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# Alleviation of Postharvest Skin Dimpling of MN55 Apple Fruit and a Possible Association with *Apple stem pitting virus*

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***Abstract.*** **MN55 is an apple (*Malus ×domestica* Borkh.) cultivar recently released by the University of Minnesota apple breeding program, with fruit marketed in the U.S. as Rave®. When stored for 4 months at 0 to 4 °C, MN55 fruit can develop several storage disorders, including skin dimpling. Skin dimpling incidence was greater for fruit harvested 1 week later than those harvested earlier. Dimpling was not alleviated by prestorage treatments of 1-methylcyclopropene or diphenylamine or by holding fruit at room temperature for 1 day before long-term cold storage. However, dimpling incidence was very low when fruit were stored at 6 to 7 °C. Because viruses have been implicated in other fruit dimpling disorders, the presence of viruses in MN55 leaves and fruit was studied. *Apple stem pitting virus* (ASPV) was detected by microscopy, reverse transcriptase polymerase chain reaction (RT-PCR) methodology, and high throughput sequencing (HTS) in peel of fruit from MN55 trees that exhibited skin dimpling after 4 months of storage at 0 to 1 °C. ASPV was also detected in supermarket-purchased fruit of other cultivars with noticeable skin dimpling. Although ASPV was not conclusively demonstrated to cause skin dimpling in our work, its prevalence indicates that further investigations are warranted to determine the relationship between viruses and skin deformities in stored apples.**

Apple (*Malus ×domestica* Borkh.) fruit are susceptible to a variety of postharvest disorders (Meheriuk et al., 1994; Plagge et al., 1935) that may or may not be present at harvest but are typically manifested after several months of cold storage (Smock, 1977). Common examples of these disorders include superficial scald, soft scald, bitter pit, soggy breakdown, water core, and various types of flesh browning. Prestorage treatment, such as diphenylamine (DPA) dips, 1-methylcyclopropene (1-MCP) fogs, heat treatment (Lurie et al., 1991), or preconditioning at a warm temperature, can alleviate development of superficial scald (Johnson et al., 1980; Jung and Watkins, 2008), senescent breakdown (Nock and Watkins, 2013), and soft scald (Moran et al., 2010), respectively. Susceptibility to these disorders varies by cultivar, and modern cultivars are no less susceptible than older ones (Howard et al., 2018).

MN55 is a recently released variety from the University of Minnesota apple breeding program (Bedford and Luby, 2014). It was developed from a cross between 'Honeycrisp' and AA44, a cultivar from the University of Arkansas, and is sold in the United States as Rave®. In preliminary studies at the University of Minnesota, it was found to be susceptible to superficial scald, soft scald, and skin dimpling when stored at 4 °C.

While superficial and soft scald are known to be chilling injuries, there are few data on the effects of storage temperature on skin dimpling. Al Shoffe et al. (2016) documented a chilling disorder that they labeled "wrinkly skin" in 'Honeycrisp' apple fruit, which may be the same disorder as skin dimpling. In previous studies, skin dimpling was associated with several viruses and viroids, such as apple dimple fruit viroid (He et al., 2010), *Apple chlorotic leafspot virus* (ACLSV), *Asian Prunus virus*, *Peach latent mosaic viroid*, *Nectarine stem-pitting–associated virus* (Xu et al., 2019), *Zucchini yellow mosaic virus* (Coutts et al., 2011), and *Pepino mosaic virus* (Moerkens et al., 2015). ACLSV, *Apple stem grooving virus* (ASGV), and ASPV are widely distributed and commonly occurring in apples. All three are single-stranded RNA viruses, typically latent (symptomless), and spread through vegetative propagation by grafting, and top-working on infected rootstocks or interstems. ACLSV can elicit diverse symptoms of apple fruit, including sunken spots on fruit skin, and is often detected in coinfection with ASPV and ASGV (Cieniewicz and Fuchs, 2016a). In apple, no fruit symptoms of ASPV infection have been reported, but pitting and deformity can occur in fruit of susceptible pear cultivars (Cieniewicz and Fuchs, 2016b). Similarly, no reports of apple fruit disorder have been reported with ASGV infection, although swelling of pale areas of mandarin fruit rind (intumescence) has been associated with this virus (Lovisolo et al., 2003).

The project described in this report was undertaken to characterize the postharvest performance of MN55 apple fruit, and investigate the skin dimpling disorder, the extent to which postharvest treatments can alleviate its development, and its possible associations with apple viruses.

