Microstructure of Soft Scald in ‘Honeycrisp’ Apples

Yin Xu¹, Yizhou Ma, Nicholas P. Howard, Changbin Chen, and Cindy B.S. Tong²

Department of Horticultural Science, University of Minnesota, Saint Paul, MN 55108

Gail Celio

University Imaging Center, University of Minnesota, Saint Paul, MN 55108

Jennifer R. DeEll

Ontario Ministry of Agriculture, Food and Rural Affairs, Simcoe, ON, Canada N3Y 4N5

Renae E. Moran

Department of Plant, School of Food and Agriculture, University of Maine, Monmouth, ME 04259

ADDITIONAL INDEX WORDS. cold storage, epidermis, hypodermis, microscopy, peel

ABSTRACT. Soft scald is an apple (*Malus ×domestica* Borkh.) fruit disorder that appears in response to cold storage after about 2–8 weeks. It appears as a ribbon of dark tissue on the peel of the fruit, with occasional browning into the flesh. Several apple cultivars are susceptible to it, including Honeycrisp. The objectives of this study were to examine the cellular microstructure of fruit exhibiting soft scald and determine if any aspect of the peel microstructure at harvest could be indicative of future soft scald incidence. Light and electron microscopy were used to examine the peel microstructure of ‘Honeycrisp’ fruit that were unaffected or affected by soft scald. Tissue with soft scald had brown pigmented epidermal and hypodermal cells, whereas unaffected fruit peel epidermal cells were unpigmented. Cuticular wax of unaffected peel had upright wax platelets or clumps of wax, but peel surfaces with soft scald exhibited flattened granules and were more fragile than that of unaffected fruit. Epidermal cells of fruit with soft scald were more disorganized than that of unaffected fruit. Light microscopy was used to examine peels of ‘Honeycrisp’ fruit from four growing locations and fruit from a ‘Honeycrisp’ breeding population at harvest. ‘Honeycrisp’ and ‘Honeycrisp’ progeny fruit were also stored at 0 °C for 8 weeks and scored for soft scald incidence. Cross-sections of unaffected peel of stored ‘Honeycrisp’ fruit looked similar to that of freshly harvested fruit. No significant correlations were found between soft scald incidence and measured microstructural attributes of ‘Honeycrisp’ fruit at harvest, suggesting that peel microstructure cannot be used to predict possible soft scald incidence after storage.

Soft scald is a chilling-related, abiotic disorder of apple and pear (*Pyrus communis* L.) fruit in which the peel develops soft, brown, sunken lesions, but the interior initially looks fine (Brooks and Harley, 1934). The fruit flesh may eventually brown and have a soft and spongy texture and later turn dry. The disorder often appears after about 6–8 weeks of storage at temperatures below 2 °C. Apple cultivars susceptible to soft scald include Delicious, Fuji, Honeycrisp, Jonathan, McIntosh, Monark, and Rome Beauty (Watkins et al., 2004).

The metabolic reactions leading to soft scald remain unclear, and methods to mitigate its occurrence have not always been effective. Wills (1972) found that some hexyl compounds injected into apple cores induce the disorder. Tissue from fruit exhibiting soft scald contains less linoleic acid than unaffected tissue, and low linoleic acid content increases fruit susceptibility to soft scald (Hopkirk and Wills, 1981). Although post-harvest application of some antioxidants decrease the incidence of soft scald, ascorbic acid, a water-soluble antioxidant, is not

effective. In addition, the antioxidant diphenylamine reduces, but does not eliminate soft scald incidence in ‘Honeycrisp’ fruit (Watkins et al., 2004). Preconditioning fruit by holding them at a warm temperature before cold storage is the most effective method used by the industry, but can be ineffective under some conditions (Moran et al., 2010). Increasing success in predicting the probability of its occurrence would allow growers to better manage susceptible fruit.

