STRESS PHYSIOLOGY OF PEDICEL BROWNING IN SWEET CHERRIES

by

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ABSTRACT

The overall objective of this research was to investigate the influence of some physiological stress factors on postharvest pedicel browning in sweet cherry (*Prunus avium* L.). While the research focused on examining the effects of stresses on stem quality; decay, pitting and soluble solids content (SSC) in the fruit were also monitored. Because PPO is a major enzyme involved in browning, PPO activity under the influence of some stress factors was also examined. Recommendations aimed at improving commercial sweet cherry quality were made as a result of this work.

The use of a reflective tarp was assessed for its potential to improve quality retention of sweet cherries. The tarp was applied as cover to bins of harvested cherries in the orchard and also during open-truck transport to the packing house. Controls were bins that were left uncovered in the orchard and during transport. The results show that reflective tarps, applied with a white painted side facing the sun and the shiny metallic surface next to the cherries, reduced pedicel browning, fruit decay and pitting, when cherries were stored in modified atmosphere packages (MAP) for several weeks.

Reflective tarps helped to retain cherry pedicel quality through modification of temperature and relative humidity (RH). Fruit that was covered remained up to 5 °C cooler than fruit that was uncovered and the RH in the immediate air space surrounding the fruit was increased by 15-20%. Under these modified conditions the pedicels of covered fruit retained more water than those that were uncovered. UV-absorbing substances leaking from pedicel tissue was measured . From this inferences were made regarding damage to pedicel cellular membranes. RLR always negatively correlated with pedicel browning ($R = -0.92$, $P \leq 0.01$). This indicated that browning was related

to some structural damage to the plant tissue.

The effects of temperature, RH and light on pedicel browning were examined separately in several laboratory experiments. RH had the single largest effect on pedicel browning. As RH decreased, browning increased. Higher temperatures also resulted in more pedicel browning. Radiation from metal halide lamps and fluorescent bulbs had no effect on pedicel browning.

The effect of postharvest handling at 0 °C, 10 °C, 20 °C and 30 °C on 'Van', 'Bing' and 'Lapins' cherry pedicels was investigated at two stages of maturity. 'Van'pedicels were more resistant to browning than 'Bing' or 'Lapins' pedicels regardless of temperature or maturity. 'Lapins' pedicels showed the highest amount of bruising. Less browning occurred in more mature 'Lapins' and 'Bing'pedicels than in less mature 'Lapins' and 'Bing' pedicels. Cherries that are harvested too early may be more susceptible to handling injury. Cherry pedicels were most susceptible to bruising at 30 °C and least susceptible at 10 °C. These results suggest that optimum harvest time for 'Lapins' and 'Bing' for pedicel quality was at the mahogany maturity stage and the best handling temperature was 10 °C.

Preharvest water stress had no effect on pedicel browning. Cherry trees were stressed by withholding water for four weeks and two weeks before harvest. Controls were cherries from trees that were watered weekly. Overall soil moisture content and pressure bomb readings indicated that the water stressed trees were without sufficient irrigation. Withholding water for up to four weeks before harvest did not result in higher rates of pedicel browning.

Some properties of cherry pedicel polyphenol oxidase (PPO) were examined by kinetic analysis and electrophoresis. PPO activity did not correlate with pedicel browning. The optimum pH for PPO activity was 5.5. Two PPO isozymes were isolated from 'Lambert' and 'Lapins' pedicels by SDS-PAGE on 4-15% gradient gels under partially denaturing conditions. A smaller,

more intense band with an apparent molecular weight of 43.5 kD and a less intense larger band with apparent molecular weight of 37.5 kD, were isolated from both brown and green 'Lambert' and 'Lapins' pedicels. More PPO was expressed in the pedicels of 'Lapins' than those of 'Lambert'.

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I. GENERAL INTRODUCTION

1. Pedicel browning in sweet cherry

Sweet cherry (*Prunus avium* L.) is an important commercial crop in British Columbia (B. C.) and the U. S. In 1996, the Washington State sweet cherry crop was estimated at 31,500 tons and the yearly average for the previous three years was 44,655 tons (Haas, 1996). In Washington and B. C., the current trend is towards a significant increase in sweet cherry production (Hansen, 1997).

The key economic value of the sweet cherry crop is in fresh marketing. Sweet cherries are highly perishable and strategies are being developed to extend the shelf life in order to reach larger, more distant markets. Modified atmosphere packaging (MAP) is now being used on a commercial scale to a limited extent (Reed, 1995; Thompson *et al.*, 1997). Although it has been shown that the quality of the fruit remains high for as long as 6 weeks in storage, the pedicels often become dried and shrivelled. Brown pedicels seriously affect the acceptance and sale of fresh cherries (Ian Robertson, Sunfresh Co-op, personal communication). It is therefore desirable to keep the pedicels looking as green and turgid as possible.

MAP systems are designed to alter the ambient levels of O_2 and CO_2, inside sealed gas-permeable packaging material. Low levels of O_2 have been shown to reduce the postharvest respiration rates in fruits and vegetables (Weichmann, 1987). High levels of CO_2 (10% to 20%) have also been shown to prevent browning by decreasing polyphenol oxidase (PPO) activity and phenolic content (Buescher and Henderson, 1977). The recommended optimum atmosphere for sweet cherries is 3% to10% O_2 and 10% to12% CO_2 (Reed, 1995).

Extensive research has attempted to develop cherries with high quality agronomic characteristics such as large size, lasting firmness, uniform color, characteristic flavor and resistance

to rain cracking. *Prunus avium* L. 'Lambert' is a major cultivar in the Okanagan Valley, with good yields and firm fruit (Hansen, 1997). It is somewhat susceptible to rain splitting but pedicel quality is generally good. *Prunus avium* L. 'Lapins' is a new cultivar that was developed at the Pacific Agriculture and Agri-food Canada Station (PARC), Summerland, BC. The flavor and size is considered to be as good as *Prunus avium* L. 'Bing' (generally used as a standard for quality in the United States) without the problem of rain splitting (Hansen, 1999). The planting trend for 'Lapins' is currently increasing both in BC and Washington (BCMAF, 1998). Unfortunately, pedicels of 'Lapins' seem to be more susceptible to browning (Hansen, 1999).

'Lapins' pedicels are long and very thin and could be more susceptible to physical and physiological injury. They may be more sensitive to chemicals than 'Bing' and they may be more photosensitive. Pedicel quality is one characteristic that gives 'Bing' a clear advantage over 'Lapins' (Hansen, 1999). 'Lapins' tend to grow in huge, tightly held clusters, where wind can damage the pedicels when they rub against each other. Very little light filters through to pedicels in the middle of these tight clusters. This may cause the pedicels to be more vulnerable, immediately postharvest, when exposed to bright sunlight in open bins.

Several factors have been implicated in pedicel browning. Siegelman (1952) reported that browning was a function of temperature and humidity. Radiation from the sun and respiration both increase the temperature of the cherries during harvest (Siegelman, 1952). Keeping fruit temperatures low, immediately after harvest, results in firmer fruit, reduced decay and shrivel rates, and greener pedicels (Drake *et al.*, 1988; Young and Kupferman, 1994).

In order to market cherries that are commercially acceptable, it is imperative to prevent immediate postharvest heat loading. Reflective materials have been used by foresters in remote areas

to protect seedlings from heat stress (Deyoe *et al.*, 1985). Reflective tarps, made up of a laminated polyethylene material with Mylar on one side and a white coating on the other side, have been shown to keep temperature down to a range equivalent to that of deep shade. This may be one step towards optimizing pedicel quality that could be implemented easily in the field by commercial orchardists.

Conventional breeding can be used to improve agronomic characteristics, but is often a long, arduous task. Sometimes biotechnology can be used to significantly decrease the time to incorporate improved quality characteristics into new cultivars. It was evident at the International Cherry Symposium recently held in Norway that very few molecular genetic techniques have been used in cherry breeding and research (Lang, 1998). The use of molecular genetic techniques to incorporate quality characteristics into pedicels of new cultivars has not been fully investigated. If pedicel browning is more prevalent in some cultivars, it may be possible to map related genes and use them as molecular markers to screen for seedling populations that have potential for high expression of components that are involved in browning. Isoenzyme analysis can be used to identify the proteins that are more prevalent in brown tissue.

2. Oxidative enzymes involved in browning

Enzymatic browning of fruits and vegetables is well known and is generally considered to be caused by the oxidation of phenolic compounds into highly reactive quinones (Vasmos-Vigyazo, 1981; Zawitowski *et al.*, 1991; Fraignier *et al.*, 1995). These molecules then react with proteins and amino acids, polymerize, and form the characteristic brown pigments. One major reaction is catalyzed by PPO in the presence of O_2.

PPO is an intracellular enzyme that is reported to occur in plastids (Zawitowski *et al.*, 1991). Phenolic compounds are normally restricted to the vacuole, which explains why intact tissues do not turn brown. Following intracellular damage, substrates and enzymes mix freely in the presence of O_2 and browning occurs. Intracellular damage occurs when tissues are under physical stress, pathogen attack or other environmental stress.

PPO is ubiquitous in plant tissues and is thought to serve mainly in plant defense mechanisms (Zawitowski *et al.*, 1991). PPO is activated during infection and mechanical injury which results in quinone formation. Quinones then combine to form the insoluble colored pigment polymers (Mayer and Harel, 1979). In plant tissues these serve as a physical barrier and thus prevent the spread of infection in the plant. It has also been suggested that intermediates formed during polymerisation of phenolics can bind to or inactivate plant viruses and limit their virulence (Zawitowski *et al.*, 1991). Melanins and quinones have bacteriostatic properties and can partially inactivate some potato viruses. Quinones have also been shown to prevent the spread of virus infection in apples and broad beans (Zawitowski *et al.*, 1991).

Browning is responsible for serious economic losses in the agriculture and food industries (Vasmos-Vigyazo, 1981). Techniques that have been used to deal with the problem include the use of browning inhibitors and breeding for cultivars that are less susceptible to browning (Mayer and Harel, 1979; Zawitowski *et al.*, 1991). Attempts have been made to study PPO expression using molecular techniques. Cloning and sequencing of PPO genes from vegetative tissues of tomato (Newman *et al.*, 1993), potato (Hunt *et al.*, 1993) and broad bean (Cary *et al.*, 1992) indicate that PPO genes code for native proteins with molecular masses ranging from 57 kDa to 62 kDa. Bachem *et al.* (1994) used antisense gene strategy to inhibit PPO activity in potato tubers. This approach may

be beneficial in preventing oxidative browning in other tissues like cherry pedicels.

3. Plant cellular membranes in response to stress

Plant cellular membranes control diffusion and determine what leaves and enters plant cells (Sussman and Harper, 1989). Diffusion across membranes is inversely proportional to resistance, and thus affected by any alterations to the cell membrane (Whitlow *et al.*, 1992). A measure of either UV-absorbing substances or electrolytes leaking from plant cell tissues is often used as a measure of membrane integrity in plant tissues (Pooviah and Leopold, 1976; Redman *et al.*, 1986; Finlayson *et al.*, 1989; Whitlow *et al.*, 1992). Environmental stresses, growth and development, and genotypic variation all contribute to variable membrane integrity (Whitlow *et al.*, 1992). Redman *et al.* (1986) examined salt stress injury to leaf tissue of woody species by measuring leakage of UV absorbing substances (amino acids, nucleosides, nucleotides) diffusing through the plant cell membranes into the surrounding bathing media.

Cellular membranes are composed of three layers each containing proteins and lipids (for a summary see Salisbury and Ross, 1992). Proteins make up approximately 60% of the dry weight of the membrane. The lipid component consists of a 3-carbon glycerol backbone (hydrophilic) with attached long chain fatty acids (hydrophobic). The principal lipids of plant cell membranes are phospholipids, glycolipids and sterols. The proteins are either embedded in the lipid bilayer or are strictly on one of the surfaces. Some of these proteins make up channels that are structured to be selective for solutes depending on cellular conditions. They can remain open, closed or partly open. Plant cell membranes are differentially permeable as they allow water (osmosis) and selected solutes to pass through. Once organic molecules are absorbed into cytoplasm or vacuoles they do not readily

leak out. Rapid leakage can be induced by damaging cell membranes with heat, poisons, lack of O_2 and by removing calcium (Levitt, 1972).

Stresses can alter membrane systems or membrane-associated protein patterns. For example heat shock proteins are produced by plants in response to rapid exposure to heat. These proteins are observed in abundance in plants that show heat tolerance, perhaps as a defense mechanism by protecting sensitive enzymes and nucleic acids from denaturation (Lindquist and Craig, 1988).

4. Objectives

Because pedicel color is an important quality index and brown pedicels is often a primary reason for rejection of sweet cherries by produce buyers., it is important to design research that addresses pedicel browning in order to make recommendations. PPO is the enzyme that is generally associated with the browning of tissues and can be activated by mechanical injury (Mayer and Harel, 1979). Further investigation into the properties and behavior of PPO is justified then by adding to the general body of scientific knowledge regarding this enzyme. If it can be shown, by molecular techniques, that PPO correlates with browning in certain cultivars or with brown tissue, then it may be possible to silence the PPO gene through antisense gene technology. Another application may be as a probe to screen out populations of new cultivars that are more susceptible to browning.

The goal of this research was to investigate the influence of some abiotic stress factors on postharvest browning in cherry pedicels and examine PPO activity under the influence of these stress factors. The specific objectives were to determine if:

1. preharvest water stress influences postharvest pedicel browning and shrivel rates,
2. covering cherries with reflective tarps during harvest and transport reduces pedicel browning in

storage under MAP conditions,

3. modelling potential effects of temperature, relative humidity, and light on pedicel browning to obtain a better understanding how tarps might influence pedicel quality in the field,
4. temperature affects susceptibility of cherry pedicels to bruising,
5. PPO activity and isoenzyme patterns in cherry pedicels correlate with their browning.

II. STRESS FACTORS INVOLVED IN PEDICEL BROWNING

1. Preharvest water stress

1.1 Introduction

Water stress is an important consideration for researchers in agriculture because it can severely limit growth and productivity (Hanson and Hitz, 1982). It is difficult to know how often and how much water should be applied to a crop to balance economy and maximize production. Plant water relations often provide the most cost effective approach to obtaining information regarding physiological status (Levitt, 1972). Low soil water potentials are generally indicative of insufficient water and this may be associated with stress. The water status of the plant tissue when measured with a pressure bomb reveals whether water deficit stress exists (Jones and Tardieu, 1998).

Whether low levels of soil moisture lead to plant water stress depends on many factors. The most important factors are the relative size of the root system compared to the canopy, climate (wind, air temperature, humidity) and soil texture (Vereecken *et al.,* 1990). Finer textured soils have a lower water potential for a given water content than coarse textured soils, and therefore provide water to plant roots much more slowly. Considering the complexity, it is difficult for orchardists to know how much water is required.

Because horticultural crops are sold by weight, an attempt is generally made to maximize water content, without sacrificing quality (Jones and Tardieu, 1998). For sweet cherries, too much water at a critical time can lead to problems (Lang, 1998; Seske, 1996). During the latter part of fruit maturation, tissues become fully turgid and when rain droplets remain on the fruit surface, conditions leading to cracking can occur. In an attempt to reduce cracking, some orchardists reduce or eliminate watering for a period prior to harvest (Norm Filipenko, personal communication). During hot and

dry conditions, the physiological status of the cherries may be altered (Kays, 1999) causing the pedicels to be more susceptible to bruising during harvest and packing line procedures (Crisosto *et al.*, 1993). A secondary effect of water deficit stress is that it renders produce more susceptible to thermal injury (Kays, 1999).

An experiment was set up to subject cherry trees to water stress by withholding water for 14 d and 28 d prior to harvest. The objective of this study was to determine if these levels of water stress would cause an increase in pedicel browning. The hypothesis was that "cherries harvested from trees undergoing water stress results in higher rates of pedicel browning compared with cherries from trees watered on a weekly schedule".

1.2 Materials and methods

Twelve 'Lambert' cherry trees were randomly selected from a mixed block planting at PARC. Water deprivation was accomplished by placing 6 mil polyethylene vapor barrier on the soil surface and extending it to beyond half of the radius of the canopy (6.1 m x 6.1 m). This is considered to be the area under the cherry tree where most of the root system is likely to occur (Facteau *et al.,* 1996). The vapor barrier was put in place under four trees 28 d before harvest (DBH) and under an additional four trees 14 DBH. The sprinklers in these plots were also turned off at the same time. The four control trees were watered each week for 8 to10 h.

