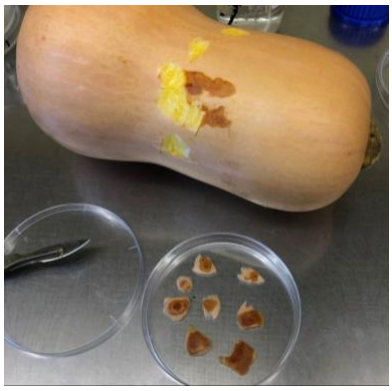
Fruit were scored on a final occasion when they had incubated for 21 days after agar plugs were applied. All negative control fruit showed no rotting and the plant tissue around the inoculation disc had callused and healed. Fruit inoculated with the *F. solani* isolate and the culture filtrate of *D. bryoniae* treatments also failed to develop any *BE* or *BFR* symptoms. A varying proportion of fruit inoculated with either *D. bryoniae* isolate did develop *BE* and/or *BFR* symptoms (Figure 4 D, E & F). More of the green fruit were clearly affected by both diseases (Table 2). Treatments of either *D. bryoniae* isolates were associated with approximately 50% of fruit with *BE* symptoms. *D. bryoniae* isolate #2 also caused 50% of the green fruit to develop *BFR* symptoms. *D. bryoniae* isolate #1 was less aggressive with only 8.5% of green fruit affected with *BFR*. At this time *BFR* symptoms had developed to almost cover some fruit (Figure 4E). The *BFR* lesions had a halo of brown tissue with a developing soft rot. None of the ripe fruit inoculated with either *D. bryoniae* isolates was affected by *BFR* (Table 2).

Table 2. Proportions of fruit affected by Brown Etch (BE) or Black Fruit Rot (BFR)

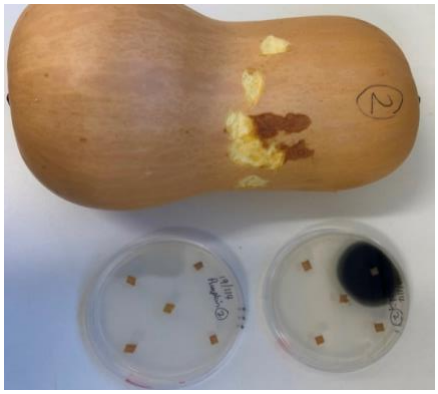
	Green fruit		Mature fruit	
Treatment	**Brown Etch (%)**	**Black Fruit Rot (%)**	**Brown Etch (%)**	**Black Fruit Rot (%)**
Control	0	0	0	0
D. bryoniae isolate 1	**33**	8.5	17	0
D. bryoniae isolate 2	**42**	50	8.5	0
Fusarium solani	0	0	0	0
GSB 1 & 2 culture filtrate	0	0	0	0

Diagnostic analysis of the two fruit which had been placed aside before treatments were applied failed to detect any significant plant pathogens. A single isolate of the common airborne fungus *Alternaria* sp. was recovered from one fruit (Figure 5A and B).

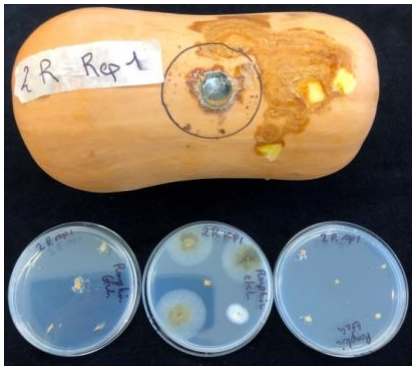
Of the 12 fruit that developed etch during the experiment only 2 (approximately 17%) yielded *D. bryoniae* from affected tissue. Recovery of *D. bryoniae* was also low when it was detected, particularly from tissue near the margin of the *BE* symptoms (Figure 5C). There were also several cases (5/12) where the *BE* symptoms were remote from the inoculation zone suggesting an independent cause of *BE* (Figure 5D). In contrast, *D. bryoniae* was recovered from **all** fruit with *BFR* symptoms.



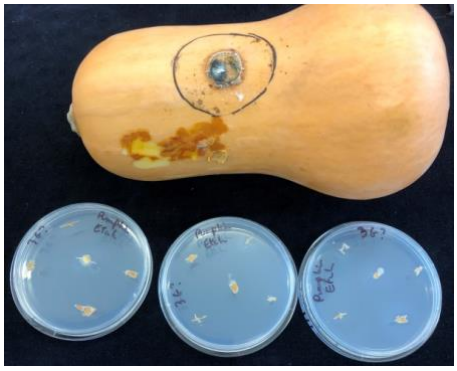
A. Pieces of BE-affected tissue aseptically excised from fruit set aside from the experiment.



B. Agar plates with a single isolate of the fungus Alternaria sp. recovered from BE-affected tissue



C. D. bryoniae recovered from three of fifteen BE-affected tissue pieces(middle plate) and only from the middle of three tissue areas sampled



D. BE symptoms developed remotely from the inoculation zone and no D. bryoniae was recovered.

Figure 5. Recovery of fungi from fruit with BE symptoms

Discussion

This experiment has confirmed fungal isolates of *D. bryoniae* can cause *BE* and *BFR* of butternut pumpkin. It confirms findings from a preliminary pathogenicity trial from this project with immature detached fruit. It should be noted that the fruit used in the preliminary trial were greener and were inoculated sooner after being harvested. This might explain why about double the proportion of fruit developed *BE* in that trial.

It was clearly demonstrated here that green fruit are more easily infected by *D. bryoniae,* which is consistent with the findings of Johnson (1976). Despite fulfilling Koch's postulates in some cases, the low recovery rate of *D. bryoniae* from symptomatic tissue in this experiment and previous diagnostic pathology results casts some doubt over causality in all cases. **The key question remains: is *D. bryoniae* the only cause of *BE* symptoms?**

Some of these results can be explained by the fact that *D. bryoniae* is a necrotrophic pathogen. It releases metabolites that break down tissue ahead of colonisation[10,11]. This could explain why *D. bryoniae* was not recovered from all affected tissue in this experiment and previous attempts at isolation. However, it does not explain why it was not recovered at all on many occasions despite tissue being sampled across lesions.

One explanation is that *D. bryoniae* may trigger a plant response that spreads across fruit epidermal tissue even if infection is not successful. Johnson (1976) had suggested *BE* was a plant response to infection by *D. bryoniae* and several *Fusarium* species isolates in his report. That could imply *BE* symptoms result from a more generalised plant response of the pumpkin epidermis to fungal pathogen attack. In this experiment an isolate of *F. solani* failed to infect fruit or cause *BE* symptoms suggesting any generalised plant response is not universal, at least not if the fungus is a non-pathogen.

Given the use of a similar inoculation technique to those described by Johnson (1976) there were some notable differences in our results. One difference was a slower development of disease symptoms apparent in this study. More notably, in this study, several fruit developed typical *BFR* symptoms whereas none was described by Johnson. These differences could be due to the genetics of fungal isolates used in the two studies, although isolates used in this study had been isolated from fruit with *BE* symptoms and yet they also produced *BFR*.

If *D. bryoniae* causes both *BE* and *BFR*, what governs development into either *BE* or *BFR*? Our previous storage and diagnostic studies indicate that *BFR* rarely appears as a consequence of *BE* symptoms. More detailed research of the infection process is required to understand how *BE* or *BFR* symptoms develop in response to infection. Applying deep genome sequencing to 'affected' versus 'healthy' tissue would identify differences in their respective microbial genetic profiles. This approach would also identify other potential pathogens.

There may be environmental factors that also trigger *BE* symptoms. High humidity was applied in this experiment to all treatments and failed to incite *BE* symptoms in negative control fruit. It was noted that a few fruit from different treatments developed corky oedemas on their surfaces which did turn brown. However, this symptom was distinct from *BE* and exposure to high humidity is known to elicit oedema formation. Further research should also attempt to identify other specific environmental conditions that may cause BE symptoms. Diurnal applications of cold packs to fruit while they are developing could test the assertion of Cox (1971) for a 'cold pox' disorder.

If *D. bryoniae* is the primary cause of *BE* then there needs to be more research to develop effective management options. Fungicide sprays have been used to manage *GSB* in several cucurbit crops. However there are several overseas reports of decreased sensitivity in key activity groups such as the strobilurins (group 11), DMIs(Group 3) and SDHIs (Group 7) [12,13]. The fungicide sensitivity status of *D. bryoniae* isolates in Australia is unknown and needs to be assessed.

D. bryoniae is known to be seed-borne and therefore seed disinfestation is a key preventative strategy. Unfortunately, dry heat treatments are known to decrease seed germination in larger-seeded cucurbits despite them being useful for other important pathogens[14]. Wet disinfectant treatments may be more useful in this case, or applying lower temperatures / duration of dry treatments than are used for bacterial and viral pathogen disinfection.

Previous overseas research has demonstrated that *D. bryoniae* can survive for up to two years in infected crown tissue left on the soil surface[15]. Burial of residues from affected crops together with a minimum two year rotation are therefore recommended. There is potential to use microbial products that accelerate decomposition of the crop residues, and there are also microbial biocontrols registered oversea for *GSB* control. These options should be explored in further research under Australian condition. Given *GSB* also significantly affects melons and greenhouse cucumbers a broader project might be envisaged.

References

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Appendix 3. RNA analysis and microscopy

Summarised from the thesis submitted by Firdause Al Haj Hasson, University of Sydney for fulfilment of the requirements of a Masters of Philosophy degree.

RNA analysis

Aim

To determine whether brown etch symptoms of the disease are caused by a biotic factor, eg fungi or bacteria, or by an external abiotic environmental condition.

Method

Multiple mature butternut squash samples (n =3 fruits) were collected from a farm in Queensland (Amaros Farm 2km west of Mareeba), Fruit skin tissues were dissected into three replicates in a molecular laboratory at the Plant-Breeding Institute, University of Sydney. Each sample weighed 1 mg and was identified as an unaffected area (Un), affected area (A) and the edge between the two areas (E) (fig 3.5). The samples were stored at –80ºC until RNA extraction.

Total RNA was extracted from three separate butternut squash samples that represented three biological replicates of mature fruits. Samples were taken from the unaffected, affected and edge areas. RNA extraction was performed by using the TRizol reagent according to the manufacturer's instructions, and purification was performed using the Bioline RNA extraction kit following the manufacturer's protocol. Agarose gels, Nanodrop and Qubit were used to determine the quality and quantity for the extracted RNA samples. Samples were electrophoresed using a 1.25% agarose gel in a 1X TAE buffer with 1% bleach. The samples were separated by electrophoresis at 100 V, first for 35 minutes and then for 10 minutes in the red gel.
The RNA samples were submitted to the Ramaciotti Centre for Genomics at the University of New South Wales in Sydney, Australia, for quality control (QC) testing and library construction. Individual samples containing 1000 mg RNA were placed in a 12-cycler PCR. Libraries were sequenced on NextSeq 550, using the NextSeq v2 2x75 bp kit mid output.

DESeq was used detect differentially expressed genes, from the RNA-seq analysis. Pairwise comparisons were made between all three types of tissues, i.e. affected vs unaffected, affected vs edge, and edge vs unaffected. This DE gene analysis was used to reveal differences in gene expression between the tissue types examined.

Results

The tissue samples produced over 181 million uniquely mapped reads (Table 1). It is interesting to note that the number of reads was similar in all of the tissue samples examined. Had a fungal pathogen been present in these samples, it would be expected that the number of reads would be substantially increased.

Table 1. Summary of RNA sequence data.

	Affected	Edge	Unaffected
Reads uniquely mapped	61,423,417	56,548,036	63,275,083
Reads unmapped	3,151,388	3,470,095	2,944,017
Reads mapped more than once	13,801,088	15,545,782	15,486,320
Assigned	108,118,466	98,880,515	110,921,902

A number of genes (11 of total 162 identified) were detected which were differentially expressed in etched and non-etched tissue. Ten genes had significantly up-regulated activity, while one had significantly down-regulated activity, in etch affected tissue compared to unaffected tissue. In general, the edge area of tissue was intermediate between these two values, suggesting that this region was transitioning from one state to another. All of these genes were associated with the phenylpropanoid pathway.

Phenylpropanoids are involved in plant defence against, for example, UV light, herbivores and pathogens. They include natural products such as lignins, flavonoids, coumarins, catechins and stilbenes. For example, expression of 4-coumarate 3-hydroxylase increases lignification of cell walls. Lignin can help protect the cell from external stresses, such as cold or drought, as well as against fungal attack.

Table 2. Expression levels for phenylpropanoid pathway genes in the three tested areas (affected, unaffected and edge)

Gene description	Affected vs Unaffected	Affected vs Edge	Edge vs Unaffected
Cinnamoyl-CoA reductase	4.54 ± 1.04*	0.30 ± 1.03	4.24 ± 1.04*
Cinnamoyl-CoA reductase 4	4.50 ± 0.81*	1.13 ± 0.81	3.37 ± 0.81*
4-coumarate-CoA ligase 1	3.73 ± 0.82*	1.05 ± 0.82	2.68 ± 0.82
4-coumarate-CoA ligase 2	6.01 ± 1.48*	0.29 ± 1.46	5.73 ± 1.48*
4-coumarate-CoA ligase	5.46 ± 1.44*	2.92 ± 1.40	2.54 ± 1.45
4-coumarate:CoA ligase 1	5.05 ± 0.94*	3.61 ± 0.91*	1.44 ± 0.96
4-coumarate:CoA ligase 1	5.11 ± 1.32*	-0.27 ± 1.27	5.38 ± 1.32*
Cinnamoyl-CoA reductase	7.37 ± 1.00*	3.34 ± 0.99*	4.03 ± 1.00*
4-coumarate-CoA ligase	4.04 ± 0.61*	2.18 ± 0.61*	1.85 ± 0.61*
Caffeoyl-CoA O-methyltransferase	3.58 ± 0.57*	2.59 ± 0.57	0.99 ± 0.58
Caffeoyl-CoA O-methyltransferase	-2.45 ± 0.70*	-1.55 ± 0.70	-0.90 ± 0.70

*	Genes with significantly lower expression
	Genes with non-significantly lower expression
	Genes with non-significantly higher expression
*	Genes with significantly higher expression

The results of the RNA analysis therefore suggest that etched areas have an increased level of phenylpropanoid biosynthesis, resulting in secondary cell wall deposition.

Microscopy

Aim

To examine physical changes in the cell structure in etched, non-etched and edge areas of butternut pumpkin skin.

Sample preparation

Specimens (3–6 pieces) of the three types of butternut squash skin tissues were removed (1 × 1 × 1 mm^3) and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer followed by washing with 1% osmium tetroxide in 0.1 M phosphate buffer. The osmium solution was removed, and the specimens rinsed in Milli-Q water for 5 min with three changes of water. The fixed tissues were then dehydrated using a series of ethanol wash solutions (30%, 50%, 70%, 95%, and 100%). Resin (1 mL, 25%) in 3 mL of ethanol (100%) was added to the specimens, which were then incubated with rotation for 2–3 h. Then, 50%, 75%, and 100% resins were added sequentially after removing the 25% resin. The cells were incubated overnight and embedded in paraffin (58–60°C) for the microtome stage.

Specimens were embedded in labelled capsule moulds. Then, 1–2 drops of 100% resin were added to the moulds, and one piece of tissue was carefully placed on each mould. The mould was filled with 100% resin and placed in an oven at 60°C for overnight polymerisation to prepare samples for sectioning.

Light microscopy

The resin blocks were removed from the mould. A microtome (Leica RM2255, Germany) was used to cut sections with a thickness of 1.0 µm or 0.50 µm. The sections were collected, and samples for all examined parts (A, Un and E) were placed on slides. Toluidine blue was used to stain the sectioned samples for examination with a Leica DMIL light microscope (Leica Microsystems Pty Ltd., Germany) under 100× magnification with normal bright field optics. Samples were photographed with a Nikon Photo Coolpix 900 camera mounted on the same microscope in the tissue culture laboratory of the Plant Breeding Institute, University of Sydney.

Scanning electron microscopy

Ultra-thin samples of butternut squash skin tissues (70 nm) were sectioned by microtome and placed on copper grids for post staining. The specimens were floated over a droplet of uranyl acetate (2%) and incubated in the dark (OL0K cover with Al foil) for 10 min. Thereafter, the specimens were placed over a droplet of lead citrate surrounded by NAOH pellets to reduce CO_2 contamination, which could lead to carbonate precipitates on the sections. Three beakers were filled with warm MilliQ/distilled water, and the grids were rinsed gently (15–20 dips) in each beaker. Specimens with lead citrate were stained once more for 10 min, and the grids were rinsed gently as described above. The mounted specimens were observed with a JEOL Neoscope benchtop scanning electron microscope (SEM) (Nikon Instruments Inc., NY, U.S.A) operating at an acceleration voltage of 5 kV, and digital images were captured by a camera installed with the equipment. The image pixel size was 215.8 nm.

Results and Discussion

Light microscopy

Numerous cells in the affected tissue were deformed, with thickened walls, irregular shapes and disrupted contents, possibly due to increased turgor pressure. This was in clear contrast to the cells in the unaffected area, which were regularly shaped, with normal nuclei and vacuoles clearly visible.

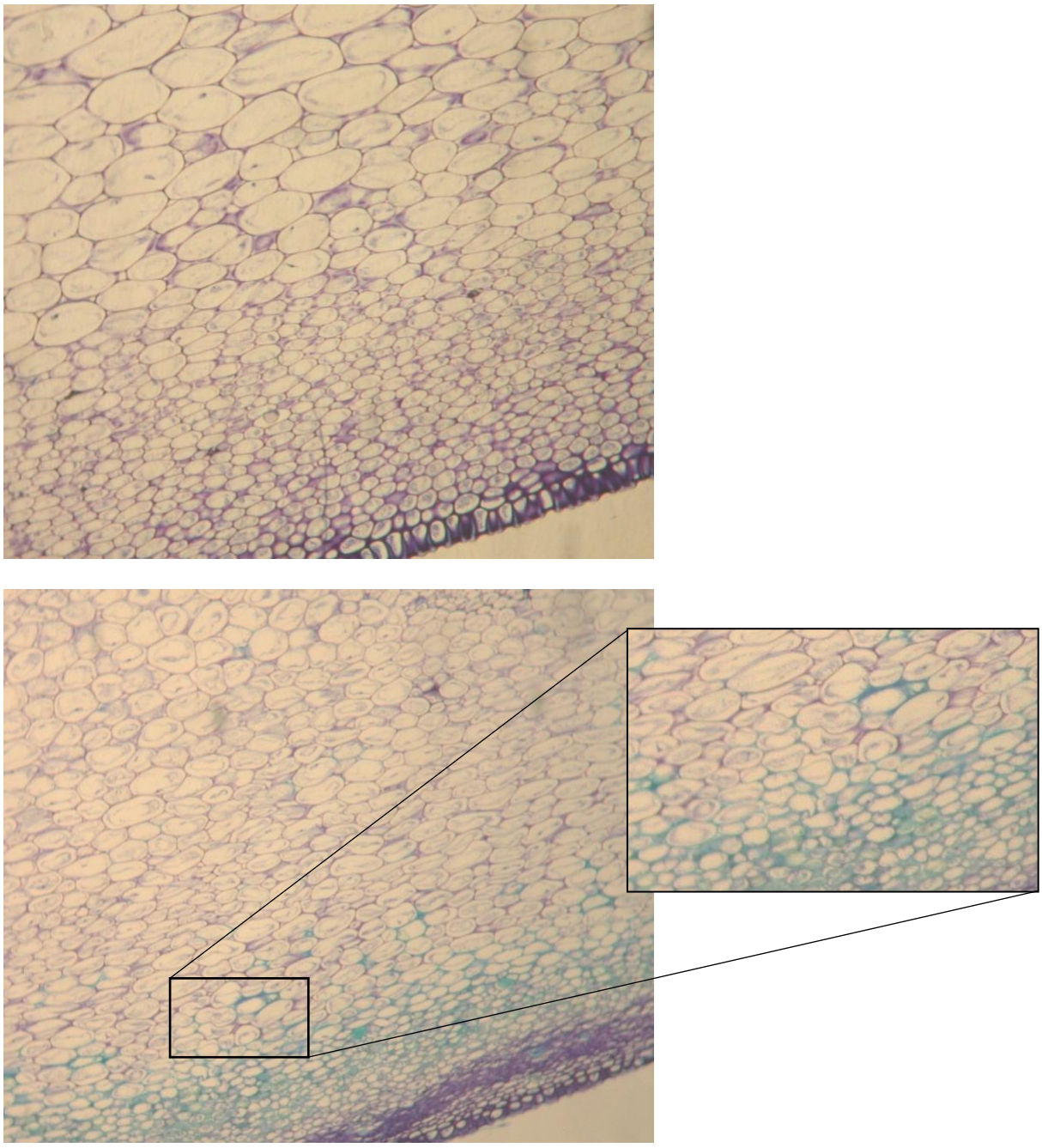


Figure 1. Normal (top) and etch affected (below) epidermal cells.

Scanning electron microscopy (SEM)

The SEM revealed clear differences between the etched and unaffected tissue. The etched cells have developed extremely thickened cell walls. The contents are clearly disrupted, with some showing evidence of detachment of the inner membranes and coagulation of the cell contents.