Predicting the incidence of soft scald is difficult partly because of fruit-to-fruit, tree-to-tree, orchard-to-orchard, and year-to-year variation (Hopkirk and Wills, 1981; Lachapelle et al., 2013; Moran et al., 2009; Watkins et al., 2005). Because of the large variability in occurrence, identification of these unknown orchard factors may improve prediction of susceptibility. Lack of precipitation 30 d before harvest accounted for 53% of the year-to-year variation in soft scald incidence in Maine and 28% of the variability in Ontario (Moran et al., 2009). Precipitation early in the growing season, from full bloom until fruit reached 10 mm in diameter, was associated with increased soft scald incidence in Canada (Lachapelle et al., 2013). Predictive factors leading to tree-to-tree and fruit-to-fruit variation remain unknown. Not every fruit from a tree will develop soft scald, so soft scald incidence is scored as a percentage of a batch of stored fruit. Labor and storage space could be saved if growers could avoid harvesting susceptible individual fruit or to cull fruit at harvest that might develop soft scald during cold storage.

To determine whether commonalities exist in fruit susceptible to soft scald, the peel microstructure of ‘Honeycrisp’ fruit

Received for publication 30 Aug. 2017. Accepted for publication 4 Oct. 2017. We are grateful for funding from the Jiangsu Provincial Department of Education (Jiansu Overseas Research & Training Program for University Prominent Young & Middle-aged Teachers and Presidents) for Yin Xu. Further support was provided by the Minnesota Agricultural Experiment Station for project MN 21-028, the Maine Agricultural and Forest Experiment Station Project for ME031404, and the Ontario Apple Growers.

We thank John Krause, Hsueh-Yuan Chang, and Lorie Walker for their technical assistance and Wood Orchard for collaboration.

¹Permanent address: Department of Bioengineering, Jiangsu Vocational College of Agriculture and Forestry, Jurong City, Jiangsu Province 212400, China.

²Corresponding author. E-mail: c-tong@umn.edu.

from different growing locations and of different genotypes from a 'Honeycrisp' breeding population with varying susceptibilities to soft scald were compared in this study.

Materials and Methods

FRUIT AND STORAGE. 'Honeycrisp' fruit exhibiting soft scald used for microscopic analyses of the disorder, as well as corresponding unaffected fruit, were grown and harvested in 2016 at the University of Maine Highmoor Farm in Monmouth (lat. 44°13'51"N, long. 70°4'5"W) or the Horticultural Research Center of the University of Minnesota in Chanhassen (lat. 44°51'30"N, long. 93°39'41"W). The fruit had been stored at 0 ± 1 °C for at least 8 weeks. Fruit from Maine were shipped overnight to Minnesota.

In 2014, 'Honeycrisp' fruit were grown and harvested from the University of Maine Highmoor Farm in Monmouth, the Horticultural Research Center of the University of Minnesota in Chanhassen, two commercial orchards near Simcoe, Ontario, Canada (lat. 42°52'44"N, long. 80°15'22.6"W and lat. 42°49'22"N, long. 80°20'3"W), and a commercial orchard in Egg Harbor, WI (lat. 45°3'51"N, long. 87°15'60"W). Fruit were harvested three times, at 1-week intervals, during the harvest season at each location, except that there were only two harvests at orchard 1 in Ontario. In Maine, the first harvest occurred when the starch index reached 5 (Blanpied and Silsby, 1992), and flavor was beginning to develop. In Minnesota and Wisconsin, the first harvest occurred when fruit background color changed from green to yellow. In Ontario, the first harvest occurred when the starch index neared 6, and background color started to change from green to yellow. About 30–40 fruit from each harvest from Maine, Ontario, and Wisconsin were shipped overnight to Minnesota, where 10 fruit were processed immediately for light microscopy, and the remaining fruit were stored at 0 ± 1 °C. In Minnesota, at least 10 fruit per harvest were processed immediately for light microscopy, and 30–40 fruit were stored at 0 ± 1 °C.