Water content in the soil was monitored with tensiometers (Essert and Hopmans, 1998), 0.61 m in length (Model R, Irrometer Co., Riverside, CA). They were installed 2.13 m from the trunk and on the same side of each tree. The tensiometers consisted of a porous ceramic cup glued into a PVC pipe, which was filled with water after installation into the soil. Soil water potential (SWP)

was measured by negative pressure which was monitored by an attached pressure gauge.

The water potential (ψ) in the cherry pedicels and leaves was measured by a pressure bomb (PMS, Model 610, Corvallis, OR). Measurements were taken for seven consecutive days before harvest date, between 8:00 AM and 9:00 AM. Cherry leaves and pedicels (with the fruit attached) were selected randomly from the shaded side of each tree. The proximal portions of the leaf petioles and cherry pedicels were severed with a razor blade and placed in the pressure vessel of the pressure bomb with only the cut end protruding from the specimen holder through a silicone rubber seal (Herppich *et al.*, 1999). The tank contained pressurized nitrogen gas which was slowly released until the xylem sap started to emerge from the water conducting system. ψ values are the means of 10 readings from one tree per treatment, selected randomly from the mixed block.

A separate set of samples were carefully harvested by hand and collected in 1 kg hinged berry baskets. Eight 1 kg samples were harvested from each of the twelve trees, refrigerated immediately and stored at 1 °C, ~75% RH overnight. The following day all the samples were heat sealed with a vacuum bag sealer (Neue Transvac Maschinen AG, Lucern, Switzerland) in a 75 gauge polyolefin material (Cryovac PD-941bag, Grace & Co., Mississauga, ON) and returned to 1 °C, ~75 % RH for storage evaluations. The plastic allowed for 16,544 mL m^{-2} 24 h O_2 transmission rate, which is suitable for packaging high-respiration produce (Moyls, 1998). Two samples from each replicate were evaluated for quality characteristics at 2 wk intervals over a 6 wk period.

Twenty-five fruits from each sample were scored for cracking, pitting and decay. Soluble solids content (SSC) in the juice, obtained by hand crushing 10 cherries in a plastic bag, was measured with a refractometer as an additional indicator of fruit quality. Pedicels were assessed for browning using a four point pedicel colour index; 1 = 75-100% brown, 2 = 50-75% brown, 3 = 25-

50% brown and 4 = 0-25% brown. Fresh pedicels were weighed (FW), dehydrated (24 h) at 60 °C in a vacuum oven, and reweighed (DW) until no further weight loss occurred. The percent water content was calculated as [(FW - DW) ÷ FW] x 100.

The experimental design was a randomized complete block design with four blocks. Each block consisted of three trees and treatments were assigned randomly within each block. Data were subjected to ANOVA using SAS GLM procedure (SAS, Cary, NC). Duncan's Multiple Range Test was used to analyze the pressure bomb data.

1.3 Results

Water stress in this experiment had no effect on pedicel browning through 6 wk of storage (Fig. 1). Throughout the first four weeks of storage all pedicels rated 3 or better. It wasn't until week 6 that the pedicels of cherries from trees that had water withheld for 28 d scored slightly below 3. At week 6 there was also a slight increase in browning in untreated (control) cherries and those that had water withheld for 14 d. There were no significant differences between treatments.

The amount of water deprivation used in this experiment had no effect on the water content of the pedicels (Fig. 2). The percent of fresh weight water content decreased during storage but there were no significant differences between treatments.

Water tension in the soil, as measured by tensiometers, rose from approximately - 10 kPa to almost - 60 kPa when water was withheld for 28 d (Fig. 3), indicating that less water was available as the experiment progressed. In the control plot, the water tension ranged between - 10 kPa and - 15 kPa. Some seepage occurred at the edges of the plastic during the weekly irrigation sessions as indicated by the dips in water tension in all treatments on four occasions (Fig. 3).

FIG. 1. Pedicel browning in 'Lambert' cherries that had water withheld for 14 d and 28 d before harvest (DBH). Normal irrigation (control) occurred on a weekly basis for 8 to 10 h. Pedicel color index: 4 = 0 - 25 % brown, 3 = 25 - 50% brown, 2 = 50 - 75% brown and 1 = 75 - 100% brown. There were no significant differences between the treatments.

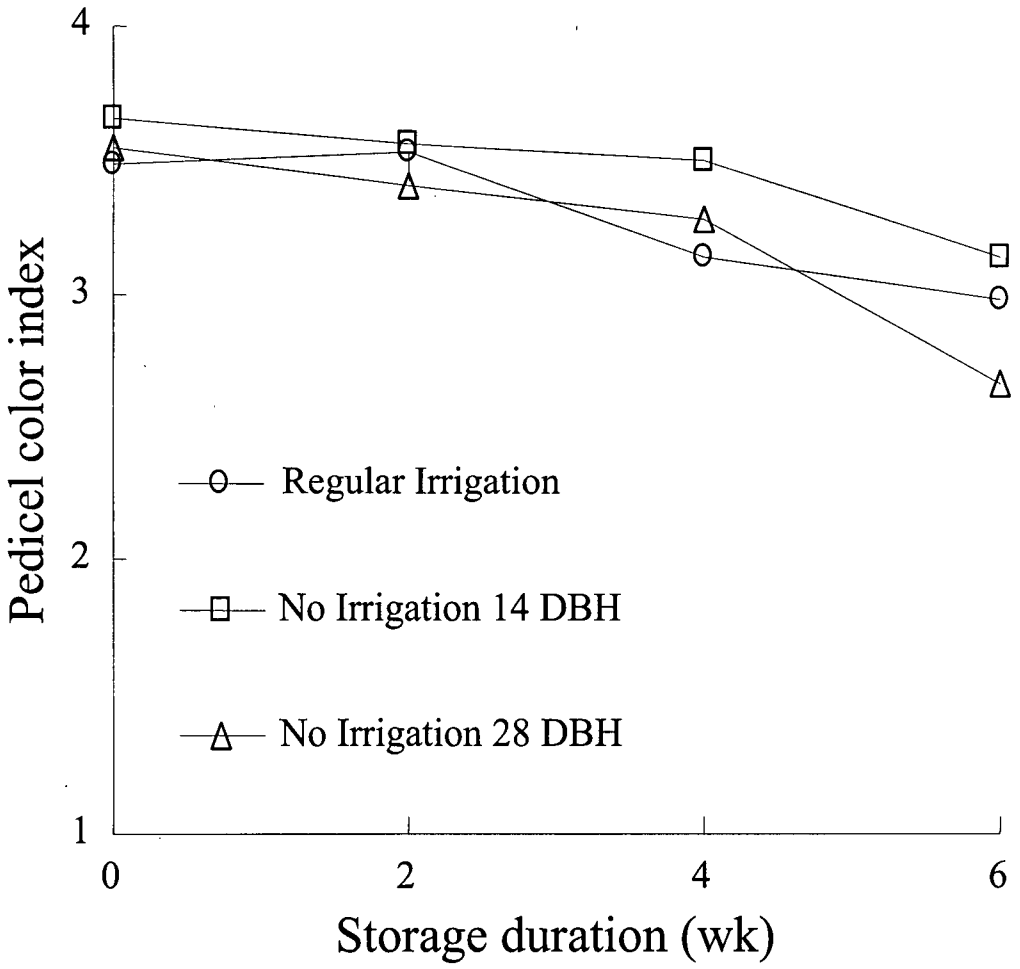

4
3
2
1
Pedicel color index
Regular Irrigation
No Irrigation 14 DBH
No Irrigation 28 DBH
0
2
4
6
Storage duration (wk)

FIG. 2. Pedicel water content in 'Lambert' cherries that had water withheld for 14 d and 28 d before harvest (DBH). Normal irrigation (control) occurred on a weekly basis for 8 to10 h. The percent water content was calculated as [(FW - DW) ÷ FW] x 100. There were no significant differences between the treatments.

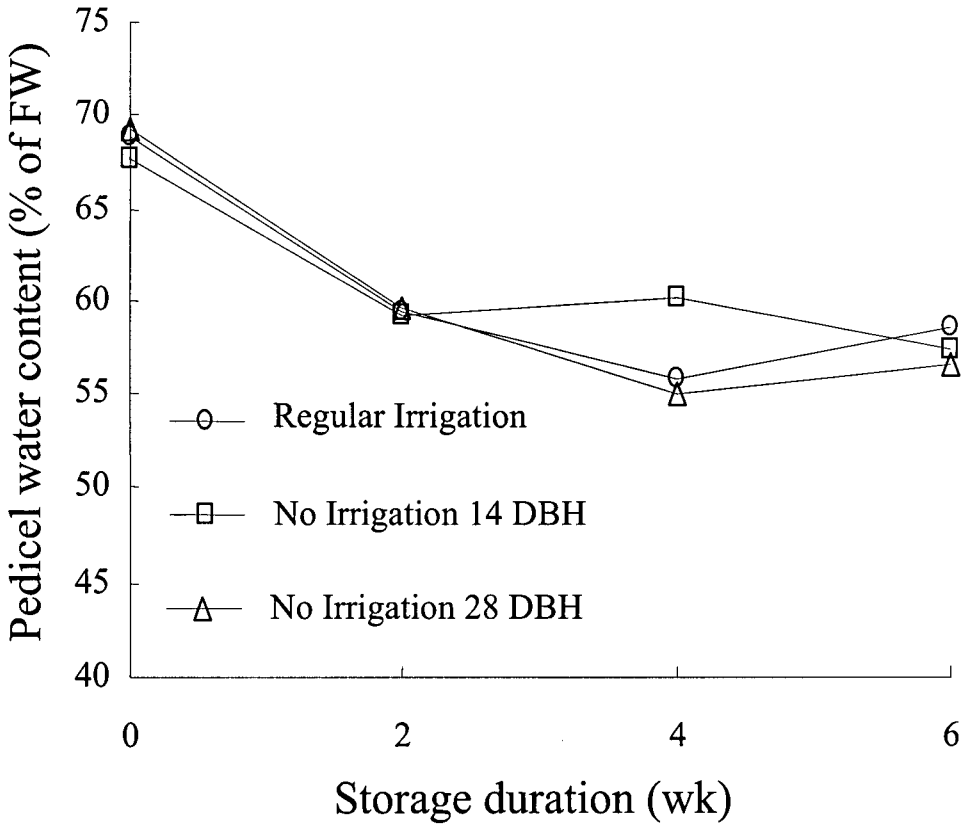
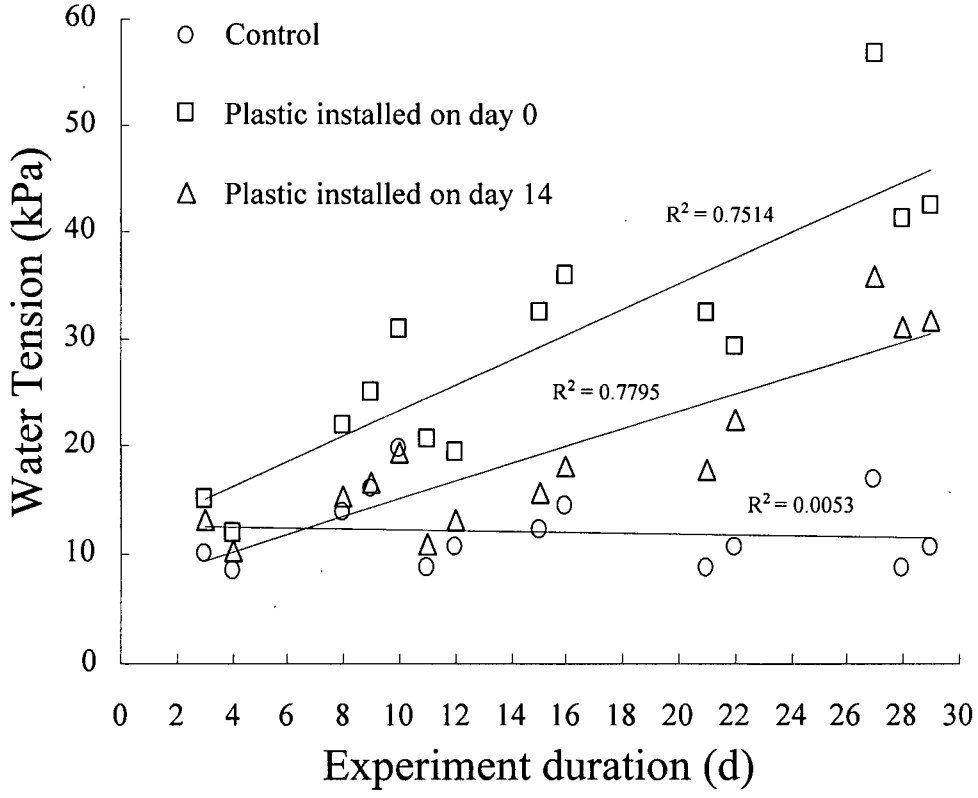

75
70
65
60
55
50
45
40
Pedicel water content (% of FW)
Regular Irrigation
No Irrigation 14 DBH
No Irrigation 28 DBH
0
2
4
6
Storage duration (wk)

FIG. 3. The water tension of the soil under cherry trees was measured by tensiometers, 2.13 m from the trunk of the tree. Values are the absolute values of means to indicate the drying trend in the soil under three levels of water stress. Control trees were watered on a weekly basis for 8 to10 h. Linear regressions for each treatment and R^2 values are indicated on the graph.


Control
Plastic installed on day 0
Plastic installed on day 14
$R^2 = 0.7514$
$R^2 = 0.7795$
$R^2 = 0.0053$
Water Tension (kPa)
Experiment duration (d)
0
10
20
30
40
50
60
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

Pressure bomb readings (Table 1) indicated that the control trees were under mild water stress (Hanson and Hitz, 1982; Jones and Tardieu, 1998). In general, plants are under mild stress when ψ is in the range of -0.3 MPa to -0.8 MPa (Bradford and Hsiao, 1982). The ψ for cherry pedicels and leaves was highest in trees that had water withheld for 28 d (Table 1). There were no visible signs of wilting in any of the trees.

Withholding of water reduced the percentage of fruit that was cracked. The percentage of cracked fruit was 40% for the control compared with 33% and 27% for the fruit that had water withheld for 14 d and 28 d respectively (Appendix A). For fruit decay, fruit pitting and soluble solids data, see Appendix A.

1.4 Discussion

SWP is an indicator of the amount of water potentially available to plant roots in the soil (Levitt, 1972). If the water available is insufficient, the plant will become stressed. One way to assess the water stress level inside a plant is by measuring the ψ in the leaves or other vegetative tissue with a pressure bomb (Scholander *et al.*, 1966). In this experiment the pressure bomb readings indicated that even the control (regular irrigation) trees were under some stress (Bradford and Hsiao, 1982).

If there is adequate water available, transpiration contributes to cooling during hot weather. Excessive transpiration can lead to drought stress in plants in spite of high moisture levels in the soil due to the fact that the tissues cannot replenish water fast enough to meet evaporative demand (Levitt, 1972). Plants can be protected against drought injury by suppressing transpiration (Levitt, 1972). Antitranspirants (wax and oil emulsions) have been used on cherry trees in an attempt to

Table 1. The water potential (ψ) measured in cherry pedicels and leaves from trees under three levels of water stress treatment. Pressure bomb readings were recorded for seven consecutive days before harvest (DBH) and the means are shown.

Treatment	ψ (MPa)	
	Leaves	Cherry pedicels
Control	-0.55 b[Z]	-0.86 c
No irrigation 14 DBH	-1.20 a	-1.00 b
No irrigation 28 DBH	-1.30 a	-1.20 a

[Z]Mean separation in columns by Duncan's multiple range test ($P \leq 0.05$)

increase fruit size by reducing water stress (Uriu *et al.*,1975; Mitchell *et al., 1975*). Mitchell *et al.* (1975) found that wax emulsions, applied before harvest, reduced pedicel browning by 50%.

In general plants with ψ in the range of - 1.0 MPa to - 2.0 MPa are under high levels of stress and have increased respiration rates (Hsiao, 1973). The ψ in leaves from trees that had water withheld for 14 d or 28 d indicated that they were under relatively high levels of stress. The objective in this experiment was to determine whether this level of stress could lead directly to pedicel browning, but the results indicate that this was not the case. However, cherry pedicel susceptibility to bruising, under these levels of stress, was not tested here because of the way the cherries were handled. During commercial production cherries are handled quite differently and damage in the form of pitting and bruising occurs during harvest, transport and in packing line operations (Thompson *et al.*, 1997). Further investigation is required to establish if there is a relationship between water stress and pedicel browning resulting from any increased susceptibility to bruising.

