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HIGH TUNNEL PRODUCTION OF RED RASPBERRY (*Rubus idaeus* L.): COLD HARDINESS AND AN ECONOMIC ANALYSIS

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HIGH TUNNEL PRODUCTION OF RED RASPBERRY (*Rubus idaeus* L.): COLD HARDINESS AND AN ECONOMIC ANALYSIS

By

Michael Douglas Von Weihe

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ABSTRACT

HIGH TUNNEL PRODUCTION OF RED RASPBERRY (*Rubus idaeus* L.): COLD HARDINESS AND AN ECONOMIC ANALYSIS

By

Michael Douglas Von Weihe

Interest in using high tunnels for producing red raspberries is increasing in temperate climate regions of the United States. High quality fruit can be produced under tunnels but the profitability of this system has not been investigated in Michigan under current economic conditions. Additionally, no work has been done to describe how the use of a 3-season tunnel during the growing season may influence cold hardiness during the winter. Objectives of this work were to: 1) determine the profitability of floricane- (FF) and primocane-fruiting (PF) raspberry cultivars in tunnels under certain costs and berry prices, 2) characterize cold acclimation of high tunnel-grown raspberry plants, and 3) determine carbohydrate levels in acclimating plants and describe their relationship to cold hardiness. Crop values at full production for one acre of FF and PF tunnel raspberries were $60,060 and $71,520, respectively. Break-even yields (yields necessary to cover costs) for PF types were lower than for FF types, indicating PF cultivars have a higher potential for profit under tunnels. Cold hardiness of tunnel and field raspberry tissues were similar on most dates in the autumn, suggesting cold acclimation under covered, 3-season tunnels and in the field may be similar. Bud primordia and cambium tissues of 'Nova' were typically more cold hardy than 'Canby' and 'Encore' during acclimation and in mid-winter. Concentrations of the soluble carbohydrate raffinose were higher in 'Nova' than the other two cultivars. All soluble carbohydrate levels were positively correlated with hardiness while starch was negatively correlated. Early high tunnel plastic removal increased hardiness of bud primordia but had no effect on cambium hardiness.

DEDICATION

To my parents, Doug and Becky Von Weihe for their unwavering support in all I do

and to Mike J.

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CHAPTER 1

REVIEW OF LITERATURE

Fresh Raspberry Production

Fresh raspberry consumption has increased considerably in the U.S. during the last two decades. Per capita consumption of fresh raspberries in the United States was 0.37 pounds in 2007, a value over three times what it was in the early 1990s (USDA-ERS, 2009). Strong demand has kept the price of fresh raspberries high, encouraging greater production for the fresh-market. In Oregon, fresh-market raspberry prices averaged twice that of processing berries during 2001-2005 and in Washington, three times that of processing during the same five year period (Pollack and Perez, 2006). One can quickly observe that crop values are much larger when producing raspberries for the fresh-market.

Michigan is one of the leading fruit producing states in the United States. Michigan ranks fifth in the nation for the value of its fruit and prepared fruit products exported internationally based on the state's share of U.S. production in 2007 (USDA, 2008). Commercial red raspberry production is already occurring in the state with 500 acres under cultivation in 2006 (USDA, 2007). Michigan is in close proximity to many large population centers such as Chicago and Detroit and has the potential to meet a greater quantity of the demand for fresh raspberries in these population centers.

Growing raspberries in Michigan for the fresh market has several limitations. Michigan summers are typically warm and humid with frequent rain. Diseases like grey mold (*Botrytis cinerea*) and penicillium rot (*Penicillium spp.*) are increased by rain and humid conditions where water on leaf surfaces evaporates slowly. Primocane-fruiting (PF) raspberry cultivars (those that produce flowers on the current season's shoots called primocanes) fruit from early August through the first killing frost in Michigan. Full production often is not realized due to early frosts, and loss of fruit is common in northern regions (Hoover et al., 1989). Floricane-fruiting (FF) raspberry cultivars produce fruit on canes during their second season of growth. These cultivars do not fruit at the time when fall frosts occur, but must be able to survive cold winter temperatures since fruit is produced on a two-year-old cane.

Due to these limitations, Michigan fresh raspberries often lack the consistent quality and volumes required to supply grocery store chains. Major raspberry growing regions in the western United States, particularly California, have more consistent weather conditions, less rain and humidity, and a longer growing season. Growers in Michigan are beginning to adopt the use of a structure called a high tunnel to extend the growing season and produce high quality raspberries with the potential to compete with fresh raspberries from California.

High Tunnels

The term high tunnel is used to describe a variety of structures that protect horticultural crops. High tunnels can differ somewhat in size and shape although they share many common characteristics (Lamont, 2009; Carey et al., 2009). High tunnels are hoop houses with metal frames that are covered in a single layer of plastic. These structures are large enough to allow for small equipment to enter from the ends. High tunnels are generally unheated and as such, plastic is placed on the ends and sides of tunnels in early spring and late fall to trap heat accumulated during the day. During warm periods of the year, plastic covering the ends of tunnels is removed and plastic along the sides is vented to allow for air movement. Additionally, high tunnels lack a foundation and raspberry plants are planted directly in the ground. High tunnels also do not typically have electricity and are considered nonpermanent structures. As such, venting of the tunnel plastic is done by hand.

Two categories of high tunnel structures exist: 4-season and 3-season structures. A 4-season structure is often covered with plastic year round and is strong enough to withstand heavy snow loads. Four-season structures usually have a peaked frame, cover a smaller area per tunnel than 3-season structures, and single tunnels typically stand alone. Research growing red raspberries and or blackberries under 4-season structures has been conducted at the Penn. State High Tunnel Research and Education Facility (Lamont et al., 2003; Demchak, 2009), Cornell University (Heidenreich et al., 2008), Iowa State University (Domoto et al., 2007), and the University of Massachusetts (Schloemann, 2007).

Three-season high tunnels differ from 4-season in that plastic is removed during the winter months. Plastic removal is necessary because the structure often has increased distances between its structural supports and cannot support the weight of snow (Lang, 2009). The 3-season high tunnel has a rounded frame with vertical legs which can be linked to form multi-bay units. Multi-bay structures can become quite large and cover several acres. The dimensions of 3-season high tunnels range from 5.5 to 9 m (18 to 30 feet) wide, 3 to 4.5 m (10 to 15 feet) tall and lengths are designed to fit grower specifications (Gaskell, 2004; Haygrove, 2009).

Research efforts using high tunnels to producte red raspberries are increasing. Studies assessing yield under tunnels have been conducted in the United States as well as abroad (Demchak, 2009). For instance, a multi-national project was started in 1993 in which several European countries set out to determine proper agronomic management of primocane-fruiting cultivars for winter production using tunnels in southern regions of Europe (Rosati et al., 1999). Also, researchers in Belgium demonstrated season extension potential for high tunnel raspberry production (Meesters and Pitsioudis, 1999).

Gaskell (2004) discussed the rise in protected agriculture in Southern California during the early 1990s. The author noted that off-season raspberry production under protected structures in California occurred largely in San Diego, Ventura, and Santa Barbara counties in 2004. Scientists at universities in the Northeast have been conducting multi-year studies producing red raspberries in high tunnels (Lamont et al., 2003; Heidenreich et al., 2008). A 2007 survey of extension agents across the U.S. indicated that there were over 4,000 acres of raspberries under tunnels in California (Carey et al., 2009). The survey also noted raspberries were grown as a tunnel crop in other states such as Florida, Maine, Michigan, New Jersey, Oregon, Pennsylvania, Utah, and Washington but in much smaller numbers than in California (Carey et al., 2009).

Economics

The economic feasibility of a high tunnel red raspberry production system is an important consideration for a fruit grower. The venture must be profitable in order for a grower to make the large high tunnel investment and for the operation to be sustainable. Several economic analyses have been conducted for raspberry production in high tunnels (Heidenreich et al, 2008; Yan and Du, 2003) and in the field (Bolda et al., 2005; Bushway et al., 2007). Bolda et al. (2005) composed a detailed extension publication covering the costs to produce red raspberries in California; however, this analysis did not incorporate high tunnel production. Moreover, the potential returns a grower can realize in California are different from those in Michigan. Yields in California are higher than in Michigan because of a longer growing season, resulting in the potential to generate more revenue per acre. Many of the costs associated with field production of raspberries can be used in determining high tunnel expenses; however, additional expenses must be considered for high tunnel production which include the cost of the structure, plastic, as well as additional labor costs associated with the more intensive system.

The analysis conducted by Heidenreich et al. (2008) used 4-season high tunnels with dimensions of 30 x 90 ft. (2,880 sq. ft.). These structures require space between individual tunnels which results in unused land around the high tunnel structure. The cost to purchase one structure was $6,400, or over $2.00 per square foot. This is more expensive than 3-season tunnels, which usually cost less than $1.00 per square foot, depending on the size of the structure.

An economic analysis conducted in Ontario, Canada, investigated the financial feasibility of producing raspberries under Haygrove (Ledbury, Herefordshire, UK) 3-season high tunnels and greenhouses (Yan and Du, 2003). The net present value of the cash flow for a one acre 3-season tunnel operation was calculated to be $67,300 with an internal rate of return equal to 34.9% considering a baseline interest rate of 5% (Yan and

Du, 2003). Net present value evaluates investments by considering opportunity costs of using funds for capital items as well as the time value of money while internal rate of return describes the interest rate relating the present value of cash inflows to outflows (Boehlje and Eidman, 1984). The authors determined producing red raspberries under a high tunnel system was practical and profitable. However, this information is not completely applicable to Michigan growers. The capital costs included in the analysis (Yan and Du, 2003) represented Canadian prices for goods. Capital costs were listed as a lump sum; however, it would be beneficial to itemize costs so that growers can determine for themselves what items are necessary for individual operations. The analysis also used a standard yield estimate of 10,000 $lb{\cdot}acre^{-1}$ for both PF and FF raspberries, not taking into account yield differences between the two types. It currently is not clear whether this level is realistic for Michigan producers. Studies in southwest Michigan indicate that yields can be higher (Hanson et al., 2008). Also, fresh raspberry prices fluctuate throughout the year as the supply of fresh raspberries changes. A distinction between prices during harvest periods for FF and PF raspberries would be beneficial. An additional economic analysis using current prices and yield data would be helpful to growers considering 3-season high tunnel raspberry production in Michigan.

High Tunnel Benefits and Concerns

High tunnels offer a variety of benefits to raspberry producers. High tunnels exclude rainfall and modify temperature, light quality, humidity, and wind around the plant. Rainfall exclusion from the raspberry canopy reduces the incidence of diseases spread by water on both foliage and fruit. The duration of leaf wetness and temperature have the greatest effect on producing *Botrytis* inoculum in strawberry (Legard et al., 2000). The incidence of *Botrytis* and anthracnose was negligible on strawberry under tunnels (Chandler et al., 2005), likely because of lower leaf wetness. Growers also can use high tunnels to hasten or prolong harvest seasons by covering the sides and ends to trap heat from sunlight in the spring or late fall. Sealed high tunnels are warmer than ambient temperatures during the day, causing raspberry plants to remain active longer in the fall (Kadir et al., 2006).

Keeping raspberry plants active longer in the fall can be beneficial. Elevated daytime temperatures under sealed high tunnels allow PF raspberry cultivars to produce fruit longer in the fall, increasing yields and extending the growing season (Demchak, 2009). Canes of PF raspberry cultivars are not needed for fruit production the following year and are pruned to the ground to allow new primocanes to emerge in the spring.

Management of FF raspberry cultivars differs from that of PF cultivars. Floricanes of FF cultivars are removed following harvest in late July and the remaining primocanes continue growth. These primocanes initiate floral buds in their leaf axils in response to short days and low temperatures in the fall (Williams, 1960) and buds must overwinter before they emerge and flower. Extending the growing season and keeping plants active longer in the fall could delay acclimation to freezing temperatures in FF raspberry plants (winter injury in PF cultivars is avoided by pruning). Conversely, raspberry plants that remain active longer may accumulate more total carbohydrates. Carbohydrate accumulation has been shown to increase the cold hardiness of other woody species in some studies (Stergios and Howell, 1977) but not others (Wample and Bary, 1992). Cold hardiness is defined as the ability of a plant to survive or resist freezing temperatures (Fuchigami, 1996). The cold hardiness of FF raspberry canes grown under a tunnel has yet to be studied in depth and only anecdotal comments indicating improved plant survival under 4-season tunnels have been made (Heidenreich et al., 2008).

Freezing Stress and Cold Hardiness

Woody plants in temperate climates experience seasonal stresses caused by freezing temperatures. Freezing stress sets the northern limits of raspberry production and restricts the cultivars that can be grown. The process by which plants are damaged by freezing temperatures, remain uninjured, or are injured but recover is complex.

Mechanisms of Plant Injury by Freezing Temperatures

Freezing injury can be classified into several categories based on the location and mechanism of injury in the plant: (1) primary direct freezing injury, and (2) secondary freeze injury (Levitt, 1980). Primary direct freezing injury is the result of intracellular ice crystal formation and is assumed to always be fatal (Levitt, 1980). Ice crystals formed in cells cause damage to the protoplasmic structure possibly by puncturing membranes (Levitt, 1980). When intracellular freezing is very rapid (100°C s^{-1} or more) some cells may survive because the ice crystals are extremely small; however, these rapid rates are not typically found in nature (Levitt, 1980).

Secondary freezing injury is caused by processes other than ice crystal formation. An important form of secondary freezing injury in deciduous woody plants results from extracellular freezing. Ice that forms outside cells has a dehydrating effect on the unfrozen solution of the cell, increasing solute concentrations and lowering osmotic potentials (Guy, 1990). Liquid water then moves out of the cell to the extracellular solution or the ice crystal being formed (Guy, 1990; Sakai, 1982). The contents of cells are then concentrated and cell volume reduced as liquid water leaves the cell. It is hypothesized that extracellular freezing can cause cell injury through cell volume contraction, concentration of solutes, and possible pH changes (Steponkus, 1984); however, concentration of cell solutes (Burke et al, 1976) and cell dehydration (Ishikawa and Sakai, 1981) are suggested to increase tolerance to freezing temperatures as well.

Weiser (1970b) suggested that freezing injury in woody stems is not a continuous process but occurs at specific temperature points. In controlled freezing experiments with woody plant material, as many as three exotherms (points at which heat is given off as water freezes) were observed as temperature declined (Weiser, 1970b). The first exotherm coincides with the extracellular freezing of water. The second occurs as protoplasmic water moves out of the cell in response to extracellular ice causing a vapor-pressure deficit (Weiser, 1970b). The third exotherm is associated with the freezing of protoplasmic constituents necessary for life, what Weiser termed as 'vital water', and results in cell death. Exotherms have been studied in a variety of woody species including apple (Quamme et al., 1972; Quamme et al., 1973), grape (Pierquet and Stushnoff, 1980), and blackberry and raspberry (Warmund and George, 1990).

Freezing Resistance and Mechanisms of Plant Survival

The strategies which allow plants to survive freezing stress can fit into one of two categories: 1) freezing avoidance and 2) freezing tolerance (Levitt, 1980).

Freezing avoidance

Supercooling of plant tissue water is one method utilized by woody plants to avoid freezing temperatures. It is the result of plant temperature dropping below its freezing point without ice formation (Burke et al., 1976; Levitt, 1980). Flower bud meristematic tissues of azalea, blueberry, apricot, cherry, and plum have been shown to supercool (Weiser, 1970b; George et al., 1974; George and Burke, 1977). Wood ray parenchyma cells of apple have also been shown to deep supercool down to temperatures as low as -38° to -47°C (Quamme et al., 1973). The degree to which plant tissues can supercool is limited since the lowest possible supercooling point for pure water is -38°C in the absence of ice nucleators (Burke et al., 1976). Concentrations of aqueous solutions likely to be found in plants have been shown to supercool to about -47°C (Rasmussen et al., 1975; George and Burke, 1976). Supercooling is likely responsible for setting the northern limit of production for several species in the Rosaceae family (apple and pear) in North America (Quamme, 1976). It also has been found in a variety of other cultivated fruit crops including apricot, blueberry, cherry, currant, grape, and raspberry (Quamme, 1995).

In supercooling tissues, ice crystals do not form even though temperatures fall below the freezing point of cellular water (Chen et al., 1994). In all freezing situations, ice nucleation must take place in order for an ice crystal to form on or within a plant (Wisniewski et al., 2003). The process of converting water to a more stable state (ice) is initiated by nucleation, or the first appearance of a very small volume of ice (Vali, 1995). Ice nucleation can be either heterogeneous or homogeneous. Heterogeneous ice nucleation takes place when small clusters of water molecules attach to a foreign surface, a substrate, and the stability of that cluster is increased in response to having part of its surface area attached to the substrate (Vali, 1995). Homogeneous ice nucleation occurs without the presence of a substrate as water molecules spontaneously combine due to reduced thermal motion (Vali, 1995).

Research has been conducted to investigate the control of ice nucleation events on the plant surface, thus supercooling plants below 0°C to avoid freezing (Lindow, 1995). The protein coat of certain bacteria are sites of ice nucleation (Hirano and Upper, 1995). The first release of a genetically modified organism for agricultural use was an ice-nucleating active (INA) strain of bacteria (*Pseudomonas syringae*) where much of the nucleation protein gene was deleted (Lindow, 1990). The resulting bacteria lacked ice nucleation activity (non-INA) and were sprayed onto plants to compete with and reduce the presence of INA bacteria thus reducing ice nucleation. Potato plants sprayed with non-INA bacteria tolerated temperatures -2 to -5 °C to a greater extent than control plants (Lindow, 1995).

Infrared (IR) video thermography has been used to visually determine points of ice nucleation and propagation in plants (Fuller and Wisniewski, 1998; Ceccardi et al., 1995). Using this technology, the heat of fusion released as water freezes is imaged and the location and timing of freeze events in plant material can be determined. Workmaster et al. (1999) demonstrated that in cranberry, water droplets containing ice-nucleating bacteria would only freeze if located on the abaxial side of leaves, providing evidence that stomata are likely to facilitate ice propagation into plant leaves. A lag time existed between when the droplet froze on the abaxial side of a cranberry leaf and when ice would enter the leaf. The authors also noted that the adaxial cuticle may provide an adequate barrier to ice propagation. Other possible entry points for extrinsic ice include wounds, lenticels, and cracks in the cuticle while internal ice propagation is thought to proceed through extracellular spaces and xylem vessels (Levitt, 1980).

Freezing tolerance

Freezing tolerance in plants can only be the result of extracellular freezing, as intracellular freezing presumably disrupts cell membranes (Levitt, 1980). During extracellular freezing in plants, liquid water moves out of the cell to ice crystals forming in extracellular spaces (Guy, 1990). Ishikawa and Sakai (1981) propose that during a freezing event, the cell water of *Rhododendron* flower buds moves from florets to ice forming in the bud scales, increasing cell sap concentration and lowering the freezing point of the florets. Extracellular ice formation can be tolerated in plants if the accompanying dehydration of cells can be avoided or tolerated (Chen, 1994). The resistance of cell walls to collapse in some plants creates a negative pressure potential as water moves toward extracellular ice, possibly decreasing cell dehydration during extracellular freezing (Rajashekar et al., 1982). The result of any dehydration of cells in response to extracellular ice formation is the concentration of intracellular and extracellular solutes (Steponkus, 1984).

Some researchers suspect that cold acclimation can be induced by plant dehydration. The killing temperature of cells frozen extracellularly has been shown to vary based on the cell's ability to withstand freeze-dehydration when cooled slowly (Sakai, 1982). The promoters of several cold response genes are activated by both low temperature and dehydration (Thomashow, 2001). Several genes that are induced by water deficits in Arabidopsis seedlings as well as prior to seed desiccation may contribute to freezing tolerance (Thomashow, 1998).