Fruit from progeny of a 'Honeycrisp' × 'Monark' family, which segregates for soft scald incidence, were grown at the University of Minnesota Horticultural Research Center and are described in further detail by McKay et al. (2011). Scions were budded onto Budagovsky 9 (B.9) rootstock in 2008. Ten to 25 fruit of 37 genotypes were harvested in 2014 and 2015 at a starch index of 5 (Blanpied and Silsby, 1992). Ten apples were processed immediately for light microscopy, and the remaining fruit were stored at 1 ± 1 °C (different cooler as used for 'Honeycrisp' fruit) to induce soft scald and subsequent soft scald incidence assessment.

SOFT SCALD ASSESSMENTS. 'Honeycrisp' fruit from the four locations were assessed for soft scald after 8 weeks of storage at 0 °C. Fruit were removed from cold storage and held at room temperature for 7 d, after which the percentages of fruit per location exhibiting soft scald were determined. The presence or absence of soft scald, rather than degree of injury, was assessed. Soft scald was scored as positive only if the typical brown or black spots or ribbons were detected, regardless of lesion size. Fruit from the 'Honeycrisp' × 'Monark' cross were evaluated after 10 and 20 weeks of cold storage, on the day of removal from storage without incubation at room temperature. Only the presence or absence of soft scald was scored. Disorder incidence was determined as number of fruit with the disorder divided by the total number of evaluated fruit.

MICROSCOPY. To compare fresh and stored 'Honeycrisp' fruit with light microscopy, 8-mm discs of peels were removed from three fresh or three stored Minnesota-grown fruit and placed in a fixative. Similarly, to compare stored 'Honeycrisp' fruit with and without soft scald using light microscopy, 8-mm peel discs were removed from stored 'Honeycrisp' fruit with and without soft scald and placed in a fixative. Fruit used for these comparisons were grown in Maine or Minnesota and stored for 8 weeks at 0 ± 1 °C. The fixative used for these experiments contained 4% paraformaldehyde, 62% ethanol, 5% acetic acid, and 29% distilled water (v/v).

In different light microscopy experiments of peel from only freshly harvested 'Honeycrisp' fruit from multiple locations and 'Honeycrisp' × 'Monark' progeny, 8-mm discs of peel from the border of the sun-exposed and shaded areas of each fruit were removed and immediately placed into a fixative containing 10% paraformaldehyde, 70% ethanol, 5% acetic acid, and 15% distilled water (v/v). Three peel samples were taken from each of three replicate fruit from each location or genotype.

All fixed samples were dehydrated in an ethanol series from 50% to 100% ethanol, cleared in a Histo-Clear series (Sigma-Aldrich, St. Louis, MO) from 25% Histo-Clear:75% ethanol (v/v) to 75% Histo-Clear:25% ethanol with a final incubation in 90% Histo-Clear:10% chloroform (v/v). The samples were then infiltrated in 50% paraffin (Paraplast, Sigma-Aldrich):45% Histo-Clear:5% chloroform (w/v/v) at 56 °C for 12 h and cleared three times in 100% paraffin at 56 °C for 2 h after the paraffin had been melted at 70 °C. After clearing, the samples were embedded in paraffin, sectioned to 10-μm thicknesses, dewaxed at 37 to 40 °C from 100% Histo-Clear to 67% Histo-Clear:33% ethanol (v/v) and 33% Histo-Clear:67% ethanol (20 min each), and rehydrated with 2 min incubations in 100%, 90%, 70%, 50%, and 30% ethanol, followed by two incubations of 2 min duration in distilled water. The samples were sectioned using a microtome (Spencer Lens Co., Buffalo, NY). Some sections of freshly harvested fruit were stained with 0.15% methyl green and 0.03% pyronin Y. The samples were visualized using a light microscope (Laborux K; Leitz, Wetzlar, Germany) equipped with a digital camera (Insight; SPOT Image Solution, Sterling Heights, MI). Three images were taken of each peel sample.

Cuticle smoothness for 'Honeycrisp' samples was scored visually, as either rough or smooth, based on evenness of the cuticle surface across several cells. For the 'Honeycrisp' × 'Monark' population, smoothness was judged using a 3-point scale, in which 1 = undulating and uneven surface across 10 or more cells in an image and 3 = smooth, with an even surface spanning 10 or more cells. Cuticle, epidermal, and hypodermal thicknesses, as well as epidermal and hypodermal cell widths were measured for 8–10 cells per image using ImageJ software version 1.48 (Schneider et al., 2012).