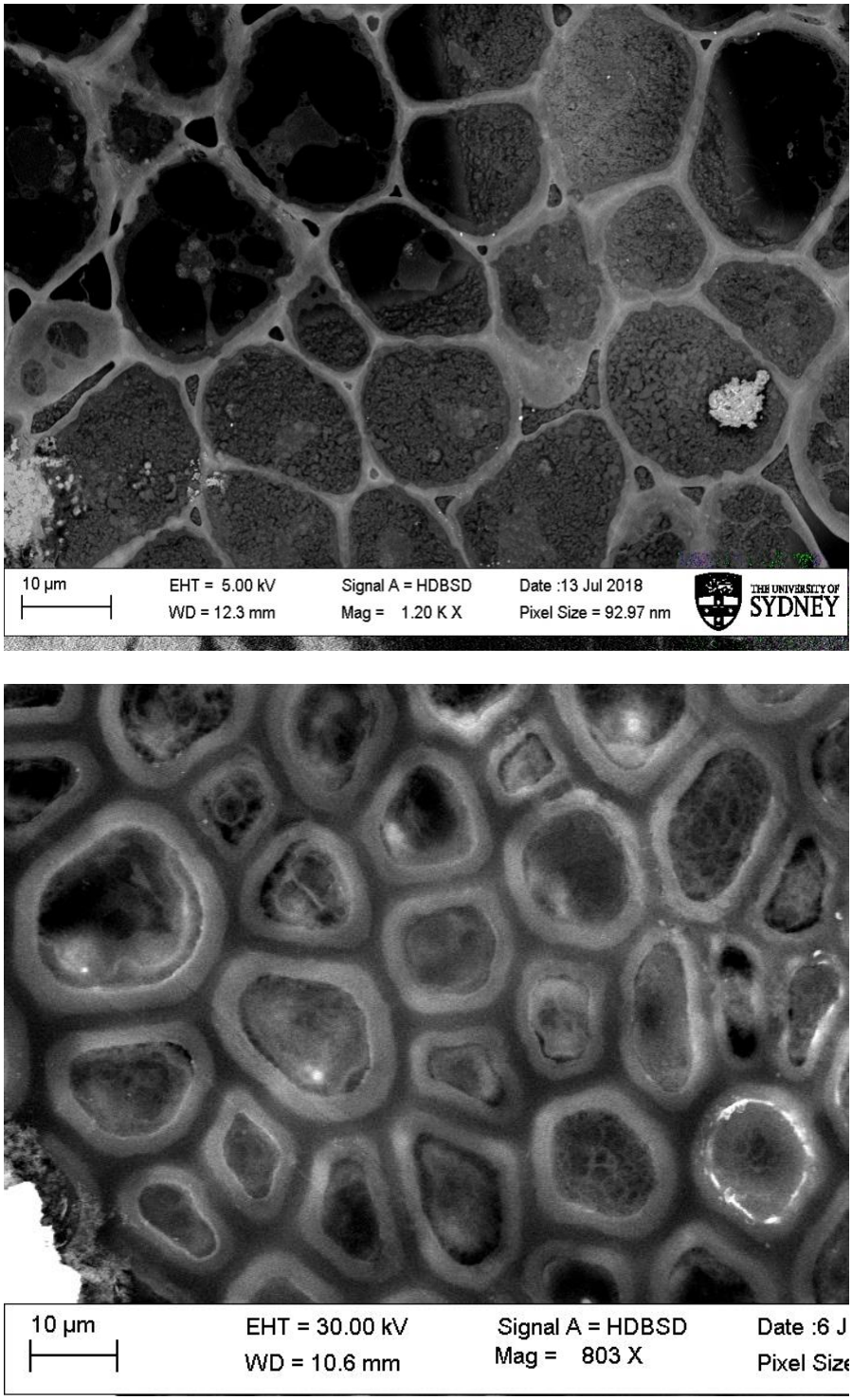


Figure 2. Normal (top) and etch affected (below) epidermal cells. Photographs have been adjusted to provide the same scale in both.

This change in the cell structure is readily apparent when examining the edge area between the two zones. As the cell walls thicken, the cells themselves distort. As previously, it appears that as the etch spread the cells develop increased internal pressure, whether simply due to the thickening of the wall or from osmotic forces.

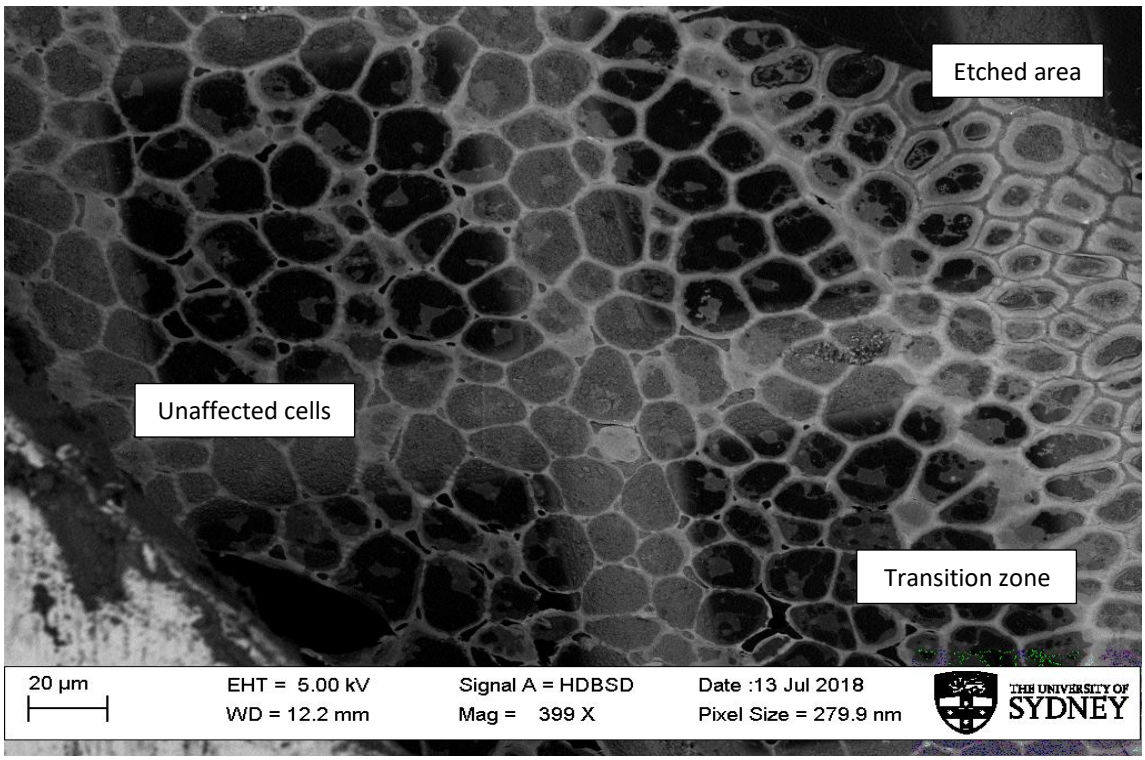


Figure 3. SEM image of the edge between etched and unaffected areas. There is a clear gradation from normal cells to etched tissue with grossly thickened cell walls

Conclusions

The results from the microscopy and the RNA studies consistently indicate a massive increase in cell lignification in etched areas of the pumpkin. This is likely to be due to a form of biotic or abiotic stress, which triggers defence strategies inside the plant tissue.

However in this case the degree of lignification appears out of proportion to any threat to the plant tissue itself. The cell walls are thickened to the extent that the cell contents are completely disrupted. It appears likely these cells will die.

This lignification, followed by cell death, may account for the transition of etched areas from a rich, brown colour to the whitish, “petrified wood” appearance of old, etched areas on the fruit. Field observations of mature pumpkins commonly find fruit where there is evidence that etch has occurred (whitened areas on the fruit skin), but that the pumpkin has then recovered and developed normally.

The results of both the RHNA analysis and microscopy are therefore consistent with other observations of the development of brown etch. A biotic or abiotic stress triggers expression of genes that code for lignification of the cell walls. As the walls expand, cells become distorted and deformed. Their contents are disrupted and normal function is likely to cease. Excessive lignification results in the rich, brown colour typical of etch. After a time these cells die, leaving behind the whitened skeletons of the empty cell walls.

The results of the pathology tests indicated that etch can be triggered by presence of a pathogen, particularly *S. cucurbitacearum*. However, attempts to re-isolate the pathogen from etched areas were only moderately

successful. This is consistent with the observation in these trials that no fungal hyphae were visible in the cells expressing symptoms of etch, and the number of genes present was similar for etched, non-etched and edge areas on individual fruit.

Figure 4. Etched pumpkin showing fresh, brown etch and old, whitish etch.

Appendix 4. Field trials and monitoring on commercial crops

These trials were conducted at commercial pumpkin farms. The primary purpose of these activities was to monitor occurrence of etch in the crop at the same time as recording climate parameters including temperature, relative humidity, leaf wetness, soil moisture and rainfall.

Climate monitoring was provided by six automated weather stations constructed by Pacific Data Systems in Brisbane. Each station uploaded to the web at hourly intervals, so could be interrogated remotely. Initially the weather stations worked well, with . Unfortunately, as the project proceeded, the stations progressively failed. Attempts to fix them had limited success, particularly as connection issues were frequently intermittent.

Another issue was that little or no etch was recorded in a number of these trials. This is likely due to the dry conditions experienced over much of the project timeframe. On the positive side, this confirms the strong positive influence of wet conditions on incidence of brown etch.

Only field trials with significant etch are reported here. Although the data from other trials is not reported, this information will be used in the modelling work currently underway developing a predictive model for etch.

Griffith – 2016 to 2017

A farm in Griffith was monitored over the summer of 2016 to 2017. The grower at this property furrow irrigates, so fruit develops in contact with the soil (not on plastic). Pumpkins were harvested in March, allowing a detailed examination for etch. No etch was found.

To try to trigger etch, a number of pumpkins were packed in plastic bags with a small amount of water for transport to the Sydney Markets. No etch developed.

No rain occurred in the time leading up to harvest. While dewpoint was reached a number of times during this period, warm, dry days mean that any dew on the fruit would have rapidly evaporated.

Ayr – 2017

Ayr is a major production area for butternut pumpkins. The season runs approximately from May to December. Commercial pumpkin fields were monitored weekly for occurrence of brown etch and data collected on condition where etch is found. A weather station is set up on a commercial farm in production.

Method

The trial site was marked out within a commercial field and monitored weekly during the month leading up to harvest. Weekly observations were conducted by inspecting 120 to 150 pumpkins along transects through the site. Information was recorded on:

- Soil contact
- Rotten/live vegetation contact

- Moisture around the pumpkin
- Fruit maturity
- Colour of etch
- Shape of etch/ rings present
- Nearby fruit affected

Figure 1. Trial site in Ayr

Results

Weekly inspections in the month leading to harvest showed that the percentage of etch on pumpkins increased dramatically as the pumpkins matured. The severity of etch also increased, with etch spreading from its original damage point.

Figure 2. Etch progression on a pumpkin. Photographs taken one week apart.

By harvest, approximately 10% of the crop was etch affected (Figure 3). The majority of etch occurred where the fruit was in contact with a stem, petiole, soil or other fruit. Only two of 30 observations of etch occurred at non-contact points.

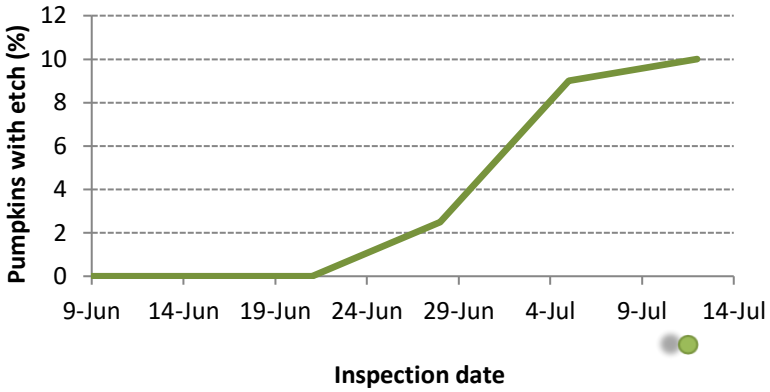


Figure 3. Incidence of etch in a pumpkin crop in Ayr. A total of 120 to 150 fruit were inspected weekly at the trial site.

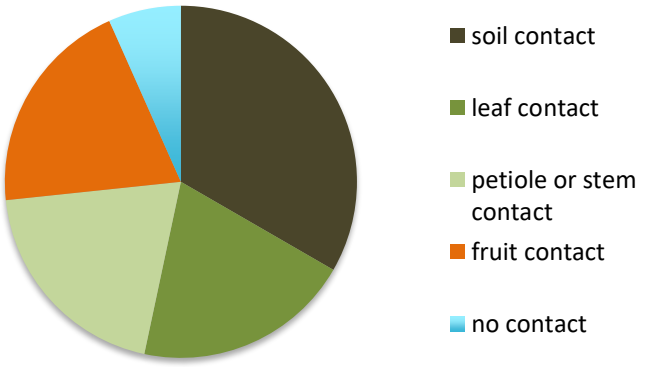


Figure 4. Material in contact with etch at its apparent centre of origin.

Figure 5. Etch development at the trial site in Ayr, showing contact points with vegetation

In the month leading to up to harvest in Ayr, RH averaged 77% and reached a maximum of 100% RH several times. This was higher than Griffith with an average of 65% RH, where no etch occurred. However, it is also far lower than Somersby, where approximately 30% of pumpkins had etch and RH averaged close to 92% over the same period.

It seems likely that etch is more likely at points where the pumpkins are in contact with vegetation (stems, petioles), rotten vegetation (dead flowers, leaves) and the soil due to the creation of microclimates at the point of contact. Saturation RH at such points, combined with high turgor pressure within the plants, may increase turgor pressure inside skin cells in the maturing fruit. This could be the key factor that results in formation of etch.

Lockyer Valley – 2018

Two farms were monitored in the Lockyer Valley. Both were using overhead irrigation and growing direct onto soil.

Methods

In addition to their normal production methods, both farms were provided with potassium silicate (3L/Ha of 1% solution) and SettEnhance Ca/B (5L/Ha as 1% solution). The growers were asked to apply the products three times during the cropping cycle.

Unfortunately, heavy rain (80mm on one day) occurred during the cropping cycle so only the initial treatments were applied. There were no differences due to the treatments, so only the overall mean values are presented.

- Farm A was assessed pre-harvest (300 fruit sample). Postharvest assessment was not conducted because splits and oedemas made the crop unsaleable; it was simply ploughed back in.
- Farm B was surveyed pre-harvest (300 fruit). Five bins of harvested fruit (total of 1459 fruit) were then sorted postharvest to record rates in the bins. It is understood that rates in the harvested product may be lower as etched fruit would normally be left in the field.

Etch was graded as major if it affected an area greater than $5cm^2$, as this would result in rejection at retail. Smaller markings of etch were graded as minor.

Results

Despite wet conditions early during production, rates of etch were relatively low. Etch that would result in market rejection remained below 5% of fruit at all assessments. It was observed that one bin at farm B had a higher percentage of etch than the other four examined. Pumpkins in this bin were also quite muddy, suggesting they had grown in a wet area of the paddock.

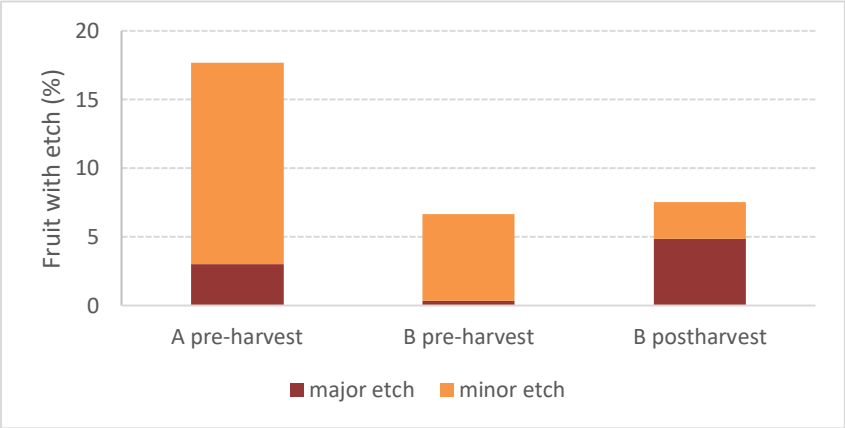


Figure 6. Percentage of pumpkins with minor or major etch at farms in the Lockyer Valley

Although approx. 15mm of rain occurred in the 30 days leading up to harvest, only 3mm occurred in the final two weeks and temperatures were above 30°C nearly every day. As a result, humidity was relatively low. This data is being added to the overall model.

Donnybrook – 2018

Pumpkins were planted in December 2017 and grown as per the grower's commercial practice, using plastic mulch and t-tape for irrigation. Soil moisture, rainfall, leaf wetness, temperature and RH were monitored using a weather station installed on site. The air vapour pressure deficit was calculated using air temperature and humidity.

The farmer regularly inspected the crop for etch, and a crop assessment was made on 14 May by inspecting 100 pumpkins along a transect through the field.

Figure 7.Donnybrook trial site with weather station

Results

Etch was first noted by the grower at the start of April. A field assessment in May found 9% of fruit affected by etch. By that stage much of the etch was old, with the damage visible as whitish "petrified wood" over the affected area. Some of these fruit also showed signs of fresh etch, particularly where the fruit was in contact with the soil.

The onset of etch occurred soon after an increase in RH, with corresponding drop in the vapour pressure deficit (VPD) towards the end of March. The VPD remained low through April, when etch continued to develop in the crop.

Figure 8.Despite the pumpkins growing on gravelly soil (left), the soil was still very damp. Most etch appeared on the ground side of the fruit (right).

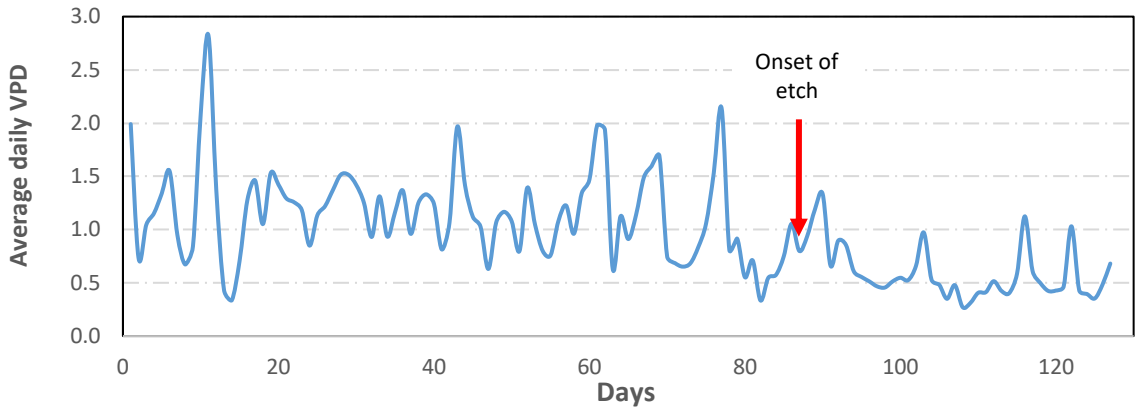


Figure 9. Average daily vapour pressure deficit in Donnybrook, WA from January to May 2018

A rapid drop in the VPD was associated with the onset of brown etch. The VPD remained low for a period when etch most likely continued to develop in the crop. This supports the hypothesis that turgor pressure within pumpkin plants, combined with inhibition of transpiration, is a key factor contributing to development of etch.

Mareeba – 2018

Although Mareeba has often experienced high levels of etch, scouting of early crops (July through to September) revealed that etch was at extremely low levels (<2%). Examination of pumpkins from Mareeba on arrival at Sydney markets confirmed that etch was a minor issue, with minimal culls from bins of transported fruits.

We hypothesised that the low level of etch was due to the extremely dry conditions during much of 2018. In the three months from July to September this year, Mareeba received only 0.2mm of rain (BOM weather station, Mareeba airport). This resulted in relatively high vapour pressure deficits between the pumpkins and the surrounding air. This is in contrast to previous years, where light rainfall continued sporadically during the growing season; mean rainfall for this period is around 18mm.

It therefore appeared possible that increasing humidity around individual pumpkins could increase development of etch.

Methods

Fifty large, clear plastic bags were prepared by punching a series of 24 holes along the top and bottom quarter folds. Holes were created due to concerns about the fruit rotting if fully enclosed.

A total of 100 pumpkins were selected, randomly distributed within an area of the crop. A bag was placed over every second green / cream butternut pumpkin and secured using a section of plastic coated twist tie. In total, 50 pumpkins were bagged, with 50 adjacent, pumpkins left un-bagged as untreated controls. The bags were installed on the 11th of September and removed one month later on 8th October.

Two temperature and humidity data loggers (Hobo Pro v2) were placed inside bags with the pumpkins, while another two were attached to short posts within the crop nearby.

Just before commercial harvest, all of the bags were removed and the pumpkins examined for signs of etch.

Results and Discussion

Surprisingly, maximum relative humidity (RH) was not increased inside the bags, but actually decreased. This appears to be due to an increase in night-time temperatures inside the bags, presumably due to buffering from cooler evening air as well as entrapment of respiration heat from the pumpkins.