## Materials and Methods

*Plant material.* MN55 trees harvested for these studies were all grown at the University of Minnesota Horticultural Research Center in Chanhassen (lat. 44°51′30″ N, long. 93°39′41″ W) in 2016–20. The types of trees used were 1) original seedling grafted onto B9 rootstock ≈6 months after germination, 2) second test trees in the breeding program's evaluation orchard that were grafted onto B9 or M.26 rootstocks, 3) top-worked trees, and 4) rootstock trial trees in a block, all of which had been grafted onto B.118 rootstock (Table 1). Second test trees had been grafted from the original tree started from seed (original seedling), had passed initial quality evaluations, and were selected for further evaluation before commercial testing. Top-worked trees have scion from the original MN55 seedling tree grafted onto existing trees of NY 66305-139 grafted onto M.26 rootstock.

To investigate harvest maturity effects on skin dimpling development, fruit were harvested from five or more rootstock trial trees on 12, 17, and 23 Aug. 2016; and 17 and 24 Aug. 2017 after fruit background peel color had turned yellow-green. Fruit from the different trees were pooled, and then randomly divided into four lots of 8 to 15 fruit each. For experiments testing various postharvest treatments, fruit were harvested from rootstock trial trees on 24 Aug. 2016; 17 and 24 Aug. 2017, 26 Aug. 2019; and 20 and 27 Aug. 2020, also when fruit background peel color was yellow-green. Fruit were sprayed twice with 10 to 18 ppm naphthaleneacetic acid (Fruitone L, Valent Biosciences, Libertyville, IL) about 7 d before each harvest occurring after 17 Aug. of each year to prevent preharvest fruit drop.

For virus testing via RT-PCR, five leaves per tree were harvested from one original seedling, two top-worked, and three rootstock trial trees on 20 July 2018. Fruit were harvested from the original seedling and the

Table 1. Trees harvested for experiments in this study.

| Type of tree | Years harvested | Tissue sampled | Experimental objective |
|---|---|---|---|
| Original seedling | 2018 | Leaves and fruit | RT-PCR for virus detection |
| | | Fruit | HTS for virus detection |
| | 2020 | Leaves | HTS for virus detection |
| Top-worked | 2018 | Leaves | RT-PCR for virus detection |
| | 2020 | Leaves | HTS for virus detection |
| Second test | 2020 | Leaves | HTS for virus detection |
| Rootstock trial | 2016, 2017 | Fruit | Harvest date effects |
| | 2018 | Leaves and fruit | RT-PCR for virus detection |
| | | Leaves | HTS for virus detection |
| | 2019, 2020 | Fruit | Storage temperature effects |

HTS = high throughput sequencing; RT-PCR = reverse transcriptase polymerase chain reaction.

three rootstock trial trees (same trees used for leaf harvests) on 20 July (five fruit per tree), 9 Aug. (three fruit per tree), and 27 Aug. (five fruit per tree). To determine whether virus could be detected in fruit of other cultivars showing varying levels of skin dimpling, five fruit of three commercial apple cultivars were purchased from a local grocery store on 6 Dec. 2019.

For HTS, five fruit from the original seedling tree and five fruit from each of three rootstock trial trees were harvested on 30 Aug. 2018. Fruit were stored at 0 °C before overnight shipment to the U.S. Department of Agriculture—Agriculture Research Service laboratory in Beltsville, MD, where they were stored upon arrival at 4 °C until processed for HTS. Leaves were also collected on 24 June 2020 from the original seedling, and four second test, two top-worked, and three rootstock trial trees (eight leaves per tree), and sent overnight to Beltsville, MD, via overnight shipment.

*Maturity assessments.* At every harvest, 10 fruit were assessed for fresh weight, firmness, soluble solids content, percentage of red peel coloration, and starch index. Fruit dry matter content was only measured in 2016 and 2017. Fruit were weighed the day of harvest after transport from the orchard to the laboratory. Flesh firmness was measured by a puncture test using a drill press-mounted FT30 penetrometer (Wagner Instruments, Greenwich, CT) fitted with an 11-mm tip. Measurements were made on two opposing, equatorial sides of each fruit on peeled areas. Soluble solids content was measured on expressed juice from penetrometer measurements of each fruit using an ATC-1E refractometer (Atago Co. Ltd., Tokyo, Japan). Red peel coloration was estimated subjectively. Starch index was rated using the iodine test and the McIntosh scale (Blanpied and Silsby, 1992). Dry matter was measured after pieces of peeled fruit flesh were weighed and dried at 65 °C until dry weight no longer changed.