For scanning electron microscopy (SEM), four 'Honeycrisp' fruit exhibiting soft scald after at least 8 weeks of cold storage were used. Paired samples of unaffected and soft-scalded tissue were taken from the same fruit. Apple peels were cut from fresh tissue and oven-dried on filter paper at 42 °C for at least 48 h. The peels were cut with a razor blade, mounted onto aluminum stubs with double-sided carbon tape, and sputter coated with 2 nm of gold–palladium. Images were visualized using a variable pressure scanning electron microscope (S3500N; Hitachi High-Technologies, Schaumburg, IL) with an accelerating voltage of 10 kV.

Fig. 1. Soft scald of Minnesota-grown 'Honeycrisp' apple fruit as indicated with the arrow in (A). Light micrographs were taken of peels from three each unaffected (B) and scalded (C, D) Minnesota- or Maine-grown 'Honeycrisp' fruit after 8 weeks of storage at 0 °C. Discs (8 mm) of peels from three each unaffected and soft-scalded fruit were removed and placed in fixative containing 4% paraformaldehyde, 62% ethanol, 5% acetic acid, and 29% distilled water (v/v), then dehydrated in an ethanol series and embedded in paraffin (Paraplast, Sigma-Aldrich, St. Louis, MO). Figures shown are typical examples of each type of peel. Images in B and C are at the same magnification. Cu = cuticle, Ep = epidermal cell, Hy = hypodermal cell. Arrows in C and D point to areas of pigmentation.

For transmission electron microscopy (TEM), pairs of unaffected and soft-scalded 'Honeycrisp' peel samples were removed from the same fruit as used for SEM. Six different fruit were sampled. The samples were fixed for at least 1 h under vacuum at room temperature then overnight at 4 °C in 0.1 M sodium cacodylate buffer containing 2.5% glutaraldehyde. The samples were rinsed in buffer, postfixed in 1% osmium tetroxide in buffer, dehydrated in an ethanol series, and embedded in resin (EMbed 812; Electron Microscopy Sciences, Hatfield, PA). Ultrathin sections of 80–100-nm thickness were cut on a microtome (Ultracut UCT; Leica Microsystems,

Fig. 2. Scanning electron micrographs of cuticles of unaffected (A–C) and soft-scalded (D–F) 'Honeycrisp' fruit after 8 weeks of storage at 0 °C. Peel samples were taken from areas with and without soft scald from each of six stored fruit from Maine or Minnesota. Magnifications of A, C, D, and F are the same. B and E are close-ups of A and D, respectively.

Fig. 3. Representative transmission electron micrographs of peel and cortex tissue of unaffected (A) and soft-scalded (B, C) 'Honeycrisp' fruit after 8 weeks of storage at 0 °C. Peel samples were taken from areas with and without soft scald from each of three stored fruit from Maine or Minnesota. Images shown on A and B are of the same magnification.

Buffalo Grove, IL) using a diamond knife, collected on formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Hatfield, PA) and stained with 3% uranyl acetate for 20 min, followed by Sato's triple-lead stain (Sato, 1968) for 3 min. The sections were examined with a transmission electron microscope (Philips CM 12; FEI, Hillsboro, OR) operating at 60 kV. Images were recorded with a digital capture system (Maxim DL; Diffraction Limited, Ottawa, ON, Canada).

STATISTICAL ANALYSES. All statistical tests were done using RStudio (version 1.0.136; RStudio, Boston, MA). Tests for normality of data distribution were done by plotting histograms and residuals (using qqnorm) of data, and Shapiro–Wilk normality tests. Analysis of variance (ANOVA) was used to examine contributions to soft scald variation by different normally distributed variables. Kruskal–Wallis H tests were used to determine differences among nonparametric microstructural attributes of progeny from the 'Honeycrisp' × 'Monark' population. Mean soft scald incidence was used because not enough fruit were available for all genotypes in the year that microstructural attributes were measured, because of alternate bearing of some genotypes. Associations between paired variables were determined using Kendall's rank correlation test for nonparametric data.

