Canadian Journal of Plant Science

Summer fruitlet thinning enhanced quality attributes of Ambrosia™ apple at harvest and after four months of cold air storage

Journal:	<i>Canadian Journal of Plant Science</i>
Manuscript ID	CJPS-2021-0080.R2
Manuscript Type:	Article
Date Submitted by the Author:	03-Jun-2021
Complete List of Authors:	Lu, Changwen; Agriculture and Agri-Food Canada, Xu, Hao; Agriculture and Agri-Food Canada, Summerland Research and Development Centre
Is the invited manuscript for consideration in a Special Issue?_:	Canadian Horticulture Special Issue
Keywords:	fruitlet thinning, rootstock, Dry matter content, at-and post-harvest quality, fruit maturation

SCHOLARONE™
Manuscripts

Title: Summer fruitlet thinning enhanced quality attributes of Ambrosia™ apple at harvest and after four months of cold air storage

Authors: Changwen Lu^{1*} and Hao Xu¹

¹Agriculture and Agri-Food Canada, Summerland Research and Development Centre, Summerland, British Columbia V0H 1Z0

* Correspondence: changwen.lu@canada.ca

Abstract

Summer fruitlet thinning is implemented as a routine orchard practice to produce apple fruits with good quality. However, its impacts on the dynamics of fruit quality metrics during the growing season and in the postharvest storage, remain unclear. In this study, summer hand thinning on fruitlets of Ambrosia™ apple (*Malus x domestica* Borkh.) was conducted on two dwarfing rootstocks, Malling 9 (M.9) and Budagovsky (B.9), in an organic orchard and a conventional orchard under the semi-arid climate in Similkameen Valley, British Columbia, Canada. Adequate thinning ("AT", in which 70% of fruitlets were removed) and light thinning ("LT", in which 30% of fruitlets were removed) were implemented in randomized plots in eight weeks after full bloom. Fruit development and dry matter content (DMC) were then monitored during the growing season; fruit quality was subsequently evaluated at harvest and after four-month of air storage at 0.5 °C. Relative to LT, AT enhanced fruit quality attributes in DMC, surface blush coverage and intensity, and soluble solids content at harvest. The apples with higher DMC under AT also possessed higher compositional quality and lower incidences of fruit disorder in the postharvest stage. This study suggests that summer fruitlet thinning of Ambrosia apples can have significant impacts on fruit composition during subsequent on-tree fruit development, on the onset of

ripening and eventually on the retention of quality and minimization of disorders over 4 months of cold air storage. This effect is found for OG and CV production systems and with both dwarfing rootstocks.

Key words: Fruitlet thinning, rootstock, dry matter content, fruit maturation, at-and post-harvest quality

Introduction

Summer fruitlet thinning is a routine orchard practice that plays an important role in producing quality fruit and sustaining tree health. Generally, hand thinning of fruitlets is applied after “June drop” in early summer, i.e., about 7-9 weeks after full bloom in commercial apple orchards, as an extra measure to fine tune the crop load upon subsequent to earlier applied chemical thinning during blossoms. Apple cropping varies from year to year and from orchard to orchard, and therefore, the impacts of fruit thinning often vary under different circumstances. It is well-known that fruit thinning alters various physiological and metabolic processes, and subsequently influences fruit development and tree growth (Fernandez et al. 2018; Musacchi and Serra 2018). However, knowledge is still lacking in understanding how crop load impacts the development of fruit quality attributes during the growing season and the consequent dynamics changes of quality metrics in postharvest storage.

Rootstocks influence many aspects of apple scion performance, including phenological characteristics, scion vigor, canopy shape and size, plant water and nutrient relations, carbohydrate assimilation and allocation, tree responses to environmental conditions and abiotic stresses, and various fruit quality attributes (Ai-Hinai and Roper 2004). Suitable rootstocks can enhance tree resilience, sustain yield (Xu et al. 2021), and improve fruit quality (Musacchi and Serra 2018). In turn, rootstock performance is under the impacts of scion cultivars, tree age, crop load, climatic and edaphic conditions, and horticultural management (Musacchi and Serra 2018). Since the environmental conditions and horticultural practices affect how different rootstocks interacted with the scion and how they influenced

fruit quality (Musacchi and Serra 2018), it is necessary to unveil the interactive effects of rootstocks and summer fruitlet thinning on the fruit quality development on-tree in conventional and organic growing systems, which highly relevant in British Columbia, Canada.

Crop load is determined by thinning intensity and rootstock vigor, and is a primary factor in tree growth and fruit production of apple (Bound 2019). Crop load represents the quantity of carbohydrate sinks per trunk cross-section area, and therefore, it influences the source-sink dynamics at the tree level. In Gala apple, it has been shown that crop load altered the photosynthetic capacity and carbohydrate partitioning amongst sources and sinks within the tree; under heavier crop load, leaf gas exchange increased and leaf starch content decreased (Yang et al. 2021). It also affected floral bud differentiation, initiation and development (Wilkie et al. 2008; Serra et al. 2016; Fernandez et al. 2018). Fruit size is one of the most important determinants for market acceptability and value, which is highly correlated with crop load (Bound 2019; Cline 2009). Sugars, starch and structural carbohydrates in apple fruits are derived from leaf photosynthesis and are the main components in fruit DMC. DMC also contains organic acids, amino acids and mineral nutrients, contributing to 10-20% of the fruit fresh weight (Musacchi and Serra 2018). As fruits expand and mature, dry matter accumulates in fruits; this process is influenced by physiological processes of net photosynthetic gain, carbohydrate allocations between source and different sinks, and water and nutrient transport (Heim et al. 1979; Schechter et al. 1993). DMC in mature fruits was found to be relevant to apple quality attributes such as fruit maturation and skin coloration (Toivonen and Lannard 2020; Bound 2019), soluble solids content (Peiris et al. 1999; Palmer et al. 2013; Travers et al. 2014) and firmness (Saei et al. 2011); therefore, it has become a widely used metric in evaluating apple quality (Trimble 2019; Palmer et al. 2010). These external appearance and internal characteristics in texture and flavor at harvest, as well as the retention of the quality attributes during postharvest storage, determine the consumer acceptance, market value and accessibility of the apple variety (DeEll 2020). Recent studies showed that pre-harvest and at-harvest DMC was highly