Dormancy and Cold Hardiness in Woody Plants and Raspberry

Tissues of woody plants have the ability to adapt to seasonal changes. During late summer and fall woody plants begin entering an inhibited state termed endodormancy (Lang et al, 1987), or rest. During endodormancy, plant meristems cease visible growth and buds will not open. Buds are released from endodormancy following the accumulation of a certain amount of time at low temperatures (Couvillon, 1995). The number of hours needed at low temperature (chilling requirement) to overcome endodormancy varies depending on plant species and cultivar. Estimates of the chilling requirement for raspberry cultivars vary from 250 to 780 hours (Dale, 2008). Dale et al. (2003) suggests a model for calculating the number of chilling hours raspberry plants accumulate using a weighted scale. Plants experiencing temperatures between 0 and 5.6 °C accumulate one chilling hour while temperature ranges slightly higher account for fewer chilling hours and temperatures below 0 °C account for no chilling hours (Dale et al., 2003; Richardson et al., 1974). In research conducted with primocane-fruiting 'Heritage' red raspberry, flowering was hastened by increasing the number of chilling units (hours between 0 and 7 °C) (Takeda, 1993). Once the chilling requirement is met and endodormancy is released, plant growth can commence when temperatures adequate for growth occur. This state is called ecodormancy.

Raspberry plants also undergo processes during the winter dormant period that increase their cold hardiness (Fuchigami, 1996). The dormant period can be divided into three stages of cold hardiness: cold acclimation, mid-winter hardiness, and deacclimation (Proebsting, 1970). Cold acclimation for woody perennials occurs in two phases. The first is induced by photoperiod (Fuchigami et al., 1970; Irving and Lanphear, 1967b; Van Huystee et al., 1967) and the second by frost or low, non-freezing temperatures (Howell and Weiser, 1970; Gusta et al., 2005).

Photoperiod

Photoperiodic induction of cold acclimation is controlled by phytochrome (McKenzie et al., 1974). Phytochrome exists in one of two photoreversible forms; P_{fr} or P_r. P_r is biologically inactive and absorbs red light, after which it is converted to P_{fr}. P_{fr} on the other hand, is the biologically active form, absorbs far red light, and is subsequently converted to P_r (Smith, 2000). Additionally, P_{fr} can be slowly converted to P_r during dark periods (Smith, 1995). It has been suggested that the ratio of P_{fr} to P_r determines physiological responses in plants (McDonald, 2003).

In *Cornus stolonifera*, short days and end-of-day far red light treatment after long days promotes growth cessation, cold acclimation, and increased cold hardiness in response to low temperatures (McKenzie et al., 1974). Perception of light quality, the relative amounts of red and far red light, occurs in plant leaves. Fuchigami et al. (1971) indicates that the short-day leaf is the source of a hardiness promoting factor and that it is translocated through the phloem. Interestingly, photoperiod appears to have no effect on cane height in the red raspberry cultivar 'Autumn Bliss' (Carew et al., 2003) and may only minimally influence growth cessation in the fall if temperatures remain high. A photoperiod response to short days can account for some increased cold hardiness during the first stage of acclimation, however, low temperature plays a primary role in increasing plant cold hardiness (Howell and Weiser, 1970).

Low temperature

The second stage of cold acclimation is brought about by low temperatures (Weiser, 1970a; Gusta et al., 2005). Weiser (1970b) suggested that frost is the triggering stimulus for the increase in cold hardiness. Temperatures of 0 to 5 °C induced greater hardening than 5 to 10 °C (Levitt, 1980). Proebsting (1978) noted that subfreezing temperatures greatly increased the cold resistance of dormant, acclimated woody plants. It was shown in apple that low temperature alone can fully harden plants exposed only to long days (Howell and Weiser, 1970).

Growth cessation is also noted as a prerequisite for cold acclimation (Fuchigami et al., 1971); however, plants do not need to be physiologically dormant. Fast growing raspberry cultivars have been shown to be more susceptible to frost injury in the fall than slower growing cultivars (Van Adrichem, 1966). Several woody plant species have the ability to cold acclimate as well as resume growth under favorable conditions (Irving and Lanphear, 1967a).

Cessation of growth and development in red raspberry is an indicator of cold hardiness. Early raspberry cane ripening (change in color) and early defoliation were positively correlated with cold hardiness (Sako and Hiirsalmi, 1980). However, the defoliation of raspberry canes must occur naturally. Doughty et al. (1972) showed that canes of 'Puyallup' manually defoliated in early September displayed more damage after freezing tests during the winter than non-defoliated canes. Freeze damage was attributed to a lack of photoperiodic induction as well as inadequate carbohydrate reserves. In eastern thornless blackberry (*Rubus sp.*), premature defoliation appeared to decrease mid-winter hardiness of stem tissue but had no effect on bud hardiness (Kraut et al., 1986).

Shoot growth in red raspberry requires both high temperature and long photoperiods (Sønsteby and Heide, 2008). Growth of 'Malling Promise' occured continually at 21 °C under short (9 h) and long (21 h) photoperiods, while at 15.5 °C growth ceased under a 9 h photoperiod (Williams, 1959). Shoot lengths in early October for 'Glen Ample' grown under natural day length were 85, 190, and 350 cm at 9, 15, and 21 °C, respectively and corresponding node numbers on the same day were 30, 45, and 80 (Sønsteby and Heide, 2008). At 21 °C, growth slowed but continued into October when the effective photoperiod was 11 h, although plants grown at 9 and 15 °C ceased growth in September (Sønsteby and Heide, 2008). Temperatures of 18 °C were adequate to maintain growth through 2 Nov. in 'Glen Ample' when the natural photoperiod was less than 9 h (Sønsteby and Heide, 2008). The critical photoperiod at 15 °C is ≤15 h to reduce elongation growth and leaf formation in 'Glen Ample' (Sønsteby and Heide, 2008). The previous research suggests that temperature plays a greater role in growth cessation than photoperiod.

Methods to Evaluate Cold Hardiness

Early studies of cold hardiness in woody plants were based on field assessments of plant growth during the following spring (Brierley et al, 1950; Van Adrichem, 1966; Jennings and Carmichael, 1975). This method has been employed recently to compare the cold hardiness raspberry of cultivars (Zatylny et al, 1996; Hanson et al., 2005). While field observations are non-destructive and straightforward, they vary with yearly weather and may not give information about when freeze injury occurred. Controlled-freezing tests have been used to simulate natural freeze events (Wolpert and Howell, 1985). Plant tissues are placed in a freezer and the temperature lowered at a consistent rate similar to what occurs in the field (<5 °C hr^{-1}) or as desired. Plant tissues are wrapped in aluminum foil to facilitate even temperature distribution and prevent desiccation. Samples can also be wrapped in moist cheesecloth to inoculate with ice and avoid supercooling if warranted by the experiment. The temperature of specific tissues can be measured by insertion of a thermocouple. The thawing rate after freezing must also be standardized to ensure uniformity.

To identify temperatures that cause injury, subsamples are frozen to a range of test temperatures. Five to nine test temperatures are typically selected and the range between the temperatures varies with the species and time of year. The temperatures are chosen so that no injury occurs at the warmest and all tissues are killed at the coldest. This can be accomplished by removing subsamples from the freezer once they reach the predetermined test temperature and placing them into 0 to 5 °C conditions for approximately 24 h.

A number of methods can be used to determine injury after freezing stress including regrowth tests (Cortell and Strik, 1997), tissue browning (Hummer et al., 1995; Warmund et al., 1992), electrical conductance/resistance (Boorse et al., 1998), triphenyl tetrazolium chloride (TTC) (Irving and Lanphear, 1968), differential thermal analysis

(DTA) (Warmund et al., 1992; Ishikawa and Sakai, 1981), and chlorophyll fluorescence (Stushnoff, 1972; Jiang et al., 1999). The appropriate method to use depends on the plant material to be tested and the objectives of the research.

Tissue browning is perhaps the most widely used method to evaluate cold hardiness. After samples are frozen and thawed, they are placed in a humid chamber for 4-7 days at room temperature. During this time, freeze-injured tissues develop a brown color from the oxidation of cellular contents which can be visually assessed by assigning ratings to the degree of browning (Warmund et al., 1986; Doughty et al., 1972) or using values of alive and dead (Sterigos and Howell, 1973; Palonen and Lindén, 1999). This method takes 1 to 2 weeks before results and is qualitative in nature, but is considered reliable and uses minimal equipment (Stergios and Howell, 1973).

Cold hardiness was, at one time, reported in terms of percent kill at a given temperature (Proebsting and Fogle, 1956). This made comparisons throughout a winter difficult because a temperature appropriate during cold acclimation may be too warm to kill sampled tissue during mid-winter. The temperature estimated to be lethal for 50% of sampled tissue (LT_{50}) has since been used to quantify and compare cold hardiness (Bittenbender and Howell, 1974). LT_{50} can be calculated in a number of ways due to the sigmoidal shape of the temperature vs. survival curve. The Spearman-Kärber method (Bittenbender and Howell, 1974) as well as logit and probit models (Lindén et al., 1996) can be used to estimate LT_{50}.

Carbohydrates

Soluble carbohydrates are known to accumulate in woody plants during cold acclimation and are positively correlated with cold hardiness (Ashworth et al., 1993; Hamman Jr. et al., 1996; Palonen, 1999; Steponkus and Lanphear, 1968; Sakai and Yoshida, 1968). Starch levels in woody plants have been negatively correlated with cold hardiness (Jennings and Carmichael, 1975; Lasheen and Chaplin, 1971; Raese et al., 1978). The role of carbohydrates in cold hardiness is complex and although much research has been conducted addressing the subject, direct relationships have not been well established (Ashworth et al., 1993).

Soluble Carbohydrates

A variety of soluble carbohydrates occur in plants depending on the species, plant tissue, environment, and developmental stage of the plant. Soluble carbohydrates found in red raspberry plant tissue include sucrose, glucose, fructose, as well as minor amounts of raffinose and stachyose (Palonen, 1999). The disaccharide sucrose is the major soluble carbohydrate in raspberry tissue during the winter (Palonen,1999; Kaurin et al., 1981), and has been reported to be a more effective cryoprotectant than its monosaccharide components glucose and fructose (Crowe et al., 1990). Raffinose, a trisaccharide composed of galactose, fructose, and glucose, has been correlated with increased cold hardiness in *Cornus sericea*, *Lonicera caerulea*, and *Forsythia* (Ashworth et al., 1993; Flinn and Ashworth, 1995; Imanishi et al., 1998).

Soluble carbohydrates may affect cold hardiness in different ways. Sugars lower the freezing point and increase the osmotic potential of cells. This could reduce the amount of dehydration that occurs during extracellular freezing (Levitt, 1980). The accumulation of low-molecular weight carbohydrates also appears to have a protective effect on cell membranes (Santarius, 1973). Evidence suggests that sugars and sugar alcohols act as colligative cryoprotectants for cell membranes and proteins by preventing an increase in electrolyte and toxic compound concentrations that accumulate during freezing (Santarius, 1982). During freezing stress, there is evidence that sucrose interacts directly with the polar head groups of phospholipids in membranes and may replace water by hydrogen binding to the polar heads (Crowe et al., 1987). Sakai and Yoshida (1968) suggest that while the degree of freezing resistance is not explained by cell sugar content alone, differences may be attributed to changes in the conformation of protein in the plasma membrane and the degree to which sugars protect the membrane.

Soluble carbohydrates are correlated with cold hardiness in many plant species. In the buds and rhizomes of Cloudberry (*Rubus chamaemorus* L.), cold hardiness is positively correlated with the amount of soluble carbohydrates in tissues (Kaurin et al., 1981). The author also states that sucrose content rose quickly after low temperature exposure in the fall. Soluble carbohydrates were positively correlated with increased cold hardiness in grape and it was found that leaving fruit on the vine did not significantly affect soluble carbohydrate and starch reserves in bud or cane tissues (Wample and Bary, 1992). Increases in the levels of sorbitol, a sugar alcohol, have been associated with low temperature and cold hardiness in apple (Ichiki and Yamaya, 1982). Cold hardy shoots and flower buds of peach (*Prunus persica* L.) accumulate more soluble sugars than tender ones as a percent of sample dry weight (Lasheen and Chaplin, 1971).

Several genetic studies indicate raffinose is related to the degree of cold hardiness in herbaceous plants. Transgenic petunia plants were created with reduced activity of α-galactosidase, an enzyme involved with the hydrolysis of raffinose during deacclimation. These transgenic plants had much higher levels of raffinose and greater cold hardiness than wildtype plants when exposed to acclimating temperatures (Pennycooke et al., 2003). However, in *Arabidopsis thaliana*, increased raffinose levels did not influence cold hardiness (Zuther et al., 2004). Mutants constitutively overexpressing galactinol synthase as well as mutants lacking a raffinose synthase gene were used to study raffinose levels and cold hardiness. Overexpressing galactinol synthase resulted in greatly increased levels of raffinose. Mutants lacking raffinose were as cold hardy as wild types or genotypes overexpressing galactinol synthase, indicating that raffinose is not essential in basic freezing tolerance of *A. thaliana* (Zuther et al., 2004).

Despite the evidence that soluble carbohydrate levels are positively correlated with cold hardiness in plant tissues, the relationship has not been shown to be causal. In *Hedera helix* L. cv. Thorndale, both light- and dark-acclimated plants exhibited increased cold hardiness; however, only the light-acclimated plants increased in sucrose levels while dark-acclimated sucrose levels decreased (Steponkus and Lanphear, 1968). Sakai and Yoshida (1968) found that poplar trees acclimated at 0 °C for two months had dramatic increases in freezing resistance accompanied by increased sugar levels. However, trees acclimated at 15 °C had similar increases in freezing resistance but no appreciable increase in sugar levels.

Starch

Soluble carbohydrates are known to accumulate in woody plants during cold hardening as starch is hydrolyzed to low-molecular weight carbohydrates (Sakai and Yoshida, 1968). Palonen (1999) reports that starch almost disappeared from red raspberry canes and entirely disappeared from bud tissues during the winter months. Keller and Loescher (1989) observed an apparent interconversion of starch and soluble carbohydrates between November and January in shoot and trunk wood of sweet cherry. While a decline in starch concentration may occur in overwintering plant tissues, it is not always accompanied by increases in total soluble carbohydrate content (Steponkus and Lanphear, 1968). Many authors indicate that soluble carbohydrates increase simultaneously with the hydrolysis of starch (Alberdi et al. 1989; Ashworth et al., 1993; Flinn and Ashworth, 1995), although it is unlikely hydrolysis of starch can account for the full increase in soluble carbohydrates during cold acclimation.

Summary

Fruit growers in northern climates like Michigan are increasingly using high tunnels to extend the growing season for raspberries and improve fruit quality. The economic considerations of a high tunnel raspberry operation are of great importance for growers of any size. Costs associated with production under 3-season high tunnels would be expected to differ from field production as well as 4-season structures due to modified management practices and tunnel purchase. The potential markets for increased yields under tunnels also must be taken into account, when investigating the profitability of a high tunnel raspberry operation.

The cold hardiness of raspberry cultivars is also an important consideration in northern climates. Red raspberry plants grown under 3-season high tunnels remain active longer in the fall if plastic is kept on late which may leave them more susceptible to cold temperatures after plastic removal. Study of how raspberry cultivars acclimate to cold temperatures under 3-season tunnels as well as understanding how removing plastic from the 3-season structure in the fall influences cold hardiness is necessary.

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CHAPTER II

THE ECONOMICS OF 3-SEASON HIGH TUNNEL RASPBERRY PRODUCTION

Abstract

The potential profitability of raspberries under multi-bay 3-season high tunnels (Haygrove Tunnels) was evaluated using yield data from studies in southwest Michigan, adjusted costs for field raspberry production, and recent prices for fresh raspberries at the Detroit Wholesale Market. Marketable yield for primocane-fruiting (PF) cultivars was 2,100 lb·acre^{-1} in year 1 and 14,900 lb·acre^{-1} thereafter, whereas yields of floricane-fruiting (FF) cultivars were 1,700 lb·acre^{-1} in year 2 and 15,400 lb·acre^{-1} in years 3 and following. Costs were estimated by modifying field production budgets and using Michigan prices. Depreciation schedules assumed 3, 7, and 15 year life for the polyethylene plastic, raspberry plants, and tunnel structures, respectively. Berry prices were $3.90 and $4.80 per lb for FF and PF harvest seasons, respectively. These analyses indicate that high tunnel raspberry production results in revenue above listed costs, particularly for PF cultivars. However, some costs were not considered, such as land cost and taxes, fruit cooling/storage and transport to market, and labor recruitment, housing, and management. Other risks/unknowns that need be considered are future raspberry prices and competitiveness of Midwest-grown berries, longevity of raspberries under tunnels, and unforeseen problems that may emerge with this new production system.

Introduction

High tunnel production of fresh red raspberries is increasing in the United States. Carey et al. (2009) conducted a survey of U.S. extension agents in 2007 in which raspberries were grown under tunnels in key fruit producing states such as Florida, Maine, Michigan, New Jersey, Oregon, Pennsylvania, Utah, and Washington. California has the largest acreage of high tunnels producing small fruit in the United States (Demchak, 2009), with high tunnel raspberry production estimated at over 4,000 acres (Carey et al., 2009).

High tunnels are made in a variety of shapes and sizes, but share several common characteristics: 1) structures are hoop houses with metal frames covered with a single layer of plastic, 2) structures are large enough for small equipment to enter from the sides, 3) structures lack a foundation, 4) structures generally do not have electricity, 5) considered nonpermanent structures, and 6) structures are generally unheated and plastic is placed on the ends and sides in early spring and late fall to passively warm the tunnel during the day. The warmer daytime temperatures increase the number of growing degree days accumulated under high tunnels (Lang, 2009). Plastic is removed from the ends of tunnels during warm periods of the year and sides are vented to provide air movement and heat dispersion.

High tunnels can be classified as either 4-season or 3-season structures. Four-season structures have a peaked frame and tunnels are constructed individually. These structures are often relatively small, ranging form 4.6 to 9.1 m wide by 18.3 to 29.3 m long (Heidenreich et al., 2008; Lamont et al., 2003). The 4-season high tunnel is strong enough to tolerate the weight of snow and will generally be covered in plastic year round.

Research conducted in Pennsylvania (Demchak, 2009), New York (Heidenreich et al., 2008), Iowa (Domoto et al., 2007), and Massachusetts (Schloemann, 2007) demonstrated that yields of raspberries or blackberries are higher under 4-season high tunnels.

Unlike 4-season high tunnels, the plastic of 3-season tunnels is removed in the winter months. Increased distances between structural supports necessitate plastic removal because the structure cannot support a heavy snow load (Lang, 2009). The 3-season tunnel has a Quonset-shaped frame and vertical legs. Three-season tunnels also form multi-bay units which can cover several acres.

Raspberry plants benefit from the protected high tunnel environment. High tunnels exclude rainfall from the raspberry canopy, reducing the incidence of water-dispersed disease on foliage and fruit. Grey mold (*Botrytis cinerea*) and penicillium rot (*Penicillium sp.*) can be minimized when leaf and fruit surfaces remain dry (Legard et al., 2000). High tunnels also alter light quality, humidity, wind, and temperature. Elevated temperatures under sealed tunnels can extend the growing season for red raspberry plants by as much as 3 to 4 weeks in the spring and the fall (Demchak, 2009).

