TEMPERATURE EFFECTS ON OVARY SWELLING IN SWEET PEPPER: PHYSIOLOGY AND ANATOMY

By

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To my wife Miriam and my daughter Diana, with love

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In bell peppers (*Capsicum annuum* L.), low night temperature and high source:sink ratios during the preanthesis stage cause swollen ovaries, *i.e*., abnormally enlarged ovaries, resulting in low quality fruits. The physiological reasons for this ovary response are still unclear, but excess carbohydrate accumulation in ovaries has been implicated. In this research, it was found that low night temperature (LNT, 12°C) induced ovary swelling in three types of sweet peppers (cherry, elongated, and blocky bell), with the greatest response occurring in bell peppers. Three to four weeks of continuous LNT were required for maximum response, which coincides with the timing required for flower bud initiation in pepper. This suggests that flowers must be exposed to LNT soon after initiation in order for this response to occur. A second set of experiments showed that both LNT and fruit removal (*i.e.* increasing the source:sink ratio) increased the incidence of swollen ovaries in sweet pepper. Fruiting plants under LNT and non-fruiting plants under optimum night temperature (20°C) produced an intermediate-sized ovary, suggesting that night temperature of 20°C combined with high source:sink ratio or night temperature of 12°C combined with low source:sink ratio can partially overcome the

detrimental effects of low night temperature or high source:sink ratio on ovary swelling in pepper. Both LNT and fruit removal decreased net carbon exchange rate without affecting total plant dry weight. This suggests that excess availability of current photosynthate (via maintaining similar carbon exchange rates and reducing plant growth) is not the mechanism that results in the increase in swollen ovaries observed under both LNT and high source:sink conditions. Ovary carbohydrate analysis and anatomical analysis revealed marked differences between swollen ovaries produced under LNT vs swollen ovaries produced under high source:sink ratios. Low night temperature increased floral ovary reducing sugar and starch concentration, while high source:sink ratios had no effect on ovary carbohydrate concentrations. Ovaries developed under LNT had thicker ovary walls and greater transverse area, with only slight increases in cell size and number. In contrast, ovaries developed under high source:sink ratios increased floral ovary size mainly through increased cell size. Finally, both LNT and high source:sink ratio increased ovary size through mechanisms that appear to be different.

CHAPTER 1
INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important cultivated species worldwide. World production is led by China, Mexico, Turkey, Indonesia, Spain and the U.S. The U.S. ranks sixth and produces 3.5% of the world's sweet and pungent (chile) peppers (FAO, 2007). In 2007, harvested area in the U.S. was 21,974 ha for bell pepper and 9,996 ha for chile pepper, accounting for about 7.5% of the total harvested area of vegetable crops (USDA - Economic Research Service, 2008). After California, Florida is the second largest pepper producing state (USDA - Economic Research Service, 2008). In 2007, harvested area in Florida was 7,365 ha of bell peppers (USDA - National Agricultural Statistics Services, 2008), and ~1200 ha of hot peppers (Li *et al.*, 2006). Peppers also have a cultural importance in countries like Mexico because of their variety of uses. In addition to the traditional use of peppers in the cuisine of several cultures, peppers are also important as a source of natural pigments (paprika pepper), capsaicin (hot peppers, used in pharmaceutical industries and food preparation) (Surh and Lee, 1995; Duarte *et al.*, 2004), antioxidants (colored bell peppers) (Ishikawa, 2003; Mateos *et al.*, 2003; Sun *et al.*, 2007) and as ornamental plants (Stummel and Bosland, 2006). Bosland (1999) described pepper's importance based on three aspects: historic, general use (fresh vegetable, condiment, ornamental), and medicinal use.

Pepper fruit development depends on proper flower development, successful pollination, fertilization and fruit set, suitable fruit growth, and ripening in pigmented varieties. Therefore, favorable conditions for the proper growth and development of the sexual organs are a requirement to ensure high fruit set, yield and quality (Testa *et al.*, 2002). Adverse environmental conditions, such as extremes in temperature, light,

and/or water availability negatively affect early flower development, fruit set and fruit growth. These effects can include altered pollen and/or ovary development and flower bud and flower abortion (Rylski and Spigelman, 1982; Polowick and Sawhney, 1985; Rylski, 1985; Aloni *et al.*, 1991a; Mercado *et al.*, 1997b; Mercado *et al.*, 1997c; Aloni *et al.*, 1999).

Fruit size and uniform shape are two important characteristics that determine fruit quality. These characteristics are determined by a number of factors, including assimilate accumulation in the developing flower/fruit and flower/fruit cell number and cell size. In turn, assimilate accumulation and cell number/size are determined by several factors, including temperature and source:sink ratios.

Previous research has shown that pepper grown under cool night temperatures develop a high percentage of malformed fruits that are not marketable. Low temperatures induce flower deformation, including swollen flowers and ovaries (*i.e.*, flowers and ovaries that are larger than the ones developed under optimum temperature), resulting in fruit malformation (Mercado *et al.*, 1997c; Aloni *et al.*, 1999). Swollen ovaries also develop under high source:sink ratios in pepper (Aloni *et al.*, 1999). In both cases (*i.e.* cool night temperatures and high source:sink ratios), swollen flower buds are reported to have increased reducing sugars and starch concentration compared to normal (*i.e.* not swollen) flower buds (Aloni *et al.*, 1999). Although several studies have been done, the relation between assimilate availability and ovary swelling in pepper remains unclear. Furthermore, the anatomical basis for ovary swelling and deformation under these conditions is unknown.

The central hypothesis of this study is that both low night temperature and high source:sink ratio increase ovary size and therefore ovary swelling via effects on assimilate availability/accumulation and/or cell size/number. The objectives of this research were to determine the effects of night temperatures and source:sink ratios on: 1) ovary size in sweet pepper cultivars that differ in fruit size and shape; 2) net carbon exchange rates, growth, and ovary swelling in two cultivars of pepper; 3) ovary soluble sugar and starch concentration in bell pepper ovaries; and 4) cell number and cell size of bell pepper floral ovaries at anthesis.

CHAPTER 2
LITERATURE REVIEW

General Description of the Plant

Pepper is the common name for several species from the genus *Capsicum*. There are five botanical species that are commercially cultivated. Four of these species (*C. frutescens, C. chinense, C. baccatum*, and *C. pubescens*) play a role in local economies but are not widely cultivated. *C. annuum,* which includes the long-fruited bell varieties, is the most widely cultivated around the world, both in temperate and tropical areas (Bosland, 1996; Wien, 1997).

Bell pepper plants are herbaceous and either annual or perennial in their native habitat. Their shoot architecture is determined by their sympodial growth habit (Elitzur *et al.*, 2009). Plant height varies from 50 to 90 cm under field conditions, but under greenhouse conditions, plant height may vary from 0.7 to 2.5 m. (Vilmorin-Diaz, 1977; Nuez-Viñals *et al.*, 1996).The main stem bears 8 to 15 leaves forming a helix around the stem, with a 2/5 phyllotaxy, and terminates in a flower (Child, 1979). The two or three nodes below the terminal flower in the main stem bear sympodial shoots (Elitzur *et al.*, 2009) or first lateral branches (Child, 1979). A sympodial shoot is composed of a leaf and the internode connected to the previous node. The sympodial shoot terminates in a flower, and contains a bud at the leaf axil that develops two opposite leaves. The two opposite leaves and the terminal flower from the preceding shoot compose a sympodial unit (Figure 2-1A). As the two leaves expand, the corresponding stem internodes elongate and ‘carry up’ the leaves, which are finally located above the preceding flower (Figure 2-1B). Again, shoots end in a terminal flower, new leaves develop from the leaf axil, and internodes elongate (Figure 2-2A). After the first branching point, every new

leaf axil below the floral terminus of a sympodial shoot develops two opposite new leaves. The cycle of development repeats itself and could, in theory, continue for an indefinite period of time. (Elitzur *et al.*, 2009).However, differential growth between the two leaves developing from the same axilar bud may result in asymetric sympodial development, *i.e.*, one sympodial shoot has a smaller leaf and a shorter internode compared to the other shoot (Figure 2-2B). The suppression of one sympodial shoot that leads to the suppression of subsequent sympodial shoots may occur at any time. Such suppression is especially common in sympodia 3 and greater, and results in a great variability of final pepper plant architecture (Steer and Pearson, 1976; Child, 1979; Elitzur *et al.*, 2009). Because of the flowering pattern, leaf and flower number ratio is close to one (Heuvelink and Marcelis, 1996).

Fruit Anatomy/Morphology

The pepper fruit is a berry composed of three or four carpels (Steer and Pearson, 1976). The main parts are the peduncle, calyx, pericarp, placenta, and seeds (Figure 2-3).

The peduncle is comprised of the epidermis (rectangular-square or oblong cells), hypodermis (one to three layers of cells that are partially lignified), cortex (parenchyma cells with irregular form), vascular tissue and pith (in large fruits). The peduncle has internal and external phloem tissue, and in the outer side of the external phloem tissue, phloem fibers occur singly, in pairs, or in small groups separated by parenchyma tissue. There is a ring of xylem tissue, broken by radial xylem rays (Parry, 1969; Rylski, 1986).

The calyx is the connection between the peduncle and the pericarp and placenta itself (Rylski, 1986). The pericarp represents more than 80% of the fruit's fresh weight and is the edible part. The pericarp is comprised of the epidermis, the hypodermis, the

mesocarp (with chloroplasts), the inner mesocarp which includes fibrovascular bundles (xylem and phloem tissues), and the endocarp. Between the mesocarp and endocarp there are cells called “giant cells”, responsible for the blisters seen on the inner surface (Parry, 1969; Rylski, 1986). Pericarp thickness appears to be related to the number of cell layers in the ovary wall (Munting, 1974; Ali and Kelly, 1992).

Fruit Growth and Development

Fruit production is the final phase of a continuous physiological process that begins with successful pollination and fertilization of flowers and ends with fruit maturation and ripening. Fertilization is usually required for successful fruit set and development, otherwise the flower senesces and no fruit is formed (Gillaspy *et al.*, 1993; Testa *et al.*, 2002). In some cases, however, parthenocarpic fruits may develop due to genetic or environmental factors (e.g. extreme temperatures, low relative humidity) or by application of plant growth regulators to the flower (Fos and Nuez, 1996; Fos *et al.*, 2000; Heuvelink and Korner, 2001; Sato *et al.*, 2001; Gorguet *et al.*, 2005). In tomato, an increased level of auxins and gibberellins in the ovary can substitute for pollination and trigger fruit development, therefore those hormones are considered as the key elements in parthenocarpic fruit development (Gorguet *et al.*, 2005).

Fruit shape, fruit size, pericarp thickness, and carpel number are important characteristics used to differentiate pepper types and varieties. In sweet pepper, fruit shape, fruit size and uniformity of carpels are the main determinants of fruit quality (Aloni *et al.*, 1999). Fruit development comprises several stages, including flower and early ovary development, fruit abscission/fruit set, and fruit growth.

Flower Development

Flower initiation in bell pepper starts when the main stem reaches ~the sixth leaf (Choi and Gerber, 1992). At day/night temperatures of 28/15°C, it takes approximately 30 days from the sixth leaf to anthesis of the first flower (Cruz-Huerta, 2001). There is no specific information about the duration of cell division and cell enlargement in sweet pepper ovary. The ovary in a bell pepper flower comprises the ovary wall, placental tissue, and the ovules, which will develop into seeds after fertilization. Based on morphological observations, Munting (1974) suggested that most cell division in the ovary takes places before anthesis, similar to some other crops. In cucumber (*Cucumis sativus* L), for example, approximately 70% of cell division takes place before anthesis (Marcelis and Baan-Hofman-Eijer, 1993) and in tomato (*Lycopersicon esculentum* Mill.) fruit, cell division ends about two weeks after anthesis (Mapelli *et al.*, 1978).

Flower Abscission and Fruit Set

Flower bud and flower abscission is a serious problem in sweet pepper, especially in the large-fruited varieties. Consequences of flower abscission vary from delayed fruit production to low fruit yield depending on when the stress occurs (Wien, 1997). Factors that trigger flower abscission are high temperature (Bakker, 1989b; Turner and Wien, 1994a), low light intensity (Turner and Wien, 1994b, a), water stress (Wien, 1997), low relative humidity (Bakker, 1989a), developing fruits on the plant (Marcelis and Baan-Hofman-Eijer, 1997; Marcelis *et al.*, 2004), and biotic factors like pests and diseases (Johnstone *et al.*, 2005).

Of these factors, the most common cause of flower bud and flower abscission appears to be high temperature (> 21°C during the night or > 32°C during the day) (Rylski and Spigelman, 1982; Aloni *et al.*, 1991b), as occurs in tomato (Sato *et al.*,

2001; Gorguet *et al.*, 2005). In general, optimum temperature for fruit set in sweet pepper ranges from 22 to 26 °C during the day and from 15 to 18 °C during the night (Rylski and Spigelman, 1982; Bakker, 1989b; Bhatt and Srinivasa-Rao, 1993b; Turner and Wien, 1994a). Combinations of high temperature with other stress factors increase abscission. For instance, high air temperature combined with low soil moisture (Cochran, 1936 cited by Wien, 1997) or low light intensity (Turner and Wien, 1994b) increased flower bud and flower abscission in pepper plants compared to high temperatures alone. Low night temperatures, on the other hand, cause low pollen fertility and swollen ovaries resulting in production of small, deformed and/or parthenocarpic fruits (Rylski and Spigelman, 1982; Polowick and Sawhney, 1985; Pressman *et al.*, 1998a; Aloni *et al.*, 1999). Detailed description of the effects of low night temperature on flower and fruit development is given in the section “Abnormal Flower and Fruit Development” on page 39.

Factors related to flower bud and flower abortion in pepper can be grouped into two main categories. The first group comprises those factors, such as extreme temperatures (Pressman *et al.*, 1998a; Aloni *et al.*, 2001) and low relative humidity (Bakker, 1989a), that impair pollination. Pollination and resultant seed number are important for fruit set. Well-pollinated fruits grown under optimal conditions have between 150-300 seeds (Aloni *et al.*, 1999), although Marcelis and Baan-Hofman-Eijer (1997) found that 50-100 seeds/fruit was sufficient for maximal fruit set. Furthermore, they found that fruit set decreased as seed number fell below 50 seed per fruit. The second group comprises those factors that reduce carbohydrate availability. High temperature (Turner and Wien, 1994a), water stress (Aloni *et al.*, 1991a), low light

intensity (Turner and Wien, 1994b; Aloni *et al.*, 1996), some pests or diseases (Johnstone *et al.*, 2005), and developing fruits on the plant (Marcelis and Baan-Hofman-Eijer, 1997; Marcelis *et al.*, 2004) may reduce photosynthesis and/or sugar availability in pepper plant. Pepper cultivars that exhibit high flower abscission generally have a lower capacity to accumulate sugars and starch in their flowers compared to cultivars that exhibit low abscission (Aloni *et al.*, 1996). Marcelis *et al.* (2004) reported that when source strength was decreased in pepper by shading, high plant density, or leaf pruning, the rate of flower bud abortion increased linearly. Similar results have been reported in other species. For example, in maize (*Zea mays* L), water deficits inhibit photosynthesis, and the decrease in photosynthate flux to the developing organs appears to trigger abortion (Boyer and Westgate, 2004).