correlated with postharvest DMC (Toivonen and Lannard 2021; Travers et al. 2014), and, that the apples with higher DMC attributed to the improved sensory perception (Palmer et al. 2010).

Although it has been recognized that apple fruit development is impacted by crop load intensity, there is still a lack of knowledge regarding the interactive effects of fruitlet thinning, rootstock type, and orchard management on pre-harvest development, on-tree compositional quality, and on postharvest quality retention of fruit. In particular, there is less information about the integrated effects associated with fruitlet thinning on the Ambrosia™ apple (*Malus xdomestica* Borkh.), a relatively new cultivar that has become popular rapidly in recent years (Toivonen 2015). The aim of this study was to investigate the respective roles and combined impacts of summer thinning intensities, dwarfing rootstocks and orchard horticultures, on fruit development, DMC accumulation and fruit maturation progress during the growing season; and to assess the profile of surface coloration and compositional quality of the fruit at harvest. The subsequent carry-over effects on quality retention and disorder incidence of the fruit were further investigated after four months of cold air storage.

Materials and Methods

Trial conditions and thinning treatments

The trials were conducted in a conventional orchard ("CV": 49.196° N, -119.755° W) and an organic orchard ("OG": 49.184° N, -119.755° W) in Cawston, British Columbia, Canada. The trial plots comprised of 14-16 year-old healthy trees which were trained to super spindle structure in a four-wire trellis system with 10-foot row spacing. Tree spacing was approximately 1.5 feet apart in CV and 3 feet apart in OG. Though these two orchards are not in the same locations and are different in tree spacing; however, they are located 1.5 km apart, on the same type of sandy loam soil, with very similar yield in the last two years (23.5 ± 0.8 ton per hectare). In each orchard, two dwarfing rootstocks, i.e., Malling 9 (M.9) and Budagovsky 9 (B.9), were selected; two summer thinning treatments were implemented on each rootstock.

Fruitlet thinning was planned based on tree vigor, initial crop load, fruit spacing, leaf area, canopy size (capacity), and canopy light conditions and air flow, at the onset of summer (about 8 weeks after full bloom). Prior to hand thinning treatments, there were two to six fruitlets in a cluster. In CV, each tree had 75-90 fruitlets. In OG, each tree had over 150 fruitlets because flower thinning with chemical sprays are not permitted in organic orchards. Deformed, diseased and insect damaged fruitlets, and fruitlets smaller than average, were first removed. Then hand plucking was applied on random lateral fruitlets. Adequate thinning (AT) was carried out to keep one fruit with at least five fully expanded leaves per cluster, and, maximum two fruits of 15-20 cm apart per branch; about 30% of the initial crops were kept after thinning. Under light thinning (LT), two fruitlets were kept per cluster, and three to six fruitlets were kept per branch; about 70% of the initial crops remained after thinning. After adequate thinning, fruit were kept as 32 - 35 fruit per tree which equivalent to 64 - 70 fruit per 3-feet tree spacing in CV, and 65 - 70 fruit per tree per 3-feet tree spacing in OG. In each orchard, there were three randomized plots (replications) for each of the four thinning $\times$ rootstock combinations (AT $\times$ M.9, LT $\times$ M.9, AT $\times$ B.9 and LT $\times$ B.9); each plot consisted of six trees each.

Fruit development and DMC measurement

Starting at 2 weeks after fruit thinning, the diameter of fruitlet was measured bi-weekly using a digital caliper. Beginning at late August, fruit maturation was assessed weekly using the I_{AD} index measured by a DA meter (Sinteleia, Bologna, Italy), following the protocol modified for Ambrosia™ apples (Toivonen et al. 2016). The DA meter measures the apple nondestructively and gives an index of absorbance difference (I_{AD}) which is calculated as the subtraction of absorbance at 670 nm minus the absorbance at 720 nm. The measurement of a fruit's chlorophyll index gives an indication of the maturation and ripeness state (Ziosi et al. 2008).. A single measurement was taken on the transition zone between sunlit side and shaded side on each apple (Toivonen et al. 2016). I_{AD} levels were measured on 20 fruits per plot weekly to predict the timing of harvest.

Fruit DMCs were measured bi-weekly during the growing season until harvest, using Felix F-750 Produce Quality Meter (Felix Instruments, Camas, WA, USA), a handheld visible-near infrared spectrometer that can predict fruit DMC levels non-destructively (Toivonen and Lannard 2021; Kaur et al. 2017). Fruit on the tree were measured on the sunlit side, with the lens of the meter tightly placed against the fruit surface. After harvest, the reddest portion on the blush side of the fruits was scanned. In each plot, 20 fruit were measured on each day of investigation (i.e., 60 fruits per treatment combination in each orchard).