Raspberry plantings have performed well under high tunnels (Demchak, 2009; Hanson et al., 2008) although replicated experiments comparing yields between tunnel and field environments are lacking. Yields and fruit quality of floricane-fruiting (FF) and primocane-fruiting (PF) raspberries appear to be improved when produced under high tunnels. Cultivars that are FF produce fruit from July to early August under natural conditions in the Northeast U.S. (Hanson et al., 2005; Weber et al., 2005b) on canes in their second year of growth (floricanes). Canes of FF raspberries must overwinter before they produce flower buds, resulting in no fruit production in the planting year. Cultivars that are PF begin fruiting in August or September under natural conditions in the Northeast U.S. (Hanson et al., 2005; Weber et al., 2005a; Goulart and Demchak, 1999) on canes grown during the current season. Since PF cultivars fruit on current season's growth, they will produce some fruit the same year they are planted.

High tunnels are a large investment, making it important to determine if the additional cost of the structure and associated management costs can be recovered. Economic analyses of raspberry production have been conducted under field conditions (Bolda et al., 2005; Bushway et al., 2007) and high tunnels (Heidenreich et al, 2008; Yan and Du, 2003). Bushway et al. (2007) assembled a budget which listed the expenses for each year associated with field production of a one acre bramble planting in the Northeast. Profits from one-acre commercial and pick-your-own summer raspberry plantings were estimated at $4,660 and $2,200, respectively assuming yields of 2,000 pounds per acre. In a high tunnel raspberry production system, yields are likely to be much higher. Additionally, the high tunnel structure and associated management costs will change the potential income and profit that can be generated from a one acre planting, making it necessary to consider both a field and a high tunnel situation separately.

Heidenreich et al. (2008) assembled a budget for producing PF raspberries using 4-season high tunnels with dimensions of 30 x 90 ft. (2,880 sq. ft.). The cost of each tunnel was $6,400; over $2.00 per square foot. The cost per square foot for 3-season tunnels is less expensive at under $1.00 per square foot. Additionally, 4-season tunnels require space between individual tunnels resulting in unused land. The authors predicted that a single 4-season tunnel producing red raspberries would have a positive cash flow after four years with the first year being an establishment year having no revenue. They assumed the raspberries would be sold for $3.00 per half pint. However, the analysis did not consider differing yields prior to full production or differentiate between FF and PF yields.

The economic feasibility of raspberry production with 3-season Haygrove (Ledbury, Herefordshire, UK) high tunnels and greenhouses was conducted in Ontario, Canada (Yan and Du, 2003). One acre of 3-season tunnel production had a net present value of the cash flow of around $67,300. The internal rate of return was 35% using a baseline interest rate of 5%, which the study notes is not realistic. The net present value procedure is a method to evaluate investments which takes into account the opportunity costs associated with having funds tied up in capital items as well as the time value of money (Boehlje and Eidman, 1984). Internal rate of return describes the rate of discount that equates the present value of the cash inflows with the cash outflows (Boehlje and Eidman, 1984). This analysis showed that producing red raspberries could be profitable under 3-season tunnels with the use of successful production and marketing systems. Additional information could help growers in Michigan make better informed decisions about investing in 3-season tunnels. Yan and Du, 2003 summarized capital costs instead of itemizing them and considered Canadian prices for goods. Listing individual costs would be beneficial for growers to determine what expenses are important for their operation. A single estimate of 10,000 pounds per acre was used to approximate both FF and PF raspberry yields. Studies in southwest Michigan indicate greater high tunnel yields at full production for both raspberry types (Hanson et al., 2008).

The importance of considering the economics of raspberry production under high tunnels for a fruit grower is undeniable. An additional economic analysis that uses current costs associated with production of FF and PF raspberry types, specific yield data, and wholesale raspberry prices would be helpful to growers considering 3-season high tunnel raspberry production in Michigan. Consequently, the objective of the following study was to estimate the potential profitability of 3-season high tunnel red raspberry production in Michigan.

Materials and Methods

This study considered the scenario of a one-acre high tunnel operation producing either FF or PF fresh red raspberries to be marketed wholesale. Haygrove (Ledbury, Herefordshire, UK) 3-season high tunnels covered in Luminance THB plastic were used. The costs of producing FF and PF raspberry cultivars under 3-season high tunnels in Michigan were calculated, and wholesale raspberry prices calculated from the Detroit Terminal Market. This data was used to determine the break-even yield necessary to cover costs and potential revenue from one acre of high tunnel raspberries based on yield data from a replicated high tunnel raspberry planting.

Costs

The costs of producing FF and PF red raspberry cultivars on one acre were calculated for each year in the 3-season high tunnel production system. Costs incurred during the period prior to and at the time of planting were considered establishment costs (Table 2-1). Since establishment costs would not occur again in the life of the planting, they were summed and depreciated over the planting's productive lifespan, estimated at 7 years. Prior to high tunnel construction, establishment costs for a high tunnel raspberry planting will be the same as those for a field planting. Soil sampling, herbicide applications, plowing and disking, fertilizer application, as well as ground cover planting and incorporation the following spring were taken from Bushway et al. (2007). The cost of bare root raspberry plants was $1,950 ($0.715 per plant). Rooted plants were placed 0.6 m apart in rows on 2.4 m centers. It was assumed that 5 % of the plants would need replacing the following year, costing approximately $90. The trellis and irrigation purchase and installation costs were taken from Bushway et al. (2007) and assumed to remain in place for the life of the planting.

Production costs for years 1, 2, and 3-7 for a one acre high tunnel raspberry planting are presented in tables 2-2, 2-3, and 2-4, respectively. Purchase of the one-acre high tunnel structure ($30,000) and plastic ($6,500) were one time costs and were depreciated over the lifespan of each item using the straight-line method (Boehlje and Eidman, 1984) for which the purchase cost was divided by its expected life. Labor to install the high tunnel (1600 h) was also depreciated. It was assumed that the high tunnel structure would last 15 years, the plastic 3 years, and each asset would have no value after those time periods. An interest rate of 8 % was charged on invested capital.

The remaining costs in Tables 2-2, 2-3 and 2-4 represent activities necessary to produce one acre of red raspberries under high tunnels. All were calculated using Michigan prices in 2008, except for irrigation which had previously been calculated by Bushway et al. (2007) for field raspberry production. The costs taken from Bushway et al. (2007) are for field raspberry production in the Northeast U.S. and would be similar to costs in Michigan. Costs for pruning and harvest are specific to FF and PF raspberry cultivars. Harvest laborers were paid a piece rate of $0.50 per ½ pint container. Other labor costs were fixed at $10 per hour, which included base wages and payroll expenses. Fruit were packed in half pint plastic clamshell containers that hold 6 ounces of fruit ($0.145 per container) in cardboard flats holding 12 clamshell containers ($0.50 per flat). These packaging costs are listed under fixed and material costs (Tables 2-2, 2-3, and 2-4).

Production costs were also organized (Table 2-5) as fixed and variable annual allocated costs at full production. Fixed and variable costs at full production were determined as annual costs in order to calculate break-even yields. The cost of bumblebees is $250 per year; however, FF cultivars lack flowers in year 1 and do not need bumblebees. To account for this, the cost of bumblebees was depreciated over the life of the planting for FF raspberry cultivars.

Yields

Expected yields were based on data from a trial of four FF and PF raspberry varieties under high tunnels in southwest Michigan from 2005 to 2008 (Hanson et al, 2008). Average high tunnel yields of the two best FF cultivars (Encore and Nova) and PF cultivars (Caroline and Heritage) in the second and third production years were used to approximate full production for each type. Yield data was collected in 2008 to determine the percent of the total yield for each cultivar that would be culled. Total yields for all years were then reduced by 20% to account for cull fruit, resulting in an estimate of fresh marketable yield. Fresh marketable yield at full production for FF and PF raspberries was estimated at 15,400 and 14,900 pounds per acre, respectively. Full production was reached during different years after planting for FF and PF raspberry cultivars and it became necessary to estimate fresh marketable yield for those years. The fresh marketable yield of the two best FF cultivars ('Encore' and 'Nova') was 1,700 lbs during the second year after planting and the two best PF cultivars ('Caroline' and 'Heritage') was 2,100 lbs in the fall of the planting year. Crop values were calculated for each year after planting (Table 2-6).

Berry Prices

Expected berry prices were estimated from recent fresh red raspberry prices at the Detroit Terminal Market (USDA, 2008). The price data provided by the USDA are prices received by wholesalers for sales of less than a carload or truckload of product (USDA, 2009). While red raspberries arrive at the Detroit Terminal Market year round, the periods that FF and PF raspberries grown in Michigan would be sold were estimated from high tunnel yield trials in southwest Michigan to be 22-June to 2-Aug (6 weeks) and 10-Aug to 31-Oct (12 weeks), respectively. The high and low price per flat (twelve, 6 ounce clamshell containers) of red raspberries was recorded daily for fruit termed large. An average of the high and low price per flat for fruit having a large size was calculated for each day. These daily values were averaged for each selling period from 2006-08 and the average price per pound for FF and PF raspberry cultivars was calculated to be $3.90 and $4.80, respectively. These prices were estimates of what a grower could receive at the Detroit Terminal Market and did not reflect the cost of transportation and handling of fruit on its way to the market.

Wholesale raspberry volume

The shipping volume and place of origin for fresh red raspberries shipped to wholesale markets in the United States are also available from the USDA marketnews website (USDA, 2008). Weekly volume totals for 2006-08 were summarized based on their place of origin (Figure 2-1).

Break-even analysis

Break-even analysis was conducted using the following formula:

$$\text{Break-even yield} = \frac{\text{Fixed cost}}{\text{Selling price per pound - Variable cost per pound}}$$

The break-even yield is the number of pounds of fresh marketable fruit necessary to recover costs at full production. Prices for FF and PF raspberry types were considered the selling price. Fixed costs were those listed in Table 2-5 that are independent of the level of production. Variable cost per pound was the same for each raspberry type and was calculated by dividing the labor and annual non-labor cost to harvest raspberries by yield. The annual non-labor cost to harvest raspberries includes the cost of clamshell containers and cardboard flats. Variable cost per pound was $1.83.

Results and Discussion

Total fixed costs at full production for FF raspberry cultivars were greater than those for PF cultivars (Table 2-5). Different pruning techniques made up the majority of the difference in fixed costs between the two raspberry types. Pruning of FF raspberries requires hand thinning of canes in the spring leaving 4 to 6 canes per linear foot of row as well as removing spent floricanes at the end of harvest. Cultivars of PF raspberries were assumed to be pruned once in the spring by hand removing all canes. In field plantings, PF cultivars are typically mowed to the ground annually. A high tunnel production system differs in that an extensive trellis is used to support canes which can become quite tall. The cost to prune PF cultivars reflects hand pruning with a trellis in place. The cost of removing spent floricanes for FF raspberry cultivars is the major pruning cost difference between the two raspberry types.

Total variable costs differed for FF and PF raspberry cultivars (Table 2-5). A slightly higher yield at full production for FF raspberry cultivars under high tunnels (15,400 vs. 14,900 pounds per acre) increases harvest labor and packaging costs. As a result of higher fixed and variable costs, FF raspberry cultivars at full production under high tunnels were estimated to cost close to $4,800 per acre more to produce than PF cultivars.

All costs were not considered in this study. Specific costs not included were land values, property taxes and insurance, cooling facilities, marketing and shipping costs, sanitation services (portable toilet and washing equipment), irrigation pump/well, and any new buildings required. Cost assumptions were made considering a fruit grower wanting to incorporate high tunnel raspberry production into an existing operation.

Costs of raspberry production under 3-season and 4-season high tunnels as well as in a field setting are difficult to compare directly. Costs associated with management of a 9.1 m x 27.4 m, 4-season tunnel growing PF raspberries have been reported previously (Heidenreich, 2008). The total labor and materials cost for one 4-season tunnel was $9,500 (Heidenreich, 2008). Approximately 15, 4-season tunnels cover one acre of land. Using the depreciation schedule described in the present analysis (15 year life, 8%

interest), the annual cost to cover an acre with 4-season tunnels was $15,550 compared to $3,370 to cover an acre with 3-season high tunnels.

A number of other costs vary between 3- and 4-season tunnels. Irrigation can be more efficiently constructed with long drip tapes under multi-bay, 3-season tunnels compared to 15 individual 4-season tunnels. The design of 3-season tunnels allows for increased labor efficiency. The ends of 3-season tunnels remain open during the growing season while 4-season tunnels have doors that make removing pruned canes difficult. Although plastic needs to be installed and removed annually for 3-season tunnels ($560 per acre), plastic remains on 4-season tunnels. However, Heidenreich et al. (2008) noted that 4-season plastic needs to be retightened each year($600 per acre). Both tunnel types need to be vented, but comparative labor costs are difficult to determine. Other costs such as fertilization, pesticide applications, and purchase of pollinators might be similar under 3- and 4-season tunnels.

Crop values at full production in the present analysis were greater for PF raspberry cultivars ($71,520/acre) than FF ($60,060/acre), largely due to higher berry prices during the PF harvest period (Table 2-6). Primocane-fruiting cultivars yield around 600 pounds less than FF cultivars under tunnels but the higher fall berry prices compensate for this reduced yield. An important aspect to consider with PF raspberry cultivars is that the harvest season can be nearly twice as long as that of FF cultivars. Daily or weekly fruit volumes are much lower for PF cultivars as a result. Fresh raspberry prices increase as the PF harvest season progresses and the supply of fresh raspberries is reduced.

Bushway et al. (2007) estimated that reasonable gross sales for field-grown FF raspberries in the Northeast were between $8,000 and $12,000 per acre. This is well below the calculated crop values for 3-season tunnel production of FF raspberries in the present analysis ($60,060/acre). Heidenreich et al. (2008) estimated yields of PF raspberries under a 4-season tunnel at full production would be 4,000 half pint containers, or about 22,500 lbs per acre. Using PF berry prices in the present analysis, 4-season tunnels would have a crop value per acre of $108,000 at full production. It is apparent that higher yields under 3- and 4-season tunnels result in higher crop values per acre than field production.

Break-even yields were calculated in the present analysis for FF and PF raspberries using a range of prices (Table 2-7 and Figure 2-2). Break-even yields were lower in all price scenarios for PF raspberry cultivars than for FF. This is due to a lower fixed cost incurred for PF cultivars since pruning took place once. Break-even yields are below the estimated annual fresh marketable yields for FF and PF at full production (Table 2-7) except when prices are near $2.00 per pound. When using estimated berry prices from the Detroit Terminal Market, the break-even yield was well below what has been estimated for replicated high tunnel plantings (Hanson et al., 2008). These results indicate that FF and PF cultivars would begin to generate revenue above the costs included in the study when berry prices are at $2.72 and $2.49 per pound, respectively.

Raspberry prices change in response to supply and demand. Average price per pound during harvest periods from 2006-08 were used in this study, but there was substantial fluctuation in prices between and within these years. Most fresh red raspberries produced in the United States come from California. California shipped

36,500 tons of fresh red raspberries in 2008, representing 49% of all fresh red raspberries sold in the United States (Long and Maxwell, 2009). The remaining fresh red raspberries were imported, largely from Mexico and Chile during the U.S off season (Long and Maxwell, 2009). Usual harvest dates for red raspberries in California are 10 May to 5 August (USDA, 2006). This time period overlaps the production season for FF raspberry cultivars as well as the beginning of PF cultivars in Michigan. The supply of California raspberries has a large influence on weekly prices. An increase in volume during the fall would likely depress prices during the Michigan PF picking season. Future raspberry prices cannot be predicted with certainty, but supply of fresh red raspberries during the fall is increasing, which may result in lower prices in future years during this currently profitable harvest window.

This study indicates that PF cultivars begin recovering investment costs earlier than FF cultivars. Floricane-fruiting raspberries produce no fruit in the planting year (year 1), have partial yield in year 2, and reach full production in year 3 (Table 2-6). Primocane-fruiting raspberries have a partial yield in year 1 and reach full production in year 2. No income is earned during year 1 for FF cultivars and costs will not be covered by revenue.

Other risks should be considered before investing in high tunnels. It has been observed that many disease and insect pests are reduced under tunnels, but additional problems may develop over time. Plants in this study were estimated to remain healthy and productive for seven years, but shorter or longer plant longevity would substantially alter costs and revenues. Soils may require renovation with cover cropping or other practices to prepare for replanting. This would add non-productive years to the replant cycle. Fluctuations in the two largest costs in the analysis, labor and the high tunnel structure, would change the profitability of raspberry production in this system. Steel prices affect the cost of the high tunnel structure. Adequate labor availability is essential for this labor-intensive enterprise.

The calculations for break-even yield in this analysis indicate that 3-season high tunnel red raspberry production can be profitable above the costs in this analysis, with PF cultivars being more profitable than FF. Crop value for PF cultivars was greater at full production than for FF cultivars due to higher fall berry prices. Fresh marketable yield was achieved during the planting year with PF cultivars while FF cultivars produced marketable yield in their second year. Full production was estimated to be attained in year 2 and year 3 for PF and FF cultivars, respectively. Break-even yields were lower for PF raspberry types than for FF. Using berry price averages from the Detroit Terminal Market, break-even yields for both PF and FF raspberries were below observed yields from replicated trials, indicating revenue above costs. Several costs not considered in this analysis included land values, property taxes and insurance, cooling facilities, marketing and shipping, sanitation services, irrigation pump/well, and new buildings. These costs need to be considered before investing in high tunnel raspberry production. It is possible that some of the costs left out of the analysis could alter revenues enough to noticeably increase the break-even yield. However, based on the costs and recent berry prices used in this study, high tunnels are a promising technology for producing high quality red raspberries in Michigan.

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Table 2-1. Pre-plant and establishment costs associated with planting one acre of red raspberries under 3-season high tunnels.

Activity	Labor	Fixed Costs	Material Costs	Total	Depreciation and Interest[zy]
		Year 0 (pre-plant year)			
Soil samples and tests (5 at $5.00 ea)	$5		$45	**$50**	**$9**
Herbicide application	$10	$5	$78	**$93**	**$17**
Plow field	$20	$15	$3	**$38**	**$7**
Disc field	$15	$25	$5	**$45**	**$8**
Fertilizer	$10	$5	$83	**$98**	**$18**
Lime	$10	$5	$103	**$118**	**$22**
Disc field	$20	$15	$5	**$40**	**$7**
Harrow field	$10	$10	$5	**$25**	**$5**
Plant ground cover	$10	$5	$53	**$68**	**$12**
Incorporate cover	$20	$15	$5	**$40**	**$7**
		Year 1			
Disc field	$15	$25	$5	**$45**	**$8**
Lay out and stake rows	$24		$26	**$50**	**$9**
Purchase and plant raspberries	$950		$1,950	**$2,900**	**$530**
Pruchase and install trellis	$350	$10	$1,005	**$1,365**	**$250**
Purchase and install irrigation	$120	$25	$558	**$703**	**$129**
		Year 2			
Replant (5%)	$50		$90	**$140**	**$26**
Total	$1,639	$160	$4,019	**$5,818**	**$1,064**

[z]Interest rate = 8%

[y]Depreciated 7 years over the life of the raspberry planting

Table 2-2. Year 1 costs for producing one acre of red raspberries under 3-season high tunnels.