Under optimal growing conditions, the petals and ovary of pepper contain the highest amount of starch and sucrose per organ compared with the rest of flower structures, and the highest absolute and relative activity of sucrose synthase (Aloni *et al.*, 1996). Although a continuous flux of sucrose into the developing flower is required for successful fruit set (Aloni *et al.*, 1997), conditions that result in excess sugar accumulation in the flower bud may cause swollen ovaries (see “Abnormal Flower and Fruit Development” on page 41) that develop into abnormal fruits.

Fruit Growth

The fruit growth period in sweet pepper depends on the cultivar and the temperature. Fruit reached the mature stage approximately 65 days after anthesis (DAA) in ‘Mazurka’ (Marcelis and Baan-Hofman-Eijer, 1995b) and ‘Ariane’ (Cruz-Huerta, 2001), but this period was 75 days in ‘Domino’ (Tadesse *et al.*, 2002) and more than 100 days in ‘California’ (Pretel *et al.*, 1995). Temperature also affects the fruit growth

period. The growth period of pepper fruit ‘Delphin’ decreased as daily mean temperatures increased, regardless of the day/night temperature amplitude (Bakker, 1989b). In tomato, Walker and Ho (1977) found that carbon import was inhibited by fruit cooling (5 °C) and enhanced by fruit warming (35 °C) compared with controls (25 °C).

Both fresh and dry matter accumulation in pepper follow a single sigmoid curve, but the pattern differs among cultivars (Munting, 1974; Nielsen *et al.*, 1991; Marcelis and Baan-Hofman-Eijer, 1995b). In ‘Mazurka’ (Marcelis and Baan-Hofman-Eijer, 1995b), fruit fresh weight reached its maximum value between 40 and 45 DAA (stage of marketable green); but the fruit continued gaining dry weight until 60-65 DAA (stage of marketable red); ‘Domino’ (Tadesse *et al.*, 2002) required 56 days and 77 days, while ‘California’ (Pretel *et al.*, 1995) required 80 and 100 days to reach its maximum fresh and dry weight, respectively.

The absolute growth rate in ‘Mazurka’ fruit followed a bell-shaped curve (Marcelis and Baan-Hofman-Eijer, 1995b). In this cultivar, the maximum rate of fresh (>6 g d^{-1}) and dry (>0.4 g d^{-1}) weight gain occurred about the fourth week after anthesis. However, in ‘Domino’ there was no period of peak growth because fruit fresh weight and volume were predominantly linear until 8 weeks after anthesis (Tadesse *et al.*, 2002). Hall (1977) reported the maximum rate of dry weight gain (0.2 g d^{-1}) occurred approximately 40 DAA, while the maximum relative growth rate (RGR, g DW g^{-1} d^{-1}) occurred during the first days after anthesis. Based on the data reported by Marcelis and Baan-Hofman-Eijer (1995b), the maximum RGR in pepper fruit takes place in the first three days after anthesis, and Nielsen *et al.* (1991) found the maximum RGR during the first 11 days after anthesis.

Growth pre- and post-anthesis determines fruit shape and size in pepper. Fruit shape is defined primarily in the preanthesis stages of flower development, as a result of genetic and environmental control (Munting, 1974; Perin *et al.*, 2002; Weiss *et al.*, 2005). In bell pepper, as well as spherical and ovoid pepper cultivars, fruit shape is defined before pollination occurs, by the pattern and rate of cell division, most of which occurs before bloom (Munting, 1974; Aloni *et al.*, 1999; Meijer and Murray, 2001). However, in long-fruited pepper genotypes, fruit shape is defined by both cell division (pre and postanthesis) and cell elongation (postanthesis) (Munting, 1974).

The shape of the ovary at anthesis may not be related to the final fruit shape (Munting, 1974; Wien, 1997). One parameter to measure the shape is the length:diameter ratio (LDR). In bell peppers, the LDR in the ovary is about 0.9 and it increases to about 1.1 at fruit maturity. In long-fruited genotypes, however, the ratio may change from about 1.0 to 2.2 or even as high as about 9 in mature fruits (Munting, 1974; Wien, 1997). During the first two weeks after anthesis, relatively higher growth rates were observed in the base of the ovary of long and ovoid pepper fruits compared to the growth rates in the tip. This basal growth was attributed to cell division and elongation (Munting, 1974). Ultimately, final fruit shape and regularity is the result of processes that take place preanthesis (locule number) and postanthesis (uniformity of the locules in each fruit) (Rylski, 1986; Ali and Kelly, 1993; Wien, 1997; Aloni *et al.*, 1999; Perin *et al.*, 2002).

As with shape, final fruit size and weight (fresh and dry) are determined mainly by cell number at anthesis and cell elongation during anthesis and postanthesis (Munting, 1974; Coombe, 1976; Rylski, 1986; Ali and Kelly, 1993; Bertin *et al.*, 2002). Smaller

contributions to weight are due to cell division after anthesis and increases in cell solute concentration (Coombe, 1976).

Fruit cell number and cell size are influenced by several factors, including water supply (Ho *et al.*, 1987) and assimilate supply (Bertin *et al.*, 2002). Cell expansion and therefore cell size is influenced by water relations (Ho *et al.*, 1987). Mild water deficit (Ho *et al.*, 1987) or high vapor pressure deficit (vpd) conditions (Leonardi *et al.*, 2000), both of which decrease water import during cell elongation, may reduce final fruit size and fresh weight. Sugar import; however, may not be affected, resulting in smaller fruit with increased soluble solids.

Assimilate supply also influences fruit size by affecting source sink relations. Flowers and the small fruits just after anthesis represent a small sink (Archbold *et al.*, 1982; Aloni *et al.*, 1991b). In young pepper plants bearing one small fruit, one flower, and one flower bud, for example, the flower bud imports up to 3.6 mg of sucrose per day, which represents 3.2% of the exported sucrose, while the flower imports 3.7% and the fruit 45.9% of those carbohydrates. However, in fruitless plants of pepper, about 18.3% of the exported sucrose is allocated to flowers and flower buds (Aloni *et al.*, 1991b).

Developing fruits, however, rapidly become an important sink. In sweet pepper, when fruit growth rate is close to its maximum, fruit may represent up to 90% of the dry matter accumulation in the plant for a short period of time (≈ 4 days) and more than 80% for a period of 20 days (Hall, 1977). Over the entire development period, fruit represents between 30 and 65% of the dry matter accumulation (Nielsen and Veierskov, 1988; Bhatt and Srinivasa-Rao, 1993b; Cruz-Huerta *et al.*, 2005). Similar values have

been found for tomato (Ho, 1984). Photosynthate accumulation in the fruit depends on sink strength rather than on the capacity of the peduncle to transport assimilates (Zhang *et al.*, 2005). Small tomato fruits (≈ 20% of their maximum volume) imported carbon at an absolute rate nearly twice that of the larger fruits (80-90% of its maximum volume) even though the peduncle's phloem area in the smaller fruits was also smaller (Walker and Ho, 1977). Walker and Ho (1977) concluded that in tomato fruit, sink strength depends more on sink activity than on sink size, especially when the fruit is small. Presence of fruits also modifies dry matter distribution in the plant. Rapidly growing fruits decrease growth rate of all other organs (Hall, 1977).

Ali and Kelly (1992) found that defruiting the first and second nodes in pepper plants increased the number of cell tiers in the ovary wall, the final fruit weight, and pericarp thickness of the fruit in the third node, but had no effect on the fruit length or diameter. However, when the sink competition was alleviated in the flower bud stage, fruit length, diameter, weight and pericarp thickness were increased. Assuming the final cell size was similar in the fruits of all treatments, these findings suggest that decreased competition during the entire period of ovary development increased both periclinal and anticlinal cell divisions in the ovary wall; however, decreased competition in the later stages of growth increased only anticlinal cell division. It remains unknown whether in pepper, cell number is more important than cell size in defining fruit size under carbohydrate limiting and non-limiting conditions.

Position of the fruit on the plant also influences final fruit size. The first fruits, which are borne in the lower part of the plant, are usually larger, and fruit size decreases as they are formed in the upper levels (Khah and Passam, 1992; Cruz-Huerta, 2001).

However, this influence seems to be related to fruit cell number (Bertin *et al.*, 2002). Within a tomato truss, proximal fruits are bigger and already contain more cells at anthesis than distal ones (Bangerth and Ho, 1984). Bertin *et al.* (2002) found that the tomato fruit cell number was similar in fruits within the same truss under low sink competition conditions. However, when competition increased, cell number declined more in the distal than in proximal fruits.

Even though the pepper fruit is the main sink in the plant, its capacity to metabolize assimilates, and therefore to grow, seems to be affected by its previous assimilate supply (Koning and Marcelis, 1998). When source-limited fruits were changed to non-limiting source conditions, it took 2 to 3 weeks before fruit growth rate was as fast as that from fruits growing continuously under non-limiting conditions.

Carbon Economy in the Fruit

The assimilate supply to fruit is determined primarily by carbohydrate allocation, as carbon is one of the most important components of the fruit's dry matter. Fruit carbon accumulation is the balance between carbon loss (via fruit respiration) and carbon gain (via fruit photosynthesis and imported carbon).

Respiratory Losses

Respiration rates per unit weight are higher in young fruits of sweet pepper compared with older fruits (Pretel *et al.*, 1995; Tadesse *et al.*, 2002), especially during the first three weeks of growth. This is similar to other fruits such as tomato (Xu *et al.*, 1997), cucumber (Marcelis and Baan-Hofman-Eijer, 1995c) and blueberry (Birkhold *et al.*, 1992). In some fruits, there may be a second peak in respiration when maximum fresh weight or maturation approaches. For example, in 'Domino' bell pepper (Tadesse *et al.*, 2002), CO_2 production increased when fruit reached the maximum fresh weight;

however, in ‘California’ bell pepper (Pretel *et al.*, 1995) CO_2 liberation remained low until fruit was red. As an indirect way to measure respiration, Villavicencio *et al.* (2001) measured CO_2 concentration inside the ‘Camelot’ pepper fruit and found that the concentration increased from 33 to 66 mg L^{-1} from the mature green stage to the red stage, and then decreased.

Respiration losses in the fruits may represent an important proportion of the total carbon requirement. For instance, in cucumber, respiration losses accounted for 13 to 15% of the total cumulative carbon requirement, irrespective of temperature and number of fruits competing for assimilates (Marcelis and Baan-Hofman-Eijer, 1995c), and in tomato, such losses are 25% (Tanaka *et al.*, 1974a). In blueberry, respiration losses were 37% of the total carbon requirement (Birkhold *et al.*, 1992). There is no specific information for pepper, but the respiratory costs might be lower than they are in tomato and cucumber because of carbon fixation in pepper fruit (Steer and Pearson, 1976) and the possibility of CO_2 refixation (Bower *et al.*, 2000).

Carbon Gain

Fruit photosynthesis

Fruit carbon fixation or refixation has been reported for several species, particularly in the early stages of fruit development. In general, carbon fixation in fruit is 10 to 100 times lower than that of the respective leaves (Blanke and Lenz, 1989; Bower *et al.*, 2000) and comprises only a small percentage of the total carbon requirement for fruit development. Steer and Pearson (1976) calculated that pepper fruits were able to fix up to 13% of the required carbon. Tomato fruit photosynthesis contributions ranged from 10 to 15% (Tanaka *et al.*, 1974a; Hetherington *et al.*, 1998), in cucumber the range was from 1 to 5% (Marcelis and Baan-Hofman-Eijer, 1995a), and in blueberry about

15% (Birkhold *et al.*, 1992). Blueberry fruit photosynthesis supplied 50% of the carbon required during the first 10 days after flowering and 85% during the 5 days after petal fall (Birkhold *et al.*, 1992). Some small fruits even exhibit net CO_2 fixation under light conditions. Xu *et al.* (1997) found that small tomato fruits were net photosynthesizers under photosynthetic photon flux (PPF) greater than 300 µmol m^{-2} s^{-1}. Birkhold *et al.* (1992) found that blueberry fruits exhibited net photosynthesis from petal fall through fruit color break. In *Cinnamomum camphora*, CO_2 refixation accounted for 22.9% of the carbon balance of the fruit (Ogawa and Takano, 1997). In addition, results of Smillie *et al.* (1999) suggested that the photosynthetic activity found in the calyx, green shoulder, pericarp and locular parenchyma of tomato fruits plays a significant role in providing carbon assimilates to the fruit.

Fruit photosynthesis differs from leaf photosynthesis in several aspects. Pepper fruit enzymatic activity of ribulose bisphosphate carboxylase - oxygenase (Rubisco) on a fresh weight basis is only one fifteenth of the activity found in leaves (Steer and Pearson, 1976). Chloroplasts in the outer fruit layers are typically sun chloroplasts but evolve to shade chloroplasts in the inner tissues as light penetration decreases (Blanke and Lenz, 1989). Stomata in the fruit, if present, are 10 to 100 times fewer in number than in the respective leaves (Blanke and Lenz, 1989). In pepper fruits no stomata were found (Blanke and Holthe, 1997).

Blanke and Lenz (1989) and Blanke (1998) proposed that fruit photosynthesis is mainly intended to refix CO_2 from mitochondrial respiration of predominantly imported carbon. Gas exchange through the fruit epidermis in fruits like apple and pepper is low, which favors higher CO_2 concentrations in the center of the fruit, since seeds are an

important source of respired CO_2 (Blanke and Lenz, 1989; Blanke and Holthe, 1997; Villavicencio *et al.*, 2001). Part of this CO_2 may be refixed by phosphoenolpyruvate carboxylase (PEPC) and possibly phosphoenolpyruvate carboxykinase (PEPCk) by β-carboxylation and accumulation of malic acid in the vacuole (Blanke and Lenz, 1989); however, no diurnal variation in the pH has been measured. Pepper fruits remain green during most of the growing period. In addition, the large cavity and relatively thin pericarp may favor a higher light intensity throughout the tissue, increasing photosynthetic activity and possibly the capacity to refix more carbon than other fruits.

Imported carbon

Carbon imported by the fruit comes from either current photosynthesis or previously stored carbohydrates. There is no specific research in pepper addressing translocation of previously stored carbon to the fruit. In non-fruiting plants of pepper, the stem becomes the main sink, but there is no evidence that it stores soluble sugars or starch that are later used by the fruit (Hall and Milthorpe, 1978). In tomato, the stem is also a major sink for assimilates and it functions as a storage organ. However, there is little evidence to suggest that these assimilates are used in fruit production, except when the plant is in the final stages of its growth cycle, or if premature leaf loss occurs (Hocking and Steer, 1994).

