ABSCISSION, STORABILITY, AND FRUIT QUALITY OF MECHANICALLY HARVESTED FRESH MARKET STEM-FREE SWEET CHERRY

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Abstract

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A prototype mechanical sweet cherry harvester has been developed by the USDA in response to industry's need to improve labor efficiency. Commercial acceptance of the harvest system will require knowledge of cultural practices, stem-free fruit, and fresh market fruit quality. This research program sought to 1) identify effects of ethephon (2-chloroethyl phosphonic acid) on sweet cherry pedicel-fruit retention force and fruit quality, 2) study the effect of ethephon and mechanical harvesting on fruit storability, 3) study the role of the fruit pedicel on storability and weight loss, and 4) document metabolic changes in fruit tissue induced by ethephon. Ethephon applied at rates as low as 1.2 L ha^{-1} at 10 days prior to harvest was effectively reduced the pedicel-fruit retention force (PFRF) to ca. 400 g in 'Bing', facilitating mechanical harvest. Further, the cultivar 'Skeena' exhibits a naturally decline in PFRF to levels suitable for mechanical harvest. Conversely, 'Chelan' is non-responsive to ethephon and not suitable for mechanical harvest.

The effect of the harvester on 'Bing' and 'Skeena' fruit quality is minimal in comparison to hand harvested fruit. Commercially assessed fruit cullage was ca. 23% for

both harvest methods and cultivars. Generally, fruit storability was positively related to fruit size. In 2007, untreated stem-free ‘Bing’ lost 29% more weight than fruit with stems. However, the weight loss averaged ca. 6.5% for both stem-free and stemmed fruit in 2008. There was no difference in postharvest weight loss between ‘Skeena’ fruit with and without pedicels in both years. ‘Bing’ pedicels appear to be non-conductive to water transport. In contrast, ‘Skeena’ exhibited dye transport through the pedicel from the stylar incision in 2007 but not in 2008. Pedicels are susceptible to higher rates of browning, particularly in response to refrigeration in the commercial cold chain. Finally, metabolic profiling of ethephon-treated fruit reveal metabolic shift in simple sugars, malic acid, and color (cyanidin 3-glucoside/korumanin). Overall ethephon had little effect on quality. In general, effects of mechanical harvest are minimal upon fruit quality and storability showing promise for stem-free fresh market sweet cherries.

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INTRODUCTION

Origins and Production Trends

Sweet cherry (*Prunus avium* L.) is a temperate forest tree species that appears to have originated in the Causasus, between the Black and Caspian seas. Sweet cherry migration from the Causasus region was slow and initially performed by birds, hence the second half of the binomial *avium,* which is Latin for bird. Subsequent migration of sweet cherry is anthropocentric due to desirable fruit. Sweet cherry trees are large, deciduous, and bear fruit between 60 to 80 days after full bloom. Undisturbed seedling trees can be acutely vigorous and develop an erectly branched growth habit. Trees are intensely acrotonic consequently producing few lateral branches; through selective breeding this trait can be variable amongst variety and rootstock combinations. Sweet cherry cultivation is considered to be over 2000 years, with the majority of cropping from home orchards for personal consumption due to the high perishablity of the fruit (Webster and Looney, 1996, Westwood, 1993). High perisablility and desirable flavor combined with advancements in the industrial age have brought sweet cherry to global markets yielding tonnage values up to $2440.00, circa 2005 (NASS/USDA).

Sweet cherry production in the Pacific Northwest and especially Washington State is vital to the economic well being of the region. Washington State, as of 2006, is the largest producer of sweet cherry nationally with a total production of 171,000 tons harvested. Washington's contribution to the total national production in 2006 was 58%. Oregon was the closest competitor producing 50,000 tons or 17% of total national production. Further illustration of Washington's dominance in national sweet cherry

production is reflected in fresh market utilization, where, 136,000 tons were placed into the supply chain. The second largest harvest was in California where producers placed only 32,400 tons into the fresh market supply chain in 2006. The fresh market value, to Washington State, in 2006 was $1,700.00 per ton which contributed $231M to the state economy. (NASS/USDA)

In world competition, the USA, in 2004, was the leading producer of sweet cherries at 14%. Turkey and Iran are close competitors at 13% and 12%, respectively. Germany (7%), Russia (5%) and Italy (5%) are the last three major contenders in northern hemisphere production (FAO). The close production levels of these counties places fierce competition in world markets. For continued success, Washington State's cherry producers must identify restrictions in the supply chain.

A major concern to Pacific Northwest cherry producers is labor. Problematic areas that create general concern, in labor, are availability, cost, and safety. Availability of skilled labor to pick, prune, sort, pack and perform general production tasks has been a developing crisis (Warner, 1997; Hansen, 1999; Morgan, 2002). As accessibility is gained to global markets, sweet cherry acreage is increasing, which is creating a notable demand on a limited labor resource. Orchard managers are forced to compete for labor through increased piece rates, which causes fruit to be harvested outside of optimal maturity and/or the utilization of unskilled workers. These factors contribute to increased costs and injuries in the workplace.

Labor associated increases in production costs, through the utilization of unskilled workers, are witnessed through increased injury to fruit and personnel. Damage to fruit and fruiting spurs reduce income through higher cullage rates and reduced cropping in

the preceding year, respectively. Cullage is increased by rough handling and collection of undesirable fruit, whereas, personnel injury can be attributed to inexperience in the use of ladders. Ladder injuries, within the orchards of Yakima Valley (Region 5), between 1996 and 2001 were an estimated 31% of total tree fruit production Labor and Industry compensation claims (Hofmann et al. 2006). Injuries related to ladders were sprains (38%), contusions (26%), fractures (12%), cuts (6%) and others (18%) totaling 4020 claims at a payout of more then $21.5M (Hofmann et al. 2006).

Mechanization

Throughout history people have endeavored to make a difficult and arduous task simple with labor saving devices. In sweet cherry production, adoption of chemical inputs, genetic improvements in crop varieties and operational tactics have increased quality and yield. Presently, sweet cherries are predominately picked by hand for the fresh market (Peterson, 2005). In the late 1950's, processed and canned tree fruit products were beginning to be harvested using a shake & catch system. Shake & catch harvesters use inertia with varying amplitude, which is based on trunk and/or limb diameter combined with fruit retention force, to remove fruit. In California, prunes were harvested mechanically to assess harvest rate (Adrian and Fridley, 1958) for processed product. The harvest costs were appreciably lower than utilizing hand-harvest techniques. At 20 acres, machine harvest equaled $0.72 per box whereas hand-harvest cost equaled $1.79 per box (monetary amounts adjusted to 2007 Consumer Price Index, U.S. dollar). The inefficiencies, discussed, by Adrian and Fridley (1958), were movement from tree to tree, inadequate seal about the tree trunk, and box removal. Fruit quality was not accounted for.

Through further investigation, Adrian and Fridley (1965) worked to establish a formula for inertia-type tree shakers to minimize bark damage. This work demonstrated the shaker mass ratio and eccentricity required to develop a certain stroke, force, and torque development relating to power requirements for efficient vibration of the limb. This work resulted in amplitude modeling, allowing engineers to design shakers for varying growth habits of other commercial crops i.e. apple, apricot, cherry, olive, peach, and nuts.

In cherry, mechanized harvest was first researched at Michigan State University in 1958 (Gaston et al., 1967). Both tart and sweet cherries were under mechanized harvest for process use. By 1963, in Michigan's tart cherry production, over 60 harvesters were in commercial use, yielding approximately 1.1M Kg. Today, the vast majority of tart cherry harvest is performed by shake & catch harvesters. Sweet cherry production has not witnessed the same success in mechanized harvest adaptation. Michigan predominantly grows sweet cherries for brining. The fruit falling unto the harvester bruises easily passing through limbs and landing upon other fruits that collect at low points in the catch basin. Bruising reduces quality and pack-out yield through the development of scald, loss of red color, poor character and texture, and faulty pit removal (Whittenberger, 1967).

Maraschino or brined sweet cherry packers prefer a slightly immature fruit with a light red color and the stem attached. Mechanical harvest systems are woefully inadequate to handle this product. Immature fruit are firmly attached to the tree causing excessive amplitude to be applied to the tree. In turn, the excessive force increases fruit and tree damage. However, immature harvest yields greater stem retention than

harvesting fruit at standard color maturity (Thienes et al. 1969). The problems associated with mechanical harvest were further highlighted through Oregon State University Cooperative Extension Service Fact Sheet 166 (1970), where harvest management tactics to obtain greater pack-out yield are:

1.) Harvest early in the season for greater stem retention, reduced culls, and harvest at soluble solids of 13% -15%.

2.) Harvest early in the day to retain stems and maintain firmness.

3.) Attach shakers correctly, to avoid bark damage

4.) Do not over shake the tree, which results in fruit and tree damage.

5.) Keep harvester and bins free of dirt, cherry juice and dirt make mud that accumulates in the cherry fruit bowl and will be culled.

6.) Brine immediately, mechanically harvested fruit will have a greater percentage of bruised fruit. Quick immersion in brining solutions can minimize discoloration and other defects caused from bruising.

Minimizing fruit damage, efficient movement from tree to tree and successful tree to bin transfer are objectives for establishment of a mechanized fresh market harvest system. Research identifying substrates that absorb the kinetic energy of falling fruit saw the development of multilevel catch-frames with inflatable receiving surfaces (Millier et al., 1973). The key principle of the multilevel apple-harvester prototype is to reduce the distance the fruit drops and utilize an air cushion to transfer the kinetic energy into. Major difficulties to adoption were down-gradation of fruit due to fruit removal force applied and fruit descent through the canopy structure. Eventually, a model to determine proper cushioning material and thickness to absorb kinetic energy was tested on freestone

peaches (Hemmat et al. 1978). When applied, the modeling allows for spherical objects, of a given mass, to be analyzed over varying substrates that absorb the energy developed from the object's fall.

Due to the size of catch frames, the difficulty involved in sealing about the base of the tree and attaching the shaker efficiently, increasing harvest speed became the focus of further research. Roll-out catching frames, utilizing kinetic absorbing substrates, were introduced (Tennes and Levin, 1976, Zocca, 1984, Zocca et al., 1985) showing there is potential to reduce the number of workers, increase tree to tree set-up time and improved catch surface (Zocca, 1984). The roll-out frames were improvements to previous systems, yet they were not self propelled and continued to be for processed fruit harvest. In 1976, a shake & catch harvester prototype that consisted of two independent self-propelled halves was reported to have a 50% increase in harvest speed compared to conventional shake & catch units (Peterson et al. 1977). The prototype is capable of sealing the tree trunks utilizing foam blocks with a rigid under-frame. When the blocks are perpendicular to the tree row, they push out to fill the space between harvest units. This design was built for high density orchards.

By the mid 1980's, the trend in tree fruit production was increased tree density from traditional plantings of 75 to 300 trees/ha to 400 to 700 trees/ha (Proebsting, 1982, Peterson, 1984). Development of size-controlling rootstocks, higher hectare yields, improved cultural practices and reduced time to economical production created the potential for an over-the-row harvester in cherry production. The over-the-row prototype developed by Peterson (1984) could continuously harvest fruit through the shake & catch

mechanism at a pace of 300 trees per hour. Again, the major drawback is fruit damage, which relegated the over-the-row harvester to processed fruit end use.

Eventually, the over-the-row harvester was modified to harvest apples on dwarfing rootstocks (Peterson and Miller, 1989) for fresh market. Again, the critical issue is fruit damage. Peterson (1992) addressed the problem of fruit damage by attempting to decelerate the free-falling fruit through counter-rotating foam cylinders. The action of this system gently delivers the fruit to the conveyers, which deliver the fruit to a bin. Peterson identified the arched belt component as the contributing factor to bruising, at a drop of 900 mm at 43 G of acceleration. Other components of the catch platform contributed significantly less damage, demonstrating the possible application of fresh market harvest in apple.

Much of the problem regarding fruit damage is the shaker, which causes violent oscillation of the fruit before detachment. Upadhyaya et al. (1981a, b, and c) modeled and tested a new approach for fruit removal, where the energy is placed in lower laterals of fruit carrying branches. The impact upon the lateral is equivalent to a swing with a padded baseball bat. The impact causes a whip-like snap that detaches fruit without oscillation and releases the fruit within 0.1 s of application (Upadhyaya et al. 1981b). Ultimately, the conclusions from the limb impact model noted that stem pull is dependent upon the direction of impact, angle of fruit attachment (i.e. spur to limb and stem to spur), and elasticity of the stem. Due to the nature of impact harvesting, branches trained or trellised in a Y-shaped open tree at angles between 45º to 60º from horizontal are most effective for fruit removal allowing for vertical descent, unimpeded, to the catch frame.

Peterson (1992) highlighted the potential labor problems to deciduous tree fruit and bramble growers and identified the lack of engineering research in fresh market mechanized fruit harvest. By 2001, Peterson (2001) introduced a novel mechanized harvest system for fresh market sweet cherry. The prototype harvester is a culmination of engineering advancements utilizing kinetic energy absorption (Hemmat et al. 1980), self-propulsion (Peterson, 1976), limb impact harvesting (Upadhyaya et al. 1981a, b and c) and fruit catch-frame deceleration (Peterson, 1992). The harvest system consists of two units on opposite sides of the tree row.

The machines (Peterson et al, 2003) are configured as mirrored units that are self-propelled, utilizing all-wheel hydraulic drive. The three-wheeled machines are front wheeled steered with the operator stationed at the aft position facing forward. The fruit removal system consists of an articulated hydraulic arm that allows the operator to position a small hydraulic cylinder, with a rubber puck mounted to the cylinder head, called the rapid-displacement-actuator (RDA). The RDA is placed on the lower portion of a fruiting lateral. When engaged, the RDA transfers 8300 kPa (~1200 psi) from a 3.8 L accumulator into the lateral to generate the required acceleration and displacement for effect fruit removal.

The catch surface is a conveyor placed at an incline of 24º with open-cell foam polyester guides angled to effectively place fruit on to the conveyer. The conveyer design incorporates 2032 mm long pipes that are supported by B-1-1 attachments of 2050 roller chain spaced every 127 mm. The pipes are covered with 0.56 mm thick polyurethane pipe insulation, which is coated with nylon fabric formed into pockets to collect and transport fruit to the collecting conveyer. The collecting conveyer is a plastic perforated belt that

moves the fruit aft to a decline conveyer. Atop the decline conveyer is mounted a cleaning fan that pulls debris (i.e. leaves and small twigs), through suction, overboard. Fruit, on the decline conveyer, are deposited into a rotating bin for even distribution. The angle of the decline conveyer can be adjusted to minimize fruit drop into the bin.

The mirrored units move through the orchard as one machine, harvesting opposite sides of the same tree. The units can effectively seal the tree trunk though utilization of spring loaded catch-pans similar to catch-pans used in mechanized berry harvest. Trials, in south-central Washington State, using the prototype harvester system (Peterson and Wolford, 2001, Peterson et al. 2003) have demonstrated potential for fresh market sweet cherry production. In 2000, trials showed the prototype harvester has a significant advantage over traditional inertia shaker, where packout marketable quality fruit, of 'Van', was 86.3% compared to inertia shaker at 79.0%. The key influence noted was bruising; 5.3% RDA compared to 10.7% inertia shaker. Also, in 'Lapin', RDA single impacts were equal in packout marketable quality when compared to hand harvest however the fruit is stem-free. When multiple impacts of the RDA were applied the quality of the mechanized system significantly decreased (92.3% hand harvest compared to 85.0% RDA multiple impacts) (Peterson and Wolford, 2001).

In 2002, 'Bing' and 'Van' were harvested using the prototype in south-central Washington State. These trials, again, demonstrated no significance between hand and machine harvest. Yet, consideration of appropriate training system, fruit detachment and fruit quality were issues that hinder full-scale adoption of mechanized harvest.

Abscission

Effective mechanization of sweet cherry harvest is dependent upon applying a minimal amount of energy into the tree to release fruit. Effective release of fruit is determined by the strength of attachment of the pedicel to either the fruit or the spur. Upadhyaya et al. (1981c) specifically stressed that, for successful fruit removal, the energy sent into the limb correlates positively to the elasticity and diameter of the stem. In apple, the limb impact modulus did not provoke pedicel release from an abscission zone. To reduce the force applied, understanding how and when the abscission layer is responsive, either naturally or through manipulation, is paramount to mechanized harvest success.

Abscission occurs after the formation of an abscission zone at the point of separation. The abscission zone is a thin layer of cells, which become weakened and break down through the conversion of pectin to pectic acid. Consequently the leaf, fruit or other organ can be easily dislodged by wind or rain. The process is thought to be controlled by the amount of auxin present; a phytohormone that is responsible for apical dominance in growth (Taiz and Zeiger, 2002; Westwood, 1993). At elevated levels, auxin suppresses the biosynthesis of ethylene, a phytohormone that is responsible for ripening and senescence of plant tissue. Yet at high levels of auxin, ethylene is produced, which is evident through the usage of synthetic auxins i.e. 2,4-D or dicamba. As levels of auxin decrease, the potential for up-regulation of ethylene is increased. Ethylene then binds to receptor sites on abscission cells, which in turn initiates a signal transduction creating cell wall degrading enzymes eventually causing cell death (Buchanan et al., 2000).

Auxin, inhibits abscission, as first noted by Charles and Francis Darwin. The Darwin's identified the tip of the coleoptiles, in seedling canary grass (*Phalaris*

canariensis), as the area of origin for differential growth that causes plants to bend towards light. This phenomenon is called phototropism (Taiz and Zeiger, 2002; Buchanan et al., 2000). Frits Went, by 1926, showed there is a specific chemistry involved with growth promotion. Went removed the tips of oat (*Avena sativa*) coleoptiles, placed them on gelatin, after time removed the tips from the gelatin, cut the gelatin into blocks, and placed the block on half of the coleoptile stump. The excised coleoptiles with the blocks attached were placed in total darkness; the resulting reaction of the plant showed a curvature with resulting elongation of the cells associated to the gelatin applied half. Eventually by the 1930's, the chemical structure of auxin was determined to be indole-3-acetic acid (IAA) (Taiz and Zeiger, 2002; Buchanan et al., 2000).

Biosynthesis of IAA is tryptophan (Trp) dependent. The main path consists of the reversible reaction of tryptophan to indole-3-pyruvic acid (IPA) by Trp transaminase, followed by a committed step to indole-3-acetaldehyde (IAld) by IPA decarboxylase. IAld is reacted to IAA by IAld dehydrogenase. Other less conspicuous paths to IAA include the indole-3-acetonitrile (IAN) path where IAN's nitrile is replace with a carboxylic acid by nitrilase; and the tryptamine (TAM) path where TAM is oxidized by amine oxidase to form IAld (Taiz and Zeiger, 2002; Buchanan et al., 2000).

Transport of auxin exhibits apex to base structural polarity (Taiz and Zeiger, 2002) defined as movement through plant tissue by apoplast pathway from apical to basal end or basipetally known as polar auxin transport. Because auxin moves through the apoplast pathway, a mechanism for uptake or influx is explained through a chemiosmosis. Where, the solute is than carried through the plasma membrane by proton

motive force. Two specific means of uptake are: 1) passive diffusion across the phospholipid bilayer is achieved by the protonated form (IAAH) and 2) active transport uptake of deprotonated IAA and two hydrogen ions via a hydrogen-auxin symporter. Passive transport is dependent upon apoplastic pH; at acidic conditions IAAH is permeable. If apoplastic pH is neutral (7.0) and above then IAA will be in ionic form, ionic IAA is than dependent upon active transport to pass through the plasma membrane.

Ethylene (C_2H_4), the regulating phyotohormone, is an olefiant (double bonded carbon) gas with low molecular weight (28.05) (Budavari, 1989). Ethylene's effects were first noted in 1886 by Dimitry Nikolayevich Neljubow, a graduate student in St. Petersburg, Russia. He observed that etiolated pea seedlings grew horizontally in the laboratory, however when grown outside the seedlings exhibited vertical growth. Dimitry worked to eliminate any cultural practice, light, and temperature as causal agents and eventually discovered that the gas from oil burning lamps created the hooking response in the greenhouse (Buchanan et al., 2000). Although ethylene was known to elicit such responses as gravitropism and abscission it was not until the 1960's, coupled with the use of GC techniques, did ethylene begin to be accepted as a plant hormone.

Adams and Yang (1979) utilized L-[U-^{14}C]Methionine feeding into apple (*Malus sylvestris* Mill, var. Golden Delicious) and discovered when the substrate was left in air, ^{14}C labeled ethylene was produced. However, when the substrate was held in a strict N_2 atmosphere ^{14}C 1-aminocyclopropane-1-carboxylic acid (ACC) was formed. The elucidation of the formation and oxidation of ACC to ethylene was the final step in determining the biochemical pathway to ethylene.

The Methionine (Yang) cycle where S-adenosyl-L-methionine (SAM) is converted to ACC via ACC synthase (ACS). ACC is either oxidized to ethylene by ACC oxidase (ACO) or sequestered to N-malonyl-ACC. ACC is formed from the cleavage of SAM at the methionine carbon-sulfur bond. Without the recycling, the amount of reduced sulfur would be rate limiting to the availability of methionine (MET). Further cycling results in a reaction yielding 5'-methylthioadenosine, which proceeds to form 5-methylthioribose through enzymatic removal of adenine. The next step consumes energy in the form of phosphate from ATP to ADP, which yields 5-methylthioribose 1-phosphate. This compound is oxidized to release hydrogen phosphate and formate to form α-keto-γ-methylthiobutyric acid. Next, ammonia from an amino acid is covalently bonds, replacing the double bonded oxygen of the carboxyl, to form MET. With the reduction of an ATP, SAM synthase bonds MET to S-andenosyl to form SAM, which completes the cycle (Taiz and Zeiger, 2002; Buchanan et al., 2000).

Thorough understanding of abscission is not complete (McManus, 2008), yet advancements in technology and analytical tools in the field of molecular biology and biochemistry are increasing our knowledge of cellular pathways and their relationships. Work at Michigan State University in the late 1960's and into the 70's has attempted to identify the mechanism for fruit abscission in cherry (Stösser et al., 1969, Bukovac, 1971, Bukovac, et al., 1971, Wittenbach and Bukovac, 1972a, Wittenbach and Bukovac, 1972b, Poovaiah, et al. 1973, Wittenbach and Bukovac, 1973, Wittenbach and Bukovac, 1974, Wittenbach and Bukovac, 1975a, Wittenbach and Bukovac, 1975b). Early histological studies identified a distinct abscission layer in sour cherry (*Prunus cerasus* L.) at the pedicel-fruit connection, whereas the same continuity was not observed in sweet cherry

(Stösser et al., 1969). At maturity, both sweet and sour cherry lacked development of the abscission layer in the pedicel-spur abscission zone. Further histological investigations showed that development of the pedicel-spur abscission zone is dependent upon timing (Bukovac, 1971).

Sweet cherry develops in a double sigmoidal pattern, where there are three distinct stages: 1) cell division, 2) pit hardening and 3) fruit bulking. During cell division both abscission zones are not developed and when a separation force is applied the pedicel usually breaks before separation occurs from either the fruit-pedicel or spur-pedicel abscission layers (Bukovac, 1971). During pit hardening and into early fruit bulking the pedicel-spur abscission layer will develop in fruit that are over-cropped. This response is due to low leaf to fruit ratio (Blanusa, 2006) or embryo underdevelopment and/or damage (Wittenbach and Bukovac, 1975), which causes a phenomenon called "June-drop". During the latter stages of fruit bulking and through maturity, fruit tend to release at the pedicel-fruit abscission zone (Bukovac, 1971).

In sour cherry, the cells in the pedicel-fruit abscission zone first show development approximately 10 days before harvest. Whereas, sweet cherry is cultivar specific in abscission development and the degree of development (Stösser et al., 1969). The variability in abscission zone detachment force in mature sweet cherry effectively minimizes mechanical means as a harvest method. Previous work has shown that mechanical removal of fruit can be accomplished if the retention force between fruit and pedicel is near or below 400 g (Cain, 1967, Peterson et al., 2003). Ethephon (2-chloroethyl phosphonic acid), an ethylene releasing compound, was shown to advance maturity and increase abscission in sweet cherry cultivars 'Emperor Francis',

Heldelfingen', 'Napoleon', 'Schmidt', 'Vega', 'Venus', 'Vic', 'Vista', and 'Windsor' (Bokovac et al., 1971). The effect of ethephon is concentration and time dependent and appears to have the greatest efficacy early in the fruit bulking stage. When ethephon is applied at high concentration (i.e. 1000 to 4000 ppm) adverse effects such as leaf abscission, pedicel-spur abscission, terminal dieback, and gummosis occur, rendering concentration a critical and determinate factor (Bukovac et al., 1969). However, temperature and timing of low ethephon concentrations are critical to gain the appropriate efficacy (Bukovac et al. 1971, and Olien and Bukovac 1978).

During abscission zone formation, there is specific enzymatic activity that reduces auxin, increase cell wall and membrane degradation. Peroxidase activity is associated with auxin degradation in sweet and sour cherry (Poovaiah et al. 1973) and is increased with ethephon application (Wittenbach and Bukovac, 1975). However, peroxidase is not the only factor controlling abscission. In transgenic plants where peroxidase levels were increased or decreased by tenfold, auxin levels remained unchanged (Normanly et al. 1995).

Cell wall hydrolases, e.g. endo-β-1,4glucanase (EG), polygalactronase (PG), pectin-methyl esterases (PME) and expansins, have been implicated in middle lamella and primary cell wall softening and degradation (Cho H-T and D. Cosgrove, 2000, Tasuki, et al., 2006, Costa, et al., 2006, and Sharova, 2007). For abscission layer cells, signaling for upregulation of hydrolytic enzymes is ethylene induced (González-Carranza, et al., 2002, Rasori, et al., 2002) and controlled by the receptablility of a family of membrane bound receptor proteins. Ethylene receptor proteins are categorized into two subfamilies. Subfamily 1 contains ethylene-resistant 1 (ETR1) and ethylene-related

sequence 1 (ERS-1) proteins that consist of a ethylene binding site coupled to a GAF (acronym derived from the 3 class of proteins involved in its structure: G = cyclic guanosine 5'-monophosphate, A = adenylyl cyclase, and F = *E. coli* transcription factor *FhlA*), and a histidine kinase, whereas the ETR-1 also has a receiver domain with a carboxylic acid tail coupled to the histidine kinase. Subfamily 2 consists of three proteins, ethylene insensitive 4 (EIN4), ETR2, and ERS2. All three proteins share a similar structure of an ethylene receptor site coupled to a GAF with a degraded histidine kinase domain, EIN4 and ETR2 also contain a receiver domain (Taiz and Zeiger, 2002; Buchanan et al., 2000).

Ethylene receptor proteins are membrane bound to the endoplasmic reticulum and are in the active state, meaning, that in the absence of the ligand (ethylene), the signaling pathway is 'on' and when ethylene binds to the receptors the signal is 'off' causing pathway response to proceed. Further, all receptors must be active and able to receive the ligand to cause signal transduction particular to ethylene sensitivity.

Olefins, such as ethylene, have a high affinity for transition metals i.e. copper, zinc, or nickel, and in the binding protein of ETR1, identified as RAN1, Hirayama et al. (1999) showed copper is required to bind ethylene. As RAN1 assembles the copper co-factor and ethylene binds, the histidine kinase is autophosphorylated which takes a phosphate from ATP to form ADP. The energetic reaction transfers the phosphate from the histidine kinase domain to the receiver domain causing activation of CRT1 (constitutive triple response 1), this protein appears to be related to RAF-1, a MAPKKK serine/threonine protein kinase (mitogen-activated protein kinase kinase kinase). Here again the CRT1 is negatively regulated by ethylene: when the ethylene is absent the

MAPKKK is active, so when CRT1 is activated by ethylene the MAPKKK is deactivated. The loss of MAPKKK signaling activates another membrane bound protein called EIN2. EIN2 resembles a protein from a family of cation transporters called N-RAMP (natural resistance-associated macrophage protein), suggesting EIN2 acts like a channel or a pore sending signal molecules or elements from the inner ER into the cytoplasm (Taiz and Zeiger, 2002; Buchanan et al., 2000).

The activation of EIN2 turns on the family of transcriptional factors, named EIN3, within the nucleus. EIN3 then induces expression of ERF1 (ethylene response elements 1) family of transcription factors. The activation of the ethylene transcriptional cascade leads to large-scale changes in gene expression that alter cell function. Expressly within the abscission layer, the result of gene expression can be witnessed by cell wall breakdown, protoplast swelling, and increased aerial space (Taiz and Zeiger, 2002; Buchanan et al., 2000).

Ethylene is a developmental signal of the abscission zone; utilization of chemicals that "turn-on" the methionine cycle is crucial in horticultural and agronomic production. Various chemicals have been applied to promote a reduction in fruit removal force (FRF). Ethephon is registered specifically for fruit loosening in cherry, wine grape (*Vitis vinifera*) and macadamia nut (*Macadamia sp.*). Whereas in citrus, the efficacy of ethephon will reduce the FRF to facilitate mechanized harvest, yet causes significant defoliation to warrant research into wound induced compounds such as methyl jasmonate (MeJA) (Hartmond et al., 2000), traumatin (12-oxo-trans-10dodecenoic acid) (Zimmerman and Coudron, 1979) and a phytotoxin from *Pseudomonas syringae* called coronatine which mimics MeJA (Burns et al., 2003). Other compounds identified as

potential FRF agents, in citrus, were 5-chloro-3-methyl-4nitro-1*H*pyrazole (CMNP) which induces expression of genes with high similarity to allene oxidase synthase and 12-oxophytodienoate reductase which are lipid degradation enzymes (Alferez et al., 2005), dikegulac, which is the common name of a monosaccharide-related compound (2,3:4,6-di-O-isopropylidene-α-L-xylo-2-hexulofuranosoic), whose sodium salt has been shown to be a potent disruptor of apical dominance (Pozo et al., 2004) and 6-benzyladenine (BA), a cytokinin-like compound, has been utilized in an attempt to disrupt the auxin/ethylene ratio to entice abscission in olive (Burns et al., 2008). Interestingly, ethephon was the only compound to show significant efficacy in fruit abscission in black olive (*Olea europaea* L.) when compared to the other abscission agents utilized from the Burns et al. (2008) study. Noting the variety of chemistries, excitation of abscission is derived from varied responses to stimuli and leads to potentially numerous pathways for upregulation of the methionine cycle and degradation of abscission cells.

Fruit Quality

Sweet cherry fruit is classified as a drupe, where the exocarp and the mesocarp are consumed and the stony endocarp is discarded. In the last phase of fruit development, fruit bulking, metabolic changes are occurring that characterize maturity. Acids i.e., malic and citric, remain constant (Spayd et al., 1986), whereas monosaccharides, anthocyanins and xanthopylls are increasing and chlorophyll is decreasing (Webster and Looney, 1996). In the fruit, chemical changes are a combination of metabolite translocation via photosynthesis of the leaves and enzymatic assembly of aroma and color chemicals (Kays and Paull, 2004). Translocated photosynthates are the sole source of fruit sugars. Once a fruit is removed from the parent plant there will be no further accumulation. The lack of

postharvest respiration, where oligosaccharides would be reduced to monosaccharides, classifies sweet cherry as non-climacteric (Kays and Paull, 2004). Climacteric fruit are denoted by an increase in respiratory pattern during the final stages of ontogeny of the organ (Kays and Paull, 2004). The climacteric rise in respiration was described as early as 1908 (Müller-Thurgan and Schineider-Orelli, 1908) in apple and pear fruit. Later, Kidd and West (1925) detailed the relationship between changes in respiratory rate and changes in quality attributes occurring during the climacteric period. For non-climacteric fruit, respiration spikes after 2-3 days postharvest and declines steadily and predictably over time to final senescence (Kays and Paull, 2004). It should be noted that non-climacteric produce will be similar to climacteric CO_2 evolution as fungal infection accelerates, the discriminating difference between infection and natural climacteric respiration is an ever increasing CO_2 concentration and signs of fungal mycelia (Kays and Paull, 2004).

Horticultural maturity indices, for fresh market sweet cherry, consist of primarily two criteria in the orchard: color and sweetness. Color is cultivar dependent, varieties such as ‘Royal Anne’, ‘Rainier’ and ‘Early Robin’ are yellow to blush in coloration and maturity is characterized by the reduction of chlorophyll and increase in xanthophylls pigments with a slight red blush on the cheeks of the fruit. Further, red cultivars mature at varying stages of color, examples such as ‘Sweetheart’ are considered ripe when exocarp color reaches a light mahogany and in contrast ‘Regina’ is considered harvestable at mahogany to dark mahogany. Yet, soluble solids (sweetness) of both cultivars are from 19 to 22 ºBrix for fresh market quality fruit (Long et al., 2007). Anthocyanin pigments in dark sweet cherry are predominated by cyanidin-3-glucoside and cyanidin-3-rutinoside,

but some cultivars contain lesser amounts of peonidin glucoside and rutinoside (Mazza and Miniati, 1993).

The relationship of harvest timing and commodity quality is controlled by supply chain demand, storage potential, and consumer preference. In the orchard, fresh market quality is judged using color chips i.e., Centre Techique Interprofessionnel des Légumes (CTIFL), which is a seven chip color scheme utilizing light red (1) to dark mahogany or almost black (chip 7). Further, hand held refractometers are used to identify the level of soluble solids. Fruit size and firmness usually are noted through visual inspection, but do not deter harvest timing when compared to color and soluble solids. Fruit are harvested considering firmness, where early morning harvest and on farm refrigerated trailers are used to minimize the effects of field heat (Webster and Looney, 1996).

In the packing shed, fruit quality is further refined. Packing lines separate fruit through mechanical and anthropocentric means. Initially, fruit are removed from the field collection bin in a water bath, because sweet cherries sink, this allows for debris removal before the fruit enters the sorting line. A submerged conveyer transfers the fruit to a sorting line where a cluster cutter removes multiple fruit attached to a single spur. The cluster cutter is at the beginning of the sizing rollers. Mechanical separation sizes fruit to the appropriate 'Row' size, which is a measurement of uniform fruits that, when packed side-by-side, fit across a 10.5-inch (276 mm) container e.g. 10 Row = 67/64' (26.8 mm) and is equivalent to a fruit weighing 8.7-10.4 g (Webster and Looney, 1996). The segregated fruit travel through water troughs and are deposited to other conveyers that pass under personnel that visually inspect the fruit and remove the damaged, off-sized,

and off-colored product. The inspected fruit receive a second hydrocooling and are resized, packed and placed into cool storage.

Ultimately, quality is judged by the consumer. Organoleptic studies have shown that sweetness is of primary importance (Webster and Looney, 1996); however flavor consists of aroma and taste. Aroma depends upon the content of volatile organic compounds i.e., hydrocarbons, alcohols, aldehydes, ketones, acids and esters. In sweet cherry, 28 volatile compounds were identified in commercially acceptable 'Bing' (Mattheis et al., 1992), where 2-propanol was of highest concentration, acetic acid was the only volatile acid, and hexanal and benzaldehyde (cherry characteristic flavor compounds) were reported. Volatile compounds vary between cultivars, Girard and Kopp (1998) showed significant variation in 12 sweet cherry cultivars of hexanal, (*E*)-2-hexanal, and benzaldehyde. Predominately, sensory evaluations isolate sugar and acid content as the key indicators of acceptable quality (Turner et al., 2008); further the volatile chemicals are not noted for varying desirability until fermentation in storage i.e., acetaldehyde and ethanol production (Esti et al., 2002).

Texture is related fruit firmness or crispness. Firmness is difficult to assess in the field, yet is a characteristic that is crucial in cultivar selection. Early firmness testing devices were held as suspect; there was serious question if the apparatus was measuring tensile strength of the skin over the rigidity of the mesocarp cells (Proebsting and Murphy, 1987). Later, firmness measuring utilizes a constraint gauge that measures the force necessary to crush the fruit a percentage of its equatorial width. This style of measuring instrument has allowed correlations that identify mechanical firmness with organoleptic acceptability (Blažková et al., 2002, Esti et al., 2002, Bernalte et al., 1999).

Sweet cherry is an acidic fruit, which gives the fruit a tart or sour taste. Utilizing the ratio between sugars and acids helps researchers identify consumer perception for a quality eating experience. In a study performed by Kappel et al. (1996) a minimum acceptable soluble solid concentration (SSC) was established at approximately 15% with titratable acidity of 425 mg of malic acid per 100 ml of cherry juice. Kappel et al. (1996) also identifies the taste of a sweet cherry becomes bland at 4.0 pH and the 'Just Right Rating' associates a pH of 3.8 with a 17-19% SSC with a sugar/acid ratio of 1.8-2 SSC/ml NaOH.

Stem damage can be extreme even before the fruit reach the packing-shed. Shrivel and browning deterioration are problems caused from excessive heat and detained removal of field heat. Traditionally, the stem of the fresh market sweet cherry is a significant attribute that consumers prefer (Long et al., 2007). However, Long et al. (2007) showed that the consumer would be willing to purchase a stem-free product at the same price as a stemmed fruit. Peterson and Wolford's (2001) prototype sweet cherry harvester effectively removes stem-free fruit eliminating concerns over pedicel preservation in storage.

Northwest Mechanical Sweet Cherry Harvest

As stated before, fresh market sweet cherry harvest is dominated by hand-harvest, in a mature high density orchard yielding 19.75 metric tons/ hectare (7 tons (US)/acre) harvest cost is estimated at $0.59 per kg ($0.27 per pound) (Seavert et al., 2008). It has been suggested that the cost to man, operate and maintain the USDA prototype is $0.04 per kg ($0.02 per pound, US) (Lehnert, 2008). This results in an approximate cost reduction of 93% or $0.55 per kg ($0.25 per pound). Further, the orchard design must

accommodate this harvest system. In early mechanical harvest trials, the greatest fruit damage was caused by fruit passing through the canopy, therefore an architecture that allows the fruit to fall unobstructed to the catch-frame is desirable. Y-trellis systems, in sweet cherry, provide a system where the laterals are trained to hang over the row and the fruit to hang below the wood.

High density orchards trained in vase-shape and Y-trellis have been shown to facilitate mechanical harvest in cultivars 'Bing', 'Lapin' and 'Van' (Peterson and Wolford, 2001, and Peterson et al., 2003). However, vase-shaped training systems had wispy lateral fruiting limbs that mechanical removal proved difficult to harvest (Whiting, personal comm.). 'Bing' fruit harvested by hand and machine from the Y-trellis system showed hand harvest having a slight advantage in percent fresh market packout with no significant difference between the two harvest methods (Peterson et al., 2003). Noted in the Peterson et al. (2003) study is the need to manage the tree structure and orchard design to accommodate the harvest machinery.

Sweet cherry trees on a precocious rootstock i.e. 'Damil' (hybrid *P.* x *dawyckensis*), 'Gisela 5' [(148/2) *P. cerasus* x *P. canescens*)], or 'Gisela 6' [(148/1) *P. cerasus* x *P. canescens*)] have been shown to produce fruit a minimum of one year before traditional Mazzard (*P. avium* L.) or Mahaleb (*P. mahaleb* L.) plantings (Whiting et al., 2005; Meland, 1998). Precocity is important for gaining a return on investment (ROI), by reducing timing to market. The ROI has been estimated at 22.1% over a 25-year period for high density orchard (672 trees per ha) of 'Bing' on 'Gisela 6'; this compared to a traditional planting system of 337 tree per ha ('Bing' grafted to Mazzard) yielded 12.6% ROI (Seavert and Long 2007). Maximizing ROI is dependent upon marketability and

fruit quality. In Y-trellis systems on precocious rootstocks, fruit quality has been compared to various training systems, namely fruit weight and size, where Y-trellised compared favorably in comparison to the other tree training systems (Spanish bush, central leader and palmatte) (Whiting et al., 2005). Whiting et al. (2005) reported the Y-trellis system, where the scion 'Bing' was grafted to 'Gisela 6', yielded $63.8 per tree as gross crop value over the nine year trial.

Following research of training systems and USDA prototype harvester competency, the need to understand relationships into fruit removal force and fruit quality must be explored to further advance the understanding of cause and effect of the mechanical harvest system. The objectives of my research are to: 1) Identify rates and timing of ethephon to facilitate successful fruit removal of popular northwest commercial cultivars of sweet cherry i.e. 'Bing', 'Chelan' and 'Skeena'. 2) Identify effects of ethephon and mechanical harvest on fruit quality through chemical and physical measurements. And 3) evaluate the role of the pedicel in postharvest

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CHAPTER II

EFFECT OF ETHEPHON APPLICATION ON SWEET CHERRY PEDICEL-FRUIT RETENTION FORCE AND QUALITY ARE CULTIVAR DEPENDENT

Abstract

Due to the threat of labor shortages for sweet cherry (*Prunus avium* L.) harvest in the Pacific Northwest, our research program is investigating the potential for mechanized harvest of fresh market quality fruit. To date, very little research has investigated the role of ethephon (2-chloroethyl phosphonic acid) on pedicel-fruit retention force (PFRF) and quality of new cultivars. Ethephon treatments were made at different timings and rates to 'Bing' and 'Chelan' during the 2006 season. Ethephon applications to 'Bing' trees more than 10 days prior to harvest were effective at reducing PFRF and facilitating mechanical harvest, irrespective of rate (1.2, 3.5, 5.8 L ha^{-1} [1, 3, 5 pt A^{-1}]). Ethephon applied fewer than 10 days prior to harvest did not reduce PFRF sufficiently. In contrast, no rate or timing of ethephon studied induced a reduction in 'Chelan' PFRF sufficient for mechanical harvest. Accompanying PFRF analyses, fruit quality was assessed by measuring firmness (g mm^{-1}), soluble solids (ºBrix), weight (g) and color (CTIFL, scale 1-7). Ethephon applied 22 days before harvest at a rate of 3.5 L ha^{-1} enhanced color in 'Bing' by 27%, while reducing firmness in both 'Bing' (-19%, 22 days prior to harvest) and 'Chelan' (-15%, 20 days prior to harvest). We observed a significant natural decline in 'Skeena' PFRF to levels acceptable for mechanical harvest. This research shows great

potential for mechanical harvest of fresh market quality sweet cherry fruit, especially utilizing cultivars that exhibit a natural decline in PFRF.

Introduction

Producing sweet cherries (*Prunus avium* L.) is a costly and labor-intensive endeavor. Harvest costs are highest among tree fruits, approximately 55% of the costs of production (Seavert et al., 2002, 2008), largely because of the number of fruit per tree (e.g., 10,000+), large unwieldy tree architectures, and the lack of mechanical harvesters. Indeed, the general harvest process for sweet cherry has not evolved appreciably. Availability of skilled harvest labor is declining (USDA/NASS, 2006) and this creates competition for fruit pickers, further increasing the cost. In a short labor situation, higher rates are paid and fruit quality may suffer as fruit are harvested outside their optimal maturity. Further, sweet cherry harvest is fraught with hazards associated with heavy ladder use. The perils of ladder-intensive harvest operations are documented and underscore the need to improve harvest technology. From a study of workers' compensation claims from Washington State orchards, Hoffman et al. (2006) concluded that "there is a strong and compelling need to develop interventions to reduce the number of ladder-related injuries". Ladder-related claims accounted for nearly half of all compensable claims and claims related to ladders were the most frequent and expensive (Hoffman et al., 2006). To remain competitive on a local and global scale, the sweet cherry industry in the Pacific Northwest of the U.S. must reduce costs, improve worker safety, and improve fruit quality.

The traditional sweet cherry system is labor-intensive due to the high fruit number per tree, high crop perishablilty, and large trees that require the use of tall

ladders, carried by workers to access fruit. In apple (*Malus domestica* Borkh.), innovative cultural practices and size-controlling rootstocks have dramatically improved the efficiency of modern orchard systems (Marini et al. 2001). New orchards are planted in compact hedgerows designed to facilitate access to fruit and minimize ladder use. Harvest mechanization was adopted decades ago, but only for lower grade fruit for brining and processing (Webster and Looney, 1996). More recently, attempts to automate or mechanize harvest of fresh market quality fruit have shown promise (Peterson, 2005 a). A harvest aid utilizing narrow inclined trellises and an auto-steer mechanism allows worker freedom of movement that improves productivity by 22% compared to hand harvest (Peterson, 2005 b). Since 2002, the sweet cherry research program at Washington State University has cooperated with USDA-ARS to test a novel mechanical harvester for its potential to harvest fresh market quality fruit (Peterson et al., 2003). In contrast to the traditional shake-and-catch harvest systems (Whittenberger et al. 1967, Peterson, 1992), the USDA prototype utilizes a rapid displacement actuator (RDA) to target individual fruiting limbs. This harvest system removes fruit from the fruit-pedicel abscission zone, harvesting a stem-free product. Successful and uniform fruit removal is related to the retention force required to separate fruit from pedicel (PFRF). Previous work has documented near complete removal of sweet cherry fruit at a FRF (fruit detachment at either fruit-pedicel or spur-pedicel abscission zone) near or below 400 g (Cain, 1967, Bukovac, 1979, Peterson, et al., 2001). At this FRF, the harvester is capable of removing 90% of the fruit in the cultivar ‘Bing’ (Peterson et al., 2003).