Postharvest handling and storage

Fruit samples were harvested when I_{DA} index decreased to 0.5 to 0.7 (Toivonen et al. 2016) and average fruit size reached the commercial grade of 68–75 mm in diameter (Cline 2009). Sampling was scheduled to target the optimal fruit maturation for each thinning × rootstock treatment combination; three plots for each treatment combination were picked on the same day. In total, 24 batches of samples were harvested from the two orchards; each batch included 25 apples per each of three plots from a treatment combination were placed in one of two dividers per storage tote (total of 12 totes were filled). To simulate the local commercial procedure of precooling (personal communication in September 2018 with Dan Taylor, Operation manager of Cawston Cold Storage, Ltd, British Columbia) storage conditions, the totes were held at 10°C for three days and then placed in air storage at 0.5°C for four months. Delaying application of precooling is considered a simple and efficient protocol to inhibit or mitigate physiological disorders in cold storage of susceptible apples (Watkins et al. 2004).

Postharvest quality assessments

Blush coverage was assessed visually in a estimated percentage. Red blush intensity was ranked visually from 1 for light pink to 6 for fully red or “perfect red”, in reference to the color chart of New Tree Fruit Varieties Development Council (NVDC) of British Columbia (Toivonen et al. 2019; Cline 2009).

Flesh firmness was measured with a 11 mm diameter plunger mounted on Fruit Texture Analyzer (FTA) (Model GS-25, Güss Manufacturing Ltd., Strand, South Africa). Two punches were made on the opposite sides of each apple, on two peeled areas in the sunlit-shaded transition zone at the equator; the plunger was punched into the flesh at the speed of 200 mm/min to a depth of 8 mm; the firmness was calculated as the mean of the two readings on each fruit.

For the measurements of titratable acidity (TA) and soluble solids content (SSC), two non-peeled slices from the opposite sides on each of eight fruits, i.e., 16 slices in total per sample, were juiced in bulk using a masticating juicer (model 2000, Plastaket Manufacturing Co., inc./Champion Juicer Lodi, CA, USA). SSC (% w/v) was determined using the Refracto 30PX refractometer (Mettler Toledo, Columbus, OH, USA). For TA, 15 mL of the clear juice was diluted with 60 mL of distilled and deionized water; the diluted juice was then titrated with 0.1 N NaOH solution using an automated titrator (Model OrionStar T940, Thermo Scientific, Waltham, Massachusetts, U.S.A.); the original concentration of organic acids was calculated in mg/L of malic acid.

The storage disorders in soft scald, skin bruise, and decay were inspected visually after 4 month storage (DeEll 2020). Because the appearances of fruit bruising are changed in postharvest (Opara and Pathare 2014; Toivonen et al. 2007), the bruising fruit were counted in 4 months of storage and were presented in section of 'Storage disorder' in this report. The incidence of disorders was calculated in percentage.

Mass loss, an important criterion that represents fruit freshness in air storage, was monitored by weighing 10 apples per plot of treatment at harvest and after four months of cold air storage; the discrepancy was expressed in the percentage of weight reduction.

Data analyses

Changes in red blush coverage and intensity, DMC, I_{AD} , firmness, titratable acidity, and soluble solids were measured using three replicates of twenty fruit at harvest; changes in firmness, titratable acidity, soluble solids, decay, soft scald incidence, bruising, and mass loss were assessed using three replicates

of twenty five fruit in 4 months air storage. The difference between pairs of means amongst treatments were separated by Fisher's protected LSD using Proc mixed model in SAS ($P \leq 0.05$) (SAS Institute Inc., Cary, NC, USA). Data on fruit diameter and DMC were collected bi-weekly, and data on I_{AD} were collected weekly. Regression of the changes in these measures was accomplished using the regression function of Microsoft Excel 2016, and the linear regression lines are shown as dashed lines on the graphs for these data. Pearson's correlation analysis comparing the fruit size and fruit quality attributes, and comparing the trunk cross-section area between the two rootstocks was conducted using the PROC Corr routine of the SAS statistical package.

Results and Discussions

Fruit development after summer thinning

Changes in fruit size

Compared to light thinning (LT), adequate thinning (AT) led to about 25% larger fruit size in the CV plots (Figure 1A) and 15% larger in the OG plots (Figure 1B). Based on data across all apple samples in this trial, regardless of differences in orchard type, rootstock, or level of fruitlet thinning, our correlation analysis shows that fruit size was associated with blush color coverage ($r = 0.76$) and was highly correlated with SSC ($r = 0.92$). In contrast, fruit size was correlated to neither flesh firmness ($r = 0.08$) nor TA ($r = -0.09$). The positive correlations of size with surface color and SSC may be due to the high DMC in these apples, which is further discussed below