By far, the most important source of imported carbon to developing fruit comes from current photosynthesis. Although leaves supply the majority of photosynthate for fruit development (Steer and Pearson, 1976), green stems and petioles may also fix CO_2. Xu *et al.* (Xu *et al.*, 1997) found that net photosynthesis in petioles of young tomato leaves (5^{th} and 10^{th} node from the apex) was positive when PPF was higher than 100 µmol m^{-2} s^{-1}, but in petioles from older leaves (15 and 18^{th} nodes) net CO_2

exchange was close to 0 even at higher PPF. In contrast, there was no net photosynthesis in the stem, but the higher the irradiance, the lower the rates of net CO_2 efflux regardless of stem age, indicating some carbon fixation was occurring.

Leaf and plant photosynthesis are affected by a variety of factors including leaf area, leaf age, presence of fruits, light intensity, and temperature.

a) *Leaf area*. Pepper plants can compensate photosynthetically for certain amounts of defoliation. Defoliation of up to 50% in young pepper plants increased net photosynthesis in the remaining leaves such that final fruit fresh and dry matter were not affected (Bhatt and Srinivasa-Rao, 1993a). In tomato, partial leaf removal increased net assimilation rate of the remaining leaves, so fruit growth was not affected (Tanaka and Fujita, 1974).

b) Leaf age. Photosynthetic rates vary with leaf age. Very young leaves have a negative carbon exchange rate and may represent the major sink for photosynthates manufactured within them (Tanaka *et al.*, 1974b). In pepper 'California Wonder', maximum photosynthetic rate occurs when leaf area is ~ 10 cm^2 (Steer, 1971), the time at which stomatal density is highest (Steer, 1972). The photosynthetic rate decreases slightly until the leaf reaches its maximum size, and then remains steady for a period of time (Steer, 1971). Hall (1977) reported that the photosynthetic rate of pepper leaves that expand during or immediately after anthesis in fruiting plants remains steady throughout fruit growth. Older leaves generally exhibit lower photosynthetic rates compared to younger leaves (Steer and Pearson, 1976). In tomato, Bolanos and Hsiao (1991) reported that maximum leaf photosynthesis was reached near the time of full expansion and declined steadily thereafter. Also in tomato, Xu *et al.* (1997) found that

net photosynthesis of the 10th, 15th and 18th leaves from the apex were only 50, 21 and 7% of that of the 5th leaf, and suggested that leaves below the 18th node can be eliminated to improve air circulation and reduce humidity within the crop without affecting CO_2 fixation. Photosaturation may also differ with leaf age, where old leaves photosaturate at lower light intensities. In addition, the surplus of photosynthates from young, upper leaves may depress the photosynthetic activity of old leaves (Tanaka *et al.*, 1974a).

c) Developing fruits. Developing fruits may affect plant photosynthesis by decreasing leaf area and/or increasing leaf photosynthetic rates. Compared with non-fruiting plants, plants with developing fruits often have reduced total leaf area (due to fewer and smaller leaves) and increased life span of functional leaves (Hall, 1977; Kläring *et al.*, 1996). Decreases in leaf area of fruiting pepper plants compared to non-fruiting plants were significant as soon as 2 weeks after anthesis (Hall, 1977; Bhatt and Srinivasa-Rao, 1989). By 80 DAA (2 weeks after maturation of first fruit), functional leaf area in fruiting plants was about 50% that of non-fruiting plants. However, in non-fruiting plants, leaf senescence and abscission rates were seven-fold or more greater than in fruiting plants (Hall, 1977). This phenomenon is not seen in all pepper cultivars. Bhatt and Srinivasa-Rao (1997) reported no differences in total leaf area in bell pepper with different fruit loads.

Developing fruits may also affect photosynthesis by increasing leaf CO_2 assimilation rates or by causing a partial recovery of the leaf photosynthesis rate in older leaves (Hall and Brady, 1977). Leaf CO_2 assimilation rates may increase up to 65% in fruiting plants compared to non-fruiting plants (Hall and Brady, 1977; Nilwik,

1980; Bhatt and Srinivasa-Rao, 1989), or in the same plants before and after fruit set and development (Cruz-Huerta *et al.*, 2005). In addition, removing pepper fruits during rapid growth reduced the rate of CO_2 assimilation by up to 30% in the leaves close to the fruits, mainly due to changes in both the leaf and the intracellular resistance (Hall and Milthorpe, 1978). However, removal of the first two flowers or fruits in pepper plants had no consistent effect on the photosynthetic rates of fully expanded and exposed leaves (Bhatt and Srinivasa-Rao, 1997). Bertin *et al.* (2001) reported that decreasing sink competition during tomato fruit cell elongation decreased fruit dry matter content, suggesting that reduced sink strength inhibited leaf photosynthetic rates.

d) Light. Light is one of the most important factors in plant productivity (Papadopoulos and Pararajasingham, 1997). It is generally accepted that most C_3 species photosaturate between 500 and 1000 PPF (full sun light intensity is ~2000 PPF) (Taiz and Zeiger, 2006); however, there is great variation, even within species. For example, Xu *et al.* (1997) found that young tomato leaves reached their maximum photosynthetic rate between 750 and 800 µmol m^{-2} s^{-1} while older leaves saturated at about 300 µmol m^{-2} s^{-1}. In pepper, E-Jaimez *et al.* (2005) reported that light saturation in bell pepper grown in a greenhouse occurred ~ 1500 µmol m^{-2} s^{-1} PPF. Decreasing light intensity from 1800 to 1000 µmol m^{-2} s^{-1} PPF decreased photosynthesis ~20% on sunny days; however photosynthesis decreased ~40% on cloudy days due to higher stomatal resistance (E-Jaimez *et al.*, 2005).

Photoperiod also affects plant productivity. Some *Capsicum annuum* genotypes have the ability to acclimate to supplementary light, even to continuous light, by increasing chlorophyll content (Murage and Masuda, 1997), carboxylation capacity,

electron transport capacity, and the light saturation point (Dorais *et al.*, 1995). Demers *et al.* (1998) reported that continuous light increased plant growth (fresh and dry basis) and yield for the first five weeks in pepper compared to a 14 h photoperiod; however, after five weeks, growth under continuous light decreased,. The authors suggested that the negative effects of continuous light on plant growth were due to a limitation in photosynthate export, as evidenced by high leaf starch content and leaf deformation. However, Dorais *et al.* (1996), reported that after 15 weeks of growing bell pepper under extended photoperiod (18 h) or continuous lighting, fruit yield increased compared with plants grown under 8 or 12 h photoperiods, indicating increased photosynthate allocation to fruits.

e) Temperature. Temperatures above or below the optimal range for photosynthesis reduce net photosynthesis and alter biomass partitioning, favoring vegetative over reproductive growth (Ahmed *et al.*, 1993; Wang *et al.*, 1997; Diczbalis and Menzel, 1998). Temperature effects on photosynthesis depend on several factors, including the plant organ exposed (root *vs* shoot), the time of the day (day *vs* night), and the duration of the event.

Some species tolerate root temperatures up to 35°C without decreasing photosynthetic rates (Du and Tachibana, 1994; Nada *et al.*, 2003); however, root temperatures above 38°C generally result in severe decreases in photosynthesis (Du and Tachibana, 1994; Diczbalis and Menzel, 1998; Nada *et al.*, 2003). The reduction in photosynthesis is initially caused by stomatal closure triggered by water stress (He *et al.*, 2001) or abscisic acid (ABA) accumulation (Nada *et al.*, 2003). Other physiological disorders, including impaired activation of Rubisco and decreased chlorophyll content,

also play an important role under prolonged high root temperatures (Du and Tachibana, 1994; Nada *et al.*, 2003).

Variable root temperatures (from 23°C to 40°C) decreased photosynthesis in lettuce (He *et al.*, 2001) and dry matter accumulation in pepper (Dodd *et al.*, 2000), However, Gosselin and Trudel (1986) reported that maximum dry weight in pepper was found at root temperatures of 24°C, while maximum photosynthesis occurred at root temperatures of 36°C. In tropical rambutan (*Nephelium lappaceum*), maximum photosynthetic rates occurred at 28°C root temperature, and photosynthesis decreased significantly at lower (15°C) or higher (38°C) root temperatures (Diczbalis and Menzel, 1998).

Excessively high shoot temperatures decrease photosynthesis and increase respiration, reducing carbohydrate availability (Taiz and Zeiger, 2006). Decreases in photosynthetic rates in C_3 plants are often attributed to stomatal closure (Taiz and Zeiger, 2006), followed by the inactivation of Rubisco and/or denaturation of Rubisco activase, which facilitates the carbamylation and maintenance of Rubisco activity (Hendrickson *et al.*, 2008, and references there in).

Low temperatures also reduce shoot photosynthesis. This effect is more serious in chilling-sensitive species, such as cucumber, tomato, and sweet pepper (Li *et al.*, 2003b). Studies in cucumber, sweet pepper (Li *et al.*, 2003b), and Arabidopsis (Zhang and Scheller, 2004) showed that low temperature (4°C) combined with low light intensity (100-150 PPF) damaged PSI without damaging PSII. However, cold temperatures and high irradiances damaged PSII (Liu *et al.*, 2001). Pepper plants that were cold-hardened

(5 d at 14°C and 250 PPF) were more tolerant to chilling stress, but tolerance was only expressed by a faster recovery (Liu *et al.*, 2001).

Night shoot temperature also affects photosynthesis. Night temperatures between 15 and 25 °C decrease photosynthesis in grapevine due to a delay in stomatal opening the next morning, although stomatal conductance recovers as temperatures increase during the day (Hendrickson *et al.*, 2004a; Hendrickson *et al.*, 2004b). Low night temperatures, especially below 10°C, may also decrease photosynthesis by decreasing ribulose-1,5-bisphosphate (RuBP) regeneration and Pi availability for recycling (Hendrickson *et al.*, 2004a; Hendrickson *et al.*, 2004b), reducing activity of PSII (Sundar and Reddy, 2000; Bertamini *et al.*, 2005), and decreasing leaf chlorophyll content (Sundar and Reddy, 2000; Bertamini *et al.*, 2005). Reduction in Rubisco amount and activity, as well as other photosynthetic/carbohydrate metabolizing enzymes, such as fructose bisphosphatase (FBPase), and sucrose phosphate synthase, may also occur in response to low night temperature (Sundar and Reddy, 2000; Ramalho *et al.*, 2003; Hendrickson *et al.*, 2004a; Hendrickson *et al.*, 2004b; Bertamini *et al.*, 2005), However, species/cultivars tolerant to low night temperatures increase the activity of Rubisco and other photosynthetic enzymes, including phosphoenolpyruvate carboxylase, NADP-malate dehydrogenase, NADP-malic enzyme, and stromal FBPase (Du *et al.*, 1999).

In several tropical crops, including sugar cane, bean, soybean, coffee, maize, and peanut (Wolfe, 1991; Ying *et al.*, 2000, and references there in; Ramalho *et al.*, 2003; Wang *et al.*, 2004), one cold night may decrease photosynthetic rates 5 to 80%. Sweet pepper seems to be very sensitive to low night temperatures. Bhatt and Srinivasa-Rao (1993b) reported that decreasing night temperatures from 22 to 17°C decreased net

CO_2 assimilation rates up to 30%. During maize grain-filling, one night of exposure to 4°C decreased the carbon exchange rate 14 to 30%, depending on the genotype (Ying *et al.*, 2000). Greater reductions were found when maize plants were exposed to two or three cold nights at 4°C. Maintaining plants in the dark at 15 to 18°C for an hour before light exposure reduced the negative effect of cold night temperature on maize photosynthesis (Ying *et al.*, 2002). The reduction in maize leaf photosynthesis after cold exposure was linearly related to the incident PPF level the next day, but fluorescence and CO_2 exchange readings suggest that the reduction in photosynthesis is not associated with photoinhibition (Ying *et al.*, 2002). Similarly, Hendrickson *et al*. (2004a; 2004b) found that chilling-induced reduction in grapevine photosynthesis was not due to photoinhibition. Initial inhibition appeared to be related to stomatal closure, followed by a biochemical restriction due to limitation of RuBP regeneration and sucrose and starch synthesis limitation (end-product limitation) (Hendrickson *et al.*, 2004a; 2004b).

Some species have the ability to acclimate to low temperatures. In such cases, exposure to low temperatures for long period (weeks) markedly increased the maximum activities of Rubisco, stromal and cytosolic fructose-1,6-bisphosphatase, and sucrose-phosphate synthase, and increased RuBP regeneration and carboxylation in leaves of several species, leading to significant increases in whole plant photosynthetic capacity (Holaday *et al.*, 1992; Hurry *et al.*, 1994; Du *et al.*, 1999; Yamori *et al.*, 2005). Although net photosynthesis in *Parthenium argentatum* initially decreased when night temperature was decreased from 20°C to 15°C, photosynthetic rates began to recover after 30 days of low night temperatures, reaching values equal to or higher than initial values (Sundar and Reddy, 2000). In cotton, Singh *et al*. (2005) reported that after 30

days of growing plants under decreasing night temperatures from 16 to 11°C (at day temperatures of 28°C), CO_2 assimilation, stomatal conductance, carboxylation capacity, electron transport capacity, and triose-phosphate utilization capacity were similar between leaves developed under low night temperature and leaves developed on control plants (28/24°C day/night). Mercado *et al.* (1997a) reported that sweet pepper plants grown under low night temperatures (14 °C) showed improved chilling resistance upon exposure to night temperatures of 6 °C compared with plants grown under higher night temperatures (20 °C).

Abnormal Flower and Fruit Development

In pepper, as in most horticultural crops, yield of high quality fruit is as important as total yield. Fruit weight, size, shape, firmness, color, and disease/pest incidence are used to evaluate pepper fruit quality (Aloni *et al.*, 1999; Navarro *et al.*, 2002; Kissinger *et al.*, 2005; Lim *et al.*, 2007). Both final fruit size and shape in pepper are directly affected by early flower and fruit development (Ali and Kelly, 1992; Aloni *et al.*, 1999). Early development, in turn, is very sensitive to several factors, including ambient temperature and carbohydrate availability in the plant.

High temperatures induce flower abortion and impair pollen fertility in several crops, including tomato (Bertin, 1995; Sato *et al.*, 2001; Sato *et al.*, 2002), pepper (Polowick and Sawhney, 1985; Turner and Wien, 1994a; Aloni *et al.*, 2001), and *Brassica napus* (Young *et al.*, 2004). In tomato and pepper, high temperature stress also reduces pollen grain number and germination (Aloni *et al.*, 2001; Sato *et al.*, 2004; Sato and Peet, 2005). In pepper, additional morphological changes are associated with high temperatures. Day/night temperatures of 28/23°C decreased ovary diameter and increased style length compared to a day/night temperatures of 23/18°C (Polowick and

Sawhney, 1985). High temperature after anthesis also reduces fruit and seed growth and seed quality in chili pepper (Pagamas and Nawata, 2008).