*Postharvest treatments.* In 2016 and 2017, fruit were treated with 1-MCP or DPA (Sigma-Aldrich, St. Louis, MO) before storage at 0 to 1 °C. 1-MCP in the form of SmartTabs donated by AgroFresh was applied at room temperature at the rate of 1 $\mu L \cdot L^{-1}$ according to the AgroFresh recommended protocol. DPA was solubilized to 6 mM according to the method of Padfield (1959), except that 2.5% (v/v) ethanol was used instead of isopropanol. The control treatment was not treated with 1-MCP or DPA and was placed in storage at 0 to 1 °C directly after harvest. Three lots of five to nine fruit per lot were used for each treatment. Lots were stored in separate plastic boxes and randomly placed in different areas of a 0 to 1 °C cold room. All fruit were assessed after 4 months of storage.

In 2019 and 2020, 120 fruit were pretreated with 1-MCP according to the SmartFresh protocol and then separated into replicate lots of 10 fruit per lot before storage. Three lots each were stored at 0 to 1 °C, 4 to 5 °C, or 6 to 7 °C. Three lots were kept at room temperature for 1 d before storage at 0 to 1 °C. All fruit were assessed after 4 months of storage.

*Microscopy.* Apple peel was removed from three undimpled and three dimpled fruit using a razor blade, placed on a glass slide, and viewed with a Nikon SMZ1500 stereo microscope (Nikon Instruments Inc., Melville, NY) equipped with an Olympus Evolt E-330 digital camera (Olympus America Inc., Center Valley, PA) at 1× magnification.

Virus particles in MN55 fruit were imaged using transmission electron microscopy (TEM). Virus particles were partially purified from apple skin tissue and leaf tissue as described by Lockhart et al. (1997). Briefly, apple skin or leaves were powdered in a mortar with liquid nitrogen, and the powder extracted 3:1 (v/w) with 250 mM Tris-HCl, pH 7.4, containing 1% (w/v) $Na_2SO_3$ and 0.5% (v/v) 2-mercaptoethanol. The extract was filtered through cheesecloth and clarified by low-speed centrifugation at 18,000 $g_n$ (max) for 15 min. Triton X-100 was added to the supernatant to a final concentration of 2% (v/v), the mixture was shaken for 1 min, layered over 6 mL of 30% (w/v) sucrose in extraction buffer, and centrifuged for 2 h at 148,000 $g_n$ (max). The resulting pellets were resuspended overnight in 50 mM Tris-HCl, 150 mM NaCl, pH 7.4, and clarified by shaking with one-half volume of chloroform and centrifuged at 18,000 $g_n$ (max) for 10 min. The aqueous phase constituted a partially purified virus suspension. Partially purified suspensions were examined by TEM using samples mounted on carbon-coated formvar grids negatively stained with 2% phosphotungstic acid, pH 7.0.

*Molecular virus detection.* Leaves of MN55 original seedling, and top-worked and rootstock trial trees, as well as fruit from the MN55 original seedling, MN 55 rootstock trial trees, and of various cultivars purchased from local grocery stores, were tested for the presence of three viruses using RT-PCR. The viruses were ACLSV, ASPV, and ASGV. RNA was extracted the same day as harvest, using 100 mg of each peel or leaf sample. Fruit peel was removed using a scalpel, and all samples (peel and leaf) were ground in liquid nitrogen. RNA was extracted from all samples using RNeasy kits (Qiagen, Germantown, MD). One step RT-PCR reactions were prepared using Illustra Ready-To-Go RT-PCR Beads (GE Healthcare Bio-Sciences, Pittsburgh, PA) per manufacturer instructions. Twenty-five µL of the reaction solution was mixed with 3 µL of total RNA.

The reaction mixtures were incubated at the following conditions: 45 min at 25 °C for reverse transcription, 5 min at 95 °C to activate Taq polymerase, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for ACLSV and ASPV or 58 °C for ASGV for 30 s, and extension at 72 °C for 45 s for ACLSV and ASPV or 30 s for ASGV, and final extension at 72 °C for 5 min. PCR products were separated with agarose gel electrophoresis and visualized using ethidium bromide dye.