In addition, the RH inside the pumpkin crop appears to be much higher (during the evening) than that in the surrounding air. During the trial period RH in Mareeba rarely exceeded 70%, whereas inside the pumpkin crop it approached 100% virtually every night. Transpiration by the crop, combined with low wind speeds, is likely to have produced this effect.

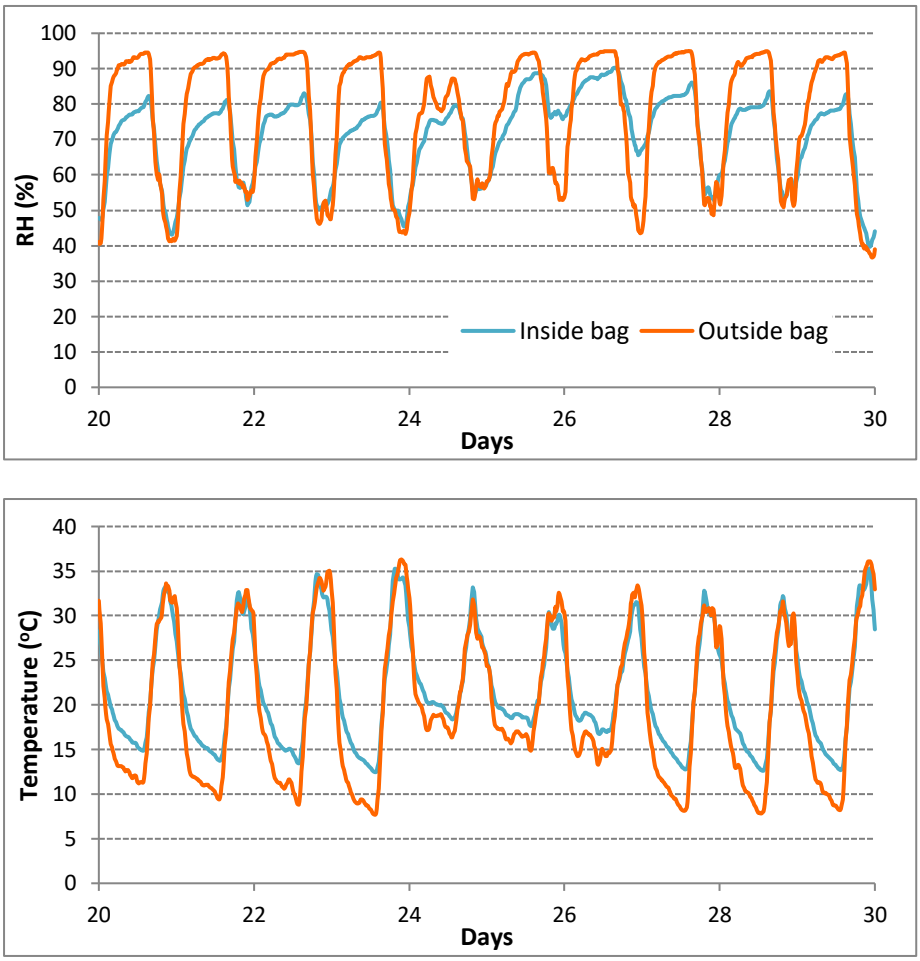


Figure 10. RH (top) and temperature inside and outside bags placed over pumpkins. Lines are average of two loggers.

While a few etched pumpkins were observed, numbers were too low to draw any conclusions as to the effect of bagging on etch. This is perhaps unsurprising, given the lack of effect on the VPD of bagging. The bags were perforated due to concerns about the fruit splitting and/or rotting inside sealed bags. However, it seems likely that the number of perforations in the bag allowed water vapour to equalise between the outside and inside during the day, but restricted air movement

during the evening. If this trial were to be repeated then fewer – or even zero – perforations should be added to the bags, so as to significantly increase RH around the pumpkins.

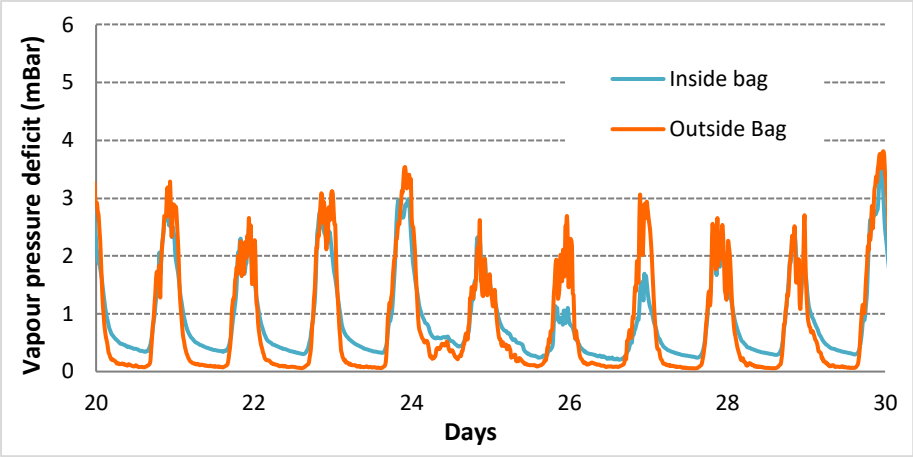


Figure 11. Vapour pressure deficit inside bags and within the pumpkin crop

Figure 12. Splitting of a bagged pumpkin (left) and etch on an unbagged pumpkin (right)

Appendix 5. Field trials with experimental crops

Somersby – 2016 to 2017

Methods

Pumpkins were planted at the Somersby field station, located around an hour north of Sydney on the central coast. The soil at this site is a rich loam, high in organic matter with good water holding capacity.

The site was cultivated and formed into 8 x 40m long beds. Half of the site was planted with butternut cv. Jacqueline, with the remainder planted with cv. Sunset QHI. Jacqueline and Sunset QHI are current commercial varieties which are regarded as susceptible and resistant to etch respectively. The pumpkins were planted directly into the soil, without addition of plastic mulch, and irrigated using sub-surface t-tape.

By February 2017 young pumpkins were developing and maturing on the vines. Pumpkins were chosen which were in good condition and at the white to pale orange maturity stage (not green or orange). A number of treatments were then tested, using equal numbers for the two varieties. Treatments aimed to keep pumpkins wet, keep them dry, or challenge them with a pathogen previously associated with brown etch;

- Pumpkin wrapped in wet hessian and placed on the soil, to maintain a wet environment (10 fruit)
- Pumpkin placed on a plastic stand to keep it off the ground (10 fruit)
- Pumpkin scratched then moistened with a spore solution of *Fusarium* sp. (30 fruit)
- Pumpkin scratched then moistened with a spore solution of *S. cucurbitacearum* (Gummy stem blight, GSB) (30 fruit)
- Pumpkin scratched then inoculated with distilled water only (10 fruit)

Figure 1. Pumpkin wrapped in wet hessian sacking with unwrapped control.

Treated pumpkins were marked with flagging tape and pegs (Figure 2). Temperature, humidity and soil moisture were recorded at the site using a combination of Hobo dataloggers and Wild Eye soil moisture probes.

Figure 2. Pumpkin trial at Somersby. Flags indicate locations of treated pumpkins.

Mature pumpkins were harvested on 9/3/2017 then again on 29/3/2017. On each occasion the location and size of etched areas (eg in contact with soil or vegetation), pumpkin maturity, presence of disease and whether rings were present in the etched area was recorded.

On the early harvest, the pumpkins which were on stands or wrapped in hessian were harvested, along with matching 'controls' that were located next to each treated pumpkin. Inoculated pumpkins were picked and evaluated at the late harvest.

Results

Average RH inside the crop was at least 90% during February, increasing to 95% during March. This reflects the damp conditions in the field due to a high rate of irrigation several rainy days and the luxuriant foliage of the pumpkins. Temperatures were warm, rarely falling below 15°C and regularly rising over 30°C. Soil temperatures were also warm, averaging 20–25°C during the growth period.

As the dew point that was only slightly below ambient temperatures, the pumpkins were regularly wet with dew during development. As a result, many of the fruit developed oedemas, warty outgrowths caused by excess water pressure inside the fruit.

Figure 3. Oedema on a developing pumpkin

Figure 4. Soil moisture content in pumpkin crop at Somersby.

From the start on March, significant numbers of etched pumpkins started to appear in the crop. Etch was commonly observed on the side of the pumpkin in contact with the soil, or where the pumpkin was pressing against stems or vegetation.

Figure 5. Symptoms of brown etch observed in the Somersby crop. Etch was in contact with the soil.

Figure 6. Pumpkin with slight symptoms of brown etch where it has been covered with dead vegetation.

Pumpkins were found which had been damaged by deer, or were infected with another pathogen (mainly *Sclerotinia*), however there was no observed association between either of these and etched fruit. Other symptoms included oedemas. Some of these did appear to be associated with development of etch.

Figure 7. Symptoms of disease (left and centre) were noted in the crop, but were not associated with etch. In contrast oedemas (right) were often brown, and/or surrounded by etch.

Initial harvest

At the first harvest, 28.2% of Jacqueline and 28.0% of Sunset QHI had symptoms of brown etch. In nearly all (~95%) of these pumpkins the etched area was in contact with the soil, or with dead vegetation. The estimated area affected was an average 26cm^2 for Jaqueline and 33cm^2 for Sunset QHI, but the extremely high variability in this data suggests this difference is not significant. Etch could be simply a small light brown spot, to large brown splotches covering large parts of the pumpkin skin.

Wrapping in a wet hessian sack and raising the pumpkin on a plastic guard both appeared to reduce the incidence of etch. Only one of the pumpkins on a stand, and two of those wrapped in hessian, developed etch. While the numbers of each treatment were very low, limiting the inferences that can be drawn from this, it does suggest that separating the pumpkin fruit from the soil or vegetation reduced risk of etch.

Late harvest

The incidence of brown etch increased by the second harvest. More than half of the harvested pumpkins had some symptoms of brown etch, many of them severe. Again, the estimated area affected was highly variable, with an average of 31cm^2 for Jacqueline and 28cm^2 for Sunset QHI.

Pumpkins had been inoculated with pathogens suspected of causing brown etch. In this case, inoculation with a pathogen appeared to **reduce,** rather than increase, incidence of brown etch. The matching controls (scratched then 'inoculated' with water only) were similar to untreated fruit.

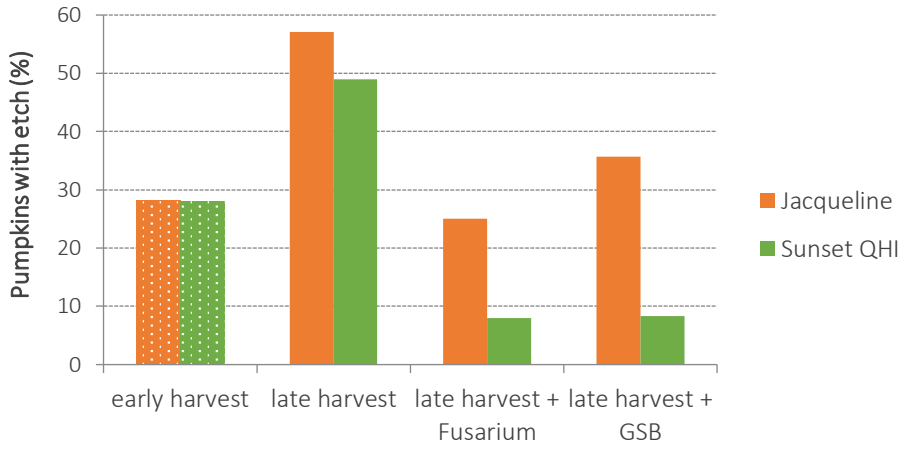


Figure 8. Incidence of etch at two harvests for pumpkins cvs. Jacqueline and Sunset QHI. Pumpkins were left untreated or inoculated during development with Fusarium sp. or gummy stem blight (GSB).

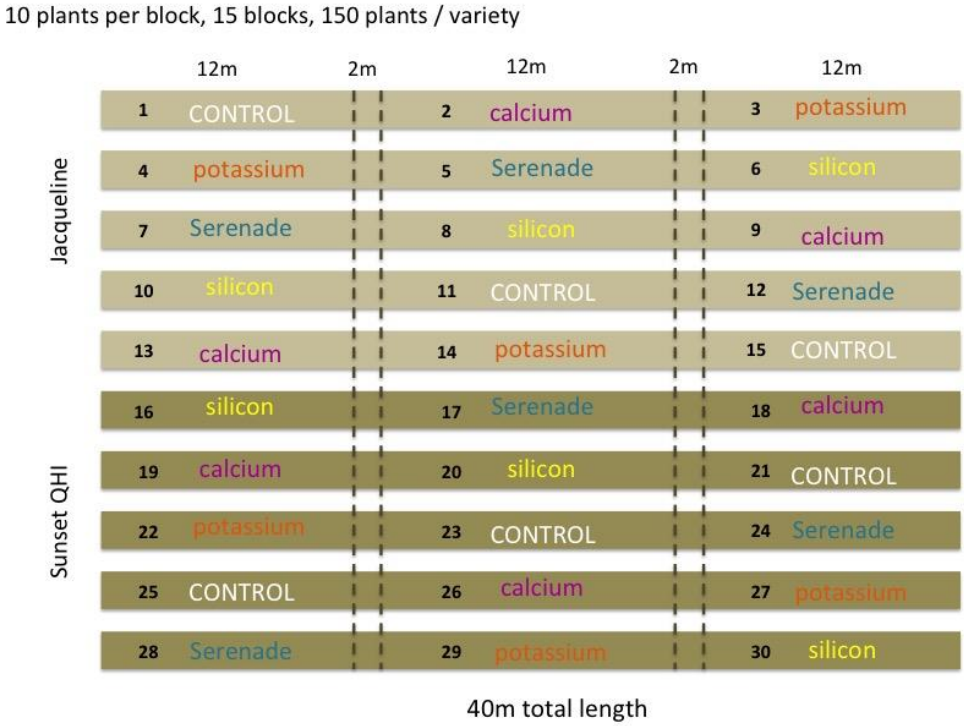
Visible signs of rots such as hyphae, spores and sclerotia were not associated with brown etch.

Somersby – 2017

Methods

A block comprising ten rows was planted half each with butternut pumpkins cv. Jaqueline or Sunset QHI, these representing nominally susceptible and resistant varieties respectively. Each row was divided into three then treated with;

1. Silicon, applied as AgriSil liquid, a.i. 25g/L ortho silicic acid (3 applications, 1ml/m^2)
2. Calcium applied as SettEnhance, a.i. 11% Calcium + 1.4% Boron (3 applications, 0.5ml/m^2)
3. Serenade, a.i. *Bacillus subtilis* (drench at planting + 3 additional applications, 0.35ml/plant)
4. Foliar potassium, a.i. K_2SO_4 (2 applications, 2% solution)



Somersby trial plan, planted in October 2017.

Pumpkins were planted directly into the soil and watered with a combination of sub-surface drippers and overhead irrigation. Pumpkins from each block were assessed for etch at harvest, with each pumpkin classed as clean, small (<5cm^2 etched); medium (5–10cm^2 etched) or large (>10cm^2 etched).

Results

Unfortunately the trial was cut short due to excessive growth of weeds in the plot. This severely affected some blocks and made it difficult to distinguish treatment units as the vines grew. A single harvest was conducted of all fruit.

Differences between the two varieties were not significant. The data could therefore be combined, giving a total of six blocks/treatment.

The results suggested there was a significant positive effect of SettEnhance in reducing etch, with <5% of these pumpkins affected compared to 11-15% for all other treatments. While this result appeared promising, issues with clearly identifying pumpkins by treatment and potential interference by weeds suggest this result should be treated with caution, and the treatment repeated under normal production conditions.

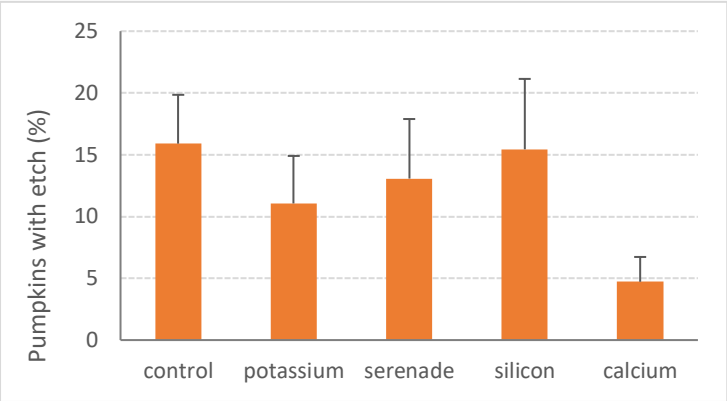


Figure 9. Average incidence of etch in plots treated with water only (control); foliar potassium, silicon or SettEnhance (calcium); or soil drenching with *Bacillus subtilis* (Serenade). Bars indicate std errors of means (n=6).

Somersby – 2018

Method

Butternut pumpkins (*cv*. Jacqueline) were sown into black plastic mulched beds on the 13th of February 2018 at a field research station site in Somersby, NSW (33°37′S, 151°30′E).

Somersby trial site before planting (left), and two months later.

The crop was routinely irrigated and fertilised as required. No fungicide applications were made prior to experimental treatment applications. Plots were 2 x 12 metres (60m^2) with three planted rows per plot. Treatments were:

1. Untreated control
2. Inoculated with *Fusarium* spp.
3. SettEnhance (Calcium)
4. Chitosan

Each treatment was replicated three times in randomised positions.

A total of four treatment applications were made from early fruit set at intervals and application rates as presented below. Calcium (Metalosate® 6% amino acid chelate liquid) and chitosan (Taikang® 0.5% liquid chitosan) were applied at twice the label rate. *Fusarium* inoculant was made by scraping pure isolates of *Fusarium* spp. grown on agar plates, sourced from NSW DPI, Ourimbah, into water.

Application rates

	Treatment	Rate	Application volume	Field mix per plot	Application timing
1	Untreated control	-	-	-	-
2	Inoculated with *Fusarium* spp.	-	-	-	A only
3	Calcium (6%)	4L/ha	833 L/ha	24ml in 5L	A,B,C,D
4	Chitosan (0.5%)	6L/ha	833 L/ha	36ml in 5L	A,B,C,D

Pumpkins were harvested on 8th June, 37 days after the last treatment application.

Pumpkins were assessed in the field for incidence and severity of etch lesions. Severity was rated based on lesion size with larger than a 50-cent coin (32mm wide) classified as major etch and less than a 50-cent coin as minor etch. When an etched lesion was found whether it had contact with the ground (plastic mulch) or leaf material was recorded. The maturity of the etched fruit, assessed as green, cream, pale orange or orange was also recorded.

Results

At harvest approximately 20% of the pumpkins in the untreated control plots had etch. In contrast to the previous results the highest level of etch was in calcium treated plots (average 28% etch) and lowest in chitosan treated plots (average 7%). While the difference between SettEnhance and chitosan was statistically significant, the previous positive results with SettEnhance suggest that, again, this result should be treated cautiously. The severity of lesions was similar across all treatments with approximately 50% major lesions and 50% minor lesions.

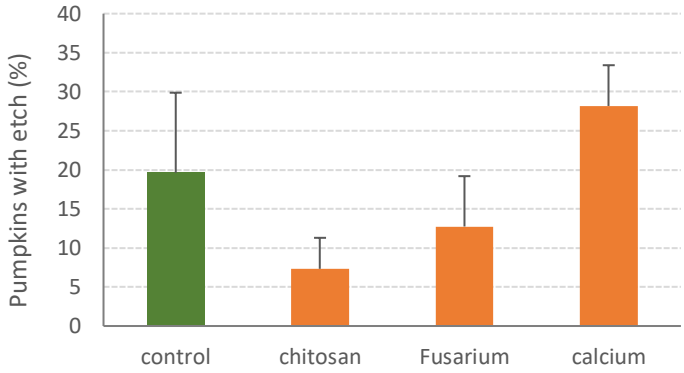


Figure 10. Average incidence of etch in plots treated with water only (control); foliar chitosan; inoculated with *Fusarium* spp.; or foliar SettEnhance (calcium). Bars indicate standard errors of means (n=3).

The majority of etch occurred where the pumpkin was resting on the plastic mulch (**Error! Reference source not found.**). Etch generally appeared on fruit as they were maturing; no green pumpkins, and only a few cream pumpkins, were found with etch (**Error! Reference source not found.**).

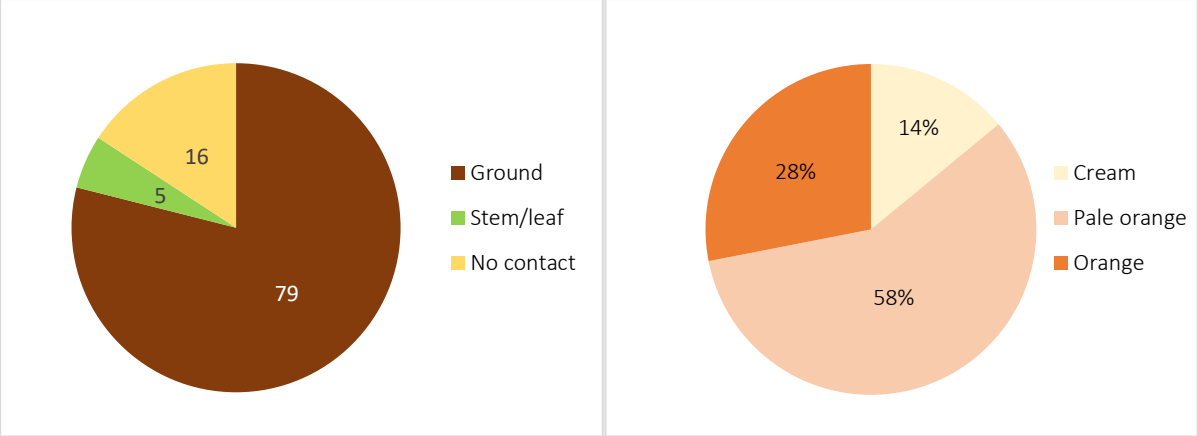


Figure 11. Contact points of etched lesions in the field (left) and maturity of etched fruit at harvest (right)

There appeared to be some correlation between plot position and level of etch, with plots at the southern side of the trial generally having lower levels of etch compared with northern plots (see figure 12). This may be due to slope, the southern side being lower and potentially damper.