Low temperatures also induce abnormal flower and fruit development in bell pepper. In winter, cooler temperatures and shorter day lengths increase the percentage of small, flattened parthenocarpic fruits. Pollen fertility is the main reason for this condition, but female organs (Polowick and Sawhney, 1985; Pressman *et al.*, 1998a; Shaked *et al.*, 2004) and other parts of the flower (Aloni *et al.*, 1999) are also affected. Night temperatures of 15°C or less (day temperature of 20 to 25°C) increase ovary diameter in pepper without increasing locule number, creating “swollen” ovaries and malformed fruit (Polowick and Sawhney, 1985; Rylski, 1985; Bakker, 1989b; Aloni *et al.*, 1999; Shaked *et al.*, 2004). Low night temperatures also produce abnormalities in the petal and stamen, and reduce style length. The longer the duration of low night temperatures, the higher the percentage of swollen flowers and ovaries (Polowick and Sawhney, 1985; Aloni *et al.*, 1999). Aloni *et al.* (1999) found that 15 days at night temperatures of 12 or 18°C resulted in 21 and 7% swollen ovaries, respectively. By 49 days, these percentages increased to 78 and 14%, respectively. Cell volume in swollen ovaries is greater than that in normal ovaries, but cell number remains the same (Pressman *et al.*, 1998b) suggesting that swollen ovaries develop after cell division ends.

Although low night air temperatures result in swollen pepper ovaries and malformed fruit, low night root temperatures do not appear to have the same effect. Pepper plants grown at night air temperatures of 12°C produced low fertility pollen and small fruits at root night temperatures of either 12° or 20°C (Mercado *et al.*, 1997c; Aloni

et al., 1999). These findings suggest that flower deformation and ovary swelling are favored by low air temperature during the night, regardless of root temperature.

Very high day temperature may reduce negative effects that low night temperatures have on final fruit shape and size in pepper. Pressman *et al.* (2006) found that increasing the day temperature to ~ 35°C compensated for some of the deleterious effects of low night temperature on vegetative and reproductive development, particularly on stamen and pollen development. However, no information on the effects of these high day temperatures on ovary development was reported.

Excess carbohydrate availability during early flowering/fruiting also results in abnormal flower and fruit development in pepper. Increased source/sink ratio by fruit removal increases the proportion of swollen ovaries (≥7 mm diameter) compared with fruiting plants (Aloni *et al.*, 1999). Fresh weight of swollen flowers that developed in defruited plants was three to four times that of the flowers on fruiting plants (Aloni *et al.*, 1999). In addition, the percentage of swollen flowers was inversely related to the number of growing fruits on the plant, and directly proportional to the concentration (fresh weight basis) of reducing sugars and starch in the flowers developed in those plants (Aloni *et al.*, 1999). Flowers that develop on defruited plants accumulated four-fold more $^{14}CO_2$ (600 *vs* 150 µg d^{-1}) and fresh weight (180 *vs* 60 mg) than flowers that develop on fruiting plants (Aloni *et al.*, 1991b; Aloni *et al.*, 1999). These data suggest that excess assimilates are transported to flower buds on defruited plants, resulting in ovary swelling and deformation (Aloni *et al.*, 1999).

In summary, flower and ovary swelling in pepper is favored by exposing the shoot to low night temperature or by removing actively growing fruits. However, the

physiological changes in the plant and flower buds that induce such swelling and deformation are still not clear.

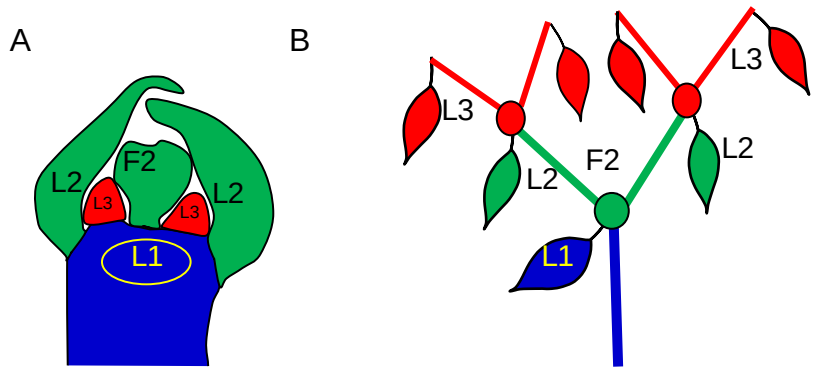

Figure 2-1. Schematic drawing of pepper branching and flowering sequence. A) Meristematic stage. B) Well developed branch. A sympodial shoot bears a leaf (L1, blue), and terminates in a flower (F2). From the leaf axil, two leaves (L2) develop. The flower and the two leaves developed from the same sympodial shoot compose a sympodial unit (F2 and L2, green). L2 leaves are 'pushed up' by internode elongation, so that the leaves appear above the preceding flower. The shoot terminates in a flower, which is part of the subsequent sympodial unit. From each of the L2 axils, two new leaves (L3, red) develop. Each color represents sympodial units of a different order (Drawing A based on micrograph from Elitzur *et al.*, 2009).

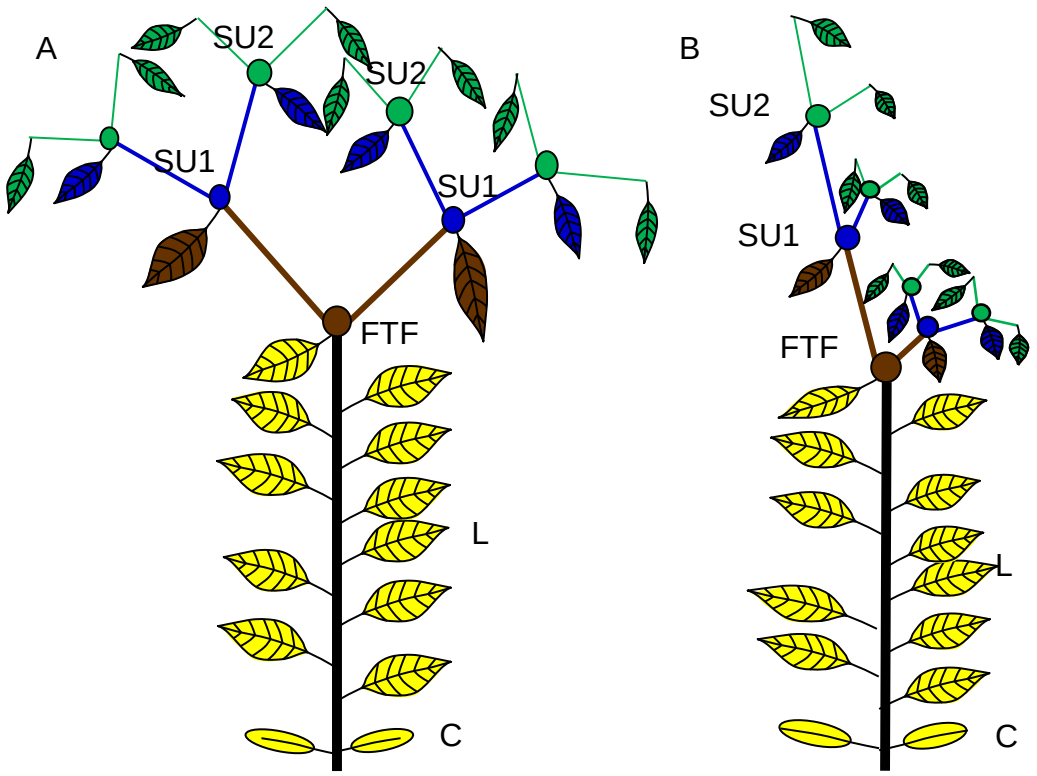

Figure 2-2. Schematic diagram of a pepper plant. A) Pepper plant has a main stem with cotyledonary (C) and true leaves (L, yellow). The main stem ends in a terminal flower (FTF, first terminal flower, brown circle). The terminal flower and the first two leaves above it (brown) originate from the main stem and form the main stem sympodial unit. Each new sympodial unit is also composed of two leaves and a flower (circle), forming sympodial unit 1 (SU), 2 (SU2), and so forth. B) Asymmetric growth of the sympodial components results in different plant architecture.

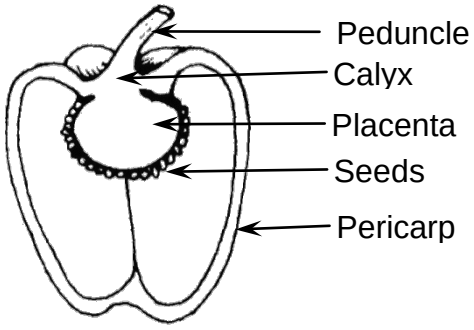

Figure 2-3. Pepper fruit and its parts (Modified from Rylski, 1986).

CHAPTER 3
LOW NIGHT TEMPERATURE INCREASES OVARY SIZE IN SWEET PEPPER

Introduction

Pepper (*Capsicum annuum* L) is an important vegetable crop that belongs to the Solanaceae family, and whose edible part is botanically a fruit. Historically, peppers have been grown in the tropics as well as in temperate climates (Eshbaugh, 1993). Hot peppers are consumed fresh, dried or processed, mostly as spice, or as a source of food colorants or capsaicin. Sweet peppers, a genetic recessive non-pungent form, are an important green vegetable crop worldwide, especially in temperate regions (Eshbaugh, 1993; Bosland, 1996). There is large variability in sweet pepper fruit size and shape, ranging from cherry (spherical, ~2.5 to 3.5 cm in diameter), to elongated (banana or cubanelle), to bell (blocky) peppers, which are the most important from an economic standpoint (Bosland, 1996; Bosland, 1999). In the U.S., per capita consumption of bell peppers increased from 1 kg in 1970 to ~3 kg in the late 1990s and has remained steady during the last 10 years (USDA - Economic Research Service, 2008).

Pepper plants develop a main stem that bears leaves on the first 8 to 15 nodes and terminates in a flower. The two or three nodes preceding the terminal flower bear sympodial shoots (first lateral branches) (Child, 1979; Elitzur *et al.*, 2009). Each sympodial shoot has a leaf, and terminates in a flower. Two new leaves emerge from the leaf axil below the floral terminus, and are “pushed” above the terminal flower by elongation of the new internode (Figure 3-1). From every sympodial shoot, a new sympodial unit is formed, consisting of the two sympodial shoots, the flower in the vertex of the shoots (*i.e.* the terminal flower from the preceding shoot), and the single

leaf at the distal end of each sympodial shoot (*i.e.* two leaves, due to the presence of two sympodial shots). This cycle of development repeats itself and could, in theory, continue for an indefinite period of time (Elitzur *et al.*, 2009). Thus, flowering and fruiting in pepper is continuous as long as plants are growing.

Fruit shape, size and regularity are important quality components in sweet peppers. Final fruit shape is the result of genetic and environmental factors (Perin *et al.*, 2002; Weiss *et al.*, 2005), and it is defined in the early stages of fruit development (Munting, 1974). In bell, spherical, and ovoid fruited pepper varieties, shape definition takes place before pollination occurs (Munting, 1974; Aloni *et al.*, 1999; Meijer and Murray, 2001). However, in long-fruited varieties, fruit shape is defined during both the preanthesis and postanthesis stages (Munting, 1974).In all genotypes, pollination and fertilization are key factors in attaining the ideal size, shape and uniformity (Mercado *et al.*, 1997c). Under optimal growth conditions, bell pepper fruit requires 150-300 seeds to develop the uniform blocky shape (Aloni *et al.*, 1999). After fertilization and fruit set in bell pepper, changes in assimilate supply can change the growth rate and therefore the size, but do not affect fruit shape (Aloni *et al.*, 1999).

Sweet peppers, as with other fresh fruits and vegetables, are a commodity in demand all year. In order to fill that market need, sweet pepper production during the winter has been developed in areas with warm or mild winters or even in cold winters under greenhouse conditions. However, when grown in cold winters under greenhouse conditions, bell peppers can develop up to 60% of flattened and deformed fruits, and thus, unmarketable (Rylski, 1973; Ali and Kelly, 1993; Aloni *et al.*, 1999).

Several reports have previously addressed the effect of low night temperature (LNT) on flower and fruit development and malformation in sweet pepper. However, they have mainly focused on pollen quality (Mercado *et al.*, 1997b; Pressman *et al.*, 1998a; Shaked *et al.*, 2004), pollination (Mercado *et al.*, 1997c; Pressman *et al.*, 1998a), number of seeds (Rylski, 1973; Kato, 1989), and fruit quality (Rylski, 1973; Kato, 1989; Pressman *et al.*, 2006). Only a few studies have focused on flower and ovary malformation and swelling, and these studies have been limited to effects in bell pepper (Pressman *et al.*, 1998b; Aloni *et al.*, 1999). Flower deformations due to LNT include abnormal and curled petals that do not fully expand, shorter stamens with low pollen content and low germinability, and larger ovaries with shorter styles compared with flowers developed under intermediate or high temperatures (Polowick and Sawhney, 1985; Rylski, 1985; Mercado *et al.*, 1997b; Pressman *et al.*, 1998a; Aloni *et al.*, 1999; Shaked *et al.*, 2004). The ovary enlargement observed under cool temperatures is referred to as ovary swelling (Pressman *et al.*, 1998b; Aloni *et al.*, 1999). Ovaries developed under LNT are impaired in fruit development, even when pollinated with viable pollen, and result in smaller, irregularly shaped fruit compared with fruit developed under higher night temperatures (Pressman *et al.*, 1998a).

In addition to LNT, high source:sink ratio can also result in swollen ovaries in bell pepper (Aloni *et al.*, 1999). High source:sink ratio can be caused by harvesting all the fruits or by a massive flower drop. When bell pepper plants bearing several fruits in different stages were artificially defruited, 90% of the flowers that reached anthesis 15 days after defruiting were swollen, compared to 20% in the non-defruited control. Although partial defoliation of completely defruited plants did not reduce ovary swelling,

some reduction in ovary swelling was observed in subsequently formed flowers when two developing fruits were allowed to remain on the plant (Aloni *et al.*, 1999).

Although the effects of LNT and high source:sink ratios on ovary swelling in bell peppers are documented, LNT and high source:sink ratio effects in other types of sweet peppers are unknown. In addition, in pepper cultivars with blocky or spherical fruit, ovary shape and cell number are determined in the preanthesis stages; however, in long-fruited cultivars, cell number and ovary shape are modified in the postanthesis stages (Munting, 1974). Thus, LNT and/or source sink ratio might affect ovary shape differently depending on the final fruit shape. Therefore, the hypothesis tested in this work is that low night temperature increases ovary size and causes ovary swelling in a range of pepper cultivars differing in final fruit shape and size, but low source:sink ratio can counteract the effect of LNT on ovary swelling. The objectives were to determine the effects of LNT and two source sink ratios on ovary size in different sweet pepper cultivars and to determine the parts of the ovary that are most affected by LNT.