Results

MICROSTRUCTURE OF SOFT-SCALDED 'HONEYCRISP' FRUIT.

Soft scald was apparent as areas of darkened peel tissue (Fig. 1A). Under light microscopy, tissue with soft scald exhibited brown pigmentation in epidermal and hypodermal cells (Fig. 1C). This pigmentation was not evident in unaffected stored

Fig. 4. Representative light micrographs of 'Honeycrisp' fruit peels from freshly harvested Minnesota fruit (A) and Minnesota fruit stored at 0 °C for 8 weeks (B). Peel discs (8 mm) of three each fresh or stored apples were removed and placed in fixative containing 4% paraformaldehyde, 62% ethanol, 5% acetic acid, and 29% distilled water (v/v), then dehydrated in an ethanol series and embedded in paraffin (Paraplast, Sigma-Aldrich, St. Louis, MO). Images in A and B are of the same magnification.

Table 1. Mean wax, epidermis, and hypodermis cell sizes and abbreviated analyses of variance of factors affecting peel microstructural traits of 'Honeycrisp' apple fruit from multiple harvests in Maine, Minnesota, Ontario, and Wisconsin.

Location	Harvest no.	Mean wax thickness	Mean epidermal thickness	Mean epidermal cell width	Mean $\pm$ SE (μ m)		Mean hypodermal thickness	Mean hypodermal cell width
Maine	1	9.7 $\pm$ 1.0	11.2 $\pm$ 3.2	14.3 $\pm$ 3.9			12.0 $\pm$ 3.4	23.0 $\pm$ 7.1
	2	10.8 $\pm$ 2.0	11.5 $\pm$ 2.9	16.0 $\pm$ 4.8			15.2 $\pm$ 5.4	26.8 $\pm$ 7.5
	3	10.6 $\pm$ 2.3	13.5 $\pm$ 2.8	15.7 $\pm$ 3.4			13.5 $\pm$ 4.0	24.6 $\pm$ 6.7
Minnesota	1	11.7 $\pm$ 1.5	12.0 $\pm$ 2.7	13.2 $\pm$ 2.7			12.4 $\pm$ 2.8	19.9 $\pm$ 5.0
	2	10.3 $\pm$ 1.2	12.4 $\pm$ 2.5	17.1 $\pm$ 3.1			12.6 $\pm$ 4.0	20.6 $\pm$ 4.6
	3	12.6 $\pm$ 1.7	12.6 $\pm$ 2.4	15.6 $\pm$ 3.7			12.1 $\pm$ 2.5	22.9 $\pm$ 4.2
Ontario 1	1	10.9 $\pm$ 1.9	11.1 $\pm$ 2.0	14.2 $\pm$ 2.5			12.8 $\pm$ 3.1	18.9 $\pm$ 4.8
	2	10.4 $\pm$ 1.1	11.7 $\pm$ 2.8	13.2 $\pm$ 3.1			12.2 $\pm$ 3.7	20.6 $\pm$ 5.2
Ontario 2	1	9.8 $\pm$ 2.1	11.1 $\pm$ 2.1	13.4 $\pm$ 2.3			11.3 $\pm$ 2.9	17.6 $\pm$ 4.0
	2	9.5 $\pm$ 1.0	10.7 $\pm$ 2.6	13.6 $\pm$ 3.5			11.8 $\pm$ 3.9	21.7 $\pm$ 6.8
	3	11.8 $\pm$ 1.0	12.8 $\pm$ 3.4	15.3 $\pm$ 3.6			14.5 $\pm$ 3.8	24.1 $\pm$ 6.4
Wisconsin	1	11.0 $\pm$ 1.4	14.1 $\pm$ 3.0	15.6 $\pm$ 2.9			16.2 $\pm$ 3.8	24.5 $\pm$ 6.3
	2	10.2 $\pm$ 1.9	12.6 $\pm$ 3.3	16.2 $\pm$ 3.7			14.6 $\pm$ 3.8	24.6 $\pm$ 5.1
	3	10.5 $\pm$ 1.5	13.5 $\pm$ 2.3	15.9 $\pm$ 2.9			14.7 $\pm$ 4.0	21.4 $\pm$ 4.8
Source	df	Sum of squares	Sum of squares	Sum of squares	df	Sum of squares	Sum of squares	Sum of squares
Location	4	8.9	19.8**	24.8***	4	47.1*		137.5*
Harvest	2	8.8*	10.6*	11.4**	2	3.0		37.2

.. *, **, ***Significant at $P \leq 0.10, 0.05, 0.01$, or 0.001 , respectively.