Activity	Labor	Fixed and Material Costs	Depreciation and Interest[z]	Total
Pre-plant and establishment costs			$1,064	**$1,064**
Tunnel purchase and construction			$3,371	**$3,371**
Purchase plastic (3 year life span)			$2,427	**$2,427**
Install plastic	$280			**$280**
Bumblebees		$250		**$250 PF[y]**
Irrigate	$18	$214		**$232**
Fertilize		$465		**$465**
Hand hoe/Weed	$300			**$300**
Harvest (Piece rate $0.50 per 1/2 pt)	$2,800	$1,045		**$3,845 PF**
Remove plastic	$280			**$280**
Total (PF)	**$3,678**	**$1,974**	**$6,862**	**$12,514**
Total (FF)	**$878**	**$679**	**$6,862**	**$8,419**

[z] Labor as well as fixed and materials cost is included in this figure

[y] PF = primocane-fruiting raspberry cost

Table 2-3. Year 2 costs for producing one acre of red raspberries under 3-season high tunnels.

Activity	Labor	Fixed and Material Costs	Depreciation and Interest[z]	Total
Pre-plant and establishment costs			$1,064	**$1,064**
Tunnel purchase and construction			$3,371	**$3,371**
Purchase plastic (3 year life span)			$2,427	**$2,427**
Install plastic	$280			**$280**
Prune weak floricanes	$1,200			**$1,200 FF[y]**
Prune all floricanes	$1,000	$15		**$1,015 PF[x]**
Fertilize		$465		**$465**
Irrigate	$18	$214		**$232**
Bumblebees		$250		**$250**
Hand hoe/weed	$300			**$300**
Harvest (Piece rate $0.50 per 1/2 pt)	$2,267	$846		**$3,113 FF**
Spider mite spray	$90	$61		**$151**
Prune spent floricanes	$750			**$750 FF**
Harvest (Piece rate $0.50 per 1/2 pt)	$19,867	$7,417		**$27,284 PF**
Remove plastic	$280			**$280**
Total (PF)	**$21,835**	**$8,422**	**$6,862**	**$37,119**
Total (FF)	**$5,185**	**$1,836**	**$6,862**	**$13,883**

[z] Labor as well as fixed and materials cost is included in this figure

[y] FF = floricane-fruiting raspberry cost

[x] PF = primocane-fruiting raspberry cost

Table 2-4. Year 3-7 costs for producing one acre of red raspberries under 3-season high tunnels.

Activity	Labor	Fixed and Material Costs	Depreciation and Interest[z]	Total
Pre-plant and establishment costs			$1,064	**$1,064**
Tunnel purchase and construction			$3,371	**$3,371**
Purchase plastic (3 year life span)			$2,427	**$2,427**
Install plastic	$280			**$280**
Prune weak floricanes	$1,500			**$1,500 FF**[y]
Prune all floricanes	$1,000	$15		**$1,015 PF**[x]
Fertilize		$465		**$465**
Irrigate	$18	$214		**$232**
Bumblebees		$250		**$250**
Hand hoe/weed	$300			**$300**
Harvest (Piece rate $0.50 per 1/2 pt)	$20,534	$7,666		**$28,199 FF**
Spider mite spray	$90	$61		**$151**
Prune spent floricanes	$3,400			**$3,400 FF**
Harvest (Piece rate $0.50 per 1/2 pt)	$19,867	$7,417		**$27,284 PF**
Remove plastic	$280			**$280**
Total (PF)	**$21,835**	**$8,422**	**$6,862**	**$37,119**
Total (FF)	**$26,402**	**$8,656**	**$6,862**	**$41,919**

[z] Labor as well as fixed and materials cost is included in this figure

[y] FF = floricane-fruiting raspberry cost

[x] PF = primocane-fruiting raspberry cost

Table 2-5. Annual allocated costs for producing one acre of red raspberries under 3-season high tunnels at full production.

Activity	Labor	Annual non-labor costs	Total
FIXED COSTS			
Establishment costs depreciation and interest[z,y]	$300	$764	**$1,064**
High tunnel depreciation and interest	$171	$3,200	**$3,371**
Plastic depreciation and interest		$2,427	**$2,427**
Plastic installation	$280		**$280**
Prune weak floricanes	$1,500		**$1,500 FF**[x]
Prune all floricanes	$1,000	$15	**$1,015 PF**[w]
Fertilize		$465	**$465**
Irrigate	$18	$214	**$232 FF**
Bumblebees		$250	**$250 PF**
Bumblebees		$214	**$214 FF**
Hand hoe/weed	$300		**$300**
Spider mite spray	$90	$61	**$151**
Prune spent floricanes	$3,400		**$3,400 FF**
Plastic removal	$280		**$280**
Total fixed costs (FF)	$6,339	$7,381	**$13,719**
Total fixed costs (PF)	$2,439	$7,396	**$9,834**
VARIABLE COSTS			
Harvest (piece rate at $0.50/cont.)	$20,534	$7,666	**$28,199 FF**
Harvest (piece rate at $0.50/cont.)	$19,867	$7,417	**$27,284 PF**
TOTAL FIXED AND VARIABLE COSTS			
Total (FF)	$26,872	$15,047	**$41,919**
Total (PF)	$22,305	$14,813	**$37,118**

[z] Interest rate = 8%

[y] Annual land costs are not included in establishment costs

[x] FF = Floricane-fruiting raspberry cost

[w] PF = Primocane-fruiting raspberry cost

Table 2-6. Total allocated costs, marketable yield, and crop value from one acre of primocane-fruiting (PF) and floricane-fruiting (FF) raspberries under 3-season high tunnels (F.O.B. farm).

	Costs[z]		Marketable yield (lb/acre)[y]		Crop value[x]	
Year	**PF**	**FF**	**PF**	**FF**	**PF**	**FF**
1	$12,510	$8,420	2,100	0	**$10,080**	**$0**
2	$37,120	$13,880	14,900	1,700	**$71,520**	**$6,630**
3-7	$37,120	$41,920	14,900	15,400	**$71,520**	**$60,060**

[z] Costs not included: land purchase and taxes, water, cooling/storage facilities, marketing and transportation, labor recruitment, housing, and management.

[y] Estimated from yield trials at SWMREC minus 20% to account for cull fruit.

[x] Based on PF and FF fresh raspberry prices of $4.80 and $3.90, respectively.

Table 2-7. Break-even yield[z] (lbs) for one acre of floricane-fruiting (FF) and primocane-fruiting (PF) raspberries under 3-season high tunnel production at various wholesale prices.

	FF: $3.90[y] PF: $4.80	$2.00	$3.00	$4.00	$5.00
FF	6,630[x]	80,700	11,730	6,320	4,330
PF	3,310	57,850	8,410	4,530	3,100

[z] Costs not included: land purchase and taxes, water, cooling/storage facilities, marketing, labor recruitment, housing, and management.

[y] Prices are 3-year wholesale averages of Detroit Terminal Market prices during harvest periods for FF and PF raspberries.

[x] Break-even yields represent the necessary annual yield to break even and are calculated by the following formula: annual fixed cost / (selling cost - variable cost)

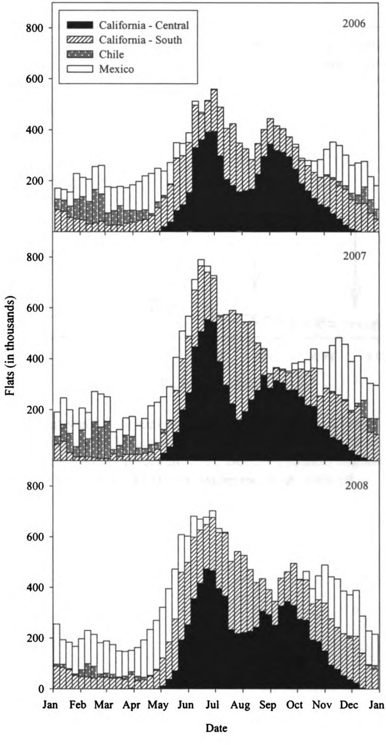

Figure 2-1. Number of flats (in thousands) of twelve, 6 oz clamshell containers of fresh red raspberries shipped in the United States from 2006-08. Stacked bars represent volumes and their place of origin (California-Central, California-South, Chile, and Mexico). Data compiled by Laura Havenga.

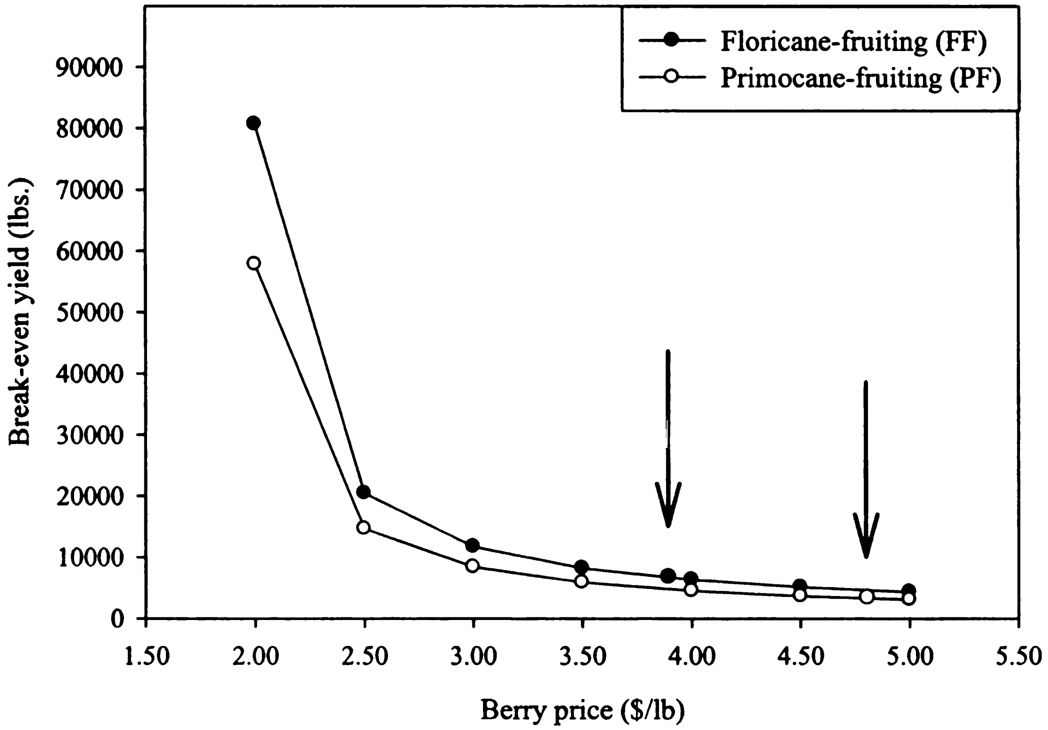

Figure 2-2. Break-even yield (lbs) for one acre of floricane-fruiting (FF) and primocane-fruiting (PF) raspberries under 3-season high tunnel production at various wholesale prices. Arrows indicate average prices from the Detroit Terminal Market during harvest periods for FF ($3.90/lb) and PF ($4.80/lb) raspberries from 2006-08.

CHAPTER 3

COLD HARDINESS AND CARBOHYDRATE COMPOSITION OF RED RASPBERRY (*Rubus idaeus* L.) GROWN UNDER 3-SEASON HIGH TUNNEL AND FIELD CONDITIONS

Abstract

High tunnels alter the microclimate around plants of red raspberry (*Rubus idaeus* L.) and influence many aspects of growth. If cold acclimation is inhibited, raspberry plants may be damaged by early fall freezes. This study investigated cold acclimation of raspberry plant tissues grown in 3-season high tunnels. Cold hardiness (LT_{50}) and nonstructural carbohydrate concentration were determined in three floricane-fruiting (FF) red raspberry cultivars during cold acclimation under high tunnel and field conditions for two growing seasons. Bud primordia and cambium tissues of 'Nova' were typically more hardy than those of 'Canby' or 'Encore'. Soluble carbohydrates (fructose, glucose, sucrose, and raffinose) in canes were positively correlated with cambium cold hardiness while starch was negatively correlated with hardiness. Raffinose concentrations were higher in canes of 'Nova' than in 'Canby' on all sampling dates for field canes. Removing high tunnel plastic after FF cultivars finished fruiting had no effect on cambium cold hardiness but slightly increased early November hardiness in bud primordia. High tunnel environments did not strongly affect the cold hardiness of bud primordia and cambium tissues during cold acclimation or affect maximum winter hardiness levels.

Introduction

Horticultural crop producers in the Midwest and Northeastern U. S. are increasingly becoming interested in high tunnel technology to produce red raspberries. High tunnels differ in size and shape but they are typically defined as unheated hoop houses covered in a single layer of plastic. Tunnels can be warmed during the day in spring and fall by covering the ends and sides with plastic. In warm parts of the year, ends and sides are removed and plastic covering the top of the structure is vented to release heat. High tunnels lack foundations and crops are often planted directly into the ground.

High tunnels are often described as 4-season or 3-season structures. A 4-season high tunnel is covered with plastic year round while a 3-season high tunnel must have its plastic removed during the winter because it cannot support heavy snow loads (Lang, 2009). A common type of 3-season tunnel forms multi-bay units and covers multiple acres while 4-season tunnels are single bay units and cover smaller areas. A 4-season tunnel costs over $2.00 per square foot to purchase (Heidenreich et al., 2008) where a 3-season high tunnel is often under $1.00 per square foot.

High tunnels modify plant microclimate by excluding rainfall and modifying temperature, light quality, wind, and humidity. The incidence of water-dispersed disease on foliage and fruit is reduced by keeping rain off the raspberry canopy. The occurrence of grey mold (*Botrytis cinerea*) and penicillium rot (*Penicillium sp.*) is minimized when leaf and fruit surfaces are not wet for extended periods (Legard et al., 2000). A major benefit of raspberry production under high tunnels is that the growing season can be extended by as much as 3 to 4 weeks in the spring and fall (Demchak, 2009; Meesters and Pitsioudis, 1999). The number of growing degree days accumulated under high tunnels is increased because of elevated daytime temperatures (Lang, 2009), particularly if tunnel ends and sides are sealed in the spring and fall. High tunnels have been increasing in use since the early 1990s (Gaskell, 2004; Carey et al., 2009) and berry production under plastic tunnels is projected to increase in the future (Carlen and Krüger, 2009).

The modified environment under high tunnels can hasten growth of raspberry plants in the spring and prolong it in the fall. Floricane-fruiting (FF) raspberries produce fruit during July and early August in Michigan (Hanson et al., 2005) on canes in their second year of growth. Primocane-fruiting (PF) red raspberry cultivars produce fruit from mid-August through the first killing frost in Michigan (Hanson et al., 2005) on canes in their first year of growth (primocanes). The prolonged physiological activity of PF raspberry cultivars in the fall under sealed 3-season high tunnels allows them to continue producing fruit longer than otherwise possible.

After the harvest of FF raspberries concludes in early August (Hanson et al., 2009), floricanes senesce and primocanes continue growth until they enter endodormancy. Plant meristems cease visible growth and buds fail to open under weather conditions favorable for growth during endodormancy. Meristems are released from endodormancy after remaining at low temperatures for an adequate amount of time which varies by species and cultivar (Couvillon, 1995). The number of hours required to release raspberry plants from endodormancy ranges from 250 and 780 hours depending on the cultivar (Dale et al., 2003; Dale, 2008).

The induction of endodormancy in red raspberry is controlled by temperature and photoperiod (Sønsteby and Heide, 2008). Continual growth occurs at 21°C under short (9 h) and long (21 h) photoperiods in 'Malling Promise' red raspberry, but growth ceases at 15.5 °C under a 9 h photoperiod (Williams, 1959). Growth at 21 °C is slowed but continuous in 'Glen Ample' into October where the photoperiod was 11 h (Sønsteby and Heide, 2008), suggesting higher temperatures negate the effect photoperiod has on inducing endodormancy.

Raspberry plants also undergo a transition concomitantly with endodormancy in which tolerance of freezing temperatures increases, a process called cold acclimation. Cold acclimation of woody plants occurs in two phases. Photoperiod initiates the first phase (Fuchigami et al., 1970; Irving and Lanphear, 1967; Van Huystee et al., 1967) while the second is induced by low, non-freezing temperatures or frost (Howell and Weiser, 1970; Gusta et al., 2005).

Cold hardiness is defined as the ability of plants to survive freezing temperatures (Fuchigami, 1996) and is commonly expressed as LT_{50}, or the temperature at which 50% of tissue is estimated to be killed (Levitt, 1980). The Spearman-Kärber method (Bittenbender and Howell, 1974) as well as logit and probit models (Lindén et al., 1996) can be used to estimate LT_{50}.

Growth cessation is a prerequisite for cold acclimation in woody plants (Fuchigami et al., 1971) and early growth cessation has been associated with greater winter survival of raspberry canes (Fejer, 1973; Van Adrichem, 1966 and 1970). Early raspberry cane "ripening" (change in color) and early natural leaf senescence were positively correlated with hardiness (Sako and Hiirsalmi, 1980). However, premature defoliation appears to reduce hardiness. Canes that were manually defoliated in early September were less hardy than naturally defoliated canes during the winter (Doughty et al., 1972). Starch and sugar content of raspberry canes were reduced when defoliated by two-spotted spider mite (Doughty et al., 1972). In eastern thornless blackberry (*Rubus sp.*), premature defoliation appeared to decrease mid-winter hardiness of stem tissue but not buds (Kraut et al., 1986).

Anecdotal evidence indicates that 4-season high tunnel use allows FF cultivars that are considered to be less hardy to overwinter in climates where they would otherwise be killed (Heidenreich et al., 2008). Research indicating if better overwintering is due to increased plant cold hardiness or protection from harsh winter conditions under the 4-season high tunnel is lacking. The carbohydrate status of overwintering plants plays an essential role in their ability to survive freezing temperatures. Overwintering tissues such as shoots, buds, and roots become sinks and must contain sufficient carbohydrates to survive winter and resume growth the following spring. Plant carbohydrate status can be influenced by various cultural practices (Howell, 1988). Reducing carbohydrate supply by manual defoliation reduced cold hardiness in raspberry (Doughty et al., 1972; Jennings and Cormack, 1969), tart cherry (Howell and Stackhouse, 1973), and grapevines (Mansfield and Howell, 1981; Stergios and Howell, 1977).

Relative levels of carbohydrates change as plants acclimate to cold. Soluble carbohydrate levels increase in acclimating raspberries (Palonen, 1999) and other woody plants (Ashworth et al., 1993; Hamman Jr. et al., 1996; Steponkus and Lanphear, 1968; Sakai and Yoshida, 1968). Red raspberry plant tissues contain the following soluble carbohydrates: sucrose, glucose, fructose, and low concentrations of raffinose and stachyose (Palonen, 1999). The soluble carbohydrate in greatest concentration during winter is the disaccharide sucrose (Palonen,1999; Kaurin et al., 1981). Sucrose is composed of the monosaccharides glucose and fructose and was noted as a more effective cryoprotectant (Crowe et al., 1990). Galactose, fructose, and glucose, form the trisaccharide raffinose which has been associated with greater cold hardiness in *Cornus sericea*, *Lonicera caerulea*, and *Forsythia* (Ashworth et al., 1993; Flinn and Ashworth, 1995; Imanishi et al., 1998). Starch concentrations in raspberry plant tissues have been shown to be negatively correlated with hardiness (Palonen, 1999).

The effect of high tunnels on cold acclimation and hardiness of PF raspberry cultivars is normally not a concern because canes are pruned to the ground each spring. However, the effect of modified tunnel environments on cold acclimation of FF raspberry canes that must overwinter has not been studied. Elevated tunnel temperatures may cause plants to grow later in the fall, possibly increasing storage carbohydrate reserves but delaying the cold acclimation process. Since FF raspberry cultivars finish fruiting by early August, high tunnel plastic is no longer needed to protect fruit after this time and could be removed. Removing plastic after harvest may promote growth cessation and cold acclimation of canes.