Material and Methods

Plant Material and Growing Conditions

Seedlings of six (Experiment 1) or four (Experiment 2) sweet pepper varieties were germinated using a mix of commercial potting media (SunGro Metro-Mix Ag-Lite Mix[1]) and perlite (4:1 v:v) in 40-cell flats (60 cm^3 each cell) in 1.4 m^2 growth chambers (E15 Conviron, Winnipeg, Canada). Chamber temperature was set to 22/20°C (day/night) and PPF was maintained between 450-500 µmol m^{-2} s^{-1} above the canopy with a 14-

[1] Composition: Canadian Sphagnum peat moss (55-65%), horticultural grade vermiculite, dolomite limestone, starter nutrient charge, and wetting agents (www.sungro.com/products.php, 12/17/2008).

hour photoperiod. Light was provided by fluorescent and incandescent lights. There was no control of CO_2 concentration or relative humidity.

Plants were watered with tap water until the beginning of expansion of the first true leaf (4 weeks after seeding), when fertigation began. The fertigation protocol differed with the experiment and is described below. Pests were controlled by using a combination of biological control (wasps for aphids) and chemical control. Insecticidal soap (Safer® Brand Insect Killing Soap, Safer® Brand, www.saferbrand.com, 12/17/2008) , dicofol (Dicofol 4E, Dicofol 42%, Makhteshim Agan of North America, NY, USA), or abamectin (Agri-Mek 0.15EC, abamectin 2%, Syngenta Crop Protection, Greensboro, NC, USA) were sprayed as needed at one-half the lowest recommended dosage until seedlings were transplanted to containers.

Seedlings at about the sixth-leaf stage (54 days after sowing) were transplanted into 1.5 L containers using the same media mix. Plants were continuously pruned once branching began. At the first branching point, two shoots were allowed to grow, forming the main stem sympodial unit. For the subsequent sympodial units in Experiment 1, the strongest shoot was allowed to grow (contributing to the main axis of the plant) and the other shoot was allowed to develop one sympodial unit (forming a lateral branch) before pruning (Figure 3-2A). In Experiment 2, one-half of the plants were pruned as in Figure 3-2A and the other half were pruned so that the less strong shoot was allowed to develop only the flower of the next sympodial unit before pruning (Figure 3-2B). Sympodial units formed on the main stem sympodial shoots were numbered SU1 (sympodial unit 1), which in turn bore SU2. Subsequent sympodial units were numbered 3, 4, …, n (see Elitzur *et al.*, 2009). For the purpose of this study, nodes above the first

branching point were also numbered according to their sympodial unit in order to describe each flower's position, *i.e.*, node 1 (corresponding to SU1), node 2, node 3, etc. Flowers from the main stem and node 1 (*i.e.* SU1) were removed at the flower bud stage.

Experiment 1

This experiment was designed to determine the response of six cultivars of sweet pepper to low night temperature. Four blocky bell-type pepper cultivars ('Ariane', 'Aristotle' with X3R®, 'Brigadier', 'Legionnaire'), one elongated-fruit cultivar ('Banana Supreme', length:diameter ratio ~4), and one cherry-type cultivar ('Red Cherry Sweet', rounded, 2.5-3.7 cm diameter) were exposed to day temperatures of 22°C and one of two night temperatures: 20° (high night temperature, HNT) or 12°C (low night temperature, LNT). Several cultivars of bell pepper cultivars allowed to test differences among bell pepper cultivars, since previous work on ovary swelling as a response to night temperature had been done mostly in 'Mazurka' cultivar (Pressman *et al.*, 1998a; Pressman *et al.*, 1998b; Aloni *et al.*, 1999). Plants were fertigated starting at about 6 weeks after seeding with 20N:6.7P:16.7K water soluble fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH, www.scottsprohort.com). Application rates were ~1 mg N per plant per day before transplanting (54 days after sowing) and from 15 to 50 mg N per plant per day after transplanting, depending on plant age. Starting at 7 weeks after transplanting, plants were fertigated as needed with the following nutrient solution (mM): 3.4 $Ca(NO_3)_2$, 1.8 KNO_3, 1.6 KH_2PO_4, 0.3 KCl, 2.7 $MgSO_4$, and (µM): 50.2 Fe-EDTA, 3.1 $CuSO_4$, 14.6 $MnSO_4$, 64.8 H_3BO_3, 4.6 $ZnSO_4$, 0.6 Na_2MoO_4 (modified from Cruz-Huerta *et al.*, 2005; and Jovicich, 2007). Plants were watered in the morning to increase root-zone temperature in the LNT treatment (Ying *et*

al., 2002) so that day temperature was similar in the root and shoot in both LNT and HNT. Three plants per cultivar were randomly distributed in each growth chamber.

Night temperature treatments began just before anthesis of flowers in node 3 (‘Ariane’ and ‘Aristotle’), 4 (‘Brigadier’, ‘Legionnaire’ and ‘Banana Supreme’) or 5 (‘Red Cherry Sweet’). Once treatments started, all the flowers at anthesis were continuously harvested during a 39-day period in ‘Brigadier’, ‘Legionnaire’, ‘Banana Supreme’ and ‘Red Cherry Sweet’ and a 64-day period in ‘Ariane’ and ‘Aristotle’. The flower harvest period was prolonged in these latter two cultivars due to a delay in their growth and development after transplanting. Plants did not bear any fruit before and during the experiment. Flower harvests occurred every 2-3 days during the first 50 days and every day during the last 13 days. A minimum of 66 flowers per night temperature and cultivar combination were harvested. The flower position in the plant (*i.e.,* node number above first terminal flower and whether it was in the main axis or in a lateral branch) was recorded. Ovary fresh weight, volume (measured by water displacement), diameter and length were recorded. Ovaries were cut in half and ovary wall thickness was also measured.

Experiment 2

Based on the results from the first experiment, four cultivars, ‘Ariane’, ‘Legionnaire’, ‘Banana Supreme’, and ‘Red Cherry Sweet’, were selected for Experiment 2. The criteria used to select the cultivars were 1) contrasting responses to LNT in bell peppers (‘Ariane’ and ‘Legionnaire’), and 2) contrasting fruit shape and size (bell, elongated and cherry-fruited varieties). Two night temperatures regimes, high (HNT, 20°C) and low (LNT, 12°C), and two levels of source supply (high and low) were studied. Source supply was modified either by leaving the complete sympodial unit on

every lateral shoot (Figure 3-2A), or removing the leaves and leaving only the flower (Figure 3-2B). Therefore, starting in sympodial unit 2, high source supply treatment had twice as many leaves as the low source supply treatment did., *i.e.*, 8 *vs*. 4 leaves per sympodial unit, as shown in Figure 3-2.

Each growth chamber was divided into 3 blocks (based on variation in light intensity within the growth chamber observed in Experiment 1) and within each block, 8 plants were randomly placed (4 cultivars and 2 levels of source supply), with one plant per treatment using a completely randomized block design. Treatments began just before anthesis of flowers on the second sympodial unit (node 2). The first terminal flower and flowers from node 1 were removed.

Flowers were harvested every day at anthesis for up to 45 days, from the second to the seventh nodes above the first terminal flower. As in Experiment 1, plants did not bear any fruit. The position of every harvested flower in the plant was recorded. Ovary fresh weight (FW), volume, diameter, and length were recorded. Ovary wall thickness and FW were also measured, as was placenta FW.

Statistical Analysis

Data from Experiment 1 were grouped into five harvest intervals (Table 3-1). For ‘Brigadier’, ‘Legionnaire’, ‘Banana Supreme’, and ‘Red Cherry Sweet’, harvest intervals were 8 days, and for ‘Ariane’ and ‘Aristotle’, harvest intervals were 13 days, since development was delayed in these two cultivars. Data were analyzed as a mixed model with repeated measures using cultivar, night temperature and harvest period as main factors, axis (*i.e.* main axis or lateral branch) nested within each plant as the repeated measures subject, and days after anthesis as the covariate.

Experiment 2 originally included three main factors (4 cultivars, 2 temperature regimes, 2 levels of source supply). Additionally, node number (number of nodes counted above the first flowering node) and the axis within the plant were included in the model during the analysis. After the initial statistical analysis, it was found that the source supply did not significantly affect the parameters evaluated, either as main or interactive effects. Thus, this factor was not considered in the final analysis.

Data from experiment 2 were also analyzed as a mixed model with repeated measures, using temperature and cultivar as the factors of the main model, and the node number and axis (main axis, lateral branch) of the harvested flower as components of the sub-model. The subject for the repeated measures was an individual plant.

Data were analyzed using SAS (SAS Institute Inc., 2008), and mean separation was done using Tukey-Kramer (multiple LSmeans) or t-test (two means). Multiple LSMeans letter grouping was done using a SAS macro developed by Saxton (1998).

Results

Experiment 1

Approximately 30 flowers per plant reached anthesis and were harvested during the experiment, with no significant difference in the number of flowers among cultivars (Table 3-2). Low night temperature (LNT) decreased the number of flowers compared with high night temperature (HNT) (27 *vs* 33 flowers, respectively). Within each cultivar, temperature reduced the number of flowers only in ‘Ariane’ and ‘Legionnaire’ ($P \leq 0.04$). The decrease in flowers harvested at LNT compared with HNT was accompanied by a concomitant decrease in number of nodes under LNT (Table 3-2).

All ovary parameters measured were affected by temperature, cultivar, and harvest interval as main effects. Interaction of temperature with cultivar and harvest interval was significant in most cases, except for ovary wall thickness and fresh weight:volume ratio (Table A-1, Appendix). For fresh weight and volume, all interactions were significant, whereas for the rest of the variables, one or more interactions were non-significant. The covariate days after anthesis (DAA) and the repeating subject component (branch within each plant) were also significant for most of the cases.

Ovary FW, volume, diameter, length, and ovary wall thickness were greater in flowers developed under LNT compared with flowers developed under HNT (Table 3-3). However, shape was not affected, as indicated by the length:diameter ratio.

There were significant differences among cultivars in ovary size (Table 3-4). Blocky varieties (bell peppers ‘Ariane’, ‘Aristotle’, ‘Brigadier’, and ‘Legionnaire’) developed larger ovaries than the cherry (‘Red Sweet Cherry’) or elongated (‘Banana Supreme’) cultivars. Within the bell pepper types, ‘Ariane’ and ‘Aristotle’ developed smaller ovaries than ‘Legionnaire’, possibly due to the delayed growth. Differences in ovary volume and diameter were similar to those in ovary FW; however, length in some blocky cultivars (‘Aristotle’ and ‘Brigadier’) was less than in the non-blocky cultivars (‘Banana Supreme’ and ‘Red Cherry Sweet’), resulting in significant differences in the length:diameter ratio (0.70 *vs* 1.1, respectively). Ovary wall thickness was greater in cherry and elongated-fruited cultivars compared with bell-fruited cultivars.

Ovary fresh weight and volume of flowers harvested at anthesis remained constant during the first two harvest intervals (*i.e.*, 16 DAT ‘Brigadier’, ‘Legionnaire’, ‘Banana Supreme’ and ‘Red Cherry Sweet’ and 26 days in ‘Ariane’ and ‘Aristotle’, see

Table 3-1 for further details) (Table 3-5), which generally corresponded to the first and second nodes harvested (Figure A-1, Appendix). Subsequently, ovary FW increased significantly at a rate of ~6 mg per harvest interval, and ovary volume increased at ~7 mm^3 per interval. Similar patterns were observed with diameter and length. Ovary wall thickness, however, decreased initially, then increased, but did not reach the initial values. Ovary shape at anthesis (as indicated by the length:diameter ratio) remained constant during the first four harvest intervals, but was more elongated in the final interval.

There was a significant interaction between temperature and cultivar on ovary fresh weight, volume, diameter, length and length:diameter ratio (Table 3-6). When comparing the average effect of the night temperature treatment on ovary characteristics, significant differences were detected only in ‘Aristotle’ and ‘Legionnaire’. However, when only the flowers harvested during the last two harvest intervals of the experiment (harvest intervals IV and V) are considered, significant differences were found in ‘Aristotle’, ‘Legionnaire’, ‘Brigadier’, and ‘Banana Supreme’ (Table 3-6).

The relationship between ovary fresh weight and volume was linear (volume = b·FW) for every combination of cultivar and temperature. In general, the slopes (b) ranged from 1.04 to 1.09 in bell peppers, slightly less than 1.00 in ‘Red Cherry Sweet’, with intermediate values for ‘Banana Supreme’ (Table 3-7). Thus, ‘Red Sweet Cherry’ ovaries were denser than ovaries of bell pepper cultivars.

There was a significant three-way interaction (temperature x cultivar x harvest interval) on ovary characteristics (Table A-1). There was no effect of night temperature during the first three weeks of treatment (*i.e.*, harvest intervals I and II for ‘Aristotle’ and

‘Ariane’ and I to III for ‘Brigadier’, ‘Legionnaire’, ‘Banana Supreme’, and Red Cherry Sweet) in any cultivar (Figure 3-3). Subsequently, however, fresh weight of ovaries from flowers at anthesis in three out of four bell pepper varieties (‘Aristotle’, ‘Brigadier’, and ‘Legionnaire’), and in the long-fruited variety (‘Banana Supreme’) increased under LNT compared with HNT. However, in this experiment, ovary FW of ‘Ariane’ (bell), and ‘Red Cherry Sweet’ (cherry), did not significantly increase under LNT compared with HNT. In general, three to four weeks of LNT were required to significantly increase ovary fresh weight compared to HNT treated plants. In four out of six cultivars, ovary FW did not change under HNT throughout the harvest intervals.

Experiment 2

Ovary characteristics of ‘Ariane’, ‘Banana Supreme’, ‘Legionnaire’, and ‘Red Cherry Sweet’ were dependent on night temperature, cultivar, and their interaction (Table A-2 in Appendix), but were not dependent on number of leaves at the node (*i.e.* potential source supply differences), as indicated earlier.

Fresh weight of ovary, placenta and ovary wall were significantly greater in flowers developed under LNT compared with those developed under HNT (Table 3-8). Ovary diameter and length were 18% and 15% greater, respectively, under LNT compared with HNT, while ovary wall thickness was 10% greater in ovaries developed under LNT. Length:diameter and ovary wall fresh weight:total fresh weight ratios were similar in both LNT and HNT.

The ovaries at anthesis were larger in the bell pepper types (‘Ariane’ and ‘Legionnaire’) than in the banana or cherry types (Table 3-9). In addition, there were differences in the percent of the total ovary FW that was contributed by the ovary wall. In ‘Ariane’ and ‘Legionnaire’, the ovary wall FW represented ~ 50% of the total FW,

while in ‘Banana Supreme’ and ‘Red Cherry Sweet’, the ovary wall represented ~65% of the total FW. There was also a significant correlation between the ovary fresh weight and ovary wall thickness for each genotype ($r^2 \geq 0.63$ for all four genotypes; $P \leq 0.01$).