The results in this experiment did not support the hypothesis that water stress might be one factor that is directly related to higher levels of pedicel browning. Overall soil moisture tension and pressure bomb readings indicated that the trees were without sufficient irrigation, but this did not translate into higher rates of pedicel browning. The observations here as well as reports on occurrence of damage in packing house operations (Thompson *et al.,* 1997) suggest that observed commercial browning, which occurs during harvest and postharvest handling, may be only indirectly related to preharvest water stress. More experimentation is required to test the hypothesis that preharvest water stress makes cherry stems more susceptible to the bruising that results from impact damage in packing house operations. In this experiment, cherries were handled carefully and very

little pedicel browning was observed in any of the treatments.

2. Considerations during harvest and immediately postharvest

2.1 Field work

2.1.1 Introduction

Maintenance of post harvest sweet cherry quality in modified atmosphere packaging (MAP) continues to be a focus of research for potential commercial consideration (Meheriuk *et al.*, 1995; Reed, 1995). Cherries are nonclimacteric with a fixed pool of resources for respiration throughout storage and therefore have a relatively short commercial postharvest storage life that depends on initial quality and storage conditions (Reed, 1995). Therefore it is imperative to optimize fruit quality through improved harvest and handling procedures. MAP is designed to maintain quality, not improve quality through storage (Reed, 1995).

Pedicel browning continues to be a problem for commercial sweet cherry production in British Columbia and Washington State (Thompson *et al.*, 1997). In 1997, a continuation of the project entitled "Optimization of modified atmosphere handling of sweet cherries" indicated that the largest factor influencing the levels of pedicel browning and loss of fruit quality was postharvest handling (Toivonen and Schick, 1997). Reflective tarps were used, coincidently, to cover cherries that were harvested since a refrigerated vehicle was not available and they could not be immediately transported to cooling facilities. Pedicel browning was not a problem under these conditions and excellent fruit quality was maintained for up to 6 wk in storage. However, samples that were taken from the packing line or from growers' bins, showed high rates of pedicel browning and deterioration in fruit quality (Toivonen and Schick, 1997). The improvement in fruit and pedicel quality were thought to be due directly to the use of these reflective tarps. A study was initiated in the spring of 1998 to determine if tarps could be beneficial in preserving quality of sweet cherries

in a commercial orchard setting.

2.1.2 Materials and methods

Reflective tarps, made up of a laminated polyethylene material with Mylar on one side and a white coating on the other side, were supplied by Bushpro® Supplies Inc. (Armstrong, B. C.) and designed to fit over commercial bins, 118 cm x 107 cm x 46 cm. The bin covers were placed over commercially grown and harvested 'Lapins' (Naramata, B.C.) and 'Lambert' cherries (Oliver, B.C.) in the field and during transport to packing houses.

During harvest, three bins were selected randomly and covered with reflective tarps. One bin was left uncovered beside each covered bin and served as a control. Fruit pulp temperature during harvest and transport was measured by HOBO data loggers (Onset Computer Corporation, Pocasset, MA). An extendable sensor was inserted inside the cherry fruit and the loggers were placed in the bins at the half-full level. The loggers were also equipped with humidity sensors, which recorded the RH in the same space within the cherry bins. Ambient orchard temperature and RH were monitored and recorded in the shade of the tree canopy. The data loggers were programmed to record data at 5 min intervals using BoxCar® Pro software (version 3.0+ for Windows, Onset Computer Corp., Pocasset, MA).

After the cherries were delivered to local packing houses, eight samples were selected randomly from each bin and packaged in 1 kg hinged berry baskets. All samples were transported back to PARC in a refrigerated vehicle (1 °C) and stored inside a cold room at 1 °C, ~75% RH overnight. Samples were also collected at the orchard site immediately after harvest and evaluated on site for pedicel browning.

All the samples that were transported back to PARC were handled, packaged and sealed as described in the previous experiment (Chapter II, Section1.2). Two samples (n = 25) from each of the three replicates were evaluated for quality characteristics at 2 wk intervals over a 6 wk period. Twenty-five fruits from each sample were scored for presence of pitting and decay. Fruit decay was assessed as a percentage of the 25 fruit sample showing any decay. Pitting was evaluated and recorded as a percentage of fruit showing any visible pitting. SSC in the juice, obtained by hand crushing 10 cherries in a plastic bag, was measured as described in Chapter II, section 1.2. Browning and per cent of fresh weight water content of the pedicels were also determined as described in the previous experiment (Chapter II, Section 1.2).

Relative leakage of UV-absorbing substances through cellular membranes was determined using modifications of the method described by Redman *et al.* (1986). Ten pedicels were cut into 1 mm thick cross sections. After rinsing off the damaged cells with 50 mL distilled water, the segments were incubated in 20 mL distilled water at room temperature for 2 h. Leakage of UV-absorbing materials into the bathing medium was determined in 2.0 mL by reading the absorbance at 280 nm (DU 640B Beckman Spectrophotometer, Fullerton, CA). This sample was added back to the original solution and the cherry pedicel tissue was cooled to - 20 °C overnight to destroy the cellular membranes. After thawing and bringing to room temperature, a second absorbance was determined at 280 nm. The relative leakage ratio (RLR) of the UV-absorbing compounds was calculated as follows: RLR = [A_{280} before freezing] ÷ [A_{280} after freezing], and this was used as a measurement to assess membrane integrity in pedicel tissue.

The CO_2 and O_2 inside the package headspace was measured by withdrawing a 2.0 mL gas sample with a syringe and injecting 0.5 mL into a gas chromatograph (Shimadzu GC - 14A,

TekScience, Oakville, ON.). The CO_2 was separated on a 1.83 m x 3.2 mm o.d. stainless steel column packed with 80/100 mesh Porapak Q (Supelco, Oakville, Ont.). The O_2 was separated on a 2.44 m x 3.2 mm o.d. stainless steel column packed with 80/100 mesh Molecular Sieves 5A. The flow rate of the carrier gas (He) was 30 mL min^{-1} and the column temperature was isothermal at 55 °C. The gases were quantified with a thermal conductivity detector.

The experimental design was nested with repeated measures over time. Data presented are means of two subsamples from each of the three replicates and pooled LSD. Data were analysed by SAS General Linear Models procedure. Correlation analyses were performed by SAS Regression procedure (SAS, Cary, NC).

2.1.3 Results

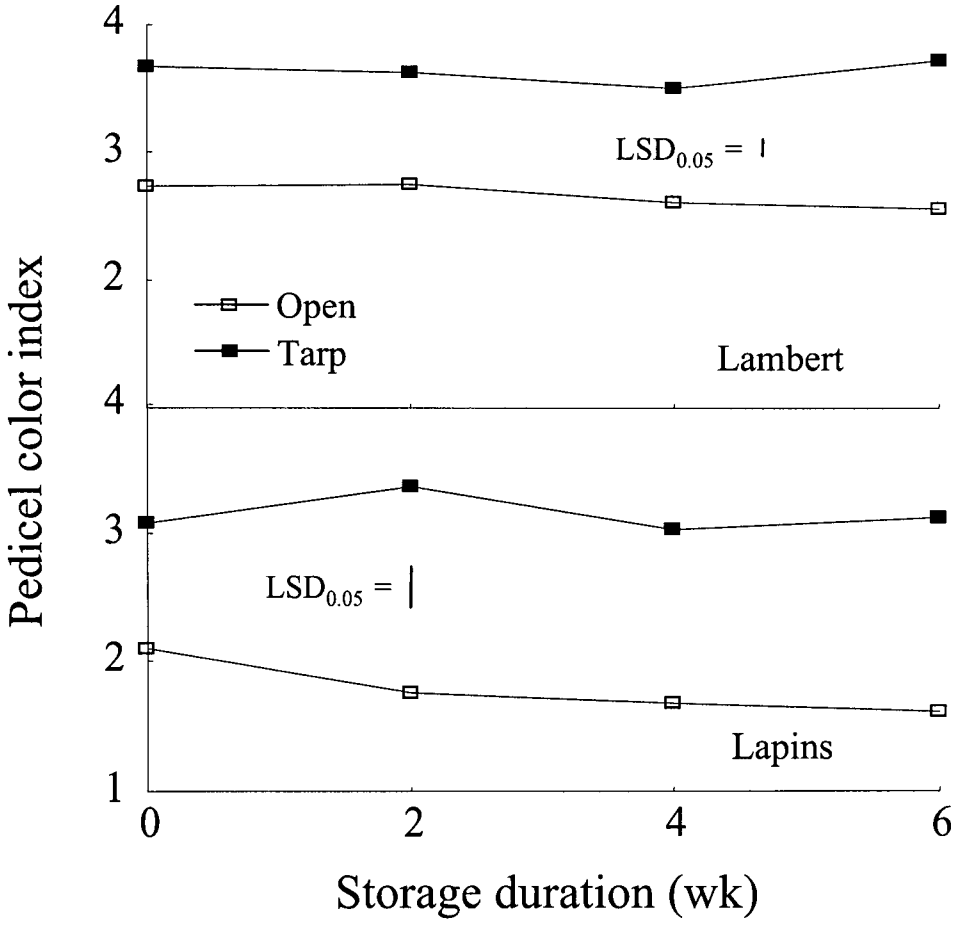
The tarps maintained lower temperatures in both the 'Lambert' and the 'Lapins' bins during harvest (Table 2). Relative humidity within the covered bins was higher than in the uncovered controls and much higher than ambient RH (Table 2). The 'Lambert' trees were large and the bins were deeply shaded during harvest. The 'Lapins' trees were much smaller and the bins were more exposed during harvest. The cherries were delivered to local packing houses in open pickup trucks. For temperature and humidity profiles during harvest, transport and overnight storage of 'Lapins' cherries see Appendix C.

Fruit kept under a tarp had better pedicel quality through 6 wk of storage (Fig. 4). Large differences were observed between pedicels of fruit from bins covered with a tarp and bins that were left uncovered. Pedicels from fruit that was covered rated at 3 or better (considered marketable) through 6 wk of storage. 'Lapins' cherry pedicels from uncovered bins rated below 2 on the four

Table 2. Fruit pulp temperature and RH within bins of covered and uncovered (control) 'Lapins' and 'Lambert' cherries. The ambient air temperature and RH in the tree canopy during harvest and postharvest. The values are maximum (max), minimum (min), means calculated from the data recorded every 5 min during 4 h at the orchard site.

		Temperature (^{0}C)			RH (%)		
Cultivar	Location	Max	Min	Mean	Max	Min	Mean
Lambert	Ambient air	35.7	23.6	27	32.7	25.4	29.5
	Control fruit	24.4	23.2	24.1	91.7	60	77
	Covered fruit	20.9	19.4	20.6	100	63.4	91.6
Lapins	Ambient air	29.9	22.5	24.9	47.3	24.5	33.2
	Control fruit	28.7	23.6	24.4	89.9	52.5	74.5
	Covered fruit	20.9	17.9	19.4	100	77.5	96.5

FIG. 4. Pedicel browning of 'Lambert' and 'Lapins' cherries covered with reflective tarps or left open in the orchard. Pedicel color index: 4 = 0 - 25% brown, 3 = 25 - 50% brown, 2 =50 - 75% brown and 1 = 75 - 100% brown. Data presented are the means of 6 samples (n = 25), two from each of the three replicates.


Pedicel color index
4
3
2
1
0
2
4
6
Storage duration (wk)
LSD0.05 = 1
Open
Tarp
Lambert
LSD0.05 =
Lapins

point color index. Pedicels scored at 2 showed greater than 50% browning and would definitely be unappealing to buyers and difficult to sell. Pedicels from cherries that were collected and evaluated at the orchard site, rated 3.8 and 3.6 for 'Lambert' and 'Lapins' respectively. Ratings for uncovered controls were 2.7 and 2.1 for 'Lambert' and 'Lapins' respectively, 48 h after harvest (wk 0). Browning did not increase during storage, indicating that the damage occurred in the field and/or during transport (Fig. 4).

Water retention in the pedicels was also significantly ($P \leq 0.001$) improved from cherries in bins that were covered with tarps (Fig. 5). Significant reductions in the initial moisture content of the pedicels can occur under direct sun and wind exposure. Pedicel water loss occurred during storage in all treatments, but fruit from covered bins had approximately 10% greater water content compared with fruit from uncovered bins. 'Lambert' cherries showed an increase in pedicel water content at 6 weeks of storage, probably due to high levels of decay in the fruit (Appendix B), which added moisture to the pedicel tissue (Fig. 5).

Tarps helped preserve membrane integrity in pedicels as indicated by RLR data (Fig. 6). Higher leakage rates indicate greater deterioration in cellular membranes (Redman *et al.,* 1986). As expected, membrane leakage increased with storage duration, regardless of treatment probably due to tissue senescence (Whitlow *et al.*, 1992). UV-absorbing material leakage in pedicel tissue was negatively correlated with pedicel browning ($R = -0.84$, $P \leq 0.01$). This suggests that browning is related to at least some structural damage in plant tissue.

Pitting is a primary defect in sweet cherries and a major cause of loss of quality grade for cherry producers (Thompson *et al.*, 1997). Pits result from damage due to injury and are characterized by small depressions due to structural distortion and cellular rupture. Pitting was

FIG. 5. Pedicel water content in 'Lambert' and 'Lapins' cherries covered by tarps or left uncovered during harvest and transport. Water content was calculated by: [(FW - DW) ÷ FW] x 100. Data presented are the means of 6 samples (n = 25), two from each of the three replicates.

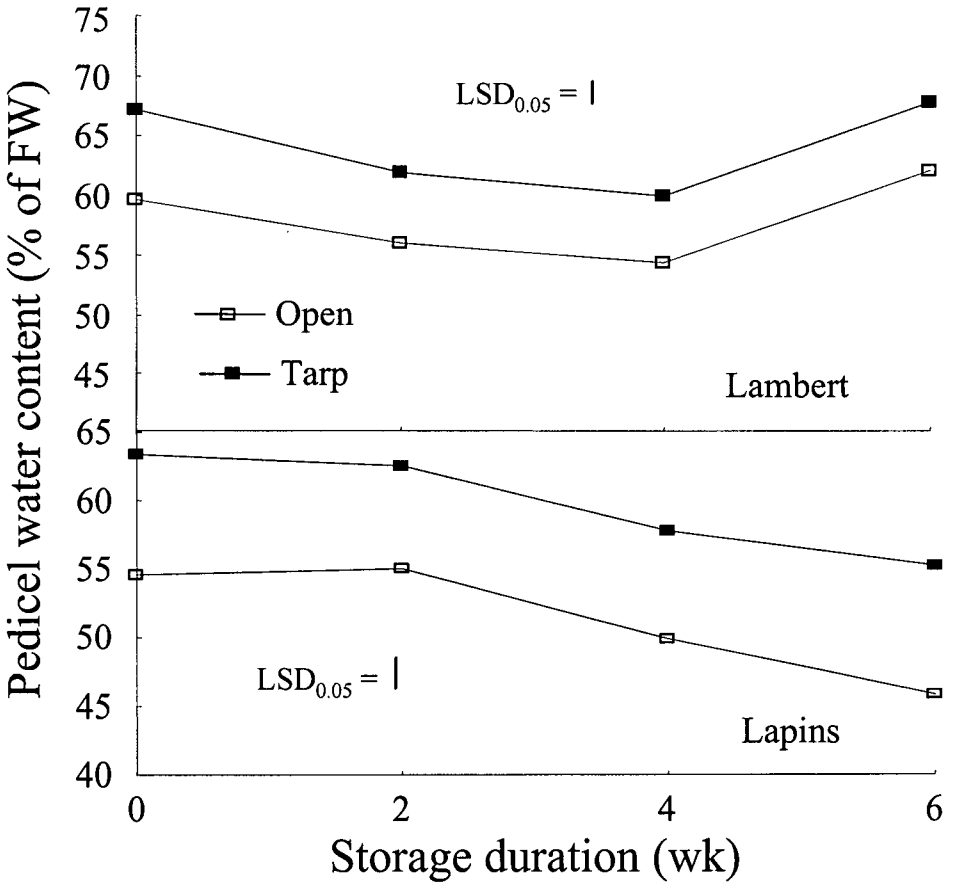
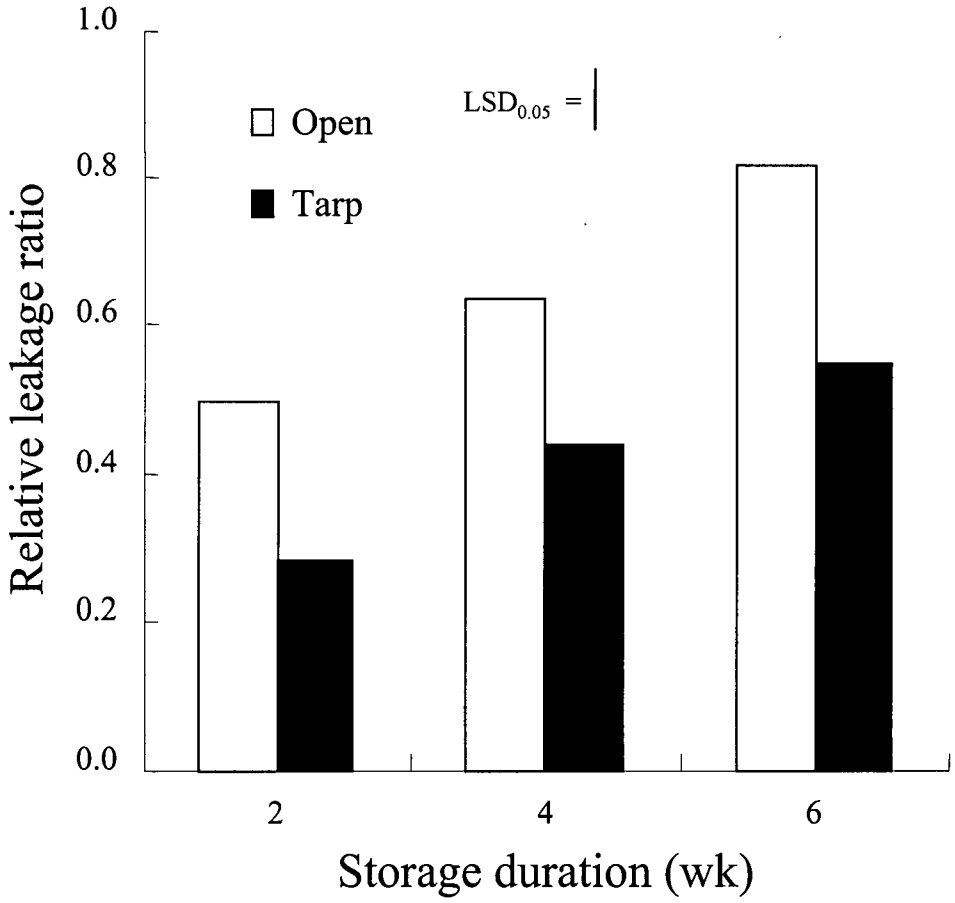