The objectives of this investigation were to characterize the acclimation pattern of red raspberry plants grown under a high tunnel in contrast to a field environment and understand how the timing of high tunnel plastic removal affects acclimation. Carbohydrate level changes occurring concomitantly with cold acclimation of plants grown under 3-season high tunnels was also investigated.

Materials and Methods

High tunnel and field cultivar comparisons

An eight bay range of 3-season high tunnels (Haygrove Ltd., Ledbury, UK) were erected at the Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, Michigan (latitude = 40.09 N, longitude = 86.36) in the spring of 2005. Each high tunnel bay measured 7.3 m x 61.0 m, was 3.8 m high, and was oriented north to south. Various raspberry cultivars were planted in one tunnel bay during May 2005 in a randomized complete block design with three blocks. Raspberries were planted in plots containing three plants with a between plant spacing of 1.3 m and rows on 2.4 m centers. The same RCBD layout was also duplicated in an adjacent field site with blocks oriented east to west. However, the field layout differed in that rows were on 3.0 m centers.

Trials included four cultivars ('Canby', 'Encore', 'Heritage', and 'Nova') pruned for floricane fruit production by removing floricanes after fruiting in the summer and thinning floricanes each spring to approximately 20 canes per linear meter of row. Plants were irrigated daily throughout the growing season and fertigated one or two times per week with a total of 19 L of 4N-0P-8K liquid fertilizer on a Spinks loamy fine sand soil. The rate applied is roughly equivalent to 224 kg $N \cdot ha^{-1}$. No fungicides or pesticides were used in the high tunnel or field plantings until 11 Jul. 2008 when an application of Acramite was applied to both plantings to reduce two-spotted spider mite populations.

Maximum and minimum outside air temperatures were recorded at 1.5 m height from the SWMREC/MAWN (www.agweather.geo.msu.edu/mawn/station.asp?id=swm) weather station (Campbell Scientific, Logan, Utah) less than 200 m from the high tunnel.

Temperatures inside the high tunnel were measured from 8 Aug. to 8 Oct. 2008 using 3 data loggers (HOBO U10-003; Onset Computer Co., Bourne, Mass.) at 1.5 m height. Data loggers were spaced equidistant down the center of the high tunnel with established raspberry plants and were within the raspberry canopy at this time. High tunnel temperatures from 10 Oct. to 29 Oct. 2008 were recorded using 3 data loggers in another high tunnel at 1.4 m height above the raspberry canopy. Temperatures from the data loggers as well as from the weather station were converted to growing degree days (GDD) using a base temperature of 10 °C (Baskerville and Emin, 1969).

Cold hardiness determination. During the fall and winter of 2007-08 and the fall of 2008, experiments were conducted using three FF cultivars ('Canby', 'Encore', and 'Nova') to evaluate cold hardiness in a high tunnel and field environment. Samples were collected during the first winter season on 8 Nov. 2007, 18 Jan., and 14 Mar. 2008. Sampling dates during the second season were 30 Oct., 13 and 29 Nov., 6, 26, and 29 Dec. 2008. Tissues were sampled from the tunnel and field on the same dates except in one instance when samples were collected three days earlier from the field (26 Dec. 2008) than the tunnel (29 Dec. 2008), due to time constraints. A more detailed time course sampling was conducted using 'Heritage' pruned to fruit on floricanes to observe changes in hardiness over an entire dormant season. Samples of 'Heritage' were collected on 8 and 28 Nov., and 27 Dec. 2007 as well as 18 Jan., 22 Feb., and 14 Mar. 2008.

Whole cane samples, ranging from two to five canes per plot, were collected the morning of each sampling date. It became necessary to collect more than two canes at later sampling dates in field plots in order to have adequate tissue. Samples were driven to East Lansing, MI within 2.5 h after collection. Field canes sampled on 26 Dec. 2008 were collected in the afternoon and not in the morning. Cane samples were kept cool in the bed of a pickup truck with a topper or inside a vehicle with air conditioning.

Once in East Lansing, samples were placed in a 4 °C cooler. Samples were prepared for controlled freezing in order of replication. The apical portion of each cane was removed near 0.6 cm diameter or below the lowest fruiting lateral if present. The basal portion of each cane was removed as well, totaling 13 to 20 nodes. The middle section of each cane was divided into segments with two healthy buds.

The controlled freezing procedure was similar to that done by Wolpert and Howell (1985). Eight two-node segments of each cultivar replication were spaced 5 cm apart and secured between two pieces of masking tape. Four of the segments were tunnel-grown canes of a single cultivar and four were field-grown. Each eight-segment group was then wrapped in moist cheesecloth to avoid supercooling and cause ice nucleation (McKenzie and Weiser, 1975), then wrapped in aluminum foil. A 40-guage copper-constantan thermocouple was inserted into the pith of one segment to measure temperature. The foil was then rolled into a loose bundle. For each cultivar x replicate combination, a total of five, six, or seven bundles were prepared to be frozen to five, six, or seven test temperatures. Temperatures were selected based on the time of year so that the warmest would cause no damage and the coldest would kill all test tissues. Bundles were placed in a programmable freezer, arranged by replication, allowed to equilibrate to 0 °C, and then cooled at 3 °C h^{-1}.

When bundles reached the desired temperature, they were removed and placed in a 4 °C cooler to thaw overnight. The next day, the foil, cheesecloth, and thermocouples were removed and cane segments were placed in a humid chamber at 18 °C for four to seven days to induce oxidative browning of damaged tissues. Bud primordia and cambium tissues were then evaluated visually for injury based on the level of oxidative browning (Stergios and Howell, 1973). Bud primordia were rated alive or dead, while cambium tissue was rated alive, undistinguishable, or dead. Ratings from each replication were used to calculate the temperature at which 50% of sampled tissue was estimated to be killed (LT_{50}). LT_{50}s were estimated using logit models (Lindén et al., 1996) in the PROBIT procedure (SAS Institute, Cary, N.C.).

Carbohydrate determination. Cane tissue was collected for carbohydrate analysis at all cold hardiness experiment dates, as well as on 14 Aug. 2008. Three 20 to 30 mm thick cross-sectional pieces were collected from the upper, middle, and lower portions of the central section of each cane used in the controlled freezing experiments. Cane sections were stored at -80 °C and later lyophilized and ground to pass through a 40-mesh screen.

Lyophilized, ground samples weighing 100 mg were extracted three times with 2.0 mL 80% (v/v) ethanol for 30 min followed by 5 min centrifugation at 3000 rpm. During each extraction, samples were placed in a 60 °C hot water bath. Supernatants were collected and evaporated to dryness (Savant Speedvac SC200) and stored over desiccant prior to analysis. Pellets were frozen for later starch analysis.

To determine soluble carbohydrates, samples were re-suspended by adding 2.0 mL of an oximation solution containing 10 mg·mL^{-1} hydroxylamine hydrochloride and 2.5 mg·mL^{-1} β-phenyl-D-glucopyranoside brought to volume with pyridine. Samples were capped, agitated on a shaker tray overnight to dissolve carbohydrates, placed in an 80 °C heat block for 1 h, and allowed to cool to room temperature. Oximes and sugars were converted to trimethylsilyl derivatives by adding 1.0 mL hexamethyldisilazane (HMDS) and 0.1 mL trifluoroacetic acid (TFAA). Samples were held at room temperature for 1 h, then transferred to vials and analyzed by gas-liquid chromatography (Aligent Technologies 6890N). Peaks for soluble carbohydrates were identified based on retention times and compared to sugar standards (glucose, fructose, inositol, sucrose, and raffinose). Peak areas were converted to percent of sample dry weight.

Pellets from extracted samples were used for starch determination. To each dry pellet, 2.0 mL of 0.1M pH 5.0 acetate buffer was added. Samples were placed in a 100 °C hot water bath for 1 h. Upon hot water bath removal, 100 µL of amyloglucosidase from *Aspergillus niger* (Sigma Chemical 10115) having 10 units of activity was added and samples were placed into a 55 °C hot water bath for 16 h. After centrifugation, a 50 µL aliquot transferred to a new test tube and brought to 1.0 mL volume with ddH_2O. Triplicate 250 µL aliquots of sample from the new test tubes were incubated for approximately 1 h in 2.0 mL of a reaction mixture containing 12.8 units·mL^{-1} of glucose oxidase, 2.6 units·mL^{-1} of peroxidase, and 3.1 µg·mL^{-1} of o-dianisidine dihydrochloride. The reaction was stopped by the addition of 2.0 mL of 12 N H_2SO_4. Samples were analyzed spectrophotomerically at 540 nm using a BioSpec-1601 (Shimadzu Scientific Inst., Japan).

Potted raspberry experiment

To study plastic removal date effects on cold hardiness, potted raspberry plants were removed from a tunnel on different dates in the fall to simulate the removal of high tunnel plastic Bare root cuttings of three FF red raspberry cultivars ('Canby', 'Encore', and 'Nova') were potted on 17 Apr. 2008 in East Lansing, MI. Two bare root cuttings of each cultivar were planted in # 5 Nursery Supply pots containing 14 L of a standard nursery potting substrate (80% pine bark screened to pass a one-inch grid, 20% Canadian sphagnum peat moss, 454 g of granulated sulfur per cubic yard). Potted raspberries were placed in a greenhouse for one week to accelerate growth, then transported to SWMREC. Cuttings were pruned to the lowest above ground node to encourage primocane growth.

Potted raspberries were placed under one of the high tunnels described earlier. Pots were arranged randomly in three-pot groups so that every group contained one pot of each cultivar. The groups were placed into socket pots buried in the ground 0.46 m apart in a row down the length of the high tunnel starting 12.2 m away from either end.

Each three-pot group was randomly assigned one of four high tunnel removal dates (14 Aug., 11 Sept., 9 Oct., 6 Nov. 2008 (not removed)), when pots were moved to a second row of socket pots outside the tunnel with the same pot spacing and order. Pots were removed from the tunnel to simulate removal of high tunnel plastic at different dates. Each treatment (cultivar x removal date) was represented by six pots, with two pots serving as one replication.

Raspberries were irrigated for approximately 1 $h \cdot d^{-1}$ using 3.8 $L \cdot h^{-1}$ micro emitters placed in each pot and top dressed with Harrell's Inc. Polyon® Acid Blend with Micros (19N-2.6P-9.9K) at 88 g per pot on 30 Apr. 2008. Substrate pH was monitored during the growing season but did not need adjustment.

On 6 Nov. 2008, all raspberry canes with a base diameter larger than approximately 1 cm were collected from each treatment to evaluate cold hardiness as described above, except the freezer temperature was lowered at 2 °C h^{-1}. The lower rate was selected to account for the larger number of foil bundles in the freezer ensuring a similar freezing rate in all bundles. Carbohydrate determination was conducted as described previously.

Statistical analysis. The high tunnel and field cultivar comparisons experiment was arranged in a randomized complete block design in a high tunnel and field setting, while the potted raspberry experiment was a 2-way factorial design. Statistical analyses in both experiments were performed for LT_{50} as well as individual and total soluble carbohydrates, starch, and total nonstructural carbohydrates (total soluble carbohydrates + starch) using percent dry weight values. Mean separation between cultivars was carried out at individual dates using the GLM procedure in SAS (SAS Institute, Cary, N.C.). Differences were declared significant at p=0.05 using Fisher's protected least significant difference test (LSD). Original data was assumed normally distributed and passed a Levene's test for equal variance at p=0.01. Mean separation of bud primordia LT_{50}s in the potted raspberry experiment was conducted with two outliers removed that fell outside of 1.5 times the interquartile range (Sokal and Fohlf, 1981). Pearson correlation coefficients between LT_{50} and carbohydrate percent dry weight were determined using the CORR procedure in SAS.

Results

High tunnel and field cultivar comparisons

Ambient air temperatures at SWMREC during the fall and winter of 2007-08 and 2008-09 are shown in Figure 3-1. Temperatures less than 0 °C occurred prior to the first cold hardiness sampling dates of 8 Nov. 2007 and 30 Oct. 2008. Freezing temperatures occurred on 28 Oct., 1, 2, and 7 Nov. 2007 as well as 22, 27, 29, and 30 Oct. 2008. High tunnel plastic was removed on 14 Nov. 2007 and 11 Nov. 2008, after which high tunnel raspberry plants were exposed to ambient temperatures.

Maximum and minimum temperatures from within the high tunnel in 2008 were compared to a weather station less than 200 m away (Figure 3-2). No tunnel temperatures were recorded in 2007. Maximum daily temperatures in the high tunnel averaged 7 °C higher than in the field during the last three weeks of August and 6 °C higher during all of September. Minimum temperatures were 0.3 and 0.2 °C higher for the same time periods, respectively. In 2008, sides and end walls were put on the tunnel on 17 Oct. Sealing the high tunnel dramatically changed the differences between high tunnel and weather station temperatures. Between 17 and 29 Oct. 2008, maximum daily temperatures were 14 °C higher in the enclosed tunnel while the minimum daily temperatures were only 0.4 °C higher in the tunnel.

Growing degree days calculated from temperatures with in the high tunnel and at a weather station are shown in Figure 3-3. Accumulated GDD for the high tunnel and weather station were 1546 and 1022, respectively. When the tunnel was sealed from 17 to 29 Oct. 2008, the tunnel and weather station accumulated 150 and 18 GDD, respectively.

Cold hardiness. Cultivars did not differ in bud primordia hardiness during the winter of 2007-08 (Table 3-1). During cold acclimation in 2008, cultivar differences in bud primordia hardiness were observed in late October and early November (Table 3-2). When differences were detected, 'Nova' had lower LT_{50} values (more cold hardy) than the other cultivars. In October, the same differences between cultivars existed in both the high tunnel and field environment. Bud primorida LT_{50} values observed in high tunnel-grown plants typically resembled those grown in the field. Mean LT_{50}s of bud primorda across cultivars on 30 Oct., 13 and 29 Nov. and 6 and 26 (field) or 29 (tunnel) Dec. 2008 were -14.2, -20.3, -25.3, -25.3, and -25.2 for tunnel plants and -15.1, -19.7, -25.2, -25.6, and -25.2 for field plants, respectively.

Significant differences in cambium cold hardiness between cultivars were observed in Jan. and Mar. 2008 (Table 3-1) and during cold acclimation later that year (Table 3-2). 'Nova' was significantly hardier than 'Canby' at all dates where statistical significance was declared. A general trend was that 'Nova' was more hardy than 'Canby' and 'Encore' in both bud primordia and cambium tissues at most sampling dates.

Bud primordia were consistently less hardy than cambium tissue. Mean LT_{50}s for tissue types across cultivar and growing environment (tunnel and field) on 30 Oct., 13 and 29 Nov. and 6 and 26 (field) or 29 (tunnel) Dec. 2008 were -14.7, -20.0, -25.3, -25.6, and -25.2 for bud primordia and -15.9, -28.1, -29.7, -31.2, and -30.2 for cambium, respectively. Bud primordia and cambium tissues were most similar on 30 Oct. 2008 and were less alike at later dates. Bud primordia reached maximum cold hardiness on 29 Nov. 2008 whereas cambium tissues reached maximum cold hardiness earlier on 13 Nov. 2008. The average LT_{50} values for bud primorida of high tunnel and field grown plants decreased by approximately 5 °C (increased cold hardiness) between 13 and 29 Nov. 2008 when cambium tissue average LT_{50} values decreased by less than 1 °C.

Tissues of 'Heritage' sampled approximately every month throughout the 2007-08 winter showed similar differences between bud primordia and cambium cold hardiness (Figure 3-4). By late December, bud primordia and cambium tissue reached maximum cold hardiness on approximately the same date in late December. While there were no differences in bud primordia cold hardiness on any date for 'Heritage' red raspberry, it appears that high tunnel cambium tissue was hardier than field cambium tissue in Jan., Feb. and Mar. 2008.

Carbohydrates. Soluble carbohydrates in raspberry cane tissue during late fall and winter include fructose, glucose, sucrose, and raffinose. Low levels of inositol were found in a few samples (data not shown). Fructose and glucose concentrations were very similar at all sampling dates in both 2007-08 and 2008 (Figures 3-5 and 3-6). Generally, glucose levels were higher in 'Canby' than other cultivars, with statistical significance being declared in high tunnel grown samples on 18 Jan. and 14 Mar. 2008 (Table 3-3). Glucose levels in 'Canby' were also higher during the winter season of 2008 when significance could be declared (Table 3-4).

Cultivars did not differ in fructose levels on any date (data not shown). Mean fructose levels across all cultivars on 8 Nov. 2007, 18 Jan., and 14 Mar. 2008 were 0.8%, 1.9%, and 1.6% of dry weight for tunnel plants and 0.7%, 1.9%, and 1.9% for field plants, respectively. Mean fructose levels across all cultivars on 14 Aug., 30 Oct., 13 and 29 Nov. and 6 and 26 (field) or 29 (tunnel) Dec. 2008 were 1.3%. 0.7%, 1.1%, 1.5%, 1.8%, and 2.0% of dry weight for tunnel plants and 1.1%, 1.1%, 1.1%, 1.6%, 1.8%, and 2.2% for field plants, respectively.

Sucrose was the most abundant soluble carbohydrate on all sampling dates. Sucrose, fructose, and glucose concentrations were very low on 14 Aug. 2008 (Table 3-4). Sucrose levels did not vary significantly between cultivars in the tunnel and field on any date during the fall and winter of 2007-08. Mean sucrose levels across cultivars on 8 Nov. 2007, 18 Jan. and 14 Mar. 2008 were 3.8%, 5.8%, and 5.9% of dry weight in tunnel plants and 2.9%, 5.4%, and 5.6% in field plants, respectively.

Raffinose was present at lower levels than all other soluble carbohydrates at all sampling dates (Figures 3-5 and 3-6). No raffinose was present on 14 Aug. 2008 in any cultivar but was present in small amounts at every other sampling date in all cultivars. Raffinose levels were significantly higher in 'Nova' from the field on all sample dates in 2007-08 and 2008 compared to other cultivars (Tables 3-3 and 3-4). When significant, raffinose levels of 'Nova' grown under high tunnels were higher than the other cultivars.

Starch levels were highest on the first sampling date each season (Figures 3-5 and 3-6). High tunnel starch levels were appeared greater than field levels on all sampling dates in the fall and winter of 2008, although statistical comparisons cannot be made. Cultivars did not vary significantly in starch concentration in the tunnel or field during the fall and winter of 2007-08. Mean starch levels across cultivars on 8 Nov. 2007, 18 Jan. and 14 Mar. 2008 were 2.2%, 0.8%, and 0.7% of dry weight in tunnel plants and 2.1%, 0.3%, and 0.5% in field plants, respectively.

Total soluble carbohydrates (fructose + glucose + sucrose + raffinose) did not vary between cultivars or between tunnel and field plants in the fall and winter of 2007-08 or 2008. Total soluble carbohydrate levels were highest in tunnel and field grown plants on 18 Jan. 2008 and 26 or 29 Dec. 2008. Mean total soluble carbohydrate levels across all cultivars on 8 Nov. 2007, 18 Jan. and 14 Mar. 2008 were 6.2%, 10.2%, and 9.5% of dry weight in tunnel plants and 4.7%, 9.3%, and 9.3% in field plants, respectively. Mean total soluble carbohydrate levels across all cultivars on 14 Aug., 30 Oct., 13 and 29 Nov., and 6 and 26 (field) or 29 (tunnel) Dec. 2008 were 3.2%, 5.0%, 6.5%, 9.1%, 10.0%, and 10.8% of dry weight in tunnel plants and 2.9%, 6.1%, 5.9%, 9.5%, 9.6%, and 11.6% in field plants, respectively.