Primers for the different viruses were: ACLSV-FWD (5'-GAG ART TTC AGT TTG CTM GA-3'), ACLSV-REV (5'-AGT CTA CAG GCT ATT TAT TAT AAG T-3')

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J.J.L. and D.B. are entitled to royalties from the MN55 cultivar described in this paper. The University of Minnesota also has a royalty interest in MN55 and related intellectual property. These relationships have been reviewed and managed by the University of Minnesota in accordance with its Conflict of Interest policies.
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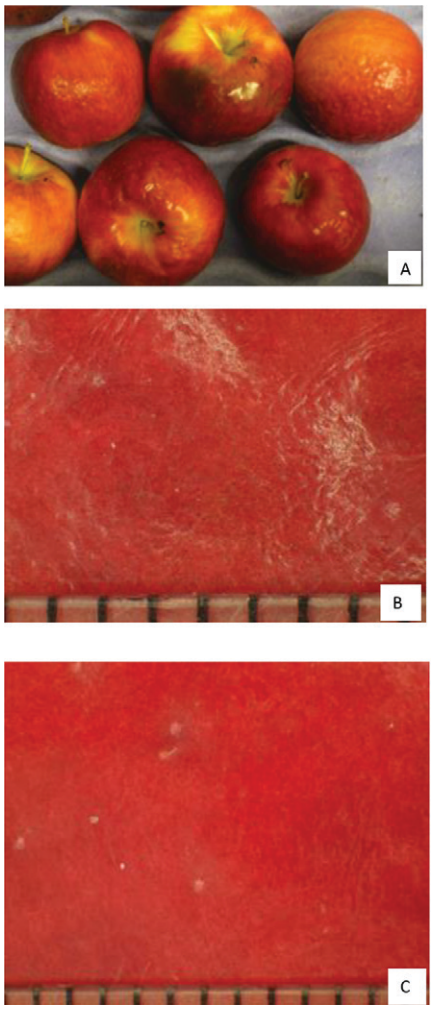


Fig. 1. (**A**) MN55 fruit 7 d after removal from 0 to 1 °C storage, showing skin dimpling symptoms. Representative stereoscopic microscopy images of affected (**B**) and unaffected (**C**) peel at 1× magnification; ruler demarcation (at bottom of microscopy images) = 1 mm.

Expected amplicon 794bp (Yaegashi et al., 2007); ASPV-FWD (5'-WGC IAA RGC IGG ICA RAC-3'), ASPV-REV (5'-RMY TCI CCI SWR AAI CKC AT-3') Expected amplicon 363 bp (Dovas and Katis, 2003); and ASGV-U (5'-CCC GCT GTT GGA TTT GAT ACA CCT C-3'), ASGV-2 (5'-GGA ATT TCA CAC GAC TCC TAA CCC TCC-3') Expected amplicon 499bp (James et al., 1997).

*HTS.* Subsamples were removed from leaves and fruit peel samples from each tree and combined to obtain 0.1 g of each sample per tree. RNA was extracted using RNeasy Plant Extraction kits (Qiagen, Germantown, MD), then outsourced to SeqMatic LLC (Fremont, CA) for DNase treatment, rRNA depletion, and cDNA library construction. The cDNA libraries were sequenced on an Illumina NextSeq500 platform as 75 bp single end reads. Sequence data were analyzed using CLC Genomics Workbench (Qiagen).

*Statistical analyses.* The experimental design of the postharvest experiments was completely randomized. Data were analyzed using analysis of variance or linear mixed-effects models using the lm function if normality of residuals could not be satisfied, with R statistical software (RStudio version 1.4.1106 for Ubuntu Bionic, PBC, Boston, MA). Data were log transformed if needed. Separation of means used Tukey's honestly significant difference tests. Redness was analyzed using the Kruskal-Wallis test for nonparametric data.

## Results and Discussion

After 4 months of storage at 0 to 1 °C, skin dimpling was evident on MN55 fruit (Fig. 1A). Stereoscopic imaging showed that the affected areas of dimpled fruit exhibited lighter colored areas among red areas of peel (Fig. 1B), whereas unaffected fruit had uniformly red peels (Fig. 1C). In 2016, the percentage of fruit showing this disorder was least for harvest 1 when scored on day 7 after removal from storage (Table 2). The percentage of dimpled fruit was equal for harvests 2 and 3 at day 7. Skin dimpling severity was similar for fruit of all harvests scored 7 d out of storage. In 2016, soluble solids content (Fig. 2C) and dry matter content (Fig. 2F) were similar among harvests, but fruit from the last harvest were less firm than those of the earlier harvests (Fig. 2B, $P = 0.02$). Fresh weights (Fig. 2A, $P = 0.04$), starch index (Fig. 2D, $P = 0.002$), and % redness (Fig. 2E, $P = 0.02$) increased with increasing harvest date. These data show that fruit continued to ripen between harvests 1 and 2 in 2016. However, in 2017 there were no differences in any of the traits between harvests 1 and 2 (data not shown), and no difference in dimpling incidence when scored 1 d out of storage. Unfortunately, these fruit were not scored 7 d after removal from storage. However, subsequent experiments verified that percentages of fruit with dimpling scored 7 d after removal from storage increased with harvest date (Table 4). The cumulative data suggest that dimpling incidence increases between harvests 1 and 2, with increasing fruit maturity, but is only evident 7 d after fruit are removed from storage. However, all of the maturity indices except for peel redness were based on flesh characteristics, whereas the disorder is manifest in the peel.