Pedicel water content (% of FW)
75
70
65
60
55
50
45
65
60
55
50
45
40
LSD0.05 = I
Open
Tarp
Lambert
LSD0.05 = I
Lapins
0
2
4
6
Storage duration (wk)

FIG. 6. The effect of tarps on relative leakage ratios of UV-absorbing substances in 'Lapins' cherry pedicels that were covered (tarp) or uncovered (open) during harvest and transport. Cherry exposure time was 4 h at the orchard site and 30 min en route to the packing house. Data presented are the means of 6 samples (n = 25), two from each of the three replicates.


1.0
0.8
0.6
0.4
0.2
0.0
Relative leakage ratio
Open
Tarp
LSD0.05 =
2
4
6
Storage duration (wk)

reduced by covering fruit with tarps (Appendix B). The largest increase in pitting was observed after 2 wk of storage regardless of treatment. This was likely due to a natural delay in pit development. Pitting evaluations were difficult to make beyond 2 weeks of storage due to high levels of decay, so values at later storage dates were considered unreliable.

Percentage of soluble solids content (SSC) was higher for 'Lapins' initially and during storage compared with 'Lambert' (Appendix B). However there were no consistent differences in SSC in either cultivar due to treatment and so tarp bin covers were not considered to be important to retention of soluble solids content.

After two weeks of storage, the head space concentration of atmospheric gases for 'Lapins' was 0.70% CO_2 and 19.42% O_2 (Table 3). At 6 weeks the atmosphere consisted of 1.16% CO_2 and 18.36% O_2.

2.1.4 Discussion

Cherries that were left exposed in the orchard after harvest and during transport to the packing house may be under stress due to elevated temperatures (Hevia *et al*, 1998), solar irradiance (Whitlow *et al.,* 1992) and/or reduced RH (Dewey, 1951) compared with cherries that were under reflective tarps. Under stress, membrane integrity is compromised (Levitt, 1972), leading to tissue breakdown and acceleration of tissue browning due to the oxidation of phenolic compounds into quinones by polyphenoloxidase (Vasmos-Vigyazo, 1981).

Plant cellular membranes control diffusion and determine what leaves and enters plant cells. Cellular membranes are highly susceptible to stresses such as high temperatures and exposure to sunlight (Whitlow *et al.*, 1992). In this experiment, cherries that were exposed to direct sun and

Table 3. Headspace analysis for 'Lapins' cherries stored in MAP bags at 1°C for 6 weeks. Data are means ± SE for 10 samples (1 kg) measured at 2 wk and 6 wk.

Storage at 1 °C (wk)	CO_2 (%)	O_2 (%)
2	0.70 ± 0.14	19.42 ± 0.49
6	1.16 ± 0.51	18.36 ± 1.4

moving air during transportation showed increased browning and higher RLR in the pedicels. The changes that occur to plant cellular membranes in response to environmental stresses are not clearly understood (Whitlow *et al.*, 1992). Cell membranes consist primarily of proteins and lipids and increased permeability in the membranes results from changes in one of these two components (Levitt, 1972). In this experiment, higher RLR were observed in pedicels in the uncovered bins where temperature was higher and RH was much lower. The increased leakage may have been due in part to such a change in membrane protein as a result of mechanical stress induced by a dehydration strain (Levitt, 1972). Dehydration subjects the proteins to a strain that leads to an unfolding or denaturation of the insoluble structural membrane proteins. This unfolding would unmask some of the hydrophobic groups formerly within the native molecule and lead to intermolecular bonding with adjacent molecules causing protein aggregations which result in loss of function in structural proteins involved in controlling membrane permeability.

Browning was visible within 48 h of harvesting and did not increase substantially during storage, so damage must be occurring in the field and/or during transport. Due to a high surface area to volume ratio, pedicel dehydration occurs almost immediately and therefore it is imperative that measures be taken to prevent this rapid evaporation. Postharvest dessication of pedicels, which is generally thought to be related to browning, can be increased by bruising injury, sun exposure and excessive air movement (Dewey, 1951; Drake *et al.*, 1988). Previous work indicated that pedicel browning is highly correlated with pedicel shrivelling ($R = 0.74$, $P \leq 0.01$) (Toivonen and Schick, 1997). Tarps prevent dehydration of the pedicels by keeping temperatures lower and by increasing RH in the immediate airspace surrounding the fruit.

Polymeric films have been shown to improve visual appeal of stored produce by preventing

shrivelling associated with moisture loss (Meheriuk *et al.*, 1995). It is therefore important to evaluate the effects of postharvest handling technologies in the context of MAP. In addition to preserving freshness by preventing evaporative water loss, another basic principle in MAP systems is to alter the ambient CO_2 and O_2 levels (Reed, 1995). It has been shown that reduced O_2 and increased CO_2 slow respiration rates in some produce and therefore extend shelf life (Weichmann, 1987). However, the resulting gas concentrations in this experiment did not approach the recommended gas concentration for sweet cherries which is 3-10% O_2 and 10-12% CO_2 (Reed, 1995). The cherries in this experiment were not flushed to a predetermined gas equilibrium before being sealed in gas permeable plastic wrap. The resulting headspace concentrations at 6 wk were 1.2% CO_2 and 18.4% O_2.

The concentration of atmospheric gases during storage was a consequence of the gas transmission properties of the packaging material and the respiration rate of the cherries inside the bag. Because the cherries were packaged under slight vacuum, the total volume of atmospheric gases in the package was reduced. According to standard MAP principle, reduced package volume results in reduced rate of moisture loss from the fruit (Reed, 1995). This may have been beneficial in preserving the quality of the cherry pedicels and the concentration of atmospheric gases may not be as important a consideration for cherry quality.

The use of reflective tarps may be an economic option which results in cherries with superior fruit and pedicel quality. Subsequent laboratory experiments were performed to determine whether tarp benefits are due to effects of reduced temperatures, higher relative humidity or prevention of exposure to direct sunlight. Knowledge of how individual components impact cherry pedicel browning may lead to a better understanding of the process and have important implications in

improving the quality of cherries at the industrial level.

2.2. Laboratory work

2.2.1 Effect of temperature, humidity and light

2.2.1.1 Introduction

The importance of proper temperature management has been well documented for cherries (Patterson, 1987; Young and Kupferman, 1994; Hevia *et al.*, 1998). The benefits of good temperature management are generally discussed with reference to cherry fruit quality. Few studies (Siegelman, 1952) report on the effects of high temperature on cherry pedicels. It is important to examine the effects of elevated temperature on cherry pedicels, since pedicel color is an important quality index. Cherries with dried and shrivelled stems are not appealing to consumers and therefore rejected by produce buyers.

The importance of proper RH management for cherries is not so well documented and may be as important to preservation of postharvest cherry quality as temperature management. Dewey (1950) and Siegelman (1952) reported detrimental effects on cherry pedicels in low humidity environments.

Direct exposure to sunlight can also damage plant tissues (Smith, 1983). The effects of direct sunlight on cherry pedicel tissue have not been previously described. By modelling individual components and their effects on pedicel quality, a better understanding can be obtained regarding how tarps may be preserving pedicel quality in the field. From this type of information recommendations aimed at optimizing cherry quality can be made to cherry industry personnel.

Preliminary field studies have shown that the reflective tarps help to maintain pedicel quality.

However, when performing experiments in the field many factors cannot be controlled and the interactions are often complex. In the laboratory, some conditions can be controlled and the effects of individual factors can be examined.

The objective of these laboratory experiments was to examine separately the effects of temperature, low humidity and light on cherry pedicel quality. These models can then be used to determine if the influence of reflective tarps on pedicel browning was due to modification of temperature, humidity or light. The following three hypotheses were tested:

1. Elevated temperatures contribute to higher incidences of pedicel browning.
2. Direct exposure to sunlight contributes to pedicel browning.
3. Low RH is a direct cause of pedicel browning.

2.2.1.2 Materials and methods

'Lambert' and 'Lapins' cherries at color stage 6 (mahogany) according to the CTIFL (Centre Technique Interproffessionnel de Fruits et Légumes, Paris, France) color chart were harvested between 8:00 AM and 9:00 AM each day. Samples were collected in 0.5 kg berry baskets and placed inside a sealed chamber, for 8 h, in the darkness and 100% RH. The chamber was constructed out of plexiglass and was 45 cm x 30 cm x 30 cm in size. The treatment temperatures were 20 °C, 30 °C, and 40 °C. They were then sealed in MAP bags, stored for 1 wk and evaluated for pedicel characteristics. The controls were cherries that were freshly harvested, sealed in MAP bags and stored at 1 °C for 1 wk. Temperature and RH were measured by HOBO data loggers and RH was also measured using a VWR Digital Hygrometer (Control Co., Friendswood, TX).

Twenty-five pedicels were evaluated for browning using the four point pedicel color index

previously described (Chapter II, Section 1.2). Leakage of UV-absorbing substances and water content were measured as described in the previous experiment (Chapter II, Section *2.1.2*).

Polyphenoloxidase (PPO) activity was evaluated in all treatments and compared with PPO activity observed in the control. To extract the enzyme, cherry pedicels were ground to a fine powder in liquid nitrogen with a 6800 Freezer Mill (SPEX Centriprep, Inc., Metuchen, NJ). The buffers and reagents used in this experiment were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified. Enzymes were extracted from 1.0 g of powdered tissue into 15 mL of 100 mM sodium phosphate buffer (pH = 7.0) containing 0.01 M ethylenediaminetetraacetic acid (EDTA). Polyvinylpolypyrrolidone (PVPP) (0.5 g) was added to the extraction mixture to remove phenolics which inactivate enzymes (Gegenheimer, 1990). The extraction mixture was left on ice for 1 h and centrifuged at 16,000 x g for 10 min at 4 °C. The supernatant was gravity filtered using Whatman no. 4 filter paper. The substrate was 200 mM catechol in 50 mM sodium phosphate buffer (pH = 6.6) at 25 °C. The absorbance of the reaction mixture (100 μL extract and 2.0 mL substrate) was measured for 2 min at 440 nm, using the kinetics option, on a Beckman DU 640B Spectrophotometer (Fullerton, CA). PPO enzyme activity was calculated from the linear portion of the curve and one unit (U) of activity was defined as a change of 0.001 absorbance unit per min. PPO activity was assayed in triplicate. Proteins were quantified using the method developed by Bradford (1976), using Bovine Serum Albumin (BSA) as the standard.

The experimental design was a randomized complete block design with five replicates. Data presented are means (n = 25) from each of the five replicates. Data were analysed by SAS General Linear Models procedure. Correlation analyses were performed by SAS Regression procedure (SAS Institute, Cary, NC).

The effect of relative humidity (35%, 55%, 75% and 95%) was determined on cherry samples prepared as described above, but kept at 20 °C in the dark for 8 h. When 5 samples of cherries were placed inside the sealed chamber, the atmosphere became completely saturated with water vapour (100% RH). The appropriate conditions to control the RH were obtained by adjusting the amount of $CaSO_4$ (W. A. Hammond Drierite Co., Xenia, OH), a hygroscopic absorbent, which was spread out on a 3.0 cm x 30 cm x 30 cm tray on the bottom of the sealed chamber. The amount of hygroscopic material required to create conditions of 35% RH (1055 g), 55% RH (776 g), 75% RH (605 g) and 95% RH (500 g) was determined by preliminary trials before beginning the experiment. A brushless fan (12 Volt, 0.16 Amp, 1.9 Watt) (Radioshack, Barrie, ON) was placed inside the chamber to ensure homogeneous conditions. Experimental design and evaluations were performed as described for the temperature experiment.

The effect of light was also determined by exposing both 'Lapins' and 'Lambert' cherries to simulated sunlight for 8 h at 20 °C and 100% RH. Broad spectrum metal halide (Sunmaster, Lamour Enterprises, NY) and fluorescent lamps were used to create artificial sunlight. Photosynthetically active radiation (PAR) was measured with a portable LI-COR LI-250 Light Meter (Lincoln, NE). Experimental design and evaluations were as described above.

2.2.1.3 Results

Cherries exposed to higher temperatures had higher levels of pedicel browning (Table 4). There was no significant increase in pedicel browning at 20 °C compared with the control (1 °C) for either 'Lambert' or 'Lapins'. However, significant differences in browning were observed for both cultivars at 30 °C and 40 °C, compared with the controls. 'Lambert' cherries had more pedicel

Table 4. The effects of temperature on pedicel browning, water content, tissue leakage ratios and PPO activity in 'Lapins' and 'Lambert' pedicels. The controls were cherries that were freshly harvested, sealed in MAP bags and stored at 1 °C for 1 wk.

Cultivar	Temperature (°C)		Pedicel Color (1-4)[Z]	WC[Y]	RLR[X]	PPO (U/μg protein)[W]
Lambert	control		3.92 a	0.70 a	0.21 b	-
	20		3.91 a	0.63 b	0.87 a	0.25
	30		3.83 b	0.62 b	0.88 a	0.26
	40		3.75 c	0.60 c	0.88 a	0.26
		Significance	***	***	***	NS
		LSD	0.05	0.01	0.06	
Lapins	control		3.87 a	0.64 a	0.50 c	-
	20		3.84 a	0.63 a	0.62 b	0.22
	30		3.68 b	0.61 b	0.85 a	0.21
	40		3.58 b	0.60 b	0.85 a	0.22
		Significance	**	**	***	NS
		LSD	0.15	0.01	0.09	

[Z]Pedicel color index: 1 = 0-25% brown, 2 = 25-50% brown, 3 = 50-75% brown and 4 = 75-100% brown.

[Y]Water content : values represent the fraction of fresh weight water content.

[X]Relative leakage ratios in pedicel tissue.

[W]Specific activity of polyphenol oxidase (PPO).

Mean values in each column not followed by the same letter are significantly different at 1% (**), 0.1% (***) or not significant (NS).

browning at 40 °C than at 30 °C. 'Lapins' cherry showed a similar trend, although there were no significant differences in pedicel browning at 30 °C and 40 °C.

Pedicel browning correlated with pedicel water content ($R = 0.74$, $P \leq 0.0001$). The percent of fresh weight water content in the pedicels from control 'Lambert' cherries was 70% compared with 64% for 'Lapins' (Table 4). Browning was not as severe in 'Lambert'. This evidence suggests a possible link between initial percent of fresh weight water content and potential for browning in cherry pedicels. This hypothesis was not tested and needs to be verified by further experimentation.