Total nonstructural carbohydrates (starch + total soluble carbohydrates) showed no consistent differences between cultivars on any sampling date. Levels of total nonstructural carbohydrates were lower on 8 Nov. 2007 in high tunnel and field plants compared to the other two sampling dates that winter. In the 2008 season, total nonstructural carbohydrate levels were relatively constant across dates; however, on 14 Aug. 2008 levels were less than half of 30 Oct. 2008 levels. Mean total nonstructural carbohydrate levels across all cultivars on 8 Nov. 2007, 18 Jan. and 14 Mar. 2008 were 8.4%, 11.1%, and10.2% of dry weight in tunnel plants and 6.8%, 9.6%, 9.8% in field plants, respectively. Mean total nonstructural carbohydrate levels across all cultivars on 14 Aug., 30 Oct., 13 and 29 Nov., and 6 and 26 (field) or 29 (tunnel) Dec. 2008 were 3.5%, 11.4%, 11.4%, 11.0%, 10.9%, and11.3% of dry weight in tunnel plants and 3.7%, 9.5%, 8.7%, 10.7%, 10.3%, and 11.9% in field plants, respectively.

Carbohydrate levels at monthly intervals are shown in Figure 3-7 for 'Heritage' grown in the fall and winter of 2007-08. Soluble carbohydrate levels were lowet on 8 Nov. 2007 and highest on 27 Dec. 2007. High tunnel and field grown plants behaved similarly except that starch levels continued to increase through early November for high tunnel grown plants, whereas that same increase was not observed in the field.

LT_{50} and carbohydrate correlations. The relationship between LT_{50} values and carbohydrate concentrations in plants from the two environments (tunnel and field) were evaluated across all cultivars at all dates, across cultivars for individual dates, and across cultivars for individual dates in the tunnel and field separately (Tables 3-5, 3-6, and 3-7). When data from all cultivars, dates, and environments (tunnel and field) were pooled, strong correlations were observed both in the fall and winter of 2007-08 as well as 2008. Pearson correlation coefficients between LT_{50} and fructose, glucose, sucrose, starch, and total soluble carbohydrates across all cultivars, dates, and environments were significant at $P \leq 0.005$ in the winter season of 2007-08, 2008, and for 'Heritage' sampled monthly. Raffinose was not as strongly correlated with LT_{50} during the winter season of 2007-08 or in 'Heritage' sampled monthly. All individual soluble carbohydrates as well as total soluble carbohydrates were negatively correlated with LT_{50} values while starch was positively correlated with LT_{50} values.

Potted raspberry experiment

Cold hardiness. Ambient air temperatures at SWMREC during the fall and winter of 2008 during the time potted raspberry plants were being moved from under the high tunnel to the outside environment are shown in Figure 3-8. Ambient temperatures were relatively warm at the time of cold hardiness sampling (6 Nov. 2008), as daily maximum temperatures in the three days prior to sampling were above 20 °C. Freezing temperatures occurred prior to cold hardiness sampling on 22, 27, 29 and 30 Oct. 2008.

Cambium LT_{50} values were not significantly affected by the removal date from the high tunnel, cultivar, or removal date and cultivar interaction. Mean cambium LT_{50} values (± SE) across cultivars removed from the high tunnel on 14 Aug., 11 Sep., 9 Oct, and 6 Nov. 2008 (control) were -19.7 ±0.3, -20.6 ±0.3, -18.8 ±0.3, and -18.5 ±0.2 °C, respectively.

When analyzing bud primordia cold hardiness, two observations were removed from the data set as outliers because the LT_{50} values calculated were greater than 1.5 times the interquartile range (Sokal and Rohlf, 1981). Interestingly, all removal date x cultivar means for bud primordia LT_{50} were lower than cambium means, indicating bud primordia were more cold hardy. Statistical analysis performed after outliers were removed showed no removal date x cultivar effect or cultivar effect, but a significant removal date effect on cold hardiness. Control plants that remained under the high tunnel through November had bud primordia that were significantly more cold hardy than those removed in August or September. Bud primordia LT_{50} least square means across cultivars removed from the high tunnel on 14 Aug., 11 Sep., 9 Oct, and 6 Nov. 2008 (control) were -22.7, -23.2, -21.7, and -20.3, respectively.

Carbohydrates. Analysis of variance indicated that the main effect of cultivar was significant for all carbohydrates measured, and cultivar means across all removal dates are listed in Table 3-8. The main effect of removal date was not significant for any carbohydrate, and the interaction of cultivar and removal date was not significant for any carbohydrate measurement, with the exception of total soluble carbohydrates (P=0.0415). In non-significant situations, the interaction effect was dropped from the model and main effects were analyzed. Because the interaction effect observed for total soluble carbohydrates was not strongly significant, cultivar means were included in Table 3-8. Across all high tunnel removal dates, 'Canby' had significantly higher levels of fructose and glucose than the other two cultivars, but lower levels of sucrose, raffinose, and total soluble carbohydrates. Starch and total nonstructural carbohydrates were not significantly different between cultivars. No consistent correlations could be made between LT_{50} values and carbohydrate levels of plants sampled on 6 Nov. 2008 (Table 3-9).

Discussion

These studies suggest that growth under 3-season high tunnels, having plastic sides and ends put on in mid-October and plastic removed in early November, do not dramatically influence the acclimation process or mid-winter hardiness of raspberries. Cold hardiness of tunnel and field tissues was often similar on the same date, although the experimental design did not allow for statistical comparison of the two environments. It was expected that high tunnel plants might be more susceptible to cold injury on the first cold hardiness sampling date each year (8 Nov. 2007 and 30 Oct. 2008) since plastic had not yet been removed from the high tunnel. The data appear to show little difference in LT_{50} between tunnel and field tissues at those dates (Tables 3-1 and 3-2), suggesting that high tunnel and field plants were equally acclimated under high tunnels at that point in time.

Simulating tunnel plastic removal times by moving potted raspberry plants out of a tunnel at different dates had inconsistent effects on cold hardiness in early November. Plants removed from tunnel conditions earlier in the fall were expected to acclimate to cold earlier than plants remaining in the tunnel until November because growth cessation would likely occur earlier as a result of lower maximum temperatures outside the tunnel. Early removal from the tunnel environment did not affect cambium cold hardiness, although bud primorda of plants moved in August and September were significantly hardier than those remaining under plastic through the beginning of November. Removing tunnel plastic in August or September increased bud primordia cold hardiness by 2.4 to 2.9 °C, respectively compared to plants remaining under cover. Sampling cold hardiness at additional dates during cold acclimation would help better describe the effect early plastic removal has on raspberry cold hardiness. If no consistent differences are observed, high tunnel plastic over FF raspberry cultivars could be removed when it is convenient and labor is available instead of waiting until November.

The similarity between high tunnel and field hardiness may be explained by the interaction of maximum and minimum temperatures. Tunnels elevate maximum daily temperatures substantially, but minimum daily temperatures are similar to those in the field (Figure 3-2). This difference in maximum and minimum temperatures under tunnels was particularly evident when tunnel sides and ends were enclosed from 17 to 29 Oct. 2008. Our temperature data trends are similar to those of other high tunnel researchers (Kadir et al., 2006; Rader and Karlsson, 2006). Perhaps the low night temperatures under tunnels allow plants to cold acclimate as normal but elevated daytime temperatures allow for continued photosynthesis and growth. Little information addressing the effect of day/night temperature differences (DIF) on cold acclimation is available. Internode length and plant height of herbaceous plants is known to be affected by DIF (Myster and Moe, 1995). Perennial plants of *Cornus stolonifera* exposed to 20/15 °C (day/night) temperatures for 28 days followed by 15/5 °C temperatures were taller and more cold resistant than plants grown continuously at the cooler regime (Fuchigami et al., 1971). This information does not directly apply to a tunnel situation because treatments were not differences between day/night temperatures.

Kalcsits et al. (2009) found that hybrid poplar species grown at 18.5/3.5 °C (day/night) ceased growth later and were also less cold hardy than plants grown at 13.5/8.5, 18.5/13.5, and 23.5/8.5 °C. The authors suggest that the warm night temperature treatment (18.5/13.5 °C) accelerates growth cessation, dormancy, and cold acclimation in the four genotypes that were trialed (Kalcsits et al., 2009). Two treatments in the study had a 5 °C DIF while two had 15 °C. Again, this study was not testing the effect of differences between day/night temperatures and characterizing the effects of positive DIF on cold acclimation in woody plants would be helpful, particularly because high positive DIF is unavoidable in high tunnel situations.

The current study showed that bud primordia and cambium tissues of 'Nova' were more hardy than those of 'Canby' or 'Encore' during early cold acclimation in 2008 (Table 3-2). Mean LT_{50} for 'Nova' was often the lowest of the three cultivars, but statistical significance could not always be declared. Based on bud primordia, 'Canby' was less hardy than 'Nova' at early sampling dates but similar in hardiness at later dates (Table 3-2), suggesting that 'Canby' may acclimate more slowly than 'Nova' but eventually reach similar mid-winter hardiness levels.

Cane dieback of these cultivars following a severe winter has previously been discussed (Hanson et al., 2005); 'Nova' was less injured than 'Canby' and 'Encore'. 'Canby' has been described as a hardy cultivar with a mid-winter LT_{50} of -30 °C (Hummer, 1995), but 'Encore' and 'Nova' were not tested for comparison. While 'Nova' was bred from the cold hardy raspberries 'Southland' x 'Boyne' (Jamieson, 1989), this appears to be the first instance of the cultivar's hardiness being characterized during cold acclimation.

In addition to hardiness during cold acclimation, mid-winter hardiness of canes grown in tunnel and field environments also was of interest. Comparison of high tunnel and field LT_{50} values showed no obvious indications that one was more cold hardy than the other during mid-winter. When cultivars differed significantly from one another, 'Nova' was the hardiest cultivar. Patterns of acclimation to cold were similar to those described by Palonen (1999). Maximum cold hardiness was reached by late November in both the high tunnel and field environments in 2007, and this level of hardiness was retained through March in 2008 (Table 3-1). From January to March in 2008, the cambium of 'Heritage' raspberry appeared to be hardier than field grown tissues but statistical replication is lacking to confirm this difference. Replicated cold hardiness sampling as plants are deacclimating would be helpful to determine if tunnel and field cultivars differ in their ability to retain hardiness in the spring until threat of frost damage is gone.

Soluble carbohydrates have been shown to increase during cold acclimation in raspberry (Bennett and Weeks, 1960; Jennings and Carmichael, 1975; Palonen, 1999) and other woody plants (Levitt, 1980) while starch decreases (Jennings and Carmichael, 1975). The present study confirms the presence and increase during cold acclimation of fructose, glucose, sucrose, and small amounts of raffinose in red raspberry canes (Burly, 1961; Palonen, 1999, Palonen et al.,2000). These associations have been observed previously in *Rubus* (Kaurin et al., 1981; Palonen, 1999).

The levels of total nonstructural carbohydrates in canes during the fall and winter of 2008 remained relatively consistent under the tunnel (Figure 3-5). The canes of cultivars grown in the field reached levels of nonstructural carbohydrates seen under tunnels by late December. Total soluble carbohydrate levels were similar in tunnel and field canes in 2007-08 and 2008. This work demonstrated strong positive correlations between individual as well as total soluble carbohydrates and cold hardiness (negative correlation with LT_{50}) as noted in Tables 3-5, 3-6, and 3-7.

Sucrose was the most abundant soluble carbohydrate throughout cold acclimation and has been shown to enhance cold hardening when added to the culture medium of micropropagated raspberry (Palonen and Junttila, 1999). Interestingly, very little sucrose was present on 14 Aug. 2008 and glucose and fructose were present in higher quantities (Table 3-4). This could indicate that glucose and fructose may be present in greater quantities when plants need readily available sources of energy and are actively growing. The levels of glucose and fructose across all removal dates in the potted experiment were significantly higher in the canes of 'Canby' than those of other cultivars (Table 3-8). Carbohydrates in the potted experiment were analyzed on 6 Nov. 2008 before plants were fully acclimated. Higher levels of glucose and fructose in 'Canby' may indicate the cultivar was less acclimated than the others. The mean LT_{50} for 'Canby' bud primordia across all removal dates was higher than for the other cultivars, although not statistically significant (data not shown).

Raffinose was observed in lower concentrations than other soluble carbohydrates in this study but was often highest in the hardiest cultivar 'Nova' (Tables 3-3 and 3-4). Raffinose has been observed to accumulate in cold acclimating woody (Keller and Loescher, 1989) and herbaceous plants (Bachman et al.,1994, Pennycooke et al., 2003), and is believed to be an important storage carbohydrate and cryoprotectant (Imanishi et al., 1998).

The concentration of starch in raspberry cane tissue was lowest when plants had the greatest cold hardiness, although starch was also low on 14 Aug. 2008. This may be explained by the fact that raspberry primocanes are in an active growth phase during late

August and would not be storing photosynthate as starch at this time. Engard (1939) noted that starch was never abundant in canes during the growing season. A strong negative correlation was observed between starch and cold hardiness (positive correlation with LT_{50}) as shown in Tables 3-5, 3-6, and 3-7. The highest levels of starch observed in tunnel and field raspberry canes were observed when plastic remained on the high tunnels on 8 Nov. 2007 and 30 Oct. 2008 (Figures 3-5 and 3-6) with high tunnel canes having relatively greater starch concentrations. Figure 3-7 shows how levels of starch in high tunnel canes of 'Heritage' increase in early November while field cane starch levels decreased. This may result from a greater ability of canes under high tunnels to photosynthesize during late fall and retain photosynthate since low night temperatures limit respiration.

Conclusions

This study investigated the cold hardiness of red raspberry cambium and bud primordia under high tunnel and field conditions during cold acclimation. Bud primordia and cambium tissues of 'Nova' were typically hardier throughout cold acclimation and at maximum mid-winter hardiness than 'Canby' and 'Encore'. Bud primordia across all cultivars appeared to be less hardy than cambium tissues, particularly in December. Removing high tunnel plastic after FF cultivars finished fruiting (August, September) increased bud primordia hardiness slightly but had no effect on cambium hardiness. Minimum night temperatures appear to have more of an effect on cold acclimation than maximum day temperatures, since cold hardiness under tunnel and field environments were similar. Replicated experiments to simulate high tunnel and field conditions with the use of growth chambers at various day/night temperature regimes could test this assumption. Soluble carbohydrates were positively correlated with cold hardiness while starch was negatively correlated. Raffinose concentrations were significantly higher in 'Nova' than 'Canby' though not statistically higher than 'Encore', on all sampling dates in field canes and on several dates in tunnel canes. These studies suggest that replicated experiments testing tunnel and field conditions could elucidate any potential statistical differences between the two environments. Hardiness during deacclimation was not investigated in these studies and the susceptibility of raspberry tissues grown in tunnel and field environments to freeze/thaw cycles in early spring should be characterized in the future.

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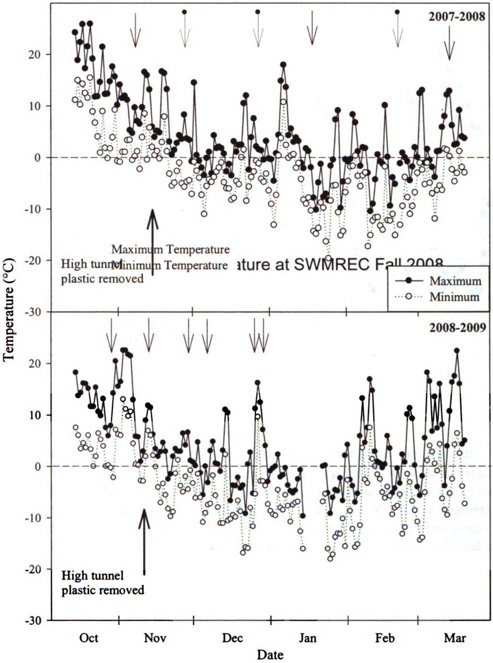

Figure 3-1. Maximum and minimum ambient air temperatures at the Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, MI from 15 Oct. to 20 Mar. in 2007-2008 (top) and 2008-2009 (bottom). Solid arrows indicate dates where multiple cultivars were sampled. Arrows with dots indicate dates where only 'Heritage' was sampled. Bold arrows indicate high tunnel plastic removal (14 Nov. 2007 and 11 Nov. 2008).

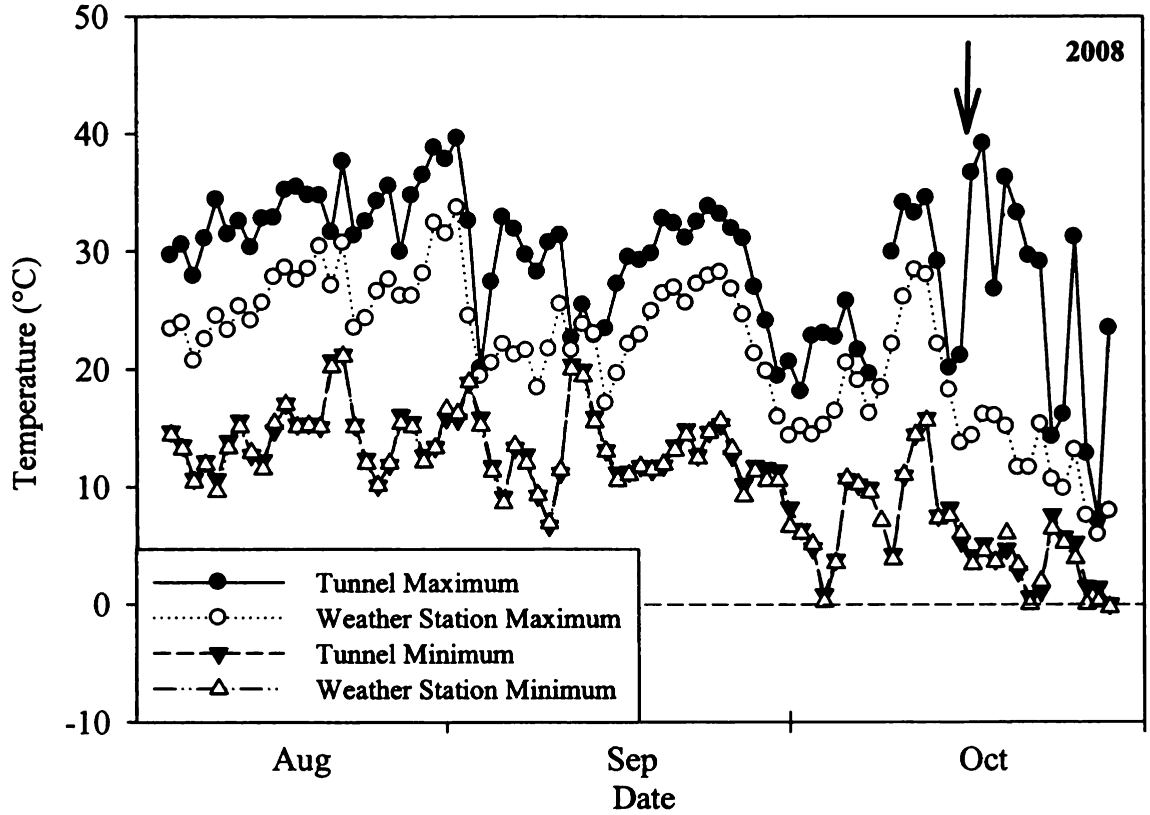

Figure 3-2. Maximum and minimum temperatures for high tunnel and ambient conditions in 2008. High tunnel temperatures from 8 Aug. to 8 Oct. are an average of 3 data loggers at 1.5 m height in the raspberry canopy. High tunnel temperatures from 10 Oct. to 29 Oct. are an average of 3 data loggers at 1.4 m height above the raspberry canopy. Ambient conditions are recorded from an automated weather station less than 200 m from the high tunnel. The arrow indicates the date tunnels were enclosed with sides and ends (17 Oct.).