SE = standard error of the mean of a sample.

fruit (Fig. 1B). The pigmentation seemed to be localized to the cytosol (Fig. 1D). Differences in cuticle smoothness were not discernible under light microscopy.

Under SEM, discernible, sharp, or bumpy wax platelets could be observed on the cuticle surfaces of unaffected areas of 'Honeycrisp' fruit after 8 weeks of cold storage (Fig. 2A–C). Surfaces of soft-scalded areas of stored 'Honeycrisp' fruit had aggregations of small or flattened granules (Fig. 2D–F).

TEM demonstrated that the wax and epidermal cells of unaffected (Fig. 3A) and scalded (Fig. 3B) areas of stored fruit differed. Wax of soft-scalded peel had tears, whereas that of unaffected peel was entire. The striations shown in Fig. 3B are most likely artifactual but not due to poor resin infiltration, as the parenchymal cells in samples of both the unaffected and affected peel were intact. The tears shown in Fig. 3B may be an indication of wax weakness. A higher magnification of the soft-scalded area showed breakdown of an epidermal cell (Fig. 3C).

In a comparison of unaffected fresh and stored 'Honeycrisp' fruit grown in Minnesota, no size or thickness differences in cuticle, epidermal cell layers, or hypodermal cell layers were discernible (Fig. 4A and B). This suggests that storage per se did not affect 'Honeycrisp' fruit microstructure.

EFFECT OF GROWING LOCATION ON FRUIT MICROSTRUCTURE. Mean epidermal thickness and cell widths, and mean hypodermal thickness and cell widths of 'Honeycrisp' fruit differed significantly by location, as determined by ANOVA (Table 1). Only the variation in mean epidermal cell width was significantly affected by the interaction of location and harvest. Location accounted for 13%, 29%, 31%, 28%, and 34%

of the variation in mean wax thickness, epidermal thickness and cell width (Fig. 5A), and hypodermal thickness and cell width, respectively. Mean scald incidence scored 7 d after removal from cold storage varied among the locations (Fig. 5B).

Because scald incidence was discrete data, Kendall's rank correlation tests were used to examine relationships between scald incidence and microstructural characteristics. A weak, negative correlation was found between mean hypodermal cell width and scald incidence measured 7 d after fruit removal from storage (Table 2).

MICROSTRUCTURE OF 'HONEYCRISP' $\times$ 'MONARK' PROGENY WITH DIFFERENT SUSCEPTIBILITIES TO SOFT SCALD INCIDENCE. The 'Honeycrisp' $\times$ 'Monark' population varied in soft scald incidence from none to 100%. They also exhibited a difference in microstructure. Some genotypes had more than one epidermal cell layer (Fig. 6A), whereas the second cell layer in fruit of other genotypes were parenchymal in appearance. Although microstructural attributes varied with genotype (Table 3), there was no correlation between any mean

Fig. 5. Mean epidermal cell width and mean scald incidence scored 7 d after removal from 8 weeks of storage at 0°C of 'Honeycrisp' fruit grown in different locations. ME = Maine, MN = Minnesota, ON1 = Ontario orchard 1, ON2 = Ontario orchard 2, and WI = Wisconsin.

Table 2. Kendall's rank correlations (tau-b) between mean scald incidences measured 7 d after removal from 8 weeks of storage at 0 °C and mean microstructural attributes of 'Honeycrisp' apple fruit measured at harvest from Maine, Minnesota, Ontario, and Wisconsin aggregated across multiple harvests.