Sweet cherry cultivars exhibit natural variability in FRF at harvest (Bukovac et al., 1971) and ethephon (2-chloroethyl phosphonic acid) has been shown to reduce the

PFRF (Bukovac et al., 1969). Interestingly, sweet cherry cultivars exhibit significant variability in their response to various rates and timings of ethephon applications (Bukovac et al. 1971). Bukovac et al. (1971) reported that ethephon applications of 500 ppm (7.8 L ha^{-1} [6.7 pt A^{-1}]) applied 7 to 10 days prior to harvest, reduced PFRF as much as 58%, to below 400g in the cultivars 'Emperor Francis', 'Napoleon' and 'Schmidt'. However, cultivars 'Vic' and 'Hedelfingen' showed a significant decrease in FRF, 53% and 49% respectively, but this decrease was insufficient for mechanical harvest.

Ethephon also affects fruit quality attributes. The sweet cherry cultivar 'Windsor' showed increasing weight and color when ethephon is applied at rates of 500 ppm (6.7 pt A^{-1} [7.8 L ha^{-1}]) or greater (Bukovac et al., 1971). The response appears to be cultivar-dependent because subsequent research revealed no significant changes in fruit quality (Bukovac, 1979). More recently, whole tree applications of ethephon by commercial air-blast sprayer have hastened sweet cherry fruit maturity – we recorded increased exocarp coloration, increased fresh weight, and elevated soluble solids (Whiting unpublished) in several cultivars.

Bukovac et al. (1971) also demonstrated that ethephon can have a significant phytotoxic effect sweet cherry including leaf abscission, terminal shoot dieback, and gummosis, though the response was cultivar dependent. 'Vic' showed significant deleterious effects at 500 ppm (6.7 pt A^{-1} [7.8 l ha^{-1}]), whereas other cultivars were unaffected at this rate. The role of ethephon rate and application timing in PFRF of fresh market quality cultivars produced in Washington is largely untested. The objectives of this research were to investigate the relationships between rate and timing of ethephon on

FRF and fruit quality for commercially significant cultivars grown in the Pacific Northwest.

Materials and Methods

Plant materials. All trees were located at Washington State University's Roza Farm, about 10 km north of Prosser, Washington, USA (46.2ºN, 119.7ºW). All trees were irrigated weekly from bloom to leaf senescence with low-volume under-tree microsprinklers and grown using standard orchard management practices.

Each trial was arranged in a complete randomized design with 6 single-tree replications per treatment. Trees were selected for uniformity of size and yield potential. 'Bing' timing and rate trials were conducted on 11-year-old trees planted on various rootstocks (Gisela® 7 (*P. cerasus* x *P. canescens*), 6 (*P. cerasus* x *P. canescens*), and 5 (*P. cerasus* x *P. canescens*) with an in-row spacing of 2.44 m (8 ft) and between row spacing of 4.27 m (14 ft). Rows were planted in a north-south orientation and trained to Y-trellis architecture. 'Chelan' timing trials were conducted on 4-year-old Gisela® 5-rooted trees. The trees were maintained on a Y-trellis with a tree spacing of 1.52 m (5 ft) in-row by 4.27 m (14 ft) between rows. 'Chelan' rate trials were conducted on 9-year-old trees trained to a central leader on Mazzard (*P. avium*) and Colt (*P. avium* x *P. psudocerasus*) rootstocks with 2.44 m (8 ft) in-row by 4.88 m (16 ft) between row spacing. 'Skeena' trials were conducted on 4-year-old trees on Gisela® 12 (*P. canescens* x *P. cerasus*) rootstock, trained in a Y-trellis system with between-tree spacing of 1.52 (5ft) and 4.27 m (14ft) between rows.

Timing experiments. The ethylene-releasing agent ethephon (formula 240 g/l [2 lbs/gal]) was used in all timing and rate trials. Within 'Chelan' or 'Bing' trials, 30 trees were selected within a single orchard. Over a period of four weeks, single applications of ethephon were applied via air-blast sprayer at 3.5 L ha^{-1} (3 pt A^{-1}) with an 1871 L ha^{-1} (200 g A^{-1}) spray volume. 'Chelan' timing trials commenced on 15 May 2006 (32 d before harvest) and subsequent applications were made on 24 May (23 d before harvest), 30 May (17 d before harvest), and 5 June (11 d before harvest). 'Bing' trials commenced on 30 May (31 d before harvest), 5 June (25 d before harvest), 12 June (18 d before harvest), and 21 June (9 d before harvest).

Rate experiments. Ethephon rate trials compared 4 treatments: control (no application), low rate of 1.2 L ha^{-1} (1 pt A^{-1}), medium rate of 3.5 L ha^{-1} (3 pts A^{-1}) and a high rate of 4.7 L ha^{-1} (5 pts A^{-1}). All applications of ethephon were made via air blast sprayer ('Bing' 1871 L ha^{-1} [200 gal A^{-1}], 'Chelan' 3742 L ha^{-1} [400 gal A^{-1}]). 'Chelan' ethephon applications were made on 31 May 2006 (15 d before harvest) and 'Bing' treatments were applied on 12 June 2006 (18 d before harvest).

Fruit retention force. In each ethephon timing and rate trial, we assessed treatment effects upon fruit retention force twice weekly until harvest. We utilized a digital force gauge (Imada DPS-11, Imada Co., Northbrook, IL) with a custom fitted polyvinyl chloride attachment to detach fruit. Trees were divided roughly into 4 quadrants (upper, lower, east, west canopy regions). On each sample date, 6 fruit per quadrant (24 fruit per tree) were selected randomly and assessed for fruit retention force. We recorded fruit retention force and from where removal occurred from the tree (e.g. pedicel-fruit separation, pedicel-spur separation).

Fruit quality. After FRF determinations, fruit quality was analyzed, firmness and diameter, (Firmtech, Bioworks, Inc., Wamego, Kans), exocarp color, [Centre Techique Interprofessionnel des Légumes (CTIFL)], soluble solids [digital refractometer (Atago Co., Ltd, Japan)], and weight. Mean CTIFL (1 to 7 scale), weight, firmness, and diameter were averaged over the 24 fruit sample. Statistical analysis of means was performed using general linear model (GLM) procedure in Statistical Analysis System (SAS) program (SAS Institute, Cary, N.C.).

Statistical Analysis of FRF. Treatments were analyzed as nonparametric ANOVA with GLM calculated in SAS program. In addition, correlations were calculated in SAS using Proc Reg procedure for simple linear regression.

Results and Discussion

Timing trials. Successful mechanized harvest of fresh market quality sweet cherries is dependent upon a retention force between pedicel and fruit (PFRF) of about 400g (Peterson et al., 2003). 'Chelan' is an early-maturing cultivar that is being planted extensively in Washington State. Natural FRF of 'Chelan' is too high for mechanical harvest at about 800+ g (Fig.1). Further, untreated 'Chelan' fruit exhibited no decline in PFRF throughout fruit maturation. However, FRF was significantly ($P \leq 0.05$) reduced by 24% lower when treated at harvest at 3.5 L ha^{-1} of ethephon applied 17 days earlier. Other applications also reduced FRF compared to the control. We recorded reductions of 13%, 11%, and 12%, from applications made 32 days, 23 days, and 11 days before harvest, respectively. However, no ethephon application reduced FRF sufficiently to facilitate mechanical harvest. The lowest FRF, at harvest, remained 239 g above the 400 g target for uniform removal. Interestingly, the results of FRF analysis in the 'Chelan'

timing trial shows an increase in FRF from 32 d before harvest to 11 d before harvest, at which point all treatments begin to decline in FRF. Olien and Bukovac (1978) suggest that ethephon has a half-life that is temperature dependent. Where temperatures of 20º C at pH of 6.1, ethephon is expected to remain active for 5.6 days, if the temperature is 30º C at pH 6.1 the effective half-life is 26.5 hrs. Determination of ethephon's half-life in the orchard is complicated by fluctuating temperature, physiological optimum to absorption, wetting and weathering of ethephon residue. 'Chelan' is an early maturing sweet cherry where optimal physiological response to ethephon may be limited by environmental factors such as temperature. At 17 days before harvest, this ethephon application had the greatest reduction in FRF, where average temp, over an 8 day period post application was, 17.9º C with an min of 6.1º C and a max of 28.6º C.

'Chelan' fruit quality was affected by ethephon. Most notably we recorded a 24% increase in mean exocarp color (i.e. equivalent of red to light dark red) and a 15% decrease in firmness in response to the application 17 days before harvest sampling (Fig. 5). Ethephon-treated fruit were significantly heavier and darker then non-treated fruit (Fig. 5). However, though firmness suffered from ethephon applications, there was a significant and consistent decrease that is notable throughout timing treatments. Samplings 11, 8, and 4 days before harvest showed a 10% (50 g/mm), 11% (39 g/mm), and 12% (33 g/mm) average reduction in firmness, respectively. Sweet cherry fruit quality response to ethephon treatments has been documented previously. Bukovac, et al. (1971) showed increased fresh weight and increased pigment formation in the cultivar 'Windsor' at a rate of 500 ppm (6.7 pt A^{-1} [7.8 l ha^{-1}]). The authors also reveal significant

variation in response to ethephon treatments among cultivars: percent weight change varied from -1 to 30% among the cultivars tested (Bukovac, et al. 1971).

For ‘Chelan’ we recorded significant spur abscission (i.e., complete spur removal instead of fruit-pedicel separation) indicating that this cultivar is not well-suited for mechanical harvest. From sampling untreated control trees we recorded 57% pedicel retention at harvest. Further, the ethephon treated fruit at harvest were significantly less in pedicel retention i.e., 38%, 32%, 23%, and 20% from treatments 32 days, 23 days, 17 days, and 11 days before harvest, respectively. These data suggest that ethephon efficacy, for ‘Chelan’, is greatest when applications are made between 17 to 11 days before harvest. For other cultivars the optimum timing of ethephon application varies (e.g., ‘Windsor’ needed longer exposure or higher rates compared to ‘Emperor Francis’ and ‘Napoleon’ (Bukovac, 1979)). Also, Bukovac, et al. (1971) showed significant reduction in FRF, at an ethephon treatment of 500 ppm (6.7 pt A^{-1} [7.8 l ha^{-1}), in sweet cherry cultivars ‘Hedelfingen’, ‘Vega’, and ‘Vic’ at 49%, 51%, and 53%, respectively, without a approaching the 400 g target to facilitate mechanized harvest.

In contrast to ‘Chelan’, ‘Bing’ exhibited a natural and gradual decline in FRF throughout final fruit growth and maturation (Fig. 1). Compared to the control, we recorded reductions in FRF of 43%, 45%, 48%, and 16% from the applications made at 31, 24, 18, and 9 days before harvest, respectively. Importantly, the earliest three applications reduced PFRF to below 400 g, only the application at 9 days before harvest was ineffective for mechanical fruit removal. Notably, the 18 days before harvest treatment’s PFRF was below 400 g by 22 June (8 d before harvest), suggesting significant PFRF response occurs within 10 days of application. In previous work, a

specific abscission zone did not form in sweet cherry cultivars ‘Napoleon’ and ‘Windsor’, whereas the sour cherry (*P. cerasus* L.) cultivar ‘Montmorency’ does form a pedicel/fruit abscission zone (Stösser et al., 1969). However, the sweet cherry cultivar ‘Schmidt’ formed localized cell separation in a distinct area similar to ‘Montmorency’ yet not at the same intensity (Stösser et al., 1969). When ethephon was applied in early to mid-stage of phase III fruit development at rates above 500 ppm (6.7 pt A^{-1} [7.8 l ha^{-1}]), the abscission zone in ‘Windsor’ forms within 8 to 10 days. When compared to the control or untreated fruit, the same degree of separation is witnessed 7-10 days past maturity (Bukovac, 1971, Wittenbach and Bukovac, 1972). This suggests ethephon advances maturity of abscission cells and timing is cultivar dependent as to application date in phase III of development.

Ethephon had subtle effects on ‘Bing’ fruit quality and most were generally associated with hastening of fruit maturation. We observed a 26% increase in red coloration of fruit exocarp in response to ethephon applications made 18 days before harvest with no significant reduction in firmness (Fig. 7). Earlier ethephon applications (i.e. 24 days before harvest) also induced a similar increase in exocarp color (+ 27%) but reduced fruit firmness by 19%, compared to the control. Again, ethephon was associated with advancing maturity by increasing color and softening fruit as in apple (Whale et al. 2008) and blueberry (*Vaccinium ashei*, cv. rabbiteye) (Ban et al. 2007).

Stem retention at harvest of the control was 21% for untreated fruit. Ethephon applications at 31, 25, 18, and 9 days before harvest retained pedicels at 5%, 4%, 4% and 10%, respectively. Interestingly, FRF of untreated ‘Bing’ fruit (587 g) was significantly less than ‘Chelan’s’ control sample (841 g) at harvest, and comparing pedicel retention,

‘Bing’ 21% and ‘Chelan’ 57%. These data suggest that FRF is an indicator of pedicel retention. Traditionally, sweet cherry harvest timing is associated to exocarp color, because ethephon increases maturity by advancing pigment development (Bukovac et al. 1971) the PFRF then becomes the indicator of maturity for successful mechanized harvest.

The results from trials on ‘Bing’ and ‘Chelan’ demonstrate a cultivar effect in response to ethephon applications. Irrespective of timing of application, ‘Chelan’ FRF did not decline sufficiently for fruit to be readily harvested mechanically. In contrast, ‘Bing’ PFRF is responsive to ethephon applications made at least 10 days before harvest. Further, the rate of decline in the timing trial of ‘Bing’ also identified the 18 days before harvest application of ethephon as optimal through reducing the PFRF by 71% over the trial period. Whereas, the control fruit decreased by only 29%. In ‘Bing’, the 31, 25, and 9 days before harvest ethephon applications had a 63%, 65% and 44% rate of decline, respectively. ‘Chelan’ timing trial rate of decline was similar to the harvest FRF comparisons to control FRF, further verifying ‘Chelan’ as non-responsive to ethephon at the timing and rate applied.

Rate trials. Ethephon applied at 1.2, 3.5, and 5.8 L ha^{-1} 15 days before harvest to ‘Chelan’ trees had little effect on FRF and fruit quality attributes. No treatment reduced ‘Chelan’ FRF by more than 11% of the control (e.g., reduction of ca. 85 g). Moreover, ethephon had no significant effect upon fruit firmness and color when compared to the control. When comparing the timing and rate trials, the neutral response to ethephon in the rate trials could be associated to tree age, tree size, crop load, an/or application timing. Plant bioregulator use has been shown to be affected by environmental conditions

(Williams et al., 1999). Also varying conditions within the orchard can have a critical effect upon efficacy (Williams et al., 1981). To ensure whole tree coverage in our rate trials, the older and larger trees had 3742 L ha^{-1} volume applied compared to the smaller trees volume of 1871 L ha^{-1} applications. With analysis of ethephon treatments, the response to ethephon is weakened in 'Chelan' with increasing age and vigor.

In 'Chelan' rate trials, spur abscission was, again, significant with 48% of the pedicels being retained on the control fruits. Treatments of 1.2, 3.5, and 5.8 L ha^{-1} are significantly different ($P \leq 0.05$) from the control at 28%, 34%, and 26% pedicels being retained on the fruit, respectively. In addition, we observed no clear relationship between rate of ethephon and reduction in FRF. The greatest rate of decline in FRF was 20% at 3.5 L ha^{-1} ethephon, where as control, 1.2 L ha^{-1}, and 5.8 L ha^{-1} showed 11%, 13%, and 16% rate of decline, respectively. In contrast, Bukovac (1971) showed that high rates of ethephon caused lower FRF in 'Emperor Francis' and 'Windsor' sweet cherry cultivars.

For 'Bing' we again documented significant reductions in FRF in response to ethephon. In contrast to 'Chelan', the reduction in FRF was rate dependent. Compared to control, 'Bing' FRF was reduced by 61%, 53%, and 42% from ethephon applied 15 days before harvest at rates of 5.8 L ha^{-1} (5 pt A^{-1}), 3.5 L ha^{-1} (3 pt A^{-1}), and 1.2 L ha^{-1} (1 pt A^{-1}), respectively. Further, the rate of ethephon affected the rate of decline in FRF. At rates of 5.8 L ha^{-1} and 3.5 L ha^{-1}, FRF declined at rates of 76% and 71%, respectively, where as 1.2 L ha^{-1} declined by 64%. Natural decline for 'Bing' was 37% over the 15 day period. FRF in the 4.7 L ha^{-1} and 3.5 L ha^{-1} was reduced sufficiently for mechanical harvest within 10 days of ethephon application. However, 1.2 L ha-1 application of

ethephon had a lower rate of decline causing a 4 day delay in suitable FRF for mechanical harvest (Fig. 1).

Pedicel retention was similar to 'Bing' timing trials. In 'Bing' rate trials, natural stem retention was 21%, where as ethephon treatments 1.2 L ha^{-1}, 3.5 L ha^{-1}, and 5.8 L ha^{-1} retained 5%, 4%, and 3%, respectively, of their pedicels. Hence, in 'Bing', the amount of FRF directly correlates to where pedicel removal happens i.e. the lower the force to remove the fruit the greater the likelihood for pedicel-fruit abscission, $r^2 = 0.88$ (Fig.2).

Similar to the timing trial, ethephon had significant effects on 'Bing' fruit quality. The greatest effects were an increase in exocarp red coloration by 25% with the 3.5 L ha^{-1} rate, and a 12% decrease in firmness by the 5.8 L ha^{-1} rate. However, Blažková et al. (2002) suggests the firmness of studied sweet cherry fruits be above 2 N (202 g mm^{-1}) for acceptable fresh market retail. None of our timings or rates of ethephon affected firmness to below the threshold reported by Blažková et al (2002). Further, regression analysis, of our data, shows there is no relationship between FRF and firmness, $r^2 = 0.11$ (Fig. 3) and the lowest recorded firmness of fruit at harvest was 229 g mm^{-1} in timing trials (31 d before harvest) and 248 g mm^{-1} at 5.8 L ha^{-1} (5 pt A^{-1}) in the rate trial. Noting the low correlation and acceptable firmness of fruit at harvest, firmness has not been dramatically affected by ethephon. Our data suggests that to facilitate mechanical removal of fruit and minimize any potential negative effects on fruit quality ethephon applications should be made 14 days before harvest at 1.2 L ha^{-1} (1pt A^{-1}).

'Skeena' Assessment of FRF. 'Skeena' is a late-maturing ('Bing' + 10 to 12 days) dark sweet cherry that is renowned among growers for its low natural PFRF at harvest

(Long et al., 2004). In traditional production systems, the loss of the pedicel at harvest or during packing is undesirable but it is a trait that may facilitate mechanical harvest without the use of ethephon. We recorded a progressive natural decline in 'Skeena' FRF over an 11 day period (Fig. 8) leading up to harvest. At commercial mechanical harvest on 11 July 2006, 'Skeena' FRF was 417 g, nearly ideal for mechanical harvest. This observation suggests that 'Skeena' is well-suited to the mechanical harvest system. In addition, 'Skeena' was mechanically harvested in 2006 season, in our experimental orchard, and our observations of fruit removal and quality show great potential. Peterson et al. (2003) showed a 90% or better removal rate on ethephon treated 'Bing'. Also, Peterson et al. (2003) noted ethephon efficacy, for reducing FRF, was lost due to cooler temperatures and calcium chloride applications.

Harvest for 'Skeena' has two potential commercial harvest dates; 1) hand-harvest (HH) with consideration of color (~ CTIFL 4), and 2) machine harvest (MH) with consideration of FRF (ca. 400 g). In comparisons of fruit quality between HH (7 Jul.) to MH (11 Jul.) in 'Skeena', we witnessed a 12.8% increase in weight and a 15.2% increase in color. The firmness decreased by 24.6% in the four day period and there was no significant difference in soluble solids. The firmness (212 g mm^{-1} at harvest MH) (Table 1.) was within acceptable limits for fresh market product as explained previously.

The timing, rate, and observational analysis of 'Bing', 'Chelan', and 'Skeena' in conjunction with previous research on other cherry cultivars (Whiting, unpublished) suggest that there are three distinct classifications for FRF in relation to mechanical harvest. The first two categories relate to the cultivar's response to ethephon, 'responsive' (e.g. 'Bing') and 'non-responsive' (e.g., 'Chelan'). The third category exhibits natural

decline in FRF and fruit from this group are ideal for mechanical harvest (e.g., ‘Skeena’). To efficiently identify cultivars suitable for mechanical harvest, future research should be directed toward identifying genetic markers for the auto-abscission trait.

Genetic improvement of sweet cherry through controlled breeding is one approach. ‘Bing’ is the Pacific Northwest industry standard developed from a chance seedling that originated in Oregon, USA more than 100 years ago. ‘Skeena’ pedigree contains ‘Bing’, ‘Stella’ and ‘Van’ (Kappel et al. 2000), whereas ‘Chelan’, a non-responsive cultivar, is a cross between ‘Stella’ and ‘Beaulieu’. Two other non-responsive varieties are ‘Benton’ and ‘Tieton’ (Whiting unpublished). ‘Benton’, like ‘Chelan’, is a cross of ‘Stella’ x ‘Beaulieu’ and ‘Tieton is a cross of ‘Stella’ x ‘Early Burlat’ (Lang et al. 1998). Interestingly, the cultivars lacking ‘Bing’ in the pedigree also lack adequate responsiveness to ethephon.

Ethephon use can have deleterious effects upon wood, leaves, and fruit. Bukovac et al. (1969, 1971, 1979) reported that rates as low as 500 ppm (7.8 L ha^{-1} [6.7 pt A^{-1}]) can cause gummosis, whereas rates of 1000 ppm (15.64 L ha^{-1} [13.4 pt A^{-1}) trigger defoliation, terminal meristem dieback and lenticel enlargement. Gummosis, in cherry, is generally a response to injury (Stösser, 1984). The ruptured tissue becomes an ideal point of entry for insects and disease. The long term impacts from repeated annual applications of ethephon to sweet cherry are poorly understood. Yet, observational and analytical analysis of ethephon-induced gummosis (Olien et al. 1982 a, b) shows reduced xylem hydraulic conductance in 1-year-old sour cherry (*Prunus cerasus* L.). To facilitate fruit loosening, ethephon is applied over the whole tree, therefore, stimulating gum influx to xylem tissue throughout the tree. Potentially, annual ethephon applications could create

water stress reducing the number of functional vessels, internode growth, and a reduction in reproductive and vegetative spurs.

Ethephon affects sweet cherry fruit quality by reducing firmness, hastening skin coloring, and increasing soluble solids (Bokovac et al. 1971). In the current study, we documented ethephon-induced reductions in fruit firmness and hastening of fruit color in both timing and rate experiments. Further investigation into the response, both long and short term, to ethephon and understanding the relationship of ethylene response could help identify genotypes and cultural practices that are adaptable for mechanical harvest of fresh market sweet cherry. Especially, breeding and identifying cultivars that are conducive to natural decline in FRF, which will eliminate any potential deleterious effects of ethephon use.

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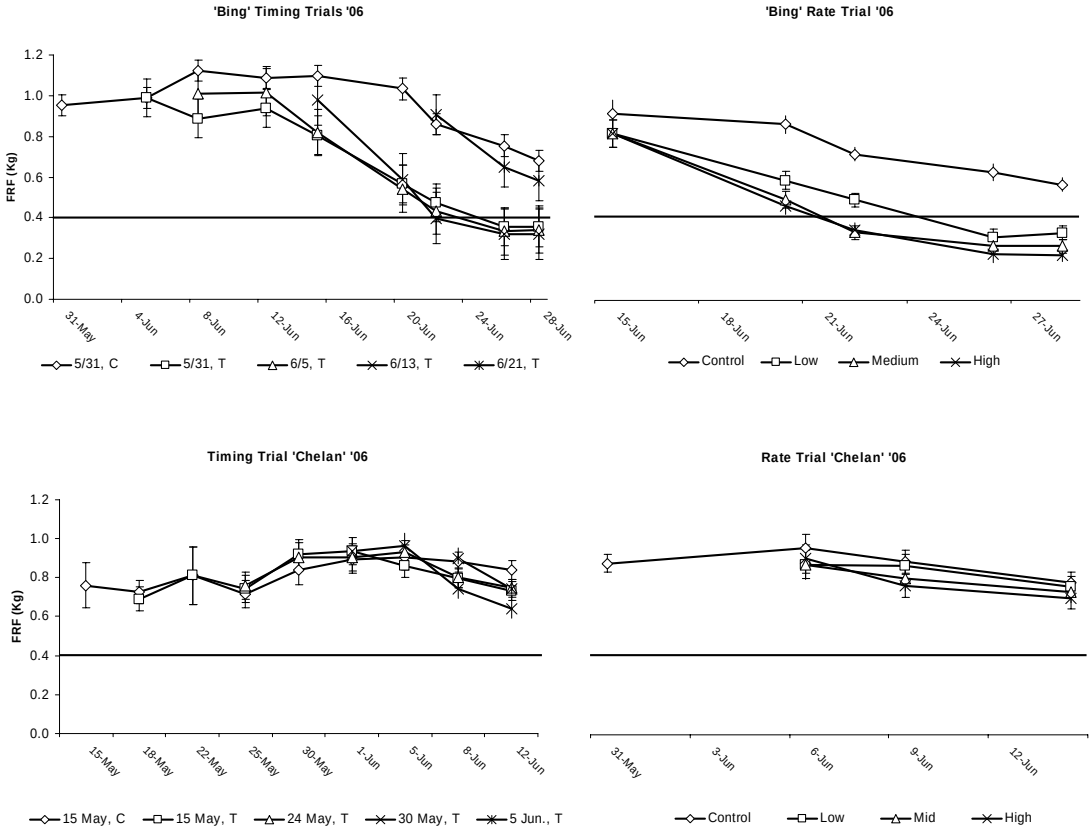

Fig 2-1. 'Bing' and 'Chelan' timing and rate trials, FRF (Kg) measurements: C = control no ethephon applied, T = treatment. In timing trials all dated points have an ethephon treatment of 3.5 L ha^{-1} (3 pt A^{-1}) applied. In the rate trials: Control = no treatment, Low = 1.2 L ha^{-1}(1 pt A^{-1}), Mid = medium rate (3.5 L ha^{-1} [3.5 L ha^{-1}]), High = 5.8 L ha^{-1} (5 pt A^{-1} 5.8 L ha^{-1}). Each sample point represents an average FRF of 144 randomly selected fruits from six trees (24 fruit per tree) measured with a digital force gauge with a custom fitted polyvinyl chloride attachment. Statistical significance at $P \leq 0.05$

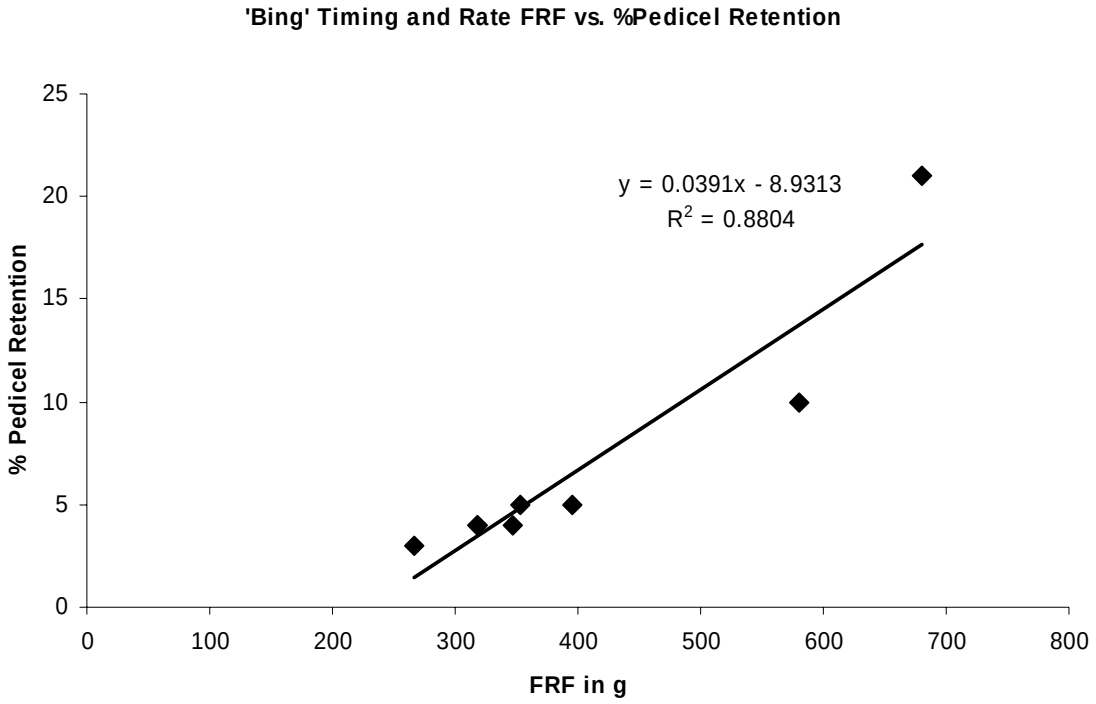

Fig.2-2 Regression analysis of 'Bing' timing and rate trials of FRF against % pedicel retention at harvest. The graph identifies the FRF correlation to pedicel retention, where less force to remove the fruit associates to removal at the pedicel-fruit abscission zone.

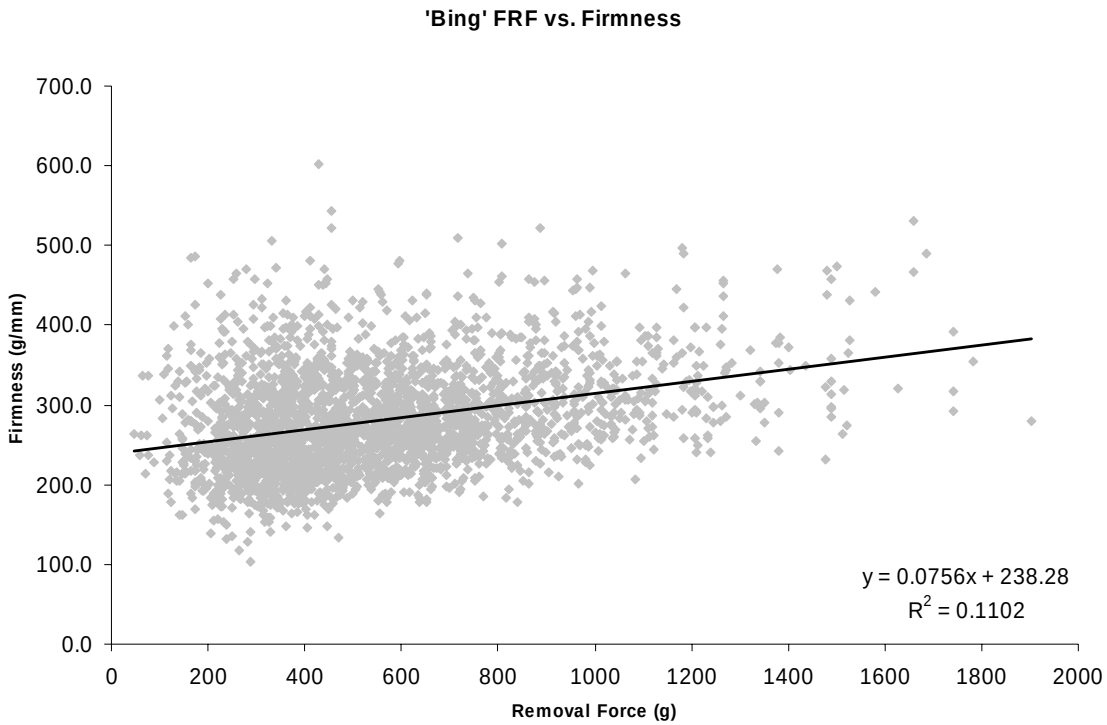

Fig 2-3. Regression analysis of 'Bing' FRF against firmness. Analysis expresses no correlation between removal force and firmness (N = 2733).

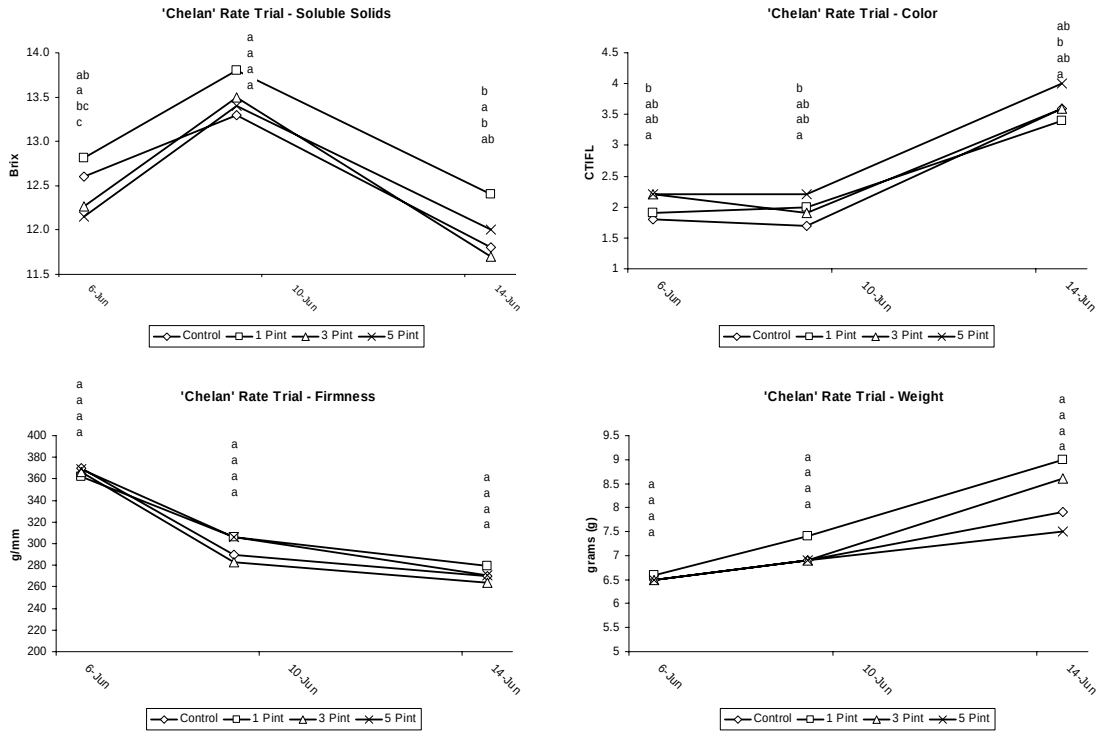

Fig 2-4. 'Chelan' rate trial quality analysis: soluble solids (ºBrix), color (CTIFL), firmness (g mm^{-1}) and weight (g). Different letters within the means of each date signify statistical separation at $P \leq 0.05$.

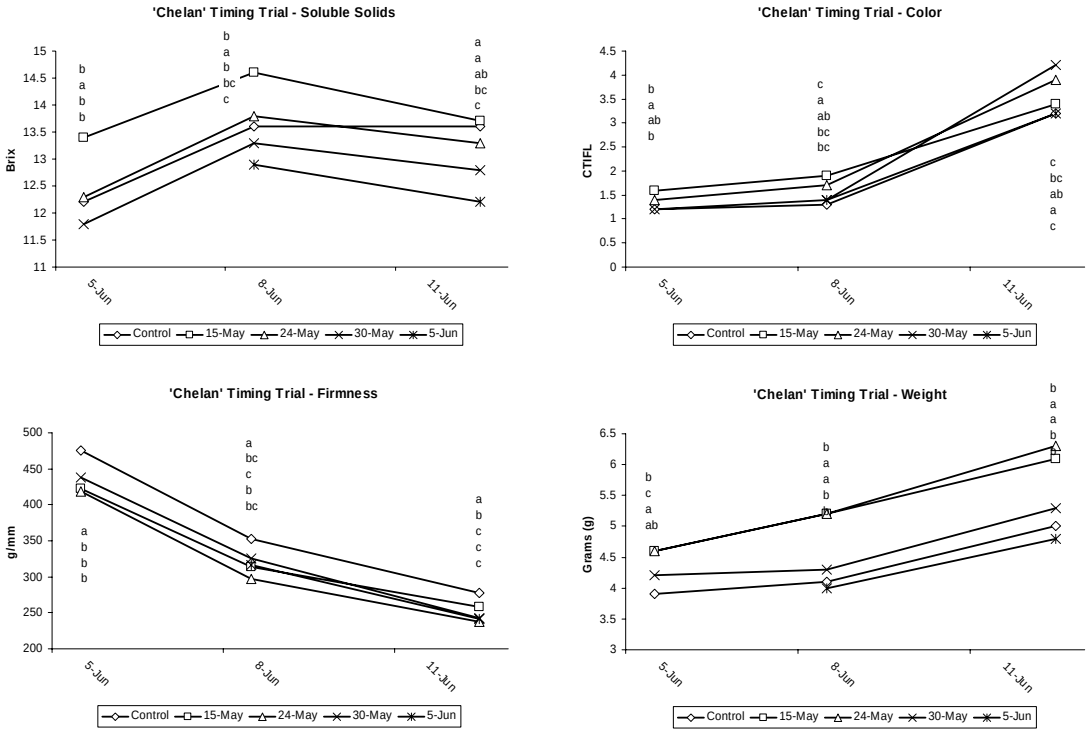

Fig 2-5. 'Chelan' timing trial quality analysis: soluble solids (ºBrix), color (CTIFL), firmness (g mm^{-1}) and weight (g). Different letters within the means of each date signify statistical separation at $P \leq 0.05$.

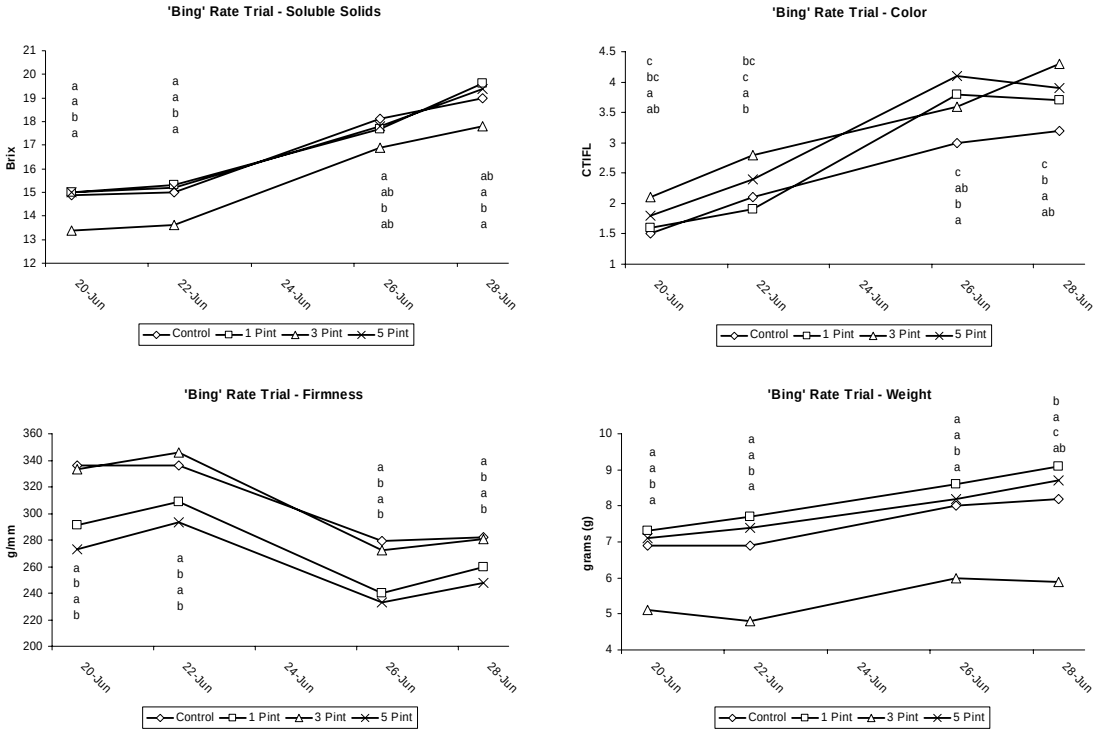

Fig 2-6. 'Bing' rate trial quality analysis: soluble solids (ºBrix), color (CTIFL), firmness (g mm^{-1}) and weight (g). Different letters within the means of each date signify statistical separation at $P \leq 0.05$.

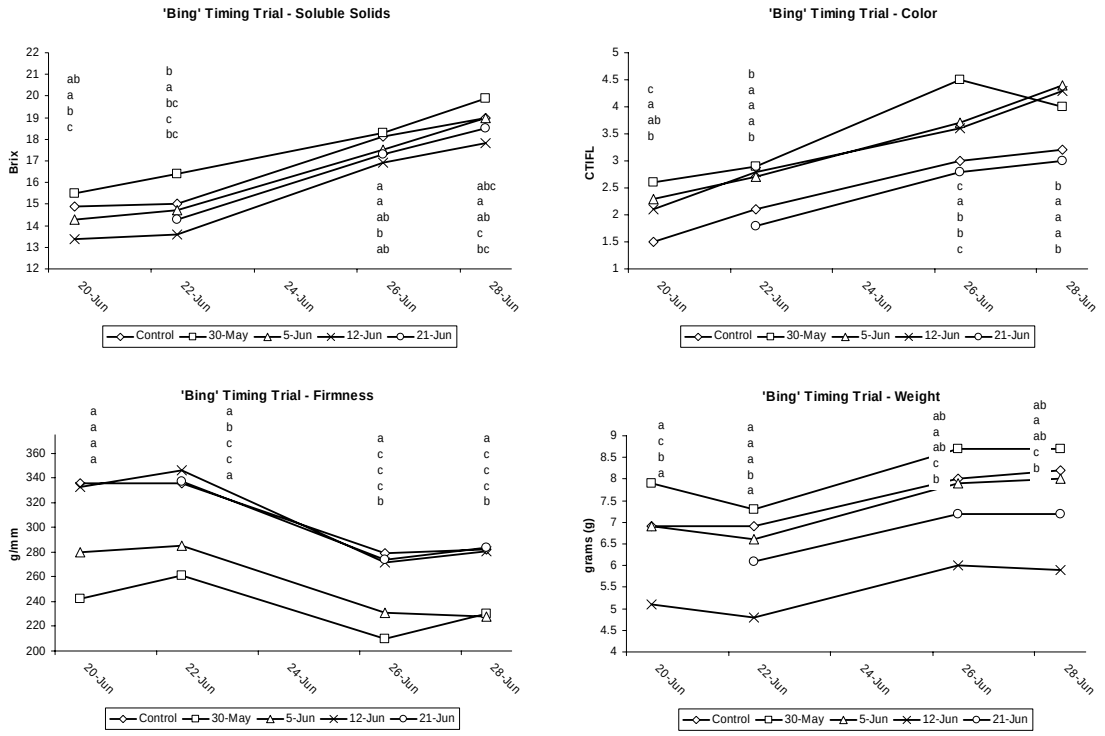

Fig 2-7. 'Bing' timing trial quality analysis: soluble solids (ºBrix), color (CTIFL), firmness (g mm^{-1}) and weight (g). Different letters within the means of each date signify statistical separation at $P \leq 0.05$.