*Postharvest treatment effects on disorder appearance.* Treating fruit with 1-MCP did not decrease the percentage of affected fruit or severity of skin dimpling compared with the untreated controls (Table 3). Although DPA treatment had no effect on the percentage of fruit developing dimpling, it did slightly decrease the mean severity of dimpling observed on day 7 after removal from storage. Some fruit of all treatments (including 1-MCP and DPA-treated fruit) also developed soft scald, but no fruit treated with 1-MCP or DPA developed superficial scald, unlike the control fruit (data not shown). Subsequent experiments included prestorage treatment of fruit with 1-MCP, but not DPA, for this reason.

In both 2019 and 2020, fewer fruit stored at 6 to 7 °C developed dimpling than those stored at 4 to 5 °C (Table 4). However, greasiness was a more likely problem at the higher than lower storage temperatures. In 2019, fruit stored at 0 to 1 °C exhibited less dimpling than expected, based on previous experiments. The reason for this observation is not clear, but fruit stored at 0 to 1 °C also developed more superficial scald than at other storage temperatures (data not shown), and this may have affected dimpling development. In 2020, a lower mean percentage of fruit harvested on 20 Aug. were dimpled compared with fruit harvested on 27 Aug., similar to results obtained with fruit harvested in 2016 and 2017 (Table 1). Fruit of all treatments in both years exhibited soft scald and superficial scald because the fruit had not been treated with DPA.

*Virus testing.* Flexuous filaments of ≈700 nm in length were observed in RT-PCR positive samples using TEM (Fig. 3).

Using RT-PCR, ASGV was not detected in MN55 leaves or fruit from any tree.

Table 2. Mean (± SD) percentages of three replicate lots of fruit displaying skin dimpling symptoms per harvest date, and mean severity of the disorder observed 1 and 7 d after removal to room temperature from 0 to 1 °C storage for fruit harvested in 2016 and 2017.

| | 2016 | | | | 2017[z] |
|---|---|---|---|---|---|
| Harvest[y] | Mean percentage on day 1 | Mean severity on day 1[x] | Mean percentage on day 7 | Mean severity on day 7 | Mean percentage on day 1 |
| 1 | 20 ± 8 | 3.7 ± 0.3 | 54 ± 5 | 3.8 ± 0.4 | 37 ± 15 |
| 2 | 39 ± 6 | 3.5 ± 0.2 | 89 ± 11 | 4.5 ± 0.3 | 50 ± 10 |
| 3 | 3 (1 of 28 fruit) | 3 | 97 ± 6 | 4.3 ± 0.7 | |
| | | | *P* values from analysis of variance | | |
| Harvest | NS[w] | 0.02 | 0.002 | NS | NS |

[z]Severity and day 7 dimpling not measured in 2017.
[y]Harvests 1, 2, and 3 were made on 12, 17 and 23 Aug. 2016 and on 17 and 24 Aug. 2017 (no third harvest), respectively.
[x]Severity scored as 1 = none, 2 = 1% to 10%, 3 = 11% to 25%, 4 = 26% to 49%, 5 = >50% of fruit surface.
[w]Mean separation by Tukey's honestly significant difference tests, NS = not significant at $P = 0.05$.