Chitosan	Calcium	Untreated	*Fusarium* inoc.
Calcium	Chitosan	*Fusarium* inoc	Untreated
Untreated	*Fusarium* inoc	Chitosan	Calcium

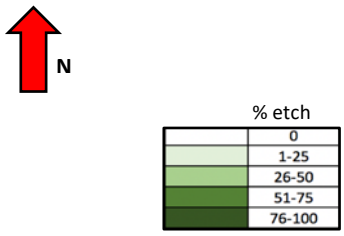


Figure 12. Heat map showing spatial distribution of etch in the field.

None of the in-crop treatments stopped etch development or expansion. Overall, position within the block appeared to have a greater effect on incidence of etch than the treatments applied. It is also noted that etch almost always occurred in contact with the ground. These factors together suggest that moist microclimates strongly favour etch development.

Cowra – 2018

This trial was conducted using a small area of a commercial pumpkin crop, which was purchased outright for this purpose. This allowed us to manage it independently.

As Cowra has a dry climate, some treatments were designed to increase incidence of etch by raising RH around the plants and reducing transpiration from the fruit. An additional treatment was therefore included using the anti-transpirant 'Envy', to test whether this would increase development of etch.

Aim

To examine the effect of foliar applied silica and calcium products on brown etch development in a commercial pumpkin crop, as well as whether increasing RH / reducing transpiration increases development of etch.

Method

Pumpkins cv. Jaqueline were direct seeded during December 2017. Plants were grown using a 1m plant spacing and commercial drip irrigation and fertiliser program. The trial block was six rows wide and approximately 80m length, divided into four sections, as per the diagram shown below.

Treatments were applied three times at three-weekly intervals (7/2/2018, 1/3/2018, 19/3/2018) during the period leading up to harvest.

- Water only
- Potassium silicate 3L/Ha
- SettEnhance, 5L/Ha
- Envy (anti-transpirant), 10L/ha

Two of the rows were covered using plastic tunnels. The tunnels were constructed by drilling bamboo stakes into the ground either side of the row, bending sections of irrigation pipe between them to form a "hoop", then stretching a section of plastic across the top and securing using cable ties and reinforced hole punches.

The tunnels were left half open until 3 weeks prior to commercial harvest in order to allow spray applications. As the site is quite windy, it was also thought that the tunnels might not be strong enough to stay intact in the longer term. To help reduce wind pressure, vents were cut into the plastic to allow some flow through.

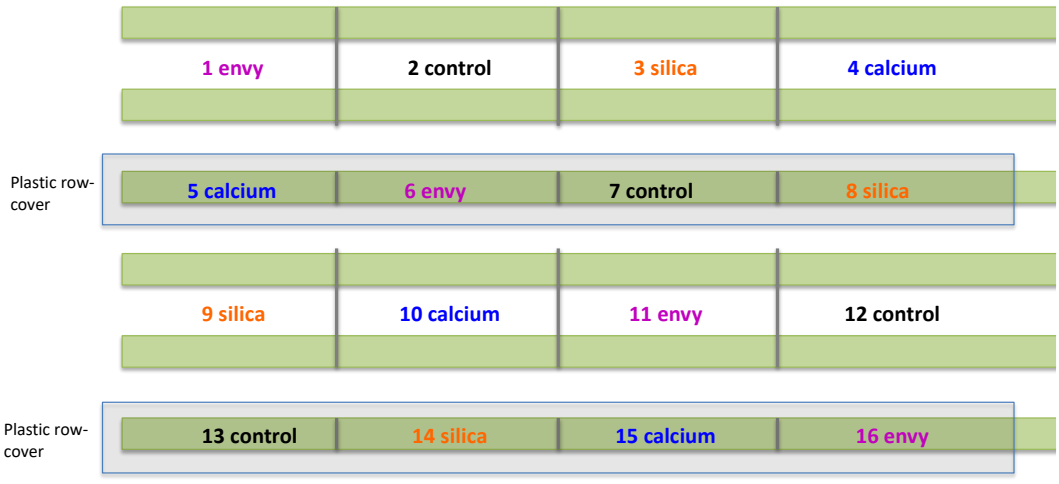


Figure 13. Trial layout at Cowra site

Figure 14. Plastic covers installed over two rows of pumpkins, showing condensation inside the tunnels

The pumpkins were assessed at harvest, being classified as clean, minor etch (still saleable, <5cm^2) or major etch (rejected by the retailers, >5cm^2 affected).

Results and Discussion

In general, levels of etch were low regardless of treatment. While the level of brown etch was slightly higher in tunnel 1 compared to the control, only one of each treatment was included. Etch was not increased in tunnel 2. It is possible this is because the covering of tunnel 2 partially blew off twice during high winds. Even though the covering was replaced each time, it meant the conditions were not modified continuously. Moreover, temperature and RH data recorded in tunnel 2 indicated that environmental conditions (temperature and RH) were barely modified compared to the field environment. This was despite there being a large amount of condensation observed inside the tunnels. Unfortunately, temperature and RH were not recorded inside tunnel 1.

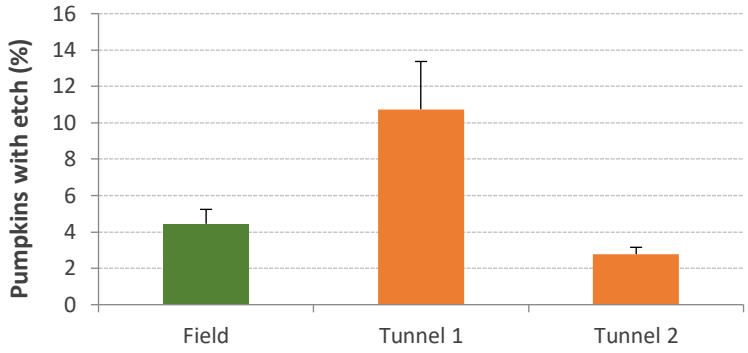


Figure 15.Percentage of etched pumpkins in the open field compared to under the two tunnels. Bars indicate the standard deviation of each mean value. Bars indicate standard errors of means (n=4/8)

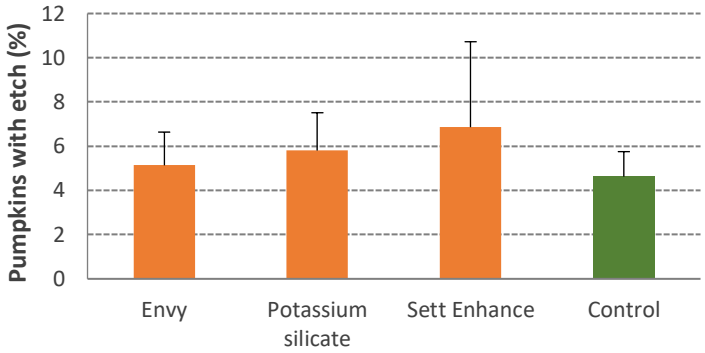


Figure 16.Percentage of etched pumpkins with foliar applications of Envy (anti-transpirant), potassium silicate, SettEnhance (calcium) or water only (control). Bars indicate standard errors of means (n=4).

The level of etch was not significantly increased by Envy® or reduced by calcium or silica. It is unfortunate that one of the tunnels was damaged by wind, as this method appeared to show some promise in terms of inducing etch.

A stronger tunnel system, without vents to allow airflow, would be more effective at raising humidity around the pumpkins than the small tunnels used in this trial. Future trials could test this strategy.

Richmond – 2018 to 2019

As the Somersby site was not available, trials in 2018 to 2019 were located at the Greater Sydney Local Land Services "River Farm" site, Richmond NSW. This site had not been used for vegetable production in recent history. The trials were designed to complement the pathology work which was investigating the link between etch and *S. cucurbitacearum*, cause of black rot / gummy stem blight (GSB).

Method

Crop 1

The cultivar *Jacqueline* was selected for its known susceptibility to etch. The trial was planted on 6 November 2018 on pre-formed 1.8 metre wide beds. A single-row of seedlings were planted in the centre of each bed. One trial plot was two beds wide (3.6m) and 10m long. Plant were irrigated with overhead sprinklers approximately twice per week.

Treatments were:

1. Untreated control
2. Inoculation with *S. cucurbitacearum* (GSB)
3. Inoculation GSB plus intensive fungicide program
4. Inoculation GSB plus chitosan (a plant defence responce elicitor)

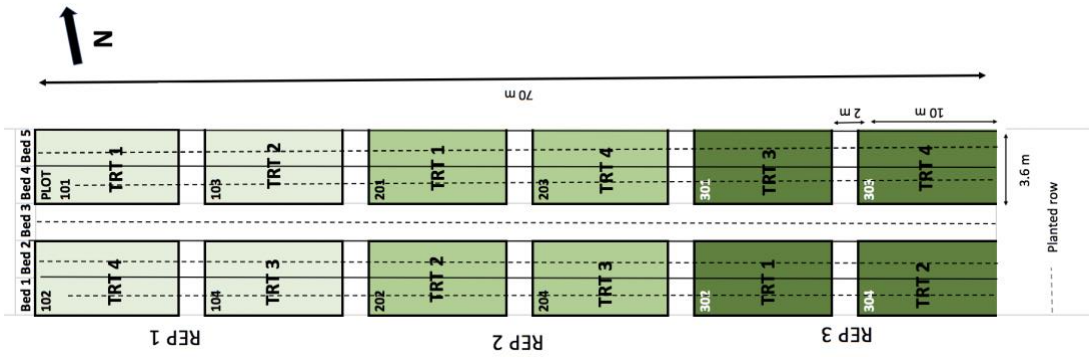


Figure 17. Layout of crop 1 planted at Richmond

Figure 18. First fungicide spray application and crop growth stage at time of application.

A spore suspension of the GSB pathogen was prepared using an isolate taken from an etched pumpkin. The inoculum was applied at late flowering/early fruit set on 9 January. The skin of ten fruit per plot were scratched and the PDA smeared over the wound to try and increase infection. Scratched fruit were marked with a pink ribbon to enable identification at harvest. Visual assessments of the leaves were conducted every 7-10 days commencing 7-days post-inoculation.

The timings of fungicide applications, inoculation and harvest are shown in Table 1.

Table 1. Schedule of treatments for crop 1, planted at Richmond in November 2018

#	Date	Application growth stage	Product	Active	Application rate (L/ha)
1	13/11/18	Vegetative	Switch	cyprodinil @375g/kg + fludioxonil @250g/kg	800g*
2	27/11/18	Vegetative	Amistar TOP	azoxystrobin @ 200g/L + difenoconazole @ 125g/L	900ml
3	6/12/18	Vegetative	Mancozeb 750	mancozeb @ 750 g/kg	2kg
4	20/12/18	Budding/flowering	Fontelis	penthiopyrad @ 200g/L	1.75L
5	2/1/19	Flowering	Amistar TOP	azoxystrobin @ 200g/L + difenoconazole @ 125g/L	1L
	9/1/19	Inoculation with spore suspension of *S. cucurbitacearum*			
I6	15/1/19	Flowering/fruit set	Switch	cyprodinil @375g/kg + fludioxinil @250g/kg	980 g
7	24/1/19	Flowering/fruit set	Bravo 720	chlorothalonil @ 720g/L	2.5L
8	31/1/18	Fruit development	Fontelis	penthiopyrad @ 200g/L	1.75L
	13/2/19	Harvest 1			
	4/4/19	Harvest 2			

The first harvest was completed on 13 February when the majority of fruit had reached harvest maturity. Fruit were assessed and incidence and severity of etch was recorded. A visual scale from 1 to 6 was used to rate severity of etch (Figure 19) where4 1=very slight and 6=severe. Grades over 3 would be unsaleable according to retail specifications.

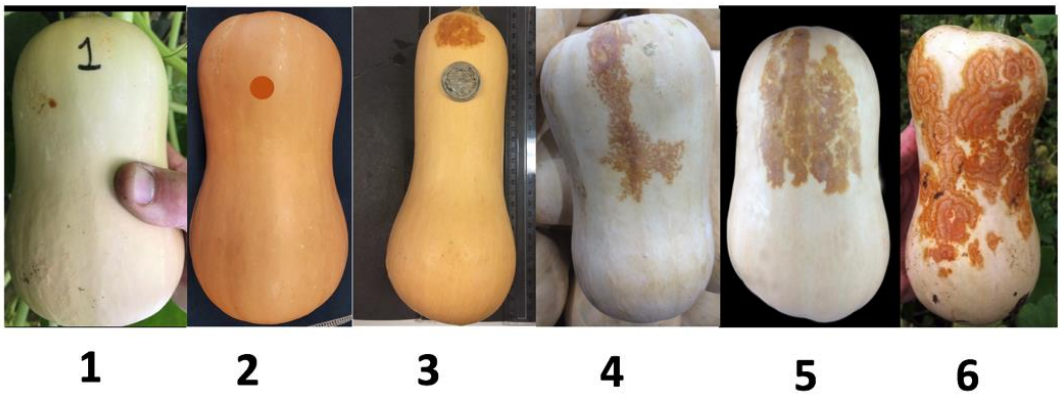


Figure 19. Severity rating scale

The pumpkins were retained in the field after the trial had been completed. On the 4th April (63 days after the last foliar application was applied) a high incidence of etch was observed on the second flush of fruit. A second harvest was conducted, with incidence and severity of etch recorded.

Crop 2

A second crop of butternut pumpkins cv. Jacqueline was planted on 8 January 2019. As previously, a single row was planted in the centre of pre-formed 1.8 metre wide beds, with each plot being two beds wide (3.6m) and 10m long. In this case chitosan was not used, but the other treatments remained the same ie.

1. Untreated control
2. Inoculation with *S. cucurbitacearum* (GSB)
3. Inoculation GSB plus intensive fungicide program

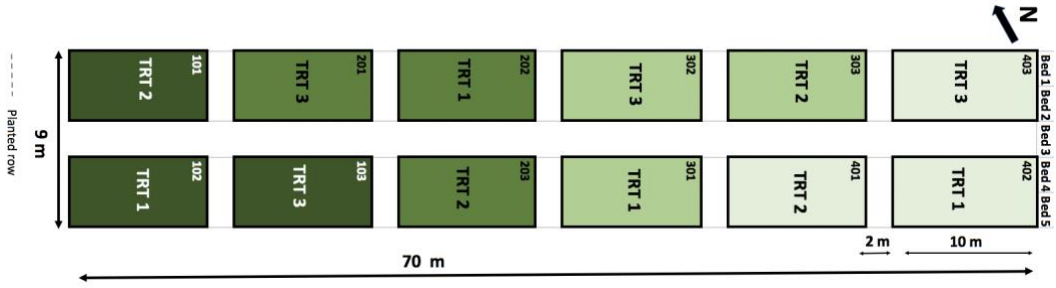


Figure 20. Layout of crop 2 planted at Richmond

The spore suspension of GSB was prepared as previously, using an isolate from an etched pumpkin. This was applied at late flowering / early fruit set, as shown in Table 2. Leaf symptoms were observed 23 days later (Figure 21), indicating that the plants had been successfully infected with the pathogen.

Table 2. Schedule of treatments for crop 2 planted at Richmond in January 2019

#	Date	Application growth stage	Product	Active	Application rate(L/ha)
1	31/1/19	Vegetative	Fontelis	penthiopyrad @ 200g/L	1.75L/ha
2	15/2/19	Vegetative	Amistar TOP	azoxystrobin @ 200g/L + difenoconazole @ 125g/L	900ml
3	22/2/19	Vegetative	Bravo 720	chlorothalonil @ 720g/L	2.5L
			Bayfidan	triadimenol@250g/L	400ml
4	4/3/19	Budding/flowering	Fontelis	penthiopyrad @ 200g/L	1.75L/ha
	13/3/19	Inoculation with spore suspension of *S. cucurbitacearum*			
6	21/3/19	Flowering/fruit set	Switch	cyprodinil @375g/kg + fludioxinil @250g/kg	980 g
	4/4/19	Harvest			

Figure 21. Foliar symptoms in the field, consistent with expression of GSB.

All pumpkins were harvested on 4th April, when the vines had started to die down and the majority of fruit were mature.

Crop 3 (variety trial)

A third crop was planted on 9 January at the same site. The objective of this crop was to examine whether there were differences in susceptibility between varieties. Four commercially available varieties were tested:

1. Hannah
2. Havana
3. Matilda
4. Tiana

A single-row of seed was planted in the centre of each pre-formed 1.8m wide bed, with each trial plot one bed wide and 16m long. The trial layout is shown in Figure 22. Note that only every second bed was planted, ensuring the varieties could be easily separated.

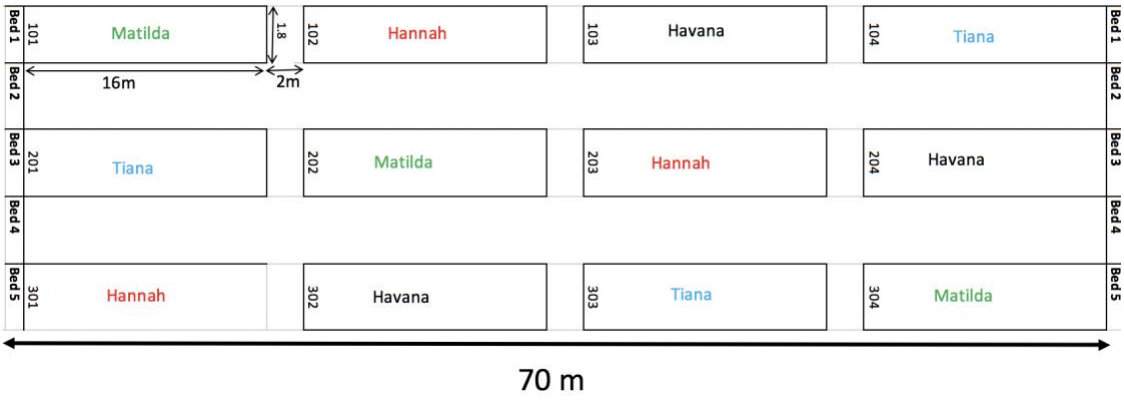


Figure 22. Layout of variety trial planted at Richmond

A spore suspension of the GSB pathogen, *S. cucurbitacearum* inoculum was applied as a foliar spray at late flowering/early fruit set, as previously described for crops 1 and 2.

All pumpkins were harvested on 4th April, when the vines had started to die down and the majority of fruit were mature.

Results

Crop 1

There was low incidence of etched fruit across all treatments at the first harvest. The maximum average incidence was found in chitosan treated plots (9%) and the lowest in fungicide (2%) treated plots. There was however no significant difference (*p=0.24*) between plots. Etch was generally minor for all treatments. No pumpkins were graded as 5 or 6 (severely etched). Pumpkins graded 3 or 4 (slight to moderately etched) were recovered only from the GSB inoculated and untreated control blocks.

Pumpkins displaying typical etch symptoms, including "petrified-wood" lesions and some with pycnidia were taken back to the lab for isolation. *S. cucurbitacearum* was always present in these lesions. Other fungal pathogens were also isolated including *Fusarium* spp. and morphology similar to *Colletotrichum spp.* No definitive diagnostics have been conducted to confirm the identity of these isolates.

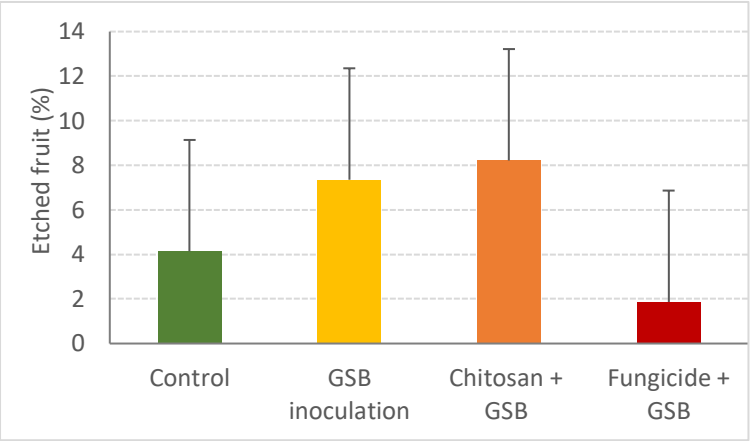


Figure 23. Incidence of etch at harvest 1. Bars indicate standard errors of means (n=3); differences between treatments were not significant.

The results from harvest 1 are in stark contrast to those from harvest 2. At the second harvest etch had increased dramatically, affecting 46% of all fruit. However, as previously, there were no significant differences between the treatments. The severity of etch was also similar, regardless of treatment.