Lower values of RLR indicate that tissues are less leaky for solutes (Redman *et al.,* 1986). Inferences are then made regarding cellular membrane integrity, since it is plant cellular membranes that control movement of solutes in and out of cells (Levitt, 1972). From the RLR data it appears then that the cellular membranes in 'Lapins' cherry pedicels were significantly affected by temperature (Table 4). RLR of 'Lambert' cherry pedicels were higher at 20 °C, 30 °C and 40 °C compared with control cherries. The trend was that RLR increased with temperature, but there were no significant differences between the temperature treatments. RLR were negatively correlated with pedicel browning ($R = -0.61$, $P \leq 0.001$) when both cultivars were combined.

Temperature did not affect PPO activity (Table 4). PPO specific activity for 'Lambert' cherry pedicels was 0.255 U/μg protein and 0.215 U/μg protein for 'Lapins' cherry pedicels. There were no significant differences in PPO activity between cultivars or between treatments within each cultivar.

Relative humidity was the single largest factor affecting browning in cherry pedicels (Table 5). Low RH led to drying of the pedicels as indicated by the percent of fresh weight water content data (Table 5). Water content in the pedicels was highly correlated with their browning ($R = 0.77$,

Table 5. The effects of humidity on pedicel browning, water content, tissue leakage ratios and PPO activity in 'Lapins' and 'Lambert' pedicels. The controls were cherries that were freshly harvested, sealed in MAP bags and stored at 1 °C for 1 wk.

Cultivar	RH (%)		Pedicel color (1-4)[Z]	WC[Y]	RLR[X]	PPO (U/μg protein)[W]
Lambert	control		3.90 a	0.62 a	0.36 c	0.17 b
	35		3.51 d	0.52 c	0.96 a	0.26 a
	55		3.67 cd	0.54 c	0.82 b	0.17 b
	75		3.70 bc	0.54 c	0.74 b	0.23 a
	95		3.88 ab	0.59 b	0.77 b	0.17 b
		Significance	**	***	***	*
		LSD	0.18	0.02	0.08	0.06
Lapins	control		3.87 a	0.64 a	0.50 d	0.19
	35		3.46 b	0.55 c	0.92 a	0.23
	55		3.73 a	0.58 b	0.78 b	0.22
	75		3.82 a	0.63 a	0.64 c	0.28
	95		3.88 a	0.62 a	0.70 bc	0.22
		Significance	**	***	***	NS
		LSD	0.18	0.02	0.09	

[Z]Pedicel color index: 1 = 0-25% brown, 2 = 25-50% brown, 3 = 50-75% brown and 4 = 75-100% brown.

[Y]Water content : values represent the fraction of fresh weight water content.

[X]Relative leakage ratios in pedicel tissue.

[W]Specific activity of polyphenol oxidase (PPO).

Mean values in each column not followed by the same letter are significantly different at 5% (*), 1% (**), 0.1% (***) or not significant (NS).

$P \leq 0.0001$). RLR was also negatively correlated with browning ($R = -0.64$, $P \leq 0.0006$). Cherries are harvested commercially during the hottest time of the year when ambient RH may be as low as 25% (Appendix C) and this is when pedicel browning is the greatest.

Light had no effect on cherry pedicels in this study (Table 6). The light levels obtained from broad spectrum metal halide lamps and fluorescent bulbs averaged around 547 $\mu E\ m^{-2}\ s^{-1}$. The intensity of direct sunlight measured at 10:00 AM was approximately 2.5 $mE\ m^{-2}\ s^{-1}$ and this was about 4.5 times brighter than the artificial light levels used in this experiment. In order to obtain the same flux density, exposure time should have been 4.5 times as long (Smith, 1983). The average exposure time for cherries in the orchard in the previous experiment (evaluation of reflective tarps) was approximately 4 h. To obtain similar flux density levels cherries should have been exposed to simulated sunlight for 18 h. This was impractical here since only one plexiglass sealed chamber unit was available and treatments were performed on consecutive days at the same time of day. In this experiment cherries were exposed to simulated sunlight for 8 h at 20 °C and 100% RH.

The greatest amount of browning occurred when high temperatures were combined with low humidity (Table 7). The 'Lapins' pedicels rated 2.5, which means that on average more than 50% of the pedicel was brown. This result was the same whether light was present or not, which also supports the observation that light had no effect on browning of cherry pedicels in this experiment. Leakage rates were correlated with browning ($R = 0.79$, $P \leq 0.0005$). The 'Lambert' pedicels at 40 °C and 25% RH scored better on the pedicel color index than the 'Lapins' at 40 °C and 25% RH (Table 7).

Table 6. The effects of simulated sunlight on pedicel browning, water content, tissue leakage ratios and PPO activity in 'Lapins' and 'Lambert' pedicels. The controls were cherries that were freshly harvested, sealed in MAP bags and stored at 1 °C for 1 wk.

Cultivar	Treatment		Pedicel color (1-4)[z]	WC[y]	RLR[x]	PPO (U/μg protein)[w]
Lambert	control		3.89	0.651 a	0.545 a	0.205
	light		3.85	0.573 b	0.918 c	0.262
	dark		3.89	0.645 a	0.709 b	0.27
		Significance	NS	***	**	NS
		LSD		0.022	0.132	
Lapins	control		3.90 a	0.634 a	0.402	0.17
	light		3.88 ab	0.602b	0.532	0.157
	dark		3.79 b	0.635 a	0.473	0.132
		Significance	*	*	NS	NS
		LSD	0.11	0.031		

[z]Pedicel color index: 1 = 0-25% brown, 2 = 25-50% brown, 3 = 50-75% brown and 4 = 75-100% brown.

[y]Water content : values represent the fraction of fresh weight water content.

[x]Relative leakage ratios in pedicel tissue.

[w]Specific activity of polyphenol oxidase (PPO).

Mean values in each column not followed by the same letter are significantly different at 5% (*), 1% (**), 0.1% (***) or not significant (NS).

Table 7. The combined effects of temperature, relative humidity and light on pedicel browning in sweet cherries.

Cultivar	RH (%)	Temperature (°C)	Light	Browning (1-4)[z]	WC (%)[y]	RLR[x]	PPO (U/μg protein)[w]
Lambert	40	20	yes	3.48 b	0.52 a	1.01 a	0.3
	90	30	yes	3.85 a	0.57 a	0.92 b	0.26
	25	40	no	3.54 b	0.53 a	1.02 a	0.31
			Significance	**	NS	*	NS
			LSD	0.21		0.07	
Lapins	50	20	no	3.73 a	0.59 a	0.73 b	0.22
	50	20	yes	3.81 a	0.59 a	0.61 c	0.2
	25	30	yes	3.22 b	0.51 b	0.92 a	0.2
	25	40	no	2.55 c	0.48 c	0.86 ab	0.21
	25	40	yes	2.51 c	0.48 c	0.92 a	0.26
			Significance	***	***	**	NS
			LSD	0.34	0.03	0.13	

[z]1- 4 scale: 1 = 75-100% brown, 2 = 50-75% brown, 3 = 25-50% brown and 4 = 0-25% brown.

[y]Water content : values represent the fraction of fresh weight water content.

[x]Relative leakage ratios in pedicel tissue.

[w]Specific activity of polyphenol oxidase (PPO).

Means within a column followed by the same letter are not significantly different at 5% (*), 1% (**), 0.1% (***) or not significant (NS).

2.2.1.4 Discussion

Temperature was identified as the principal environmental variable in loss of cherry quality in a major study by Patterson (1987). In this experiment RH had the greatest effect on pedicel browning. Dewey (1951) studied the effects of air blast precooling on the moisture content of cherry pedicels and reported that RH levels were of minor importance to moisture loss in cherry pedicels. The levels of RH that they evaluated were in the range of 70% to 90%, which is much higher than what would be observed on an average day outside during cherry harvest. Ambient RH of air can often be between 25% to 35% (Table 3).

The RH in large warehouses, where cherries are often held before sorting and packaging, is also much less than 70% . Previous experimentation indicated that during overnight cooling, which consisted of moving air by large fans, the temperature of exposed cherries, decreased rapidly from 24 °C to 6 °C in 6 h and the RH dropped to as low as 35% at one point (Appendix C). Cherry pedicels lose water much more rapidly than the fruit. Seske (1996) reported that cherry pedicels suffered a 57.3% cumulative weight loss in 7 days compared with 4.6% for the fruit in the same time period.

The loss of water content from cherry pedicels in a low humidity environment at 20 °C was greater than the loss of water content of the cherry pedicels at 40 °C and 100% RH (Tables 4 & 5). The greater water loss at higher temperature is due to increased transpiration (Crisosto *et al.,* 1993; Young and Kupferman, 1994). Respiration rates in cherries also double for every 7 °C to 8 °C increase in temperature, and this leads to further heating of the cherries (Kupferman, 1994). The greater evaporative water loss in the pedicels in a low humidity environment occurs as the vapor pressure gradient between the leaf and the external atmosphere increases (Levitt, 1972).

The density of light incident upon a surface is referred to as flux density (Smith, 1983). When attempting to simulate sunlight, using metal halide and fluorescent bulbs, flux density is governed by the inverse square law: Flux density = $(1 \div x)^2$ x flux, where x is the distance of the light source to the surface. This means that light travelling twice as far to strike a surface will be 1/4 as intense.

Irradiance, radiant energy flux received on a plane unit surface, is not only dependent on light intensity, but also on exposure time, which is the total energy or photons falling on a unit surface over an arbitrary time period. It is a measure of the total dose of radiation (Smith, 1983).

In this experiment broad spectrum metal halide lamps, in combination with fluorescent lamps, produced light levels that were about 20% as bright as sunlight. Even though exposure time was approximately double that observed in the orchard, levels may not have been high enough to have an effect on browning. Also, there was a plexiglass barrier between the light and the fruit. The levels of UV light were not measured and the plexiglass could have possibly screened out any UV wavelengths that were emitted by the fluorescent lamps (Wong and Parisi, 1996). The browning intensity observed under high temperature (40 °C) and low RH (25%) was not as large as that observed in the field (Chapter II, Section *2.1.3*). Plant tissues can be damaged by UV radiation resulting in the breakdown of membrane structure (Levitt, 1972). The differences here may be related to the UV component in the sunlight, but this would have to be investigated further in order to establish any connection.

2.2.2. Physical Stress

2.2.2.1 Introduction

In the context of this experiment physical stress is any physical force applied to a living system. In the sweet cherry industry, fruit and pedicels are subjected to impact forces. Bruising damage is caused either by: dropping fruit onto a surface, dropping objects onto the fruit, rubbing against each other or pressing fruit against a hard surface (Ogawa *et al.*, 1972; Crisosto *et al.*, 1993). This physical damage can occur during harvesting, transport or packaging.

Because temperature can have a dramatic effect on mechanical damage and shelf life, it is important to investigate the influence of temperature on bruising susceptibility. This relationship has been reported for fruit impact (Ogawa *et al.,* 1972; Couey and Wright, 1974; Lidster and Tung, 1980; Patten and Patterson, 1985 and Drake *et al.*, 1988; Crisosto et al., 1993), but very little has been reported on the relationship between maturity, temperature and mechanical damage in the pedicel.

The objective of this study was to examine the effects of temperature and maturity on 'Van' 'Bing' and 'Lapins' cherries, three major cultivars grown in B. C. and Washington. If pedicels bruise more easily at one stage of maturity than another, or at specific temperatures, adjustments at the industrial level can be made to the way cherries are handled. If one cultivar is more susceptible to bruising than another, perhaps more care is needed during harvest and packaging.

2.2.2.2 Materials and methods

Fresh 'Van', 'Bing' and 'Lapins' cherries were harvested by hand at color stage 4 (red) and color stage 6 (mahogany) according to the CITFL color chart. On six different occasions, cherries

were harvested between 7:00 AM and 8:00 AM from mature trees grown at PARC and placed into small cardboard containers. Each container held 125 cherries which were then immediately placed in Conviron growth chambers at 0 °C, 10 °C, 20 °C, 30 °C, in the darkness and 75 % RH.

The temperature of the fruit pulp was monitored by HOBO data loggers which were programmed to record data at 10 min intervals using BoxCar® Pro software. After approximately 4 h, the fruit pulp reached target temperature (0 - 30 °C) and the impact force was applied. Five replicates of 25 cherries were bruised per cultivar, stage, and temperature. Controls for each cultivar and maturity stage were cherries that were subjected to similar temperatures but without bruising treatments.

Cherry pedicels were bruised by dropping a 50 g wooden dowel, 3.8 cm diameter, through PVC pipe, 4.0 cm i.d., from a height of 8.7 cm onto single cherry pedicels which were laid out flat on a section of rubber conveyor belt material. The size of the weight dropped onto the cherries was based on preliminary tests which were done to determine minimum threshold bruising levels observed in 1998 (data not shown). Twenty-five cherries were bruised per cultivar, stage, and temperature. The samples were put in small hinged berry containers and sealed in a polyolefin material (PD-941 bag, Cryovac, Mississauga, ON.). They were stored at 1 ^{0}C for 1 wk and evaluated for browning and membrane deterioration as inferred from solute leakage measurements. Solute leakage analysis was performed on 15 pedicel sections that were under direct impact as previously described (Chapter II, Section *2.1.2*).

The cherries were evaluated immediately after they were removed from storage. Pedicel browning has been shown to increase rapidly when cherries are placed at room temperature after refrigeration (Hevia *et al.,* 1998). Browning was measured using the four-point color index

(previously described in Chapter II, Section 1.2) and also colorimetrically (in methanol) using the Minolta Chroma Meter CR-200b portable tri-stimulus colorimeter (Minolta Canada Inc, Mississauga, ON). Measurements were made by placing the 6.0-mm-diameter measuring area of the apparatus on a 2.0 cm disc Whatman no. 541 filter paper that contained homogenized and suction filtered pedicel tissue residue. Pedicel tissue color was recorded in L*, a*, and b* color space coordinates (Hunter, 1975). The L* values are a measure of lightness or darkness, where higher values indicate white and lower values indicate black. The a* and b* values are a measure of color (a* = green to red and b* = blue to yellow). The meter was calibrated with a white standard (Minolta calibration plate CR-A43) before use.

The experimental design was a randomized complete block with five replicates. The effects of temperature, cultivar and maturity stage were examined in this experiment. Pedicel bruising occurred at four different temperatures (1=0 ^{0}C, 2=10 ^{0}C, 3=20 ^{0}C, and 4=30 ^{0}C). The order of treatments was determined from a book of random numbers and were as follows: 1,4,2,3; 3,1,4,2; 1,2,3,4; 1,4,3,2. The data were analysed using GLM SAS (SAS institute, Cary, NC). Correlation analysis was performed to evaluate the relationship of pedicel browning visual scale and colorimeter values.

2.2.2.3 Results

Temperature effect

The effects of temperature on pedicel browning (1 - 4 scale and L*values) and leakage rates were significant ($P \leq 0.0001$) (Tables 8 & 9): however there were significant interaction terms so

Table 8. Mean square of quadratic comparisons for visually assessed pedicel browning, L*values and RLR for 'Van', 'Bing' and 'Lapins' cherries.

Source	df	Mean square quadratic comparison		
		Pedicel Color (1 - 4)	L*value (1 - 100)	RLR
Cultivar (C)	2	7.79 [c]	737.75 [c]	0.33 [c]
Maturity (M)	1	0.02 [a]	180.25 [c]	0.60 [c]
Temperature (T)	4	13.74 [c]	691.56 [c]	0.98 [c]
C x M	2	0.17 [b]	48.51 [c]	0.71 [c]
C x T	8	0.50 [c]	49.97 [c]	0.04 [b]
M x T	4	0.07 [a]	13.34 [b]	0.01 [a]
C x M x T	8	0.14 [c]	9.95 [b]	0.03 [b]
Error	72	0.02 [a]	1.19 [a]	0.01 [a]

[a]Not significant.

[b]Significant at $P \leq 0.05$

[c]Significant at $P \leq 0.01$

Table 9. The effects of temperature on pedicel browning (visual assessment and L*values) and RLR for 'Bing', 'Van' and 'Lapins' cherries. The data represents the mean values for both red and mahogany maturity stages combined.