In general, flowers from the lowest level nodes had the smallest ovaries, with ovary FW increasing steadily from 35 mg in node 2 to 56 mg in node 7 (Table 3-10), representing ~ a 60% increase. The ovary wall and placenta also increased by ~60%, while the diameter and length increased by 18% and 15%, respectively, as node level increased.

Interactions between night temperature and cultivar were significant for ovary, ovary wall and placenta FW, and for ovary diameter, but not for ovary length (Table A-2 in Appendix). Over the course of the experiment, plants grown under LNT produced flowers with greater ovary and ovary wall FW compared to plants grown under HNT. The increase in ovary FW under LNT ranged from 50% in ‘Banana Supreme’ to 76% in ‘Legionnaire’ (Table 3-11). Similar results were obtained in ovary wall FW, where increases ranged from 47% (’Red Cherry Sweet’) to 85% (‘Legionnaire’). However, LNT increased placenta FW only for bell peppers (‘Ariane’ and ‘Legionnaire’), not for the banana or cherry cultivars. The ovaries from plants grown under LNT increased in both diameter and length, except for the long-fruited cultivar, which increased only in length. When data from nodes 5 to 7 only are considered, the increase in ovary, ovary wall, and placenta FW under LNT is even more dramatic, resulting in greater ovary swelling under LNT compared with HNT.

Although ovary size increased as node number increased (Table 3-10), there were differences among cultivars and temperature treatments in this response (Figure 3-4).

Ovary size increased with node number in all cultivars under LNT, while ovary size increased with node number under HNT only in ‘Legionnaire’. ‘Ariane’ and ‘Legionnaire’ (bell type) responded faster to night temperature treatments than ‘Banana Supreme’ and ‘Red Cherry Sweet’. In ‘Legionnaire’, for example, LNT significantly increased the ovary size compared with HNT starting at node 2, which was harvested 8 days after the beginning of treatment (DAT) (Table A-3, Appendix). In contrast, in ‘Red Cherry Sweet’, significant differences between temperature treatments were not found until node 5 (20 DAT). In ‘Ariane’, differences in ovary FW due to night temperature treatments began by node 4 (21 DAT, Table A-3, Appendix). However, maximum ovary swelling was reached after node 5 for all cultivars (20 DAT in ‘Red Cherry Sweet’ to 31 DAT in ‘Legionnaire’).

Although ovary FW increased more than diameter and length at LNT compared with HNT within each cultivar (Table 3-11), both diameter and length responded as quickly as FW to night temperature treatments (Table 3-12). In fact, in ‘Banana Supreme’ and ‘Red Cherry Sweet’, significant differences in length were found earlier (*i.e.* node 4) than were differences in fresh weight and diameter (*i.e.* node 5).

Discussion

Most of the studies involving LNT on ovary and fruit development have been on large-fruited pepper cultivars, including bell-type (Rylski, 1973; Polowick and Sawhney, 1985; Bhatt and Srinivasa-Rao, 1993b; Mercado *et al.*, 1997b; Mercado *et al.*, 1997c; Pressman *et al.*, 1998a; Pressman *et al.*, 1998b; Aloni *et al.*, 1999; Shaked *et al.*, 2004). Limited work has been done on small fruited sweet pepper cultivars (Kato, 1989), other types of peppers such as cayenne and jalapeno (Shaked *et al.*, 2004), or other species of *Capsicum* (Mercado *et al.*, 1997c), and these studies focused on pollen development and fertility, but not on the ovary development.

In the present work, low night temperature increased ovary diameter and fresh weight in bell pepper flowers, confirming previous results (Polowick and Sawhney, 1985; Pressman *et al.*, 1998b; Aloni *et al.*, 1999). Final ovary diameter of the bell pepper cultivars used in the present experiments ranged from 5.7 to 6.8 mm under LNT, and from 4.3 to 6.4 mm under HNT, and these values are within the ranges reported previously (Pressman *et al.*, 1998b; Aloni *et al.*, 1999; Shaked *et al.*, 2004). Ovary swelling occurred in all bell, long-fruited and cherry pepper types under LNT. A previous study (Shaked *et al.*, 2004) reported that night temperatures of 10°C increased ovary diameter of sweet bell and hot long-fruited cayenne pepper flowers at anthesis compared with ovaries developed at night temperatures of 20°C; however, there was no such effect of LNT on ovary diameter of jalapeno peppers. Our results indicate that under LNT conditions, the increase in ovary fresh weight is proportionately greater than the increase in ovary diameter or length. For example, in Experiment 2, there was a 61% increase in ovary FW and only a 15 to 18% increase in ovary diameter and length under LNT compared with HNT. Most previous studies have measured night temperature effects on ovary diameter, not fresh weight or length (Polowick and Sawhney, 1985; Aloni *et al.*, 1999). Our results suggest that LNT effects on ovary swelling in peppers, and subsequent effects on fruit malformation, may be better predicted by looking at effects on ovary FW in bell peppers. However, in long-fruited cultivars such as ‘Banana Supreme’, ovary length may also be a good indicator of ovary swelling, as length increased more rapidly than did FW or volume in response to LNT compared with HNT.

Analysis of continuous harvests over time as a main effect, *i.e.* harvest intervals in Experiment 1 and nodes in Experiment 2, revealed that flowers harvested at the beginning of the experiments had smaller ovaries than the flowers harvested at the end of the experiments (Table 3-5, Table 3-10). However, when data were analyzed by cultivar and temperature treatment, there were no significant differences in ovary size among harvest intervals (Figure 3-3) or nodes (Figure 3-4) in ovaries developed under HNT, except for 'Ariane' and 'Aristotle' in Experiment 1 and 'Legionnaire' in Experiment 2. Thus, the increase in ovary size over time (harvest intervals or nodes), as observed in the present work, is primarily due to the effect of LNT. In Experiment 1, 'Ariane' and 'Aristotle' were the two cultivars that suffered delayed growth when transplanted. It is possible that the stress of transplanting reduced the carbohydrate availability in both cultivars for a period of time after transplant, resulting in smaller flowers. Carvalho and Heuvelink (2001) reported that increasing assimilate availability increased flower size in chrysanthemum. In pepper, plants bearing growing fruits developed ovaries with thinner ovary wall compared to the ovaries from plants without fruits, and the authors suggested that assimilate availability limited ovary wall thickness in fruiting plants (Ali and Kelly, 1992). Although ovary size was not recorded by Ali and Kelly (1992), the high correlation between ovary wall thickness and ovary fresh weight in our work (r^2 = 0.63 to 0.70, P<0.001; data not shown) suggests a similar limitation in assimilate availability may have occurred for 'Ariane' and 'Aristotle' in Experiment 1.

In general, sweet pepper cultivars developing under LNT required from three to four weeks to develop swollen ovaries. In the main stem of bell pepper, floral initiation occurs between the sixth and the seventh leaf and is independent of temperature (Choi

and Gerber, 1992). At day/night temperatures of 28/15°C, it takes ~ 4 weeks from the sixth leaf to anthesis of the first flower (Cruz-Huerta, 2001). This suggests that flower buds must be exposed to LNT within the first week after flower bud initiation in order to develop a large percentage of swollen ovaries. The exception to this was observed in 'Legionnaire' bell pepper in Experiment 2, in which significant increases in ovary size occurred after ~ one week of LNT compared with HNT. Even so, ~30 days of LNT were required for maximum increases in ovary size to be manifested in 'Legionnaire'.

Although a critical period for LNT exposure was revealed in our work (*i.e.* within the first week after flower bud initiation), the duration of LNT exposure required for development of swollen ovaries was not examined, as plants were exposed to low or high night temperatures continuously throughout the experiments. It is clear; however, that the percentage of flowers that exhibit ovary swelling increases as the duration of the LNT exposure increases. This has been demonstrated previously in bell pepper (Aloni *et al.*, 1999) and reflects the continuous floral initiation nature of pepper.

Previous work on ovary swelling in pepper focused only on the whole ovary. Our data show that the ovary wall FW: total ovary FW ratio varied among types of cultivars, averaging ~65% for the non-bell pepper cultivars ('Banana Supreme' and 'Red Cherry Sweet') and ~50% for the bell pepper cultivars ('Ariane' and 'Legionnaire'). However, LNT did not change the ratios, indicating that the increase in ovary FW is proportionally similar for ovary wall. The placenta FW, however, was affected by night temperature in bell and cherry-type peppers, but not in long-fruited. Therefore, increased ovary FW in bell and cherry-type peppers caused by LNT was due to increased ovary wall and placenta FW, and in 'Banana Supreme', was mainly due to ovary wall increase.

Although LNT treatments increased ovary size in both experiments, there were differences between experiments, as indicated above. Ovaries in Experiment 1 were larger than in Experiment 2, regardless of cultivar or temperature treatments. Differences in ovary size between experiments may in part be explained by harvest frequency (every 3 days in Experiment 1 *vs* every day in Experiment 2), and in part by plant density (13 and 17 plants/m^2, for Experiments 1 and 2 respectively). Previous studies have reported that higher plant densities produce smaller fruits (Cebula, 1995; Cruz-Huerta *et al.*, 2009) and that smaller fruits are correlated with smaller ovaries (Ali and Kelly, 1992).Therefore it is likely that flowers developed at higher plant densities had smaller ovaries. Relative response to LNT was also different between Experiments 1 and 2. In Experiment 1, ovary FW and volume were ~10 to 12% greater when developed under LNT compared with those developed under HNT (Table 3-3) and ~20% greater when only the last two harvest intervals were considered (Table 3-6). In Experiment 2, FW of ovaries developed under LNT were ~60% greater than that of ovaries developed under HNT (Table 3-8) or greater when data from nodes 5 to 7 within each cultivar were considered (Table 3-11). The smaller difference in ovary FW between LNT and HNT in Experiment 1 may have been due to longer exposure of ovaries to HNT before harvest, resulting in faster growth, as has been reported by Rylski (1972). As indicated, ovaries in Experiment 1 were harvested every three days, while those in Experiment 2 were harvested every day. Thus, ovaries in the HNT treatment in Experiment 1 were exposed to a longer duration of high temperatures, and likely grew faster than did ovaries under LNT, reducing the differences in ovary FW between treatments. There was also a markedly slower response to LNT with respect to

increases in ovary FW with time in Experiment 1 compared with Experiment 2, particularly in ‘Legionnaire’, ‘Red Cherry Sweet’, and ‘Ariane’. This may again reflect the larger ovary size and increased time between flower harvests in Experiment 1 compared with Experiment 2.

Although Aloni *et al*. (1999) reported that a high source:sink ratio also favors ovary swelling in bell peppers, we did not find that in the present work. The difference in leaf number we maintained (by pruning) may not have been great enough to result in a significant difference in carbohydrate supply to ovaries. Alternatively, the high plant density in Experiment 2 (17 plants/m^2) may have resulted in sufficient leaf shading such that increasing the number of leaves in a sympodial unit from four to eight did not increase whole plant photosynthesis and therefore carbohydrate supply. It is unknown, however, if under lower plant densities leaves developed under low night temperature conditions acclimate and reach similar photosynthetic rates as leaves developed under high night temperatures, as has been found in other species (Singh *et al.*, 2005).

The most visible response of bell pepper to low night temperatures during the preanthesis stages is deformed fruits caused by few or no seeds (Rylski, 1986; Pressman *et al.*, 1998a) and the formation of swollen ovaries (Rylski, 1972; Polowick and Sawhney, 1985; Pressman *et al.*, 1998b; Aloni *et al.*, 1999; Shaked *et al.*, 2004). The absence of seed or reduction in seed number is caused primarily by lack of successful pollination/fertilization (Kato, 1989; Mercado *et al.*, 1997b; Mercado *et al.*, 1997c; Shaked *et al.*, 2004). Shaked *et al.* (2004) and Pressman *et al.* (2006) reported that LNT during the 4 days prior to anthesis in bell peppers reduced pollen carbohydrate concentrations, thereby decreasing pollen viability and germinability. This may explain

why in commercial operations even 2 or 3 nights of low temperature causes deformation of the fruits developed from flowers that were exposed to LNT prior to anthesis (E. Jovicich, personal communication).

Conclusions

Low night temperature (12°C) induced ovary swelling in three types of sweet pepper (cherry, elongated, and blocky bell), with the greatest response occurring in bell peppers. Three to four weeks of continuous low night temperature were required for maximum response. This timing coincides with the time required for flower bud initiation in pepper and suggests that flowers must be exposed to LNT soon after initiation in order for this response to occur. However, the minimum duration of exposure to LNT required to elicit this response is unknown, as the present research maintained LNT throughout the course of flower development. Decreasing the source supply by leaf removal did not influence ovary size, regardless of the temperature regime. However, plant density may have resulted in plant-to-plant shading such that differences in whole plant photosynthesis (and therefore source:sink ratios) did not occur. Additional work on night temperature x source:sink ratio is necessary to further test the hypothesis that both factors interact to affect ovary swelling in sweet pepper.

Table 3-1. Relationship between days after treatment and harvest interval defined for statistical analysis in two groups of sweet pepper cultivars.

	Days after treatment[z]	
Harvest interval	Group 1	Group 2
I	1-8	1-13
II	9-16	14-26
III	17-24	27-39
IV	25-32	40-52
V	33-40	53-65

[z] Group 1: 'Brigadier', 'Legionnaire', 'Banana Supreme', and 'Red Cherry Sweet'; Group 2: 'Ariane' and 'Aristotle'.

Table 3-2. Number of flowers harvested at anthesis and number of nodes bearing flowers in six cultivars of sweet pepper grown at 22/20°C or 22/12°C day/night temperatures.

	Number of flowers/plant[z]				Number of nodes			
	Night temperature (°C)				Night temperature (°C)			
Cultivar	12	20	*P* values[y]	Mean[x]	12	20	*P* values	Mean
'Ariane'	25.0	33.3	0.04	29.2 a[w]	6.3	8.7	0.01	7.5 ab
'Aristotle'	31.3	36.0	0.29	33.7 a	8.5	9.7	0.16	9.1 a
'Brigadier'	26.7	28.0	0.82	27.3 a	6.8	7.2	0.69	7.0 b
'Legionnaire'	22.0	30.3	0.04	26.2 a	5.5	8.0	<0.01	6.8 b
'Banana Supreme'	30.0	37.3	0.12	33.7 a	7.3	8.8	0.08	8.1 ab
'Red Cherry Sweet'	27.3	34.0	0.13	30.7 a	6.2	8.3	0.01	7.3 b
Mean[v]	27.1	33.2	<0.01	30.1	6.8	8.4	<0.01	7.6

[z] All flowers were continuously harvested at anthesis during a 39-day period for 'Brigadier', 'Legionnaire', 'Banana Supreme' and 'Red Cherry Sweet' and a 64-day period for 'Ariane' and 'Aristotle'.

[y] *P* values were generated from the temperature treatment data within each cultivar; n=3 for flower number per plant and 6 for node number.

[x] Means were averaged for each cultivar across both temperature treatments; n=6 for flower number per plant and 12 for node number.

[w] Means with the same letter in the same column are not significantly different (Tukey-Kramer, $P \leq 0.05$).