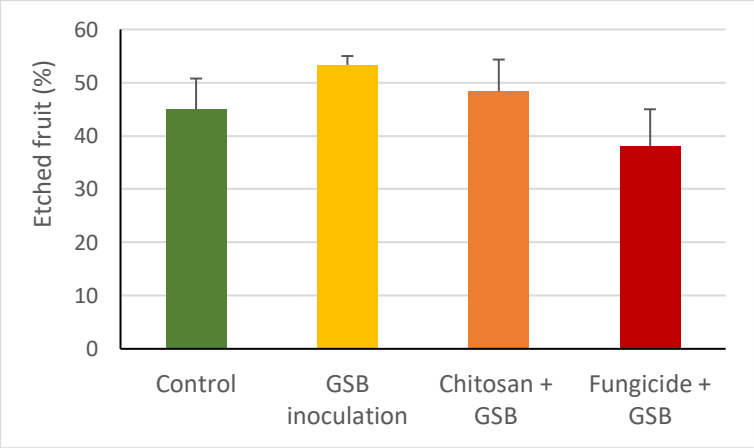


Figure 24. Incidence of etch at harvest 2. Bars indicate the standard errors of means (n=3); differences between treatments were not significant.

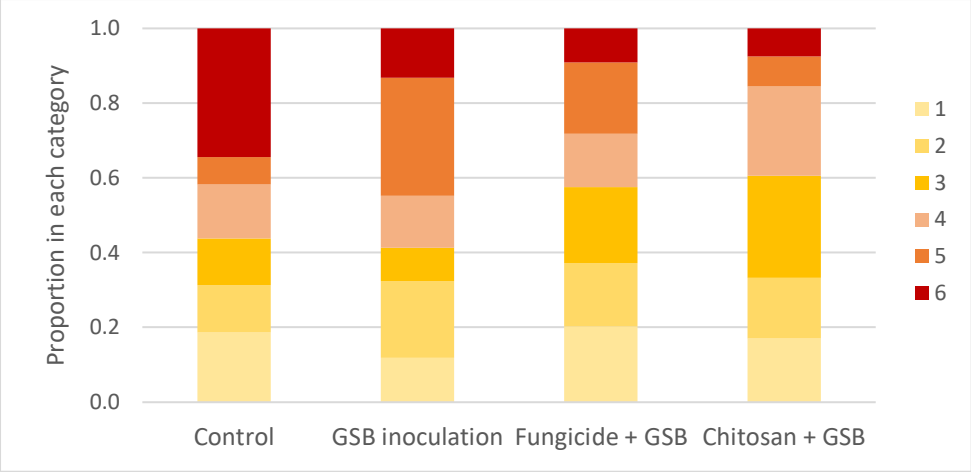


Figure 25. Proportion of etched fruit graded by severity from 1 (very slight) to 6 severe) for each treatment.

Figure 26. Severely etched fruit from harvest 2.

Pumpkins displaying typical etch symptoms, including "petrified-wood" type lesions and some with pycnidia, were taken back to the lab for isolation. *S. cucurbitacearum* was always present in these lesions. Other fungal pathogens were also isolated, including *Fusarium* spp. and *Colletotrichum spp.*.

Crop 2

The incidence of etch in crop 2 was consistent with the second harvest from crop 1, with around 40% of pumpkins affected by etch. Similarly to crop 1, differences between the treatments were not significant. However a trend to lower etch was noted for the fungicide treated plots.

The severity of etch was similar, regardless of treatment.

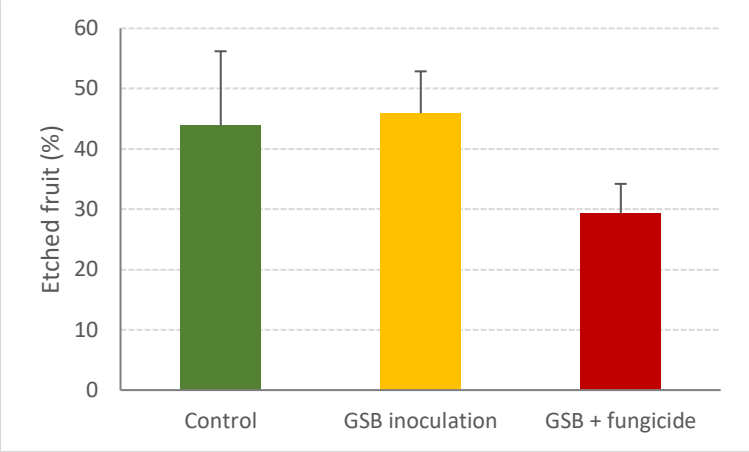


Figure 27. Incidence of etch in crop 2 at harvest. Bars indicate the standard errors of means (n=3); differences between treatments were not significant.

Crop 3

In contrast to crops 1 and 2, the incidence of etch in the variety trial was low overall, averaging only 7%. There were no significant differences between varieties. There was, however, a trend to higher rates of etch for Matilda, a variety that also produced a denser canopy cover than the other three varieties.

Figure 28. Appearance of the different pumpkin varieties two weeks prior to harvest.

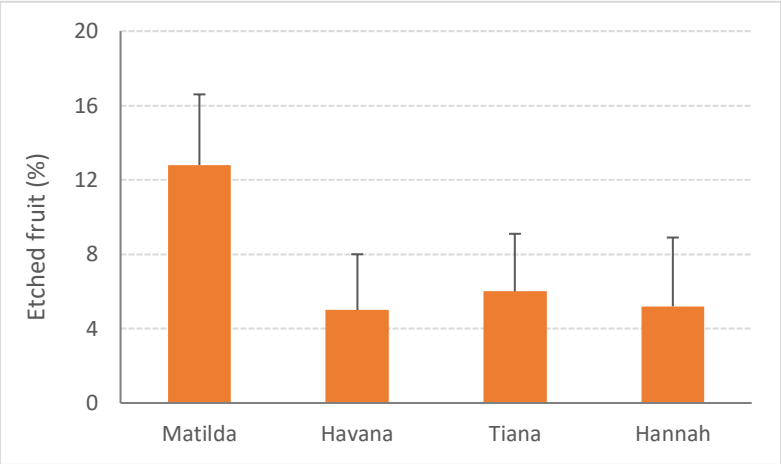


Figure 29. Incidence of etch in crop 3 at harvest. Bars indicate the standard errors of means (n=3); differences between treatments were not significant.

Discussion

It had been hypothesized that infection with the gummy stem blight pathogen *S. cucurbitacearum* was a primary cause of brown etch. However, in these trials there was no significant difference between inoculated and non-inoculated plots for crops 1 and 2 at either harvest date. However, it seemed possible that all of these plants in both crops were actually carrying the pathogen due to infection of the seed.

A sample of 100 seeds was therefore provided to the AgEtal laboratory for molecular testing for *S. cucurbitacearum*. The result was a very low level positive detection of a pathogen resembling *S. cucurbitacearum*. The laboratory suggested that this could simply be a variant compared to their known controls. However, the results are not conclusive.

While there appeared to be a slight benefit from an intensive fungicide program, differences were not significant. This would be consistent with the pathogen being already present in the plant, as foliar fungicides would be unlikely to have much effect. This is therefore not supported as a management option.

All of the tested treatments had minimal effects on etch. However there were significant differences between the initial and final harvests for crop 1, and between the first two crops of Jacqueline and the variety trial crop 3 at the final harvest in April.

The differences between the February and April harvests may be due to the weather leading up to each of these events. High RH and damp conditions appear to greatly increase development of etch. For example, the trials in Somersby recorded very high rates of etch during damp and humid conditions whereas no etch was recorded in Griffith during a hot, dry period.

In Richmond, there was 30% more rainfall during the 30 days prior to the April harvest than for the February harvest. Humidity was also higher during the month before the April harvests compared to the first harvest. Perhaps critically, RH was more than 80% for 55% of the time during the leadup to harvesting, whereas RH only exceeded this level 40% of the time during the 30 days before the February harvest. This is illustrated in Figure 30, which shows the time spent at different humidities. Of particular note is the period 14 to 21 days before harvest 2, when 68mm of rain fell and RH was almost continuously above 80%.

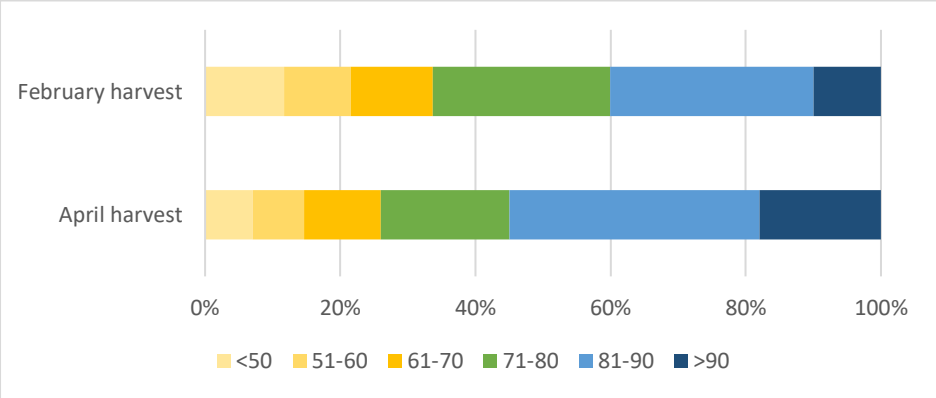


Figure 30. Time spent at <50% RH, 51-60%RH, 61-70%RH, 71-80%RH, 81-90% RH and >90% RH during the 30 days leading up to the February and April harvests in Richmond.

The difference between crop 3 and the crops of Jacqueline is more difficult to understand. Leaving the blank rows in between the different variety blocks is likely to have reduced humidity within the crop canopy, which may have had some benefit in terms of reducing etch. It is also possible that the low level of fungal infection detected by AgEtal within the Jacqueline seeds triggered an increased rate of etch in crops 1 and 2.

Figure 31. Condition of pumpkin vines cv. Jacqueline (46% etched), Matilda (13% etched) and Havana (5% etched) at the April harvest.

Conclusions

The results suggest that the causes of brown etch are complex. It is unlikely there is a simple solution to this issue. While it seems likely that the gummy stem blight pathogen can trigger etch, and certainly increase incidence, it also seems likely that this is not the sole cause of the disorder.

There is a clear influence of climate on brown etch. Wet or humid conditions definitely increase incidence of the disorder. At its simplest, simply comparing RH to rates of etch appears to give a good correlation. The results are best if using the data from the two weeks prior to harvest. As shown in

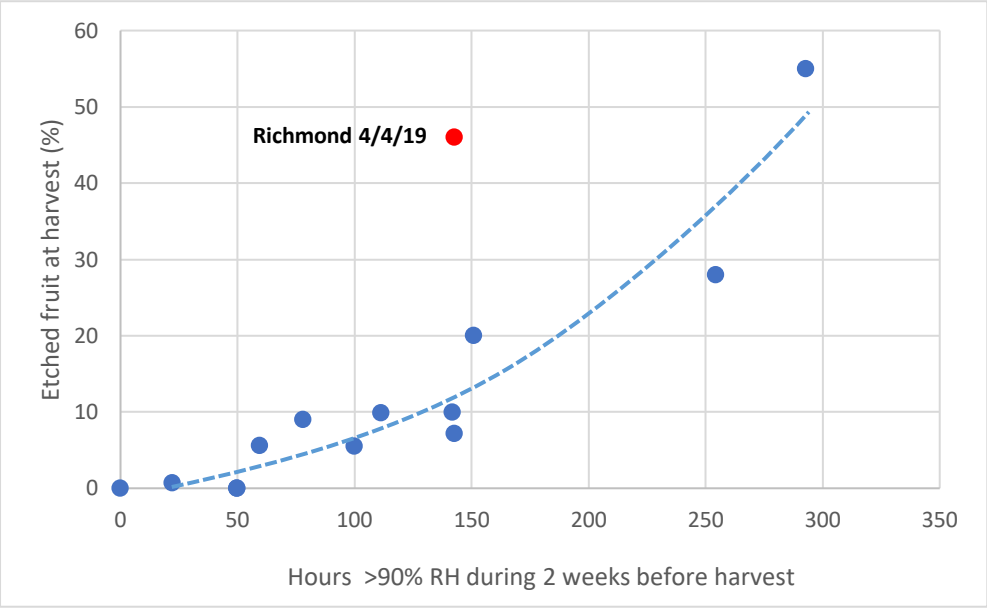


Figure 32, If RH is over 90% for more than 50 hours during the two weeks before harvest, or 15% of the time, then there is a risk that approx. 10% of fruit will be affected by etch. However, if RH exceeds 90% for >150 hours, or 45% of the time, then levels of etch may be much higher. It is interesting to note the outlier point from the April harvest in Richmond, where 46% of the pumpkins cv. Jacqueline were etched, even though RH was relatively moderate.

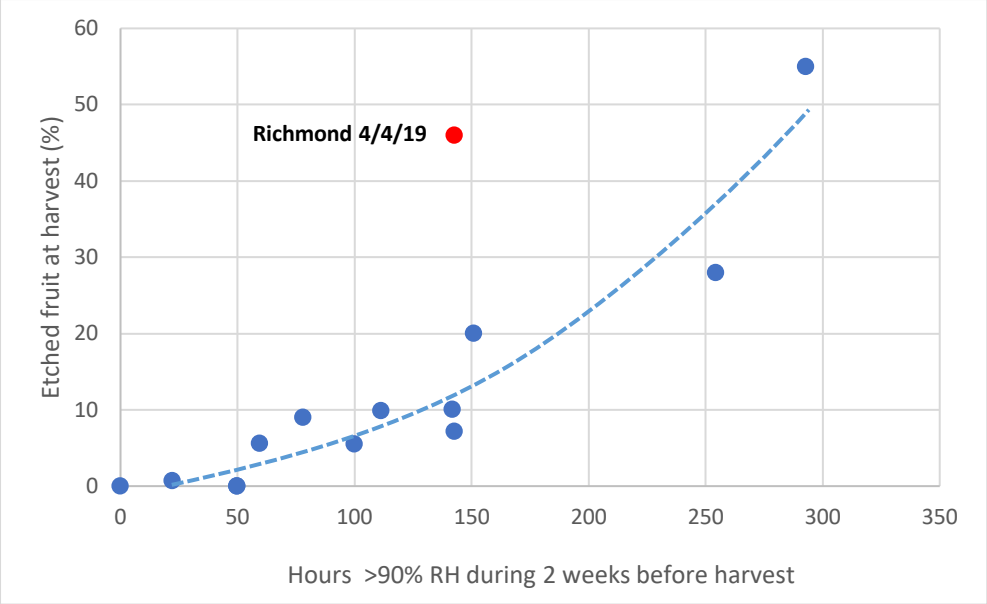


Figure 32. Correlation between RH during the two weeks before harvest and the incidence of etched fruit. Each point represents the mean values from a field trial. If the outlying value from Richmond is disregarded, then $R^2 = 0.92$

A slightly better correlation can be achieved by estimating the total time that pumpkins could potentially be wet. This was calculated by comparing dewpoint with actual dry bulb temperature (Figure 34). In this calculation, pumpkins are considered potentially wet if temperature is within 1°C of dewpoint. This may in some cases be a significant underestimate, as high soil moisture and/or thick canopy coverage are likely to increase wetness within the crop.

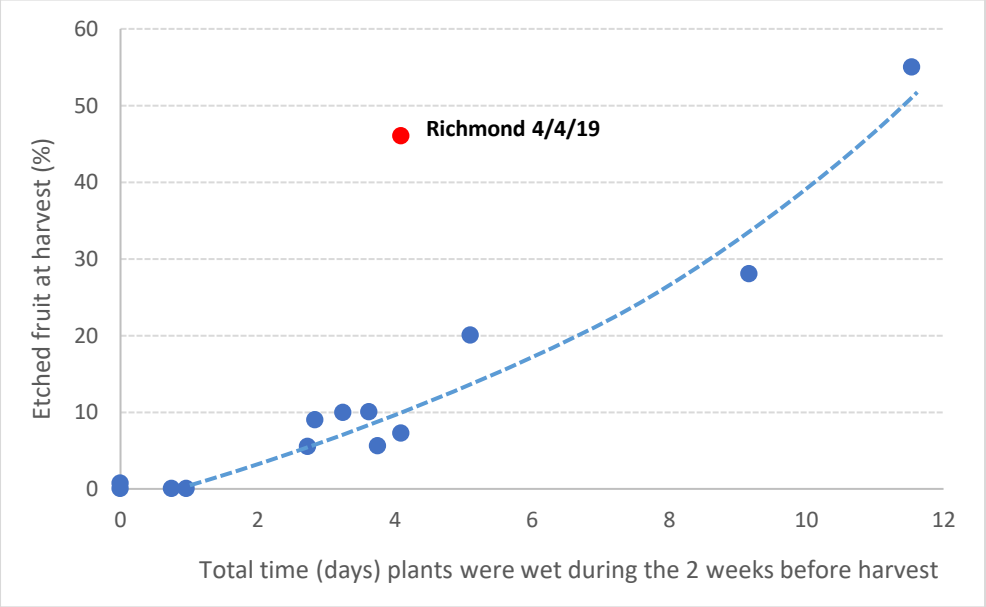


Figure 33. Correlation between wetness during the two weeks before harvest (calculated by comparing dewpoint with actual temperature) and the incidence of etched fruit. Each point represents the mean values from a field trial. If the outlying value from Richmond is disregarded, then $R^2 = 0.95$

On further examining the weather relating to the "outlier" value from Richmond, it is apparent that very wet conditions occurred for approximately one week 3-4 weeks before harvest. During this period, pumpkins were likely to have been continuously wet for approximately 3.5 days. The model shown in Figure 34 is based on the longest continuously wet period during the month before harvest. With this calculation, the Richmond crop (again shown in red) now aligns with other results.

This model suggests that even if a crop subsequently dries out, an extended wet period can trigger development of etch. The risk of etch increases if fruit stay wet for more than 1 day in the 30 days leading up to harvest. If fruit remain wet for more than 1.5 days, rates of etch are likely to exceed 20% of the crop.

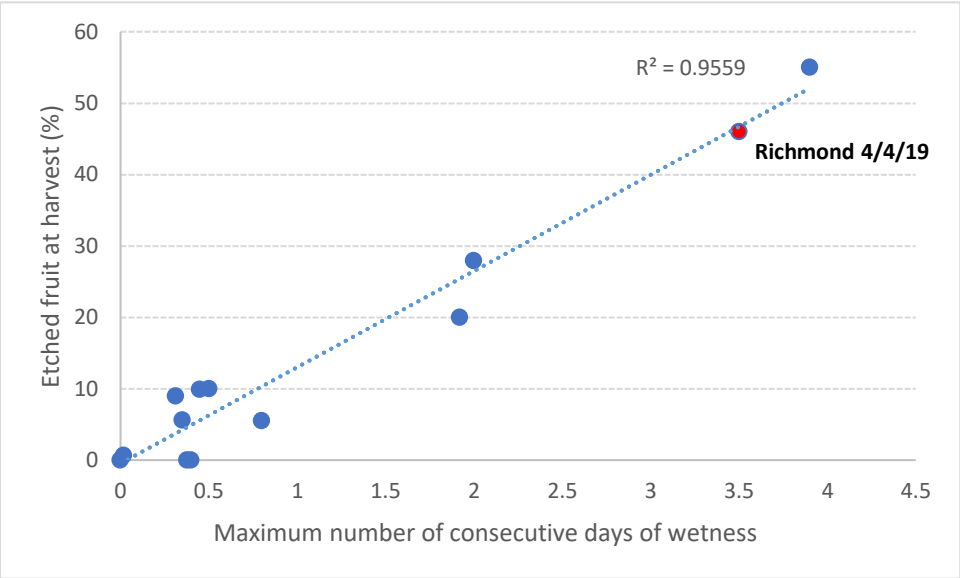


Figure 34. Correlation between the maximum consecutive time of wetness (calculated by comparing dewpoint with actual temperature) during the 30 days before harvest and the incidence of etched fruit. Each point represents the mean values from a field trial.

This suggests that an extended period of damp weather (>24 hours) during maturation therefore appears to be a primary trigger for development of etch. These models could provide a useful guide to growers as to likely presence of etch in their crops. For example, if the weather changes during the later stages of a crop, resulting in >90% RH and/or fruit remaining wet for extended periods, then harvest may be brought forward in order to avoid development of etch.

Cultural changes that reduce RH within a crop canopy may be useful if climatic conditions suggest there is high risk of etch developing. For example, reducing planting density, changing from overhead to drip irrigation, avoiding damp areas, or applying nitrogen sparingly, could potentially reduce rates of etch within the crop. However, trials are needed to determine the effectiveness of such strategies.

Appendix 6. Supply chain and postharvest studies

Supply chain monitoring – Griffith to Sydney

Method

Pumpkins cv. Jacqueline were grown using flood irrigation. After washing and sorting, they were packed into cardboard “hat bins” for transport to Sydney Markets. No etch was observed in the field or on any of the packed fruit. Temperature and RH loggers were placed into some of the bins. A number of pumpkins were packed inside plastic bags with a little moisture in order to increase RH around those fruit.

Results

Pumpkins remained at around 24^{o}C during transport, with RH averaging around 79-80% inside the bins. Conditions were likely to be fairly typical for pumpkins transported in this supply chain. No etch was observed in any of the bins, or on the bagged fruit, when the fruit arrived in Sydney. This suggests that if no etch is observed in the field, it may be unlikely to develop during harvest and transport.