Cultivar	Treatment	Pedicel color (1 - 4)	L*value (0 - 100)	RLR[Y]
'Bing'	Control[X]	3.8 a	79.2 a	0.46 c
	0 ^{0}C	2.5 c	71.2 b	0.78 b
	10 ^{0}C	2.9 b	71.4 b	0.78 b
	20 ^{0}C	2.2 d	68.6 c	0.97 a
	30 ^{0}C	2.2 d	67.9 c	1.02 a
LSD		0.16	1.59	0.09
'Van'	Control	3.9 a	80.6 a	0.43 b
	0 ^{0}C	2.9 c	75.4 bc	0.71 a
	10 ^{0}C	3.1 b	76.7 b	0.68 a
	20 ^{0}C	2.9 d	74.3 cd	0.74 a
	30 ^{0}C	2.7 d	73.1 d	0.73 a
LSD		0.12	1.91	0.13
'Lapins'	Control	3.8 a	80.7 a	0.49 d
	0 ^{0}C	2.2 b	67.1 b	0.83 b
	10 ^{0}C	2.3 b	66.6 b	0.74 c
	20 ^{0}C	1.7 c	64.2 c	0.95 a
	30 ^{0}C	1.6 c	63.2 c	0.99 a
LSD		0.18	1.43	0.08

[X]Control cherries for each cultivar consisted of freshly harvested cherries that were immediately wrapped in plastic and stored for 1 wk at 1 ^{0}C.

[Y]Relative leakage ratios for the portion of the pedicel that was directly impacted by physical stress. Values followed by different letters are significantly different at $P \leq 0.05$.

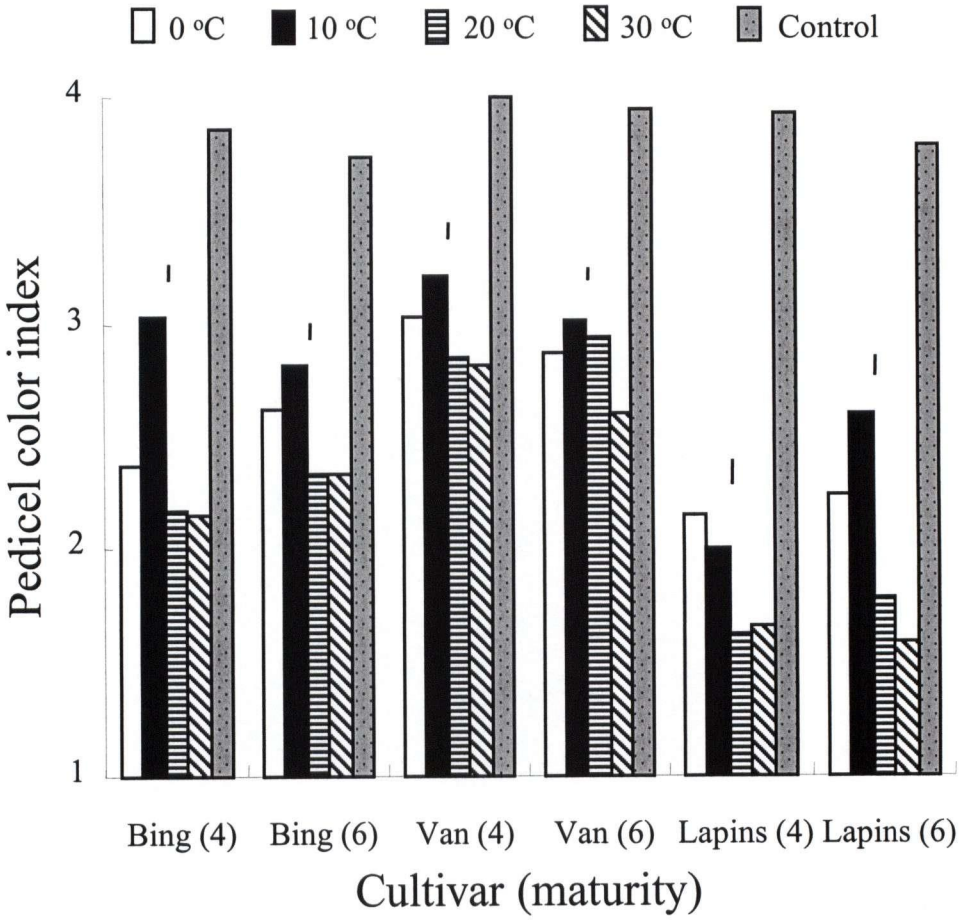
pedicel browning is presented as simple effects (Fig. 7). Cherry pedicels showed the least amount of browning at 10 ^{0}C for all cultivars and maturities, except 'Lapins', color stage 4 (Fig. 7). The levels of pedicel browning on the visual scale were highly correlated with the colorimeter (L*values) measurements ($R = 0.93$, $P \leq 0.0001$). Browning was also highly negatively correlated with RLR ($R = -0.83$, $P \leq 0.0001$).

The cultivar by temperature interaction term was significant for browning and RLR (Table 8). There were significant differences between the different temperature treatments when the two stages for each cultivar were combined (Table 9). Browning levels in 'Bing', 'Van', and 'Lapins' pedicels were almost the same at 20 °C and 30 °C. The least amount of browning was observed in all three cultivars at 10 °C. The differences in browning between 0 °C and 10 °C in 'Lapins' were not significant. However the higher RLR for 'Lapins' indicated that there was more cellular membrane deterioration at 0 °C. Higher values of RLR are indicative of greater membrane deterioration (Redman *et al.,* 1986).

Maturity effect

There were no significant differences in pedicel browning on the visual scale due to maturity (Table 8), however there were definite trends (Fig.7). At 10 ^{0}C, 'Bing' showed less pedicel browning at color stage 4 than at color stage 6. At all other temperatures (0 ^{0}C, 20 ^{0}C and 30 ^{0}C), 'Bing' was less susceptible to bruising at color stage 6 than at color stage 4 (Fig. 7). 'Lapins' at color stage 4 was more susceptible to bruising at all temperatures except 30 ^{0}C (Fig. 7). Less mature 'Van' pedicels were less susceptible to bruising at all temperatures except 20 ^{0}C (Fig. 7).

FIG. 7. The effect of temperature and maturity on visually assessed pedicel browning for three cultivars of sweet cherries. Control cherries for each cultivar consisted of freshly harvested cherries that were immediately wrapped in plastic and stored for 1 wk at 1 ^{0}C. Cherries at maturity stage 4 are red and cherries at maturity stage 6 are a mahogany color. Values are means of five replicates (n = 25). Poole LSD values are indicated on the graph.


0 °C
10 °C
20 °C
30 °C
Control
Pedicel color index
4
3
2
1
Bing (4)
Bing (6)
Van (4)
Van (6)
Lapins (4)
Lapins (6)
Cultivar (maturity)

Cultivar effect

There were significant differences in visual scale browning and leakage between cultivars (Table 9). 'Lapins' had the highest and 'Van' had the lowest rates of pedicel browning at all temperatures and both maturities (Fig. 7). The cellular membranes, as indicated by RLR, showed more deterioration at the highest temperatures (Table 9). RLR for cellular membranes of 'Bing' and 'Lapins' pedicels were almost 1, compared with 0.73 for 'Van' at the same temperature.

2.2.2.4 Discussion

Temperature management and careful postharvest handling of cherries are both critical to assure high quality fruit in the marketplace (Couey and Wright, 1974; Lidster and Tung, 1980; Patterson, 1987; Drake *et al.,* 1988; Crisosto *et al.*, 1993). Temperature was identified as the principal environmental variable in loss of cherry quality in a major study by Patterson (1987). Respiration rates in cherries can double for every 7 °C to 8 °C increase in temperature (Patterson, 1987; Kupferman, 1994). As the temperature increases, sugars vital to cherry flavor are rapidly depleted through higher respiration (Young and Kupferman, 1994). Therefore rapid removal of field heat from cherries is necessary to slow respiration in order to attain maximum shelf life.

However, while cooler temperatures preserve freshness, cold cherries have been shown to be more susceptible to impact damage (Couey and Wright, 1974). Fruit temperature at the time of impact may affect the susceptibility of the tissue to bruising by altering the elasticity of the cells (Patten and Patterson, 1985). It has been suggested that tissues of warm fruit are more elastic than that of cold fruit (Lidster and Tung, 1980). The results in this experiment indicated that 0 °C was not the optimum handling temperature for cherry pedicels, since they tended to brown to a greater

degree in response to impact. Under impact, cherry pedicel cells rupture destroying subcellular organization and physiological function (Patterson, 1987). Browning results as enzymes responsible for turning injured tissues brown can then mix freely with substrates.

The most effective temperature for maximum retention of overall quality is suggested to be just above the freezing point of the tissue (Patterson, 1987). Due to the lower SSC the pedicels freeze at a higher temperature than the fruit. Crisosto *et al.* (1993) recommended that cherries be handled at temperatures between 10 °C and 20 °C during packing to minimize bruising damage. In the sorting of cherries during packing line operations, pedicel clusters are usually first separated by spinning saw blades or sharp tines in machines called cluster cutters (Thompson *et al.,* 1997). These cutters can cause bruising of cherry pedicels, and this usually happens after they have been hydrocooled to 0 °C or precooled prior to packing. Couey and Wright (1974) also showed that water-cooled cherries were more consistently susceptible to impact injury than cherries that were air-cooled to higher temperatures.

Patten and Patterson (1985) recommended that to reduce compression damage over packing lines, that cherries be cooled to between 7 °C and 13 °C, packed shortly after harvest and then followed by cooling to just above freezing. The results in this experiment suggest that cherries should also be handled at temperatures around 10 ^{0}C to minimize bruising damage to the pedicels. They could then be cooled as suggested by Patten and Patterson (1985).

Sweet cherries are very delicate and must be handled carefully at any temperature. The internal fruit browning of 'Bing' cherries was reduced to 1% from 33%, when placed carefully in unpadded buckets compared with dropping the fruit into the same unpadded buckets (Ogawa *et al.*, 1972). Vibration and impact injury hasten postharvest deterioration and increase fruit rot (Mitchell

et al., 1980). In this experiment pedicel browning was localized and only occurred in the area directly affected by the impact force. Browning was quantified using both visual and instrumental methods and they were highly correlated.

In addition to temperature, cherry maturity was also shown to influence susceptibility of fruit to impact bruising (Couey and Wright, 1974). Mahogany cherries were less susceptible to impact injury than red, less mature cherries. Lidster and Tung (1980) attributed this phenomenon to the higher concentration of soluble solids in more mature fruit. Cherry pedicels have fewer soluble solids than fruit (Patterson, 1987) and are perhaps even more susceptible to impact injury at less mature stages. Here less mature pedicels of 'Lapins' and 'Bing' cherries showed greater amounts of bruising. Based on these results, cherries should not be harvested too early and if cherries are cooled, they should be handled with greater care to prevent bruising. Although less impact injury would occur if cherries were packed at temperatures between 7 °C and 13 °C, this would have to be considered in relation to the need for rapid cooling to slow respiration rates, the difficulty in cooling cherries after they are packed and the need to separate the clusters in order to grade and sort the cherries.

III. PARTIAL PURIFICATION AND SOME PROPERTIES OF PPO IN CHERRY PEDICELS

1. Introduction

Enzymatic browning in plant tissues is catalysed mainly by a copper-containing enzyme, polyphenol oxidase (PPO) (Vasmos-Vigyazo, 1981; Zawitowski *et al.*, 1991). The literature reflects some confusion surrounding this enzyme as many different names have been associated with it. Some examples are, tyrosinase, cresolase, catecholase, diphenolase, and phenolase. The Commission on Enzymes of the International Union of Biochemistry (IUB) placed PPO into two key categories to reflect two distinct reactions catalysed by the same enzyme. The cresolase reaction is catalysed by EC 1.14.18.1, which is also known as monophenol monooxygenase or tyrosinase, and the catecholase reaction is catalysed by EC 1.10.3.2 *o*-diphenol:O_2 oxidoreductase (Zawitowski *et al.*, 1991).

For over 100 years since it's discovery, researchers have attempted to elucidate the structure, composition and physiology of PPO (Vasmos-Vigyazo, 1981; Zawitowski *et al.*, 1991). Many plant PPO's exist in latent states and can only be detected by activation with anionic detergents (Escribano *et al.*, 1997). PPO is difficult to study because it exists in multiple enzyme forms, possibly due to associations with lipids, carbohydrates or other subcellular components (Ganesa *et al.*, 1992). In addition to this association, the form that PPO exists in is dependent on buffer ionic strength, pH, enzyme concentration and presence of dissociating agents (Zawitowski *et al.*, 1991).

The reported molecular mass of PPO is between 15 kD in 'Noble' grapes (Lamikanra, 1988) and 90 kD in suspension cultures from the tropical woody vine, *Mucuna pruriens* (Rathjen and

Robinson, 1992). Very few studies have reported PPO that has been purified, so heterogeneity probably reflects different conditions of extraction and varying degrees of purification (Zawitowski *et al.*, 1991). The conditions under which gels are run also vary from nondenaturing to partially denaturing and denaturing.

Expression of PPO as detected by SDS-PAGE has been investigated for broad beans (Ganesa *et al.*, 1992; Robinson and Dry, 1992), grapes (Rathjen and Robinson, 1992), potato (Balasingham and Ferdinand, 1970), spinach beet (Vaughan *et al.,* 1975) and apple (Oktay *et al.*, 1995). No reports were found on expression and extraction of PPO protein from woody tissues.

The objectives of this study were to develop a protocol for extraction and concentration of crude cherry pedicel proteins and subsequent separation on partially denaturing gels, to examine some properties of cherry pedicel PPO and to determine if PPO activity correlates with browning observed in 'Lapins' and 'Lambert' pedicels. PPO activity was measured spectrophotometrically and PPO isozymes were separated by electrophoresis. PPO in green pedicels was compared with PPO in brown pedicels.

2. Materials and methods

Plant material

Mature 'Lambert' and 'Lapins' sweet cherries were harvested at PARC. Fresh green pedicels were removed from the fruit and immediately frozen in liquid nitrogen and stored at -80 °C. These pedicels were labelled as 'green' in the assays. From the same harvest cherry pedicels were bruised with an aluminum square section (2.0 cm x 2.0 cm x 41 cm) that caused 100%

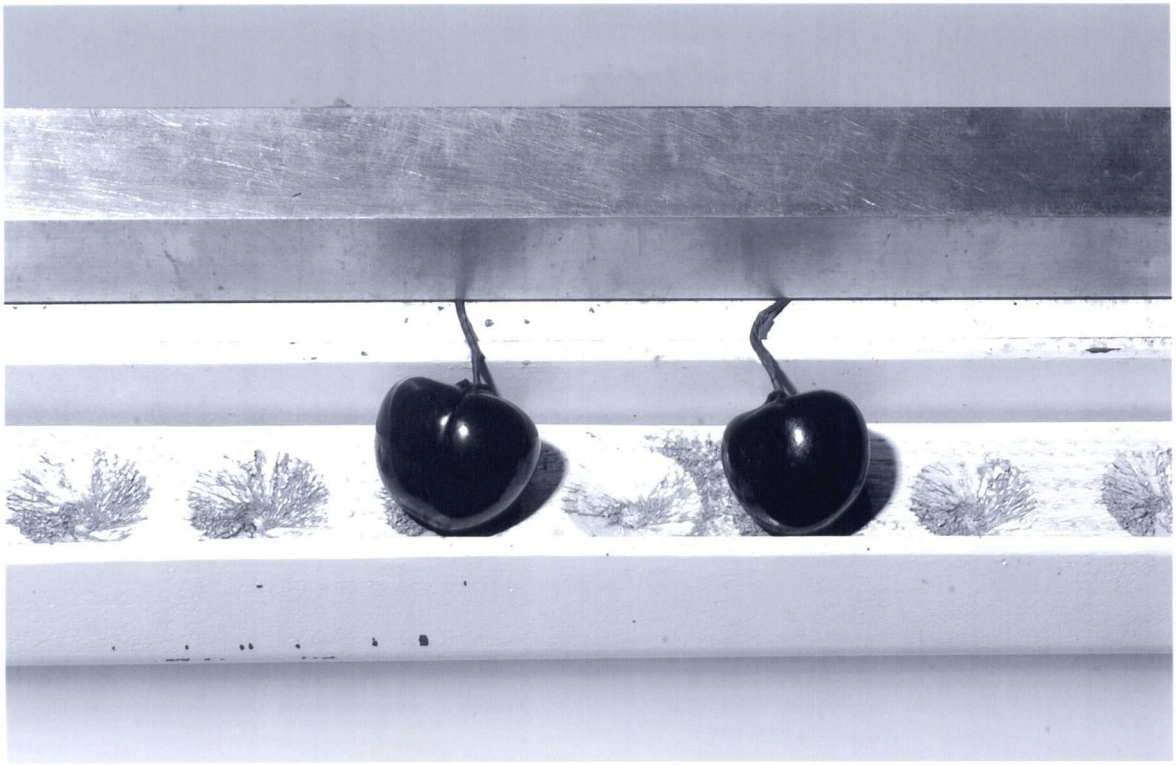
browning (bruising apparatus details see Fig. 8). The pedicels turned completely brown in approximately 30 min and were then frozen in liquid nitrogen, placed in plastic bags and stored at -80 °C. Proteins extracted from these pedicels were used for electrophoresis and in the determination of pH optimum for PPO activity. These pedicels were labelled as 'brown' in the assays.