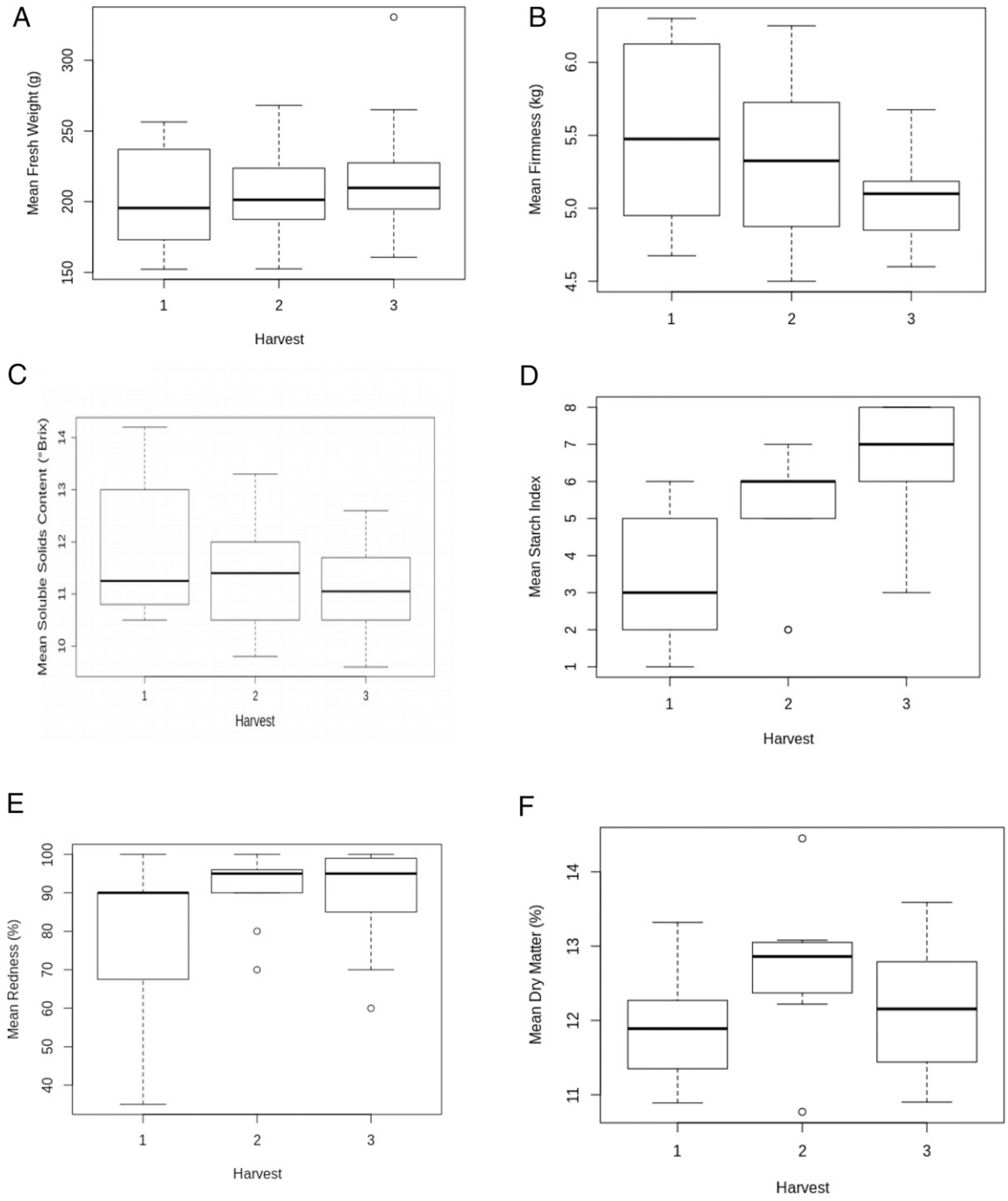


Fig. 2. Box plots of mean fresh weight, firmness, soluble solids content, starch index, peel redness, and dry matter content of MN55 fruit from three different harvests, done on 12, 17, and 23 Aug. 2016.

ACLSV was detected in fruit and leaves of rootstock trial trees, and not in leaves or fruit of the original seedling or top-worked trees. ASPV was detected in both leaves and fruit of the original seedling and rootstock trial trees (Table 5). ASPV was also detected in leaves of top-worked trees (data not shown). Fruit from both the original seedling and rootstock trial trees developed skin dimpling. Mean (of 3 replicate lots of fruit) percentage of skin dimpling observed 7 d after removal from storage was 67% for fruit harvested from the original seedling and 79%, 87%, and 82% for fruit from the three different rootstock trial trees. This suggests that if a virus is contributing to the disorder, it is most likely ASPV.