Fruit dry matter accumulation

DMC of Ambrosia™ apples was influenced by fruitlet thinning, as well as by orchards and rootstocks (Table 1). Compared to LT, AT led to an increase in DMC accumulation and maintained consistently higher levels of DMC in fruits in CV (Figure 2A) and OG (Figure 2B); the lower crop load under AT achieved 33% higher DMC on B.9 in CV, and, over 30% higher DMC on both rootstocks in OG, during

maturation (Figure 2). This OG orchard yielded overall higher DMC than CV (Table 2, 3). The dry matter content accumulation was higher in trees grafted to M.9 rootstocks as compared with B.9 rootstocks when grown in a CV orchard (Table 3), which was consistent with the previous year (data not shown). The B.9 rootstock is typically classified as small dwarfing rootstock and produces apple trees of 75%-90% in size of standard dwarfing rootstock such as M.9 (Xu and Ediger 2021). In this study, Ambrosia scions also had significantly smaller trunk cross section area at 30 cm above the grafted union on B.9 ($21.76 \pm 0.95 \text{ cm}^2$) than on M.9 ($28.31 \pm 1.00 \text{ cm}^2$) in the OG orchard ($P < 0.01$); as well, similar differences were found in the CV orchard between B.9 ($18.32 \pm 0.53 \text{ cm}^2$) and M.9 ($22.55 \pm 2.26 \text{ cm}^2$) ($P < 0.0001$). In a previous evaluation on tree-water relations of Ambrosia on different rootstocks, B.9 demonstrated more stable water status and drought tolerance than M.9 (Xu and Ediger 2021), which may contribute to more carbon assimilation over the growing season and more DMC accumulated to fruit (Xu et al. 2021).

Fruit maturation

AT treated fruit showed an earlier onset in decline of the I_{AD} Index of Ambrosia™ fruits in the late growing season on both rootstocks and in both orchards (Figure 3). In CV, the I_{AD} values collected on September 15 from AT plots were almost equal to the values collected from LT plots on September 28 (Figure 3A); similarly in OG, the I_{AD} values collected in early September from AT plots were comparable to those from LT plots in late September (Figure 3B). Overall, I_{AD} values were 0.52 under AT and 0.76 under LT at harvest (Table 2). A previous study suggested that it would take at least ten days for the I_{AD} values to drop from 0.7 to 0.5 (Toivonen 2015).

Quality profiles at harvest

The quality profiles of apple samples collected from the 2020 trials showed that summer fruitlet thinning had a primary impact on fruit development and quality metrics of Ambrosia™ at harvest (Table 1, 2, 3). Except for flesh firmness, AT conferred a better quality profile than LT, regardless of orchards and rootstocks.

The rootstocks also played an important role in determining quality attributes, except for flesh firmness in OG. B.9 was more responsive to crop load: under AT, B.9 resulted in much higher values in DMC, firmness, SSC and TA than M.9; under LT, B.9 led to higher DMC but lower firmness than M9, while SSC and TA were similar between the rootstocks in CV (Table 3).

Coloration

The market value of Ambrosia™ apples is highly dependent on the characteristic blush on the skin surface (Toivonen et al. 2019; Cline 2009). Red-blush coverage on fruit surface and the visual intensity of red-blush indicated that lower crop load from adequate summer thinning led to significant improvement of red-blush development, while rootstocks caused secondary effects on coloration (Table 3). Under AT, B.9 led to better surface color in both orchards. With regards to orchard effects, OG led to a better red-blush coverage on fruit surfaces than CV; however, the orchard effect on blush intensity was not significant (Table 2). The thinning effects on red-blush coverage, blush intensity and fruit size at harvest in the B.9 plots in OG were visualized in Figure 4: the fruits under heavier crop load from the three LT plots (top row), showed delayed skin coloration and smaller fruit size, compared to the fruits under lighter crop load from the three AT plots (bottom row). Thinning led to similar effects in M.9 in OG, and in both rootstocks in CV (photo not shown).

Internal quality at harvest

In general, OG apples had higher firmness than CV apples (Table 2). The difference in firmness between OG and CV could be attributed to the excessive nitrogen (N) fertilizer application in CV (e.g. foliar-N sprays in CV, personal communication with growers in field meeting hosted by BC Tree Fruits in Cawston, British Columbia, In 2019). Previous investigation revealed a negative correlation between fruitlet N level in the growing season and fruit DMC at harvest of Ambrosia apples on M.9 across six conventional orchards ($r^2 = 0.86$, data not shown). In the CV orchard, LT led to higher flesh firmness than AT in both rootstocks (Table 3). The lower firmness under AT concurred with the lower I_{AD} (0.49,

compared to 0.71 under LT, Table 3), suggesting a more advanced fruit maturation that possibly explaining the lower firmness under AT.

TA levels were similar under thinning treatments (Table 2), except that AT led to a higher value in B.9 in OG (Table 3). Notably, SSC, which was positively correlated with fruit DMC across the treatments and sites ($r^2 = 0.92$ based on the means in Table 3), was significantly higher under AT than LT throughout rootstocks and orchards ($P \leq 0.0001$). In addition, AT led to higher SSC : acidity ratio ($P = 0.008$, based on means in Table 3), indicating higher sweetness in the apples (Harker et al. 2002).

Postharvest quality retention

Changes in compositional quality attributes

Summer fruitlet thinning played a fundamental role on fruit quality retention of Ambrosia™ after storage (Table 4, 5, 6). Although AT showed lower firmness measurements in CV at harvest (Table 3), there was no significant difference in fruit firmness between the thinning treatments after four-month storage (Table 6) which indicated that flesh firmness in fruit from AT has better retained compared with those from LT during postharvest. AT treatment apples showed higher SSC and TA after air storage, as well as significantly less mass loss (Table 5, 6).

Storage disorders of fruit

In general, LT led to more incidence of storage disorders (Figure S1). Amongst the common postharvest disorders, skin bruise (Figure S1A) and soft scald (Figure S1B) occurred at a higher frequency in the samples collected from LT plots (Table 4). In contrast, the fruits from AT plots maintained higher quality attributes (Table 4, 5); they retained higher SSC and had less mass loss, and developed less disorders, than the fruits from LT plots, across rootstocks and orchards (Table 6). LT fruit had lower DMC indicating more water content in tissue (Musacchi and Serra 2018), which may deteriorate fruit internal quality faster during storage (Maltini et al. 2003). Fruits with lower DMC and higher water content could also be more prone to water loss during storage, leading to more fruit shrinkage (Link et al. 2004) (Figure S1C).