Protein extraction and precipitation

Crude protein extracts were made from cherry pedicel tissue that was ground to a fine powder in liquid nitrogen with a freezer mill (Model 6800, SPEX Centriprep, Inc., Metuchen, NJ). Extraction procedures were carried out at 4 °C. Finely powdered cherry pedicel tissue (10 g) was placed in 100 mL of 50 mM sodium borate buffer (pH 8.5), 5.0% PVPP (w/v), 100 mM DTT and 1 mM PMSF (Gegenheimer, 1990). The mixture was stirred at medium speed (Model 120S Thermix, Fisher Scientific, Nepean, ON) for 1 h and filtered through 2 layers of cheesecloth. The filtrate was clarified by centrifuging at 12,000 g for 15 min at 4 °C and then filtered through fluted Whatman no. 541 filter paper.

The pH of the supernatant was adjusted to 7.0 with 1.0 M HCl. Proteins were precipitated by adding and stirring $(NH_4)_2SO_4$ in increments to a final concentration of 80% w/v (0.516 g ml^{-1} at 4 °C). The mixtures were stirred for an additional 15 min and the precipitated protein pellets were collected by decanting the supernatant after centrifugation at 20,000 g for 20 min at 4 °C. The protein pellet was air-dried and stored at -20 °C.

FIG. 8. Apparatus used to bruise cherry stems which caused 100 % browning in approximately 30 min. The bruising apparatus consisted of 2 steel channels (2.4 cm x 3.0 cm x 41 cm) welded together along one side, with the open side of one facing up and the other one facing down. A hardwood support (1.0 cm x 3.0 cm x 41 cm) with 15 depressions carved with a 2.0 cm diameter drill bit was placed inside the steel channel. Bruising of the pedicels occurred by setting one cherry into each depression and laying the pedicels along the top of the other steel channel. After 15 cherries were in place, pressure was applied to the cherry pedicels with the aluminum square section.



Desalting and concentration of proteins

The pellet was washed with 20 mL of ice-cold sodium borate buffer (pH = 8.5) and concentrated in an Amicon cell (Model 8050, Grace & Co., CT), fitted with a YM 10 membrane (MW cut-off 10,000) (Millipore, Bedford, MA). Proteins were concentrated under nitrogen at 50 psi with constant stirring at 4 °C. The volume was reduced to 4.0 mL and collected in duplicate 2.0 mL microcentrifuge tubes. One sample vial was used to determine protein concentration and the duplicate sample was used for polyacrylamide gel electrophoresis (SDS-PAGE).

Electrophoresis

SDS-PAGE was conducted using a minigel system as developed by Laemmli (1970). Sample proteins were mixed with an equal volume of sample buffer (62.5 mM tris-HCl, pH 6.8, 25% v/v glycerol, 2% w/v SDS and 0.01% w/v bromophenol blue dye). Ten μg of protein was loaded into wells of 4-15% gradient tris-HCl ready gels (Bio-Rad Laboratories, Hercules, CA). The protein samples were not heated before loading, so separation occurred under partially denaturing conditions.

Electrophoresis was carried out at room temperature for approximately 45 min at 200 volts. The electrode buffer (pH 8.3) contained 1.5% (w/v) tris base, 7.2% (w/v) glycine and 0.5% (w/v) SDS. After completion of the run, the gel was cut into two symmetrical parts and stained for PPO activity. One part was immersed in a phosphate buffer (pH 7.2) containing 15 mM catechol and 0.05% *o*-phenylenediamine (Oktay *et al.*, 1995). The isoenzyme bands were developed for 30 min, rinsed with 1mM ascorbic acid for 5 min and stored in 30% ethanol before being photographed. The other half was immersed in 0.1 M phosphate buffer (pH 6) containing 1.5 mM 3,4-dihydroxyphenyl

alanine (L-dopa) substrate (Oktay *et al.*, 1995). After rinsing with 1 mM ascorbic acid, the gel was stored in dH_2O and photographed. The concentration of proteins in the extract was determined according to the dye binding method (Bradford, 1976) using bovine serum albumin as the standard.

Gels were also stained for proteins for 1 h with 0.1% Coomassie brilliant blue R-250 (CBB) in fixative (40% v/v CH_3OH and 10% v/v CH_3COOH) and destained in 40% v/v CH_3OH and 10% v/v CH_3COOH to remove background and reveal protein profile. Apparent molecular weights of proteins were compared with known molecular weight standards (Bio-Rad Laboratories, Hercules, CA): myosin (188 kDa), β-galactosidase (108 kDa), bovine serum albumin (68 kDa), ovalbumin (48.5 kDa), carbonic anhydrase (33.4 kDa), soybean trypsin inhibitor (28.5 kDa), lysozyme (20.8 kDa) and aprotinin (7.3 kDa).

Enzyme assay

PPO activity was assayed with catechol as the substrate according to a spectrophotometric procedure (Waite, 1976). Pedicels from green and brown 'Lapins' and 'Lambert' cherries were ground to a powder in liquid nitrogen. Enzymes were extracted from 1 g of powdered tissue into 10 mL of 50 mM sodium borate buffer (pH 8.5) and 5.0% PVPP. The samples were placed on ice for 1 h and then centrifuged (4 °C) for 15 min at 12,000 g. The supernatant was collected by filtering through fluted Whatman no. 541 filter paper.

The PPO assay was performed using 100 μL of enzyme extract and 2.0 mL 200 mM catechol prepared in 50 mM sodium borate buffer (pH 8.5). PPO activity was determined by measuring the increase in absorbance at 400 nm at 25 °C on a Beckman DU 640B spectrophotometer. PPO activity was calculated from the linear portion of the curve and one unit

(U) of enzyme activity was defined as a change of 0.001 absorbance unit per min. PPO activity was assayed in triplicate. The specific activity (U/μg protein) of the enzyme was calculated.

Phenol content

The phenol content in cherry pedicel tissue was determined using the method as described by Swain and Hillis (1959). Green and brown 'Lapins' and 'Lambert' powdered cherry pedicel tissue (0.5 g) was homogenised (Polytron, model PT 10/35 Fisher Scientific, Nepean, ON) at room temperature for 15 sec in 10 mL 70% methanol. The samples were refluxed for 1 h and then centrifuged at 12,000 g for 10 min at 20 °C. The resulting supernatant was used for total phenol and *o*-diphenol assays. The assay for total phenols consisted of 50 μL extract, 3.5 mL dH_2O, 250 μL Folin-Ciocolteau reagent (Sigma, St. Louis, MO). The test tubes (10 mL) were briefly vortexed and left at room temperature. After 3 min, 500 μL saturated Na_2CO_3 and 700 μL of dH_2O was added to bring the total volume to 5.0 mL. After 1 h, the absorbance at 725 nm was determined using the reaction mixture without the extract as the blank (Swain and Hillis, 1959).

The assay for *o*-diphenol concentration was adapted from Arnow (1937). An aliquot of 1.0 mL of extract was added to 1.0 mL 0.5 M HCl and 1.0 mL reagent (10% $NaNO_2$, 10% Na_2MoO_4). After vortexing briefly, 1.0 mL 1 M NaOH and 2.0 mL dH_2O was added. The samples were left at room temperature for 20 min and then read on a spectrophotometer at 530 nm. The blank was dH_2O. The total phenols and *o*-diphenols were calculated using the slope of a standard curve for chlorogenic acid (Fig. 10).

3. Results

Proteins present in the original extracts were undetectable by electrophoresis, with CBB staining, so they were concentrated by ultrafiltration. After ultrafiltration the protein concentrations in the samples were as follows: brown 'Lapins'= 19.0 μg/mL, green 'Lapins' = 12.8 μg/mL, brown 'Lambert' = 22.2 μg/mL, green 'Lambert = 23.5 μg/mL. Active PPO was detected by separating crude protein extracts on 4-15% partially denaturing gels. Under these conditions, two PPO isozymes were detected in 'Lambert' and 'Lapins' cherry pedicels with catechol (Fig. 9). A similar banding pattern resulted with L-dopa (results not shown). The smaller more intense band had an apparent molecular weight of 37.5 kD and the apparent molecular weight for the larger less intense band was 43.5 kD. Based on visual inspection, more PPO was expressed in green pedicels than in brown pedicels. PPO activity, measured spectrophotometrically, was higher in brown tissue than in green tissue (Table 10). PPO activity was also higher in "Lapins" stems compared with "Lambert" stems. Visual inspection of the gels also indicated that 'Lapins' cherry pedicels had higher expression of PPO than 'Lambert' cherry pedicels (Fig. 9).

The total phenols in the green samples was nearly double that in brown samples (Table 10). There were no significant differences in *o*-diphenols. The total phenols and *o*-diphenols were calculated using a chlorogenic acid standard (Fig. 10).

4. Discussion

PPO was extracted from cherry pedicels in an active form and therefore the enzymes could be separated and detected in partially denatured gels by staining with the substrates, catechol and L-dopa. Two PPO isoenzymes were detected. The doublet nature of PPO has been observed

FIG. 9. SDS-PAGE of crude extracts of green and brown 'Lambert' and 'Lapins' cherry pedicel proteins. The proteins were separated on 4 -15% gradient gels under partially denaturing conditions and stained for biological activity with 15 mM catechol containing 0.01% *o*-phenylenediamine. Lane1: green 'Lambert', Lane 2: brown 'Lambert', Lane 3: green 'Lapins', Lane 4: brown 'Lapins', Lane 5: protein standards.

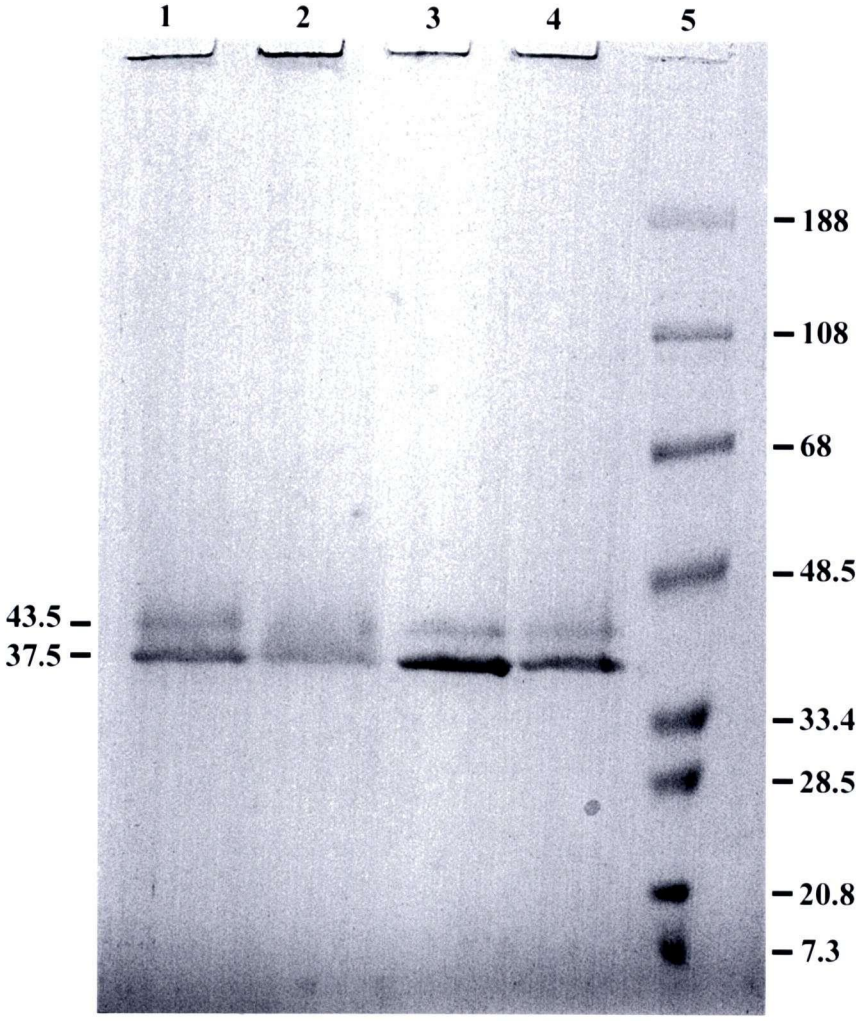

1
2
3
4
5
188
108
68
48.5
43.5
37.5
33.4
28.5
20.8
7.3

Table 10. PPO activity and phenol content in 'Lambert' and 'Lapins' pedicel tissue. SAS GLM t-test (LSD) was used to separate the means.

Cultivar	Treatment	PPO activity (U/g FW)	Protein (μg/ml)[X]	Specific activity (U/µg protein)	Total phenols (mg/g)[Y]	*o*-diphenols (mg/g)[Y]
Lapins	Brown	0.033	6.4	0.519	1.730 b	0.486
	Green	0.028	6.233	0.452	2.663 a	0.476
		NS	NS	NS	*	NS
Lambert	Brown	0.028	5.800 a	0.489	1.491 b	0.497
	Green	0.026	4.700 b	0.549	2.859 a	0.485
		NS	*	NS	*	NS

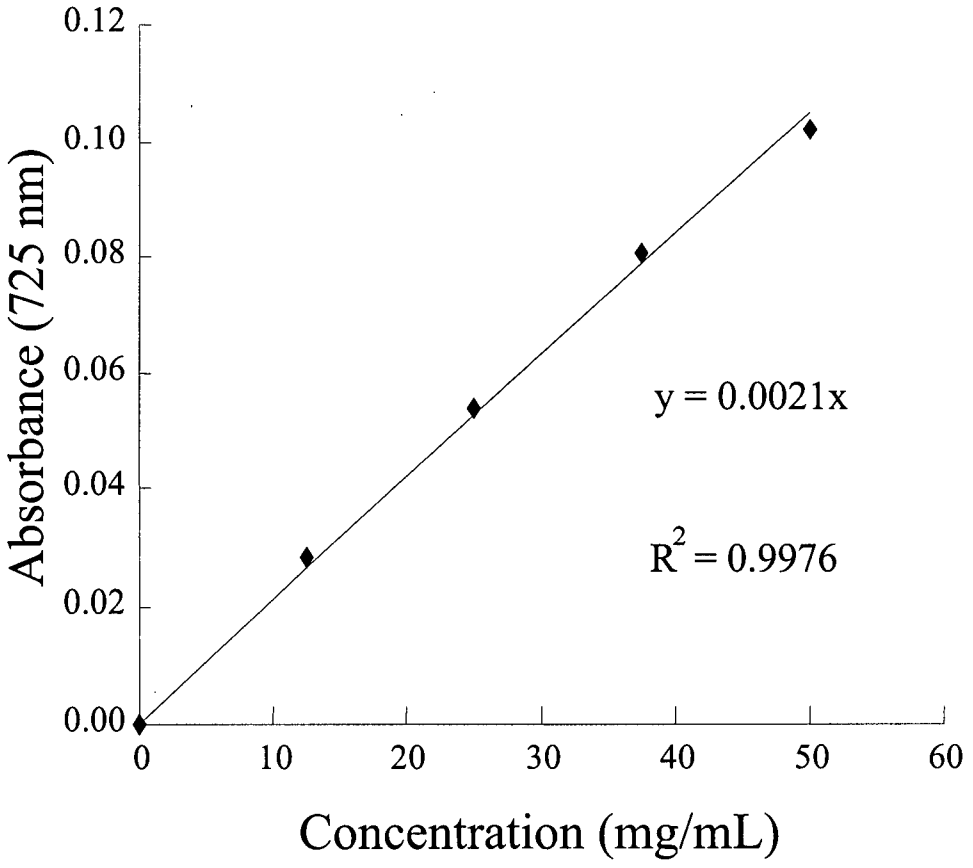
[X]Proteins were quantified (Bradford, 1976) with BSA as the standard.

[Y]Phenols were calculated using a chlorogenic standard curve.

Mean values in each column not followed by the same letter are significantly different at 5% (*)

NS = not significantly different.

FIG. 10. A standard curve of chlorogenic acid was used to calculate total phenols in samples of 'Lambert' and 'Lapins' cherry pedicels.