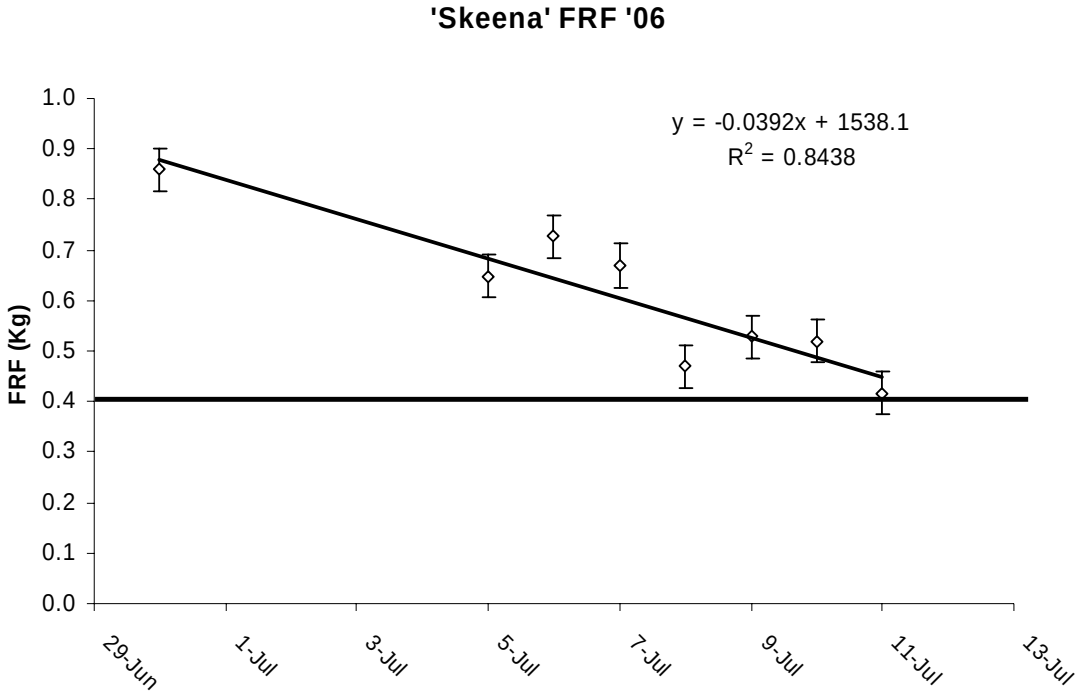

Fig 2-8. 'Skeena' FRF measured 11 d prior to harvest. Each sample point represents an average FRF of 24 randomly selected fruits from 3 trees (8 fruit per tree) measured with a digital force gauge with a custom fitted polyvinyl chloride attachment. Statistical significance at $P \leq 0.05$.

Skeena Fruit Quality at Hand Harvest (7Jul06) and Machine Harvest (11Jul06)												
Treatment	Average Weight	se		Firmness Average	se		Average Brix	se		Red Color Rating	se	
7-Jul	9.5	0.48	b	281.4	4.5	a	17.2	0.8	a	3.9	0.2	b
11-Jul	10.9	0.46	a	212	4.75	b	17.7	0.8	a	4.6	0.2	a
lsd	1.4			13.8			1.4			0.6		
% diff	12.8			-24.7			2.8			15.2		

Table 2-1. 'Skeena' fruit quality comparison at hand harvest (7Jul06) and machine harvest (11Jul06). Fruit quality measured is average weight (g), firmness (g/mm), soluble solids (ºBrix) and color (CTIFL). se = standard error: lsd = least significant difference: a and b indicate significance at $P \leq 0.05$.

CHAPTER III

SWEET CHERRY FRUIT STORABILITY IS RELATED MORE TO FRUIT SIZE THAN HARVEST METHOD

Abstract

The mechanical harvest and handling process for sweet cherry (*Prunus avium* L.) is very different from the traditional manual approach. Concerns remain over the effects of the mechanized system on fruit quality and storability. The current research compares quality of mechanically and hand-harvested cherries during storage in commercial facilities. Over two seasons for 'Bing' and three seasons for 'Skeena' hand and mechanically harvested fruit were placed in storage. In 2006, 'Bing' and 'Skeena' fruit were harvested at commercial maturity and stored directly in refrigerated storage. In 2007 and 2008, fruit were transferred to a commercial packing facility to be processed, packed, and stored. At regular intervals over a 21 to 29 day storage period, samples were analyzed for firmness, soluble solids, pH, titratable acidity, exocarp color and weight. In addition to quality analysis, the commercially-packed fruit were analyzed for cull rate. In 2006, ethephon-treated 'Bing' fruit had increased color at harvest (+14%) and decreased firmness (- 18%). Further, in 2006 mechanically harvested, ethephon treated 'Bing' fruit were not significantly different in weight, soluble solids and pH from untreated, hand-harvested fruit. In 2007, harvest method again had no effect on 'Bing' fruit quality at harvest or throughout storage. Fruit cullage rate was 12% greater at harvest for hand harvested fruit. Cullage rates were related negatively to fruit diameter, irrespective of cultivar or harvest method. In 2006, mechanically and hand harvested 'Skeena' fruit, through storage, increased in firmness and color, while pH decreased and soluble solids

did not significantly change within their respective treatment. In 2007 and 2008, ‘Skeena’ quality trended as in 2006 and cullage rates were lower for larger fruit. Quality analyses did not show hand-harvested fruit being superior to machine-harvest. In general, storability was affected by ethephon and fruit size rather than harvest system.

Introduction

Interest in improving harvest efficiency with mechanization has grown due to the significant labor requirements of sweet cherry harvest and the projected insufficient supply of skilled harvest laborers. A prototype mechanical harvest system has been developed and preliminary field-testing appears promising. Initial studies have revealed little negative impact on fruit quality from using the mechanical harvest system. Peterson and Wolford (2001) reported 2 – 6% more damage in mechanically-harvested ‘Van’ and ‘Lapin’ fruit compared to fruit that was hand-harvested. Further, mechanically-harvested ‘Bing’ were reported to have only 4% more damage than hand-harvested fruit of which the authors attribute 1% to the harvester’s effect (Peterson et al., 2003).

Critical to the efficiency of mechanical harvest is development of a pedicel-fruit zone to facilitate a clean release and limit postharvest water loss (see Chapter IV). Previous research has shown that a pedicel-fruit retention force (PFRF) of ca. 400 g is important for uniform fruit removal (Peterson et al., 2003). Ethephon (2-chloroethyl phosphonic acid) is effective for reducing PFRF and facilitating mechanical harvest of sweet cherry cultivars grown in Michigan (Bukovac et al., 1969). More recently, Peterson et al. (2003) showed significant and sufficient reductions in ‘Bing’ PFRF with applications of ethephon at 3.5 L ha^{-1} (3 pt A^{-1}). Chapter II of this volume reports on genotypic sensitivity of sweet cherry cultivars to ethephon’s effects on PFRF and defines

three categories: responsive (e.g. ‘Bing’), unresponsive (e.g. ‘Chelan’) and auto-abscising (e.g. ‘Skeena’).

Ethephon has been shown to hasten sweet cherry fruit ripening by increasing anthocyanin concentration and weight (Bukovac et al., 1971). Further, Elfving and Visser (2006) reported that ethephon at a rate of 3.5 L ha^{-1} (3 pt A^{-1}) can soften ‘Bing’ sweet cherry fruit and decrease fruit storability. In addition, histological studies demonstrate ethephon’s ability to degrade cellular tissue in both the fruit-pedicel abscission zone of the sweet cherry cultivar ‘Windsor (Wittenbach and Bukovac, 1972) and secondary xylem in current season terminal shoots of the sweet cherry cultivar ‘Emperor Francis’ (Bukovac, 1979). The effect of ethephon on fruit maturity has been demonstrated in tomato (*Lycopersicon esculetum*) by increasing soluble solids (Logendra et al., 2004), in grape (*Vitis vinifera* cv. ‘Ebony Star’) by increasing color (Avenant and Avenant, 2006), in Saskatoon (*Amelanchier alnifolia* Nutt. cvs ‘Northline’ and ‘Smoky’) by causing uniformity of ripening (McGarry et al., 2005), and in apple (*Malus x domestica* cvs. ‘Scarletspur’ and ‘Gale Gala’) by increasing starch content and reducing firmness (Drake et al., 2006).

In this study, the effects of harvest system (i.e., hand vs. mechanical) on sweet cherry fruit quality and shelf-life was evaluated. Throughout storage, fruit were subjected to a battery of quality experiments to identify response to mechanical and chemical treatment that is necessary when utilizing the sweet cherry prototype harvester.

Materials and Methods

Plant materials. All trees were located at Washington State University’s Roza Farm, about 10 km north of Prosser, Washington, USA (46.2ºN, 119.7ºW). All trees were

irrigated weekly from bloom to leaf senescence with low-volume under-tree microsprinklers and grown using standard orchard management practices for the region.

Experiments with 'Bing' were conducted on 12 and 13-year-old trees in 2006 and 2007, respectively. Trees were grafted on various rootstocks (Gisela® 7, 6, and 5) and planted at an in-row spacing of 2.44 m (8 ft) and between row spacing of 4.27 m (14 ft). Rows were planted in a north-south orientation and trained to Y-trellis architecture at various angles. 'Skeena' trials were conducted on 5-, 6-, and 7-year-old trees in 2006, 2007, and 2008, respectively. Scions were grafted to Gisela® 12 rootstock, trained in a Y-trellis system with between-tree spacing of 1.52 m (5 ft) and 4.27 m (14 ft) between rows.

2006. 'Bing'. Six harvest treatments were evaluation for their effects on fruit quality at harvest and during storage: 1) machine-harvested ethephon-treated stem-free, 2) machine harvested untreated stem-free, 3) hand harvested untreated with stem, 4) hand harvested untreated stem-free, 5) hand-harvested ethephon-treated with stem, and 6) hand-harvested untreated with stems at commercial maturity. Fruit were harvested from the same orchard block and each treatment consisted of three trees and one lug (ca. 15 kg) was collected from each tree (i.e. three lugs collected per treatment). Untreated trees were segregated from treated trees by a guard row. Fruit were harvested on 30 June for treatments 1 – 5 and on 6 July for treatment 6. To 6 trees, ethephon was applied at 3.5 L ha^{-1} (3 pt A^{-1}) 2 weeks prior to harvest.

At harvest, the lugs were taken to the Washington State University, Prosser sweet cherry laboratory and the fruit were culled for imperfections (cracks, bird pecks, insect infestation, and any other damage making the fruit unacceptable for commercial sale) and the retained marketable fruit were washed with potable water. The washed fruit were

amalgamated into their respective treatment and one lug per treatment was than placed into 4 ºC refrigerated storage. Randomized sub-samples of fruit were retrieved from each treatment lug at 3- to 4-day intervals throughout a 30-day storage period. Samples were analyzed for quality (described below) after returning to room temperature.

'Skeena'. Evaluations compared 3 treatments: 1) mechanically harvested stem-free, 2) hand harvested with stem, and 3) hand harvested stem-free (fruit separated from stem during picking). Fruit were harvested from the same orchard block and each treatment consisted of 3 trees of which one lug was collected from each tree (i.e. three lugs collected per treatment) totaling 9 trees used for the experiment. Because 'Skeena' is auto-abscising (see Chapter II), no ethephon was applied and harvest timing determined by commercial indices (e.g. color and soluble solids) plus PFRF ca. 400 g. Fruit was harvested on 11 July.

Immediately following harvest, the fruit was taken to the Washington State University, Prosser sweet cherry laboratory and analyzed for obvious imperfections (cracks, bird pecks, insect infestation, and any other damage making the fruit unacceptable for commercial sale) and the retained marketable fruit was washed with potable water. The washed fruit were separated into their respective treatment and one lug of fruit was than placed into 4 ºC refrigerated storage. Random sub-samples of 24 fruit were retrieved from each treatment/lug at 3- to 4-day intervals throughout a 20-day storage period. After returning to room temperature, samples were analyzed for quality (described below).

2007. 'Bing'. Ethephon was applied at a rate of 3.5 L ha^{-1} (3 pt A^{-1}) on 14 June to 43 'Bing' trees to facilitate machine harvest. Hand harvested trees were isolated by two

rows of guard trees to eliminate over spray effects. All trees were within the same orchard block. Harvest was on 27 June in the early morning and one bin (ca. 127 kg) of hand harvested and two bins of machine harvested fruit were collected and transferred to the Western Sweet Cherry Group in Yakima, WA by 1000 hr. The fruit were processed over a commercial sweet cherry packing line where fruit were hydrocooled and placed into cold storage until transfer to the line. Processing at the line commenced with the bin being placed into a roll-over dump tank, which reduces damage by transferring the fruit from the bin to the packing line through submergence in water. Next, the fruit are elevated to run through a stem cutter and then across a roller sizing apparatus to separate various size classes. Sized fruit were then graded for quality, packed according to size, and placed into 1.0 kg (2.25 lbs) bag with at 8 bags per box. The packed fruit were placed into cold storage at 1 ºC at 85 to 95% relative humidity.

A sub-sample of four 1 kg bags for each row size and harvest method was assessed bi-weekly for cullage rate throughout a 29-day storage period. Fruit were culled by commercial standards for any imperfections that detracted from marketability (e.g. excessive pitting, cracks, bruising, mold, bird pecks) or any other malady that detracts from consumer acceptability. Cullage was calculated as a weight percent loss against the total mass of the four bags [(g culled/g total) 100]. The fruit, that was considered marketable, was subjected to quality analyses (described below).

'Skeena'. Harvested fruit did not have an application of ethephon and harvest timing was associated to color, soluble solids, and the retention force between the pedicel and fruit (ca. 400 g). 'Skeena' fruit were harvested on 5 July, when one bin of hand harvested and two bins of machine harvested fruit were collected and transferred to the

Western Sweet Cherry Group in Yakima, WA. The fruit was processed under the same conditions as 'Bing', packed according to size, and placed into 1.0 kg (2.25 lbs) bag at 8 bags per box. Fruit were analyzed over a 21-day postharvest period for cullage and quality in the same manner as 'Bing' 2007.

2008. 'Skeena'. Again, no ethephon was applied and harvest timing was associated to the retention force between pedicel and fruit. 'Skeena' was harvested on 9 July, and one bin of hand harvested and two bins of machine harvested fruit were transferred to the Western Sweet Cherry Group in Yakima, WA. The fruit was processed according to standard practice, packed according to size, and placed into 1.0 kg (2.25 lbs) bag at 8 bags per box. Fruit were analyzed over a 25 day postharvest period for cullage and quality in the same manner as 2007. There was no 'Bing' harvest in 2008 due to heavy repeated frost events in early spring that resulted in poor pollination and crop loss.

Fruit quality. Fruit quality was analyzed as firmness (Firmtech, Bioworks, Inc., Wamego, Kans), exocarp color [Centre Techique Interprofessionnel des Légumes (CTIFL), chips 1 - 7], soluble solids [digital refractometer (Atago Co., Ltd, Japan)], pH and titratable acidity with 0.1 M KOH (Mettler Toledo DL 12 Auto-titrator, Columbus, OH), and weight. Weight and titratable acidity were considered only in 2007 and 2008, because the fruit was not sized before storage and titrations showed too much variability to be considered as quantifiable data in 2006.

Statistical analyses of means were performed using general linear model (GLM) procedure at $P < 0.05$ in Statistical Analysis System (SAS) program (SAS Institute, Cary, N.C.) for 2006. 2007 and 2008 statistical analysis was carried out in SAS using a three-way factorial design (harvest method, row size, date analyzed) with a repeated measure

on one factor (date). Due to the lack of repetition, a two-way interaction of harvest*row was used for the error term for harvest method and row size and a three-way interaction of harvest*row*date for the error term involving date. All statistical analyses are derived from interpretation of the Type Three Analysis.

Results and Discussion

2006. The experiments in 2006 were designed to assess the effects of ethephon (necessary for mechanical harvest of 'Bing') and harvest system on fruit quality at harvest and during cold storage. In previous studies, ethephon was shown to increase color and weight (Bukovac et al., 1971) and reduce firmness and shorten storage life (Elfving and Visser, 2006). In addition, work accomplished in chapter II shows that ethephon increased 'Bing' color, decreased firmness and did not significantly affect weight.

In the current study, neither 'Bing' nor 'Skeena' showed any significant detrimental effects from the mechanical harvest system compared to hand-harvest. Soluble solids and pH increased by 7% and 10% for machine and hand-harvested fruit, respectively through the duration of storage. Conversely, firmness decreased by 9% for both machine and hand-harvested fruit. However, weight increased by 7% and 13% for machine and hand-harvest, respectively, throughout storage. Further, over the same intervals, color increased by 26% and 35% for machine and hand-harvested fruit, respectively. Irrespective of harvest treatment, there was no statistically significant difference between treatments by the end of the storage period for both weight and color.

Ethephon affected 'Bing' fruit maturity and quality independent of harvest technique. Ethephon-treated fruit had significantly lower firmness and advanced red

coloration of the exocarp at harvest. Firmness of machine-harvested fruit was 18% lower than hand-harvested untreated fruit at harvest and following 17 and 31 days of storage (Fig. 3-1). The comparisons of hand-harvested treated and hand-harvested untreated fruit over the same sample points, the difference reported are 14, 19, and 15%, respectively. To further illustrate the effect of ethephon on firmness, comparisons of hand-harvested treated and machine-harvested fruit had differences of 5, 1, and 4 % over harvest, 17 and 31 days of storage, respectively. Blažková et al. (2002) suggests that marketable firmness of sweet cherry should not be lower than 2 N (200 mm g^{-1}), our study demonstrated that machine harvested ethephon treated 'Bing' was within this limit of fruit quality. Further, the practical significance of this reduction in firmness is not clear. Recent studies have shown that consumers have difficulty determining differences in firmness between sweet cherry samples (Ross and Whiting, unpublished).

Exocarp color was enhanced by ethephon (Fig 3-2). Throughout the duration of storage, ethephon treated 'Bing' fruit were at ca. 17% greater on the CTIFL scale (4.6 vs. 3.8) indicating a shift to dark red in treated fruit compared to red for untreated fruit. Treated fruit show no difference on the CTIFL scale (4.6 vs. 4.6) throughout the storage period. Sweet cherry fruit color increase in storage has been reported and storage temperature determines the rate of change (Szymczak et al., 2003). Ethephon hastens red coloration in other sweet cherry cultivars (Bukovac, et al., 1971) and Gonçalves et al. (2007) demonstrated that irrespective of harvest timing anthocyanins increase in storage considering the observations obtained, ethephon appears to have made a significant contribution to color development prior to storage. However, Li et al. (1994) showed that ethephon applied to mahogany fruit did not significantly increase respiration postharvest;

hence further study is needed to determine the metabolic nature of ethephon induced color development in sweet cherry.

Similar to the 'Bing' study, mechanical harvest had no significant effects on 'Skeena' fruit quality and storability. However, there were significant differences in soluble solids that are attributable to variation within an orchard. Even though the study was conducted on trees within close proximity, the effect of shading and heavy crop load can cause lower soluble solids (Patten et al., 1986) and reduced fruit weight (Gutzwiler and Lang, 2001). The combined effect of within orchard shading and insufficient pruning could have caused the observed variation.

2007. 'Bing'. In 2007, trials were designed to assess the storability of mechanically harvested sweet cherry in a commercial atmosphere. Fruit were harvested both by hand and machine and transferred to a packing-house to be sorted, packed and stored commercially. At delivery to the packing shed, fruit were sorted by diameter (row-size) and assessed for culls (unmarketable fruit due to bruising, stem-end tears, bird damage, polycarpy, etc.). 'Bing' segregated into 10-row (26.6 mm), 10.5-row (25.4 mm), 11-row (24.2 mm), and 11.5-row (22.6mm). Hand- and machine-harvested fruit had cullage rates of 24.7% and 14.9%, respectively, through this difference was not statistically significant at $P < 0.05$.

In general, there was a negative relationship between fruit diameter and cullage rates throughout storage (Fig. 3-11). Further, hand-harvested fruit generally exhibited higher culls than the machine-harvested fruit (Fig. 3-11). Within 5 days in cold storage, 11.5-row (22.6mm) hand harvested fruit had a 93% cullage rate. In contrast, machine-harvested fruit of the same size had 39% cullage at the same sampling date. After 8 days

in cold storage the cull rate was 88% and 21% for 11.5-row hand- and machine-harvested fruit, respectively. Conversely, the larger fruit (10-row, 26.6mm) exhibited similar rates of cullage through out cold storage irrespective of harvest method (Fig. 3-11). After 5 and 29 days in cold storage, cullage rate of hand-harvested fruit was 6% and 53%, respectively. In comparison, cullage rates of machine-harvested fruit were 14% and 46% on the same sampling intervals, respectively. Statistically, the dominate effect is time in storage, not surprising since sweet cherry exhibit high rates of respiration and lack starch reserves (Webster and Looney, 1996) causing considerable decline in fruit quality through storage. Indeed, even with prudent temperature and packaging management, storage life of sweet cherry is only 2 to 4 weeks depending upon cultivar (Mattheis and Fellman, 2004). Though hand-harvested 'Bing' fruit cull at a higher rate, the cullage trend within hand- or machine-harvested fruit is similar and larger row-size equates to greater longevity in storage.

Analyses of fruit quality attributes of marketable (i.e., not cull fruit) fruit in the IAREC lab revealed little effect of harvest system on either cultivar. Fruit juice pH, soluble solids, exocarp color, and weight remained unchanged throughout storage. In contrast, fruit juice titratable acidity declined and firmness increased significantly during storage. In 2006, 'Bing' increased in color, which was also noted in sweet cherry storage studies conducted by Szymczak et al. (2003) and Gonçalves et al. (2007). Further, color increases were noted by a reduction in hue angle values in particular L^* (the photometric parameter proportional to the light reflected by an object) (Gonçalves et al., 2007, Mozetič et al., 2005) and the loss of these values were correlated to storage temperature and humidity (Gonçalves et al., 2007, Szymczak et al., 2003). Following this rational, the

difference in our CTIFL observations from 2006 to 2007, IAREC storage temperature is 4 ºC without humidity control, whereas Western Sweet Cherry Group prescribes to the practice of managing temperature at 1 ºC with 95% humidity.

Firmness was not affected by the harvest system and ethephon-induced softening was negligible. Firmness did increase through time in storage, regardless of row size. For example hand- and mechanical-harvested 10-row fruit were 32% and 30% firmer after 29 days storage, respectively. Patterson and Kupferman (1983) reported that 'Bing' increased firmness in controlled atmosphere (CA) storage at 5 ºC and air at 0 ºC up to 6 weeks in storage. Recently, Kappel et al. (2002) reported on firmness of 'Bing' showing increases after 2-weeks of storage at 1 ºC in modified atmosphere packaging (MAP). Further, increasing firmness through storage was reported in 'Lapins' by Drake and Elfving (2002) and was associated to weight loss.

2007 & 2008. 'Skeena'. In both years, untreated 'Skeena' fruit were harvested by machine (stem-free) and hand (with stems) in early July and transferred to the Western Sweet Cherry Group for processing. Initial cullage in 2007 was ca. 22% and 20% for hand and machine harvest, respectively. Fruit size was very large and 'Skeena' packaging or packout was segregated into only 9-row (29.8 mm) and 9.5-row (28.2 mm). In 2008, the initial cullage was 18% and 21% for hand and machine harvest, respectively. Fruit size was smaller than in 2007 and the packout segregated into 9.5-row, 10-row and 11-row.

The harvest method did not affect fruit quality in both 2007 and 2008. However, a statistical effect on weight was significant by year ($P = 0.009$), year*harvest ($P = 0.0167$) and row(year) ($P = 0.0049$). These values indicate that the weight of fruit differed

between years because of environmental conditions causing frost damage and reducing pollination. Further, weight analysis was affected by row-size due to 2008 having a shift to smaller fruit. The frost event of 2008 affected other quality parameters tested, pH and titratable acidity averaged lower pH in 2008 (i.e. 9.5 row 3.55 vs. 3.7 pH, 2008 and 2007, respectively), which caused an average higher consumption of 0.1 M KOH (i.e. 9.5 row 20.9 mls vs. 12.9 mls in 2008 and 2007 respectively).

Color increased throughout storage i.e. 9.5 row hand-harvest 2007 and 2008 had a 7 and 17% increase, respectively, and machine-harvested fruit color increased by 11 and 20% in 2007 and 2008, respectively. Obviously, cullage increased throughout storage causing a notable significance in sampling overtime for both years. Soluble solids were significantly affected by harvest method i.e. hand harvested average ºBrix = 19.2 and 19.5 in 2007 and 2008, respectively, where as machine-harvested average ºBrix = 20.4 and 21.4 in 2007 and 2008, respectively. The difference in soluble solids is attributable to variation within the orchard that was evident in 2006.

In conclusion, the analyses of postharvest quality of hand- and machine-harvested ‘Bing’ and ‘Skeena’ sweet cherries over 3 years show great potential for the mechanical harvest system. Earlier work conducted with shake & catch mechanical systems showed significant damage and fruit were used for processing (Gaston et al., 1967, OSU Fact Sheet 166, 1970). Clearly, with a the advent of the prototype harvester (Peterson and Wolford, 2001) in combination with fruiting plane training system (Whiting et al., 2005) where fruit fall unobstructed to the catch surface, sweet cherry harvest can be mechanized.

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Treatment	Description	Abbreviation
1	Hand Harvest Stem-Free	HSF
2 & 7	Hand Harvest w/ Stem	HS
3	Hand Harvest w/ Stem ethephon Treated	HST
4	Hand Harvest w/ Stem Harvest on 6 July 07	HS 7/6
5	Machine Harvest Control no ethephon applied	M
6 & 8	Machine Harvest Treated with ethephon	MT

Table 3-1. Description of treatments and abbreviations for stored sweet cherry fruit in 2006

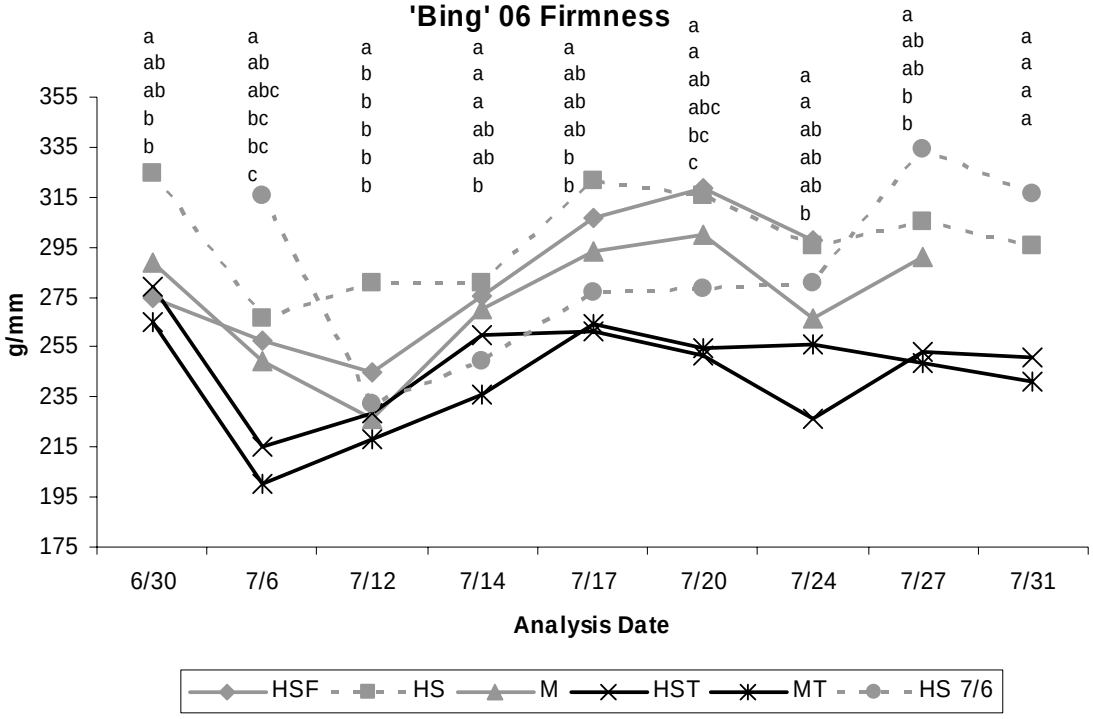

Figure 3-1. 'Bing' 2006 analysis of firmness (g mm^{-1}) 31 days of storage from harvest at 30 June to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

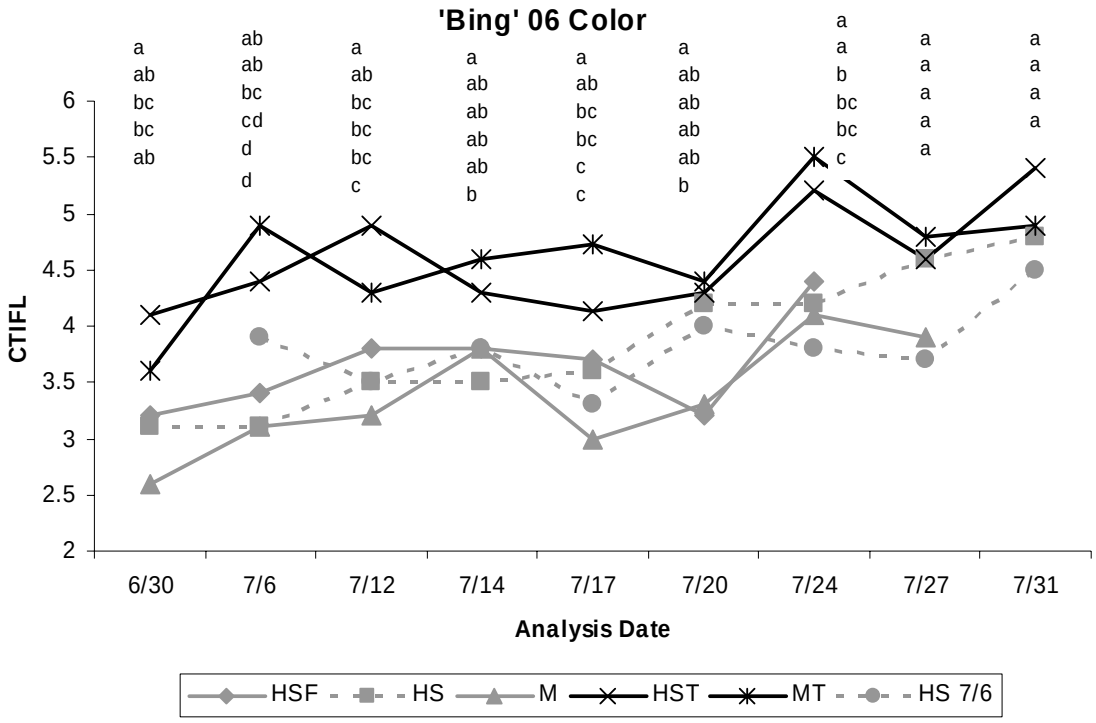

Figure 3-2. 'Bing' 2006 analysis of color (CTIFL, chips 1-7) 31 days of storage from harvest at 30 June to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

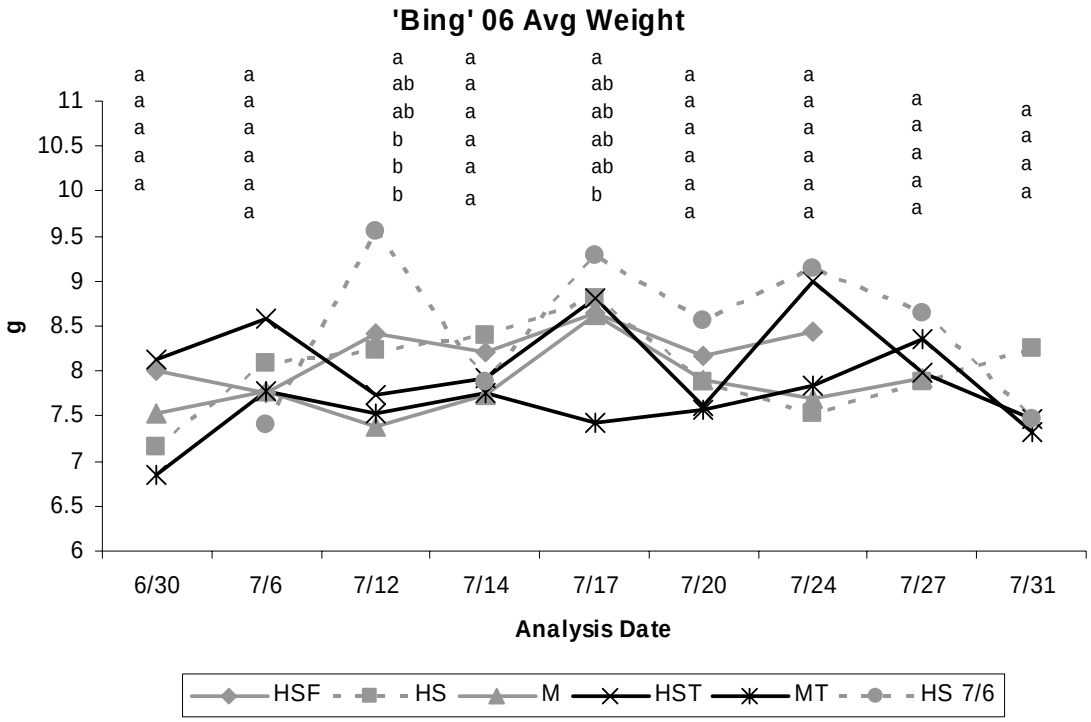

Figure 3-3. 'Bing' 2006 analysis of weight (g) 31 days of storage from harvest at 30 June to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

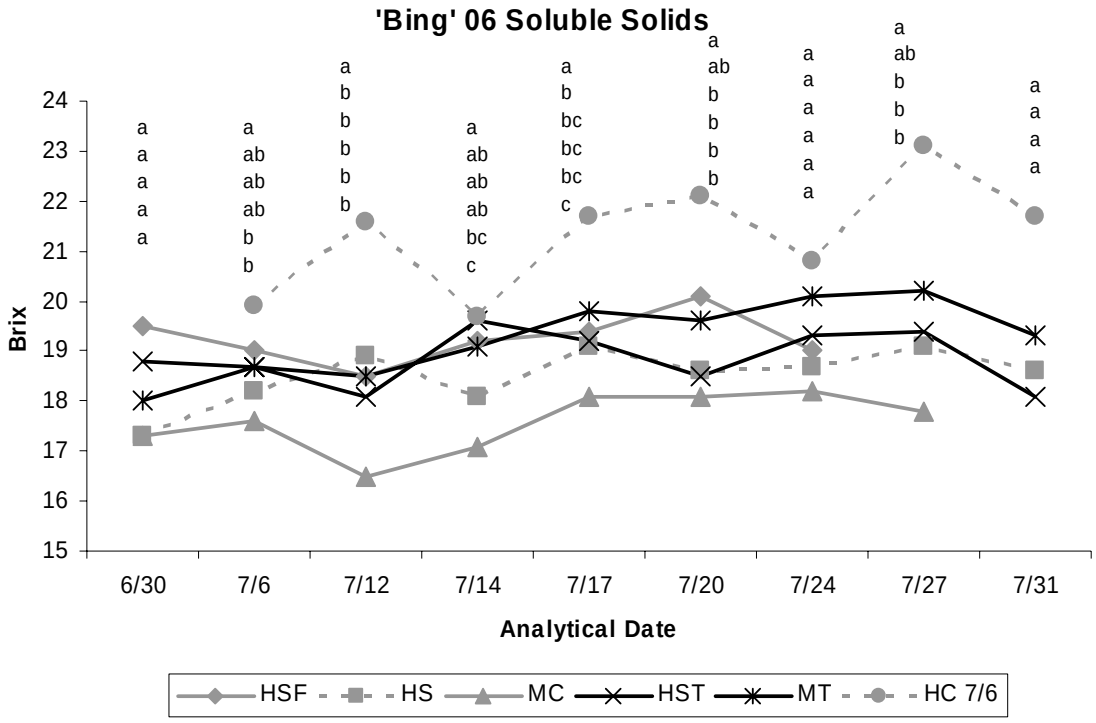

Figure 3-4. 'Bing' 2006 analysis of soluble solids (ºBrix) 31 days of storage from harvest at 30 June to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

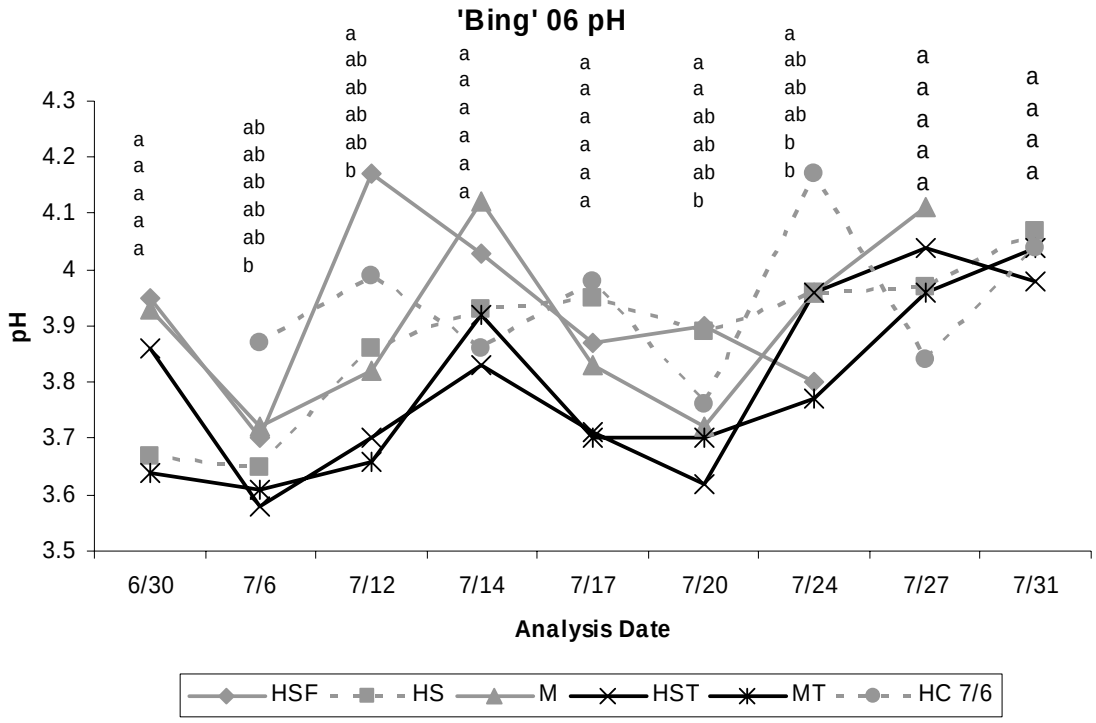

Figure 3-5. 'Bing' 2006 analysis of pH 31 days of storage from harvest at 30 June to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

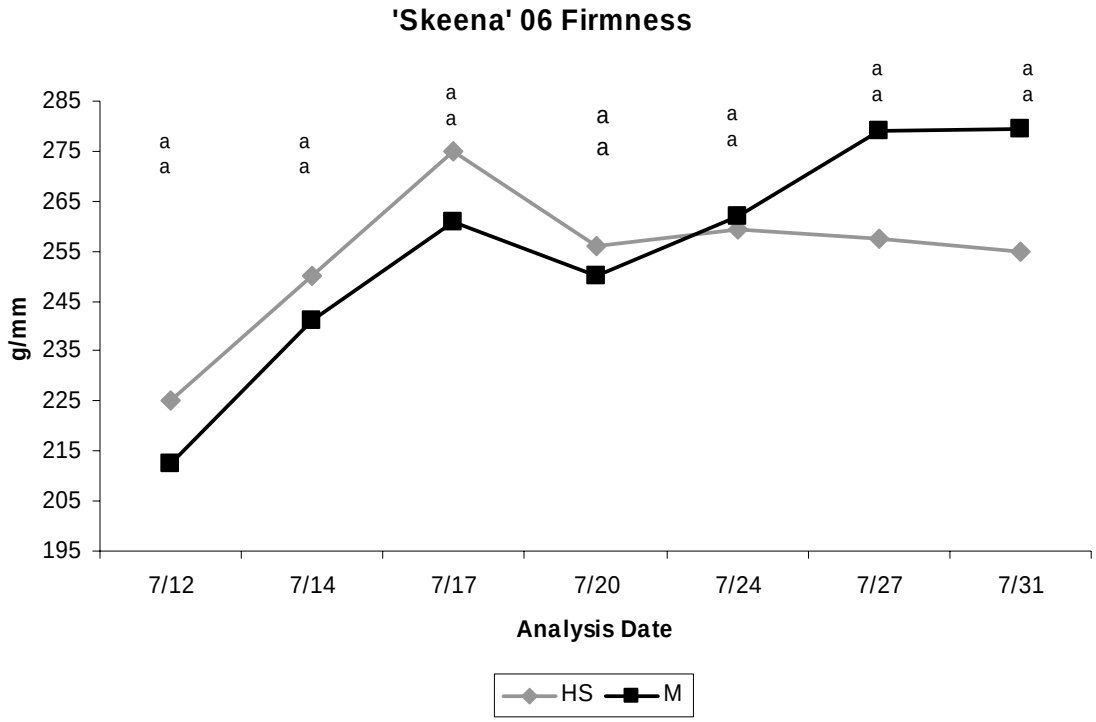

Figure 3-6. 'Skeena' 2006 analysis of firmness (g mm^{-1}) 19 days of storage from harvest at 12 July to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

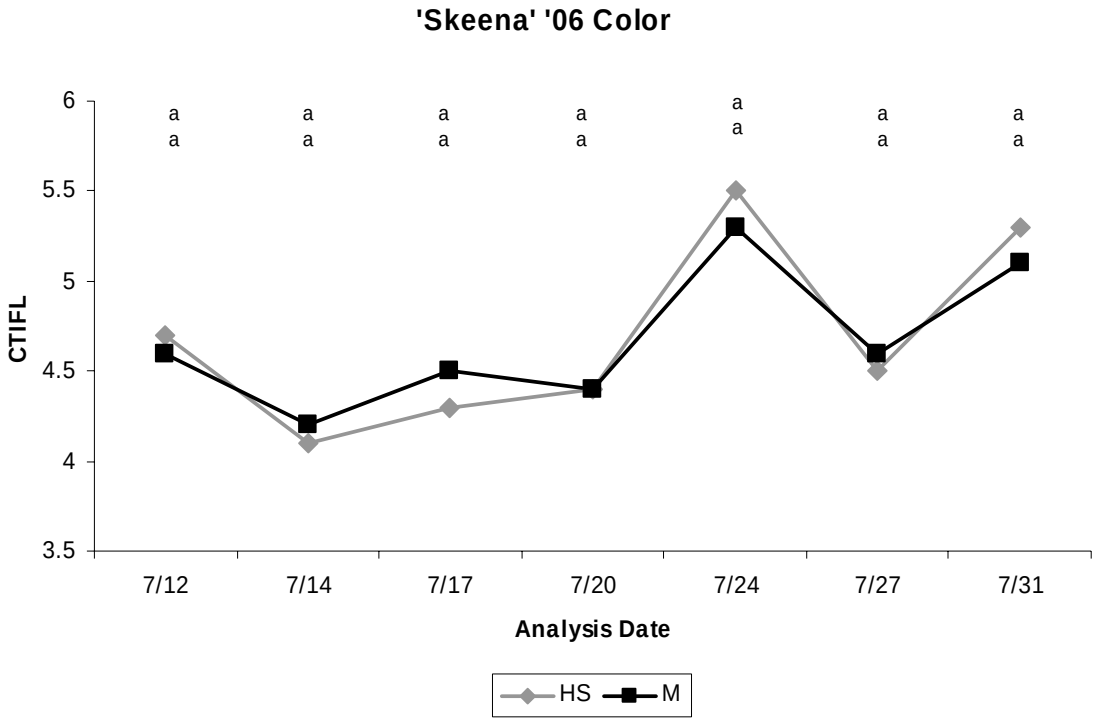

Figure 3-7. 'Skeena' 2006 analysis of color (CTIFL, chips 1-7) 19 days of storage from harvest at 12 July to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

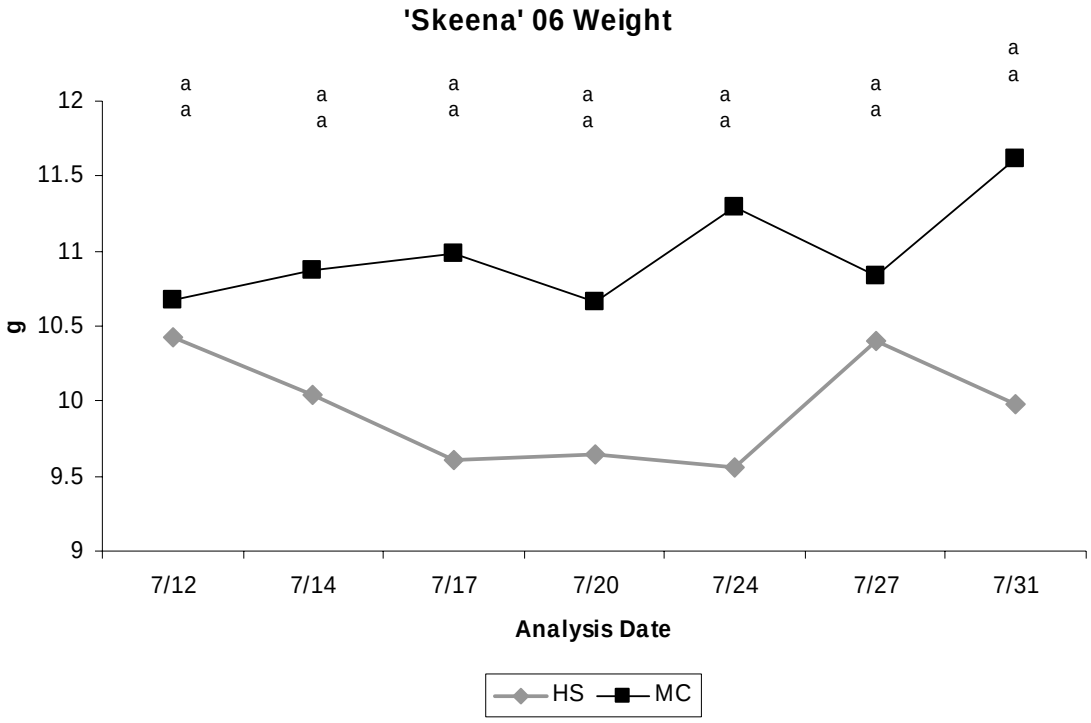

Figure 3-8. 'Skeena' 2006 analysis of weight (g) 19 days of storage from harvest at 12 July to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

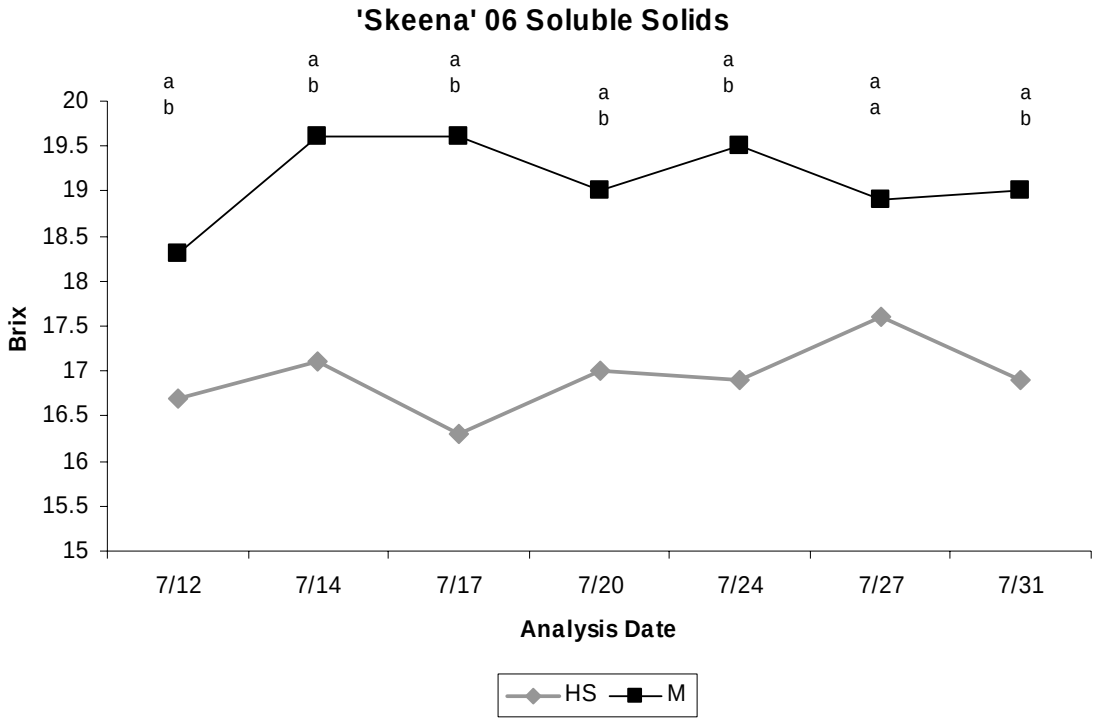

Figure 3-9. 'Skeena' 2006 analysis of soluble solids (ºBrix) 19 days of storage from harvest at 12 July to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

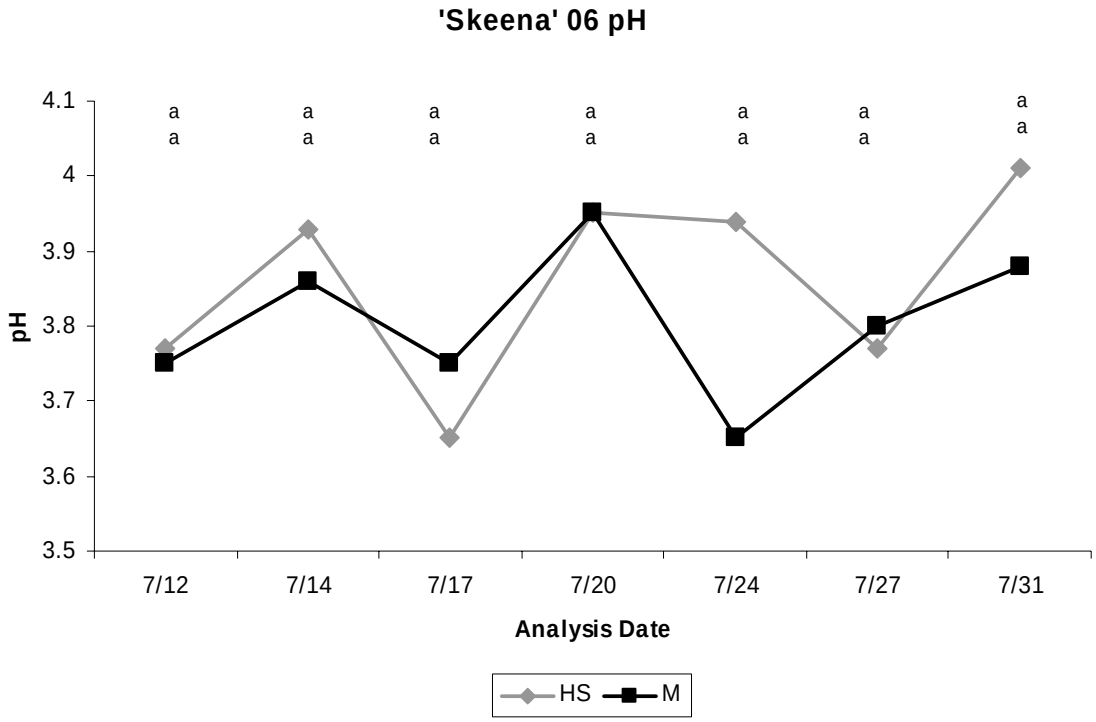

Figure 3-10. 'Skeena' 2006 analysis of pH 19 days of storage from harvest at 12 July to 31 July. Note table 3-1 for treatment description. Mean separation within each sample date is indicated by lower case letter positioned above analysis date by LSD, $P \leq 0.05$.