Microstructural attribute	Kendall's tau-b	Two-sided P value
Mean cuticle thickness	0.05	0.87
Cuticle smoothness	-0.32	0.16
Mean epidermis cell width	-0.25	0.25
Mean epidermis thickness	-0.25	0.25
Mean hypodermis cell width	-0.38	0.07
Mean hypodermis thickness	-0.25	0.25

Fig. 6. Representative light micrographs of peels from fruit of EJ128, a genotype with fruit exhibiting low scald incidence (A) and EJ138, a genotype with fruit exhibiting high soft scald incidence (B). Samples were removed from three freshly harvested fruit of each genotype as 8-mm peel discs, fixed in 10% paraformaldehyde, 70% ethanol, 5% acetic acid, and 15% distilled water (v/v), dehydrated in an ethanol series, embedded in paraffin (Paraplast, Sigma-Aldrich, St. Louis, MO), and stained with 0.15% methyl green and 0.03% pyronin Y. Images are of the same magnification.

microstructural attribute and scald incidence [data not shown ($n = 61$)].

Discussion

Differences in peel characteristics and cuticle morphology of unaffected and scalded 'Honeycrisp' peels were evident when observed using light and electron microscopy. Epidermal and hypodermal cells were brown pigmented in scalded tissue compared with unaffected tissue. Scalded tissue showed loss of plasma membrane integrity and disintegration of ultrastructure, typical of chilling injury (Parkin et al., 1989). Distinct wax platelets were observed in unaffected fruit peels, whereas the surface of scalded fruit cuticle was more amorphous.

'Honeycrisp' fruit collected from five locations differed in the microstructural attributes of mean wax, epidermal, and hypodermal thickness, as well as epidermal and hypodermal mean cell widths. It is not known what environmental factors at the different locations affected these attributes. Faust and Shear (1972) noted that growing environment affected the number and severity of cracks in wax of 'Golden Delicious' apples. 'Golden Delicious' fruit grown in a humid environment developed cracks early in the season and the cracks became more severe up to harvest, whereas fruit grown under arid conditions did not develop cracks. Differences in cuticular cracks in 'Honeycrisp' fruit among the locations were not evident, and wax smoothness did not differ among the locations (data not shown).

Based on our data, microstructural attributes at harvest cannot be used to predict scald susceptibility. The mean width of the hypodermal layer was weakly and negatively correlated with scald incidence in 'Honeycrisp' from the multiple locations.

Table 3. Kruskal–Wallis rank sum test results (χ^2) of various microstructural attributes for fruit of multiple 'Honeycrisp' × 'Monark' apple genotypes (df = 64 for all attributes).

Microstructural attribute	χ^2	P value
Mean cuticle wax thickness	121.2	2.108e-05
Cuticle smoothness	109.4	0.0004
Mean epidermal thickness	128.6	3.055e-06
Mean epidermal cell width	117.8	4.914e-05
Mean cell layer 2 thickness	113.2	0.0001
Mean cell layer 2 cell width	96.4	0.005

However, scald incidence was scored on a percentage basis of batches of fruit, and these fruit were necessarily different from those destructively sampled for microscopy at harvest, making it difficult to discern correlations between microstructural attributes and scald scored after harvest.

Microstructural attributes differed among 'Honeycrisp' × 'Monark' progeny, but there were no correlations between these attributes, and mean soft scald incidence averaged over 2 years. Differences in microstructure among genotypes have been previously reported. Skene (1963) noted that apple cultivars differed in surface wax platelet sizes, presence of globules, and appearance (corrugated, wrinkled, smooth). Differences may be due to nature of wax, developmental stage, or growing environment (not clear from the article where apples had been sourced). Variations between individual fruit of a particular cultivar were on the same order as between cultivars and between apples and pears, so Skene suggested that differences observed among cultivars were "of doubtful significance." Faust and Shear (1972) also observed differences in wax microstructure among three different apple cultivars. Homutová and Blažek (2006) reported differences in mean skin thickness in their study comparing 20 apple cultivars, although there was variation in skin thickness among slices within a single fruit. Skin thicknesses also varied by year for certain cultivars. Measurements of microstructural attributes of the 'Honeycrisp' × 'Monark' genotypes over multiple years would allow improved determination of true differences among the genotypes.