0.12
0.10
0.08
0.06
0.04
0.02
0.00
Absorbance (725 nm)
0
10
20
30
40
50
60
Concentration (mg/mL)
y = 0.0021x
R2 = 0.9976

previously (Robinson and Dry, 1992). The two bands found may represent closely related isoforms, however PPO has been previously reported to be susceptible to proteolytic cleavage and this can result in smaller PPO proteins in the extracts (Ganesa *et al.*, 1992). Many plant proteases are of the serine type, which are inhibited by sulfonyl fluorides (Beynon and Oliver, 1996). PMSF was added to the extraction buffer, so it is unlikely that proteolysis occurred here. Rathjen and Robinson (1992) also reported that active PPO existed in multiple forms in mature grapes. Three to eight bands appeared after staining for PPO activity. Three PPO isozymes were detected in apple using 15 mM catechol as a substrate (Oktay *et al.*, 1995).

Although the molecular mass of PPO has not been clearly established, an active form of PPO of apparent molecular mass of 43 kD under partially denaturing conditions is often reported (Mayer, 1987; Flurkey, 1989; Ganesa *et al.*,1992). However, molecular masses derived from partially denatured samples can be misleading (Robinson and Dry, 1992). Mobility of proteins on SDS-PAGE gels depends on charge density, so the relationship between apparent molecular mass and mobility is only valid if proteins are fully denatured and saturated with SDS. Also attachment of covalently bound carbohydrates could account for some of the apparent heterogeneous isoforms that differ in charge or size and also account for some of the anomolous behaviour of PPO in SDS-PAGE and during purification (Ganesa *et al.*,1992). Carbohydrate association with PPO has also been reported by Balasingham and Ferdinand (1970), Stelzig *et al.* (1972) and Flurkey and Jen (1980). In addition to glycosylation, enzyme heterogeneity has been attributed to association-dissociation of sub-units, polymerisation with phenolics and proteolysis during isolation/purification of the enzyme (Zawitowski *et al.*, 1991).

The mobility of PPO can also be affected by reducing agents (Ganesa *et al.*, 1992; Robinson

and Dry, 1992). In this experiment biologically active PPO was detected as a doublet of 43.5 kD and 37.5 kD, in the absence of strong reducing agents. PPO was detected as a single band of 43 kD by Robinson and Dry (1992), under non-reducing conditions. Robinson and Dry (1992) concluded from their work with broad bean leaves, that PPO was a 60 kD protein which is susceptible to proteolytic cleavage without resulting loss of activity. Apparently 15 - 18 kD can be lost and result in a biologically active 43 - 45 kD peptide. Under highly reducing conditions (100 mM DTT), the apparent molecular weight of PPO was reported to be 58-60 kD, but biologically inactive. Ganesa *et al.* (1992) reported similar results when comparing non reducing and reducing conditions. In this experiment when the reducing agent, β-mercaptoethanol was added to the extraction buffer, PPO activity was not detected in the enzyme assays.

The band intensities indicated that more PPO enzyme was present in the extract from 'Lapins' than from 'Lambert'. If higher levels of expressed PPO is indicative of more browning or higher capacity for browning, then this result is consistent with what was observed in the tarp experiment and the physical stress experiment where browning levels were higher in 'Lapins' than 'Lambert'. More PPO was found by electrophoresis in green pedicels compared with brown pedicels. However this may be due to interference by polyphenols in the protein quantification step and more proteins from the green tissue were loaded onto the gels.

It may be possible that the concentration of proteins in the brown samples was over estimated and resulted in a smaller protein load. The Bradford (1976) method was used to determine the protein concentrations and an apparent sample of 10 μg were loaded onto each lane. However there was a potential for interference from polyphenols in the Bradford protein quantification assay. Kilkowski and Gross (1998) reported that crude enzyme extracts from tannin-producing plants were

found to give a pronounced color reaction with Coomassie brilliant blue that far exceeded the values expected for the usually observed protein concentrations obtained from such sources. They thought that this effect was probably due to complexing of Bradford reagent with endogenous plant polyphenols that were present in the extracts.

It remains inconclusive whether browning correlates with PPO. More work is required to refine the quantification step and eliminate any potential interference when determining protein concentrations. Two PPO isozymes were detected in partially denaturing SDS-PAGE gels and more PPO was expressed in 'Lapins' compared with 'Lambert' cherries. If high expression of PPO is related to higher potential for browning, future development of antisense PPO technology may still provide another approach to solving the pedicel browning problem. This technology would be especially useful when applied to cultivars like 'Lapins' which possess quality agronomic characteristics (increased fruit size, heavy yield, cracking resistance and self-fertility) (Lang, 1998) but encounter some problems with brown pedicels.

IV. SUMMARY AND RECOMMENDATIONS

Pedicel browning is a problem in the cherry industry and practical solutions are essential. It is important to understand how environmental stresses are related to pedicel browning in order to make recommendations. This work was performed to examine the effect of some stress factors preharvest, during harvest and immediately postharvest on pedicel browning. Sweet cherry is a crop of significant commercial value, especially for top quality fruit. Pedicel color is an important quality index, and brown pedicels often result in rejection of cherries by produce buyers. Consumers associate brown and shrivelled pedicels with produce that is not fresh.

Cherry pedicels are extremely vulnerable to injury due to their physical structure. They are long and thin, with a large surface area-to-volume ratio. They can dry out very quickly, lack components to resist sunlight, and are easily damaged through handling. Because cherry pedicels are delicate and susceptible to environmental stresses, measures must be taken to preserve their freshness. Considerations taken to preserve pedicel quality also promote superior fruit quality. When cherry supply is high or when selling cherries to high-end markets, it is important to produce cherries with green pedicels to remain competitive.

To preserve pedicel and fruit quality in sweet cherries, the recommendations made in this chapter are based on the results of this research. Reflective tarps significantly reduce pedicel browning, decay, and pitting in cherries following storage when used during harvest and transport. Tarps reduced pedicel browning by reducing field heat exposure and by increasing RH within the bins. Maintaining water content may be an important component in preserving green pedicels. More water was retained in pedicels that were under tarps and pedicel browning was significantly reduced. UV - absorbing leakage across plant cell membranes always correlated with pedicel browning. This

would indicate that browning is related to at least some structural damage to the plant tissue.

The hypothesis that injury or water loss in the pedicels is occurring in the orchard was supported by temperature-humidity data. The tarps kept the fruit cooler, and the RH within the covered bin air space was much higher than in the control. Orchard ambient RH was measured to be as low as 25%, and this is when browning is greatest.

In laboratory experiments, light, which was substantially less intense than sunlight had no effect at all on pedicel browning; however, temperature and RH did. As RH decreased, and as the temperature increased, browning increased. The exposure temperature and RH tested in this experiment are not atypical of the conditions that would exist in the orchard on a warm sunny day. However, the level of light used in this experiment was only about 20% of bright sunlight, so the results are inconclusive regarding the effects of sunlight directly on fruit and pedicel quality.

On some occasions, cherry bins are kept overnight in large warehouses (with high airflow rates produced by cooling fans), awaiting delivery or shipping to other markets. The large fans have a tremendous drying effect, especially on the stems. Reflective tarps could be beneficial under these circumstances to reduce or eliminate evaporation of water and create an immediate surrounding airspace with optimal RH for preserving quality in cherry pedicels. Because cherry quality deteriorates rapidly at high temperatures, attempts are made to cool them as quickly as possible. Under reflective tarps cherries are prevented from heating up in the field and during transport and the necessity for rapid cooling is reduced.

There is a strong effect of cultivar on the resistance of cherry pedicels to bruising. 'Van' cherries always scored better than 'Bing' or 'Lapins' regardless of temperature or maturity. 'Van' pedicels are short and compact, and pedicel browning is not considered to be a problem. 'Lapins'

pedicels showed the highest amount of browning regardless of temperature. 'Lapins' pedicels, which are long and thin, tend to grow in tightly held clusters, with little or no light penetration during growth. For this reason they may be more susceptible to physical and physiological injury, when exposed to sunlight. In addition, 'Lapins' may be more vulnerable to high temperature and low RH immediately postharvest.

With respect to handling, both maturity and temperature should be considered. Cherries that are harvested too early may be more susceptible to handling injury. Less browning occurred in more mature 'Lapins' and 'Bing' (No. 6 CTIFL) than in less mature 'Lapins' and 'Bing' (No. 4 CTIFL). Cherries bruised more easily at 0 °C, 20 °C, and 30 °C than at 10 °C. Based on these results, the optimum harvest time for 'Lapins' and 'Bing' for pedicel quality was CTIFL color stage No. 6 (mahogany), and the best handling temperature was 10 °C.

Preharvest water stress had no effect on pedicel browning. When water was withheld for 4 wk there was 30% less fruit cracking compared with the controls and there was no increase in pedicel browning. The amount of water currently applied to cherry crops could be re-evaluated.

Future research on optimizing cherry pedicel quality may further investigate PPO, the browning enzyme. In this research PPO isozymes were isolated from both green and brown pedicels. In general, PPO exists in plastids and substrates are contained in the vacuoles. The third component that is necessary for the browning reaction to occur is molecular O_2. Browning is eliminated or reduced if any one of these three components is absent. For example, through antisense PPO technology, the PPO - polyphenol - O_2 interaction could be eliminated and tissue browning would not occur. However, PPO is ubiquitous in plant tissues and perhaps it may be more efficient to tackle the problem from one of the other two necessary components for browning to occur.

Measures can be taken to prevent tissue damage through careful handling and minimal exposure to damaging environmental stress factors. If tissues remain intact, polyphenol substrates localized in the vacuole remain separated from PPO which is located in the cytoplasm (Murata *et al.,* 1997).

Future development of antisense PPO technology or molecular techniques applied to breeding of cultivars with resistance to browning may still provide answers to solving the pedicel browning problem. As it stands, significant reduction of pedicel browning is easily obtained with the use of reflective Mylar tarps and careful handling through temperature and relative humidity modifications.

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VI. APPENDICES

Appendix A: 'Lambert' cherry fruit evaluated from trees that had water withheld for 14 d, 28 d or irrigated once a week (control). Values for pitting, decay and cracked fruit are means ± SE of four replicates (n = 25).

Treatment	Storage duration (Wk)	Pitted fruit (%)	Decayed fruit (%)	Cracked fruit (%)	SSC (%)[X]
Control	0	24.0 ± 1.6	0	25.0 ± 3.4	18.6 ± 0.7
	2	57.0 ± 5.7	14.0 ± 2.6	38.0 ± 3.8	18.8 ± 0.5
	4	75.0 ± 4.4	43.0 ± 3.0	49.0 ± 3.0	17.9 ± 0.3
	6	66.0 ± 6.2	59.0 ± 10.8	49.0 ± 5.3	17.5 ± 0.3
No irrigation					
14 DBH	0	19.0 ± 4.1	0	25.0 ± 10.1	18.3 ± 1.2
	2	56.0 ± 5.9	14.0 ± 3.8	37.0 ± 3.8	18.3 ± 0.4
	4	67.0 ± 5.7	41.0 ± 7.4	41.0 ± 7.4	17.7 ± 0.6
	6	79.0 ± 4.4	43.0 ± 5.0	29.0 ± 5.0	18.1 ± 1.0
No irrigation					
28 DBH[Y]	0	20.0 ± 3.7	0	18.0 ± 3.8	20.2 ± 0.9
	2	55.0 ± 2.5	12.0 ± 2.8	28.0 ± 6.5	18.8 ± 0.8
	4	64.0 ± 6.3	32.0 ± 8.6	41.0 ± 9.1	18.8 ± 0.4
	6	69.0 ± 2.5	21.0 ± 3.4	20.0 ± 1.6	19.1 ± 0.9

[X]Soluble solids content (SSC), in the juice obtained by hand crushing 10 cherries in a plastic bag, was measured with a refractometer.

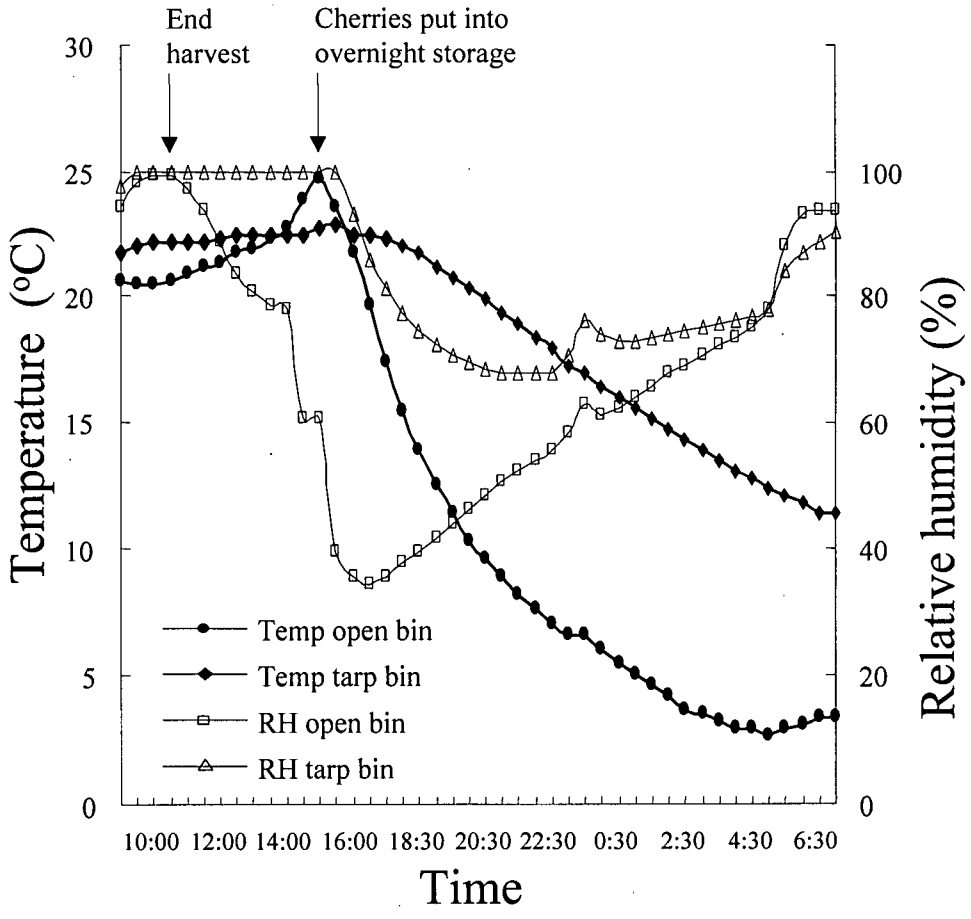
[Y]DBH = days before harvest

Appendix B. 'Lambert' and 'Lapins' cherries covered with reflective tarps or left open in the orchard. Values are means ± SE of three replicates.

Cultivar	Treatment	Storage duration (wk)	Pitting (%)	Decay (%)	SSC (%)[X]
Lambert	Open	0	39.3 ± 6.1	3.3 ± 1.6	14.4 ± 0.2
		2	81.3 ± 3.4	8.7 ± 2.2	13.8 ± 0.31
		4	82.0 ± 3.4	34 ± 3.7	13.9 ± 0.25
		6	70.0 ± 3.4	46 ± 5.3	13.7 ± 0.13
	Tarp	0	28.0 ± 2.1	0	14.1 ± 0.11
		2	64.7 ± 3.8	3.3 ± 1.2	13.6 ± 0.16
		4	66.7 ± 3.8	21.3 ± 4.1	13.2 ± 0.21
		6	64.0 ± 5.2	31.3 ± 3.6	13.4 ± 0.21
Lapins	Open	0	48.7 ± 4.5	0	16.4 ± 0.23
		2	81.3 ± 6.1	2.0 ± 0.89	16.1 ± 0.45
		4	92 ± 2.7	7.3 ± 4.2	15.6 ± 0.45
		6	74 ± 6.1	20.7 ± 4.0	15.4 ± 0.47
	Tarp	0	38.0 ± 2.9	0	16.1 ± 0.26
		2	60.0 ± 7.1	0	15.6 ± 0.21
		4	76.7 ± 5.3	4.7 ± 1.2	15.5 ± 0.64
		6	59.3 ± 3.0	9.3 ± 2.2	15.2 ± 0.37

[X]Soluble solids content (SSC), in the juice obtained by hand crushing 10 cherries in a plastic bag, was measured with a refractometer.

Appendix C: The temperature and relative humidity (RH) data for 'Lapins' cherries that were held overnight in a large warehouse cooled by fans **with high airflow rates**. The cherries were either left open or covered with reflective tarps during harvest, transport and short term storage until sampling occurred.


End harvest
Cherries put into overnight storage
Temperature (°C)
Relative humidity (%)
Time
Temp open bin
Temp tarp bin
RH open bin
RH tarp bin
0
5
10
15
20
25
30
0
20
40
60
80
100
10:00 12:00 14:00 16:00 18:30 20:30 22:30 0:30 2:30 4:30 6:30