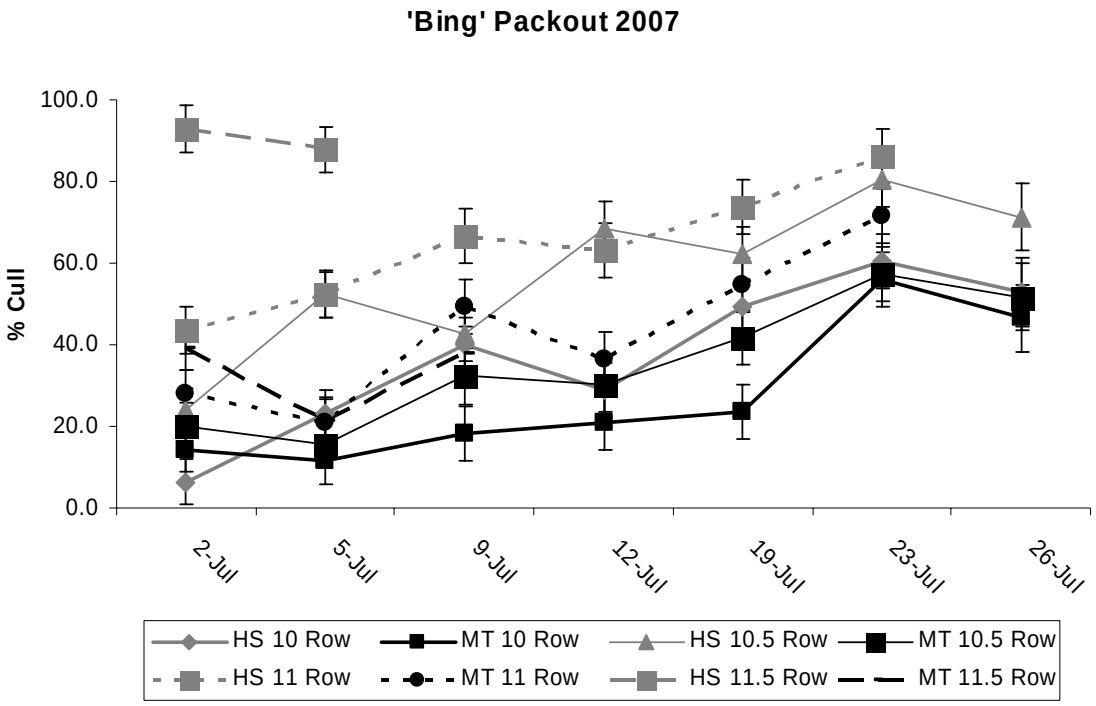

Figure 3-11 'Bing' 2007 analysis of cull rate through 29 days of storage from harvest at 27 June to 26 July. Note table 3-1 for treatment description. Row size 10 = 26.6 mm, 10.5 = 25.4 mm, 11 = 24.2 mm, and 11.5 = 22.6 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

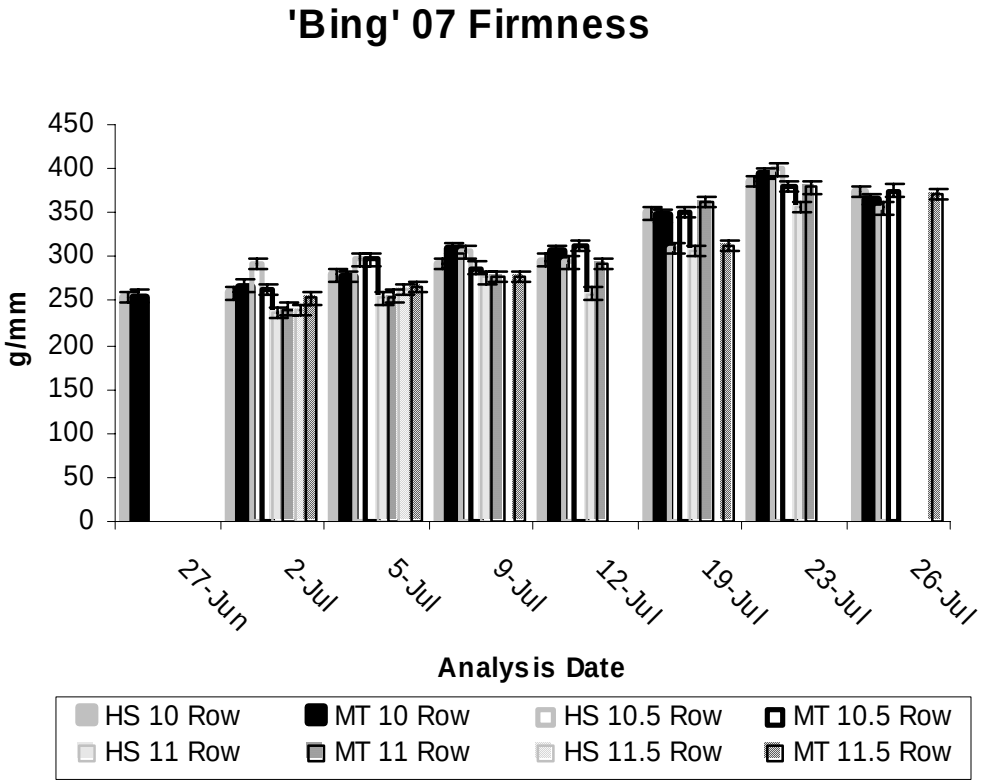

Figure 3-12 'Bing' 2007 analysis of firmness (g mm^{-1}) through 29 days of storage from harvest at 27 June to 26 July. Note table 3-1 for treatment description. Row size 10 = 26.6 mm, 10.5 = 25.4 mm, 11 = 24.2 mm, and 11.5 = 22.6 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

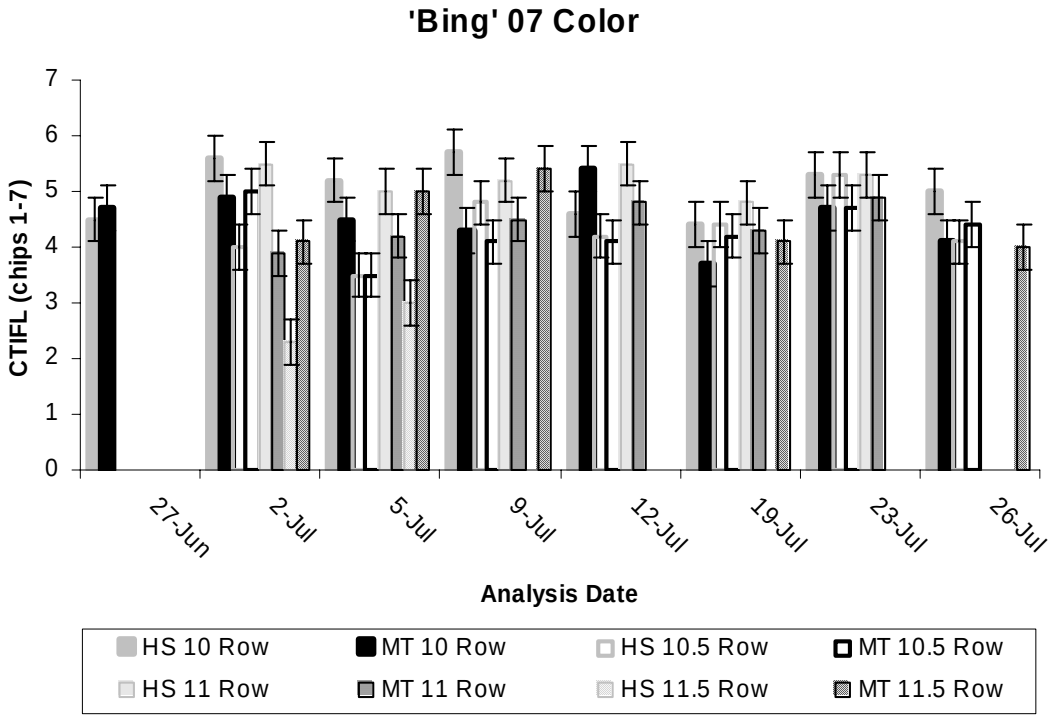

Figure 3-13 'Bing' 2007 analysis of color (CTIFL, chips 1-7) through 29 days of storage from harvest at 27 June to 26 July. Note table 3-1 for treatment description. Row size 10 = 26.6 mm, 10.5 = 25.4 mm, 11 = 24.2 mm, and 11.5 = 22.6 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

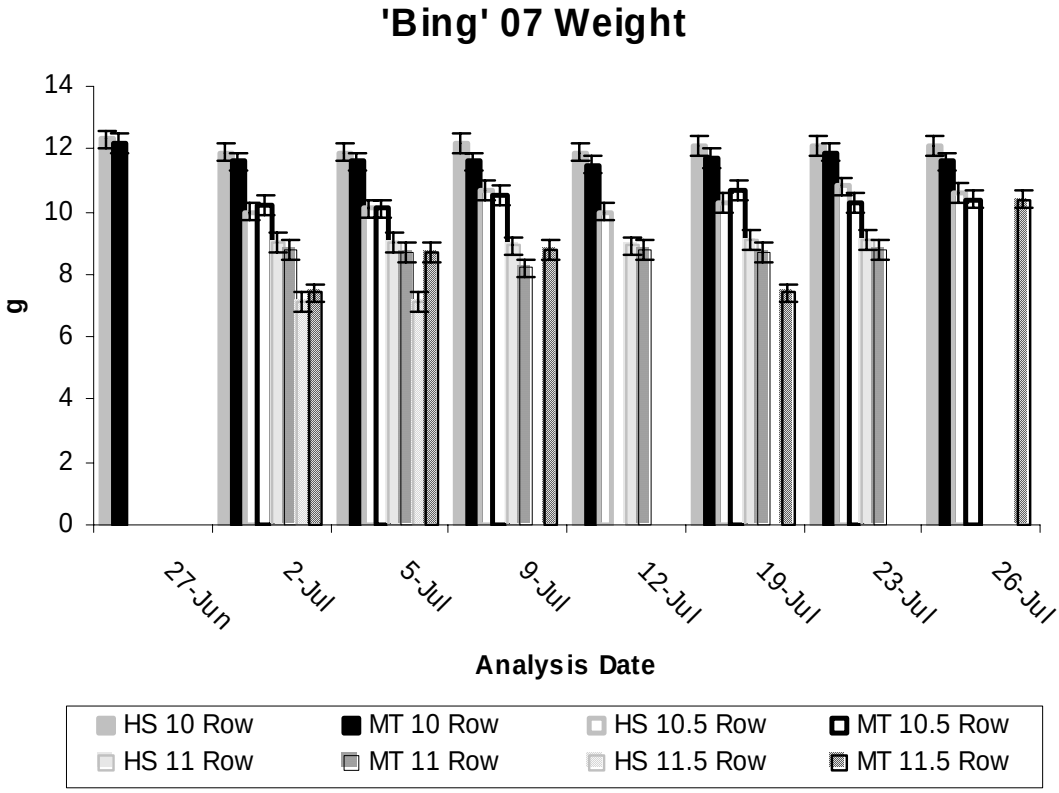

Figure 3-14 'Bing' 2007 analysis of weight (g) through 29 days of storage from harvest at 27 June to 26 July. Note table 3-1 for treatment description. Row size 10 = 26.6 mm, 10.5 = 25.4 mm, 11 = 24.2 mm, and 11.5 = 22.6 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

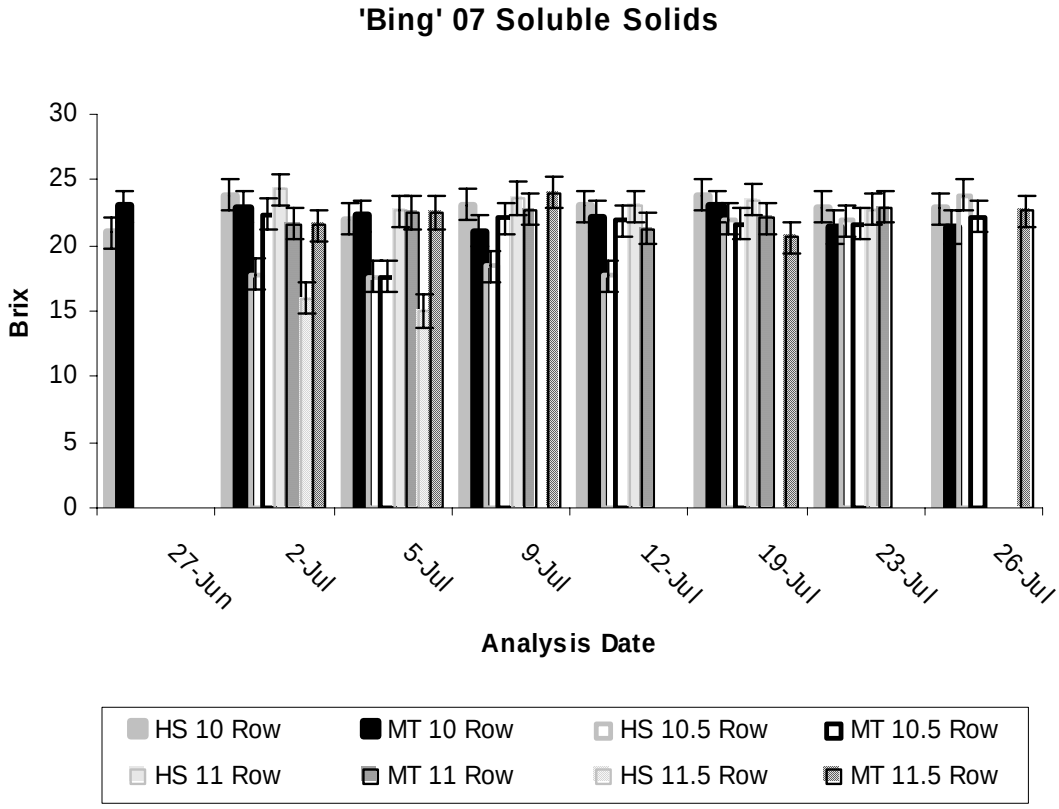

Figure 3-15 'Bing' 2007 analysis of soluble solids (ºBrix) through 29 days of storage from harvest at 27 June to 26 July. Note table 3-1 for treatment description. Row size 10 = 26.6 mm, 10.5 = 25.4 mm, 11 = 24.2 mm, and 11.5 = 22.6 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

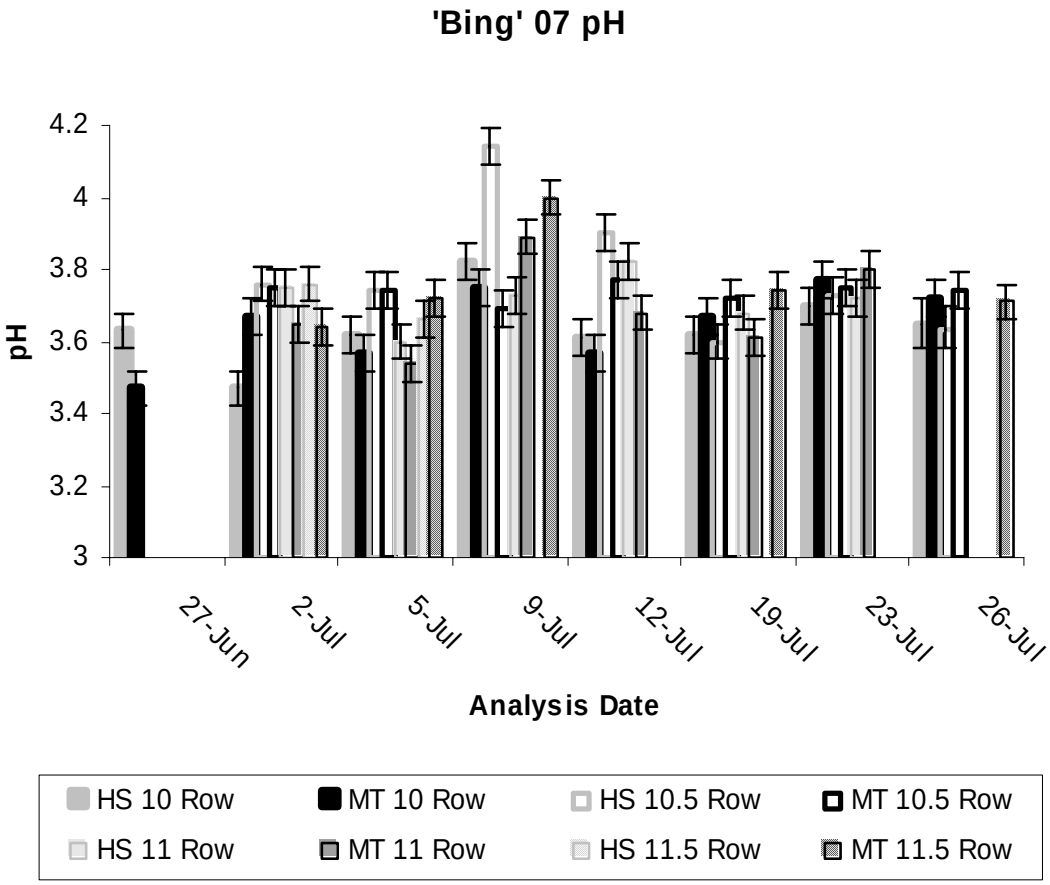

Figure 3-16 'Bing' 2007 analysis of pH through 29 days of storage from harvest at 27 June to 26 July. Note table 3-1 for treatment description. Row size 10 = 26.6 mm, 10.5 = 25.4 mm, 11 = 24.2 mm, and 11.5 = 22.6 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

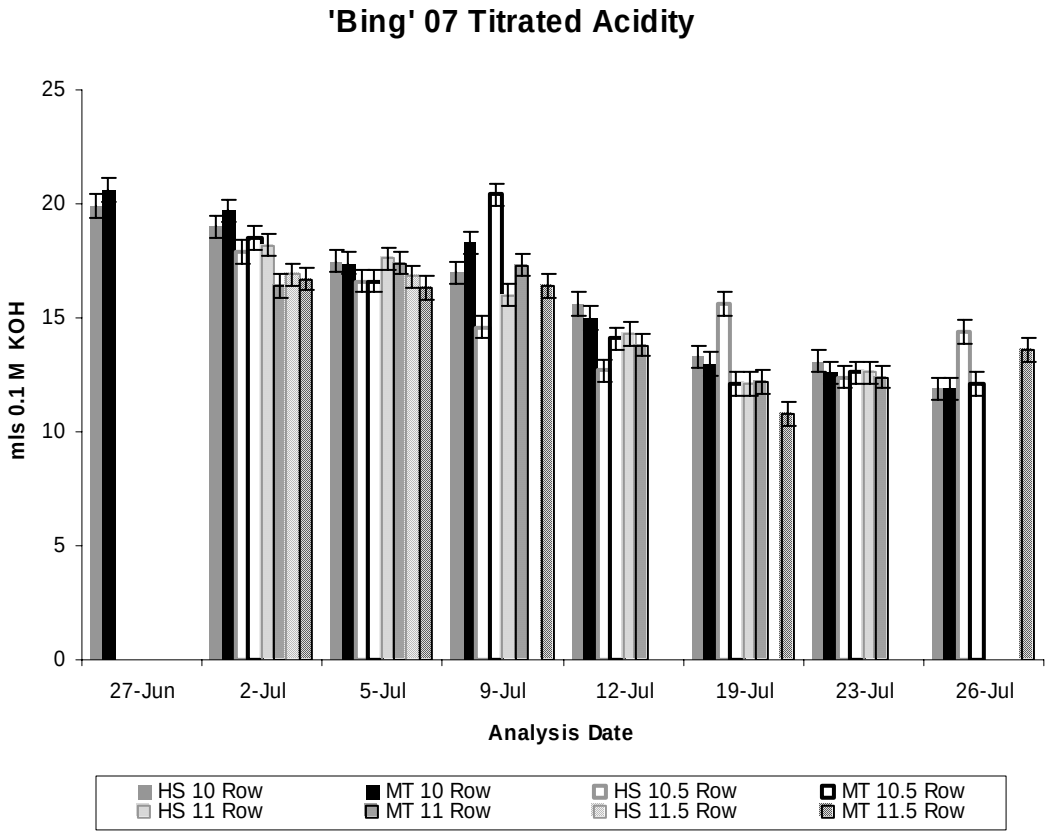

Figure 3-17 'Bing' 2007 analysis of titratable acidity (mls of 0.1 M KOH) through 29 days of storage from harvest at 27 June to 26 July. Note table 3-1 for treatment description. Row size 10 = 26.6 mm, 10.5 = 25.4 mm, 11 = 24.2 mm, and 11.5 = 22.6 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

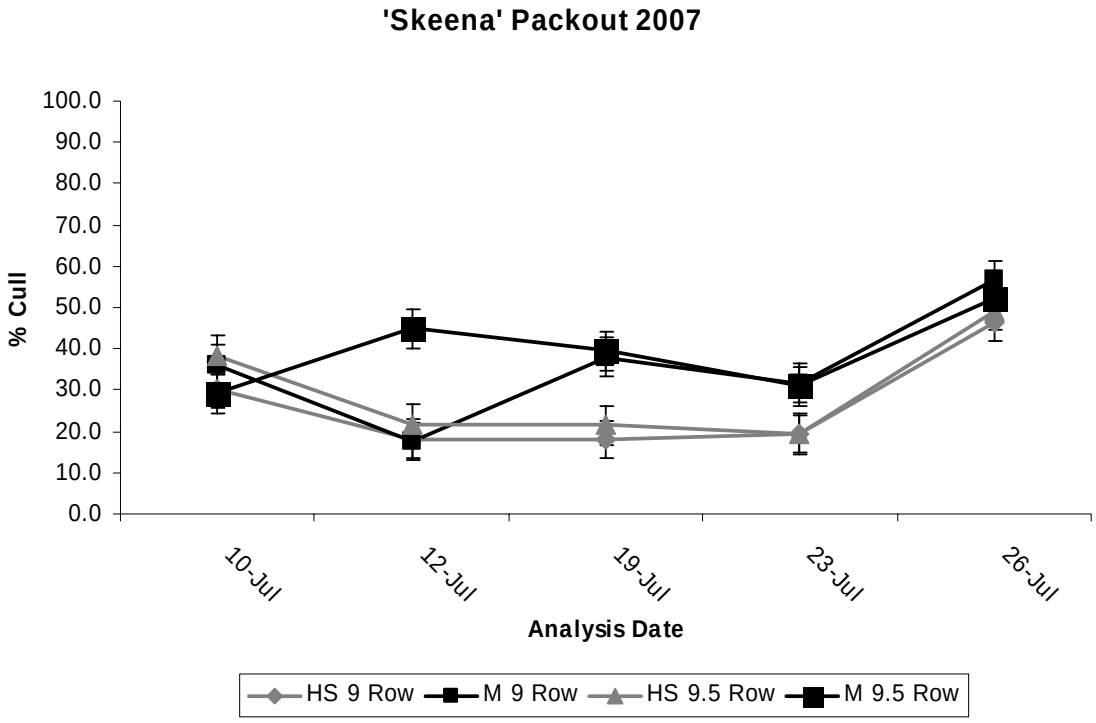

Figure 3-18 'Skeena' 2007 analysis of cull rate through 21 days of storage from harvest at 5 July to 26 July. Note table 3-1 for treatment description. Row size 9 = 29.8 mm and 9.5 = 28.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

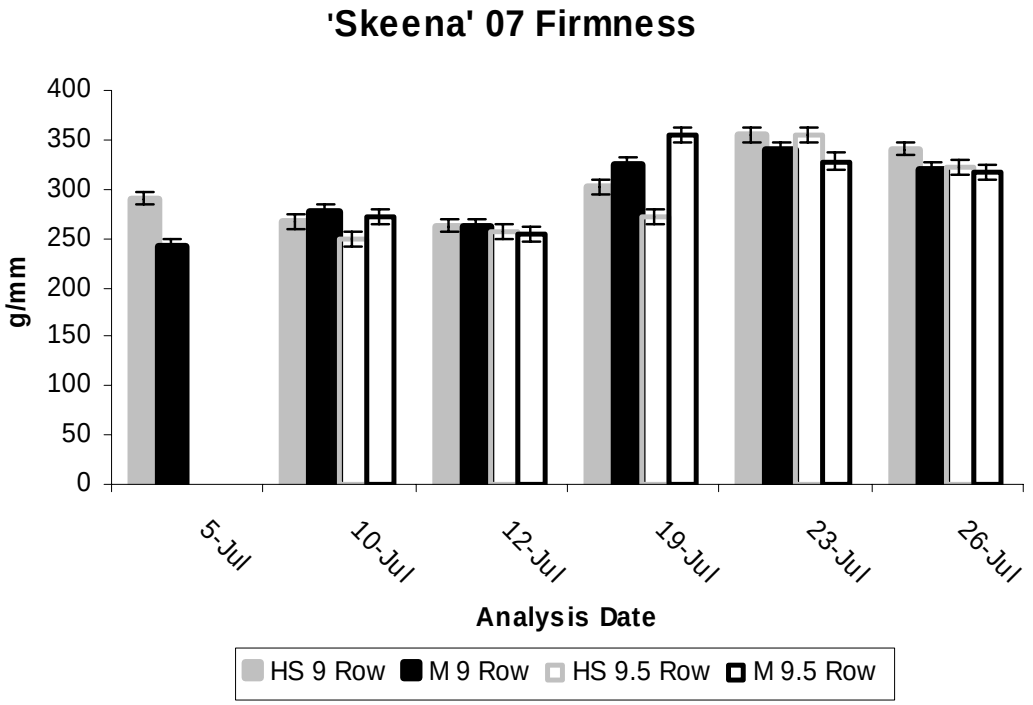

Figure 3-19 'Skeena' 2007 analysis of firmness (g mm^{-1}) through 21 days of storage from harvest at 5 July to 26 July. Note table 3-1 for treatment description. Row size 9 = 29.8 mm and 9.5 = 28.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

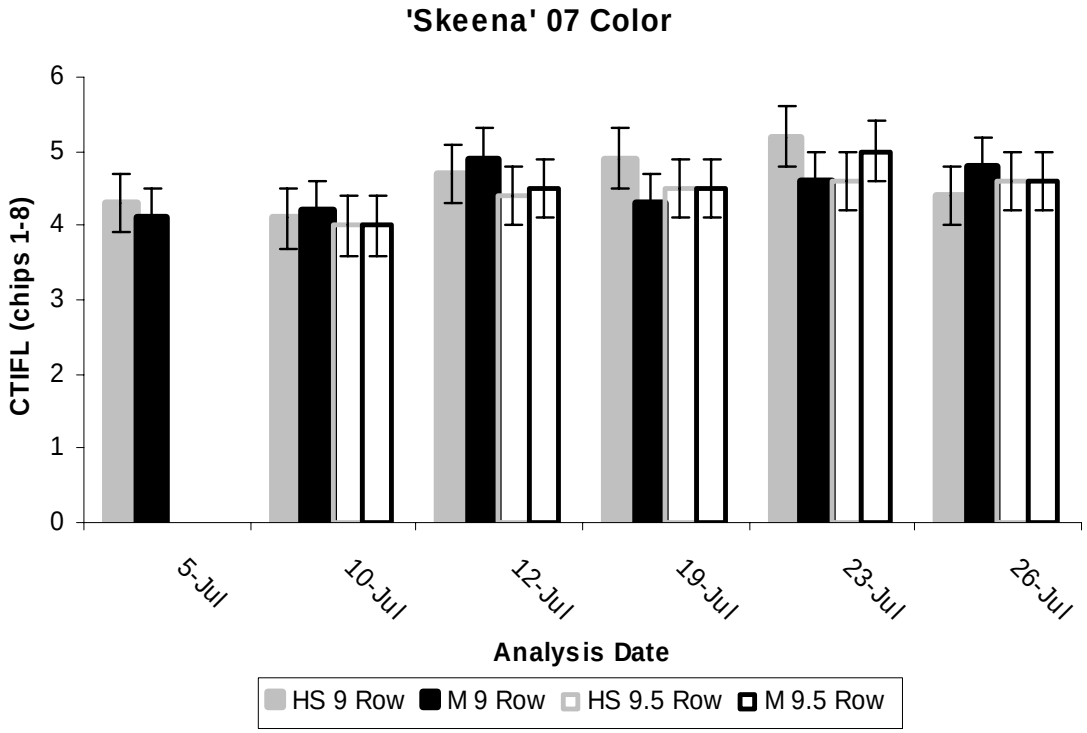

Figure 3-20 'Skeena' 2007 analysis of color (CTFIL, chips 1-7) through 21 days of storage from harvest at 5 July to 26 July. Note table 3-1 for treatment description. Row size 9 = 29.8 mm and 9.5 = 28.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

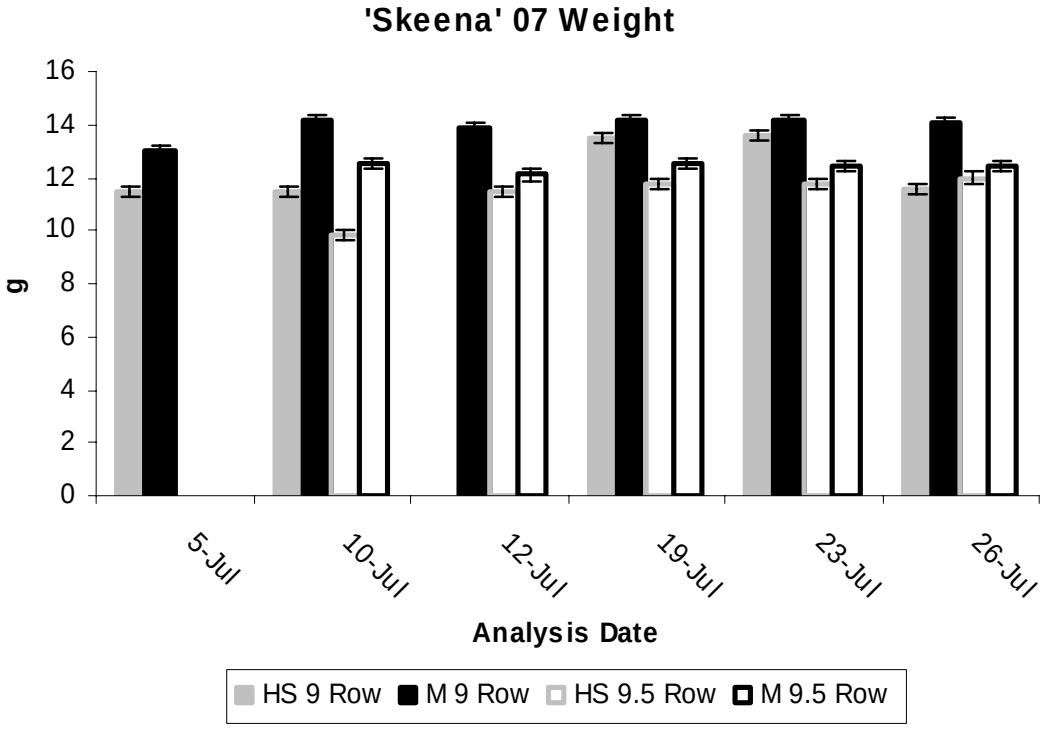

Figure 3-21 'Skeena' 2007 analysis of weight (g) through 21 days of storage from harvest at 5 July to 26 July. Note table 3-1 for treatment description. Row size 9 = 29.8 mm and 9.5 = 28.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

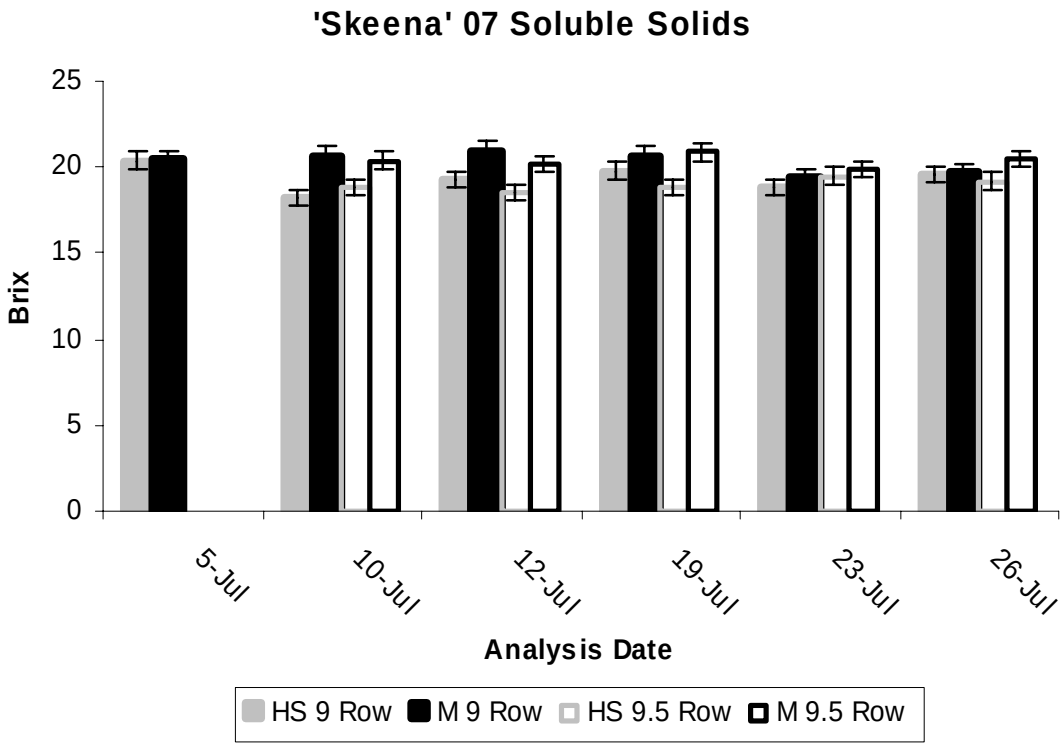

Figure 3-22 'Skeena' 2007 analysis of soluble solids (ºBrix) through 21 days of storage from harvest at 5 July to 26 July. Note table 3-1 for treatment description. Row size 9 = 29.8 mm and 9.5 = 28.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

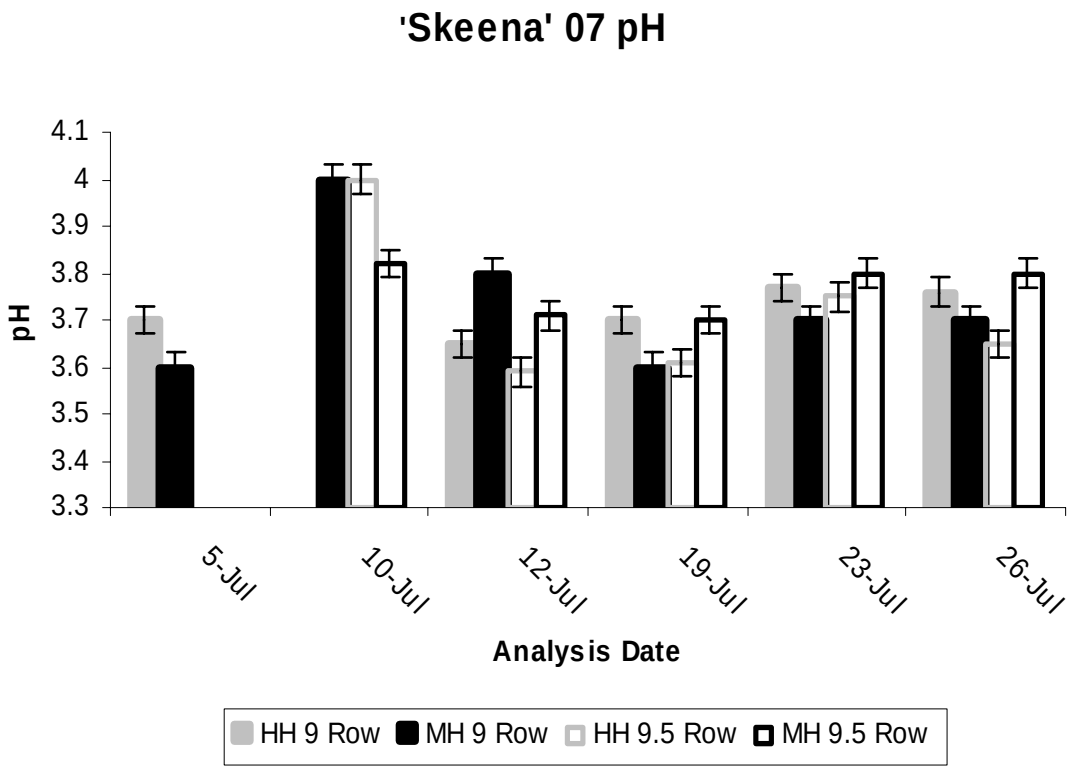

Figure 3-23 'Skeena' 2007 analysis of pH through 21 days of storage from harvest at 5 July to 26 July. Note table 3-1 for treatment description. Row size 9 = 29.8 mm and 9.5 = 28.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