In conclusion, microstructural differences were observed between unaffected and soft-scalded peel tissue of stored 'Honeycrisp' fruit. However, microstructural epidermal or hypodermal cell size differences of fruit grown in different locations or of various genotypes that were apparent at harvest before cold storage could not predict future likelihood of soft scald incidence. Other characteristics of peel tissue, such as lipid phase state or membrane permeability (Lyons, 1973), could be investigated as possible means toward identifying susceptibility to soft scald development.

Literature Cited

- Blanpied, G.D. and K.J. Silsby. 1992. Predicting harvest date windows for apples. Cornell Coop. Ext. Info. Bul. 221.
- Brooks, C. and C.P. Harley. 1934. Soft scald and soggy break-down of apples. J. Agr. Res. 49:55–69.
- Faust, M. and C.B. Shear. 1972. Fine structure of the fruit surface of three apple cultivars. J. Amer. Soc. Hort. Sci. 97:351–355.
- Homutová, I. and J. Blažek. 2006. Differences in fruit skin thickness between selected apple (*Malus domestica* Borkh.) cultivars assessed by histological and sensory methods. Hort. Sci. (Prague) 33:108–113.

- Hopkirk, G. and R.B.H. Wills. 1981. Variation in fatty acid composition of apples in relation to soft scald. *Phytochemistry* 20:193–195.
- Lachapelle, M., G. Bourgeois, J. DeEll, K.A. Stewart, and P. Séguin. 2013. Modeling the effect of preharvest weather conditions on the incidence of soft scald in ‘Honeycrisp’ apples. *Postharvest Biol. Technol.* 85:57–66.
- Lyons, J.M. 1973. Chilling injury in plants. *Annu. Rev. Plant Physiol.* 24:445–466.
- McKay, S.J., J.M. Bradeen, and J.J. Luby. 2011. Prediction of genotypic values for apple fruit texture traits in a breeding population derived from ‘Honeycrisp’. *J. Amer. Soc. Hort. Sci.* 136:408–414.
- Moran, R.E., J.R. DeEll, and W. Halteman. 2009. Effects of preharvest precipitation, air temperature, and humidity on the occurrence of soft scald in ‘Honeycrisp’ apples. *HortScience* 44:1645–1647.
- Moran, R.E., J.R. DeEll, and D.P. Murr. 2010. Effects of preconditioning and fruit maturity on the occurrence of soft scald and soggy breakdown in ‘Honeycrisp’ apples. *HortScience* 45:1719–1722.
- Parkin, K.L., A. Marangoni, R.L. Jackman, R.Y. Yada, and D.W. Stanley. 1989. Chilling injury. A review of possible mechanisms. *J. Food Biochem.* 13:127–153.
- Sato, T. 1968. A modified method for lead staining of thin sections. *J. Electron Microsc.* (Tokyo) 17:158–159.
- Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH image to imageJ: 25 years of image analysis. *Nat. Methods* 9:671–675.
- Skene, D.S. 1963. The fine structure of apple, pear, and plum fruit surfaces, their changes during ripening, and their response to polishing. *Ann. Bot.* 27:581–587.
- Watkins, C.B., M. Erkan, J.F. Nock, K.A. Iungerman, R.M. Beaudry, and R.E. Moran. 2005. Harvest date effects on maturity, quality, and storage disorders of ‘Honeycrisp’ apples. *HortScience* 40:164–169.
- Watkins, C.B., J.F. Nock, S.A. Weis, S. Jayanty, and R.M. Beaudry. 2004. Storage temperature, diphenylamine, and pre-storage delay effects on soft scald, soggy breakdown and bitterpit of ‘Honeycrisp’ apples. *Postharvest Biol. Technol.* 32:213–221.
- Wills, R.B.H. 1972. Effect of hexyl compounds on soft scald of apples. *Phytochemistry* 11:1945–1946.