Supply chain monitoring – Ayr to Sydney

Pumpkins in Ayr are grown on plastic with trickle irrigation. Sunset QHI is the most commonly grown variety, in part due to its believed resistance to etch. The Ayr supply chain was monitored to determine the development of fruit in the supply chain. There was already a high level of etch on farm, with large volumes of discarded fruit (Figure 1). The trial tested packing into CHEPs vented, folding plastic bins instead of standard cardboard “hat” bins as a way to potentially increase airflow and reduce RH during transport.

Figure 1. Etch in a paddock on plastic mulch, and reject pumpkins in a bin.

Method

Fruit were graded and packed clean into either 8 standard, unvented cardboard “hat bins” or 10 foldable vented plastic bins. Five etched pumpkins were marked to delineate the etched area and included in each bin, along with Hobo temperature and humidity recorders.

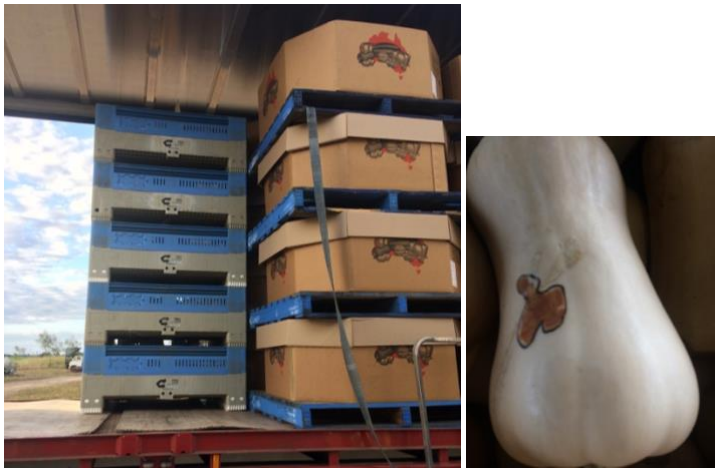


Figure 2. Plastic and cardboard bins loaded onto a tautliner truck for transport to Sydney, and a marked etched pumpkin.

The fruit in each bin were assessed on arrival at Sydney Markets (2 days in transit) and again one day later. Etch was recorded as being either minor (less than 50c piece) or major (would result in rejection). Pumpkins with markings already present were retrieved for assessment of whether the etched area had increased during transport.

Results

It had been expected that vented plastic bins would allow better cooling and reduced RH around pumpkins, potentially decreasing development and spread of etch during transport. While temperature was consistently around 4°C, the RH was initially higher in the plastic bins. This may be due to the reduced temperature as well as the cardboard absorbing some moisture from the pumpkins.

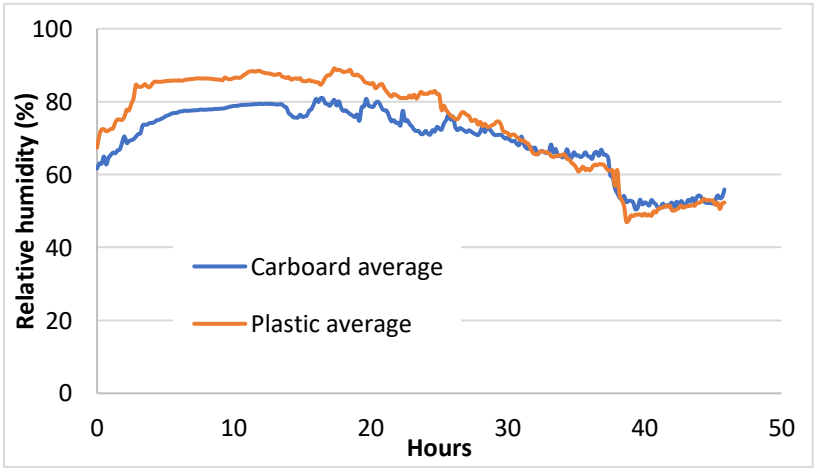


Figure 3. Average RH inside plastic and cardboard bins during transport from Ayr to Sydney.

Approximately 22% of pumpkins developed etch in transit regardless of bin type. It would have been interesting to repeat these inspections to determine if etch continued to develop during storage. However, shortages in the market meant all pumpkins were required to meet sales orders.

Table 1. Occurrence and severity of etch in cardboard and plastic bins

	Cardboard						Plastic					
	Total in bin	Major etch	Minor etch	Etch day 1 %	Etch day 2 %	Total etch (%)	Total in bin	Major etch	Minor Etch	Etch day 1%	Etch day 2 %	Total etch (%)
A	163	6	28		20.9	20.9	112	7	18		22.3	22.3
B	156	8	42		32.1	32.1	103	4	22		25.2	25.2
C	166	9	35		26.5	26.5	113	1	19	17.7		17.7
D	96	1	17		18.8	18.8	122	6	16	18.0		18.0
E	107	3	20	21.5		21.5	108	6	15	19.4		19.4
F	157	2	28	19.1		19.1	107	12	16		26.2	26.2
G	147	3	24	18.4		18.4	74	1	17	24.3		24.3
H	157	5	23	17.8		17.8	98	4	6		10.2	10.2
I							72	6	14		27.8	27.8
J							142	12	27	27.5		27.5
AVG	**144**	**5**	**27**	**19.2**	**24.5**	**21.9**	**105**	**6**	**17**	**21.4**	**22.3**	**21.9**

Etch continued to spread on three out of five pumpkins packed in cardboard and two of five pumpkins packed in plastic bins. However, effects were variable, with no significant differences between the bin types.

In this trial plastic bins did not reduce the amount of etch developed. Despite this, use of plastic bins could make it possible to air cure (using a forced air system) and/or cool pumpkins during transport.

This trial was conducted at a time of high risk; the previous week approximately 50% of the load was downgraded due to etch development during transport. The trial confirmed that if etch has been observed in the field, even clean pumpkins can develop severe symptoms during transport.

Forced air curing – Ayr

Method

Fifty etched pumpkins were selected and the edges of etched areas defined using a felt tip marker. The etched pumpkins were divided between five vented plastic bins and four normal cardboard “hat” bins, the remainder of the bins being filled with normal, clean fruit.

The plastic bins were then stacked on top of each other. An industrial fan mounted on a wooden frame was placed on top of the top bin and the sides of the stack sealed using a tarpaulin and stretchable plastic film. The fan was used to draw air through the pumpkins and out through the top of the stack in order to dry and “cure” the skins. The process was run for 24 hours, then the stack unwrapped and, together with the cardboard bins, loaded for transport to Sydney Markets.

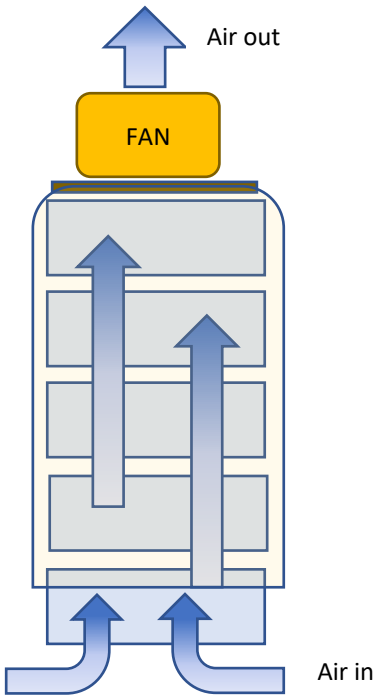


Figure 4. Forced air curing system for pumpkin bins

The bins were assessed on arrival at Sydney Markets.

Results

In this trial, temperature and RH in the plastic and cardboard bins was similar during both the curing process and transport. Etch was uniformly low, with little development on either the etched or clean fruit. Overall, 2.8% of pumpkins packed into the plastic bins were etched compared to 3.8% of pumpkins in cardboard. A trend was noted to more severe etch in the cardboard bins, but levels were too low to draw any conclusions as to the significance of this result.

Pre-harvest treatment effects – Ourimbah laboratory

Method

Pumpkins growing at the Somersby trial site during the 2017 to 2018 season were treated with SettEnhance (calcium), AgriSil (silicon), foliar potassium or a soil drench of *Bacillus subtilis* (Serenade).

Pumpkins were harvested at maturity and assessed for etch. One harvest lug of fruit from each plot was then transported to the NSW DPI Ourimbah laboratory and stored under ambient conditions. Pumpkins were re-assessed after 10 and 17 days. Etched areas were defined with a felt tip marker at each assessment so as to monitor expansion of existing lesions.

Results

There was a large increase in the percentage of etched pumpkins between harvest and the 10 day assessment. However, there was little further increase over the following week.

At harvest there was a trend to fewer etched pumpkins when treated with SettEnhance. During postharvest storage similar increases in etch were observed for all treatments. It is concluded that none of the pre-harvest treatments tested affected postharvest development of etch.

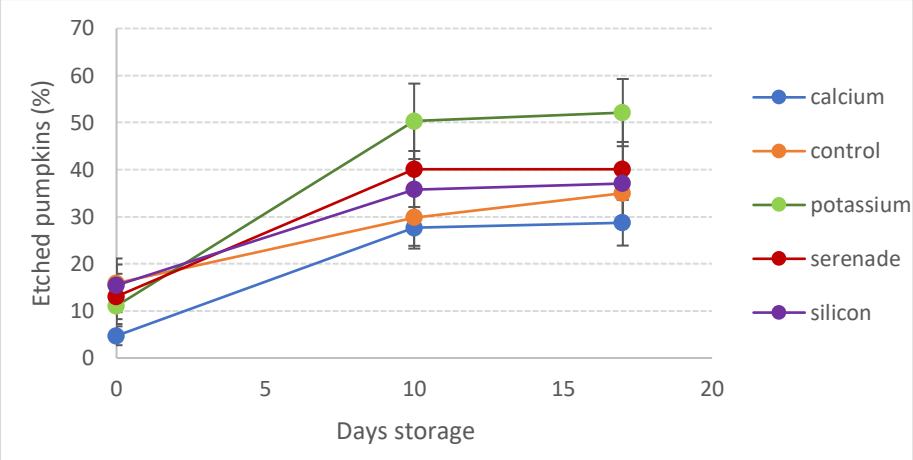


Figure 5. Percentage of etched pumpkins at harvest then after 10 or 17 days storage under ambient conditions. Bars indicate std. errors of means. Pumpkins were grown with foliar applications of calcium, potassium or silicon or a soil drench with Serenade.

Storage conditions – Sydney laboratory

Method

2017 trial; RH

Pumpkins cv. Jaqueline and cv. Sunset QHI were harvested at Somersby and classed as etched (44 x Jaqueline, 42 x Sunset QHI) or 'clean' (62 x Jaqueline, 70 x Sunset QHI). The edges of etched areas wre defined with a felt tip marker and dated so as to be able to determine whether this area was continuing to expand during storage.

Each group was then divided randomly among three different humidities;

1. Enclosed in large plastic bag with water in the bottom (95%RH).
2. Left under ambient conditions in the laboratory (70%RH)
3. Placed in an incubator (15%RH)

Temperature and RH were confirmed by including Hobo data loggers with each treatment.

The pumpkins were initially examined daily, and then every two–three days for up to two weeks. Individual pumpkins were followed so as to be able to tell which developed and when. If etched areas spread, or new etch developed then this was also marked and noted.

2018 trial; RH

The trial was repeated using pumpkins cv. Jacqueline from the Somersby crop grown in 2018. Rates of etch were lower and fewer fruit were available, so only clean fruit were used. Development of etch and expansion was monitored after 10 and 17 days of storage only.

2019 trial; temperature

A third trial was conducted during 2019 examining whether simply refrigerating the pumpkins would reduce development of etch. Etched and non-etched pumpkins were sourced from the field trial at Richmond. Etched areas were marked as previously and the pumpkins divided between refrigerated and ambient storage.

Refrigerated storage averaged 5^{o}C and 70%RH. This is colder than recommended for butternut pumpkins, which should not normally be stored below approximately 10^{o}C. Ambient storage was at 22^{o}C and 70%RH.

New areas of etch were recorded after 7 days storage.

Results

2017 trial; RH

Etch continued to develop during storage of both varieties of pumpkins. Compared to pumpkins stored at very low RH, pumpkins at high RH were more likely to develop new etch as well as expanded areas of existing etch. Results were similar for the two different varieties grown.

Figure 6. Etched pumpkin, showing initial area of symptoms, then expanded areas after 1, 2, 3 and 5 days of storage.

Pumpkins that were initially clean (no etch visible) were less likely to develop symptoms during storage but still did so, even at very low RH (Table 2).

It had been expected that expansion / appearance of new etched areas would be most likely immediately after harvest, then decline to negligible levels after a number of days. However, in this trial the greatest number of new areas of etch was recorded five days after harvest, for both varieties. A similar pattern appeared to occur for the three different storage environments, and affected initially clean as well as etched pumpkins. While development slowed after this time, new areas continued to appear even two weeks after harvest.

Table 2. Percentage of etched or (initially) clean pumpkins that developed new / expanded areas of etch during two weeks storage at 23oC with different levels of RH.

Variety	*Etched at harvest*			*Clean at harvest*		
	95%	**70%**	**15%**	**95%**	**70%**	**15%**
Jaqueline	100	56	40	28	30	10
Sunset QHI	100	93	53	17	36	7

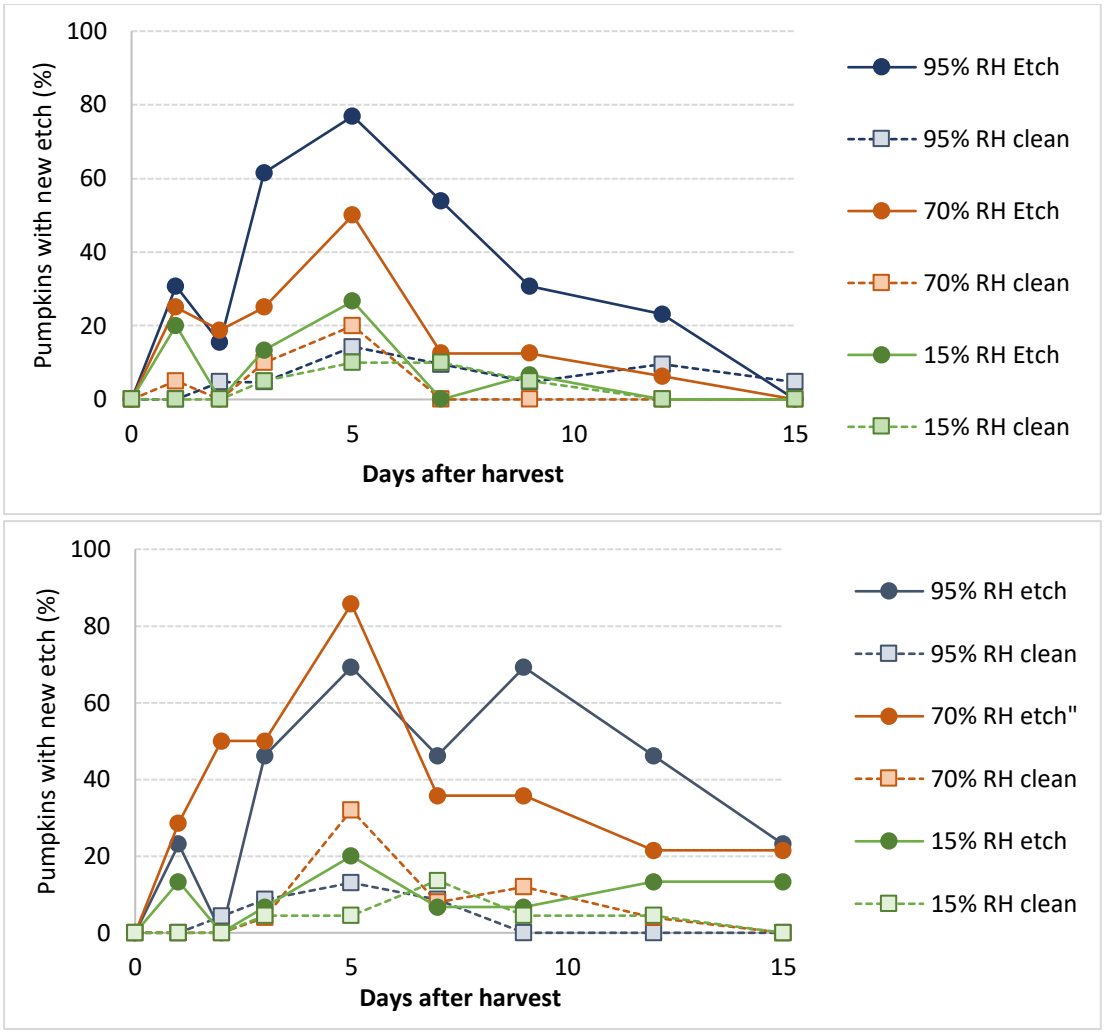


Figure 7. The percentage of pumpkins cv. Jacqueline (top) or cv. Sunset QHI (bottom) with new areas of etch, recorded every 1–3 days during storage at 23°C and 95, 70 or 15% RH.

2018 trial; RH

Results were consistent with those from the previous year. Etch development was increased under high humidity compared to drier conditions, with ambient RH intermediate. In this case, new etch continued to develop between 10 and 15 days after harvest, but at relatively low rates.

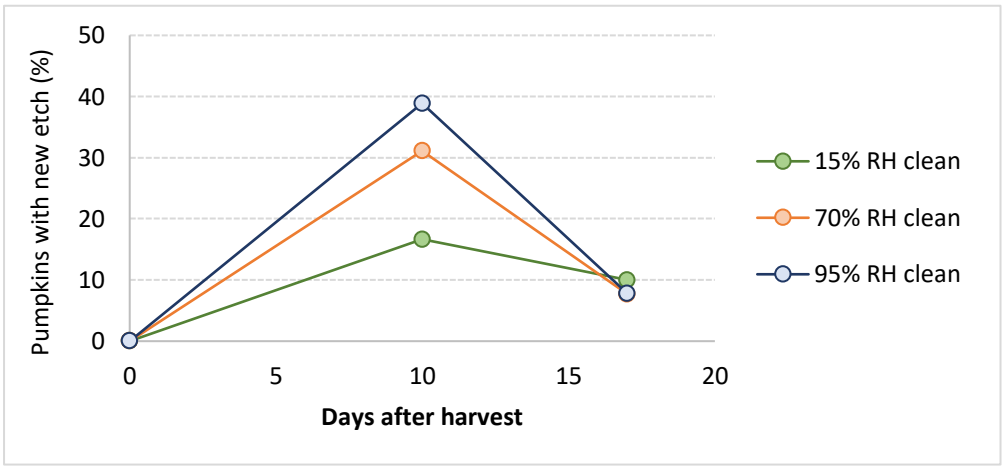


Figure 8. Percentage of clean pumpkins cv. Jacqueline that developed etch during storage at 23°C and 95, 70 or 15% RH.

2019 trial; temperature

Refrigerated storage virtually eliminated new etch on clean fruit and greatly reduced further spread of the lesions on etched fruit. Unfortunately, the temperature was damagingly low, with the result that chilling injury became apparent on these pumpkins a few days after transfer to ambient conditions.

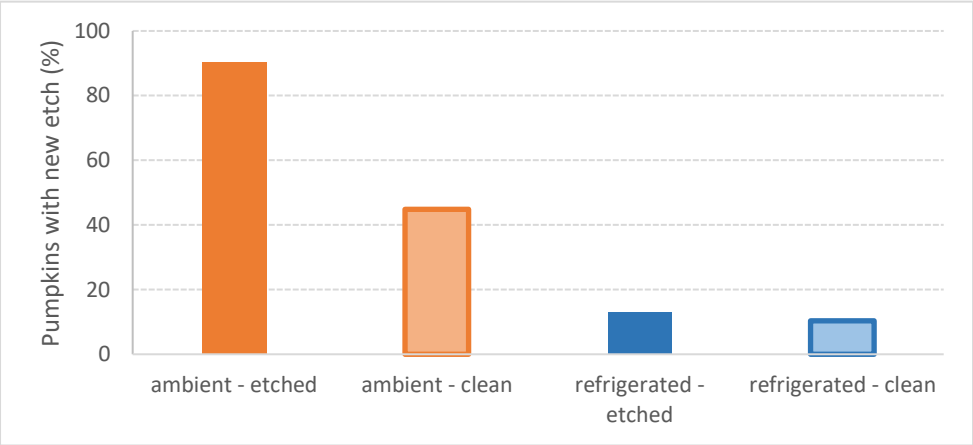


Figure 9. Development of etch during ambient and refrigerated storage of pumpkins that were either clean or etched at harvest..

Conclusions

The results demonstrate that pumpkins grown under conditions that allow etch to develop in the field can continue to develop symptoms during storage, even if they appear clean at harvest. It had been hypothesised that if etch occurs in the field, packers could store harvested pumpkins for a few days, then sort to remove blemished fruit before packing and sending to market. This would avoid the transport costs involved with rejections of etched pumpkins on arrival at wholesale.

Etch development appears to maximise three to five days after harvest, with the number of new / expanded blemishes then tending to decline. Unfortunately, etch can continue to develop for two weeks or more, so even sorting and packing one week after harvest does not guarantee that pumpkins will still be clean on arrival at market. Nevertheless, delaying re- packing from field bins could reduce the number of rejected fruit at market.