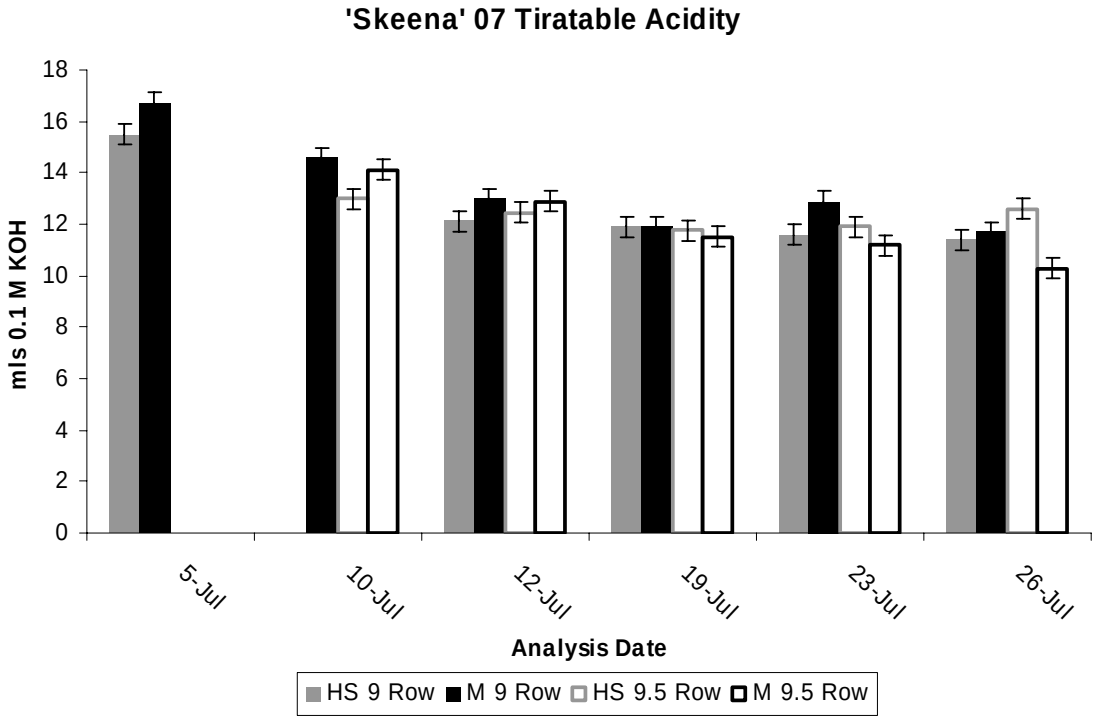

Figure 3-24 'Skeena' 2007 analysis of titratable acidity (mls of 0.1M KOH) through 21 days of storage from harvest at 5 July to 26 July. Note table 3-1 for treatment description. Row size 9 = 29.8 mm and 9.5 = 28.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

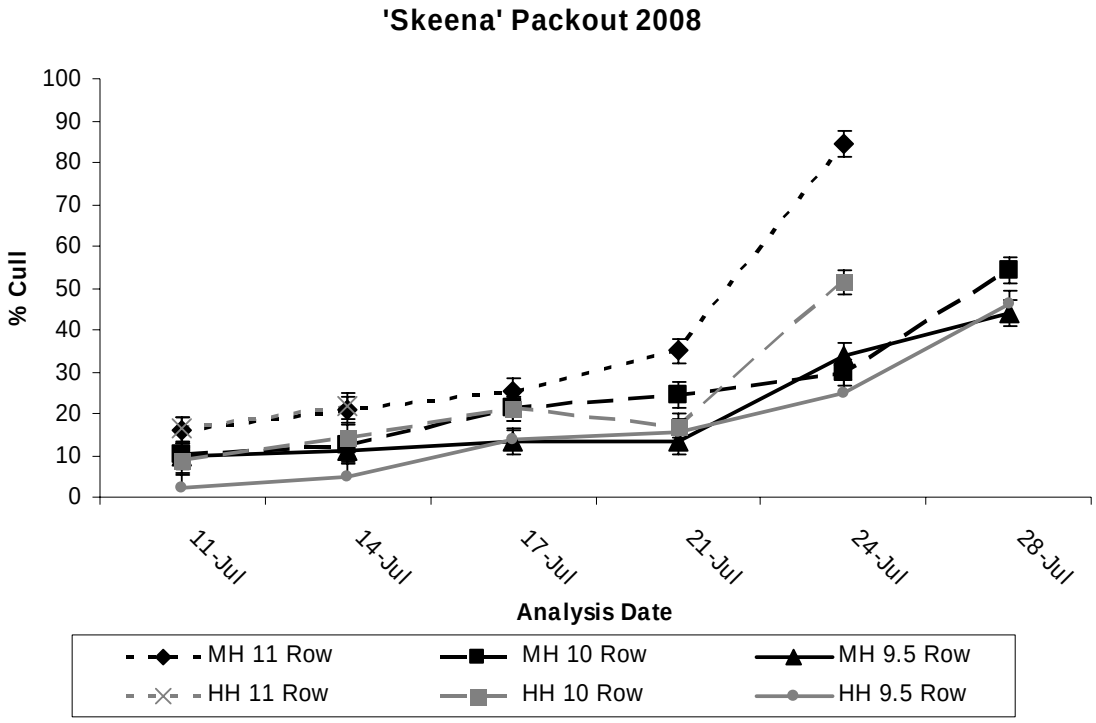

Figure 3-25 'Skeena' 2008 analysis of cull rate through 19 days of storage from harvest at 9 July to 28 July. Note table 3-1 for treatment description. Row size 9.5 = 28.2 mm, 10 = 26.6 mm, and 11 = 24.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

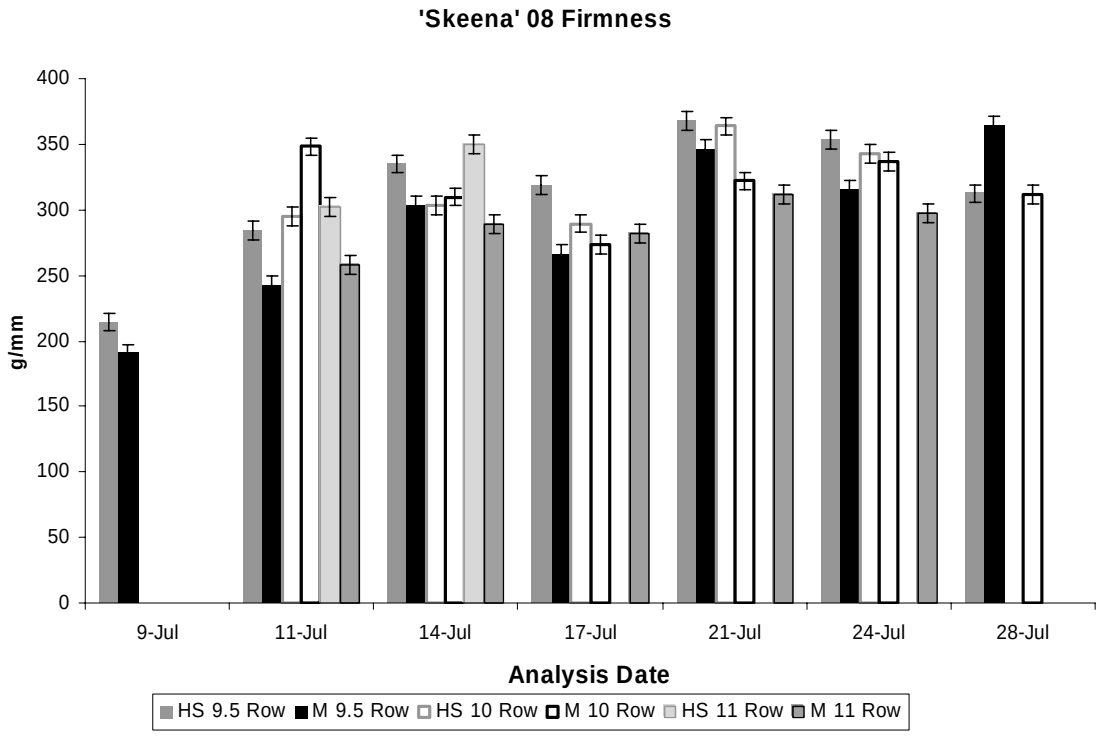

Figure 3-26'Skeena' 2008 analysis of firmness (g mm^{-1}) through 19 days of storage from harvest at 9 July to 28 July. Note table 3-1 for treatment description. Row size 9.5 = 28.2 mm, 10 = 26.6 mm, and 11 = 24.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

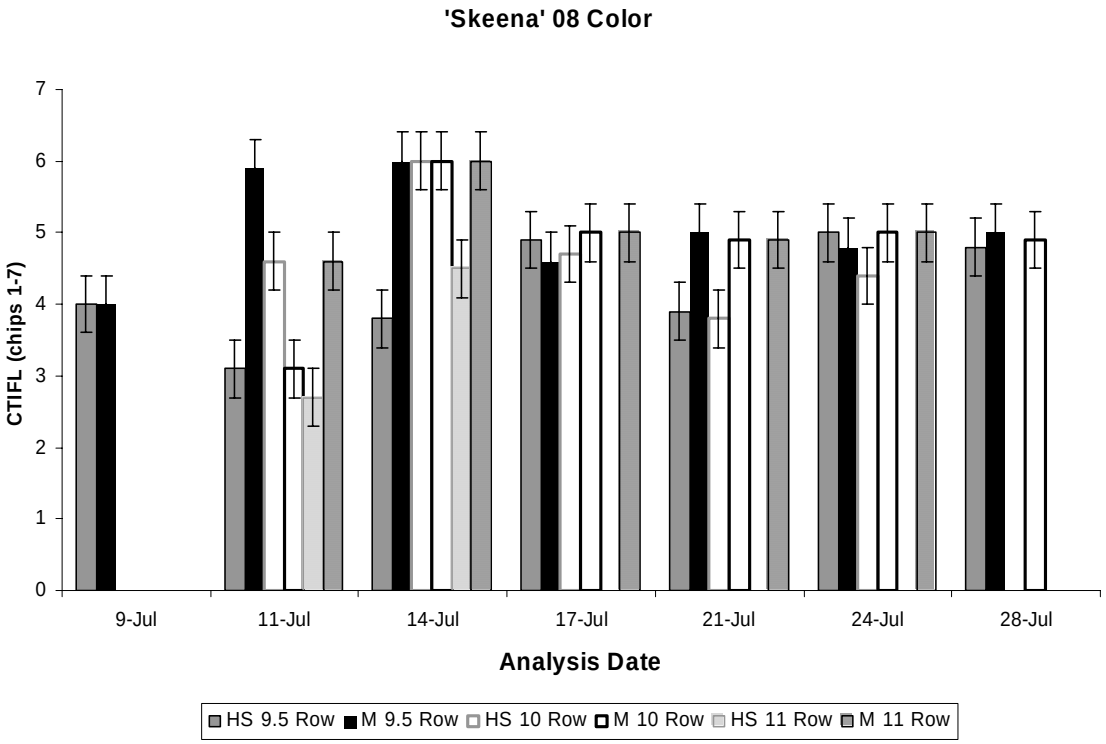

Figure 3-27 'Skeena' 2008 analysis of color (CTIFL, chips 1-7) through 19 days of storage from harvest at 9 July to 28 July. Note table 3-1 for treatment description. Row size 9.5 = 28.2 mm, 10 = 26.6 mm, and 11 = 24.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

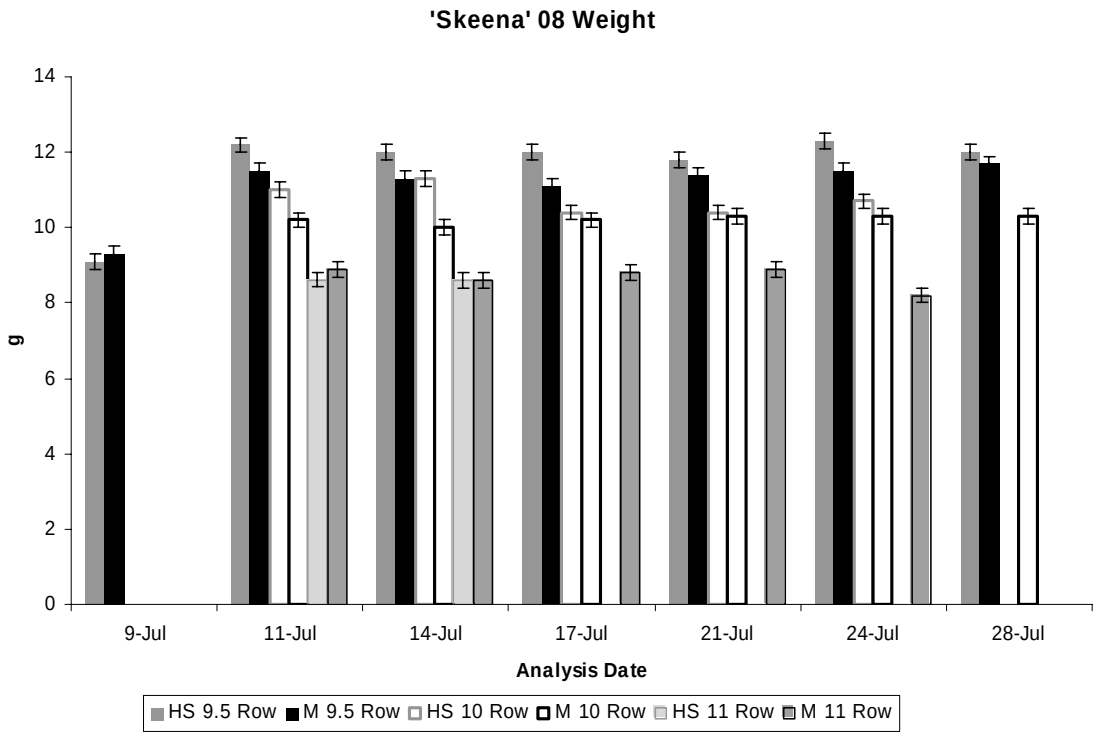

Figure 3-28 'Skeena' 2008 analysis of weight (g) through 19 days of storage from harvest at 9 July to 28 July. Note table 3-1 for treatment description. Row size 9.5 = 28.2 mm, 10 = 26.6 mm, and 11 = 24.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

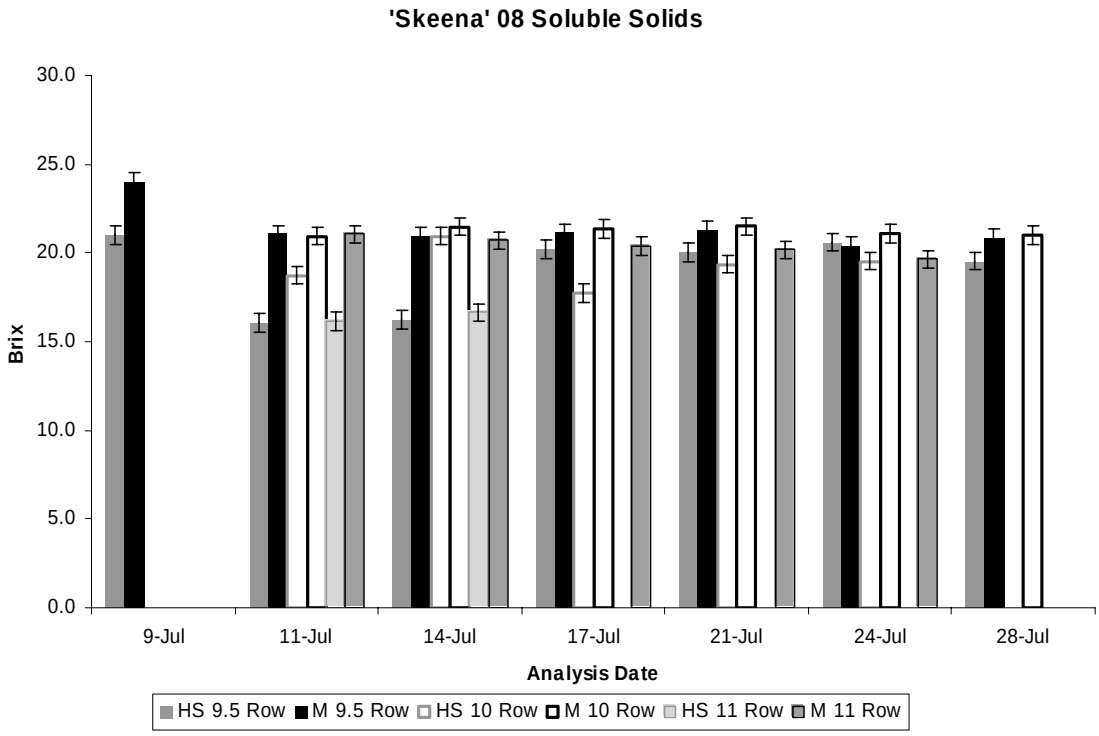

Figure 3-29 'Skeena' 2008 analysis of soluble solids (ºBrix) through 19 days of storage from harvest at 9 July to 28 July. Note table 3-1 for treatment description. Row size 9.5 = 28.2 mm, 10 = 26.6 mm, and 11 = 24.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

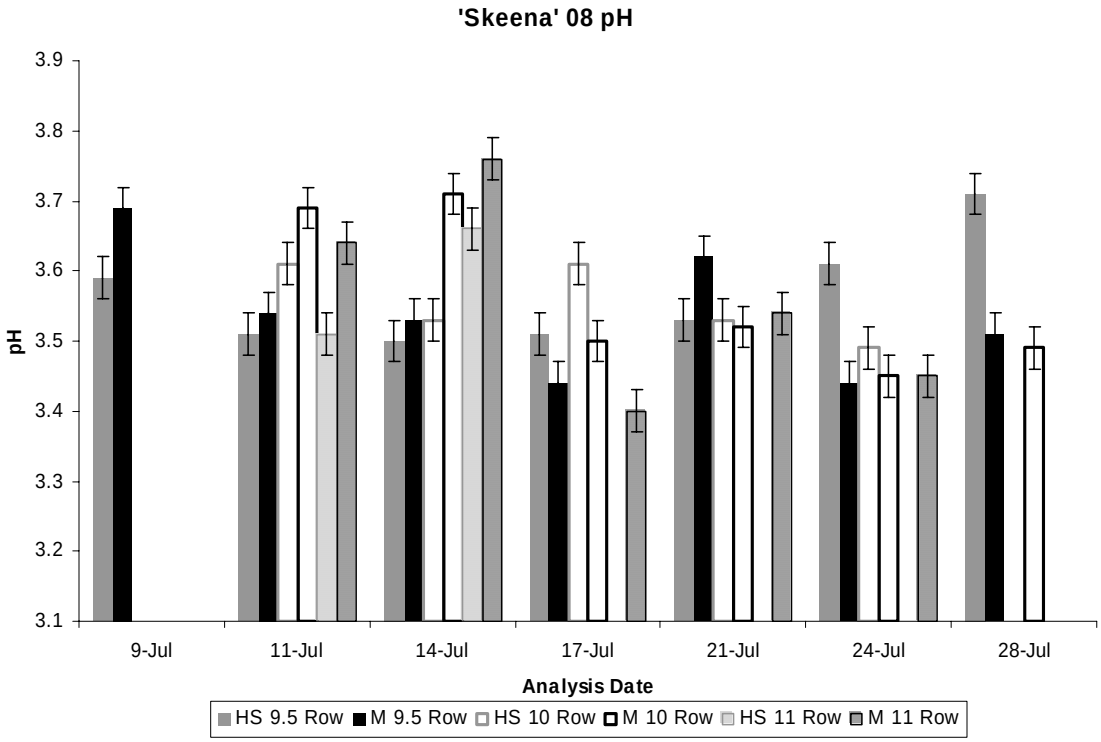

Figure 3-30 'Skeena' 2008 analysis of pH through 19 days of storage from harvest at 9 July to 28 July. Note table 3-1 for treatment description. Row size 9.5 = 28.2 mm, 10 = 26.6 mm, and 11 = 24.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

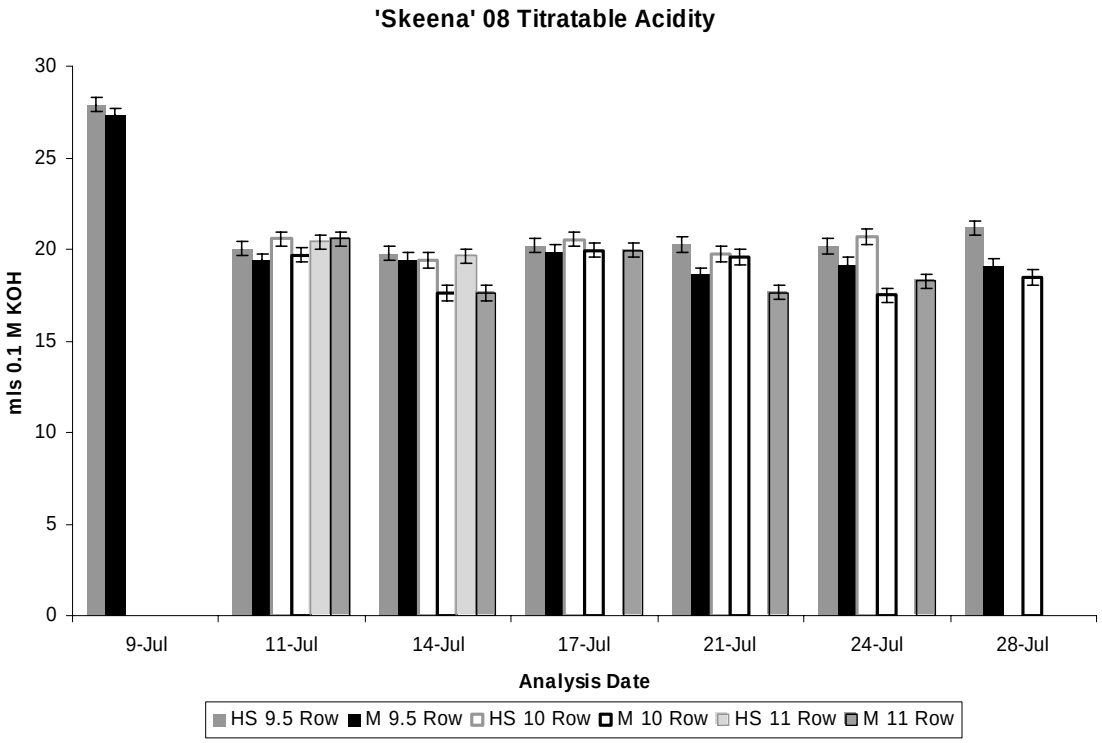

Figure 3-31 'Skeena' 2008 analysis of titratable acidity (mls of 0.1 M KOH) through 19 days of storage from harvest at 9July to 28 July. Note table 3-1 for treatment description. Row size 9.5 = 28.2 mm, 10 = 26.6 mm, and 11 = 24.2 mm. Bars indicate standard error calculated from the three way interaction of harvest method*row size*date analyzed.

Chapter IV

EFFECTS OF STORAGE ON PEDICEL QUALITY AND WEIGHT LOSS OF STEM AND STEM-FREE SWEET CHERRY FRUIT

Abstract

Mechanized sweet cherry (*Prunus avium* L.) harvest yields stem-free fruit, raising concerns over storability and the role of the pedicel. In 2007 and 2008, we evaluated postharvest weight loss, pedicel conductivity, and pedicel quality in cultivars 'Bing' and 'Skeena'. For weight loss studies, we sealed the fruit surface and/or pedicel with petroleum jelly (PJ) to form a barrier to water loss. Six (2007) and 8 (2008) treatments were compared for both cultivars: 1) no PJ, with stem, 2) no PJ, stem-free, 3) pedicel and fruit sealed with PJ, 4) pedicel sealed with PJ, 5) fruit surface sealed with PJ, 6) stem-free fruit with PJ applied to the abscission zone, 7) stem-free ethephon treated [3.5 L ha^{-1} (3 pt A^{-1})], no PJ and 8) stem-free ethephon treated with PJ applied to the abscission zone. At harvest, individual fruit were treated, weighed, placed into modified atmosphere packaging , and stored at 4 ºC for three weeks. In 2007, untreated, stem-free (2), 'Bing' lost significantly more weight (9.2%) than stemmed (1) 'Bing' (6.5%). Conversely, in 2008 untreated 'Bing' stem-free (2) and stemmed (1) fruit exhibited similar weight loss of 5.0% and 6.0%, respectively. Ethephon treatments (7 & 8) did not affect 'Bing' weight loss. 'Skeena' treatments 1 & 2 were not different (8.7% vs. 7.8% in 2007 and 6.4% vs. 6.8% in 2008, respectively). For both cultivars, treatments 4 & 6 did not significantly reduce weight loss compared to treatment 1. However, treatments 3 & 5 significantly and similarly reduced weight loss for both cultivars – mean weight loss of treatment 5 was

1.5% (2007) and 1.4% (2008) for ‘Skeena’ and 0.5% (2007) and 2.4% for ‘Bing’. These results demonstrate that weight loss occurs predominantly via the fruit exocarp and not the pedicel. In addition, static dye uptake experiments using basic fuchsin (0.1%) revealed no uptake into pedicels from either stylar or stem end of ‘Bing’ fruit for both years. In contrast, we observed dye uptake into the pedicel from stylar end immersion of ‘Skeena’ fruit in 2007 but not in 2008. Assessments of pedicel quality (i.e., color and diameter) revealed a hastening of discoloration in cold storage. Pedicel discoloration (% brown) was ca. 67% for ‘Bing’ and 81% for ‘Skeena’ in 2007 after 7 d in 4 ºC storage for ‘Bing’ and ‘Skeena’, respectively. In contrast, neither cultivar exhibited more than 10% browning after 7 days storage at room temperature in 2008. Maximum pedicel shrivel in both years was greatest by 4 days after harvest, irrespective of cultivar.

Introduction

Sweet cherry fruit harvested using the USDA/ARS prototype (Peterson and Wolford, 2001) are stem-free. Previous work has demonstrated a positive potential for harvesting and marketing fresh market quality stem-free cherries (Peterson et al., 2003, Peterson and Wolford, 2001, Drake et al., 1989, Chapter III) yet no research has investigated the pedicel’s role on storability. Anecdotal evidence suggests that pedicel quality (i.e., color and diameter) deteriorates at a faster rate than do fruit quality (L. Lancaster, per. commun.). Shriveled and brown pedicels detract from the visual appeal of sweet cherries at retail. Woodbridge and Crandell (1955) surveyed Yakima Valley sweet cherry growers for possible causes of stem shrivel and identified early morning harvest, field heat removal, and high humidity in storage as critical for minimization of pedicel shrivel.

More recently, Mattheis and Fellman (2004) underscored the importance of temperature and humidity control for maintaining sweet cherry fruit quality in storage. Fruit stored at 0 ºC with relative humidity at 95 % can expect a postharvest life of 2 to 4 weeks (Mattheis and Fellman, 2004). Further, controlled atmosphere (CA) storage can extend the shelf-life (i.e., edible qualities) of sweet cherries by up to ca. eight weeks; however the vegetative tissue of the pedicel begins to lose greenness within 3 weeks in either CA of regular atmospheric storage (Patterson and Kupferman, 2000, Drake et al., 1988). Dehydration causes the pedicel to shrink and brown, in addition, physical damage due to harvest, transport, and processing can alter the cell permeability further increasing the rate of dehydration (Horvitz et al., 2003). Even with rapid field heat removal, use of reflective tarps to cover fruit bins, and early morning harvest, pedicels dehydrate quickly due to their large surface to area volume ratio (Schick and Toivonen, 2000). The declining nature of the stem detracts the consumer, causing a perception that optimal freshness for consumption has passed (Drake et al., 1989, Schick and Toivonen, 2000).

Water loss from sweet cherries after harvest is predominantly via the exocarp though the pedicel-fruit juncture also plays a role (Weichert and Knoche, 2006, Beyer et al., 2005, and Beyer et al., 2002). Histological studies have shown cherry fruit cell expansion during stage III of development leads to increases in surface area that exceeds the rate of production for epicuticular waxes, causing microcracks that facilitate uptake and evaporation of water (Peschel and Knoche, 2005, Christensen, 1996, and Glenn and Poovaiah, 1989). However, Drake et al., (1989) reported that weight loss in stem-less sweet cherry fruit was insignificant compared to stemmed fruit. Yet, the rate of water loss through the stem is largely unknown. It appears that pedicels have capacity for

conductance after harvest. In potometry studies of cultivars ‘Van’ and ‘Sunburst’, with 1-2 mm cuts into the exocarp, showed water uptake rates of 2.4 µl fruit^{-1} h^{-1} and 1.4 µl fruit^{-1} h^{-1}, respectively (Hovland and Sekse, 2004). However, fruit that is not ruptured did not have significant conductance of water (Hovland and Sekse, 2004), which still leaves questions about passive water transport out of the pedicel.

Drake et al. (1989) showed that hand harvested stem-free fruit are comparable to stemmed fruit in quality (soluble solids, weight loss and firmness) and in chapter III of this volume; I reported that fruit quality is not reduced by mechanical harvest. Consumer and retailer acceptance of stem-free cherries may be hindered by public perception that a fresh market sweet cherry has a stem and is an indicator of freshness. In this study we attempt to answer questions about passive water conductance through the pedicel, rate of stem degradation in market environment and pedicel-fruit juncture water loss.

Materials and Methods

Plant materials. Experiments were carried out between 2007 and 2008. All trees were located at Washington State University’s Roza Farm, about 10 km north of Prosser, Washington, USA (46.2ºN, 119.7ºW). All trees were irrigated weekly from bloom to leaf senescence with low-volume under-tree microsprinklers and grown using standard orchard management practices. In 2007, ‘Bing’ fruit were harvested at commercial maturity from 13-year-old trees that are grafted to various precocious rootstocks (Gisela® 7, 6, and 5) with an in-row spacing of 2.44 m (8 ft) and between row spacing of 4.27 m (14 ft). Rows were planted in a north-south orientation and trained to Y-trellis architecture at various angles. In 2008, ‘Bing’ fruit were harvested at commercial maturity from 7-year-old trees that are grafted to Gisela® 12 with an in-row spacing of

2.44 m (8 ft) and between row spacing of 4.27 m (14 ft). ‘Skeena’ fruit were harvested in 2007 and 2008 at commercial maturity from 6 and 7-year-old trees, respectively. Scions were grafted to Gisela® 12 rootstock, trained in a Y-trellis system with between-tree spacing of 1.52 m (5 ft) and 4.27 m (14 ft) between rows.

Weight Loss. For weight loss analyses, all ‘Bing’ and ‘Skeena’ fruit were hand harvested in the standard manner; pedicels were manually removed after harvest to create the stem-free treatment. Fruit were selected for uniform ripeness utilizing color minimum of 3 (Centre Techique Interprofessionnel des Légumes [CTIFL]), minimum diameter of 24.2 mm (11 row) and lack of damage. Six groups of ten replicate fruit (i.e., 60 per treatment) were subjected to one of six treatments designed to isolate the pedicel’s role in weight loss using a petroleum jelly (PJ) barrier to water loss: 1) no PJ, with stem, 2) no PJ, stem-free, 3) pedicel and fruit sealed with PJ, 4) pedicel sealed with PJ, 5) fruit surface sealed with PJ, and 6) stem-free fruit with PJ applied to abscission zone (Table 1). In 2008, two treatments were added to ‘Bing’. Ethephon was applied two weeks prior to harvest at 3.5 L ha^{-1} (3 pt A^{-1}) on six ‘Bing’/ Gisela® 12 trees. The fruit were evaluated as stem-free treatments; 7) ethephon-treated, no PJ applied and 8) ethephon-treated PJ applied to the abscission zone. Each fruit was treated, placed into a numbered weighing cup and weighed. The treatments comprised of 6 or 8 ten-fruit sets each were placed into a standard commercial storage box and the box was sealed within standard modified atmosphere packaging (MAP [LifeSpan, PS 801 for cherry]). On each subsequent sampling date, one box of the 10-fruit sets was removed from storage, brought to room temperature, and each fruit was weighed. In addition, firmness [(g mm^{-1}) Firmtech, Bioworks, Inc., Wamego, Kans] analysis was conducted on each fruit, with the exception

of ‘Skeena’ 2007 treatments due to size limitation (i.e. fruit were too large) and data collection by the firmtech software. For ‘Bing’ in 2007, harvest was on 27 June and evaluations occurred on 29 June (2 days after harvest, DAH), 2 July (5 DAH), 6 July (9 DAH), 10 July (13 DAH), 12 July (15 DAH) and 17 July (20 DAH). In 2008, ‘Bing’ was harvested on 30 Jun and evaluated on 30 July (3 DAH), 7 July (7 DAH), 10 July (10 DAH), 14 July (14 DAH), 17 Jul (17 DAH), and 21 July (21 DAH). For ‘Skeena’ in 2007, harvest occurred 6 July and evaluations preceded on 10 July (4 DAH), 12 July (6 DAH), 20 July (14 DAH), 24 July (18 DAH), and 27 July 21 DAH) and in 2008, harvest was 7 July and evaluations occurred 10 July (3 DAH), 14 July (7 DAH), 21 July (14 DAH), 24 July (17 DAH), and 28 July (21 DAH).

For treatments 1, 3, 4, and 5, pedicel-fruit retention force was analyzed immediately following weight measurements with a digital force gauge (Imada DPS-11, Imada Co., Northbrook, IL) with a custom fitted polyvinyl chloride attachment. Statistical analyses of means were performed using a general linear model (GLM) procedure in Statistical Analysis System (SAS) program (SAS Institute, Cary, N.C.)

Dye infiltration. To study water exchange between pedicel and fruit we conducted an observational study of passive dye uptake on ‘Bing’ and ‘Skeena’. These experiments utilized a xylem mobile dye, basic fushsin at 0.1% aqueous solution (Talboys, 1955). At harvest, randomly-selected fruit were incised transversely either at the middle of the pedicel or at the stylar end, approximately 1-1.5 cm up from the stylar scar. The fresh cut was directly immersed into the dye solution and sealed using Parafilm® (American National Can, Connecticut, U.S.A.). Fruit were held in solution at room temperature,

removed after 48 hrs, and the pedicel was dissected latitudinal and longitudinally. Pedicels were observed under a light-microscope to identify extent of dye infiltration.

Pedicel Quality. Evaluation of stem browning was accomplished using APS Assess image analysis software (Lamari, 2002). In 2007, 20 'Bing' fruit, harvested on 22 June, and 20 'Skeena' fruit, harvested on 6 July were individually numbered and placed into a weighing cup. The fruit were placed into 4 ºC storage and allowed to warm to room temperature prior to evaluations. Over a 7 day storage period, 5 evaluations of each fruit occurred: digital photograph of the same half of the pedicel throughout storage period, measure for stem diameter (mm) at a marked point on each pedicel, weighed (g), and measured for firmness [(g mm-1) Firmtech, Bioworks, Inc., Wamego, Kans]. The digital photographs of each fruit were entered in Adobe® Photoshop® CS 8.0, converted to bitmap files, and the image was cropped to retain only the pedicel. Each image file was then individually entered into Assess. Using tutorial 7 (disease measurement), pedicel bitmap files were evaluated under 'leaf' tab at upper slider pointer = 105 and lower slider pointer = 31 this sets the threshold for the leaf. Next, the 'lesion' analysis is set to upper slider pointer = 87 and lower slider pointer = 72 to identify brown hue. After these steps, the 'area %' toggle is used and the reading from the 'percent' window was recorded. In 2008, stem browning evaluations were conducted on 'Bing' (harvested 30 June) and 'Skeena' (harvested 7 July) fruit addressing the same criteria as 2007 except 10 fruit from each cultivar were evaluated and no cold storage was used. Statistical analyses of means were performed using a general linear model (GLM) procedure in Statistical Analysis System (SAS) program (SAS Institute, Cary, N.C.).

Results and Discussion

Weight Loss. 'Bing'. Sweet cherry fruit deteriorate rapidly after harvest and do not always reach the consumer at optimal quality. A major cause of deterioration is water loss that appears as shrivel, softening, and surface pitting (Toivonen et al., 2004). We observed significant differences ($P < 0.05$) in weight loss among the 6 treatments in 2007 and the 8 treatments in 2008 by the last sample date 20 and 21 DAH for 2007 and 2008, respectively. In 2007, weight loss for stemmed and stem-free 'Bing' fruit (i.e., treatments 1 and 2) was 6.6% and 9.2%, respectively over 20 days of storage (Fig. 4-1). The greater weight loss from stem-free fruit suggests the abscission zone did not seal fully and that water loss occurred via the abscission scar (PFRF ca.740 g, data not shown). Conversely, weight loss of treatments 1 and 2 were similar in 2008 at 6.0% and 5.0%, respectively. In 2007, crop load was normal to large and fruit were of high quality, however in 2008 crop load was low due to spring frosts and insufficient pollination. Patten et al. (1986) reported that cherries from early blossoms were of better quality than cherries from later blossoms. Further, fruit that mature at a high leaf : fruit ratio/low crop load tend to be firmer and of better quality than fruit that mature with low leaf : fruit ratio/high crop load (Spayd et al., 1986) suggesting the variation between 2007 and 2008 is a compound effect of leaf : fruit ratio and pollination timing.

Weight loss of stemmed fruit was similar to that from treatments 4 (pedicel present but sealed) and 6 (stem-free fruit with the abscission zone sealed) in both years. In addition, weight loss from treatments in which the exocarp was sealed (i.e., 3 and 5) was negligible (1.79% and 1.21%, treatments 3 and 5 in 2007: 0.64% and 2.17%, treatments 3 and 5 in 2008) and similar, both years. These results suggest that water loss

via the pedicel is negligible and confirms findings of Beyer et al. (2005) and Drake et al. (1989).

Fruit firmness varied throughout storage but was unaffected by the presence or absence of a pedicel. In 2007, treatment 1 fruit lost 30.4% firmness 9 DAH, and by 20 DAH lost only 9.3% (Fig. 4-2). In Chapter III, 'Bing' fruit showed a similar trend in 2006 of firmness through storage where at 6 DAH firmness was 18% less then at harvest and by 20 DAH a reduction of only 3% was recorded. Conversely, in 2008 at 10 DAH fruit averaged 4.3% firmer and by 21 DAH fruit were 11.6% firmer than at harvest which is a trend noted by Spayd et al. (1986) in trees with low crop load. Stem-free fruit showed a similar trend where a 14.4% reduction and 6.8% increase in firmness for 2007 and 2008, respectively. Further, after 21 days in storage in 2008, ethephon-treated fruit increased in firmness increase by 11.5% and 9.1% for treatments 7 and 8. When comparing average firmness of the ethephon-treated fruit (treatment 7 at 180 g mm^{-1}) and stem-free untreated fruit (216 g mm^{-1}) there was only 36 g mm^{-1} difference. In addition, analysis of sample dates across the treatments within the year show no significant variation ($P < 0.05$). In 2007, between treatments 1 and 2 the difference was 6% at 2 DAH and by 20 DAH was 5%. In 2008, treatments 1 and 2 had small variation of ca. 7% and 5% at samplings 3 and 21 DAH, respectively.

Pedicel-fruit retention force (PFRF) did not vary among the treatments for 2007 and 2008 (Fig. 4-3). In general, I observed a subtle increase in PFRF throughout the storage period in both years. This may be the result of an increase in phenolic compounds in vacuoles and cell walls of abscission zone tissue (Rascio et al., 1985) potentially producing lignin strengthened vascular tissue in the abscission layer. Moreover, I did not

observe movement of dye from either the stylar end or pedicel immersions (see below). These observations further support the hypothesis 'Bing' pedicels being non-conductive after harvest.

'Skeena'. 'Skeena' is a dark sweet cherry that matures about two weeks after 'Bing' (Kappel et al., 2000) and is renowned to have low PFRF at commercial maturity – a characteristic that makes 'Skeena' highly suitable for mechanized harvest. I observed significant differences among the six treatments in rate of weight loss during storage. Weight loss of standard cherry fruit with pedicel (treatment 1) was not different from stem-free fruit (treatment 2) (Fig. 4-4). In 2007 weight loss was 8.7% for treatment 1 and 7.8% for treatment 2 while in 2008 treatment 1 lost 6.4% and treatment 2 lost 6.8% during storage. This suggests that the pedicel-fruit abscission scar is not a source of weight loss and is promising for storage of stem-free fruit.

Further, comparing rates of water loss over the duration in storage, treatment 3 lost 0.4% and 2% in 2007 and 2008, respectively whereas treatment 5 had losses of 0.6% and 3% in 2007 and 2008 respectively. As with 'Bing', the exocarp of 'Skeena' is the primary source of moisture loss. Which can be further demonstrated by comparing treatments 1 and 4 (8.7% vs. 7.1% in 2007 and 6.4% vs. 5.5% in 2008, respectively). 'Skeena' was also affected by 2008 frost events through chilling injury of blossoms causing reduced crop load. The standard fruit (treatment 1) sample showed a 27% (8.7% vs 6.4% in 2007 and 2008, respectively) reduction in water loss over the duration of storage. Suggesting, crop load has an influence over water loss in storage.

PFRF varied among the treatments over the 21 day storage period in both years. In 2007, the PFRF of treatment 1 fruit exhibited a slight but not statistically significant

increase (+ 120 g) between harvest and final analysis (Fig. 4-6). In contrast, 2008 PFRF decreased significantly from 900 g to 630 g. Further, treatment 1 (no PJ) was the only treatment to display any increase in PFRF in 2007, whereas PFRF of fruit from treatments 3 to 5 decreased both years. Also, PFRF of the treatments 1 and 4 were significantly higher than 3 and 5 in both years. This suggests that fruit dehydration can increase the amount of force needed to remove the pedicel. Our observations demonstrate that retention force is not an indicator to conductivity through the abscission zone into the pedicel after harvest.

Fruit firmness varied throughout storage with most treatments exhibiting a slight decline over the 21 day period (Fig. 4-5). This decline was apparent in fruit not sealed with petroleum jelly (treatments 1, 2, and 4 at 5.4%, 7.5%, and 5.2%, respectively). However firmness did increase in the PJ coated treatments 3 and 5 by 6.7% and 5.3%, respectively. The effect of coating on firmness was reported by Martínez-Romero et al. (2006) demonstrating that *Aloe vera* gelatinous extract is a barrier to water loss and prevents sweet cherry fruit from softening similar to the effect seen in this study.

Dye infiltration. To further elucidate the pedicel's role in fruit weight loss, an apoplastic tracer dye, basic fuchsin, was used to determine passive uptake of water into sweet cherry pedicels. Dye uptake experiments revealed contrasting response to passive uptake for 'Skeena'. In 2007, 'Skeena' fruit had uptake through the stylar end into the pedicel. However, dye uptake was not observed in 2008 from the stylar end. In addition, dye uptake was not observed when the excised pedicel was immersed in the dye solution for 2007 and 2008. Observations of 'Bing' showed no dye uptake from either the stylar end or excised pedicel immersions. Potometric studies of sweet cherry pedicels during

color development show decreasing water uptake in cultivars ‘Van’ and ‘Sunburst’ as the fruit matures (Hovland and Sekse. 2004). This study suggests that decreased conductivity in pedicels as fruit develop. This may explain why we do not see any dye in the pedicels of ‘Bing’ from stylar-end immersions. Why ‘Skeena’ has dye influx one year and not the next needs further investigation.

In an attempt to show xylem conductivity in grape (*Vitis vinifera*), pre-veraison xylem conductivity was shown to be positive from an excised pedicel into the berry of cultivars ‘Chardonnay’, ‘Cabernet Sauvignon’, ‘Pinot Noir’, ‘Shiraz’, Muscat Gordo’ and ‘Grey Riesling’ (Bondada et al., 2005). However, the authors showed that post-veraison grape does not passively uptake basic fuchsin dye. Grape and sweet cherry fruit growth are characterized as double sigmoidal with three distinct phases to maturity: 1) cell division, 2) pit-hardening (Cherry)/ lag phase (grape), and 3) fruit bulking (Mullins et al., 1992, Westwood, 1993). Keller et al. (2006) noted that passive dye uptake declined once hexose sugars accumulated in grape and at 15 ºBrix dye only penetrated to connective tissue at the brush/pedicel. Our fruit were harvested above 20 ºBrix in both 2007 and 2008 (data not shown) suggesting that sugars are a controlling factor in pedicel conductivity.

Pedicel Quality. ‘Bing’. Pedicel shrivel (diameter) and browning plus fruit firmness and weight were evaluated over a 7 day period after harvest in 2007 and 2008. Pedicel quality is often associated with quality of the fruit and research is investigating means of maintaining pedicel appearance in storage (J. Bai, pers commun.). In 2007, browning significantly increased from 6.9% to 66.5% (Fig. 4-7). Assessing the pedicels using the same scale (4 = 0-25%, 3 = 26-50%, 2 = 51-75%, 1 = 76-100%) as described by

Schick and Toivonen (2000) in 7 DAH the stem quality was reduced from 4 to 2 with cold storage. Pedicel quality declined quickly in relation to pedicel shrivel (Fig. 7-15). By 3 DAH, 31% reduction in pedicel diameter was recorded and by only 4 DAH 41% of pedicel diameter was lost. In addition, 5 DAH showed a significant decrease in fruit/pedicel weight by 7% (Fig. 4-9). However, firmness decreased by only 11% in 3 DAH and by 7 DAH decreased 17% (Fig. 4-10).

In 2008, browning did not exceed 9% (rating 4) over the trial period. However, pedicel diameter significantly decreased by 29% over a 24 hr period and by 7 DAH lost 42% of the diameter. Fruit lost about 26% of their weight between harvest and 7 DAH. Fruit firmness declined by about 14% over the 7 day evaluation period. Data from 2008 fruit showed greater mesocarp shrivel compared to 2007 (7% vs. 26% 7 DAH 2007 and 2008, respectively). Also in 2008 pedicels lost diameter at a similar rate (37% vs. 42% 7 DAH 2007 and 2008, respectively). The use of cold storage in 2007 preserved fruit quality by reducing the rate of water loss. However, cold storage appeared to accelerate the pedicel browning over the 7 day storage period. Further research into temperature management in the cold-chain to minimize weight loss and maximize quality appears warranted.