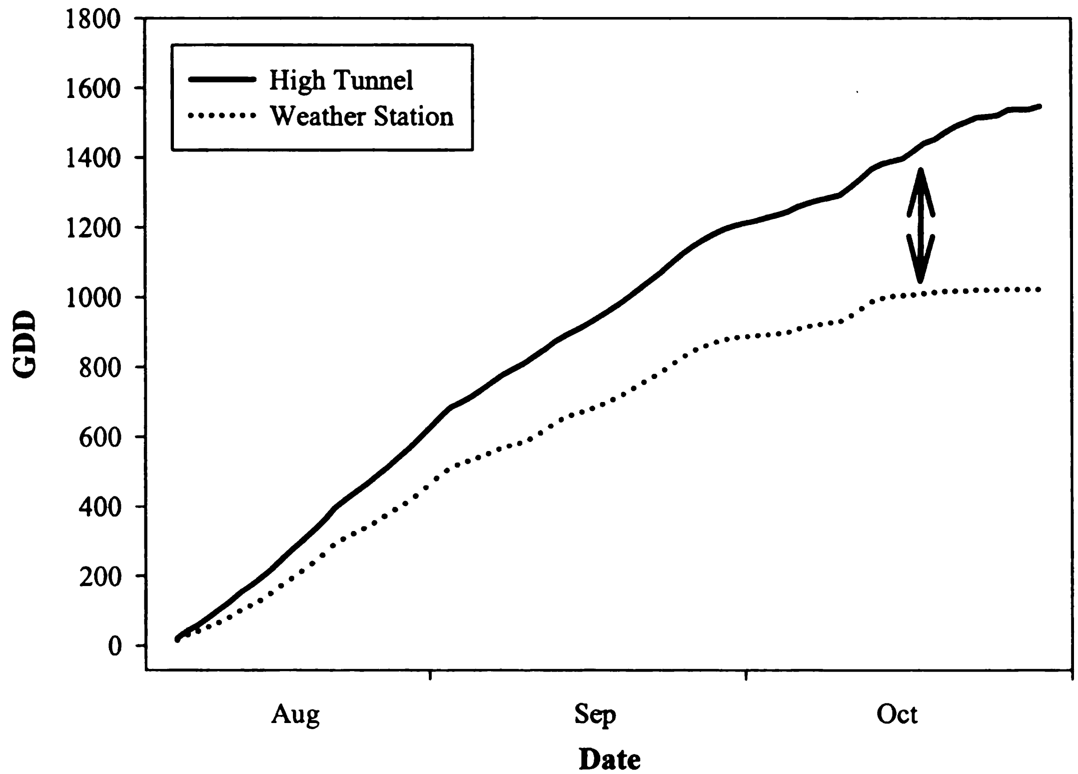

Figure 3-3. Accumulated growing degree days (GDD), with a base temperature of 10 °C, for 3-season high tunnel and ambient conditions in 2008. High tunnel GDD from 8 Aug. to 8 Oct. are an average from 3 data loggers at 1.5 m height in the raspberry canopy. High tunnel GDD from 10 Oct. to 29 Oct. are an average from 3 data loggers at 1.4 m height above the raspberry canopy. Ambient conditions are recorded from an automated weather station less than 200 m from the high tunnel. The arrow indicates the date tunnels were enclosed with sides and ends (17 Oct.).

Table 3-1. Mean LT_{50}[z] values and number (n) of observations if less than 3 for bud primordia and cambium tissues of red raspberry cultivars grown under high tunnel and field conditions in 2007-08.

	Bud primordia		Cambium	
Cultivar	Tunnel[y]	Field	Tunnel	Field
		8 Nov.		
'Canby'	-12.5 a	-13.2 a		
'Encore'	-11.7 a(2)			
'Nova'	-13.6 a(2)	-15.5 a(2)		
		18 Jan.		
'Canby'	-25.2 a	-24.6 a	-31.0 a	-31.0 a
'Encore'	-19.6 a(2)		-33.3 a	-28.6 a
'Nova'	-23.1 a	-27.1 a	-36.9 b	-34.3 a
		14 Mar.		
'Canby'	-24.9 a	-24.5 a	-34.5 a	-30.5 a
'Encore'	-22.8 a	-21.3 a	-32.8 a	-27.9 a
'Nova'	-21.9 a	-20.6 a(2)	-37.5 b	-31.7 a(2)

[z] LT_{50} is the temperature at which 50% of sampled tissue is estimated to be killed.

[y] Means within columns on the same date followed by differing letters are significantly different at P=0.05 by LSD.

Table 3-2. Mean LT_{50}[z] values for bud primordia and cambium tissues of red raspberry cultivars grown under high tunnel and field conditions in 2008.

	Bud primordia		Cambium	
Cultivar	Tunnel[y]	Field	Tunnel	Field
		30 Oct.		
'Canby'	-12.5 a	-12.6 a	-15.1 a	-12.8 a
'Encore'	-13.9 ab	-14.9 ab	-15.0 a	-17.1 b
'Nova'	-16.1 b	-17.7 b	-17.0 a	-18.4 b
		13 Nov.		
'Canby'	-19.0 a	-19.8 b	-31.0 a	-26.8 a
'Encore'	-20.4 a	-18.1 a	-31.2 a	-25.9 a
'Nova'	-21.6 a	-21.2 c	-28.8 a	-24.7 A
		29 Nov.		
'Canby'	-25.7 a	-25.5 a	-27.4 a	-25.9 a
'Encore'	-25.0 a	-24.3 a	-28.8 a	-28.7 a
'Nova'	-25.3 a	-25.9 a	-31.7 a	-35.7 a
		6 Dec.		
'Canby'	-26.3 a	-26.4 a	-29.2 a	-27.4 a
'Encore'	-24.5 a	-24.6 a	-31.5 a	-30.8 a
'Nova'	-25.2 a	-26.4 a	-35.1 a	-33.0 a
	29 Dec.	***26 Dec.***	***29 Dec.***	***26 Dec.***
'Canby'	-25.6 a	-26.6 a	-28.7 a	-25.0 a
'Encore'	-24.7 a	-23.7 a	-29.8 a	-29.0 b
'Nova'	-25.4 a	-25.0 a	-33.1 a	-35.4 c

[z] LT_{50} is the temperature at which 50% of sampled tissue is estimated to be killed.

[y] Means within columns on the same date followed by differing letters are significantly different at P=0.05 by LSD.

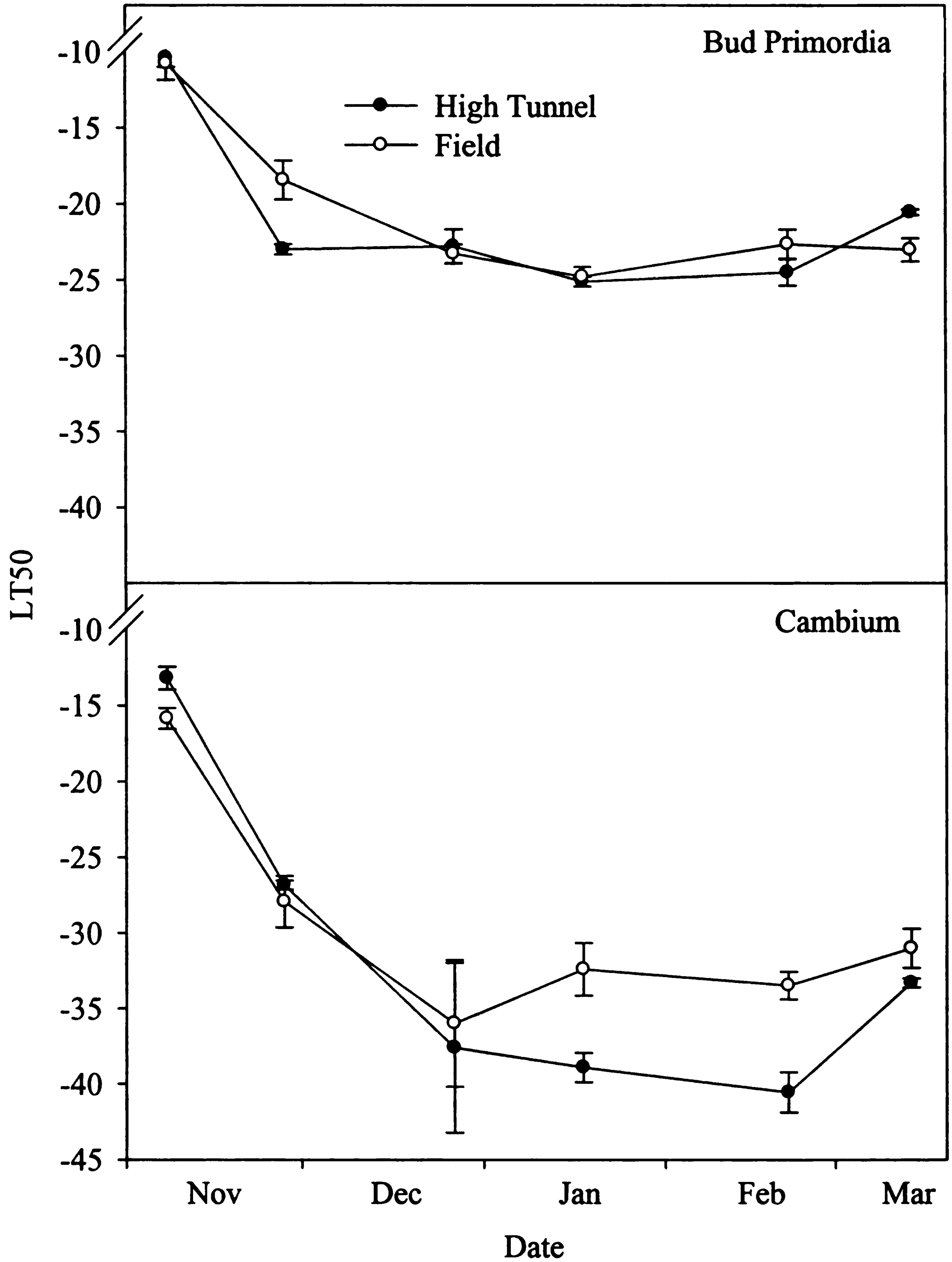

Figure 3-4. Mean LT_{50} values of bud primordia (top) and cambium (bottom) of 'Heritage' red raspberry grown under high tunnel and field conditions in 2007-08. Vertical bars indicate standard errors for individual means. Means containing two replications were: field bud primordia on 27 Dec. and tunnel cambium on 8 Nov., 28 Nov., and 27 Dec. All other means contained three replications.

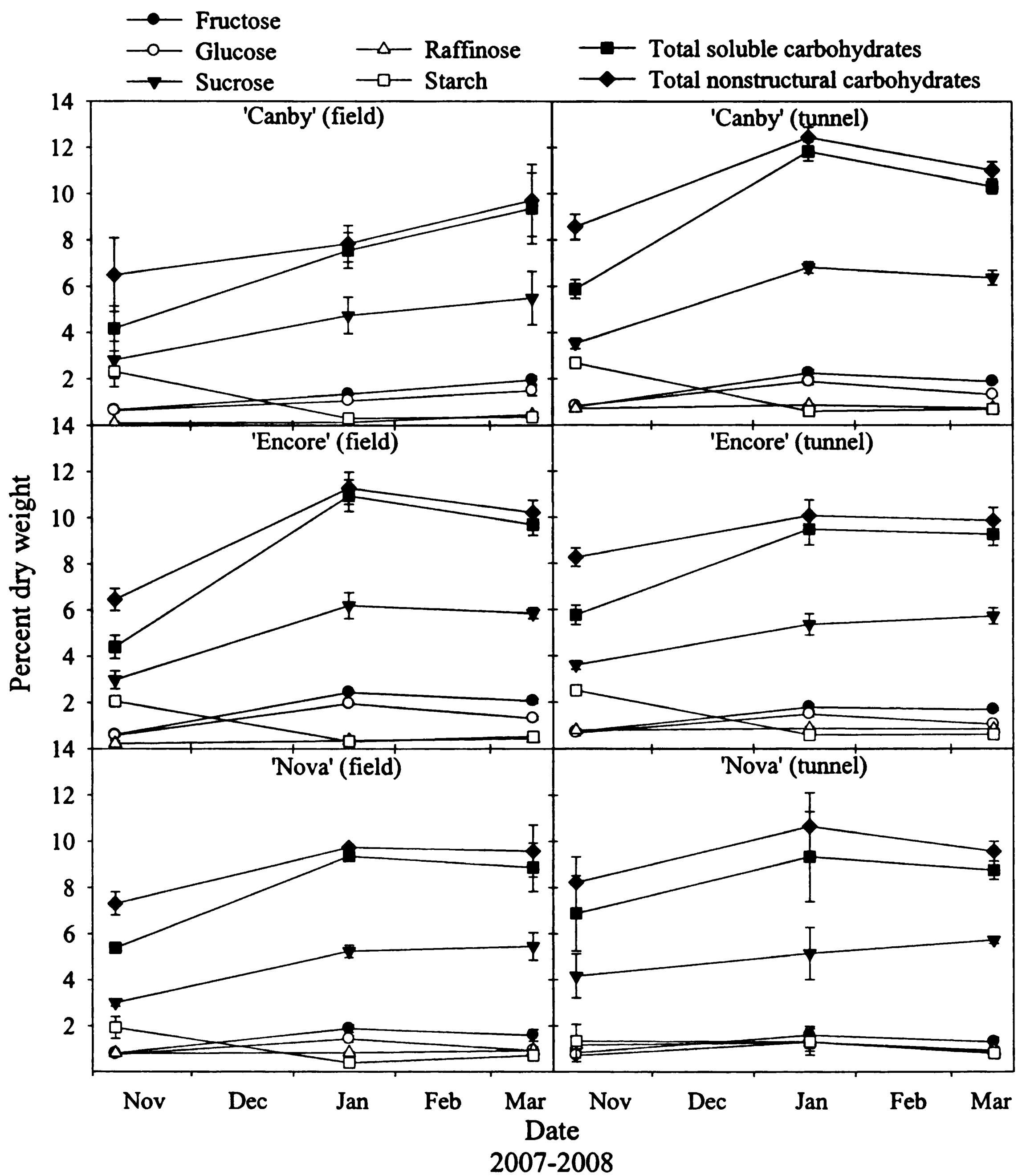

Figure 3-5. Changes in percent dry weight of soluble carbohydrates (fructose, glucose, sucrose, and raffinose), total soluble carbohydrates (sum of previous), starch, and total nonstructural carbohydrates (starch + soluble carbohydrates) in the canes of red raspberry cultivars grown under high tunnel and field conditions in 2007-08. Vertical bars indicate standard errors for individual means.

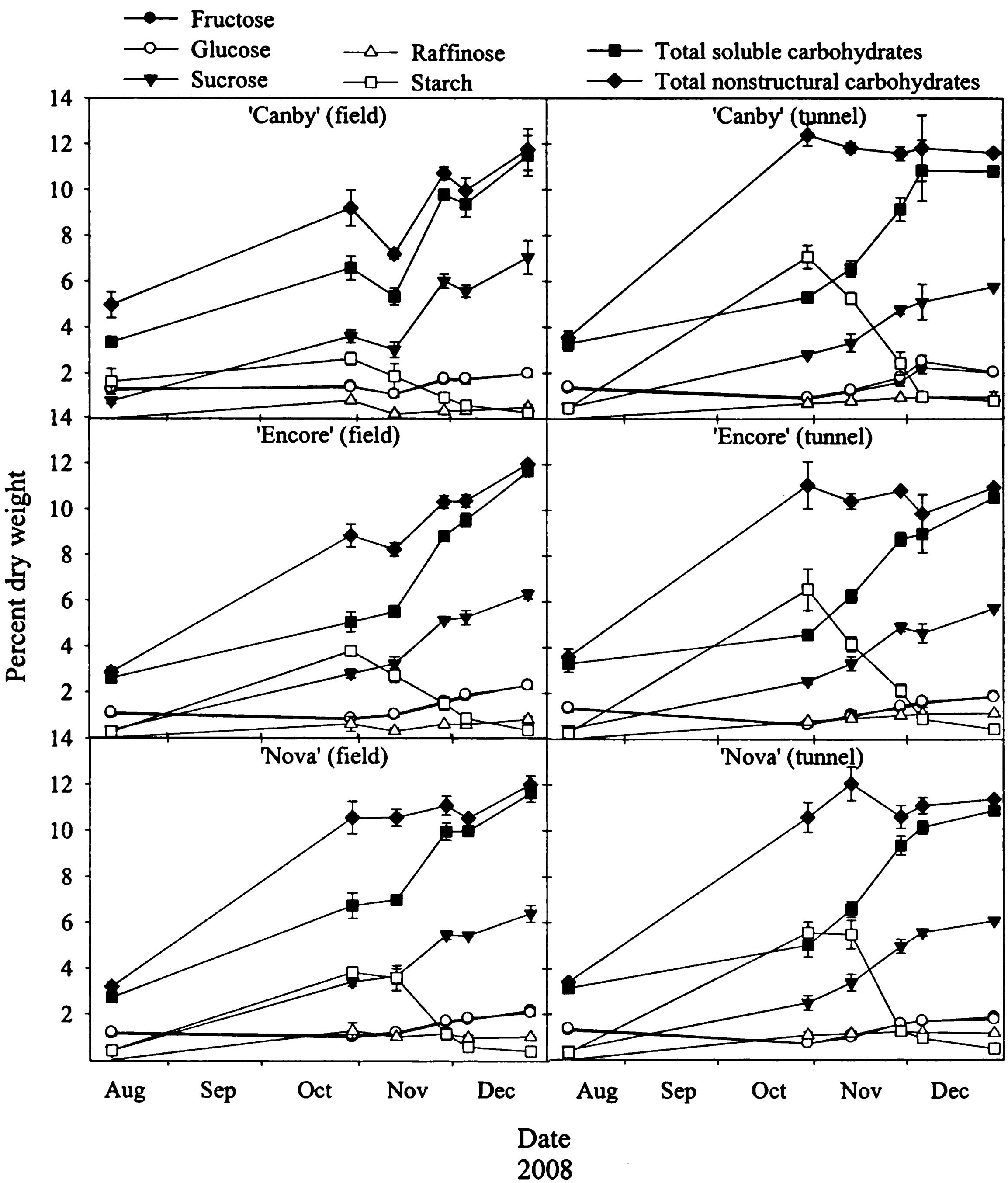

Figure 3-6. Changes in percent dry weight of soluble carbohydrates (fructose, glucose, sucrose, and raffinose), total soluble carbohydrates (sum of previous), starch, and total nonstructural carbohydrates (starch + soluble carbohydrates) in the canes of red raspberry cultivars grown under high tunnel and field conditions in 2008. Vertical bars indicate standard errors for individual means.

Table 3-3. Mean percent dry weight of carbohydrates present in cane tissues of red raspberry cultivars grown under high tunnel and field conditions in 2007-08.