Manipulating RH around the pumpkins could also help to reduce spread. Storage under only 15% RH reduced etch compared to storage under saturated conditions, with 70% RH intermediate. Although even low RH conditions did not completely prevent etch occurring on clean fruit, drying out the pumpkin skin through a curing procedure may have some promise.

Although the storage temperature used in the 2019 trial (5^{o}C) was too low for butternut pumpkins, the results suggest that managing temperature could reduce etch development during transport. This could have major benefits for growers, as it would have a major impact on waste at wholesale. Further trials should test a more moderate temperature (e.g. 12^{o}C). They should also investigate whether inhibition of etch development is permanent or only temporary, with expansion re-starting once pumpkins warm to ambient conditions.

Pumpkins that are supplied cut to retail stores must be supplied chilled due to food safety concerns. Cooling normally occurs after cutting and wrapping. Pre-cooling could be used during high risk periods to ensure against etch development in transit. If pumpkins can then be kept cold through the supply chain, total cost increase would be marginal but benefits potentially highly significant.

Effect of etch on retail sales

Etched pumpkins are typically graded out and sold at a lower price for processing, rather than to supermarkets. However the internal quality of etched pumpkins is unaffected, and these fruit could potentially be sold in supermarkets at a small discount.

Butternut pumpkins are commonly sold cut in half and over-wrapped with plastic. When displayed this way, the skin is not immediately visible, but the flesh is clearly unaffected by disease. It was thought that consumers would either not notice blemish on the skin or be unconcerned, given that the flesh was unaffected.

The aim of this trial was therefore to compare sales of discounted etched butternut pumpkins to that of clean-skin butternut pumpkins (normally priced) in a retail store.

Method

A cut butternut pumpkin display was modified at an independent fruit and vegetable grocer in Sydney, NSW.

Etched pumpkins were retrieved from graded-out fruit at from a wholesaler at Sydney Markets. To ensure the etch marking was easily visible, only fruit with blemishes greater than approx. 25cm^2 were selected.

Etched pumpkins were cut in half through the discoloured area, allowing customers to see for themselves that the flesh was unaffected. Fruit were displayed with cut-flesh facing up, on a display setup as follows (Figure 10, Figure 11):

- Clean-skin fruit on the left, etched fruit on the right, with a row of grey pumpkins separating them
- Header-cards to draw the shopper's attention to the etched skin, yet still acceptable flesh
- Clean fruit priced at $3.00/kg, etched fruit discounted to $2.49/kg for 6 days, $2.79/kg the following 12 days, and finally no discount for 9 days.

Figure 10. Etched (right) and clean-skin (left) pumpkin display

Figure 11. Display header cards

The number of fruit sold each day was recorded by store staff. Regular store visits were made by the project team to check the display was set up correctly and collect sales records.

Results and conclusion

Sales of etched pumpkins were similar to, or slightly higher than, sales of clean pumpkins when offered at a 50c/kg discount. Reducing the discount to 20c/kg appeared to marginally reduce etched fruit sales, with sales similar to that of clean pumpkins. Sales were also similar when the discount was removed (Figure 12,).

Unfortunately, only the first 3 days of sales were recorded once the discount was removed. Store staff considered sales of etched and clean pumpkins to be the same at that point, so stopped recording data.

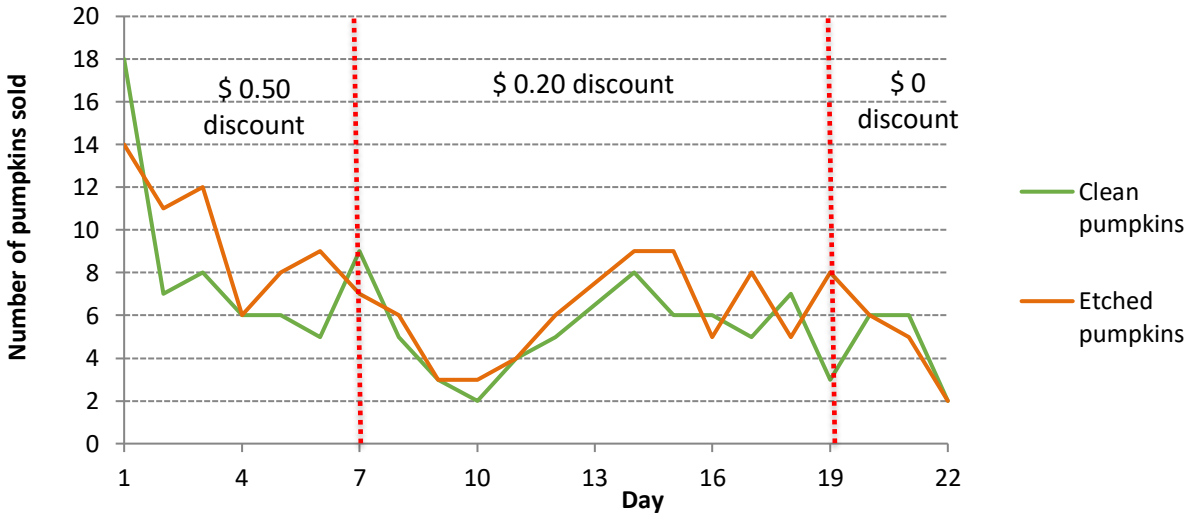


Figure 12. Daily sales of etched and clean butternut pumpkins. Clean pumpkins were sold at $3.00/kg, and etched fruit were discounted by $0.50/kg for 6 days, then by $0.20/kg for 12 days and finally $0 for 3 days.

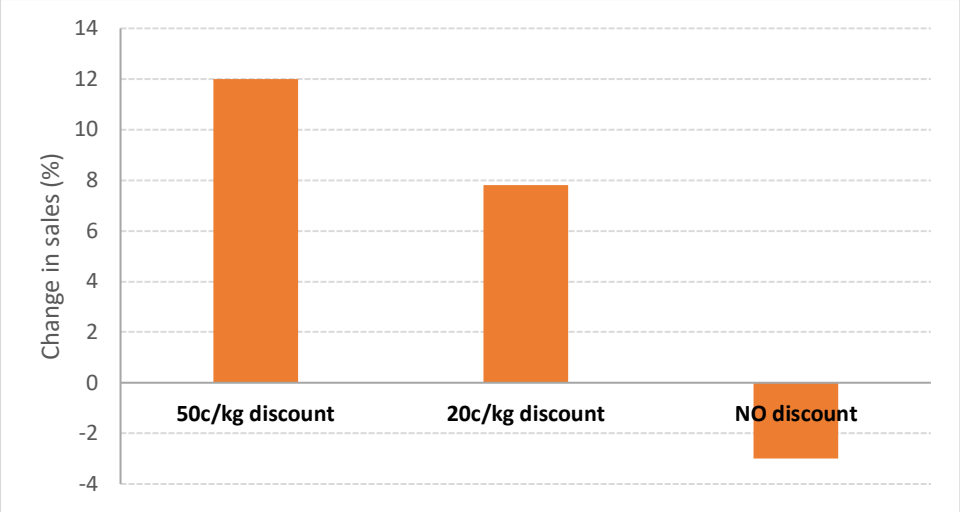


Figure 13. Average sales of cut, etched pumpkins compared to cut, clean fruit when offered with a discount of 50c/kg; 20c/kg or no discount.

Shoppers were willing to purchase etched pumpkins even at the same price. It seems likely they only checked the internal quality of the fruit, paying little regard to the appearance of the skin. This appears confirmed by store staff, who reported that customers did not question the quality of etched fruit, and that most did not turn the pumpkins over to look at the skin.

This work suggests that resistance to buying etched pumpkins is low if fruit are cut and consumers can see that the flesh is normal.

The Mysterious case of Pumpkin Etch

Brown etch, or 'Rust mark' is a big problem for Australian butternut pumpkin growers. The appearance of etch can result in major losses on farm, quality downgrades or market rejections.

In many growing regions, including Gatton, losses of 50% are not unknown. Sometimes crops may be abandoned as not worth harvesting, due to the large percentage of affected fruit.

Brown etch can develop in the field, where it often appears as concentric brown rings or patterns. As the pumpkin ages the brown area may dry and crack, or turn into a soft, rotten mess.

Etch in the field

Worse, in many ways, is when the condition develops during transport. By this time it has been picked, packed and trucked for hundreds or even thousands of kilometers, only to have to be re-graded or even rejected on arrival at the market. Some agents can find it hard to believe that the brown, unsalable pumpkins they find near the bottom of the bin were actually perfect at packing.

This type of brown etch more often has an irregular, blotchy appearance. It can appear on any part of the fruit, or all over it.

Etch that has developed in the lab, after harvest

As a cause of major economic loss, you might expect that brown etch would have been the subject of significant research effort in Australia and in other countries (eg the USA) that grow butternut pumpkins. However, surprisingly little is known about this problem.

It has not even been clear whether brown etch is caused by a disease or is actually a physiological disorder. Although many have assumed that a species of *Fusarium*, or black rot (*Didymella bryoniae*) was to blame, neither organism can be consistently isolated from the affected tissue, and attempts to induce the symptoms by inoculating with the disease have had mixed results.

In contrast, a 1971 paper in the Plant Disease Reporter describes etch as "cold pox", stating it is caused by temperatures below 50°F (10°C). It cites an example of a severe etch outbreak, where temperatures fell to the low 30's (just above 0°C).

To determine the causes of brown etch and, more importantly, find ways to minimise or prevent it, HIA have funded a project on "Improved management of brown etch". Led by Applied Horticultural Research, the project team has already conducted several field trials, as well as monitoring commercial pumpkin crops to examine the link between weather and appearance of the symptoms.

The findings so far;

- Brown etch symptoms almost always start from a contact point with soil, stem or leaf.
- Development in the field is strongly associated with warm conditions and high relative humidity, which limit transpiration by the pumpkin plant.
- Microscopic examination of the brown etch lesions reveals that the cells just under the surface have collapsed, allowing the cell contents to leak out.
- In one trial, attempting to inoculate pumpkins with potential causes of brown etch reduced, rather than increased, the appearance of symptoms.
- Brown etch is also a postharvest issue. Small spots at picking can expand rapidly in the several days following harvest, while new lesions may develop on previously unblemished pumpkins. For up to two weeks after harvest.
- Postharvest development is increased by storage in high RH.

Postharvest development of etch – lines were drawn around the etched areas daily.

From this we can conclude that, first and foremost, brown etch is not a disease but a physiological disorder. It appears to be linked to stress, and perhaps loss of integrity in the cell walls.

Initial work suggests that calcium may have an important role to play. Trials are now examining ways to enhance the pumpkin plants resistance to stress, and increase the strength in cell walls.

However, we are still searching for the real murderer in this whodunnit!

For more information contact Dr Jenny Ekman, Applied Horticultural Research

Jenny.ekman@ahr.com.au or M: 0407 384 285.

ATTENTION PUMPKIN GROWERS: HAVE YOU SEEN THIS ETCH?

Brown etch, or Rust mark, is a major problem for many Australian pumpkin growers, especially producers of butternut varieties. It regularly results in significant losses on-farm, product quality downgrades or rejections in the market. Dr Jenny Ekman from Applied Horticultural Research (AHR) explains the damage Brown etch can cause to pumpkins and the factors behind it.

In growing regions such as Mareeba, Bundaberg and Gatton in Queensland, pumpkin losses due to Brown etch can reach 50 per cent or more. Sometimes, crops may be abandoned as they are not worth harvesting, due to the large percentage of affected fruit.

Brown etch can develop in the field, where it can appear as concentric brown rings or patterns. As the pumpkin ages, the brown area dries and cracks, which allows for the growth of fungal diseases and rots.

Worse, in many ways, is when the condition develops during transport. By this time, the pumpkins have been picked, packed and possibly trucked for thousands of kilometers, only for them to have to be re-graded or even rejected upon arrival at the market.

A project on this issue, entitled *Improved management of pumpkin brown etch*, has been funded by Horticulture Innovation Australia using the research and development National Vegetable Levy and funds from the Australian Government. Led by Applied Horticultural Research (AHR), the aim is to find out what causes Brown etch and how to manage it.

A GLOBAL ISSUE

According to project leader Dr Gordon Rogers, Brown etch is not just a problem for Australian growers.

"Brown etch occurs in any area that grows butternut pumpkins and their hybrids. You would think that there would be lots of research on this issue. However, there are only a handful of references, and surprisingly little is known about what causes it," Dr Rogers said.

It is not even clear whether Brown etch is caused by a disease or if it is actually a physiological disorder. Attempts to induce the symptoms have had mixed results.

Vegetable pathologist Dr Len Tesoriero from the New South Wales Department of Primary Industries (NSW DPI) has been trying to find a causal organism on pumpkins affected by Brown etch.

"It has always been assumed that a fungus was to blame. In the past, researchers have isolated various species of *Fusarium*, while others have suggested that a type of gummy stem blight (*Didymella bryoniae*) was the cause," Dr Tesoriero said.

"However, none of these organisms can be consistently isolated from the affected tissue. Many of the samples we have worked on have yielded no live patho[illegible] this is because the fungus has alread[illegible] symptoms. Alternatively, it may not b[illegible]

GROWER DISCUSSION

In November 2016, the project team [illegible] growers from around Australia in Mareeba, Queensland to discuss when and where they had seen Brown etch.

According to Dr Jenny Ekman from AHR, humidity and prolonged wetness definitely appear to be important factors.

"Crops grown on plastic mulch or sandy sites seem less likely to get Brown etch than those grown on heavy soil, especially if overhead irrigation is used. Growers have also suggested that it is worse if there is a change in the weather, such as when a cold front comes through after a long dry spell," she said.

A series of weather stations are now being installed on farms to examine the climatic conditions associated with the onset of Brown etch. It is also planned to conduct post-harvest trials to examine whether different storage and packing methods can control Brown etch during transport.

"Pumpkins are normally packed into cardboard bins for transport, but these have no ventilation," Dr Ekman explained.

"Warm temperatures, high humidity and condensation inside the bins could be making Brown etch worse during transport. We are going to test different handling strategies, including Chep's new plastic, foldable bins, to try to reduce the problem."

The research team would like to hear from growers who have seen Brown etch in the past, or who are currently experiencing this issue in their crops. They are also particularly keen to analyse any immature pumpkins that are just starting to show symptoms of Brown etch.

R&D | Farm Productivity, Resource Use & Management

INFO

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Severe etch on a butternut pumpkin in North Queensland.

PROJECT UPDATE: MANAGING BROWN ETCH IN PUMPKINS

Brown etch, or rust mark, is a major issue for pumpkin growers in Australia – and the cause of the disorder is largely unknown. Dr Jenny Ekman from Applied Horticultural Research provides an update on a strategic levy investment looking at this mysterious pumpkin problem and how to manage it.

Any grower of butternut pumpkins is likely to be familiar with brown etch, or rust mark. Although the developing brown areas are superficial and do not reduce eating quality, the appearance of etch makes the pumpkins unmarketable. At times etch has resulted in abandonment of whole crops, while losses of 10-20 per cent are not unusual.

Initially, brown etch usually develops in the field. It can appear as either a pattern of concentric brown rings, or as irregularly-shaped brown blotches spreading across the fruit.

Etch can also develop after harvest, so that a freshly packed, clean bin of pumpkins at the farm can be riddled with etch by the time it arrives at the wholesale markets. This etch is almost always blotchy. The greatest losses can occur at this stage of the supply chain, as affected bins need to be sorted and graded.

It has always been thought that etch was caused by a pathogen, although attempts to isolate the organism responsible have had varied results. Gummy stem blight (*Didymella bryoniae*) and various species of *Fusarium* have been isolated at times, but attempts to cause the same symptoms through re-inoculating the pathogen onto healthy fruit have had little success.

A current project is attempting to find the cause of brown etch as well as minimise its occurrence. *Improved management of pumpkin brown etch* (VG15064) is a strategic levy investment under the Hort Innovation Vegetable Fund.

INCIDENCE OF ETCH (%) IN A PUMPKIN CROP IN NORTH QUEENSLAND LEADING UP UNTIL HARVEST

PROJECT FINDINGS

One thing seems clear from the results recorded so far; brown etch is not a disease, but a physiological disorder.

Project team pathologist Dr Len Tesoriero has been attempting to isolate organisms from the etched areas of pumpkins.

"There is no consistency between the samples, and often we isolate no pathogens at all. In terms of the pathogens on or just under the fruit skins, there doesn't seem to be any difference between etched and non-etched pumpkins," Dr Tesoriero said.

"We have also tried inoculating pumpkins with *Fusarium* and gummy stem blight at different points during maturation. So far, none of these inoculations have increased etch compared to the untreated controls."

This result appears to be confirmed by microscopic examination of the affected skin. Sydney University researchers have been examining the lesions and have found that no fungal structures are visible, but the cells immediately under the surface layer appear twisted and pushed out of shape.

"This suggests that either they have been exposed to heightened turgor pressure, or that the structure of the cell walls is weak. Many are partially collapsed, which may explain the brown colour of etched areas," Sydney University Associate Professor Brian Jones explained.

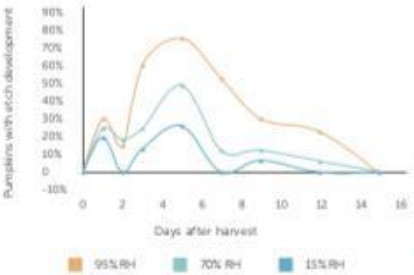
Turgor pressure is pressure within the cell of a plant that is generated by the movement of water across cell walls. Turgor pressure pushes up against the cell wall, resulting in rigidity.

Field trials have demonstrated that there is a strong relationship between development of etch and high humidity. In more than 90 per cent of cases, etch originates at a contact point with the soil, vegetation or another pumpkin. Although occasionally found on green fruit, incidence generally increases as the crop approaches maturity. This suggests that etch may be a reaction triggered by turgor pressure stress.

POST-HARVEST ISSUES

However, this still doesn't explain why etch appears post-harvest. When etched pumpkins were stored at 70-95 per cent relative humidity, the affected areas continued to spread on more than 70 per cent of fruit. Of those that appeared clean at harvest, 20-30 per cent developed etch during storage. This is consistent with observations of commercial shipments. Although only clean pumpkins were packed from one affected crop, 24 per cent of pumpkins were etched by the time the bins travelled from north Queensland to Sydney Markets.

THE DEVELOPMENT OF ETCH (SPREAD OR NEW OCCURRENCE) POST-HARVEST ON PUMPKINS STORED AT 95%, 70% OR 15% RELATIVE HUMIDITY



Moreover, symptoms can continue to appear and grow for up to two weeks after harvest. This means that even delaying packing for a few days may not guarantee that bins remain etch-free.

These trials included the popular varieties "Jaqueline" and "Sunset QHI". Although the latter was bred for etch resistance, rates of both pre- and post-harvest etch development were similar in this case.

Storing under low relative humidity reduced etch development, although it was not eliminated. Post-harvest trials are now testing whether 'curing' pumpkins, as is done for onions, can reduce post-harvest incidence.

However, prevention is better than cure, so field trials are focusing on treatments that either increase the strength of cell walls and/or turn on natural plant defences. It may also be possible to determine which genes relate to etch susceptibility, with the long-term goal of producing a truly etch-proof butternut pumpkin.

INFO

For more information, please contact Dr Jenny Ekman on 0407 384 285 or jenny.ekman@ahr.com.au

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Project Number: VG15064

Hort Innovation | VEGETABLE FUND

Article submitted to Vegetables Australia for publication in 2020.

Heading: Understanding brown etch of pumpkins

Stand-first: Brown etch, or rust mark, is a major issue for butternut pumpkin growers. A project recently completed by Applied Horticultural Research examined the causes of this disorder and what can be done to reduce the risk of it occurring in the field or after harvest. Dr Jenny Ekman provides a summary of outcomes from this strategic levy investment.

Body text: Growers of butternut pumpkins are likely all too familiar with brown etch, or rust mark. Although eating quality is unaffected (the brown areas are purely superficial) the appearance of etch greatly reduces the value of the crop. In some cases, it may not even be worth harvesting.

Figure 1. Pumpkins rejected due to etch.

Initially, brown etch develops in the field. It usually develops from a contact point with the soil, a stem or other pumpkins. Etch can appear as either a pattern of concentric brown rings, or as irregularly shaped brown blotches spreading across the fruit. Symptoms can also develop after harvest, so that a freshly packed, clean bin of pumpkins at the farm can be riddled with etch by the time it arrives at the wholesale markets.

Examination of pumpkin skin using scanning electron microscopy reveals massive thickening of the cell walls in etched areas of pumpkin skin. This is due to accumulation of lignin. Lignin is a key compound in wood and bark but also often produced to defend cells from physical stress or fungal attack. As a result of this thickening, the cell contents are squashed and disrupted. Eventually the cells die, leaving behind the whitened skeletons of their cell walls.