'Skeena'. In 2007, the pedicels showed significant browning by 7 DAH. At the onset of the trial, pedicels had 5.1% (4 rating) browning and by 7 DAH the pedicels had 80.8% (1 rating) browning. By 5 DAH, the pedicels were 55.6% (2 rating) brown (Fig. 4-11). Average pedicel shrivel was 22% by 3 DAH and over the trial period a 39% diameter loss was recorded (Fig. 4-12). Over the same 7 day storage period, fruit weight loss was 7% (Fig. 4-13) and firmness dropped by 11% (Fig. 4-14).

In 2008, the ‘Skeena’ pedicels rated 4 (0-25% brown) for the entire 7 DAH with a maximum browning of 7.1%. Pedicels shriveled by 48% over the 7 DAH though most occurred by 2 DAH (44% diameter reduction). Fruit weight and firmness loss were significantly greater than in 2007 at 22% and 35%, respectively. Similar to ‘Bing’, ‘Skeena’ fruit showed significantly greater pedicel browning when placed in cold storage. Cold shock happens when plant tissue is exposed to near 0 ºC temperature resulting in solidification of the saturated fatty acids of the lipid bilayer in the cell membrane (Williams et al., 1988, Palta, 1993). The result is inhibition of H^+-ATPase activity, of solute transport into and out of cells, of energy transduction, and of enzyme-dependent metabolism causing cell death that is visible as brown tissue (Guy, 1999). The fruit were exposed to 4 ºC storage in 2007 and brought to room temperature for analyses. In 2007, we noted significant browning compared to 2008, which is the result of electrolyte leakage from the vacuole into the cytosol causing imbalance in electrochemical potential, degradation of chlorophyll and cell death. This same response to chilling has been reported in broccoli (*Brassica oleracea*) when florets were transferred from 4 ºC to 20 ºC causing rapid reduction in chlorophyll (Paradis et al. 1996).

Further, chlorophyll degradation is cultivar dependent in bean (*Phaseolus vulgaris*). In the cultivar ‘Boby’, stored at 20 ºC, chlorophyll *a* and *b* was reduced significantly over a 6 DAH, where as the cultivar ‘Perona’ did not show a significant decline (Monreal, 1999). In 2008, fruit were not refrigerated and did not have significant browning and (stems were rated at 4 for the entire 7 day sampling period) suggesting that room temperature without prior cold storage does not cause severe degradation of chlorophyll.

This work demonstrates that pedicels of ‘Bing’ and ‘Skeena’ sweet cherries have little passive conductance of water after harvest. However, herein we document the potential for rapid degradation of pedicel quality (loss if caliper and discoloration) which likely detract from appearance of fruit at retail despite there being little degradation in quality of fruit pericarp. In the commercial supply chain, sweet cherry fruit are hydrocooled ca. 0.5 ºC and placed into 0 ºC with 95% relative humidity. If a retailer is unwilling to place sweet cherry fruit into refrigerated display, our study suggests that cold-shocked fruit will have significant and rapid pedicel degradation. Further research into postharvest temperature management is needed. Alternatively, developing a process for harvesting, processing, and packaging stem-free sweet cherries would preclude the issue of preserving pedicel quality. We report herein that the pedicel-fruit abscission zone is not a significant source of dehydration of fruit and the majority of water loss occurs via the exocarp. Future research should involve identifying potential barriers to exocarp water loss coupled with supply chain temperature management to provide the highest quality eating experience of sweet cherries.

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Treatment #	Schematic	Description
1		Standard sweet cherry fruit, no PJ, pedicel present
2 & 7		2) Stem-free fruit, no PJ 7) Stem-free ethephon-treated fruit, no PJ
3		Standard fruit, pedicel and fruit coated with PJ
4		Standard fruit, pedicel coated with PJ
5		Standard fruit, fruit surface coated with PJ
6 & 8		6) Stem-free fruit, abscission zone coated with PJ 8) same as 6 /w ethephon treatment

Table 4-1. Treatment descriptions and schematics utilized to compare the role of the exocarp, pedicel, and abscission zone in postharvest water loss of 'Bing' and 'Skeena' sweet cherry fruit. Shaded areas illustrate where a thin film of petroleum jelly (PJ) was applied.

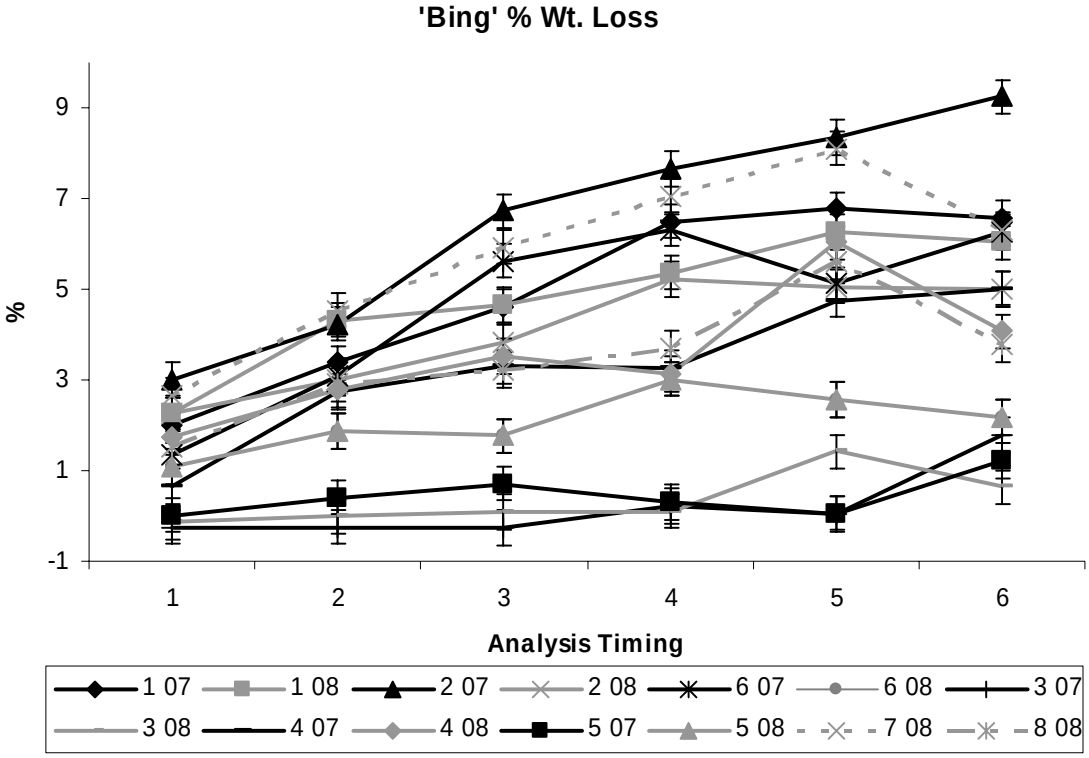

Figure 4-1. Effect of time in storage on water loss in 'Bing' fruit and pedicels using petroleum jelly as a water barrier . Data presented as percent water loss over time. Note table 4.1. for treatment code and 07 and 08 are years. Harvest was on 27 June 07 and 30 June 08 subsequent analyses timing: 1 = 29 June 07 & 3 July 08; 2 = 29 June 07 & 7 July 08; 3 = 6 July 07 & 10 July 08; 4 = 10 July 07 & 14 July 08; 5 = 12 July 07 & 17 July 08; 6 = 17 July 07 & 21 July 08. Storage period was 20 d and 21 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

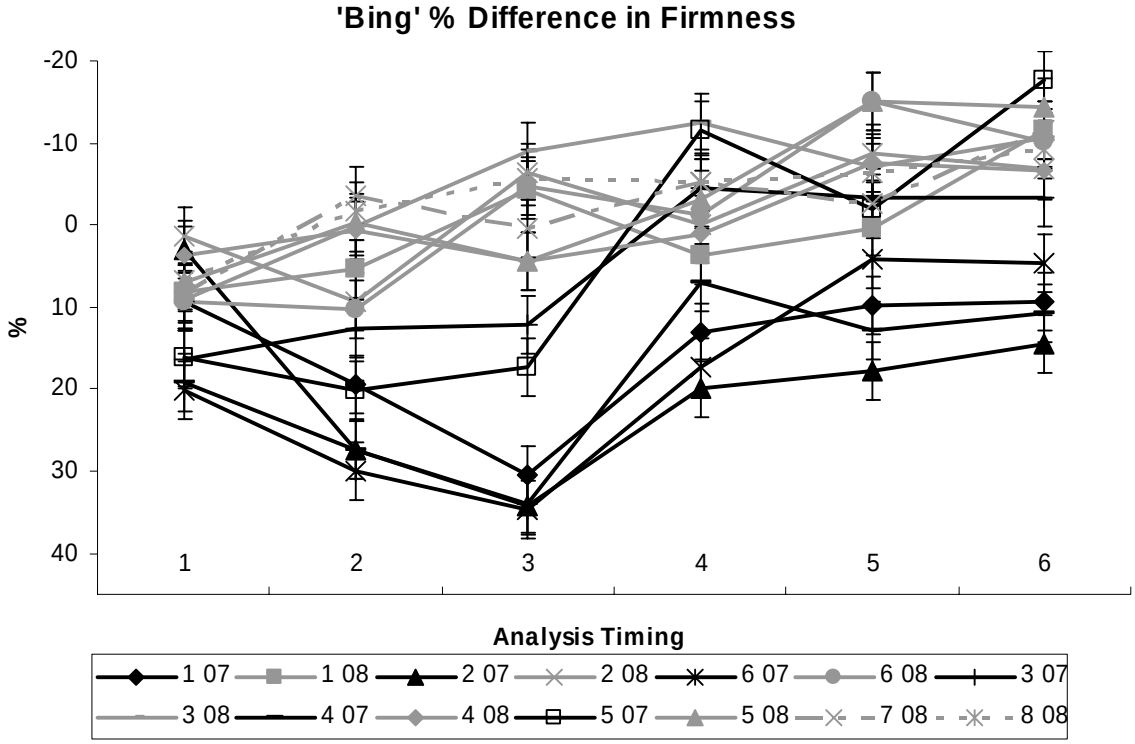

Figure 4-2. Effect of time in storage on firmness in 'Bing' fruit using petroleum jelly as water barrier. Data presented as percent firmness loss over time. Note table 4.1. for treatment code 07 and 08 are years. Harvest was on 27 June 07 and 30 June 08 subsequent analyses timing: 1 = 29 June 07 & 3 July 08; 2 = 29 June 07 & 7 July 08; 3 = 6 July 07 & 10 July 08; 4 = 10 July 07 & 14 July 08; 5 =12 July 07 & 17 July 08; 6 = 17 July 07 & 21 July 08. Storage period was 20 d and 21 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

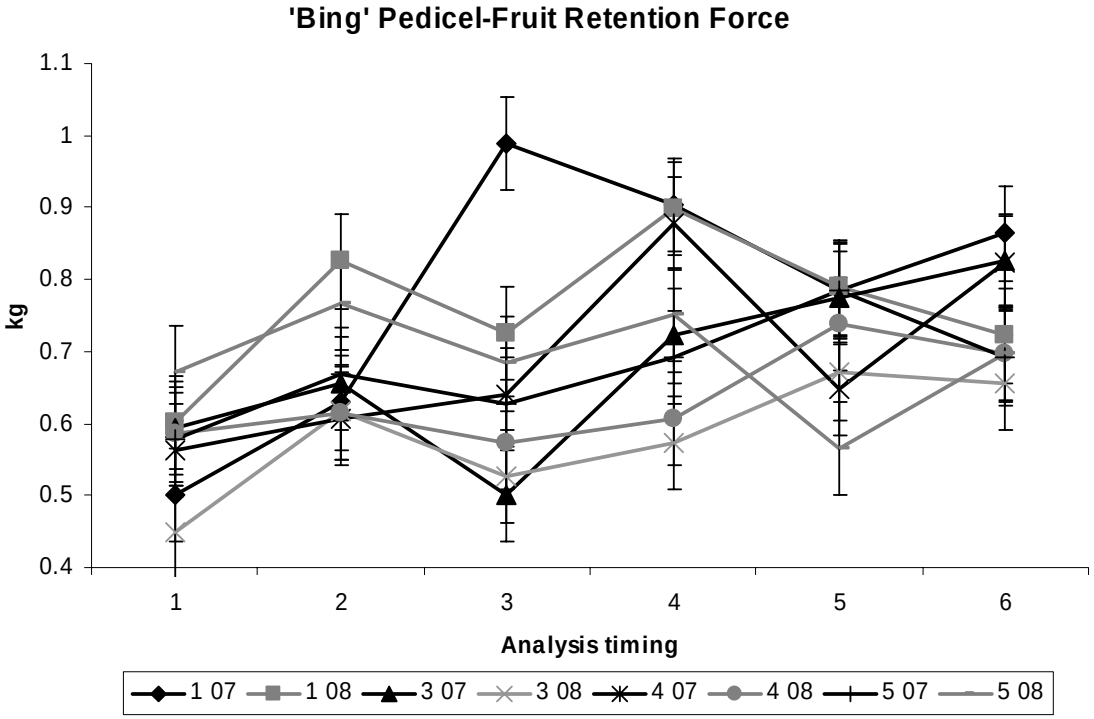

Figure 4-3. Effect of time in storage on pedicel-fruit retention force (kg) in 'Bing' fruit using petroleum jelly as water barrier. Data presented as kilograms (kg). Note table 4.1. for treatment code 07 and 08 are years. Harvest was on 27 June 07 and 30 June 08 subsequent analyses timing: 1 = 29 June 07 & 3 July 08; 2 = 29 June 07 & 7 July 08; 3 = 6 July 07 & 10 July 08; 4 = 10 July 07 & 14 July 08; 5 = 12 July 07 & 17 July 08; 6 = 17 July 07 & 21 July 08. Storage period was 20 d and 21 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

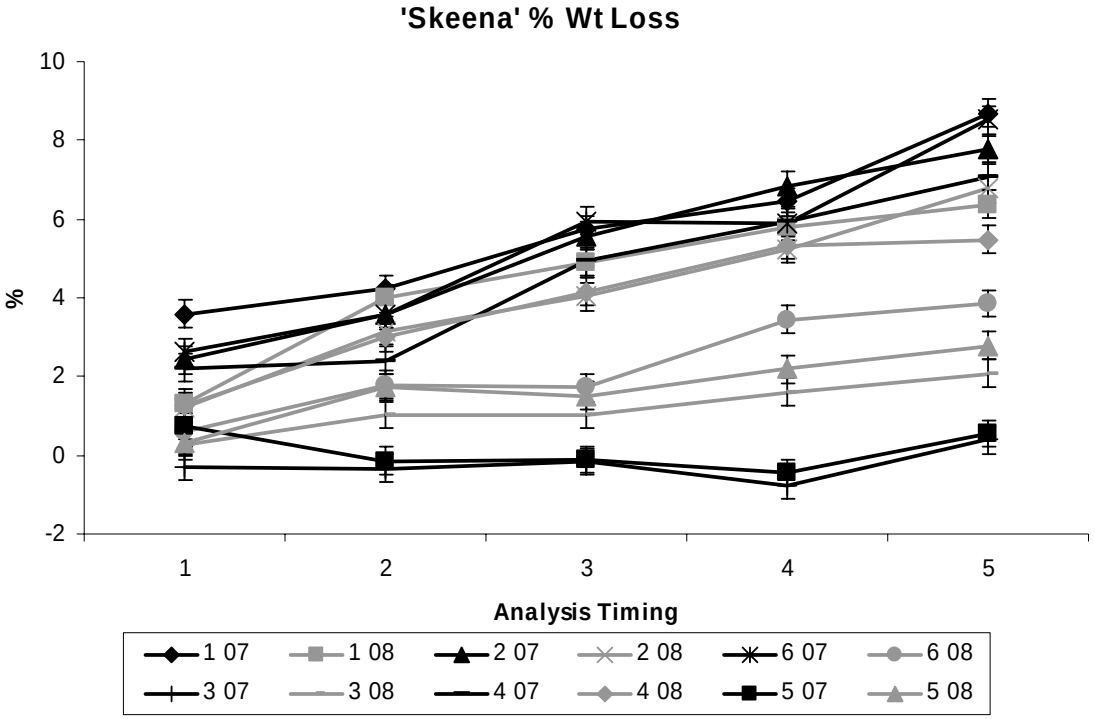

Figure 4-4. Effect of time in storage on water loss in 'Skeena' fruit and pedicels using petroleum jelly as a water barrier . Data presented as percent water loss over time. Note table 4.1. for treatment code and 07 and 08 are years. Harvest was on 6 July 07 and 7 July 08 subsequent analyses timing: 1 = 10 July 07 & 10 July 08; 2 = 12 July 07 & 14 July 08; 3 = 20 July 07 & 24 July 08; 4 = 24 July 07 & 24 July 08; 5 = 27 July 07 & 27 July 08. Storage period was 20 d and 20 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

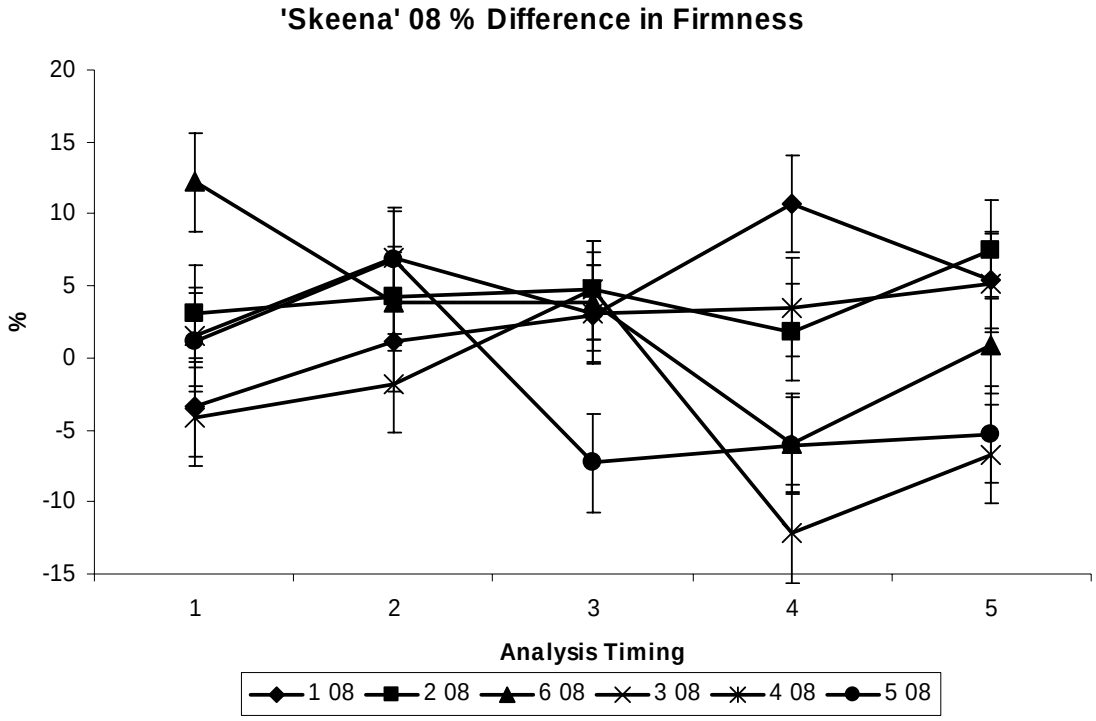

Figure 4-5. Effect of time in storage on firmness in 'Skeena' fruit using petroleum jelly as a water barrier . Data presented as percent firmness loss over time. Note table 4.1. for treatment code and 08 = year. Harvest was on 7 July 08 subsequent analyses timing: 1 = 10 July 08; 2 = 14 July 08; 3 = 24 July 08; 4 = 24 July 08; 5 = 27 July 08. Storage period was 20 d 2008. Bars are standard error and means calculated at $P < 0.05$.

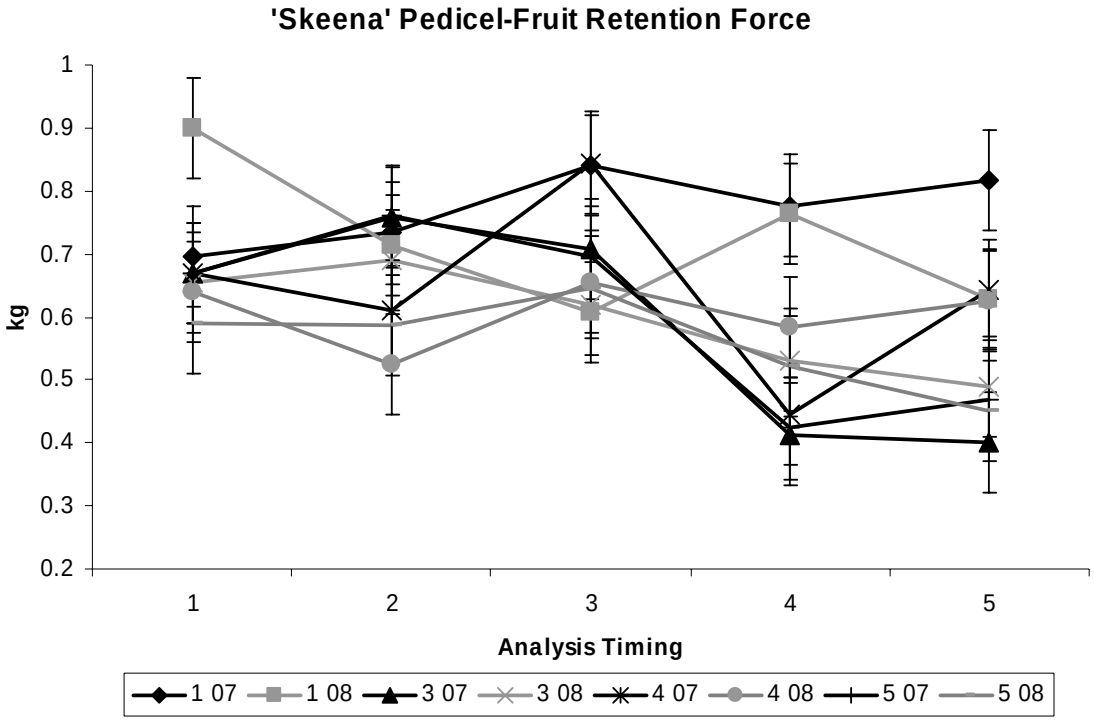

Figure 4-6. Effect of time in storage on pedicel-fruit retention force in 'Skeena' fruit using petroleum jelly as a water barrier . Data presented as kilograms (kg). Note table 4.1. for treatment code and 07 and 08 are years. Harvest was on 6 July 07 and 7 July 08 subsequent analyses timing: 1 = 10 July 07 & 10 July 08; 2 = 12 July 07 & 14 July 08; 3 = 20 July 07 & 24 July 08; 4 = 24 July 07 & 24 July 08; 5 = 27 July 07 & 27 July 08. Storage period was 20 d and 20 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

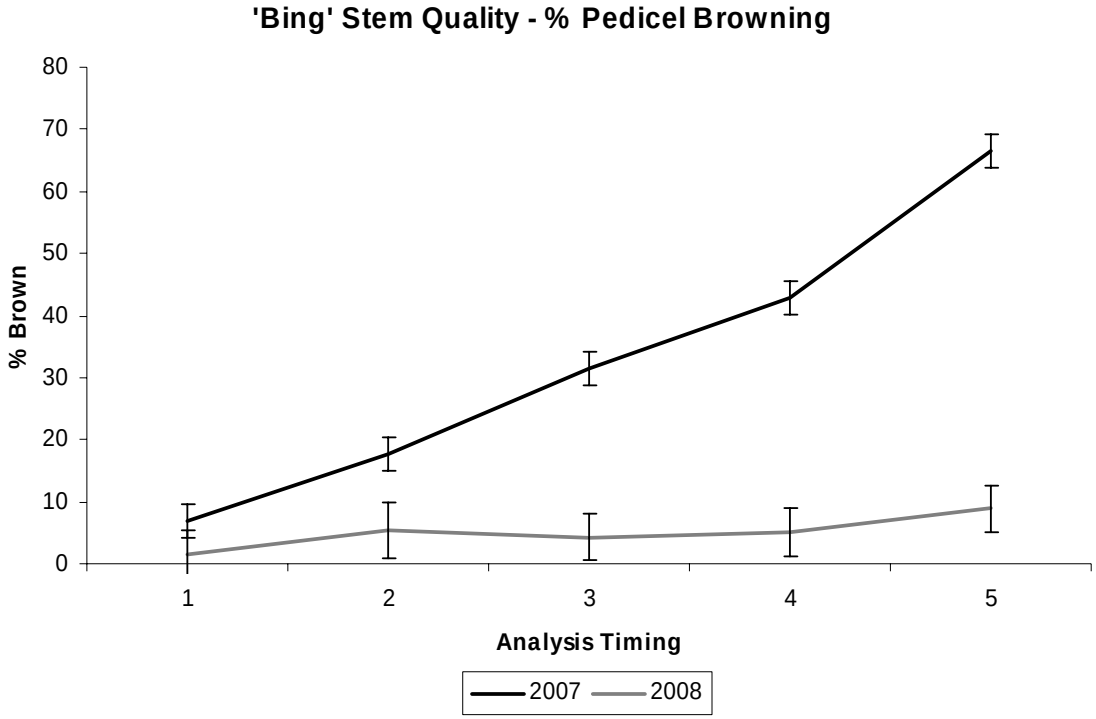

Figure 4-7. Effect of time in storage on fruit pedicel quality in 'Bing'. Data presented as % browning using APS Assess digital image analysis. . Harvest was on 22 June 07 and 30 June 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 22 June 07 & 30 June 08; 2 = 25 June 07 & 1 July 08; 3 = 26 June 07 & 7 July 08; 4 = 27 June 07 & 3 July 08; 5 = 29 June 07 & 7 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

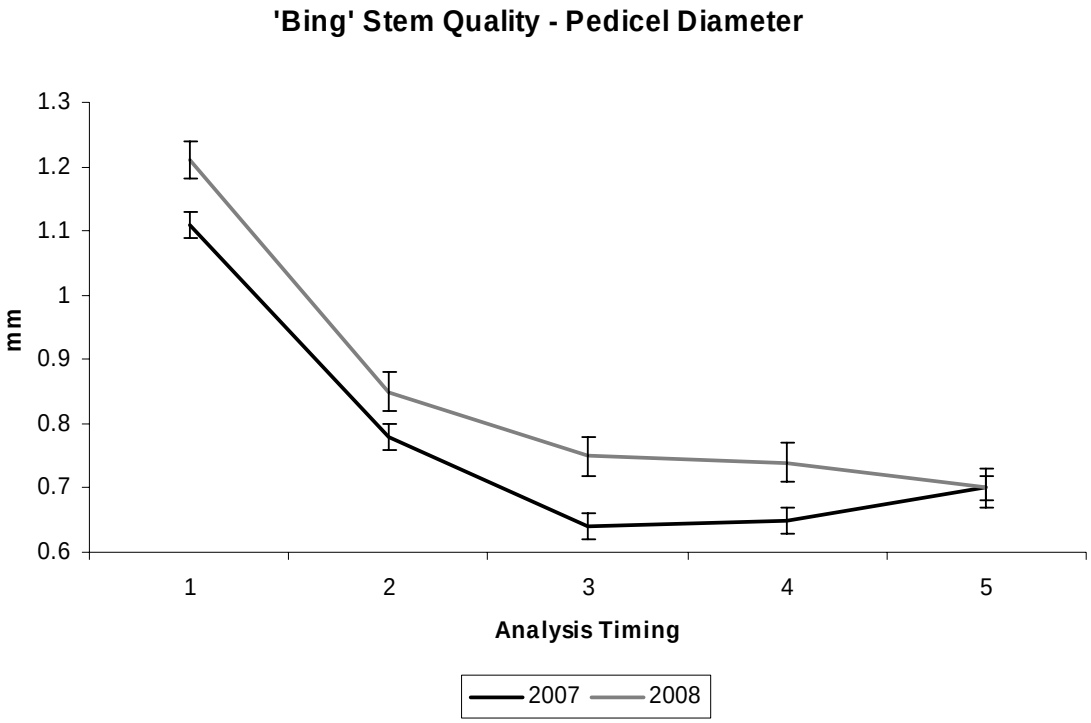

Figure 4-8. Effect of time in storage on the fruit pedicel quality of diameter (mm) in 'Bing' pedicels. Harvest was on 22 June 07 and 30 June 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 22 June 07 & 30 June 08; 2 = 25 June 07 & 1 July 08; 3 = 26 June 07 & 7 July 08; 4 = 27 June 07 & 3 July 08; 5 = 29 June 07 & 7 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

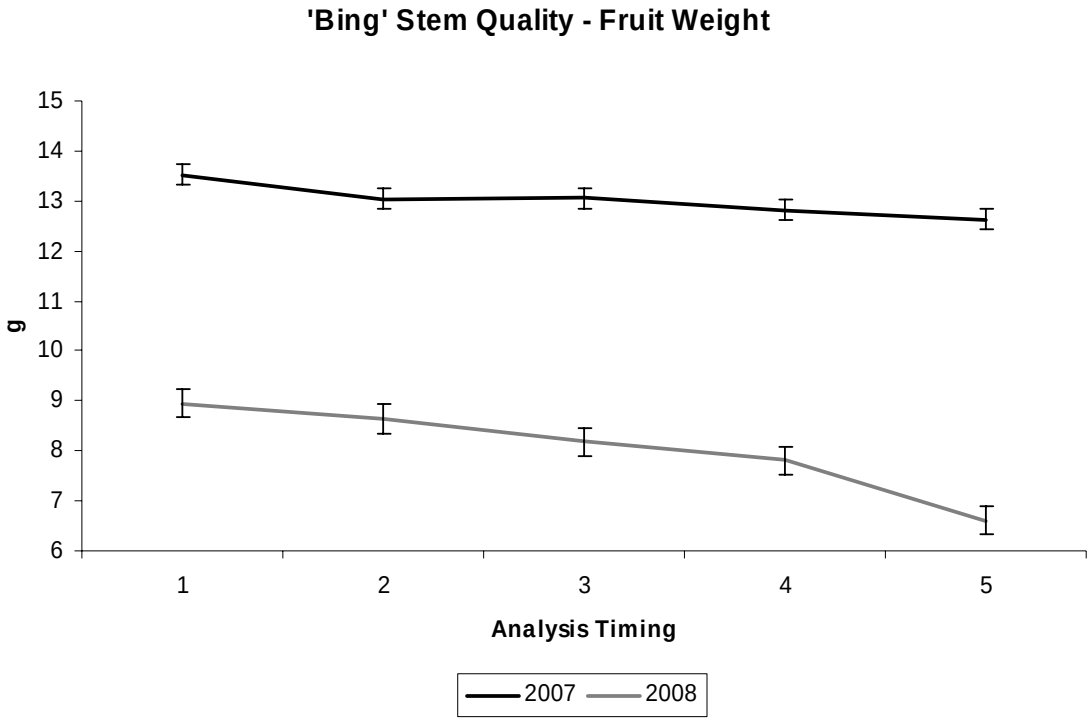

Figure 4-9. Effect of time in storage on the fruit quality of weight (g) in 'Bing'. Harvest was on 22 June 07 and 30 June 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 22 June 07 & 30 June 08; 2 = 25 June 07 & 1 July 08; 3 = 26 June 07 & 7 July 08; 4 = 27 June 07 & 3 July 08; 5 = 29 June 07 & 7 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

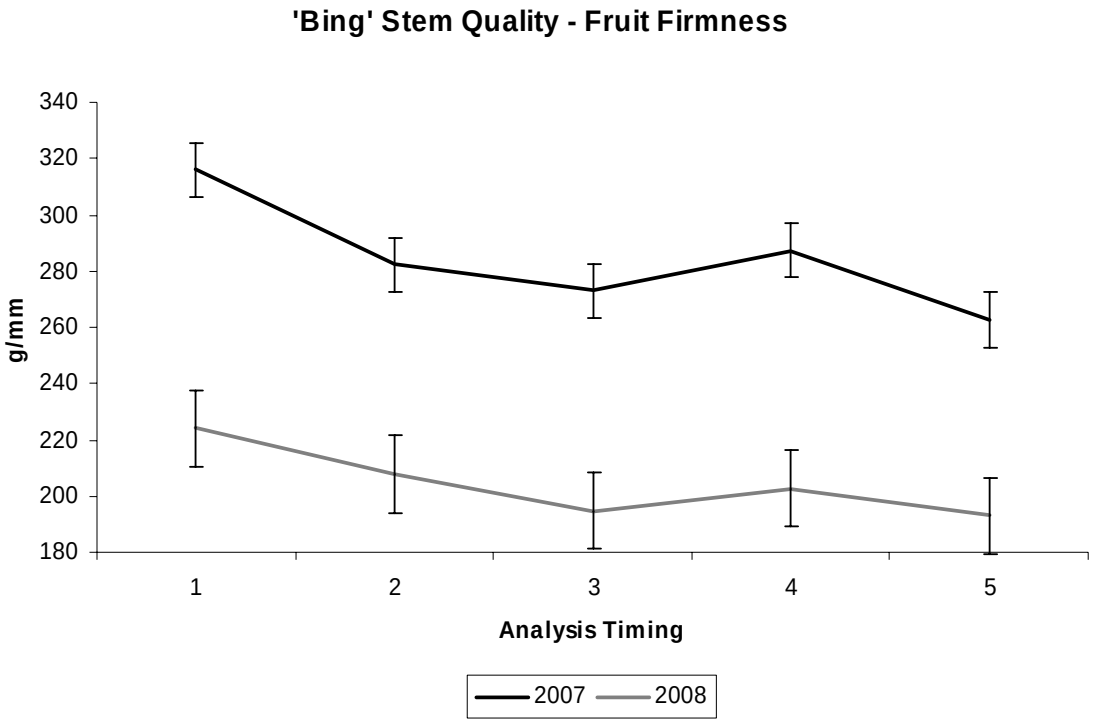

Figure 4-10. Effect of time in storage on the fruit quality of firmness (g mm^{-1}) in 'Bing'. Harvest was on 22 June 07 and 30 June 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 22 June 07 & 30 June 08; 2 = 25 June 07 & 1 July 08; 3 = 26 June 07 & 7 July 08; 4 = 27 June 07 & 3 July 08; 5 = 29 June 07 & 7 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

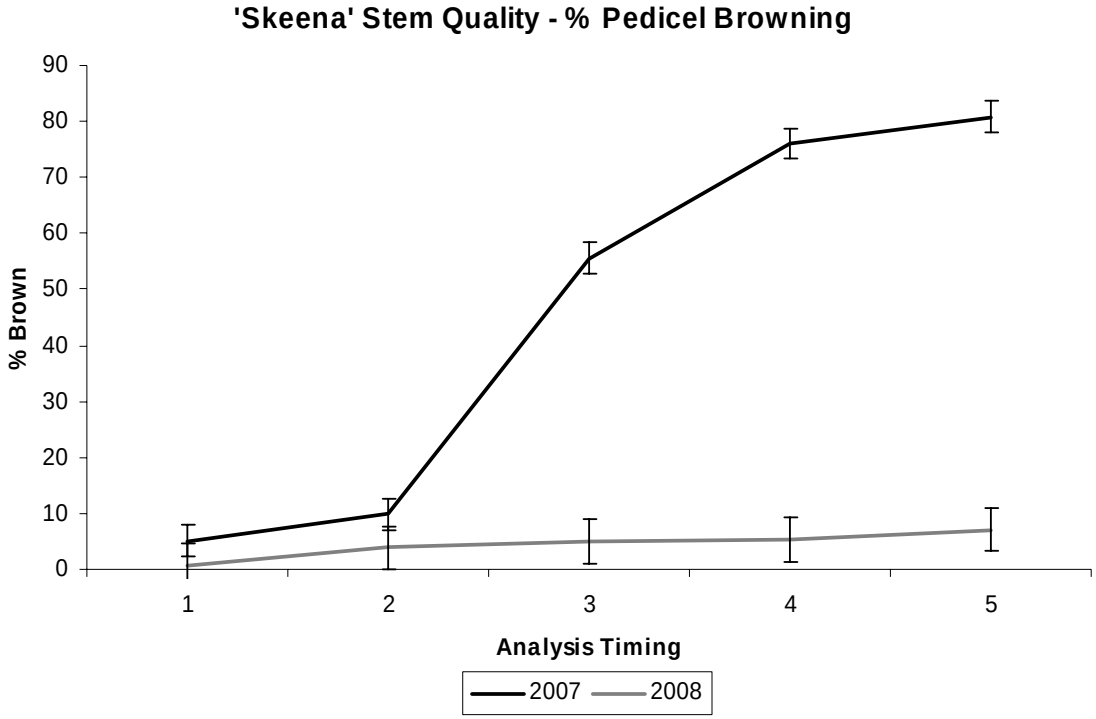

Figure 4-11. Effect of time in storage on fruit pedicel quality in 'Skeena'. Data presented as % browning using APS Assess digital image analysis. . Harvest was on 6 July 07 and 7 July 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 6 July 07 & 7 July 08; 2 = 9 July 07 & 9 July 08; 3 = 11 July 07 & 10 July 08; 4 = 12 July 07 & 11 July 08; 5 = 13 July 07 & 14 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

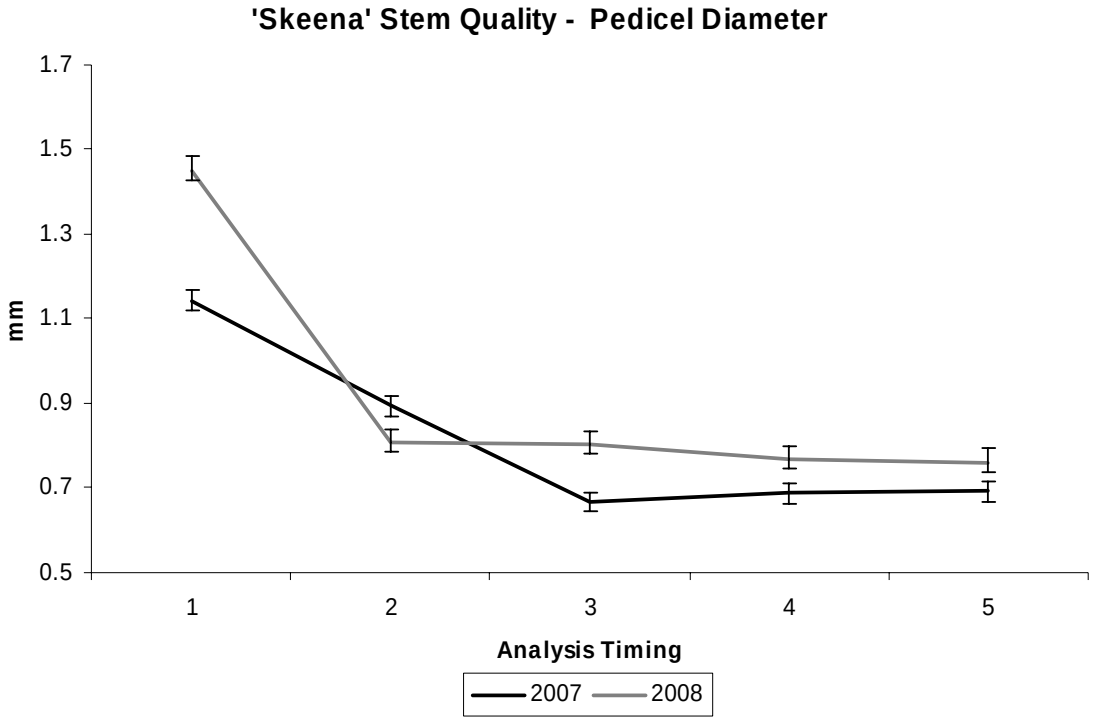

Figure 4-12. Effect of time in storage on pedicel diameter (mm) in 'Skeena'. Harvest was on 6 July 07 and 7 July 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 6 July 07 & 7 July 08; 2 = 9 July 07 & 9 July 08; 3 = 11 July 07 & 10 July 08; 4 = 12 July 07 & 11 July 08; 5 = 13 July 07 & 14 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

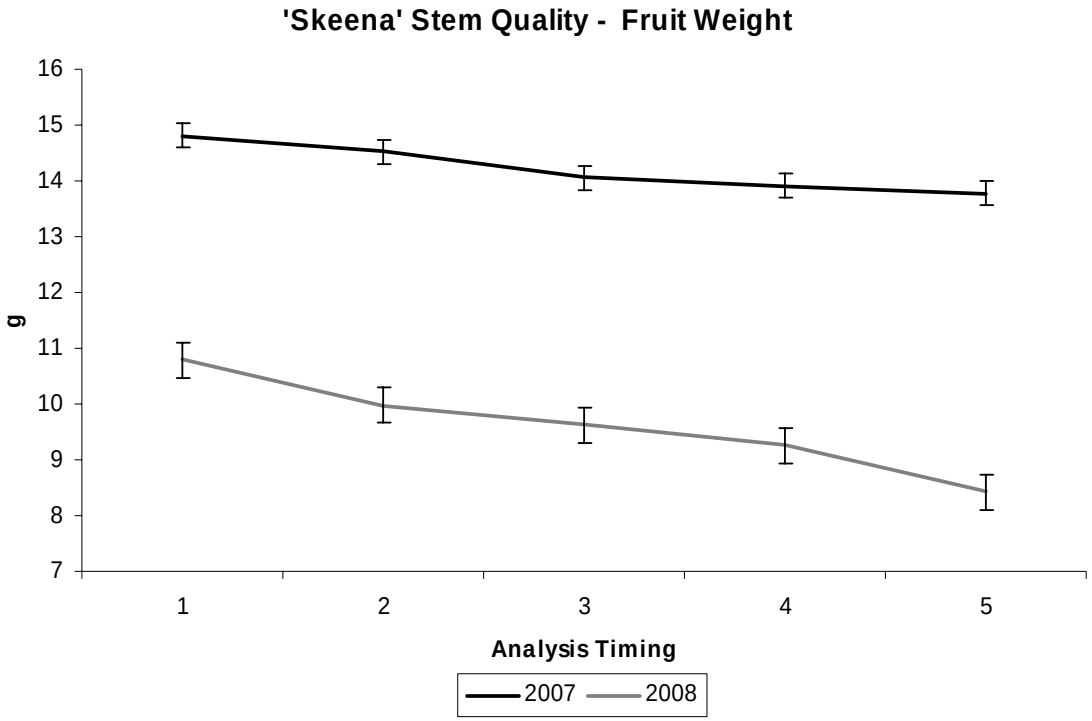

Figure 4-13. Effect of time in storage on weight (g) in 'Skeena'. Harvest was on 6 July 07 and 7 July 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 6 July 07 & 7 July 08; 2 = 9 July 07 & 9 July 08; 3 = 11 July 07 & 10 July 08; 4 = 12 July 07 & 11 July 08; 5 = 13 July 07 & 14 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

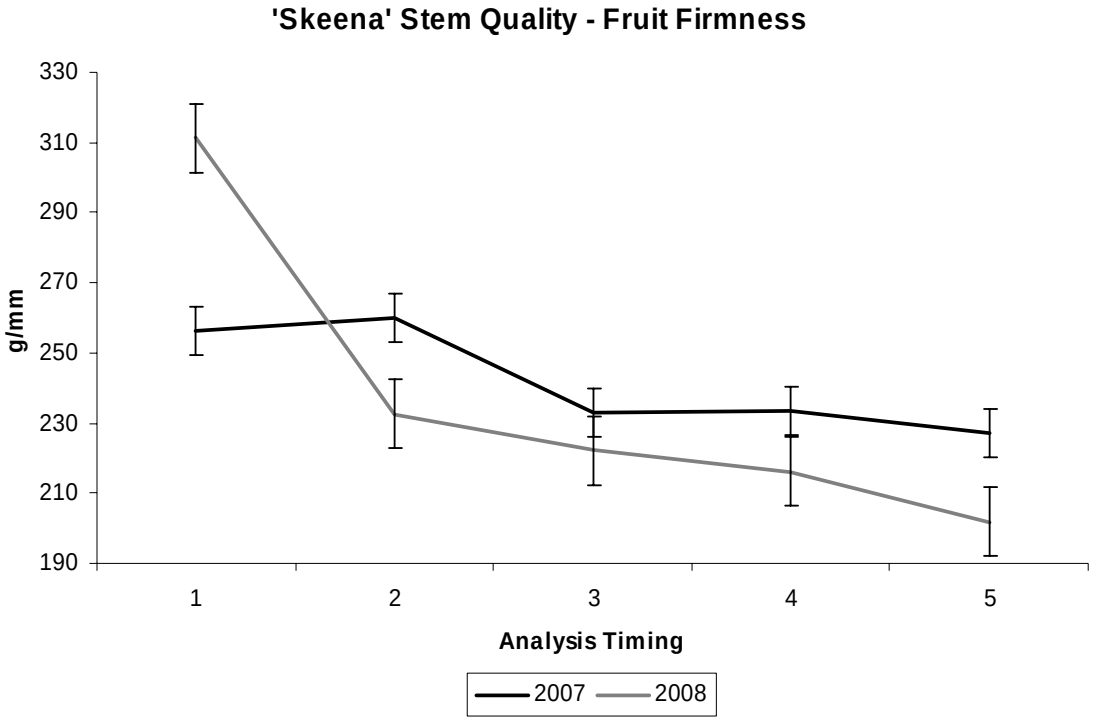

Figure 4-14. Effect of time in storage on fruit firmness (g mm^{-1}) in 'Skeena'. Harvest was on 6 July 07 and 7 July 08. Fruit in 2007 was refrigerated and brought to room temperature before analyses and 2008 fruit were stored at room temperature. The subsequent analyses timing: 1 = 6 July 07 & 7 July 08; 2 = 9 July 07 & 9 July 08; 3 = 11 July 07 & 10 July 08; 4 = 12 July 07 & 11 July 08; 5 = 13 July 07 & 14 July 08. Storage period was 7 d for 2007 and 2008, respectively. Bars are standard error and means calculated at $P < 0.05$.