RT-PCR was also used to test fruit purchased from a local grocery store. Two of the three commercial cultivars that were tested were positive for ASPV (Table 6), but only fruit of one cultivar exhibited skin dimpling at the time of purchase. However, the harvest and storage histories of these cultivars were unknown. Cultivar 1, for example, may have been stored at temperatures higher than 4 °C, which could preclude development of skin dimpling, and dimpling development may require fruit exposure to low temperature storage.

To validate RT-PCR results, HTS was done in 2018 on fruit from the original seedling and rootstock trees. HTS resulted in 18 to 27 million reads per sample. Two of the rootstock trial samples tested positive for only ACLSV, and one tested positive for both ASPV and ACLSV. The original

Table 3. Mean (± SD) percentage of MN55 fruit exhibiting skin dimpling and mean severity of the disorder after treatments with 1-methylcyclopropene (1-MCP), diphenylamine (DPA) or 5 to 6 °C preconditioning compared with no treatment before storage at 0 to 1 °C observed 1 and 7 d after removal from storage in 2016 and 1 d after removal in 2017.

| | 2016 | | | | 2017[z] |
|---|---|---|---|---|---|
| Treatment | Mean percentage on day 1 | Mean severity on day 1[y] | Mean percentage on day 7 | Mean severity on day 7 | Mean percentage on day 1 |
| 0 to 1 °C (control) | 45 ± 14 | 3.4 | 91 ± 8 | 4.5 | 37 ± 15 |
| 1-MCP | 37 ± 11 | 3.5 | 70 ± 23 | 4.8 | 33 ± 15 |
| DPA | 37 ± 20 | 2.7 | 70 ± 31 | 3.3 | 33 ± 6 |
| | | | *P* values from analysis of variance | | |
| Treatment | NS[x] | NS | NS | 0.04 | NS |

[z]Severity and day 7 dimpling not measured in 2017.
[y]Severity scored as 1 = none, 2 = 1% to 10%, 3 = 11% to 25%, 4 = 26% to 49%, 5 = > 50% surface.
[x]Mean separation by Tukey's honestly significant difference tests, NS = not significant at $P = 0.05$.

Table 4. Mean percentage (± SD) of MN55 fruit exhibiting skin dimpling 7 d after removal from storage at 0 to 1 °C, 4 to 5 °C, or 6 to 7 °C in 2019 and 2020.

| | 2019 | 2020 | |
|---|---|---|---|
| Treatment | Mean percentage (harvested on 25 Aug.) | Mean percentage (harvested on 20 Aug.) | Mean percentage (harvested on 27 Aug.) |
| 0 to 1 °C storage | 37 ± 22 | 33 ± 15 | 67 ± 15 |
| 4 to 5 °C storage | 60 ± 24 | 27 ± 23 | 27 ± 11.5 |
| 6 to 7 °C storage | 7 ± 16 | 0 | 3 ± 6 |
| Delay 1 d at RT, 0 to 1 °C[z] | 17 ± 29 | 27 ± 15 | 27 ± 11.5 |
| | | *P* values from analysis of variance | |
| Treatment | 0.02 | NS[y] | 0.001 |

[z]Delay 1 d at RT fruit were held for 1 d at room temperature before long-term storage at 0 to 1 °C.
[y]Mean separation by Tukey's honestly significant difference tests, NS = not significant at $P = 0.05$.

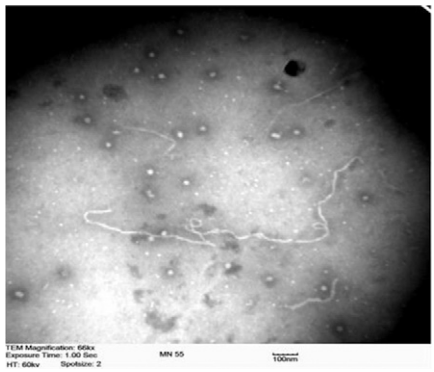


Fig. 3. Transmission electron micrograph showing virus particles in MN55 fruit.