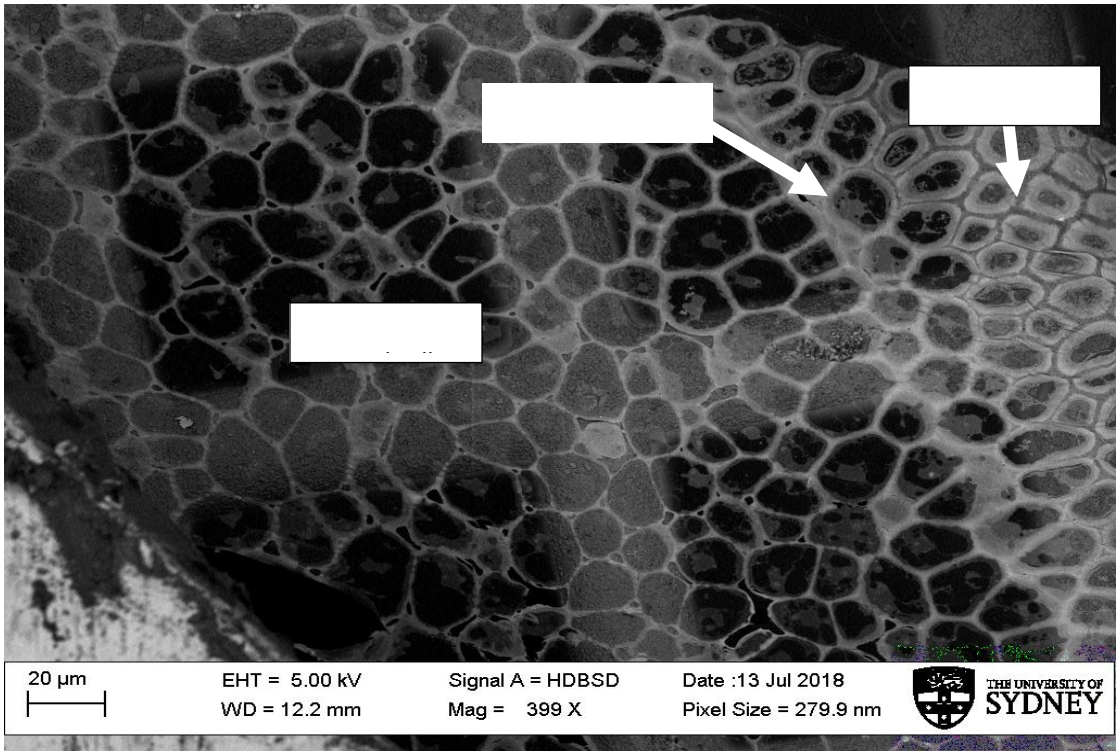

Figure 2. Scanning electron microscope image of the edge between etched and non-etched skin tissue.

Figure 3. Etched pumpkin, showing a typical pattern of concentric rings. In the centre the cells have died, leaving behind the white skeletons of their cell walls. Black fungal spores can be seen in the dead tissue.

Etch can be associated with infection by gummy stem blight or "black rot" (*Stagonosporopsis cucurbitacearum*). Immature pumpkins artificially infected with this disease often develop symptoms of etch. In this case, the pathogen can be re-isolated from the etched tissue.

However, etch also occurs when plants appear totally free of this disease, with no other symptoms of infection or evidence of fungi from RNA analysis or microscopic examination.

One thing that is certain is the correlation between etch and wet weather. Wet conditions due to rain or dew are strong predictors of the risk of etch in a crop. This is often cumulative. For example, the values shown in the table below are based on a model using the total accumulated time spent wet during fruit maturation. These are estimates only; rates of etch are also likely to vary due to other factors.

Table 1. Effect of accumulated time the plant was wet on risk of etch

Total time plants were wet during the fortnight before harvest	*Estimated* percentage of fruit likely to be affected by etch
<50 hours	0 to 5%
50 to 100 hours	5 to 10%
100 to 200 hours	10 to 30%
>200 hours	More than 30%

However, a single extended wet period can trigger increased rates of etch, even if the crop stays relatively dry before and after this event. The graph below is most useful as a guide to potential incidence after an extended rainy period.

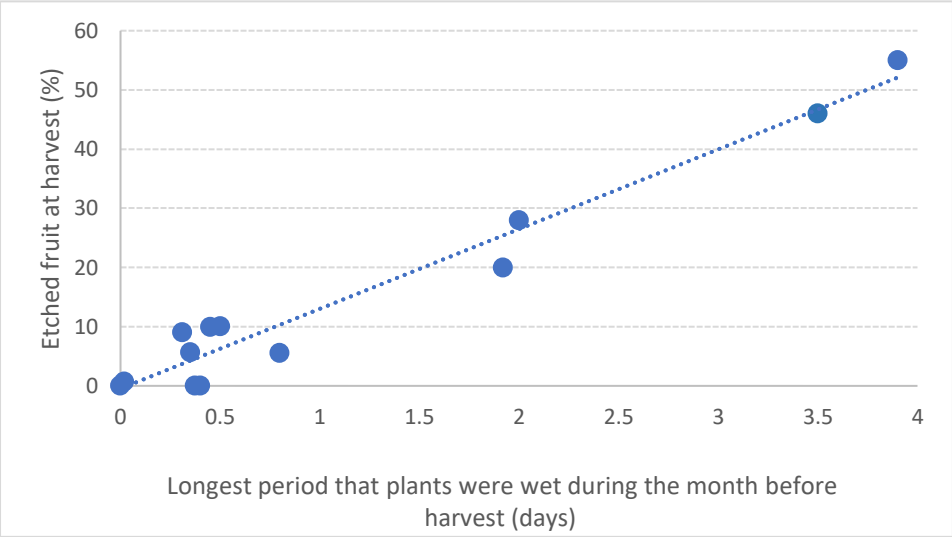

Figure 4. Effect of a single extended wet period during the month before harvest on the level of etch in the crop. Each point represents the results from one field trial or commercial crop monitoring event.

Etch therefore seems most likely to be triggered by stress. Etch can be a response to infection by gummy stem blight, but also to exposure to wet conditions.

If etch is observed in the field then more is likely to develop during transport and storage. New blemishes can appear overnight on previously clean fruit, as well as continuing to expand on already affected fruit. While new or expanded etch mainly occurs during the first week of storage, it can continue to appear for up to two weeks.

Conversely, if there is little or no etch in the field, symptoms are extremely unlikely to develop after harvest.

We tested a large range of different products and techniques to reduce etch, including fungicides, nutritional supplements and products reported to improve plant defences. None were effective. There also appeared to be little difference in susceptibility between common butternut varieties.

It seems likely that the best way to reduce development of etch is to keep RH low and the crop as dry as possible. This could mean increasing plant spacing, avoiding planting in damp areas or growing with subsurface drip instead of overhead irrigation.

If etch is present in the field, it may be best to store harvested pumpkins for at least a week before re-packing into hat bins or crates. By this time development of new etch will be minimal, allowing effective grading of the remaining fruit.

Development of etch during transport and storage may be reduced by keeping RH very low and, potentially, by cooling fruit. While 5°C storage reduced etch development by >75%, more suitable temperatures have yet to be tested using fruit at high risk of etch development.

The current drought means that etch, at least, won't be a problem for pumpkin growers. However, rain will eventually fall again, raising the question of what to do with etched fruit.

Most butternut pumpkins are sold cut in half and overwrapped - the undamaged flesh is clearly displayed, despite the brown stain on the skin. We conducted a small retail study looking at consumer preferences. Header cards were included showing etched and non-etched fruit, trying to clarify the difference between the two groups for consumers.

Figure 5. Retail header cards used with clean and etched pumpkins.

When we discounted etched fruit by 50c/kg we sold 12% more etched than clean pumpkins. Even without a discount, etched pumpkins still sold well. This suggests that if people can see the flesh is good to eat, purchasing will be minimally affected.

Perhaps simply cutting etched fruit in half could solve what is, after all, a problem that is only skin deep!

For a fact sheet on brown etch of pumpkins, or for more information on this project, please contact:

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This project has been funded by Hort Innovation using the vegetable research and development levy and contributions from the Australian Government. Project number VG15064.

UNDERSTANDING BROWN ETCH OF PUMPKINS

What does brown etch look like?

Brown etch almost always starts where the fruit is in contact with the ground, a stem, or another pumpkin. A reddish brown stain spreads across the skin, developing as a series of concentric rings or irregular, blotchy patches. Only the skin colour changes; etched areas remain firm. With time, the etched tissue dries out, developing a whitish appearance reminiscent of petrified wood. Fungal spores can sometimes appear in the centre of these dead areas.

Symptoms of brown etch are always superficial. The underlying flesh is unaffected and there is no impact on eating quality. Despite this, even a small amount of etch can lead to rejection by retailers.

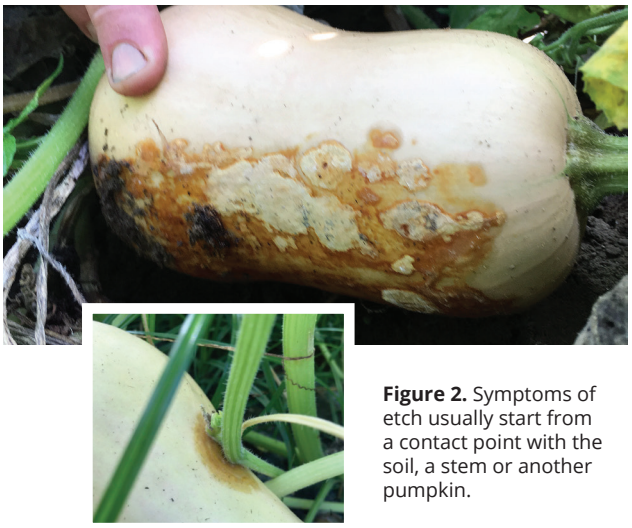

Figure 2. Symptoms of etch usually start from a contact point with the soil, a stem or another pumpkin.

Figure 1. Variable symptoms of etch in the field, showing concentric rings (left) blotchy appearance (centre) and the whitish "petrified wood" appearance of old, dried out etch (right).

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This project has been funded by Hort Innovation using the vegetable research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Microscopic examination reveals that cells in etched areas have massively thickened cell walls. This is largely due to accumulation of lignin. Lignin strengthens cells and is a key component of wood and bark. It is also often produced in response to physical or biological stress. As the wall thickens the cell contents are squashed, deformed and disrupted. Eventually they die, leaving behind the whitened skeletons of their cell walls.

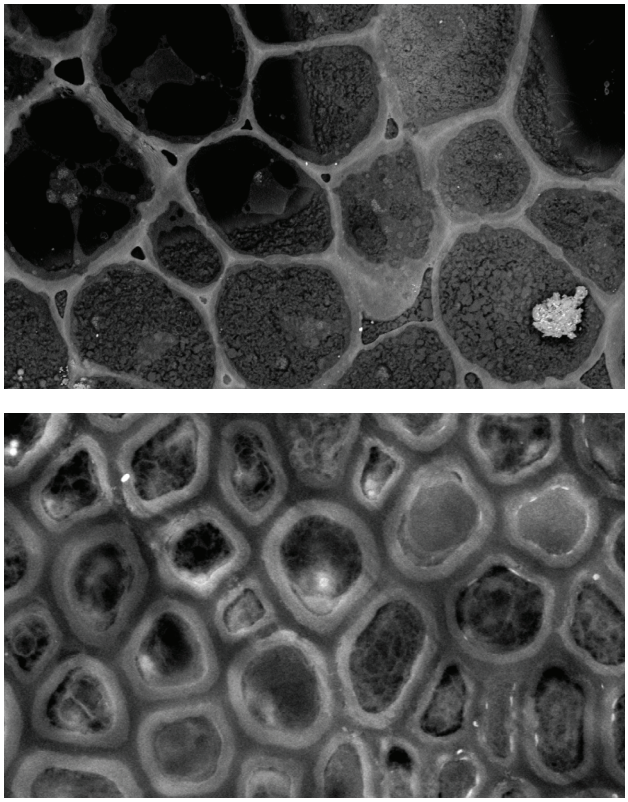

Figure 3. Normal (top) and etched (bottom) cells. Etched cells develop grossly thickened cell walls and their contents are squashed and disrupted. Scanning electron microscope image by the University of Sydney.

What types of pumpkins are affected?

Etch primarily affects butternut pumpkins (*Cucurbita moschata*) and, occasionally, related hybrids such as Kent. Etch does not generally affect long storing pumpkins such as Queensland blue or kabocha (*Cucurbita maxima*).

Is it a disease?

There appears to be an association between infection by gummy stem blight (*Stagonosporopsis cucurbitacearum*) and appearance of etch. Immature pumpkins that are wounded and then artificially infected with this disease often develop symptoms of etch. It is possible to re-isolate the fungus from the etched areas, suggesting that the fungus has caused the observed symptoms. There is some evidence that *Fusarium* also produces this response.

However, etch can also occur where plants and fruit appear totally disease-free. No fungal mycelia are seen growing through the plant tissue in microscope images of etched or borderline areas. Moreover, molecular analysis found similar amounts of DNA in etched and non-etched tissue, suggesting no fungus is present.

It therefore seems likely that although gummy stem blight can trigger etch, it is not the sole cause of the disorder.

What else causes etch?

There is a good correlation between development of etch and weather. High (>90%) relative humidity (RH), or wet conditions due to rain or dew are strong predictors of whether etch will develop in the crop. The period when pumpkins are maturing, turning from cream to orange, appears to be the most critical.

The graph in Figure 4 shows this relationship. Each point represents results from a single trial or survey of a commercial crop. The total time spent wet has been estimated using dewpoint. In actual fact pumpkins are likely to stay wet a lot longer, especially where they are in contact with the ground. This may explain why etch usually starts from these areas. This model suggests that if plants are wet for a cumulative total of 2 to 4 days during the fortnight before harvest, then 5-10% of the pumpkins will develop etched areas. If plants are wet for 5 days or more during this period, then at least 20% of fruit are likely to have etch.

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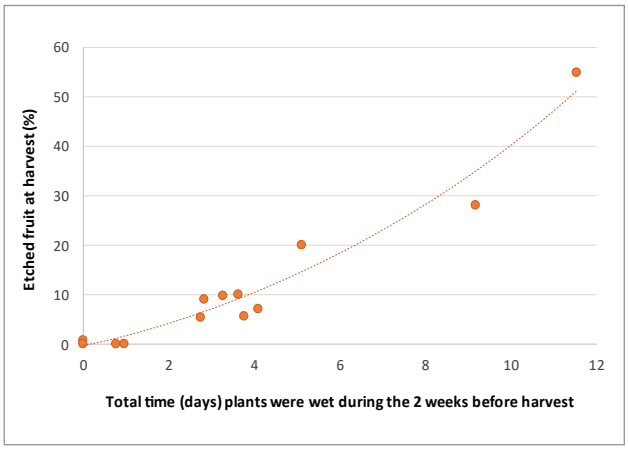


Figure 4. Relationship between time spent wet during fruit maturation and level of etch in the crop.

Even if the crop generally stays dry, an extended wet period during the month before harvest can also increase the risk of etch. Figure 5 shows that a single two day period of rain can result in more than 20% of pumpkins developing etch. If the wet weather continues for four days, half the crop may be affected.

Total time spent wet (Figure 4) is a good predictor when levels of etch are low, whereas the longest continual period spent wet (Figure 5) works best in rainy weather.

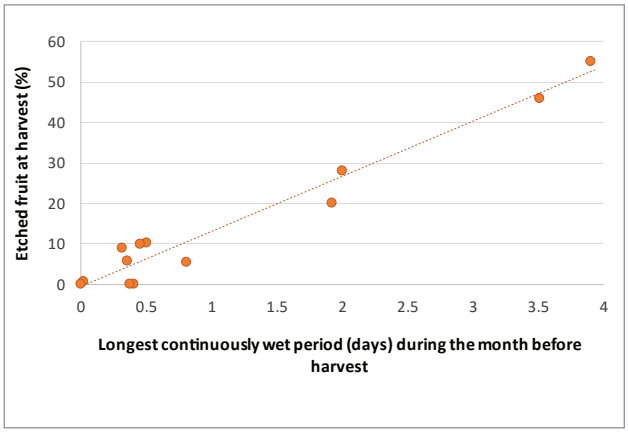


Figure 5. Effect of a single, extended wet period during the month before harvest on the level of etch in the crop.

Does etch develop after harvest?

If etch is found in the field, symptoms are likely to continue developing after harvest. Not only will affected areas expand, etch can appear overnight on previously unblemished fruit. While this is most likely during the first week of storage, new etch may continue to appear for up to two weeks.

For example, the data shown in Figure 6 was recorded for a crop where over 50% of harvested pumpkins had etch. The appearance of new etched areas peaked 3 and 5 days after harvest for initially etched and clean fruit respectively. In total, 28% of pumpkins clean at harvest developed etched areas after two weeks storage at 22°C.

Conversely, if there is no etch in the field, then etch rarely develops in harvested fruit.

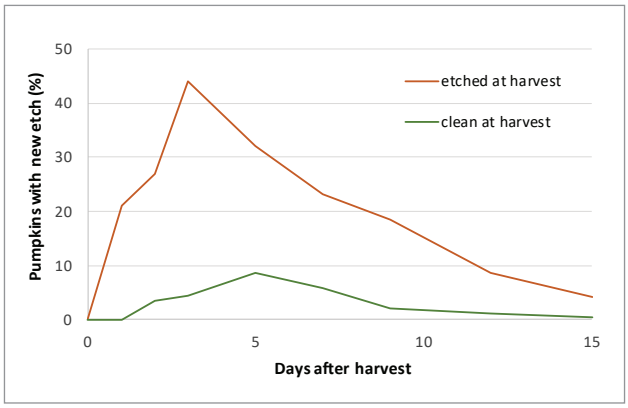
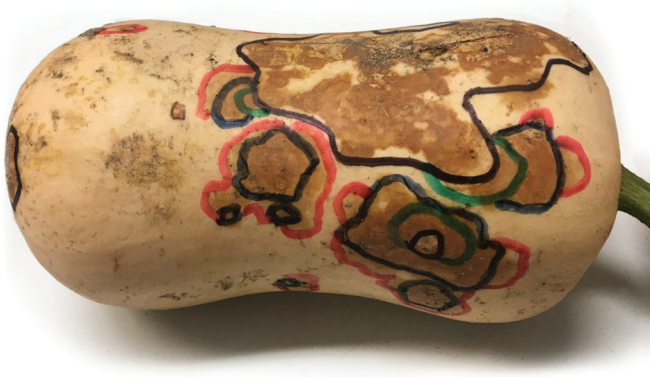


Figure 6. Development of new etch on pumpkins stored under ambient conditions with medium to high RH. While symptoms were most severe on pumpkins already etched at harvest (as in the picture shown at bottom), etch also developed on clean fruit.



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What can I do about it?

Field trials have tested a wide range of treatments to control etch in the field. These included fungicide programs, products designed to improve plant defences and foliar nutritional supplements. ***None of these products consistently reduced etch.***

In our trials we did not find any differences in susceptibility to etch between varieties, either in the field or postharvest. This includes the varieties Sunset QHI and Jacqueline.

We did find that cooling pumpkins and keeping the humidity low significantly reduced the incidence of etch.

- **Effects of cooling:** Cooling pumpkins to 5°C after harvest reduced etch development by 75% compared to holding fruit at ambient temperature.
- **Reducing humidity:** Keeping the storage environment dry, with 20% RH, significantly reduced etch development compared to storage in 70-95% RH.

Recommendations to reduce development of etch

In the field:

- Keep developing fruit as dry as possible. Strategies include increasing plant spacing to reduce humidity around the fruit and avoiding planting in damp areas.
- Irrigate crops with drip irrigation (preferably subsurface) instead of overhead irrigation.

In storage and transport:

- Cool pumpkins (12°C is optimum)
- Keep the humidity as low as possible, ideally under 20%

Note: If rates of etch in a crop are high and pumpkins are to be transported long distances, harvest the crop, store in harvest bins for at least a week, then repack for transport.

This will allow most of the underlying etch present in the fruit to appear. Discarding these pumpkins will avoid transporting low value fruit as well as the major costs associated with re-sorting at wholesale.

Can't we just tell consumers that etched fruit are still good to eat?

Yes, we can!

Most butternut pumpkins are sold cut in half and overwrapped. The undamaged flesh is clearly visible.

We conducted a small trial comparing sales of etched and non-etched pumpkins. Header cards were used to show customers the difference between the two. Most customers didn't look at the skin, and simply chose the cheapest option. As a result, a 50c/kg discount meant we sold 12% more etched than non-etched pumpkins. With a 20c/kg or zero discount, sales were generally similar regardless of skin blemish. While this may not be a solution right now, it suggests that consumers are willing to buy etched pumpkins if they can see that the fruit flesh is not diseased.

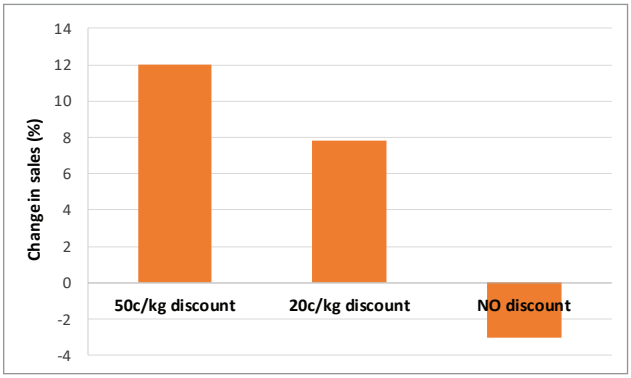

Figure 7. Sales of halved and overwrapped etched pumpkins compared to clean pumpkins when the etched fruit was sold with a 50c/kg discount, 20c/kg discount or no discount. Header cards displayed with the fruit are shown at bottom.

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