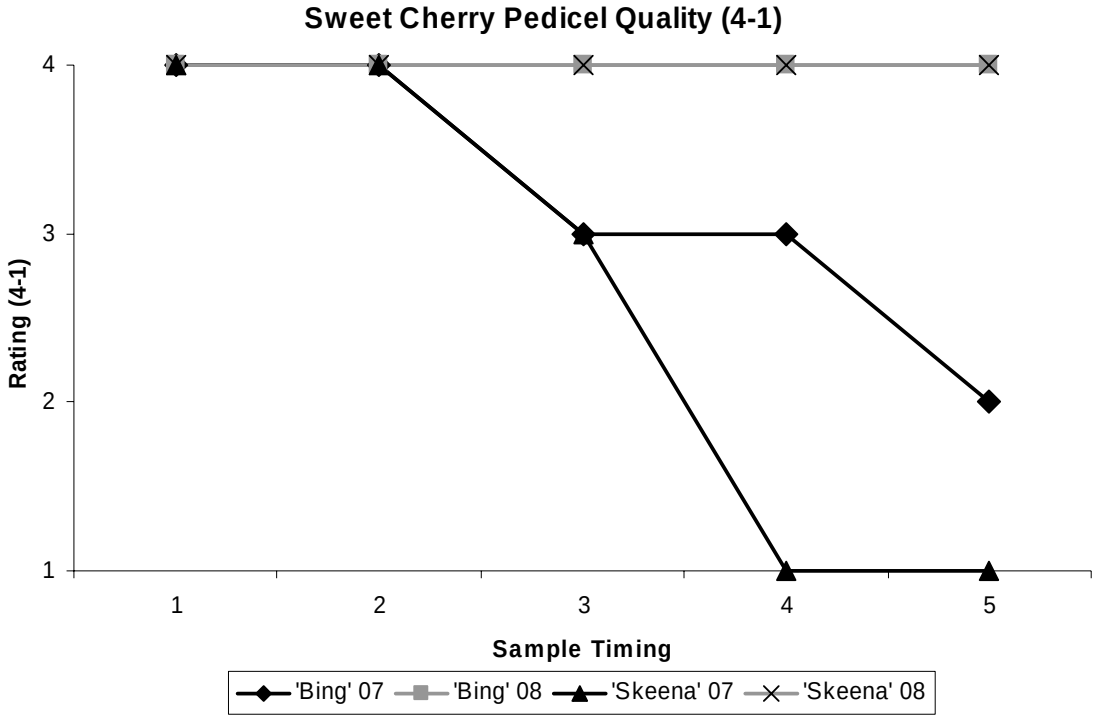

Figure 4-15. Identification of pedicel quality described by Schick and Toivonen (2000) using data from percent browning via APS Assess image analysis software. Scale is qualified as: 4 = 0-25%, 3 = 26-50%, 2 = 51-75%, 1 = 76-100%. Note previous % browning figures for sample timing of 'Bing' and 'Skeena'.

Chapter V

Metabolic profiling of ethephon-treated sweet cherry (*Prunus avium* L.)

Abstract

Metabolic profiling of the fruit or pedicel in sweet cherry cultivars 'Bing', 'Chelan' and 'Skeena' was used to evaluate metabolomic alterations resulting from ethephon (2-chloroethyl phosphonic acid) by air-blast sprayer. Trees were sprayed 13 to 14 days prior to harvest at a rate of 3.5 L ha^{-1} (3 pt A^{-1}) and samples were collected at intervals of 18 to 7 days. Trimethylsilyl (oxime) derivatized extracts from meso-exocarp and pedicel tissues were evaluated using GC-MS. The profile included more than 200 components of which 64 compounds were identified in the meso-exocarp and 55 compounds in the pedicel. This work revealed changes in the metabolome caused by the application of ethephon. Analyses of individual components of 'Bing' meso-exocarp tissue by principal component analysis models revealed distinct temporal changes through ripening and ethephon applied tissue, resulting in linkages to harvest maturity indices i.e. color, acid, and sugars. The results demonstrate metabolic pathways associated with cyanidin 3-glucoside (korumanin) synthesis, malic acid metabolism and simple sugars are altered by ethephon use. Further, analyses of malic acid and simple sugars, key metabolites related to cherry sensory quality revealed little effect of ethephon on components of flavor.

Introduction

There is strong interest to improve sweet cherry harvest efficiency due to the heavy dependence upon manual labor for harvest and predicted shortages of skilled harvest labor. At WSU-Prosser the cherry physiology research program has evaluated the efficiency of a USDA-built prototype for mechanical harvest of sweet cherries and preliminary results are promising (Peterson et al. 2003). To facilitate the mechanical harvest of sweet cherry utilizing the prototype harvester, the fruit-pedicel retention force (PFRF) must approximate 400 g or less (Peterson and Wolford, 2001). Significant reductions in PFRF can be accomplished in some cultivars by applying ethephon (at 3.5 L ha^{-1} (3 pt A^{-1}) 10 to 14 days prior to harvest (Smith and Whiting, 2007). Ethephon is known to enhance pigment development and fruit enlargement (Bukovac et al., 1971) and deteriorate pedicel-fruit abscission cells (Wittenbach and Bukovac, 1972) in 'Windsor'. In addition, ethephon slightly increases soluble solids (Chapter II), and reduces firmness (Chapter II) in 'Bing' sweet cherry. These ethephon-induced phenotypic changes are a result of altered metabolic activity that has yet to be investigated.

In sweet cherry fruit, metabolism changes associated with ripening have been conducted previously using narrowly focused analyses of targeted metabolites. Research has studied non-volatile acids (Oen and Vestrheim, 1998), volatile compounds (Mattheis et al. 1992, 1997), anthocyanins and colorless phenolics (Gao and Mazza, 1995), phytohormone gibberellin (Blake et al., 1992, Blake and Browning, 1994), and sugars (Dolenc and Štampar, 1998) during various stages of fruit growth and development. However, more comprehensive metabolite profiling techniques may be required to develop a better understanding of the role of environmental, management and/or

genotype on fruit metabolism. Recent developments in metabolomics should facilitate such studies.

Metabolomics is the systematic study of the chemistry that is specific to cellular processes of a living organism. Typically, the metabolome represents the collection of small-molecule metabolites that are the end products of gene expression. Metabolic profiling involves two main components: instrumental analysis and data analysis. A significant number of studies have been dedicated to metabolic profiling of nonvolatile compounds involved in primary plant metabolism using gas chromatography (GC) coupled to mass spectrometry (MS) (Fiehn et al., 2000, Roessner et al., 2000, Kopka et al., 2004, Desbrosses et al., 2005, Rudell et al., 2008). Compounds are identified by matching their chromatographic retention times and mass-spectral fragmentation patterns to known and predicted information available in databases. User-defined libraries are generated using the automated mass spectral deconvolution and identification system (AMDIS; National Institute of Standards and Technology). Databases of metabolite profiles have a large number of experimental data points, and are well suited for data mining. Analysis with statistical tools detects correlations and clusters that drive unbiased knowledge acquisition, by identifying unknown relationships. Principle component analysis is one method used to uncover important patterns of diffences in metabolite levels.

Metabolomics has demonstrated relationships between plant metabolite pools, genotype and phenotype, and helped to elucidate biological processes involving biotic and abiotic plant reactions in a variety of species including *Arabidopsis* (Fiehn et al., 2000), *Populus* (Jeong et al., 2004), *Solanum* (Semel et al., 2007), *Betula* (Kontunen-

Soppela et al., 2007), *Pseudotsuga* (Robinson et al., 2007), and *Triticum* (Zörb et al., 2006). An established GC-MS method for plants (Roessner et al., 2000) has been optimized for analyses of sweet cherry fruit and pedicels. In the present study, a method based upon GC-MS was used to study changes in primary and secondary metabolic pathways during ripening of sweet cherry fruit treated with ethephon.

Materials and Methods

Fruit Source and Preharvest Treatment. All trees were located at Washington State University's Roza Farm, about 10 km north of Prosser, Washington, USA (46.2ºN, 119.7ºW). All trees were irrigated weekly from bloom to leaf senescence with low-volume under-tree microsprinklers and grown using standard orchard management practices.

Each trial was arranged in a complete randomized design with 3 single-tree replications per treatment. Trees were selected for uniformity of size and crop load. 'Bing' fruit were collected from 12-year-old trees grafted on Gisela® 6 (*P. cerasus* x *P. canescens*) with an in-row spacing of 2.5 m (8 ft) and between row spacing of 5.0 m (16 ft). Rows were planted in a north-south orientation and trained to a free-standing, standard multiple leader, open-center architecture. 'Skeena' and 'Chelan' fruit were collected from 5-year-old trees on Gisela® 12 (*P. canescens* x *P. cerasus*) rootstock, trained in a Y-trellis system with between-tree spacing of 1.52 (5ft) and 4.27 m (14ft) between rows.

The ethylene-releasing agent ethephon (formula 240 g/l [2 lbs/gal]) was used in all trials. For each experiment, 3 trees were selected within a single orchard. A single application of ethephon was applied via air-blast sprayer at 3.5 L ha^{-1} (3 pt A^{-1})

('Skeena'/'Chelan' 1871 L ha^{-1} [200 gal A^{-1}], 'Bing' 3742 L ha^{-1} [400 gal A^{-1}] spray volume) on 14 June, 2007 to 'Bing'(13 days before harvest [DBH]), on 1 June (13 DBH) for 'Chelan', and on 21 June 2007 (14 DBH) for 'Skeena'. 'Bing' fruits with stems were sampled 28 DBH (31 May), 10 DBH (18 Jun), and 1 DBH (28 Jun). 'Skeena' Fruit with stems were sampled 19 DBH (18 June), 9 DAH (28 June), and harvest (5 July). 'Chelan' fruits with stems were sampled 28 DBH (17 May), 21 DBH (24 May), 14 DBH (31 May), 7 DBH (7 June), and harvest (14 June). Whole fruit material with pedicels attached were immediately frozen in liquid nitrogen and stored at -80 ºC before analysis.

Metabolite Extraction. Frozen fruit was separated from the pit retaining the mesocarp-exocarp and pedicels (six replications per treatment, one fruit per replication). The exocarp and mesocarp fruit tissue and separated pedicels were cryogenically milled to a fine powder and stored at -80 ºC prior to metabolite analysis. Frozen heterogeneous powder (100 mg per replication, six replications per treatment) was weighed in N_2(l)-cooled 1.5 ml screw-top microcentrifuge tubes to which ~100 μL of 0.5 mm (diameter) soda lime glass beads (BioSpec Products, Inc., Bartlesville. OK) was added. The tissue/bead mixture remained N_2(l)-cooled until initiation of the extraction procedure when 1 ml of MeOH was added. Samples were then shaken vigorously using a Mini Beadbeater (BioSpec Products Inc.) for 1 min for fruit tissue and 5 min for pedicel tissue. Next, the samples were floated in a 70 ºC water bath for 15 min to inhibit any sample degradation resulting from intrinsic catalytic activity (Roessner et al., 2000). Samples were than sonicated for 5 min at 25 ºC in an ultrasonic bath followed by centrifugation for 2.5 min at 162000*g*.

Derivatization and GC-MS Analyses. Methoxyamination/trimethylsilylation followed a procedure similar to that described by Roessner et al. (2000). Supernatant aliquots (10 or 100 μL) were placed into borosilicate glass test tubes followed by the addition of 45 μL of phenyl β-D-glucopyranoside (291 ng μL^{-1} in methanol) internal standard solution. The mixture was dried under a stream of N_2(g); then the residue was dissolved in 125 μL of methoxyamine (20 mg mL^{-1} in anhydrous pyridine) solution and incubated for 90 min at 30 ºC. Next, 125 μL of *N*,*O*-bis(trimethylsily)trifluoroacetaminide (BSTFA) was added and the mixture incubated for 30 min at 37 ºC. Samples were then transferred to glass vials containing deactivated glass inserts and analyzed by CG-MS.

Derivatized extract (0.2 μL) was injected into a 6890N GC coupled with a 5975B mass selective detector (MSD) using a 7683B automatic injector (Agilent Technologies, Palo Alto, CA). Samples were volatilized in a 230 ºC splitless inlet lined with an unpacked 4 mm internal diameter, deactivated, tapered-bottom glass liner. Further focusing of the sample was accomplished using a pulsed injection technique that maintained a He carrier gas linear velocity of 66 cm s^{-1} for the first 0.25 min, reducing it to 40cm s^{-1} there after. The GC column was a HP-5MS (Agilent Technologies) (30 m x 250 μm x 0.25 μm). The oven initial temperature was 40 ºC held for 2 min followed by an 18 ºC/min increase to a final temperature of 330 ºC that was held for 6 min. The detector was operated in EI mode with transfer line, source, and quadrupole temperatures maintained at 250, 150, and 230 ºC, respectively. Mass spectra ranging from *m*/*z* 30 to *m*/*z* 500 were recorded.

Data Acquisition, Deconvolution, and Peak Identification. User-defined libraries were generated using the automated mass spectral deconvolution and identification system (AMDIS; National Institute of Standards and Technology) to deconvolute GC-MS results and identify distinct chromatographic components. Retention indices (RI) were generated for each sequence by comparing the retention times of C10-C40 hydrocarbons evaluated under the same conditions as the samples with the retention times of sample components. Individual libraries made from samples from each treatment were compared and redundant components eliminated. From these libraries, mass spectral tags (MSTs) were cataloged using the RI coupled with key mass spectral features and calibration tables generated using Chemstation (G1701DA rev. D; Agilent, Palo Alto, CA). The Qedit macro was used to evaluate each compound and provide peak areas for components. Mass spectral comparison with spectra cataloged in NIST05 (National Institute of Standards and Technology) and mass spectral interpretation aided in tentative identification of many of the components. Compound identifications are based on comparison of sample compound spectra and RIs with those of authentic standards. All authentic standards were purchased from Sigma-Aldrich-Fluka (St. Louis, MO).

Statistical Analyses. Raw chromatographic data were corrected by comparison of *β*-D-glucopyranoside (ISTD) in each sample with that in an external standard and then the sample fresh weight. These values were transformed by mean centering and log transformation prior to principal component analysis (PCA) using SAS 9.1 software package (SAS Institute, Cary, NC). By modeling data using PCA, variance in a multivariate data set containing many instrument-derived components is reduced to a few orthogonal variables, called principal components, that each account for a portion of the

variance in the data set. By plotting treatment scores or component loading values derived from PCA in the space defined by the largest principal components, associations between treatment and metabolic components can be uncovered. For each cultivar, data were analyzed together and treatment differences were evaluated by plotting principal component scores against each other. Unpaired t tests were performed using SAS 9.1 proc glm (SAS Institute, Cary, NC).

Results and Discussion

For efficient mechanical harvest, sweet cherry fruit-pedicel abscission zone must have a retention force of ca. 400 g to achieve upwards of 90% fruit removal (Peterson et al., 2003). Ethephon has been shown to be effective in lowering the PFRF in many sweet cherry cultivars (Bukovac et al., 1971, Peterson and Wolford, 2001, Smith and Whiting, 2007). Further, ethephon most notably affects fruit quality through advancing maturity indices such as color (Bukovac et al., 1971) and soluble solids (Chapter II). Metabolite analyses of the mesocarp-exocarp and pedicel tissues revealed 190 and 236 components, respectively in 'Bing', 'Chelan', and 'Skeena'. Most components detected using GC-MS were trimethylsilyl or trimethylsilyl (oxime) derivatives. A total of 64 metabolites were identified in the meso-exocarp and 55 in the pedicel using retention indices RI and spectral comparison with those of authentic standards. Compounds detected in sweet cherry include many of the same compounds previously elucidated by Oen and Vestrheim (1985) in various sweet cherry cultivars, potato tuber (Roessner et al, 2000), and tomato (Semel et al., 2007). Other compounds involved in primary metabolism were present in both meso-exocarp and pedicel tissue. These include tricarboxylic acid cycle (TCA) metabolites pyruvic acid, succinic acid, fructose-6-phosphate, and glucose-6-

phosphate. Further, phenolic compounds were detected in both the meso-exocarp and pedicel and include caffeic acid, shikimic acid, chlorogenic acid (5' caffeoylquine), and benzoic acid. The compounds α-tocopherol (vitamin E) (Traber and Atkinson, 2007) and β-sitosterol (Ostlund, 2002), two important human nutrients, are present in both meso-exocarop and pedicel tissues. The one known compound identified in meso-exocarp tissue and not the pedicel was cyanidin 3-glucoside (korumanin). Other studies have identified cyanidin 3-rutinoside and cyanidin 3-glucoside as the main anthocyanin pigments in sweet cherry fruit extracts (Robinson and Robinson, 1931; Li and Wagenknecht, 1958; Lynn and Luh, 1964).

PCA Model of Metabolic Profile. Ethephon applied to 'Bing' sweet cherry trees at a minimum of 10 days (at 3.5 L ha^{-1} (3 pt A^{-1}) prior to harvest elicits a reduction in fruit-pedicel retention (Chapter II, Smith and Whiting, 2007) through accelerated degradation of the abscission zone cells (data unpublished). Principal component analyses (PCA) of ethephon-treated 'Bing' vs. untreated fruit revealed differences in metabolome were apparent from samples collected immediately following the ethephon treatment. Metabolic differences in 'Bing' due to ethephon exposure are present in the model (Fig. 5-1) based on all samples. A generation of positive movement in principal component 2 (PC1, 31.4%) suggests the compounds are associated with ripening. However, movement along principal component 1 (PC2) (22.5%) is more negative with ethephon treated samples underscoring quality indices of color and simple sugars.

Fruit ripening is reflected by physiological changes associated with alteration of gene expression, protein synthesis/activity, and metabolism (Giovannoni, 2004; Brady, 1987). 'Bing' meso-exocarp tissue content of compounds identified in this study linked to

sweet cherry ripening through evaluating the PCA include theonic acid, chlorogenic acid, caffeic acid, (-)-epicatechin and putrescine (Fig. 5-2). Conversely, immature 'Bing' is more closely associated with amino acids ala, phe, gly, asn, leu, ile, thr, val, and trp and ripening fruit are linked to asp and β-ala. Further, ripening fruit have close relations to TCA components such as fructose 6-phosphate, glucose 6-phosphate, and succinate. In addition, organic acids are identified with ripening 'Bing' like quinic, mucic, hexonic phosphoric, citramalic, and maleatic acids. However, ethephon treated fruit show associations with malic acid and simple sugars such as sucrose, fructose glucose and sorbitol, which are major components in sweet cherry flavor (Usenik et al., 2007). In addition, rhamnose, a hexapyranose, is a major cell wall deoxysugar (Buchanan et al., 2000), raffinose a phloem transported oligosaccharide, and ribose, which is associated with energy transport in the reductive pentose phosphate pathway (Calvin Cycle) and of adenosine diphosphate (De Block et al. 2005) are highlighted by the ethephon treatment (Fig 5-2). Further, the amino acid proline, a compatible osmolyte associated with plant stress (Boggess et al., 1976) and the anthocyanin kuromanin are linked to ethephon treatment.

Dilute Method Comparisons. The PCA model shows separation of essential sweet cherry quality chemistries with regard to ethephon treatment. The dominate components are simple sugars and malic acid compared to other known non-volatile compounds (Hulme, 1971, Oen and Vestrheim, 1998) . To evaluate the effects of ethephon on these fundamental components of sweet cherry quality and consumer appeal, a 10 x dilution (10 μL aliquot) of the samples were prepared and analyzed by GC-MS. A common factor

in instrumental chemical analysis is to over saturate the detection devices and dilute methods are incorporated to quantify the suspect compounds (Chapman, 1995).

Fruit acids are a key component of flavor (Kappel et al., 1996). In Previous studies, some of the acids identified are oxalic, fumaric, phosphoric, quinic, gluconic, succinic, shikimic, citric and malic acids (Hulme and Wooltorton, 1958; Oen and Vestrheim, 1998; Usenik, 2008). Total acidity in ripe sweet cherry is generally between 8 – 10 mequiv $100g^{-1}$ and 85% of total acidy is attributed to malic acid (Looney et al., 1996). 'Bing' fruit sampled 17 DBH had the greatest concentration of malic acid in the meso-exocarp and pedicel at 17.9 and 5.2 mg g^{-1}, respectively. However, malic acid concentrations did not vary from 10 DBH to harvest (Fig. 5-3) where meso-exocarp and pedicel tissue averaged 9.3 and 2.8 mg g^{-1}, respectively. 'Skeena' did not have significant concentration differences from 19 DBH to harvest when meso-exocarp and pedicel tissues averaged 19.2 and 12.3 mg g^{-1}, respectively (Fig. 5-4). 'Chelan' had fluctuation in malic acid concentrations from 28 DBH to harvest where at 14 DBH both mesocarp and pedicel tissue averaged 5.5 mg g^{-1} (fig. 5-5). Further, there was no significant difference in concentration from 7 DBH to harvest where meso-exocarp and pedicel tissue averaged 8.3 and 5.7 mg g^{-1}, respectively. For all three cultivars tested, the meso-exocarp concentration of malic acid at 10 DBH was not different from the concentration at harvest. In addition, ethephon did not affect the malic acid concentration in the meso-exocarp or pedicel tissue.

Oen and Vestrheim (1998) reported malic acid values of 1.6 to 1.4 mg g^{-1} for unripe and overripe from a composite of five sweet cherry cultivars' juices. Also, Serrano et al. (2005) reported the malic acid is ca. 12 mg g^{-1} in sweet cherry cultivar 'Marvin-

Niram' at developmental stages 10 (red) to 14 (mahogany). I report at harvest untreated 'Bing' and 'Chelan' meso-exocarp malic acid concentrations are 9.6 and 9.5 mg g^{-1}, respectively. Usenik et al. (2008) reports malic acid concentrations of 3.55 mg g^{-1} ('Burlat') to 8.12 mg g^{-1} ('Fercer') of Slavic sweet cherries. However, the malic acid concentration in 'Skeena' is reported as ca. 21 mg g^{-1}, which twice the reported concentration of 'Bing' or 'Chelan'. Showing there is a cultivar dependent variation, which is commonly noted in quality assessment studies (Girard and Kopp, 1998; Drake and Elfving, 2002; Gonçalves et al., 2004; Usenik et al., 2008).

Fructose and sucrose are the two major contributing sugars in sweet cherry with sucrose usually in higher concentration (Looney et al., 1996; Girard and Kopp, 1998; Serrano et al., 2005). Fructose levels decrease in meso-exocarp and pedicel tissue of 'Bing' from 17 DBH to harvest (Fig. 6). At 17 DBH, 'Bing' meso-exocarp tissue had 27.6 mg g^{-1} and by harvest 22.2 mg g^{-1} of fructose. Conversely, untreated 'Skeena' meso-exocarp tissue fructose concentrations increased over the 19 DBH (29.5 mg g^{-1} 19 DBH to 42 mg g^{-1} at harvest) and the pedicel did not significantly change (fig. 5-7). 'Chelan' meso-exocarp tissue varied in concentration over the 29 DBH period, whereas pedicel tissue decreased over the first 14 days and remained unchanged from 8 DBH to harvest. 'Chelan' meso-exocarp fructose concentrations ranged from 19.5 mg g^{-1} at 29 DBH to a minimum of 17.9 mg g^{-1} at 15 DBH. At 8 DBH to harvest, ethephon appears to have significantly reduced the concentration of fructose compared to control samples (harvest untreated 23.2 mg g-1 and harvest ethephon 18.4 mg g^{-1}) (Fig.5- 8). 'Chelan' is considered non-responsive to ethephon in relation to mechanical harvest and reduction in FPRF (Smith and Whiting, 2007). However, we observed a 21% decrease from ethephon

treatment on fructose concentration, whereas this effect is not seen in ‘Bing’ or ‘Skeena’. This response by ‘Chelan’ to ethephon deserves further research if the use of ethephon becomes a practical management tool.

In ‘Bing’ tissue, glucose concentrations decreased in both meso-exocarp and pedicel tissue over the 17 day sampling period (Fig. 5-9). At 17 DBH, ‘Bing’ glucose concentration was 22.7 mg g^{-1} and by harvest it was down to 15.8 mg g^{-1}, a reduction of ca. 30%. Further, concentrations of fructose 22.2 mg g^{-1} and glucose 15.8 mg g^{-1} being reported here are not in a ratio favoring glucose (Looney et al., 1996). However, the fructose/glucose concentrations are similar to values reported by Serrano, et al. (2005). The ethephon treatment did not have a significant effect upon glucose in ‘Bing’. In ‘Skeena’, we observed a ca. 40% increase in meso-exocarp tissue glucose levels between 19 DBH and harvest (34.1 mg g^{-1} and 57.2 mg g^{-1}, respectively) (Fig. 5-10). There was no significant variation of glucose levels in ‘Skeena’ pedicels. ‘Chelan’ glucose concentrations increased in meso-exocarp and decreased in pedicel tissues over the 29 day sampling period (Fig. 5-11). At 29 DBH, the glucose levels in meso-exocarp and pedicel tissues were 15.5 and 10.2 mg g^{-1}, respectively compared to untreated harvest concentrations at 18.5 and 0.6 mg g^{-1}, respectively. ‘Bing’ and ‘Skeena’ were not significantly affected by ethephon. However, ‘Chelan’ ethephon treated meso-exocarp tissue had a significant reduction in glucose when compared to control meso-exocarp tissue (17.7 mg g^{-1}control and 12.3 mg g^{-1} ethephon treated). This suggests that ‘Chelan’ has a response to ethephon that is antagonistic to mechanical harvest (Chapter II) and fruit quality.

Glucose and fructose are reported by Girard and Kopp (1998) as 63 and 51 mg g^{-1} in 'Bing'. At harvest, I report untreated 'Bing' meso-exocarp tissue glucose and fructose concentrations as 15.8 and 22.39 mg g^{-1}, respectively. There is a significant difference in concentration between these two studies. Comparing the glucose/fructose ratios the Girard and Kopp (1998) study favors glucose (ratio 1.2) whereas my work shows a ratio of 0.7 favoring fructose. Further, glucose/fructose ratios for 'Chelan' and 'Skeena' are 0.8 and 1.4, respectively. In addition, Slavic sweet cherry cultivars are reported to range in concentrations of 123 mg g^{-1} glucose (Early Van Compact) to 61.8 mg g^{-1} glucose ('Sylvia') with the glucose/fructose ratio favoring glucose (Usenik et al., 2008). These confounding results suggest that further work is needed to identify management practices, environmental factors and variation in experimental techniques.

Sorbitol concentrations in untreated 'Bing' meso-exocarp did not significantly vary significantly through the 17 day sampling period (Fig. 5-12). However, ethephon-treatment reduced meso-exocarp sorbitol concentration by ca. 31% 17 DBH (17.9 mg g^{-1}) to 10 DBH (12.4 mg g^{-1}). The ethephon treatment reduced the sorbitol concentration by ca. 14% within the 10 DBH (14.4 vs. 12.4 mg g^{-1} untreated and treated, respectively) sampling; however this reduction was not significant. Sorbitol concentrations in the 'Bing' pedicel decreased between 17 DBH to 10 DBH (21.0 mg g^{-1} to 11.8 mg g^{-1}, respectively) and then remained constant to harvest. In 'Skeena', sorbitol levels in meso-exocarp tissue increased from 16 mg g^{-1} at 19 DBH to 23.3 mg g^{-1} at 9 DBH (Fig. 5-13). The concentration of sorbitol remained statistically unchanged from 9 DBH to harvest in 'Skeena' meso-exocarp tissue. Sorbitol levels in 'Skeena' pedicel tissue varied and had significant change over the 19 DBH. At 19 DBH, sorbitol concentration was 18.1 mg g^{-1}

and by harvest was reduced to 14.9 mg g^{-1} with minima of 11.1 mg g^{-1} in the ethephon treatment 9 DBH. In 'Chelan', the meso-exocarp tissue increased in sorbitol concentration from 6.3 mg g^{-1} 29 DBH to 12.3 mg g^{-1} at harvest (Fig. 5-14). Conversely, 'Chelan' pedicel sorbitol levels decreased from 49.3 mg g^{-1} 29 DBH to 14.4 mg g^{-1} at harvest. Ethephon treatment did not have any significant effect upon sorbitol concentrations in each of the cultivars tested.

Sorbitol is considered the third highest in concentration of flavor sugars in sweet cherry (Girard and Kopp, 1998; Serrano et al., 2005; Usenik et al., 2008). Girard and Kopp (1998) reported that 'Bing' fruit sorbitol/mannitol concentration is 36 mg g^{-1} and Usenik et al. (2008) reports sweet cherry fruit sorbitol concentrations ranging from 26 and 4.5 mg g^{-1} in cultivars 'Early Van Compact' and 'Ferprime', respectively. Sorbitol concentrations reported here in untreated meso-exocarp at harvest are 16.0, 18.8, and 24.1 mg g-1 in 'Bing', 'Chelan' and 'Skeena', respectively. In addition, Girard and Kopp (1998) showed that sorbitol is 24% of total sugars, whereas reported here 'Bing' meso-exocarp sorbitol is 29% of the total sugars. 'Chelan' and 'Skeena' sobitol concentrations in the meso-exocarp were 29% and 20%, respectively.

The concentration of sucrose of the total sugars in sweet cherry is small ca. 4% (Usenik et al. 2008). I report percentages of 3, 2, and 3% for sucrose in meso-exocarp of 'Bing', 'Chelan' and 'Skeena', respectively. In 'Bing' meso-exocarp, the sucrose concentration was 0.7 to 1.5 mg g^{-1} from 17 DBH to harvest, which was an insignificant increase (Fig. 5-15). The pedicel did have a significant decrease in sucrose from 8.1 mg g^{-1} at 17 DBH to 1.6 mg g^{-1} at harvest. 'Skeena' had a significant increase in sucrose in the meso-exocarp tissue from 1.2 mg g-1 19 DBH to 3.4 mg g-1 at harvest (Fig. 16).

However, the pedicel tissue decreased in sucrose over the 19 DBH from 9.0 mg g^{-1} to 4.3 mg g^{-1}. 'Chelan' was similar to 'Bing' – sucrose in the meso-exocarp tissue did not significantly change over the 29 DBH while concentrations in the pedicel tissue decrease by 86% from 22.6 to 3.1 mg g^{-1} at 29 DBH and harvest, respectively. Ethephon treatment did not appear to have a significant effect upon sucrose concentrations.

Sweet Cherry Metabolomics. Sweet cherry maturation is a complex phenotypic event brought about by changes in gene expression, the proteome and ultimately the metabolome. Over the years various horticultural practices including PGR application have been developed with the intention of improving fruit quality (i.e. phenotype). The metabolic shift can potentially affect marketability through deviations in quality.

The current work has profiled a portion of the sweet cherry metabolome during maturation and discovered subtle metabolic alterations induced by the PGR ethephon. Important quality chemistries such as sugars and malic acid are affected by management tactics, environmental conditions and cultivar, which makes metabolic profiling an intricate addition to identifying specific effects in fruit maturation.

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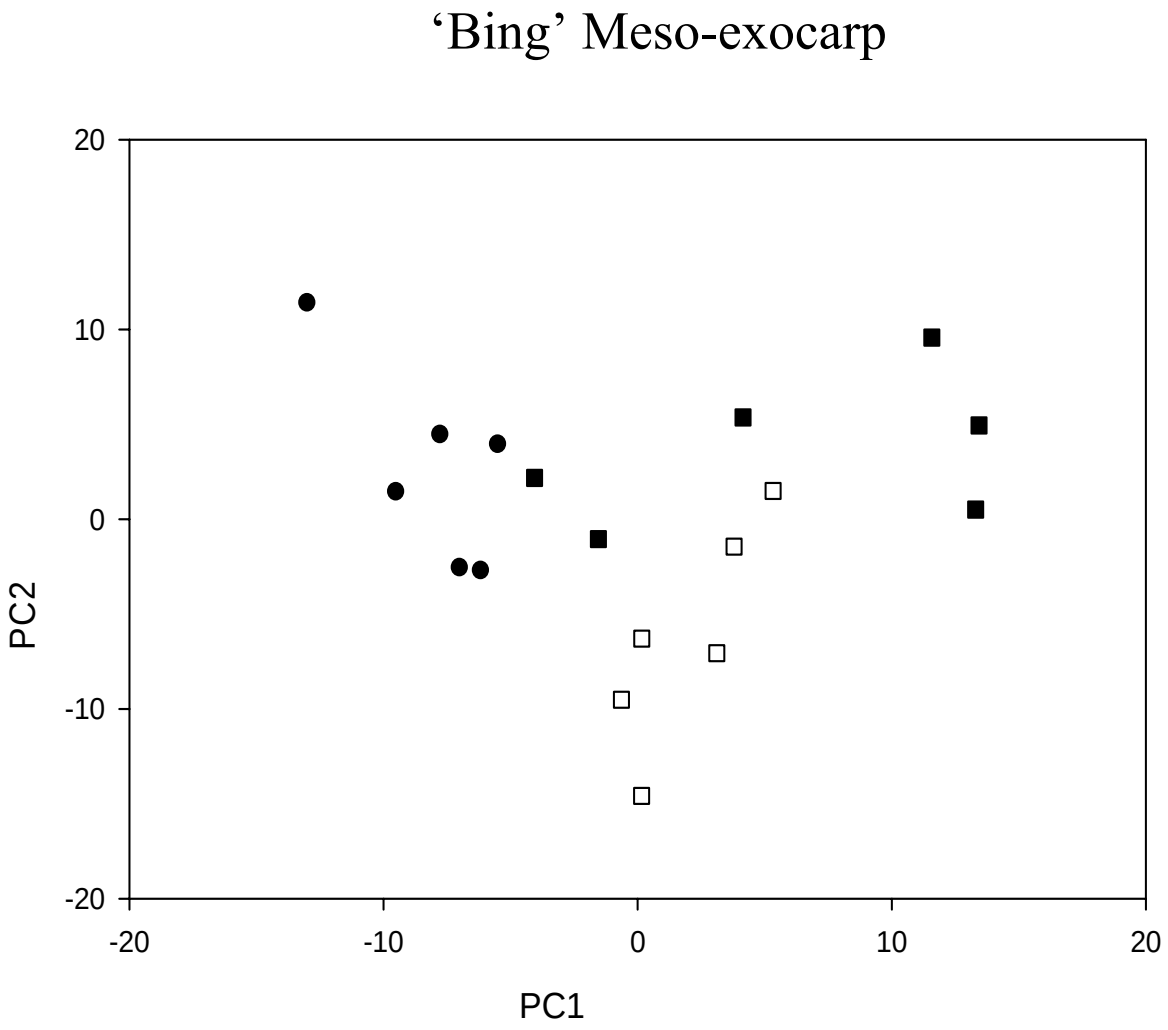

Figure 5-1. Principal component analysis of the metabolic profile of 'Bing'sweet cherry meso-exocarp following ethephon treatment of 3.5 L ha^{-1} (3 pt A^{-1}) 13 DBH against untreated control samples. PC1 = 31.4% and PC 2 = 22.5%. Circle represents 17 DBH control sample (31 May 07), darkened square is 10 DBH (18 Jun 07) untreated sample, and open square is 10 DBH (18 Jun 07) ethephon treatment.

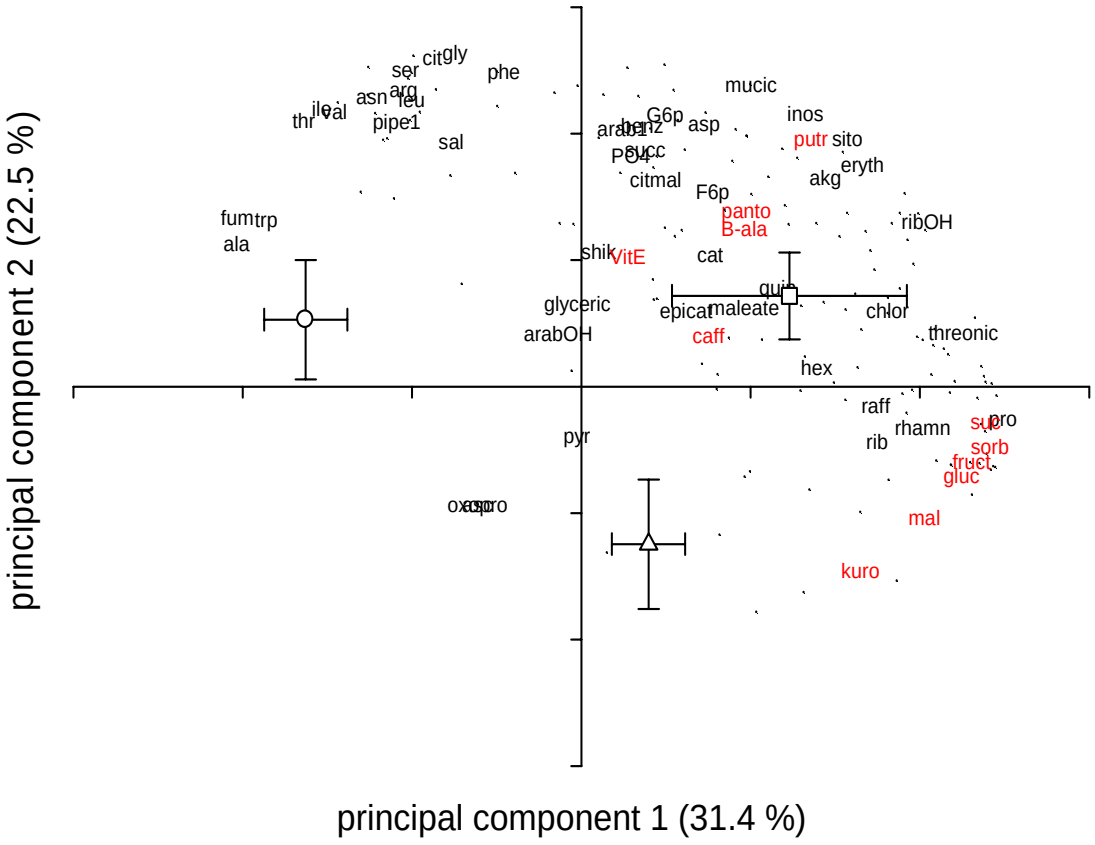

Figure 5-2. Principal component analysis of the metabolic profile of 'Bing', compound identification, sweet cherry meso-exocarp following ethephon treatment of 3.5 L ha^{-1} (3 pt A^{-1}) 13 DBH against untreated control samples. Circle represents 17 DBH control sample (31 May 07), square is 10 DBH (18 Jun 07) control sample, and triangle is 10 DBH (18 Jun 07) ethephon treatment.