	Glucose		Raffinose		Total soluble carbohdyrates		nonstructural carbohydrates	
Cultivar	Tunnel[z]	Field	Tunnel	Field	Tunnel	Field	Tunnel	Field
				8 Nov. 2007				
'Canby'	0.8 a	0.6 a	0.8 a	0.1 a	5.9 a	4.2 a	8.6 a	6.5 a
'Encore'	0.7 a	0.6 a	0.8 a	0.2 a	5.8 a	4.4 a	8.3 a	6.4 a
'Nova'	0.7 a	0.8 a	1.2 b	0.8 b	6.9 a	5.4 a	8.2 a	7.3 a
				18 Jan. 2008				
'Canby'	1.9 b	1.1 a	0.9 a	0.1 a	11.8 a	7.5 a	12.4 a	7.8 a
'Encore'	1.5 a	2.0 c	0.9 a	0.4 a	9.5 a	11.0 b	10.1 a	11.3 b
'Nova'	1.3 a	1.4 b	1.3 a	0.8 b	9.3 a	9.4 ab	10.7 a	9.7 ab
				14 Mar. 2008[y]				
'Canby'	1.3 b	1.5 a	0.8 a	0.5 a	10.3 a	9.4 a	11.0 a	9.7 a
'Encore'	1.0 ab	1.3 a	0.9 a	0.5 a	9.3 a	9.7 a	9.9 a	10.2 a
'Nova'	0.8 a	0.9 a	1.0 a	0.9 b	8.8 a	8.9 a	9.6 a	9.6 a

[z]Means within columns on the same date followed by differing letters are significantly different at P=0.05 by LSD.

[y]Percent dry weight for 'Nova' grown under field conditions on 14 Mar. 2008 is an average of two replications.

Table 3-4. Mean percent dry weight of carbohydrates present in cane tissues of red raspberry cultivars grown under high tunnel and field conditions in 2008.

	Glucose		Sucrose		Raffinose		Starch		Total soluble carbohdyrates		nonstructural carbohydrates	
Cultivar	Tunnel	Field	Tunnel	Field	Tunnel	Field	Tunnel	Field	Tunnel	Field	Tunnel	Field
							14 Aug.					
'Canby'	1.4 a	1.3 a	0.5 a	0.8 b	NA[y]	NA	0.3 a	1.6 a	3.3 a	3.3 b	3.5 a	5.0 b
'Encore'	1.4 a	1.1 a	0.4 a	0.3 a	NA	NA	0.3 a	0.2 a	3.3 a	2.6 a	3.6 a	2.9 a
'Nova'	1.4 a	1.2 a	0.4 a	0.4 a	NA	NA	0.3 a	0.5 a	3.1 a	2.7 a	3.4 a	3.2 a
							30 Oct.					
'Canby'	0.9 b	1.4 c	2.8 a	3.6 a	0.7 a	0.2 a	7.1 a	2.6 a	5.3 a	6.6 a	12.4 a	9.2 a
'Encore'	0.6 a	0.9 a	2.5 a	2.8 a	0.8 a	0.6 ab	6.5 a	3.8 b	4.6 a	5.1 a	11.1 a	8.8 a
'Nova'	0.7 ab	1.1 b	2.5 a	3.4 a	1.1 b	1.3 b	5.6 a	3.8 b	5.0 a	6.7 a	10.6 a	10.6 a
							13 Nov.					
'Canby'	1.3 a	1.1 a	3.3 a	3.0 a	0.8 a	0.2 a	5.3 a	1.9 a	6.6 a	5.3 a	11.8 a	7.2 a
'Encore'	1.0 a	1.0 a	3.3 a	3.2 ab	0.9 a	0.3 a	4.2 a	2.7 a	6.3 a	5.5 a	10.4 a	8.2 a
'Nova'	1.0 a	1.2 a	3.4 a	3.7 b	1.1 a	1.0 b	5.5 a	3.6 a	6.6 a	7.0 b	12.1 a	10.6 b
							29 Nov.					
'Canby'	1.8 a	1.8 a	4.8 a	6.0 a	0.9 a	0.3 a	2.4 a	0.9 a	9.2 a	9.8 b	11.6 a	10.7 a
'Encore'	1.4 a	1.6 a	4.9 a	5.1 a	1.0 a	0.6 b	2.1 a	1.5 a	8.7 a	8.8 a	10.9 a	10.3 a
'Nova'	1.6 a	1.7 a	5.0 a	5.5 a	1.2 b	1.1 c	1.3 a	1.1 a	9.4 a	10.0 b	10.6 a	11.1 a
							6 Dec.					
'Canby'	2.5 b	1.8 a	5.5 a	5.6 a	0.9 a	0.4 a	1.0 a	0.6 a	10.8 a	9.4 a	11.8 a	10.0 a
'Encore'	1.7 a	1.9 a	4.8 a	5.3 a	1.1 a	0.6 a	0.9 a	0.8 a	9.0 a	9.5 a	9.8 a	10.3 a
'Nova'	1.7 a	1.8 a	5.8 a	5.4 a	1.2 a	1.0 b	0.9 a	0.6 a	10.2 a	10.0 a	11.1 a	10.5 a
	29 Dec.	***26 Dec.***	***29 Dec.***	***26 Dec.***	***29 Dec.***	***26 Dec.***	***29 Dec.***	***26 Dec.***	***29 Dec.***	***26 Dec.***	***29 Dec.***	***26 Dec.***
'Canby'	2.1 a	2.0 a	5.8 a	7.0 a	1.0 a	0.5 a	0.8 a	0.3 a	10.8 a	11.5 a	11.6 c	11.8 a
'Encore'	1.9 a	2.3 a	5.7 a	6.3 a	1.2 a	0.8 ab	0.4 a	0.3 a	10.6 a	11.6 a	11.0 a	12.0 a
'Nova'	1.9 a	2.1 a	6.1 b	6.4 a	1.3 a	1.0 b	0.5 a	0.4 a	10.9 a	11.6 a	11.4 b	12.0 a

[z]Means within columns on the same date followed by differing letters are significantly different at P=0.05 by LSD.

[y]Raffinose was not present in cane tissues on 14 Aug. 2008

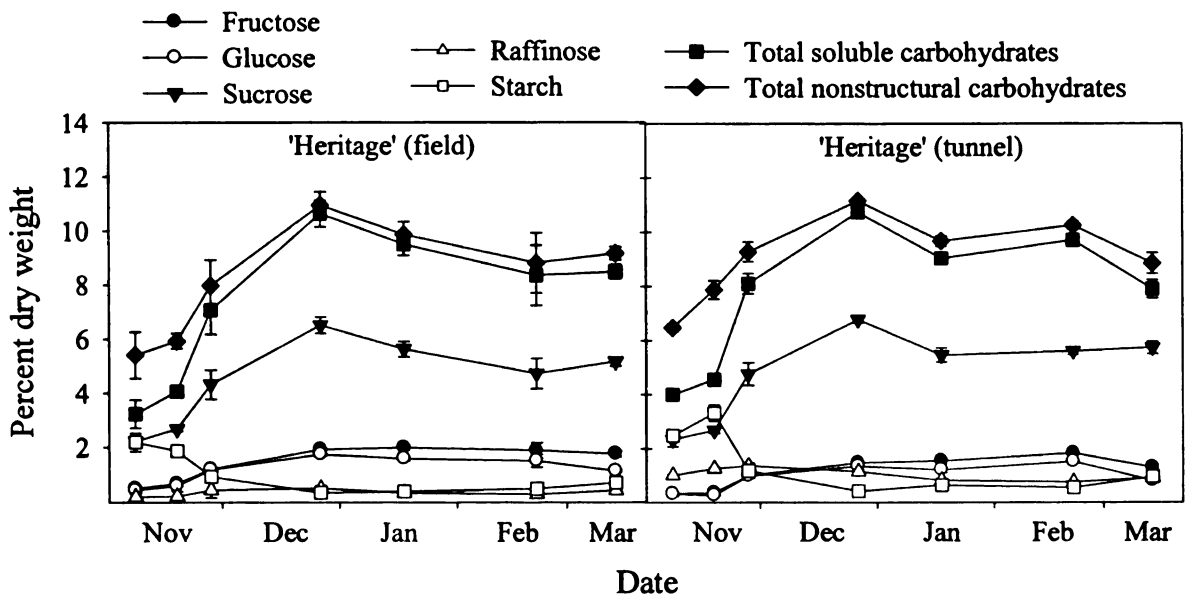

Figure 3-7. Changes in percent dry weight of soluble carbohydrates (fructose, glucose, sucrose, and raffinose), total soluble carbohydrates (sum of previous), starch, and total nonstructural carbohydrates (starch + soluble carbohydrates) in the canes of 'Heritage' red raspberry grown under high tunnel and field conditions in 2007-08. Vertical bars indicate standard errors for individual means.

Table 3-5. Pearson correlation coefficients for cambium LT_{50} values of red raspberry cultivars ('Canby', 'Encore', and 'Nova') and percent dry weight of fructose, glucose, sucrose, raffinose, starch, total soluble carbohydrates, and total nonstructural carbohydrates in cane tissue during the winter of 2007-08.

	Fructose	Glucose	Sucrose	Raffinose	Starch	Total soluble carbohydrates	Total nonstructural carbohydrates
			All cultivars, dates, high tunnel, and field				
Overall	-0.49***[z]	-0.40***	-0.66***	-0.34*	0.61***	-0.65***	-0.58***
			All cultivars, high tunnel, and field				
18 Jan. 2008	NS	NS	NS	NS	NS	NS	NS
14 Mar. 2008	0.60*	NS	NS	-0.72***	-0.62**	NS	NS
			All cultivars: High tunnel				
18 Jan. 2008	NS	NS	NS	NS	NS	NS	NS
14 Mar. 2008	NS	NS	NS	NS	NS	NS	NS
			All cultivars: Field				
18 Jan. 2008	NS	NS	NS	NS	NS	NS	NS
14 Mar. 2008	NS	NS	NS	NS	NS	NS	NS

[z]NS, *, **, and *** indicate non-significant or significant coefficients at $P \leq 0.05$, 0.01, or 0.005, respectively.

Table 3-6. Pearson correlation coefficients for cambium LT_{50} values of red raspberry cultivars ('Canby', 'Encore', and 'Nova') and percent dry weight of fructose, glucose, sucrose, raffinose, starch, total soluble carbohydrates, and total nonstructural carbohydrates in cane tissue during cold acclimation in 2008.

	Fructose	Glucose	Sucrose	Raffinose	Starch	Total soluble carbohydrates	Total nonstructural carbohydrates
			All cultivars, dates, high tunnel, and field				
Overall	-0.56***[z]	-0.53***	-0.61***	-.30***	0.61***	-0.63***	NS
			All cultivars, high tunnel, and field				
30 Oct. 2008	0.52*	NS	NS	-0.56*	NS	NS	NS
13 Nov. 2008	NS	0.56*	NS	NS	NS	NS	NS
29 Nov. 2008	NS	NS	NS	-0.62**	NS	NS	NS
6 Dec. 2008	NS	NS	NS	-.56*	NS	NS	NS
26/29 Dec. 2008[y]	NS	NS	NS	NS	NS	NS	NS
			All cultivars: High tunnel				
30 Oct. 2008	NS	NS	NS	NS	NS	NS	NS
13 Nov. 2008	NS	NS	NS	NS	NS	NS	NS
29 Nov. 2008	NS	NS	NS	NS	NS	NS	NS
6 Dec. 2008	NS	NS	NS	NS	NS	NS	NS
26/29 Dec. 2008	NS	NS	NS	NS	NS	NS	NS
			All cultivars: Field				
30 Oct. 2008	0.82**	0.74*	NS	NS	-0.75*	NS	NS
13 Nov. 2008	NS	NS	NS	NS	NS	NS	NS
29 Nov. 2008	NS	NS	NS	-0.91***	NS	NS	NS
6 Dec. 2008	NS	NS	NS	-0.67*	NS	NS	NS
26/29 Dec. 2008	NS	NS	NS	-0.77*	NS	NS	NS

[z]NS, *, **, and *** indicate non-significant or significant coefficients at $P \leq 0.05$, 0.01, or 0.005, respectively.

[y]Field and high tunnel cambium tissue were sampled on 26 and 29 Dec. 2008, respectively.

Table 3-7. Pearson correlation coefficients for cambium LT_{50} values of 'Heritage' red raspberry and percent dry weight of fructose, glucose, sucrose, raffinose, starch, total soluble carbohydrates, and total nonstructural carbohydrates in cane tissue during the winter of 2007-08.

	Fructose	Glucose	Sucrose	Raffinose	Starch	Total soluble carbohydrates	Total nonstructural carbohydrates
All dates: high tunnel and field							
Overall	-0.78***[Z]	-0.73***	-0.83***	NS	0.83***	-0.84***	-0.78***
Individual dates: high tunnel and field							
8 Nov. 2007	NS	NS	NS	NS	NS	NS	NS
28 Nov. 2007	NS	NS	0.91*	NS	NS	0.93*	0.91*
27 Dec. 2007	NS	NS	NS	NS	NS	NS	NS
18 Jan. 2008	NS	NS	NS	-0.85*	-0.83*	NS	NS
22 Feb. 2008	NS	NS	NS	-0.89*	-0.84*	NS	NS
14 Mar. 2008	NS	NS	NS	NS	NS	NS	NS
High tunnel							
8 Nov. 2007	Contained two reps						
28 Nov. 2007	Contained two reps						
27 Dec. 2007	Contained two reps						
18 Jan. 2008	NS	NS	NS	NS	NS	-1.00*	NS
22 Feb. 2008	NS	NS	NS	NS	NS	NS	NS
14 Mar. 2008	NS	NS	1.00*	NS	NS	1.00*	NS
Field							
8 Nov. 2007	NS	NS	-1.00***	NS	NS	NS	NS
28 Nov. 2007	NS	NS	NS	NS	1.00*	NS	NS
27 Dec. 2007	NS	NS	NS	NS	NS	NS	NS
18 Jan. 2008	-1.00*	-1.00*	NS	NS	NS	NS	NS
22 Feb. 2008	NS	NS	-1.00*	NS	NS	NS	NS
14 Mar. 2008	NS	NS	NS	NS	1.00*	NS	NS

[Z]NS, *, **, and *** indicate non-significant or significant coefficients at $P \leq 0.05$, 0.01, or 0.005, respectively.

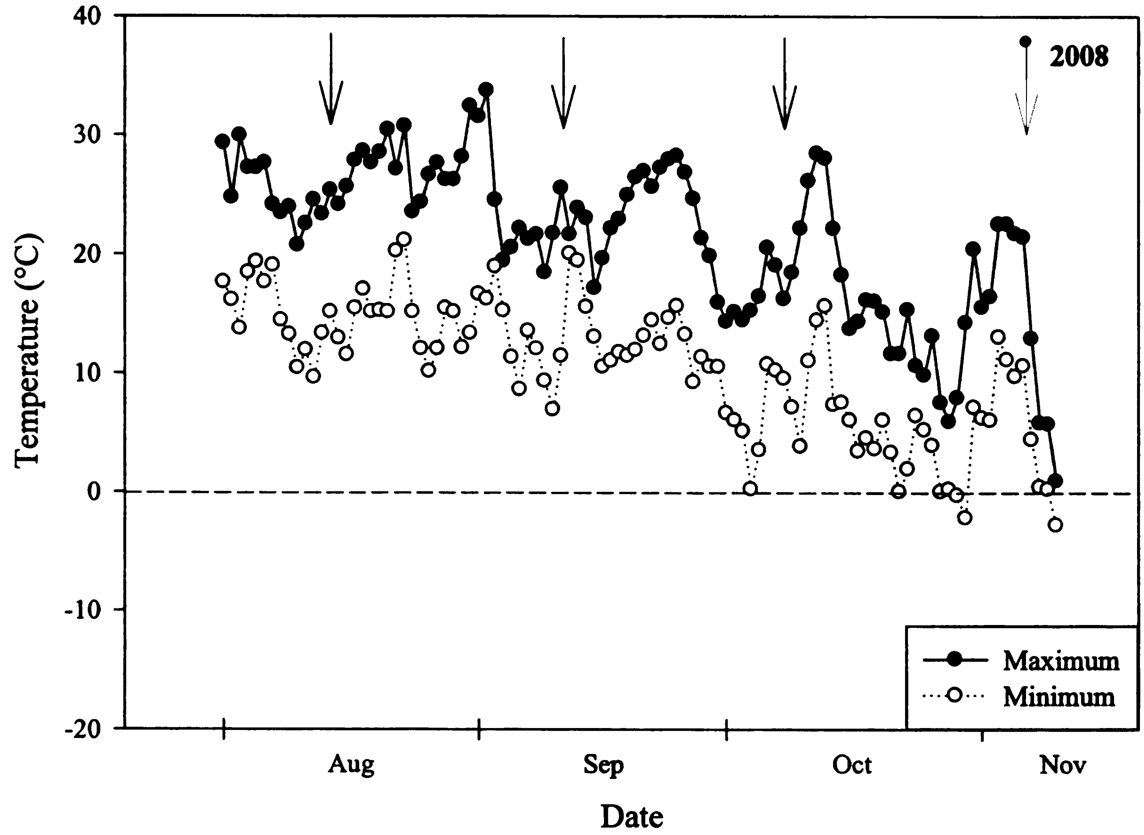

Figure 3-8. Maximum and minimum ambient air temperatures at the Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, MI from 1 Aug. to 10 Nov. in 2008. Solid arrows indicate dates potted plants were moved outside of the high tunnel. Dotted arrow indicates the date of cold hardiness sampling.

Table 3-8. Mean percent dry weight of carbohydrates present in cane tissues of potted raspberry plants grown under a high tunnel and moved outside at monthly intervals in the fall of 2008. Carbohydrates were sampled on 6 Nov. 2008 and means are across all removal dates (14 Aug., 11 Sep., 9 Oct., and 6 Nov. (not removed)).

	Fructose[z]	Glucose	Sucrose	Raffinose	Starch	Total soluble carbohdyrates[y]	Total nonstructural carbohydrates
'Canby'	1.05 c	1.13 b	2.25 a	0.51 a	4.21 a	4.19 a	9.16 a
'Encore'	0.84 a	0.81 a	2.88 b	0.64 ab	3.88 a	5.18 a	9.05 a
'Nova'	0.95 b	0.91 a	2.86 b	0.80 b	4.27 a	5.54 b	9.82 a

[z]Means within columns on the same date followed by differing leters are significantly different at *P*=0.05.

[y]Significant interaction effect (*P*=0.0415) was observed between cultivar and removal date in total soluble carbohydrates.

Table 3-9. Pearson correlation coefficients for cambium LT_{50} values of potted red raspberry plants and percent dry weight of fructose, glucose, sucrose, raffinose, starch, total soluble carbohydrates, and total nonstructural carbohydrates in cane tissue on 6 Nov. 2008.

	Fructose	Glucose	Sucrose	Raffinose	Starch	Total soluble carbohydrates	Total nonstructural carbohydrates
	All cultivars and removal dates						
Overall	NS[z]	NS	NS	NS	0.37*	NS	0.45**
	By cultivar across removal date						
'Canby'	NS	NS	NS	NS	NS	NS	NS
'Encore'	NS	NS	NS	NS	NS	NS	NS
'Nova'	NS	NS	NS	NS	NS	NS	NS
	By removal date across cultivar						
14 Aug. 2008	NS	NS	NS	NS	NS	NS	NS
11 Sep. 2008	NS	NS	NS	0.67*	NS	NS	NS
9 Oct. 2008	NS	NS	NS	NS	NS	NS	NS
6 Nov. 2008	NS	NS	NS	NS	NS	NS	NS

[z]NS, *, **, and *** indicate non-significant or significant coefficients at $P \leq 0.05$, 0.01, or 0.005, respectively.