Table 5. Number of MN55 leaves and fruits harvested in 2018 with virus detected by RT-PCR (numerator) out of total number of leaves or fruit sampled (denominator).

| Harvest date | Tree type | Tissue | ACLSV | ASGV | ASPV |
|---|---|---|---|---|---|
| 20 July | Original seedling | Leaf | ND[z] | ND | 5/5[y] |
| | Rootstock trial | Leaf | 2/15 | ND | 9/15 |
| | Original seedling | Fruit | ND | ND | 2/3 |
| | Rootstock trial | Fruit | 3/9 | ND | 6/9 |
| 9 Aug. | Original seedling | Fruit | ND | ND | 3/3 |
| | Rootstock trial | Fruit | 4/9 | ND | 8/9 |
| 27 Aug. | Original seedling | Fruit | ND | ND | 5/5 |
| | Rootstock trial | Fruit | 10/15 | ND | 12/15 |

[z]ND = not detected by reverse transcriptase polymerase chain reaction (RT-PCR).
[y]Numerator = number of fruit that tested positive for virus by RT-PCR, denominator = total number of tested fruit.
ACLSV = *Apple chlorotic leafspot virus*; ASGV = *Apple stem grooving virus*; ASPV = *Apple stem pitting virus*.

seedling sample was negative for both ACLSV and ASPV. Further verification of virus presence was done in 2020, with testing of 10 new leaf samples from each of the original seedling, top-worked trees, second test trees, and rootstock trial trees. HTS resulted in 13 to 21 million reads per sample. All samples were negative for viroids. No viruses were detected in the original seedling and second test tree leaves. Leaves from both top-worked trees that were sampled had ASPV and apple green crinkle virus. One of those trees also tested positive for apricot latent virus. One of the rootstock trial trees had only ASPV, another had only ACLSV, and the third tested negative for all viruses. No other viruses were detected by HTS in the 2018 and 2020 samples. The differing percentages of samples testing positive for ACLSV and ASPV by HTS compared with RT-PCR may be due to different locations of the leaf samples because viruses would not be evenly distributed through the tree canopies (Rott et al., 2017). However, HTS verified that virus was present in top-worked and rootstock trial trees.

When this work was initiated in 2016, it was assumed that the original seedling tree was virus-free because it had been sent to the National Clean Plant Center in Prosser, WA, in 2011 for virus testing. In 2011, it tested negative for the viruses typically assayed at the time, including ASPV. Having determined that rootstock trial trees produced fruit that developed postharvest skin dimpling, we wanted to determine the stage at which the rootstock trees became infected, so tested second test and top-worked trees for viruses. Second test trees had not been tested for viruses, and although the rootstocks used for grafting were ordered from certified programs, they also were not tested before use. In 2018, as we obtained RT-PCR data showing that the original seedling tree was infected with ASPV, the National Clean Plant Center retested stored samples from the original seedling tree, and found it to be positive for ASPV. Thus, the rootstock trial trees were probably infected when grafted using scions derived from the original seedling.

In conclusion, harvest date can affect skin dimpling appearance of stored MN55 fruit, so some problems with dimpling could be avoided by harvesting earlier rather than later, without affecting soluble solids or dry matter content, once enough fruit have developed adequate color. To avoid development of skin dimpling, soft scald and superficial scald, MN55 fruit should be treated with DPA and stored at ≈6 °C, although fruit may exhibit greasiness at this storage temperature. Keeping fruit for 24 h at room temperature before storage at 0 to 1 °C can reduce, but not eliminate, the occurrence of skin dimpling. Skin dimpling can therefore be classified as a chilling

Table 6. Presence of viruses detected using RT-PCR and skin dimpling with number of positive samples (numerator) out of the total number of fruit sampled (denominator) of three commercial cultivars purchased from a local grocery store.

| Cultivar | ACLSV | ASGV | ASPV | Skin dimpling |
|---|---|---|---|---|
| 1 | 4/5[z] | ND | 5/5 | ND |
| 2 | 3/5 | 4/5 | 5/5 | 5/5 |
| 3 | ND | ND | 1/5 | Minor |

[z]Numerator = number of fruit that tested positive for virus by reverse transcriptase polymerase chain reaction (RT-PCR), denominator = total number of tested fruit, ND = not detected.
ACLSV = *Apple chlorotic leafspot virus*; ASGV = *Apple stem grooving virus*; ASPV = *Apple stem pitting virus*.

disorder, but the extent to which it is affected by virus presence is unclear.

Although we found ASPV in fruit that developed skin dimpling, the presence of viruses in the fruit skin was only circumstantially linked with the observed skin dimpling. There was a positive relationship between the presence of ASPV and fruit dimpling observed in both MN55 fruit and apples obtained from a supermarket. However, not all supermarket-sourced fruit with ASPV developed skin dimpling. Further experiments to investigate whether virus infection correlates with skin dimpling induction can include inoculating uninfected trees with ASPV and monthly monitoring for fruit symptoms through harvest and 0 °C storage. This research is needed to determine the effects of storage conditions and viruses, singly and in combination, on apple fruit dimpling.

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