[v] Means averaged across all cultivars; n=18 for flower number per plant and 36 for node number.

Table 3-3. Main effect of night temperature during the preanthesis stage on ovary characteristics of sweet pepper flowers measured at anthesis.

	Temperature treatments[y]		
Parameter[z]	22/12°C	22/20°C	*P* value
Fresh weight (mg)	84[x]	76	0.008
Volume (mm^3)	88	79	0.002
Diameter (mm)	5.5	5.3	0.003
Length (mm)	4.3	4.2	<0.001
Length:diameter ratio	0.82	0.82	0.59
Ovary wall thickness (mm)	0.59	0.57	0.004

[z] Flowers were continuously harvested at anthesis during a 39-day period for 'Brigadier', 'Legionnaire', 'Banana Supreme' and 'Red Cherry Sweet' and a 64-day period for 'Ariane' and 'Aristotle'.
[y] Day/night temperatures.
[x] Means were averaged for each temperature treatment across all cultivars and harvest intervals; n= 597 and 487 for 22/20°C and 22/12°C, respectively.

Table 3-4. Main effect of cultivar on characteristics of ovaries at flower anthesis in sweet pepper.

Cultivar[z]	Ovary FW (mg)	Volume (mm^3)	Diameter (mm)	Length (mm)	Length: Diameter ratio	Ovary wall thickness (mm)
'Ariane'	80 c[y,x]	86 c	5.6 c	4.1 c	0.74 b	0.56 b
'Aristotle'	91 bc	96 bc	5.9 bc	3.9 d	0.66 c	0.55 b
'Brigadier'	101 ab	107 ab	6.1 ab	4.2 bc	0.68 c	0.56 b
'Legionnaire'	112 a	117 a	6.4 a	4.4 a	0.69 c	0.56 b
'Banana Supreme'	48 d	49 d	4.1 d	4.5 a	1.08 a	0.61 a
'Red Cherry Sweet'	48 d	46 d	4.1 d	4.4 ab	1.07 a	0.62 a

[z] Flowers were continuously harvested at anthesis during a 39-day period for 'Brigadier', 'Legionnaire', 'Banana Supreme' and 'Red Cherry Sweet' and a 64-day period for 'Ariane' and 'Aristotle'.
[y] Means were averaged for each cultivar across both temperature treatments and all harvest intervals. For fresh weight, n = 173, 200, 158, 152, 192, and 171 for 'Ariane', 'Aristotle', 'Brigadier', 'Legionnaire', 'Banana Supreme', and 'Red Cherry Sweet', respectively. For the remaining variables, n was equal to or greater than it was for FW.
[x] Means with the same letter in the same column are not significantly different (Tukey-Kramer, $P \leq 0.05$).

Table 3-5. Main effect of harvest interval on ovary characteristics of six cultivars of sweet pepper.

Harvest interval [z,y]	Fresh weight (mg)	Volume (mm3)	Diameter (mm)	Length (mm)	Length:diameter ratio	Ovary wall thickness (mm)
I	70 d [x,w]	74 d	5.2 c	4.1 b	0.84 a	0.60 a
II	73 d	76 d	5.3 c	4.2 b	0.82 a	0.58 b
III	79 c	82 c	5.3 bc	4.3 ab	0.84 a	0.56 c
IV	86 b	89 b	5.4 b	4.3 a	0.82 a	0.56 c
V	92 a	97 a	5.7 a	4.3 a	0.79 b	0.58 b

[z] Flowers were continuously harvested at anthesis during a 39-day period for 'Brigadier', 'Legionnaire', 'Banana Supreme' and 'Red Cherry Sweet' and a 64-day period for 'Ariane' and 'Aristotle'.

[y] Harvest intervals were either 8 (39 days total) or 13 (64 days total) days, depending on cultivar (see details on Table 3-1).

[x] Means were averaged for each harvest interval across cultivar and temperature treatment; n = 160, 207, 211, 233, and 273 for intervals 1 to 5, respectively. For fresh weight, volume, FW:volume ratio, and ovary wall thickness in interval 1, n = 122.

[w] Means with the same letter in the same column are not significantly different (Tukey-Kramer, $P \leq 0.05$).

Table 3-6. Interaction of night temperature and cultivar on ovary characteristics of sweet pepper flowers harvested at anthesis during the duration of the experiment or only during the last two harvest intervals.

	Fresh weight (mg)			Volume (mm^3)			Diameter (mm)			Length (mm)		
	Night temperature (°C)											
Cultivar[z,y]	12	20	*P* value[x]	12	20	*P* value	12	20	*P* value	12	20	*P* value
All harvest intervals [w]												
ARN	75	86	0.10	81	91	0.17	5.4	5.7	0.10	4.1	4.1	0.74
ATS	101	80	0.003	108	84	0.002	6.2	5.6	0.002	4.0	3.8	0.11
BRI	104	97	0.30	112	101	0.14	6.3	6.0	0.14	4.2	4.1	0.48
LEG	119	105	0.05	126	109	0.03	6.6	6.2	0.07	4.5	4.3	0.09
BAS	54	42	0.10	55	42	0.10	4.3	4.0	0.18	4.7	4.2	<0.001
RCS	50	46	0.57	48	44	0.57	4.2	4.0	0.24	4.4	4.4	0.72
Harvest intervals IV and V [v]												
ARN	87	97	0.22	94	103	0.34	5.7	6.0	0.15	4.2	4.2	1.00
ATS	116	79	<0.001	125	83	<0.001	6.5	5.5	<0.001	4.0	3.8	0.18
BRI	122	97	0.01	132	102	0.004	6.6	6.0	0.01	4.4	4.1	0.03
LEG	133	112	0.02	141	117	0.02	6.8	6.4	0.12	4.6	4.3	0.01
BAS	65	46	0.03	68	46	0.03	4.5	4.1	0.07	5.1	4.2	<0.001
RCS	61	52	0.34	57	50	0.46	4.5	4.2	0.17	4.6	4.4	0.10

[z] Flowers were continuously harvested at anthesis during a 39-day period for BRI, LEG, BAS and RCS and a 64-day period for ARN and ATS.
[y] ARN=‘Ariane’; ATS = ‘Aristotle’; BRI =‘Brigadier’; LEG = ‘Legionnaire’; BAS = ‘Banana Supreme’; RCS = ‘Red Cherry Sweet’.
[x] *P* values were generated from the temperature treatment data within each cultivar.
[w] Means were averaged for each cultivar across all harvest intervals; n = 75 to 107, depending on temperature treatment and cultivar.
[v] Last two harvest intervals corresponded to 25 to 39 days after treatments began for BRI, LEG, BAS and RCS or 40 to 64 days after treatments began for ARN and ARS; n = 30 to 64, depending on temperature treatment and cultivar.

Table 3-7. Coefficients of conversion to estimate ovary volume at anthesis stage as a function of its fresh weight in six cultivars of sweet pepper grown under two night temperatures. Volume (mm^3) = b · FW (mg) (R^2>0.99).

	Temperature treatment (°C)		
Cultivar	22/12	22/20	Average
‘Ariane’	1.09	1.07	1.08
‘Aristotle’	1.07	1.05	1.06
‘Brigadier’	1.08	1.05	1.07
‘Legionnaire’	1.06	1.04	1.05
‘Red Cherry Sweet’	0.97	0.96	0.96
‘Banana Supreme’	1.05	1.00	1.03
Average	1.07	1.04	1.06

Table 3-8. Main effect of night temperature during the preanthesis stage on ovary characteristics of sweet pepper flowers at anthesis.

Night temperature [z]	Ovary FW (mg)	Ovary wall FW (mg)	Placenta FW (mg)	Ovary wall FW:total FW	Diameter (mm)	Length (mm)	Length: diameter ratio	Ovary wall thickness (mm)
12°C	58** [y,x]	30**	27*	0.56*	4.5*	3.8*	0.91	0.51
20°C	36	19	17	0.55	3.8	3.3	0.90	0.46

[z] Flowers were continuously harvested at anthesis for up to 45 days from nodes 2 to 7.
[y] *, ** Means are significantly different (t-test) at $P \leq 0.05$ and $P \leq 0.01$, respectively.
[x] Means were averaged across four cultivars and six nodes; n = 514 for 12°C and 401 for 20°C.

Table 3-9. Main effect of cultivar on ovary characteristics of sweet pepper flowers at anthesis.

Cultivar [z, y]	Ovary FW (mg)	Ovary wall FW (mg)	Placenta FW (mg)	Ovary wall FW:total FW	Diameter (mm)	Length (mm)	Length: diameter ratio	Ovary wall thickness (mm)
ARN	61 a[x, w]	28 a	32 a	0.47 c	4.9 a	3.5 b	0.70 c	0.47 b
LEG	62 a	29 a	33 a	0.46 c	4.9 a	3.4 b	0.69 c	0.46 b
BAS	33 b	22 b	11 b	0.65 a	3.4 b	3.9 a	1.15 a	0.50 a
RCS	32 b	20 b	12 b	0.63 b	3.3 b	3.5 b	1.06 b	0.52 a

[z] Flowers were continuously harvested at anthesis for up to 45 days from nodes 2 to 7.
[y] ARN ='Ariane'; LEG = 'Legionnaire'; BAS = 'Banana Supreme'; RCS = 'Red Cherry Sweet'.
[x] Means were averaged for each cultivar across both temperature treatments and nodes.
[w] Means with the same letter in the same column are not significantly different (Tukey-Kramer, $P \leq 0.05$); n =229, 220, 201, and 265 for 'Ariane', 'Legionnaire', 'Banana Supreme', and 'Red Cherry Sweet', respectively.

Table 3-10. Main effect of node on ovary characteristics of sweet pepper flowers at anthesis.

Node[z, y]	Ovary FW (mg)	Ovary wall FW (mg)	Placenta FW (mg)	Ovary wall FW:total FW	Diameter (mm)	Length (mm)	Length: diameter ratio	Ovary wall thickness (mm)
2	35 e[x,w]	18 e	16 d	0.55 bc	3.8 e	3.3 c	0.91 a	0.46 c
3	39 d	22 d	17 d	0.58 a	3.9 d	3.3 c	0.90 a	0.48 bc
4	46 c	24 c	21 c	0.56 ab	4.1 c	3.5 b	0.91 a	0.48 ab
5	51 b	26 b	25 b	0.54 c	4.2 b	3.7 a	0.91 a	0.50 a
6	54 ab	28 ab	26 ab	0.54 c	4.4 ab	3.7 a	0.90 a	0.50 a
7	56 a	29 a	27 a	0.54 bc	4.5 a	3.8 a	0.90 a	0.51 a

[z] Flowers were continuously harvested at anthesis for up to 45 days from nodes 2 to 7.
[y] Days after treatment at which nodes were harvested: 2: 2-9; 3: 6-17; 4: 11-26; 5: 16-31; 6: 22-38; 7: 28-42, depending on the cultivar.
[x] Means were averaged across all four cultivars and two temperature treatments; n = 179, 154, 154, 161, 148, 119 for nodes 2, 3, 4, 5, 6, and 7, respectively.
[w] Means with the same letter in the same column are not significantly different (Tukey-Kramer, $P \leq 0.05$).

Table 3-11. Interaction of night temperature and cultivar on ovary characteristics of sweet pepper flowers harvested at anthesis.

	Ovary FW (mg)			Ovary wall FW (mg)			Placenta FW (mg)			Diameter (mm)			Length (mm)		
	Night temperatures (°C)														
CV[z,y]	12	20	*P* value	12	20	*P* value	12	20	*P* value	12	20	*P* value	12	20	*P* value
Nodes 2-7															
ARN	74[x]	47	<0.001[w]	35	22	<0.001	39	26	<0.001	5.4	4.5	<0.001	3.7	3.2	<0.001
LEG	79	45	<0.001	37	20	<0.001	42	25	<0.001	5.4	4.3	<0.001	3.6	3.1	<0.001
BAS	39	26	0.04	26	17	0.01	13	9	0.21	3.5	3.2	0.13	4.2	3.5	<0.001
RCS	38	25	0.04	23	16	0.03	15	9	0.06	3.6	3.1	0.007	3.8	3.3	<0.001
Nodes 5-7															
ARN	88[v]	52	<0.001	40	22	<0.001	48	29	<0.001	5.8	4.6	<0.001	4.0	3.3	<0.001
LEG	93	49	<0.001	43	21	<0.001	50	28	<0.001	5.8	4.5	<0.001	3.8	3.1	<0.001
BAS	45	27	0.01	30	18	0.01	15	9	0.09	3.7	3.2	0.04	4.5	3.6	<0.001
RCS	48	25	<0.001	29	16	<0.001	19	9	0.01	4.0	3.1	<0.001	4.1	3.3	<0.001

[z] Flowers were continuously harvested at anthesis for up to 45 days from nodes 2 to 7.
[y]CV=cultivars. ARN: 'Ariane', LEG: 'Legionnaire', BAS: 'Banana Supreme', RCS: 'Red Cherry Sweet'.
[x] Means were averaged for each cultivar across all nodes harvested; n = 67 to 134, depending on temperature treatment and cultivar.
[w] *P* values were generated from the temperature treatment data within each cultivar.
[v] Means were averaged for each cultivar across nodes 5, 6 and 7; n = 23 to 68, depending on temperature treatment and cultivar.

Table 3-12. *P* values of t-test comparison between night temperatures of 12 and 20°C within each cultivar and node for ovary fresh weight (FW), diameter (Diam.), and length.

	Cultivar [z,y]											
	ARN			LEG			BAS			RCS		
Node	FW	Diam.	Length	FW	Diam.	Length	FW	Diam.	Length	FW	Diam.	Length
2	0.86[x]	0.97	0.42	0.04	0.02	0.04	0.49	0.58	0.04	0.76	0.61	0.98
3	0.15	0.04	0.22	<0.001	<0.001	0.03	0.06	0.15	<0.001	0.76	0.97	0.23
4	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.10	0.10	0.002	0.23	0.13	0.003
5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.08	0.08	<0.001	0.004	<0.001	<0.001
6	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	0.14	<0.001	<0.001	<0.001	<0.001
7	<0.001	<0.001	0.002	<0.001	<0.001	0.007	0.08	0.51	<0.001	<0.001	<0.001	<0.001

[z] Flowers were continuously harvested at anthesis for up to 45 days from nodes 2 to 7.
[y] ARN ='Ariane'; LEG = 'Legionnaire'; BAS = 'Banana Supreme'; RCS = 'Red Cherry Sweet'
[x] In all cases, *P* values ≤0.05 mean that ovaries developed at 12°C had significantly greater fresh weight, diameter or length than the ovaries developed at 20°C.