Conclusion

The impacts of summer fruitlet thinning with interactive effects of rootstocks on pre-, at- and post-harvest fruit quality of Ambrosia™ apples were investigated in a conventional orchard and an organic orchard in Cawston, British Columbia, in 2020. Adequate thinning (AT) enabled a full development of fruit size, high DMC accumulation and advanced fruit maturation during growth and to harvest, achieved the improvement of quality attributes in surface blush coverage and intensity, and SSC at harvest. The apples with higher DMC under AT also possessed better postharvest quality after four months of storage in air at 0.5°C, shown as improved compositional quality attribute and minimal storage disorders. The results also shed light on the importance of rootstocks in determining fruit quality, and showed that compared to M.9, Ambrosia™ apples on B.9 rootstock possessed better quality in DMC, blush color coverage and intensity, and SSC at harvest and after four months of cold air storage. In summary, the study suggested that summer fruitlet thinning played a major role in determining fruit development and the dynamic changes of quality attributes in both M.9 and B.9 rootstocks, and in both conventional and organic orchards; rootstocks and orchard conditions had synergistic impacts on the efficacy of fruitlet thinning.

Acknowledgement

This study was part of the research project ASP-005 BCFGA Activity 8 (J-002066), funded by Agriculture and Agri-Food Canada and the New Tree Fruit Varieties Development Council. The authors were grateful to Dr. Peter Toivonen for sharing insights on fruit dry matter research, and, to Mr. Wilfred Mennell, and to apple breeding and organic fruit specialist in British Columbia, for providing study materials and technical assistance.

References

- Al-Hinai, Y. K. and Roper, T. R. 2004. Rootstock effects on growth and quality of 'Gala' apples. *HortScience* 39: 1231–1233.
- Bound, S.A. 2019. Precision Crop Load Management of Apple (*Malus x domestica* Borkh.) without Chemicals. *Horticulturae*, 5: 3. doi.10.3390/horticulturae5010003
- Cline, J. A. 2009. Commercial Production of Ambrosia™ Apples in Ontario. Agdex 211/32. August 2009. Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada.
- DeEll, J. R. 2020. Pome fruits: Apple quality and storage. In: Gil, M. I. and R. Beaudry (Eds). Controlled and modified atmospheres for fresh and fresh-cut produce. Academic Press, Chapter 14.1. pp: 293-298. doi.org/10.1016/B978-0-12-804599-2.00015-6
- Fernandez, E., Baird, G., Farias, D., Oyanedel, E., Olaeta, J. A., Brown, P., Zwieniecki, M., Tixier, A. and Saa, S. 2018. Fruit load in almond spurs define starch and total soluble carbohydrate concentration and therefore their survival and bloom probabilities in the next season. *Sci. Hortic.* 237: 269–276. doi.org/10.1016/j.scienta.2018.04.030
- Harker, F. R., Marsh, K. B., Young, H., Murray, S. H., Gunson, F. A. and Walker, S. B. 2002. Sensory interpretation of instrumental measurements 2: Sweet and acid taste of apple fruit. *Postharvest Biol. Technol.* 24: 241–250.
- Heim, G., Landsberg, J. J., Watson, R. L. and Brain, P. 1979. Eco-physiology of apple trees: dry matter production and partitioning by young Golden Delicious trees in France and England. *J. Appl. Ecol.* 16: 179–194.
- Kaur, H., Künnemeyer, R. and McGlone, A. 2017. Comparison of hand-held near infrared spectrophotometers for fruit dry matter assessment. *J. Near Infrared Spec.* 25: 267–277.
- Link, S. O., Drake, S. R. and Thiede, M. E. 2004. Prediction of apple firmness from mass loss and shrinkage. *J. Food Qual.* 27: 13–26.
- Maltini, E., Torreggiani, D., Venir, E. and Bertolo, G. 2003. Water activity and the preservation of plant foods. *Food Chem.* 82: 79–86.
- Mpelasoka, B. S. 2001. Fruit water relations, growth, yield, and quality of 'Braeburn' apple in response to deficit irrigation and to crop load. Doctoral dissertation, Massey University.
- Musacchi, S. and Serra, S. 2018. Apple fruit quality: Overview on pre-harvest factors. *Sci. Hort.* 234: 409–430.

Opara, U.L., P.B. Pathare. 2014. Bruise damage measurement and analysis of fresh horticultural produce – a review. *Postharvest Biol Technol*, 91: 9-24.

Palmer, J., Diack, R., Johnston, J. and Bolding, H. 2013. Manipulation of fruit dry matter accumulation and fruit size in 'Scifresh' apple through alteration of the carbon supply, and its relationship with apoplastic sugar composition. *J. Hort. Sci. Biotech.* 88: 483–489.

Palmer, J., Harker, F. R., Tustin, D. S. and Johnston, J. 2010. Fruit dry matter concentration: a new quality metric for apples. *J. Sci. Food Agric.* 90: 2586–2594.

Peiris, K. H. S., Dull, G. G., Leffler, R. G. and Kays, S. J. 1999. Spatial variability of soluble solids or dry-matter content within individual fruits, bulbs, or tubers: implications for the development and use of NIR spectrometric techniques. *HortScience* 34: 114-118.