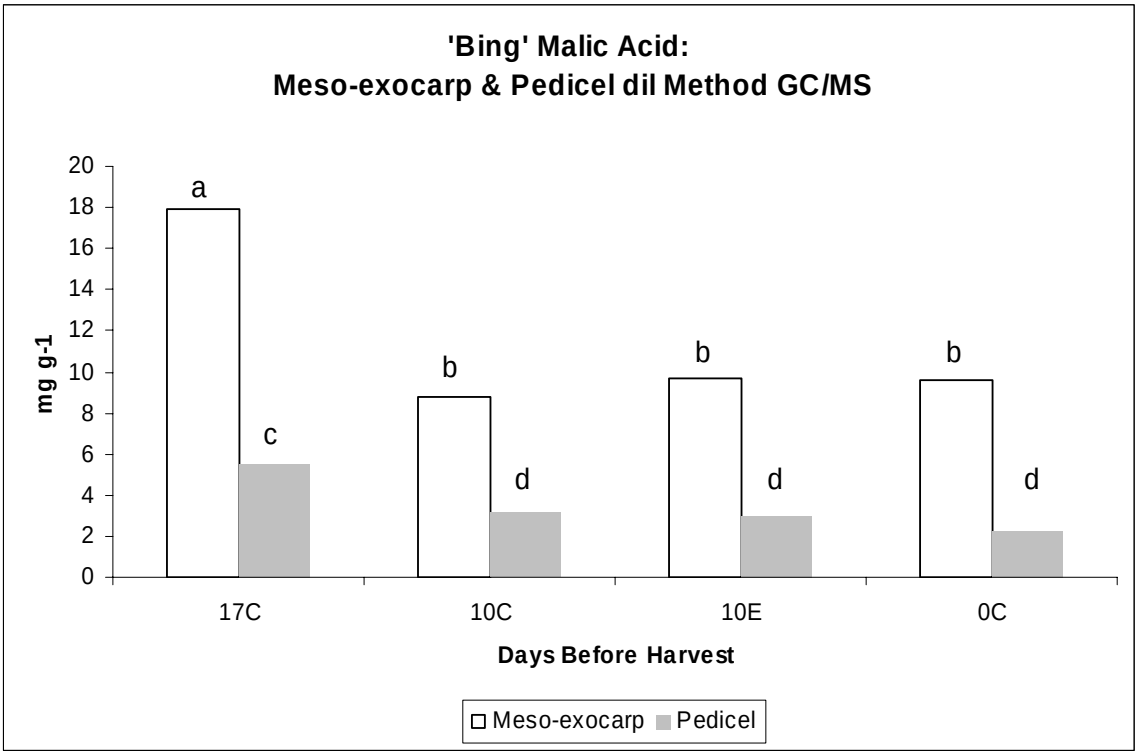

Figure 5-3. Malic acid concentrations (mg g^{-1}) in 'Bing' meso-exocarp and pedicel tissue from 17 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

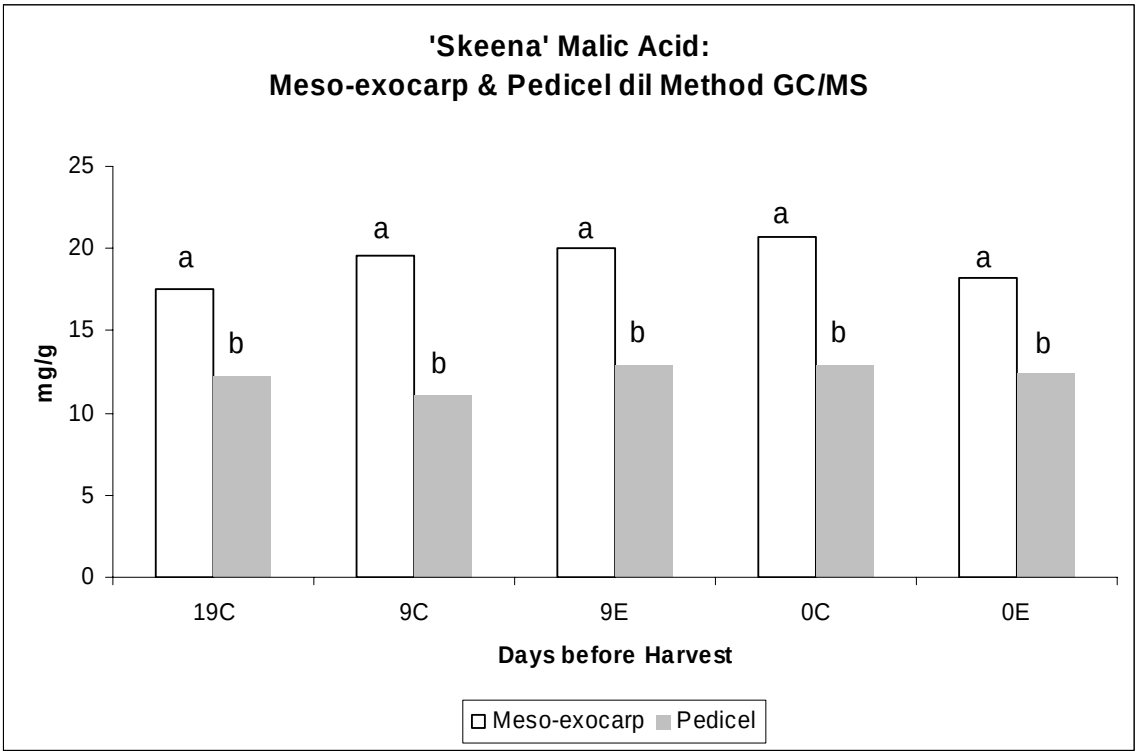

Figure 5-4. Malic acid concentrations (mg g^{-1}) in 'Skeena' meso-exocarp and pedicel tissue from 19 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

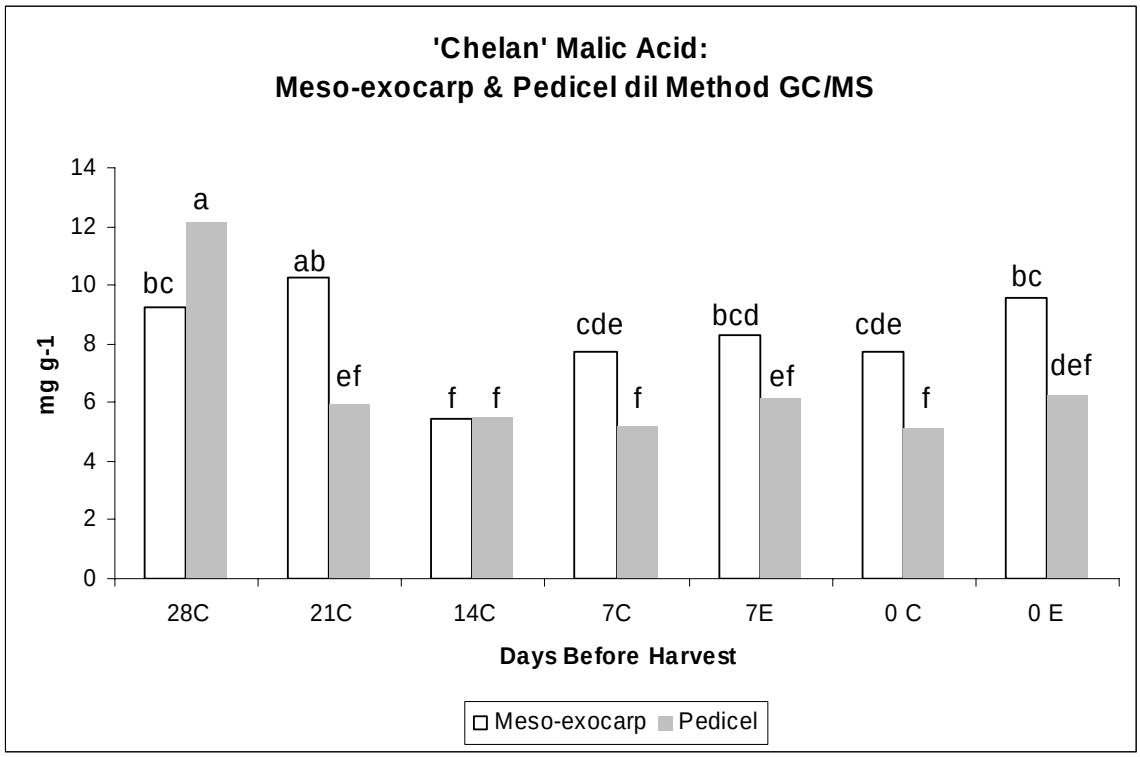

Figure 5-5. Malic acid concentrations (mg g^{-1}) in 'Chelan' meso-exocarp and pedicel tissue from 28 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure]

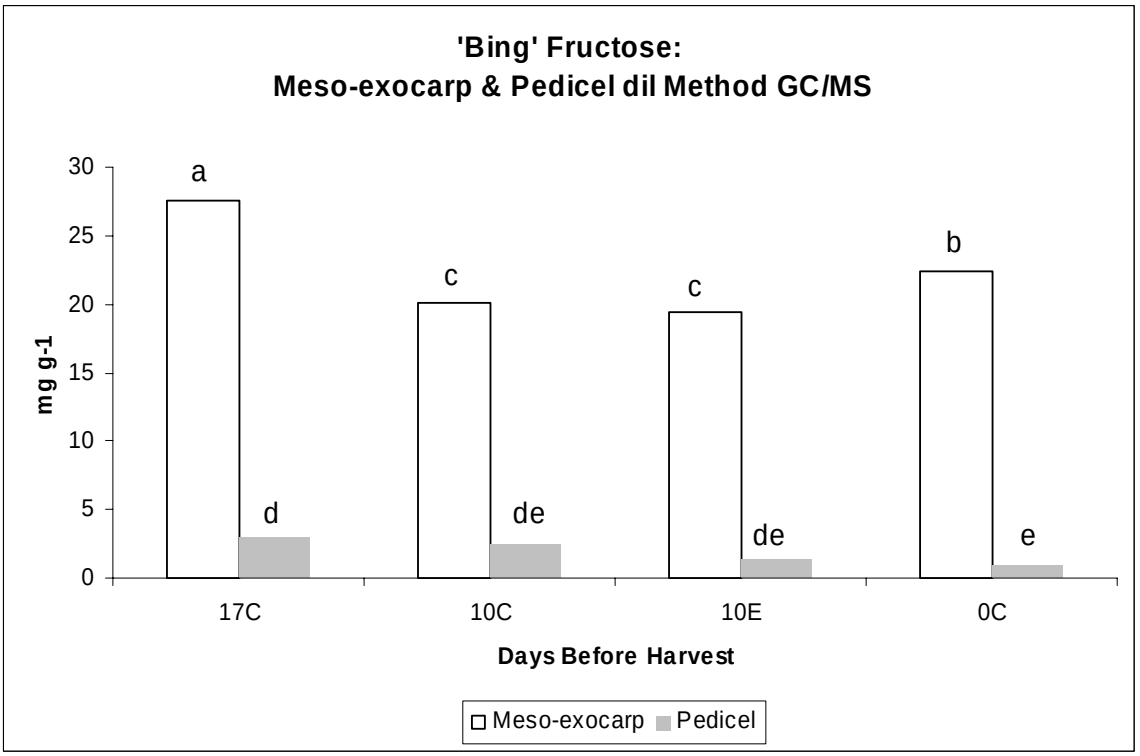

Figure 5-6. Fructose concentrations (mg g^{-1}) in 'Bing' meso-exocarp and pedicel tissue from 17 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure]

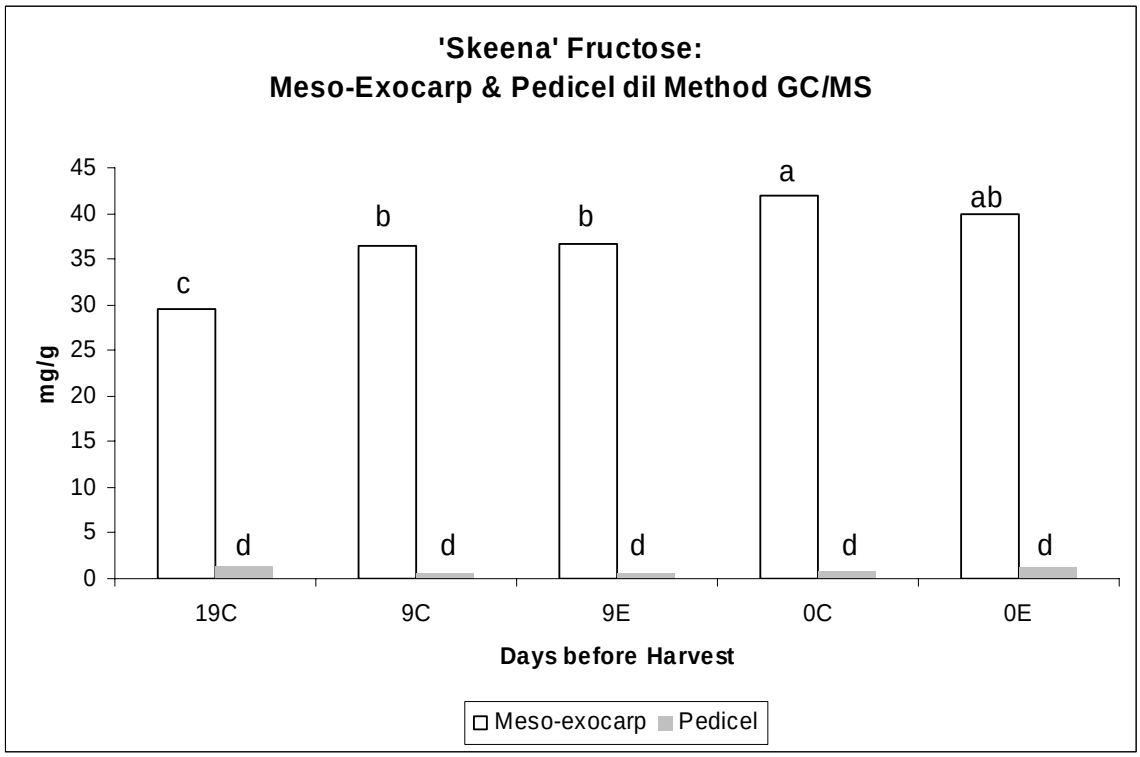

Figure 5-7. Fructose concentrations (mg g^{-1}) in 'Skeena' meso-exocarp and pedicel tissue from 19 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

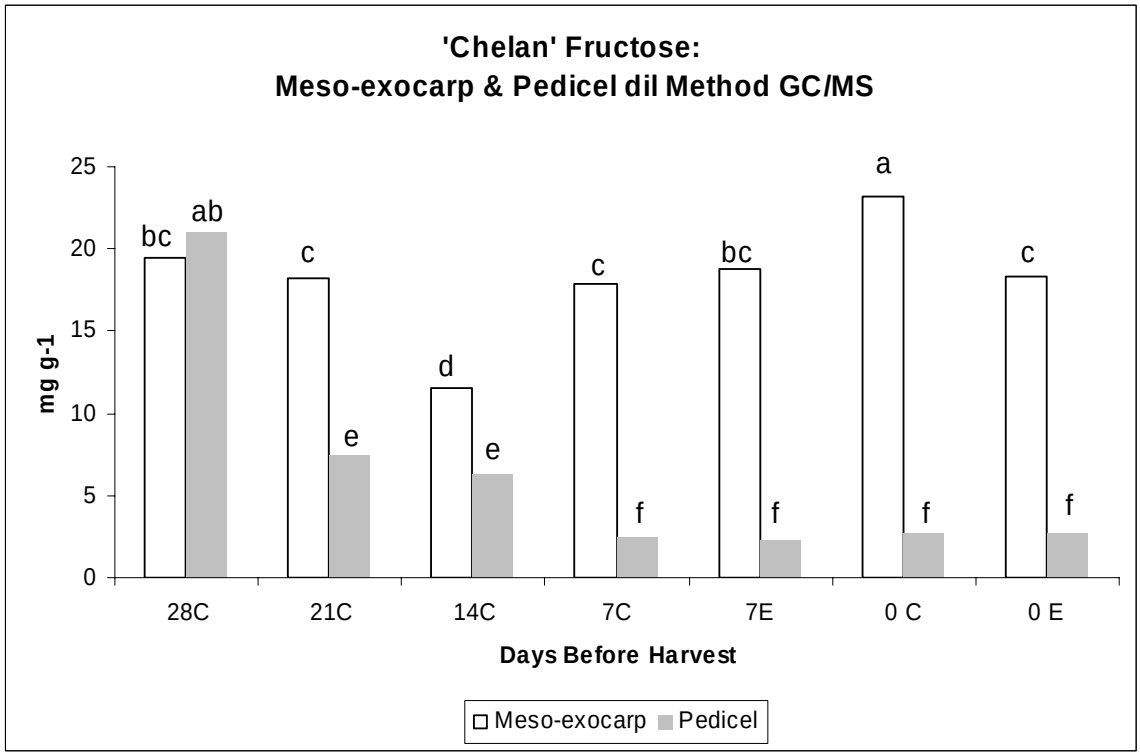

Figure 5-8. Fructose concentrations (mg g^{-1}) in 'Chelan' meso-exocarp and pedicel tissue from 28 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

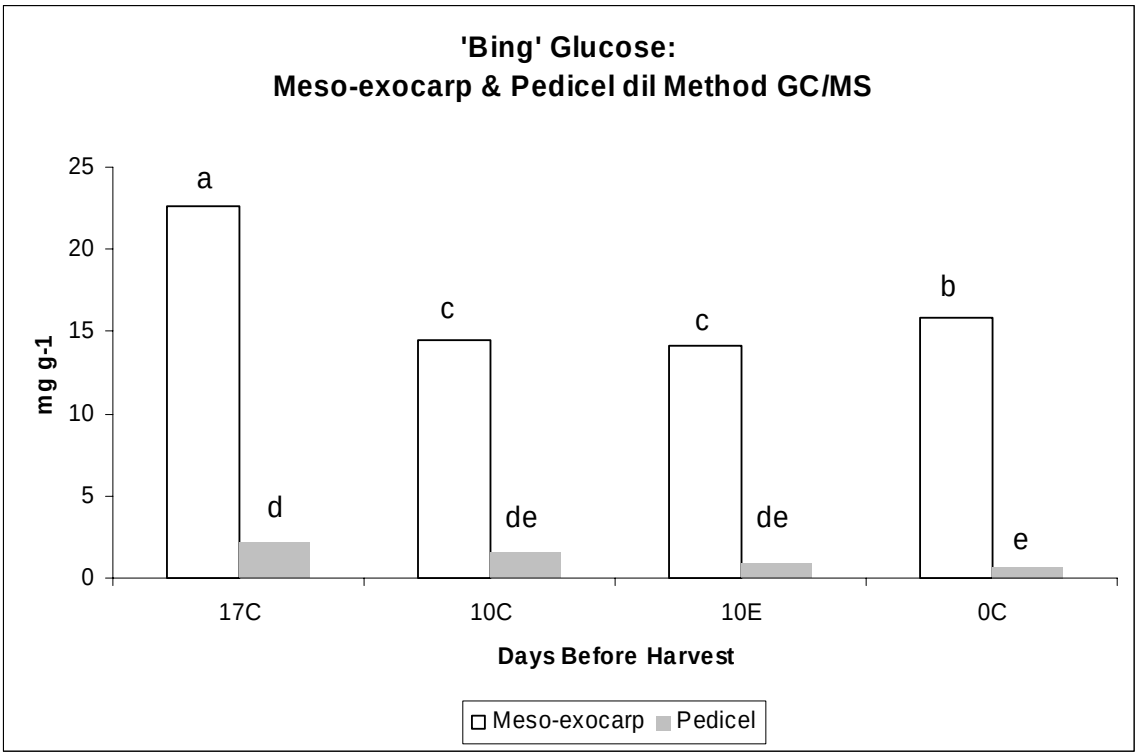

Figure 5-9. Glucose concentrations (mg g^{-1}) in 'Bing' meso-exocarp and pedicel tissue from 17 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

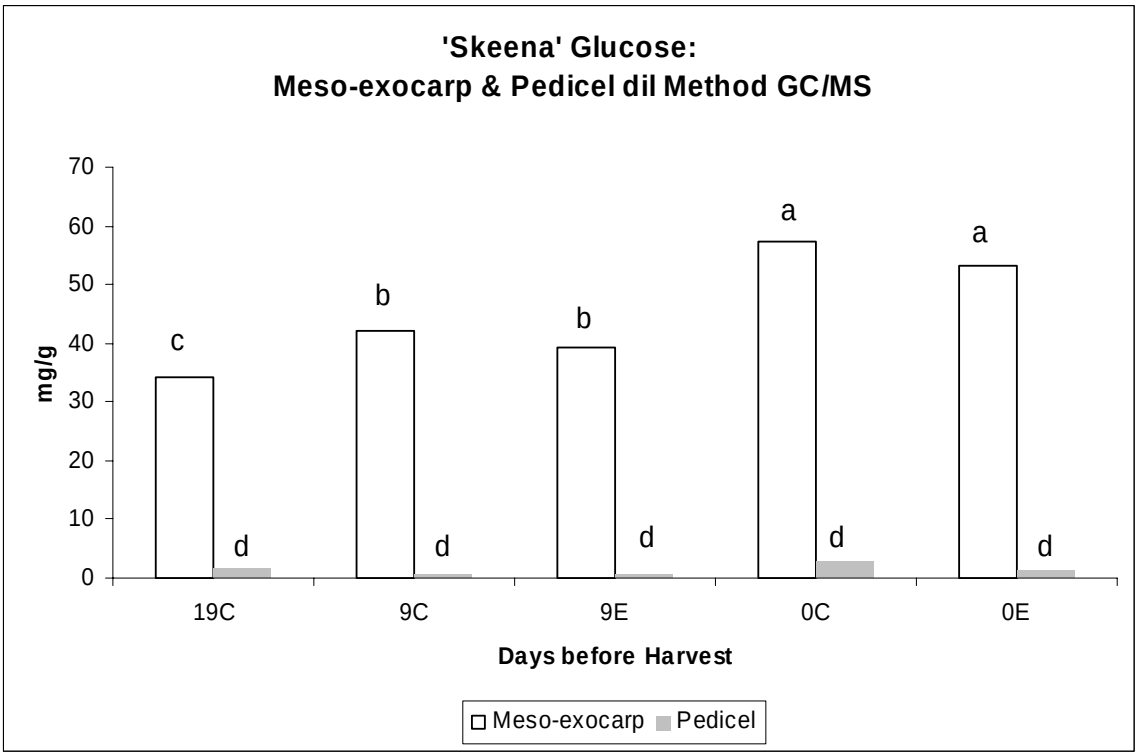

Figure 5-10. Glucose concentrations (mg g^{-1}) in 'Skeena' meso-exocarp and pedicel tissue from 19 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

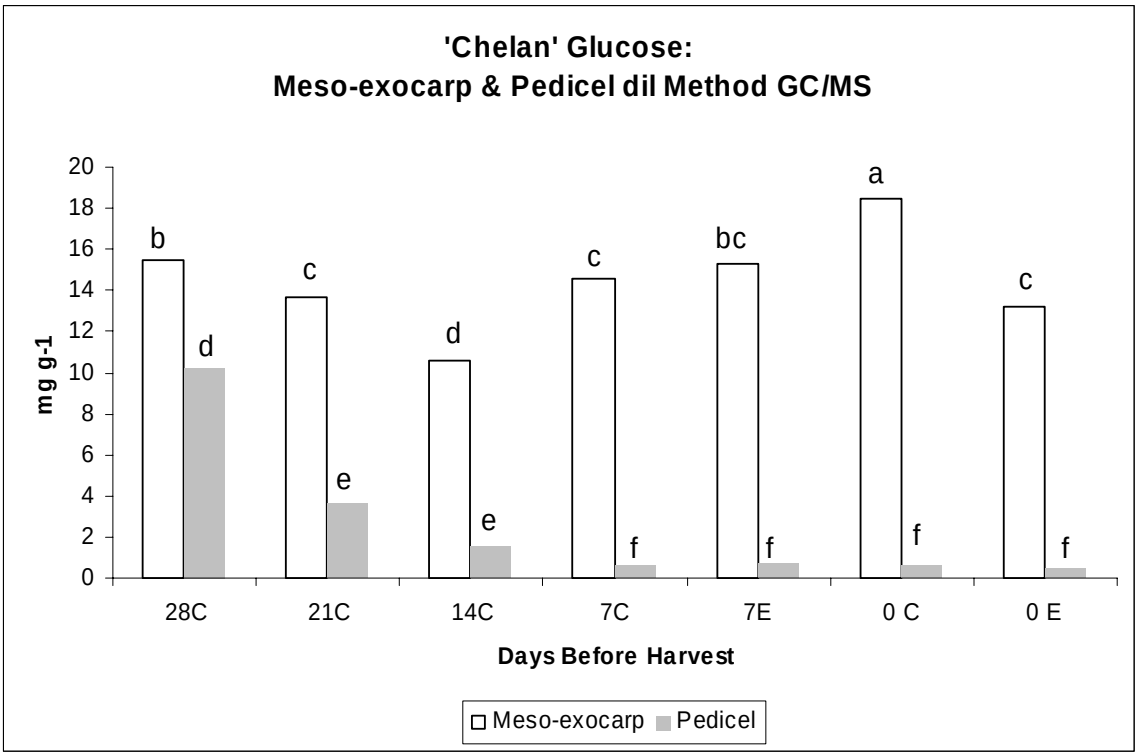

Figure 5-11. Glucose concentrations (mg g^{-1}) in 'Chelan' meso-exocarp and pedicel tissue from 28 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

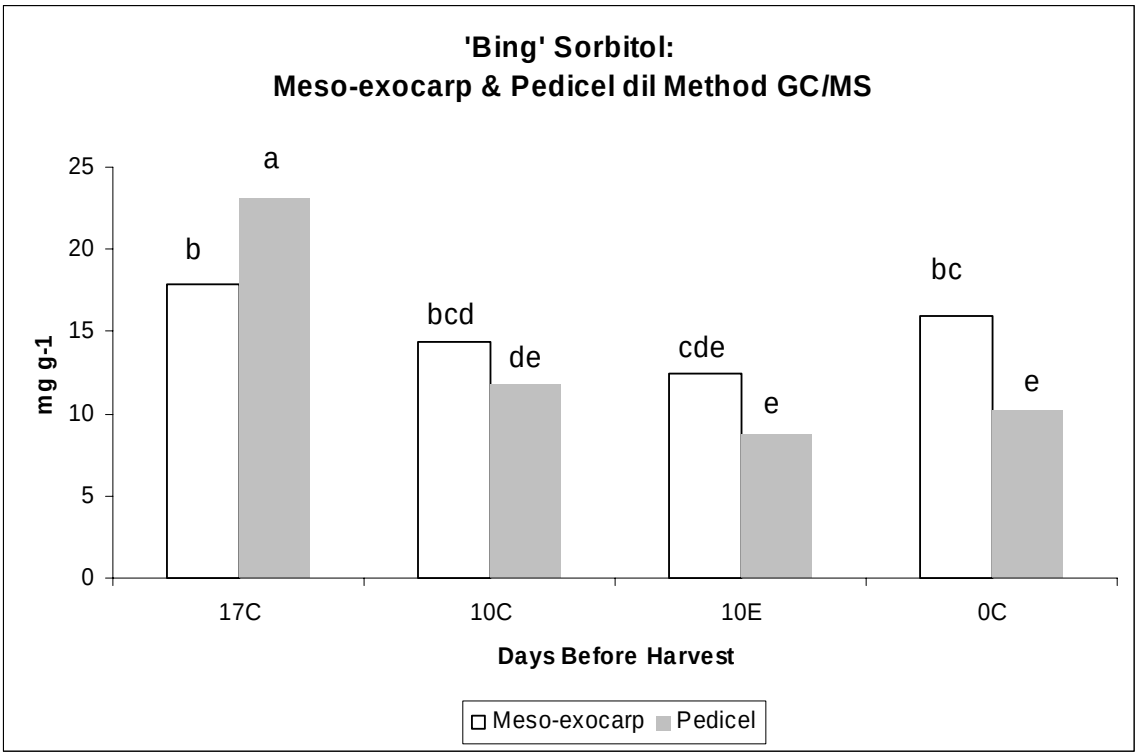

Figure 5-12. Sorbitol concentrations (mg g^{-1}) in 'Bing' meso-exocarp and pedicel tissue from 17 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

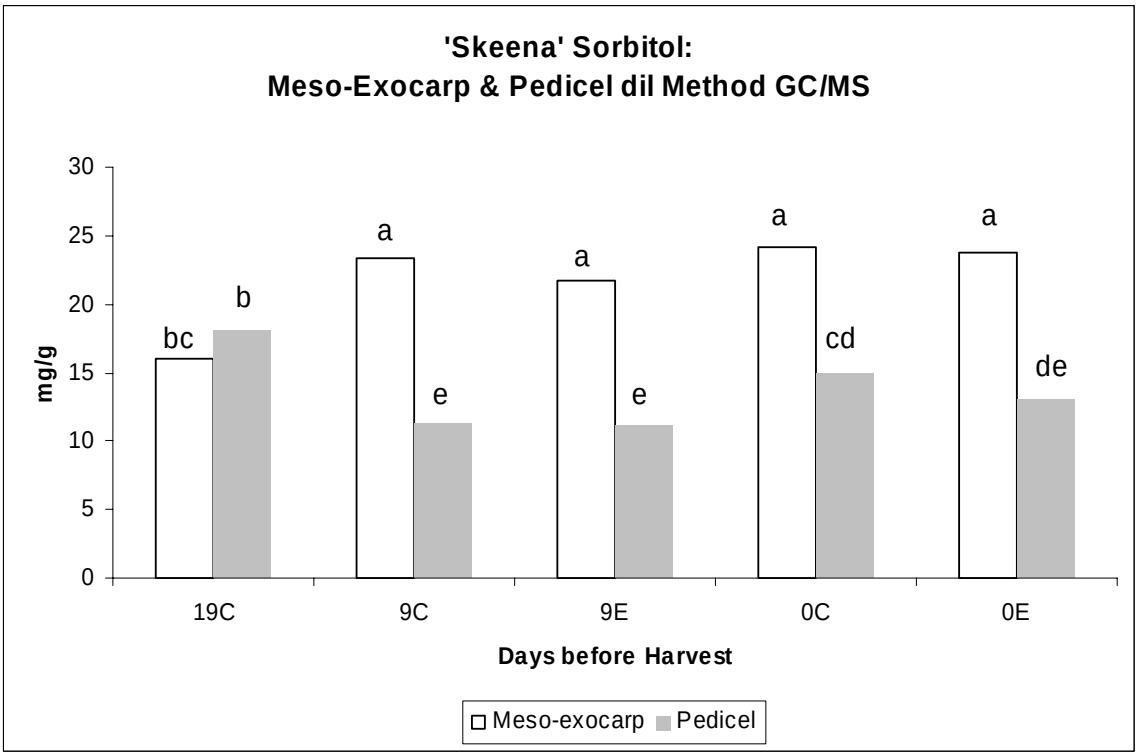

Figure 5-13. Sorbitol concentrations (mg g^{-1}) in 'Skeena' meso-exocarp and pedicel tissue from 19 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

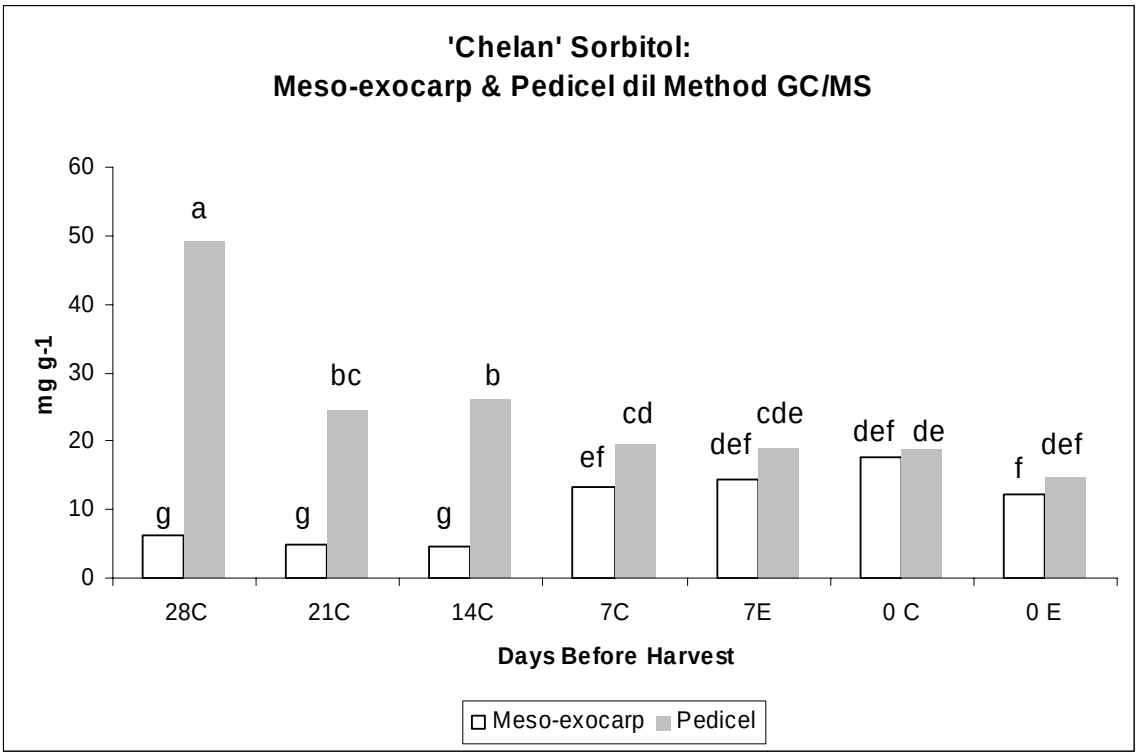

Figure 5-14. Sorbitol concentrations (mg g^{-1}) in 'Chelan' meso-exocarp and pedicel tissue from 28 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

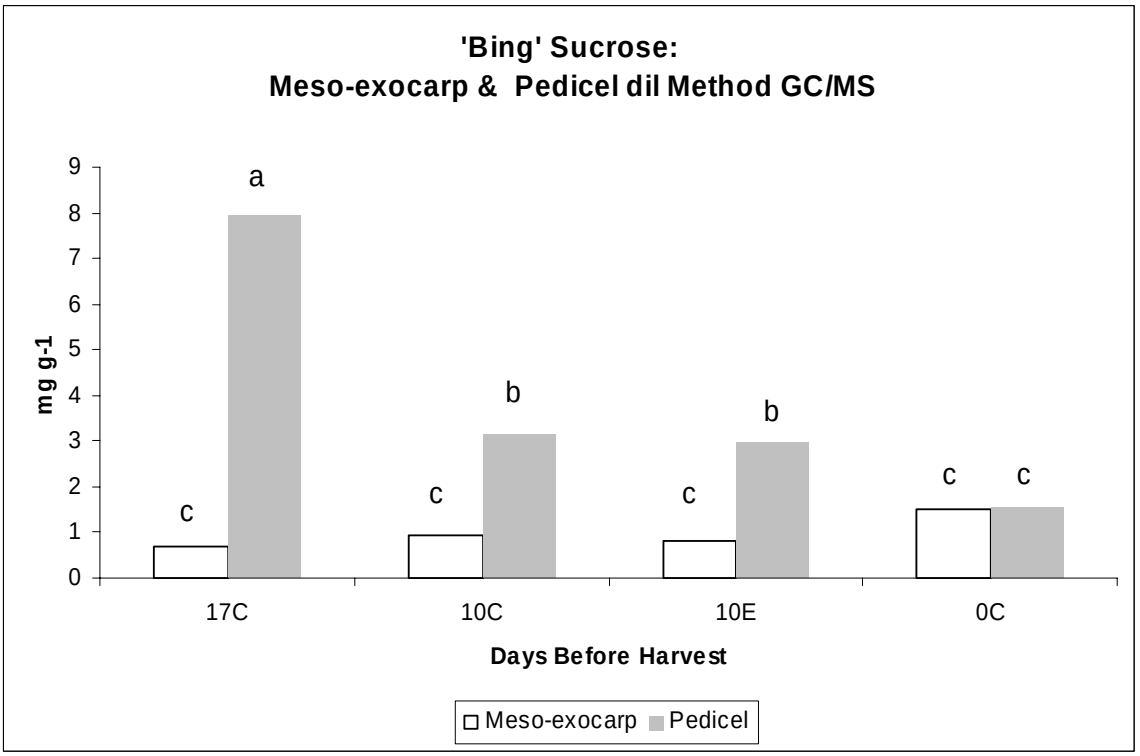

Figure 5-15. Sucrose concentrations (mg g^{-1}) in 'Bing' meso-exocarp and pedicel tissue from 17 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

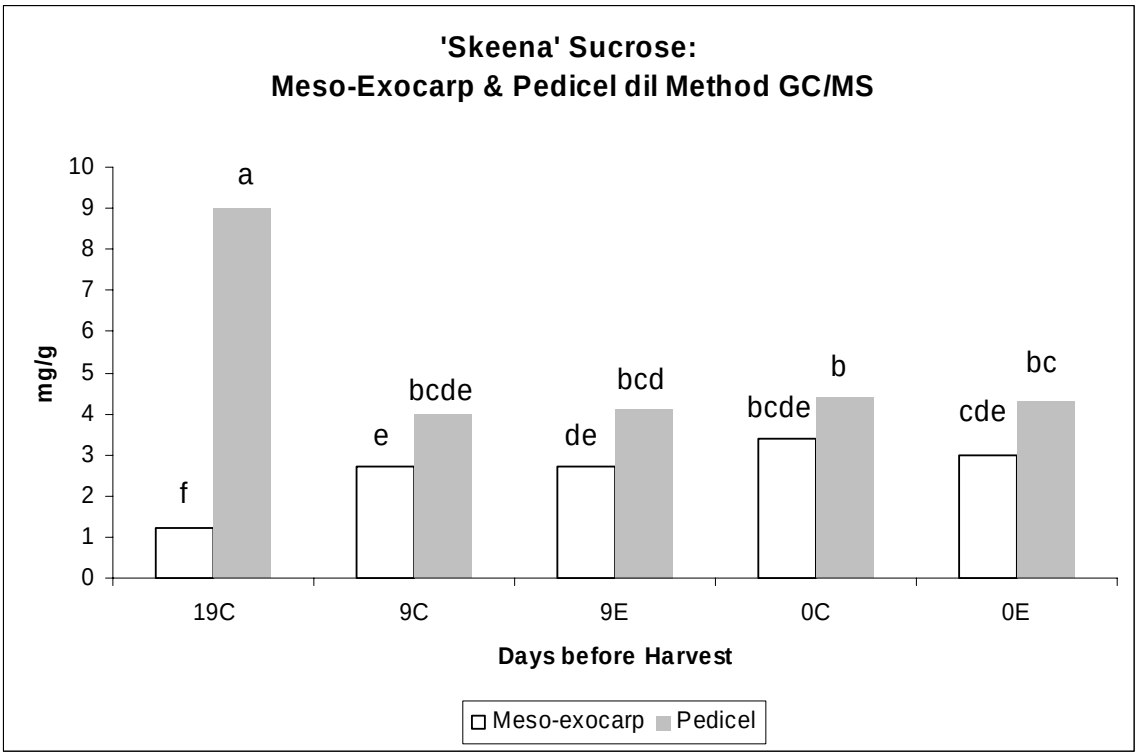

Figure 5-16. Sucrose concentrations (mg g^{-1}) in 'Skeena' meso-exocarp and pedicel tissue from 19 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

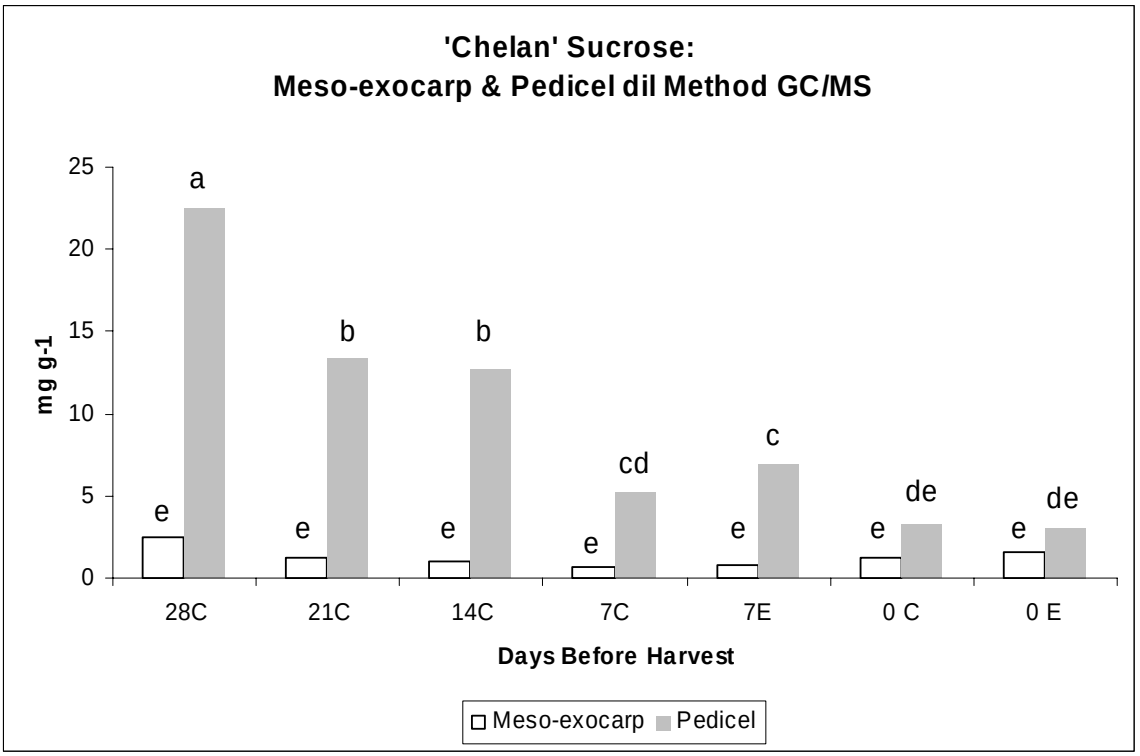

Figure 5-17. Sucrose concentrations (mg g^{-1}) in 'Chelan' meso-exocarp and pedicel tissue from 28 DBH to harvest. C = control untreated tissue; E = ethephon treated tissue. Bars followed by the same letter are not statistically significant at $\alpha = 0.05$ [SAS Proc GLM (SAS Institute, Cary, NC) using least significant difference procedure].

Appendix A-1

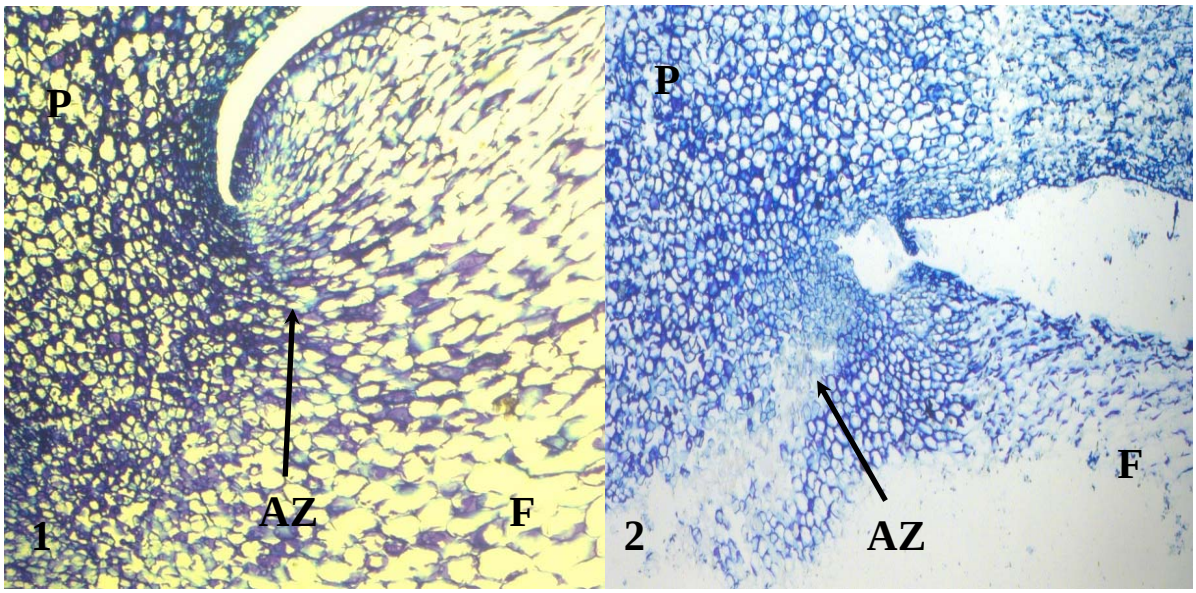

Figure A-1. Cross sections of 'Skeena' pedicel/fruit abscission zone infiltrated with paraffin and stained with toluidine blue O. Images viewed at X4 with a light microscope. 1 = sample collected on 27 June 06 with a PFRF of 859 g. 2 = sample collected on 11 July 06 with a PFRF of 417 g. P = pedicel tissue. F = fruit tissue. AZ = abscission zone tissue

Appendix A-2

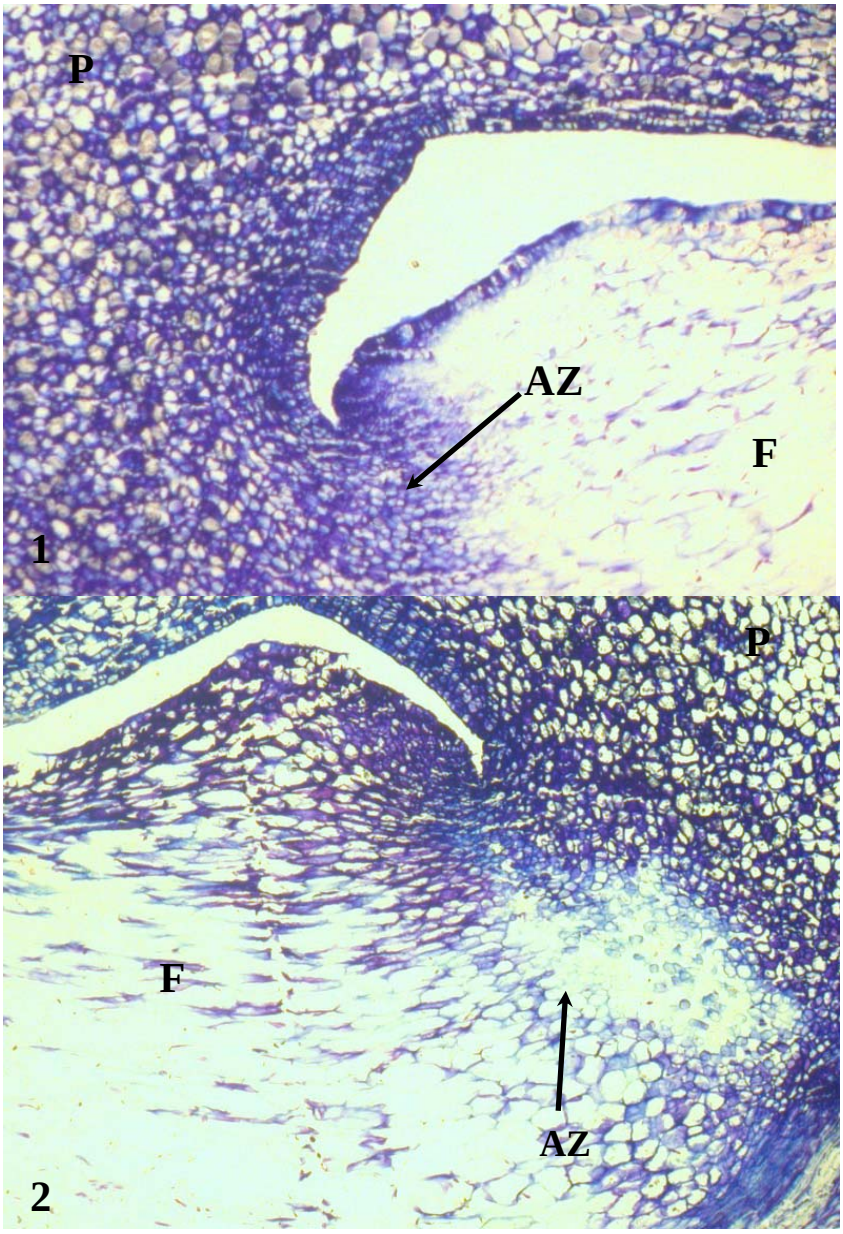

Figure A-2. Cross sections of 'Bing' pedicel/fruit abscission zone infiltrated with Paraffin and stained with toluidine blue O. Images viewed at X4 with a light microscope. 1 = sample collected on 23 June 06 with a PFRF of 850 g. 2 = sample had 3.5 L ha^{-1} ethephon applied 11 days prior and was collected on 23 June 06 with a PFRF of 418 g. P = pedicel tissue. F = fruit tissue. AZ = abscission zone tissue