Saei, A., Tustin, D. S., Zamani, Z., Talaie, A. and Hall, A. J. 2011. Cropping effects on the loss of apple fruit firmness during storage: The relationship between texture retention and fruit dry matter concentration. *Sci. Hort.* 130: 256–265.

Serra, S., Leisso, R., Giordani, L., Kalcsits, L. and Musacchi, S. 2016. Crop load influences fruit quality, nutritional balance, and return bloom in 'Honeycrisp' apple. *HortScience* 51: 236–244.

Schechter, I., Proctor, J. T. A. and Elfving, D. C. 1993. Reappraisal of seasonal apple fruit growth. *Can. J. Plant Sci.* 73: 549–556.

Toivonen, P. M. and Lannard, B. 2021. Dry matter content association with time of on-tree maturation, quality at harvest and changes in quality after controlled atmosphere storage for 'Royal Gala' apples. *Can. J. Plant Sci.* 101: 98-106. doi.org/10.1139/cjps-2020-0124.

Toivonen, P. M. A. 2019. Relation between preharvest conditions, harvest maturity and postharvest performance of apples. *Acta Hortic.* 1256: 469 – 480. DOI 10.17660/ActaHortic.2019.1256.67

Toivonen, P. M., Lu, C. and Stoochnoff, J. 2019a. Postharvest quality implications of pre-harvest treatments applied to enhance Ambrosia™ apple red blush color at harvest. *Can. J. Plant Sci.* 99: 40-49. DOI: doi: 10.1139/CJPS-2018-0193.

Toivonen, P. M., Stoochnoff, J., Usher, K., Lu, C., Wiersma, P. and Zhou, C. 2019b. Biochemical and gene expression involved in red blush color development in 'Ambrosia' apple. *J. Amer. Soc. Hort. Sci.* 144: 164–171. doi.org/10.21273/JASHS04606-18

Toivonen, P. M., Lu, C. and Lannard, B. 2016. Visible spectroscopy I_{AD} measures in apple: impact of stress and shading on maturity indexing. *Acta Hortic.* 1119: 243–249.

Toivonen, P. M. 2015. Comparison of I_{AD} and starch-iodine indices at harvest and how they relate to post-storage firmness retention in Ambrosia™ apples over three growing seasons. *Can. J. Plant Sci.* 95: 1177–1180.

Toivonen, P.M.A., Hampson, C., Stan, S., McKenzie, D.-L., Hocking, R., 2007. Factors affecting severity of bruises and degree of apparent bruise recovery in a yellow-skinned apple. *Postharvest Biol. Technol.* 45, 276–280.

Travers, S., Bertelsen, M. G. and Kucheryavskiy, S. V. 2014. Predicting apple (cv. Elshof) postharvest dry matter and soluble solids content with near infrared spectroscopy. *J.Sci. Food Agric.* 94: 955–962.

Trimble, S. 2019. NIR Vs. Penetrometer to Judge Produce Quality. <https://cid-inc.com/blog/nir-vs-penetrometer-to-judge-produce-quality>. Accessed on March 21 2021.

Watkins, C. B.; Nocka, J. F.; Weis, S. A.; Jayanty, S.; Beaudry, R. M. 2004. Storage temperature, diphenylamine, and pre-storage delay effects on soft scald, soggy breakdown and bitter pit of ‘Honeycrisp’ apples. *Postharvest Biol. Technol.* 32: 213–221.

Wilkie, J. D., Sedgley, M. and Olesen, T. 2008. Regulation of floral initiation in horticultural trees. *J. Exp. Bot.* 59: 3215–3228.

Xu, H.; Ediger, D. 2021. Rootstocks with Different Vigor Influenced Scion–Water Relations and Stress Responses in Ambrosia™ Apple Trees (*Malus Domestica* var. Ambrosia). *Plants*, 10, 614. doi.org/10.3390/plants10040614.

Xu, H.; Ediger, D.; Singh, A.; Pagliocchini, C. 2021. Rootstock–Scion Hydraulic Balance Influenced Scion Vigor and Yield Efficiency of *Malus Domestica* cv. Honeycrisp on Eight Rootstocks. *Horticulturae* 7, 99. doi.org/10.3390/horticulturae7050099

Yang, X., Chen, L. and Cheng, L. 2021. Leaf Photosynthesis and Carbon Metabolism Adapt to Crop Load in ‘Gala’ Apple Trees. *Horticulturae* 7: 47. doi.org/10.3390/horticulturae7030047

Ziosi, V.; Noferini, M.; Fiori, G.; Tadiello, A.; Trainotti, L.; Casadoro, G.; Costa, G. 2008. A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. *Postharvest Biol. Technol.* 49: 319–329.

Table 1. Statistical significance of the impacts of summer fruitlet thinning, orchards, rootstocks, and their interactions on fruit quality attributes measured at harvest.

Source	Blush coverage	Blush intensity*	DMC	I_{AD} **	Firmness	TA	SSC
Thinning (T)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0007	0.6093	< 0.0001
Orchard (O)	0.005	0.0396	< 0.0001	0.0383	< 0.0001	0.0342	< 0.0001
Rootstock (RS)	0.003	0.0022	< 0.0001	0.5894	0.3937	< 0.0001	< 0.0001
T × O	0.556	0.004	0.8728	0.0198	0.0036	0.0449	0.5717
T × RS	< 0.0001	0.0128	0.0037	0.3123	0.0014	0.8206	< 0.0001
RS × O	< 0.0001	< 0.0001	0.48	0.3578	0.9279	< 0.0001	0.2652
T × O × RS	0.556	0.4939	0.1194	0.4243	0.0812	0.331	0.0003

DMC refers to dry matter content. TA refers to titratable acidity. SSC refers to soluble solids content. *P* values are listed to show statistical significance in fruit quality attributes. $P \leq 0.05$ suggests significant different.

*Blush intensity was ranked visually according to the color chart of NVDC (New Tree Fruit Varieties Development Council, British Columbia)(Cline 2009), from 1 for light pink to 6 for fully red or “perfect red”.

**The I_{AD} is a relative measure of chlorophyll content in the apple peel; a decline in I_{AD} indicates the progression in Ambrosia™ apple maturity (Toivonen et al. 2016).

Table 2. Individual effects of each variable on fruit quality attributes evaluated at harvest.

Variables		Blush coverage (%)	Blush intensity*	DMC (%)	I_{AD} ** (DA value)	Firmness (Newton)	TA (mg/L of malic acid)	SSC (%)
Thinning	AT	61.1 A	4.6 A	16.9 A	0.52 B	73.4 B	209.4 A	14.0 A
	LT	22.5 B	2.2 B	14.1 B	0.76 A	74.4 A	208.6 A	12.0 B
Rootstock	M.9	39.7 B	3.3 B	15.1 B	0.64 A	73.8 A	213.8 A	12.7 B
	B.9	43.9 A	3.6 A	15.9 A	0.63 A	74.0 A	204.2 B	13.3 A
Orchard	CV	40.3 B	3.5 A	15.8 A	0.61B	73.2 B	207.1 B	13.4 A
	OG	43.3 A	3.3 A	15.2 B	0.67 A	74.5 A	210.9 A	12.6 B
P value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

DMC refers to dry matter content. TA refers to titratable acidity. SSC refers to soluble solids content.

Mean values of each attribute with different letters in each column are significantly different at $P \leq 0.05$ as determined by Fisher’s protected t test using Proc Mixed Models Procedure in SAS.

*Red blush intensity was ranked visually according to the color chart of NVDC (New Tree Fruit Varieties Development Council, British Columbia)(Cline 2009), from 1 for light pink to 6 for fully red or “perfect red”.

**The I_{AD} is a relative measure of chlorophyll content in the apple peel; a decline in I_{AD} indicates the progression in Ambrosia™ apple maturity (Toivonen et al. 2016).

Legend: AT = adequate thinning; LT = light thinning, CV = conventional orchard; OG = organic orchard.

Table 3. The impacts of treatment combinations on fruit quality attributes evaluated at harvest.

Treatment combinations	Blush coverage (%)	Blush intensity*	DMC (%)	I_{AD} ** (DA value)	Firmness (Newton)	TA (mg/L of malic acid)	SSC (%)
CV × M.9 × AT	51.4 C	4.1 C	15.6 C	0.49 D	71.6 D	202.6 C	13.8 C
CV × M.9 × LT	19.1 F	2.1 E	14.1 E	0.71 BC	74.7 A	207.4 BC	12.3 E
CV × B.9 × AT	67.2 A	5.1 A	17.9 A	0.55 D	73.1 C	208.8 B	14.9 A
CV × B.9 × LT	23.4 E	2.8 D	14.6 D	0.67 C	73.6 B	209.5 B	12.4 E
OG × M.9 × AT	60.2 B	4.76 B	16.2 C	0.53 D	74.1 AB	225.5 A	13.2 D
OG × M.9 × LT	27.9 D	2.2 E	13.5 F	0.84 A	74.8 A	219.7 A	11.4 G
OG × B.9 × AT	65.6 A	4.7 B	17.1 B	0.50 D	74.8 A	202.9 C	14.0 B
OG × B.9 × LT	19.6 EF	1.7 F	14.1 E	0.80 AB	74.5 AB	197.7 D	11.8 F
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

DMC refers to dry matter content. TA refers to titratable acidity. SSC refers to soluble solids content.

Mean values of each attribute with different letters in each column are significantly different at $P \leq 0.05$ as determined by Duncan's Multiple Range Test using Proc Mixed Models Procedure in SAS. Letters in alphabetical order from A to G correspond to values from high to low.

*Red blush intensity was ranked visually according to the color chart of NVDC (New Tree Fruit Varieties Development Council, British Columbia)(Cline 2009), from 1 for light pink to 6 for fully red or "perfect red".

**The I_{AD} is a relative measure of chlorophyll content in the apple peel; a decline in I_{AD} indicates the progression in Ambrosia™ apple maturity (Toivonen et al. 2016).

Legend: AT = adequate thinning; LT = light thinning, CV = conventional orchard; OG = organic orchard.

Table 4. Statistical significance of the impacts of summer fruitlet thinning, orchards, rootstocks, and their interactions on fruit quality attributes after four-month atmospheric storage at 0.5°C.

Source	Firmness	TA	SSC	Decay	Soft scald	Bruise	Mass loss
Thinning(T)	0.4303	0.0224	<0.0001	<0.0001	0.0005	<0.0001	<0.0001
Orchard (O)	0.8830	<0.0001	<0.0001	0.6239	0.0021	0.0001	0.0002
Rootstock (RS)	0.1544	0.8698	0.1266	0.0030	0.3322	0.0628	0.4897
T × O	0.0782	0.2773	0.0279	0.6239	0.0330	0.0001	0.5426
T × RS	0.5080	<0.0001	0.0053	0.0030	0.1150	0.0628	0.2746
RS × O	0.5356	<0.0001	0.3479	0.6239	0.0021	0.3322	0.0527
T × O × RS	0.8288	<0.0001	0.1266	0.6239	0.0001	0.3322	0.5609

TA refers to titratable acidity. SSC refers to soluble solids content. *P* values are listed to show statistical significance in fruit quality attributes. *P* ≤0.05 suggests significant different. where ≤0.01 and ≥0.0001 means highly significant, <0.0001 means extremely significant

Table 5. Individual effects of each variable on fruit quality attributes after four-month atmospheric storage at 0.5°C.

Variable		Firmness (Newton)	TA (mg/L of malic acid)	SSC (%)	Decay (%)	Soft scald (%)	Bruise (%)	Mass loss (%)
Thinning	AT	60.1 A	143.6 A	13.9 A	0.0 B	4.2 B	0.0 B	1.4 B
	LT	59.2 A	136.3 B	12.0 B	5.7 A	10.9 A	10.4 A	2.6 A
Rootstock	M.9	58.7 A	140.2 A	12.9 A	1.0 B	6.8 A	4.2 A	2.1 A
	B.9	60.1 A	139.7 A	13.1 A	4.7 A	8.3 A	6.3 A	2.0 A
Orchard	CV	59.4 A	129.6 B	13.3 A	2.6 A	10.4 A	2.6 B	2.2 A
	OG	59.6 A	150.3 A	12.6 B	3.1 A	4.7 B	7.8 A	1.9 B
<i>P</i> value		0.4358	< 0.0001	< 0.0001	0.0003	< 0.0001	< 0.0001	< 0.0001

TA refers to titratable acidity. SSC refers to soluble solids content. Mean values of each attribute with different letters in each column are significantly different at $P \leq 0.05$ as determined by Fisher's protected *t* test using Proc Mixed Models Procedure in SAS.

Legend: AT = adequate thinning; LT = light thinning, CV = conventional orchard; OG = organic orchard.

Table 6. The impacts of treatment combinations on fruit quality attributes after four-month atmospheric storage at 0.5°C.

Treatment combinations	Firmness (Newton)	TA (mg/L of malic acid)	SSC (%)	Decay (%)	Soft scald (%)	Bruise (%)	Mass loss (%)
CV × M9 × AT	57.8 A	106.0 D	14.3 A	0.0 B	2.1 CD	0.0 C	1.7 D
CV × M9 × LT	59.2 A	136.7 C	12.1 D	2.1 B	22.9 A	2.1 C	2.9 A
CV × B9 × AT	60.1 A	163.6 A	14.3 A	0.0 B	8.3 BC	0.0 C	1.5 DE
CV × B9 × LT	60.5 A	112.1 D	12.9 C	8.3 A	8.3 BC	8.3 B	2.7 AB
OG × M9 × AT	60.1 A	156.8 AB	13.7 B	0.0 B	0.0 D	0.0 C	1.3 E
OG × M9 × LT	58.3 A	161.3 A	11.4 E	2.1 B	2.1 CD	14.6 A	2.4 C
OG × B9 × AT	61.4 A	147.8 BC	13.6 B	0.0 B	4.2 CD	0.0 C	1.3 E
OG × B9 × LT	58.3 A	135.3 C	11.7 E	10.4 A	12.5 B	16.7 A	2.6 BC
<i>P</i> Value	0.4358	< 0.0001	< 0.0001	0.0003	< 0.0001	< 0.0001	< 0.0001

TA refers to titratable acidity. SSC refers to soluble solids content. Mean values of each attribute with different letters in each column are significantly different at $P \leq 0.05$ as determined by Duncan's Multiple Range Test using Proc Mixed Models Procedure in SAS. Letters in alphabetical order from A to E correspond to values from high to low.

Legend: AT = adequate thinning; LT = light thinning, CV = conventional orchard; OG = organic orchard.

Figure captions

Figure 1. Effects of fruitlet thinning on bi-weekly growth of Ambrosia™ apples measured in fruit diameter (mm) in a conventional orchard CV **(A)** and an organic orchard OG **(B)**, respectively. Points are LS means $\pm$ standard error.

Legend: AT = adequate thinning; LT = light thinning, CV = conventional orchard; OG = organic orchard.

Figure 2. Effects of fruitlet thinning on the dynamics of dry matter content (DMC) of Ambrosia™ apples measured bi-weekly in a conventional orchard CV **(A)** and an organic orchard OG **(B)**, respectively. Points are LS means $\pm$ standard error.

Legend: AT = adequate thinning; LT = light thinning, CV = conventional orchard; OG = organic orchard.

Figure 3. Effects of fruitlet thinning on the loss of chlorophyll in fruit skin measured bi-weekly as I_{AD} Index of Ambrosia™ apples in a conventional orchard CV **(A)** and an organic orchard OG **(B)**, respectively. Points are LS means $\pm$ standard error.

Legend: AT = adequate thinning; LT = light thinning, CV = conventional orchard; OG = organic orchard.

Figure 4. At-harvest red-blush coverage and intensity of Ambrosia™ apples on B.9 rootstock under thinning treatments. Photos were taken on-site in the organic orchard OG plots. The image showed the representative fruits from three plots under higher crop load rendered by light thinning treatment (LT) in top row, and, those from the three plots under lower crop load rendered by adequate thinning treatment (AT) in bottom row, respectively.

Figure 1.

Figure 2.

Figure 3.

Figure 4.