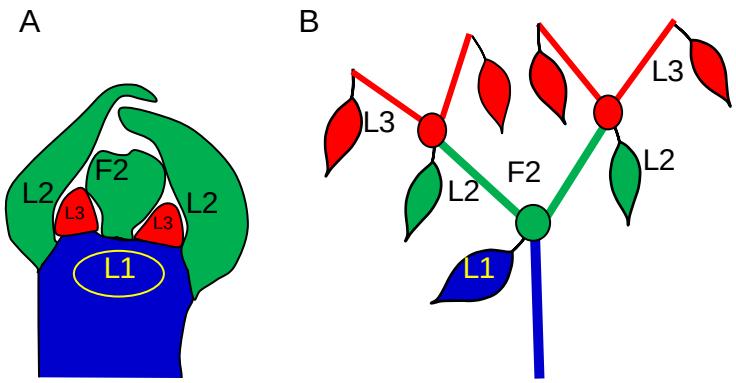

Figure 3-1. Schematic drawing of pepper branching and flowering sequence. A) Meristematic stage. B) Well developed branch. The sympodial shoot bears a leaf (L1, blue), and terminates in a flower (F2). From the leaf axil, two leaves (L2) develop. The flower and the two leaves developed from the same sympodial shoot compose a sympodial unit (F2 and L2, green). L2 leaves are 'pushed up' by internode elongation, so that the leaves appear above the preceding flower. The shoot terminates in a flower, which is part of the subsequent sympodial unit. From each of the leaf 2 axils, two new leaves (L3, red) develop. Each color represents sympodial units of a different order (Drawing A based on micrograph from Elitzur *et al.*, 2009).

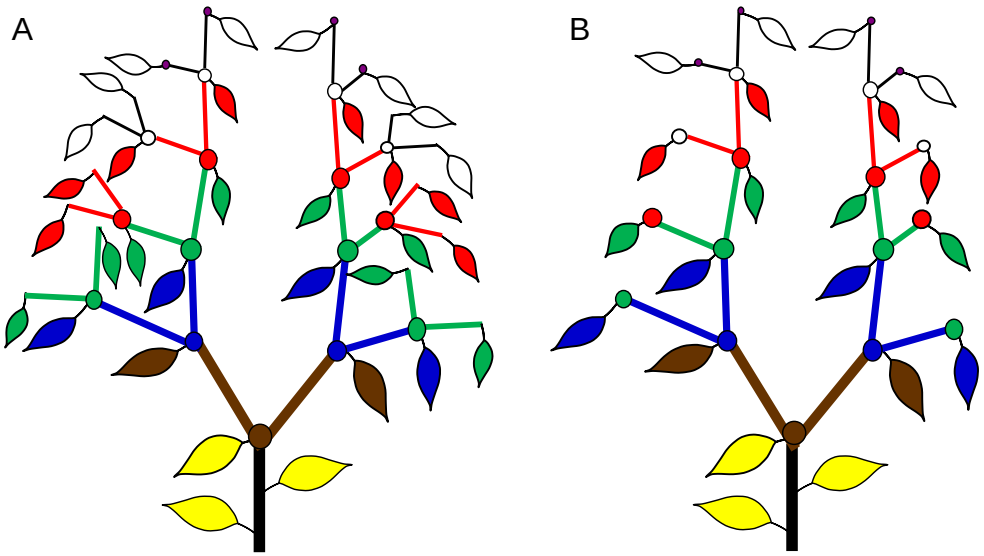

Figure 3-2. Schematic drawing of a sweet pepper plant pruned to two main axes with lateral branches. Two shoots were allowed to grow at the first branching to form the main-stem sympodial unit (SU, brown). A) Starting in SU1 (blue), the strongest shoot was allowed to grow and the second shoot was limited to development of one sympodial unit (one flower and two leaves) by pruning. This pruning system was used in all the plants in Experiment 1 and half of the plants in Experiment 2. B) The strongest shoot was allowed to grow and the second shoot was pruned so that only the flower of the next sympodial unit remained. This system was used in half of the plants in Experiment 2. In both A and B, flowers from the main stem and first SU were removed before treatment started. SU1= blue; SU2 = green; SU3= red, SU4= white.

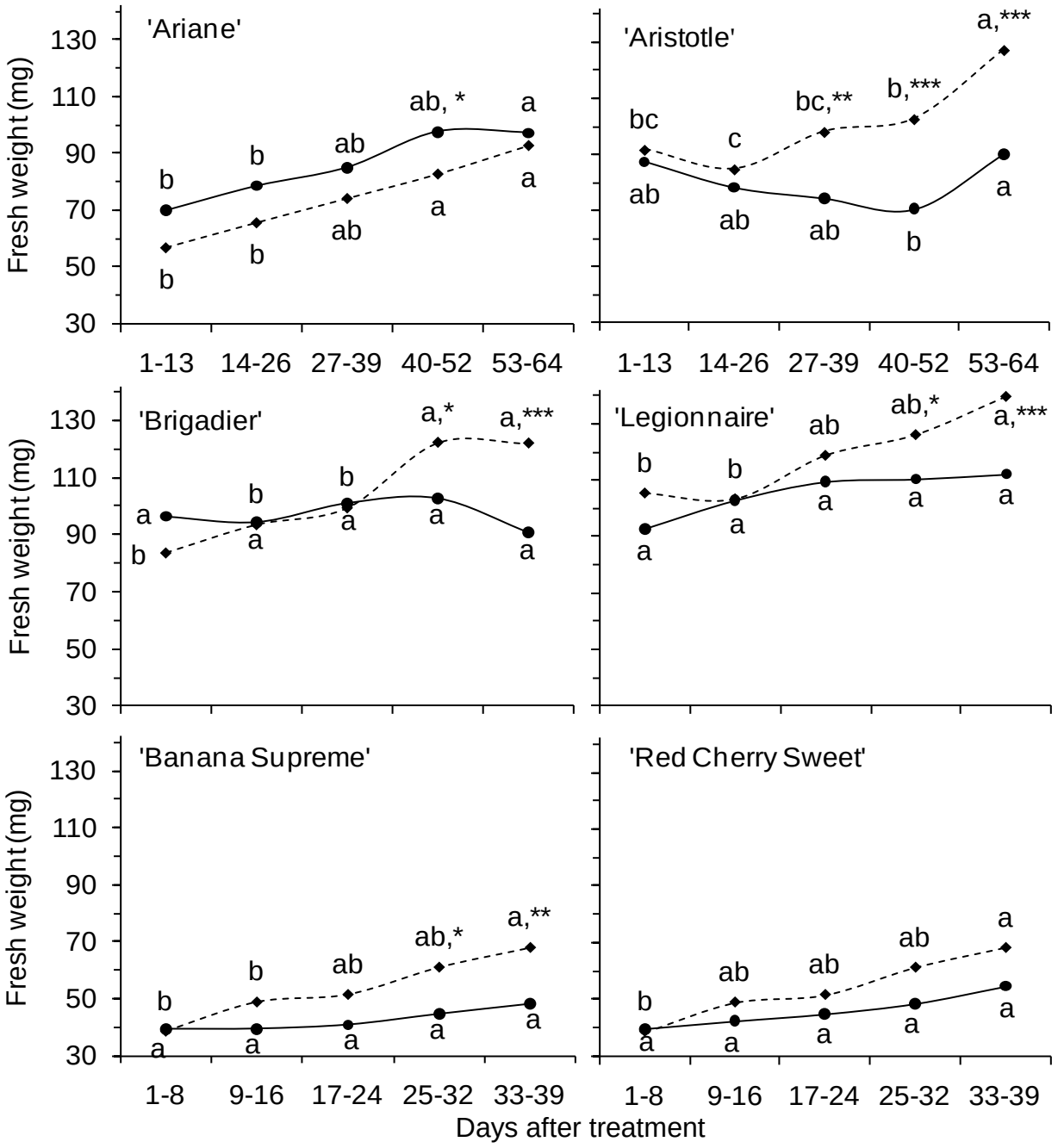

Figure 3-3. Ovary fresh weight of sweet pepper flowers at anthesis developed under 22/12°C (--♦--) or 22/20°C (—●—) day/night temperatures for discrete harvest intervals. Plants did not bear any fruit before and during the experiment. Means with the same letter in the same temperature treatment and cultivar are not significantly different (Tukey-Kramer, $P \leq 0.05$). *, **, ***: Means between temperature treatments within a harvest interval for each cultivar are significantly different at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Range of n values for harvest intervals: 1st: 7-20; 2nd: 11-25; 3rd: 12-23; 4th: 12-29; 5th: 14-35.

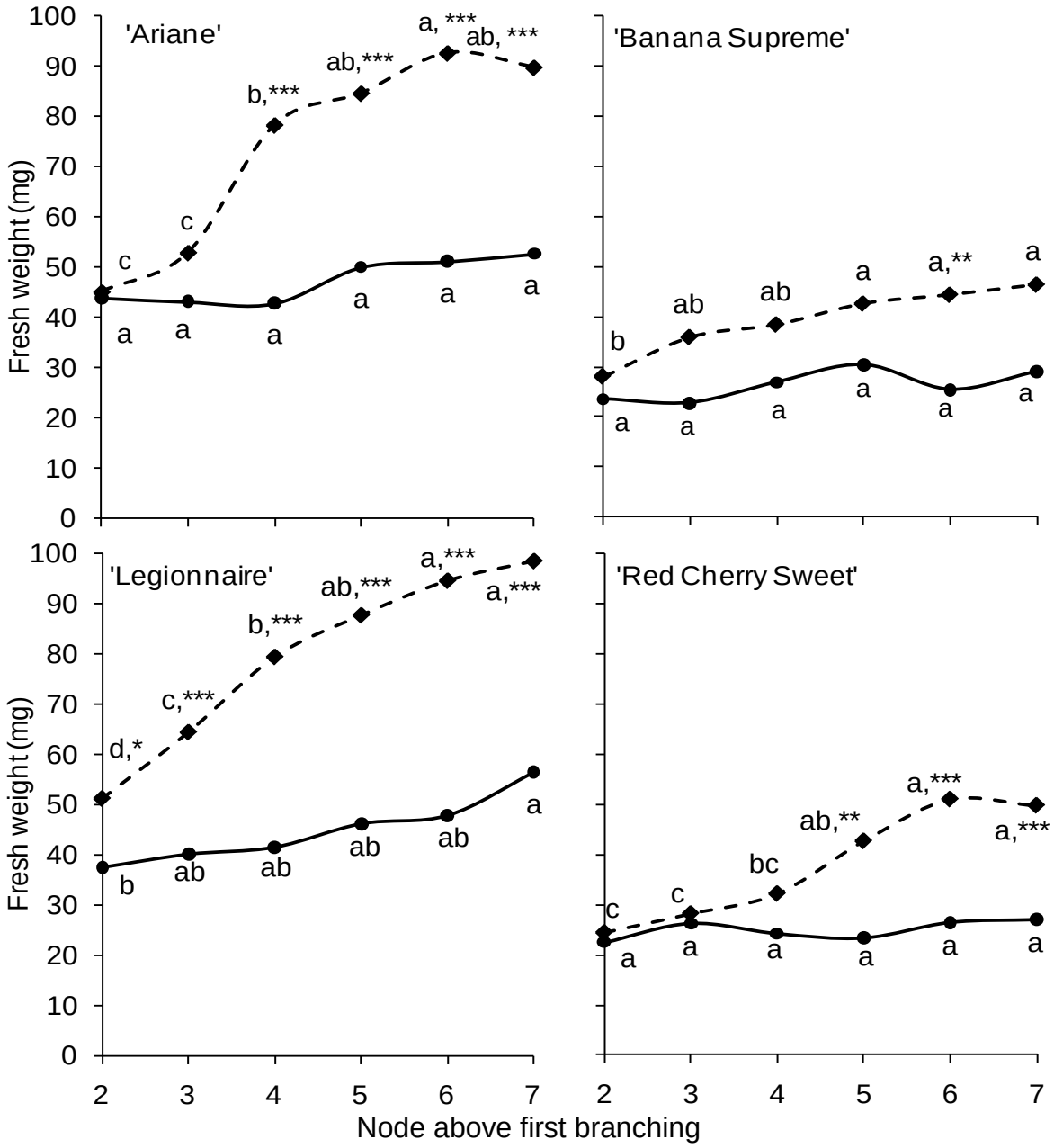

Figure 3-4. Ovary fresh weight of sweet pepper flowers harvested at anthesis from the second to the seventh nodes above the first flowering node in plants grown at day/night temperatures of 22/12°C (--♦--) or 22/20°C (—●—). Plants did not bear any fruit. Means with the same letter in the same temperature treatment and cultivar are not significantly different (Tukey-Kramer, $P \leq 0.05$). *, **, ***: Means between temperature treatments within a cultivar and node are significantly different at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Range of n values for the temperature treatments: 22/12°C: 15-23; 22/20°C: 8-24, excluding 'Banana Supreme' node 2, where n=2. Days after treatment at which nodes were harvested: 2: 2-9; 3: 6-17; 4: 11-26; 5: 16-31; 6: 22-38; 7: 28-42, depending on the cultivar.

CHAPTER 4
LOW NIGHT TEMPERATURE AFFECTS CARBON EXCHANGE RATES OF SWEET PEPPER LEAVES

Introduction

Pepper (*Capsicum annuum* L) is an herbaceous species that belongs to the family Solanaceae (Eshbaugh, 1993), and its fruit is one of the most consumed vegetables worldwide (Mateos *et al.*, 2003). Pepper fruits, especially sweet peppers, are an excellent source of essential nutrients, especially vitamin C, β-carotene, calcium and antioxidant compounds (LopezHernandez *et al.*, 1996; Mateos *et al.*, 2003; Sun *et al.*, 2007; Mateos *et al.*, 2009). Cultivated species of peppers were domesticated in tropical and temperate areas in Mexico, Central America and South America (Bolivia), and are sensitive to low temperatures (Harlan, 1971; Eshbaugh, 1993).

Low temperature is one of the major factors limiting the productivity and geographical distribution of many plant species (Li *et al.*, 2003a). Low temperature effects on physiology and growth have been studied in several agronomic and horticultural crops (Koscielniak, 1993; Gibson and Mullen, 1996; Boese *et al.*, 1997; Wang *et al.*, 1997; Roussopoulos *et al.*, 1998; Sukhvibal *et al.*, 1999; Venema *et al.*, 1999a; Adams *et al.*, 2001; Hendrickson *et al.*, 2004a; Hendrickson *et al.*, 2004b; Singh *et al.*, 2005; Limin and Fowler, 2006), including pepper (Mercado *et al.*, 1997b; Pressman *et al.*, 1998b; Aloni *et al.*, 1999; Li *et al.*, 2004; Pressman *et al.*, 2006). One of the more striking effects of low temperature, particularly low night temperature, on pepper growth is an increase in swollen floral ovaries and subsequent deformed fruit (Polowick and Sawhney, 1985; Rylski, 1985; Mercado *et al.*, 1997b; Pressman *et al.*, 1998a; Aloni *et al.*, 1999; Shaked *et al.*, 2004).

Among other physiological effects, low temperatures reduce photosynthetic rates. Day temperatures of 6-7°C may cause rapid stomatal closure in a variety of plant species (Boese *et al.*, 1997; Wilkinson *et al.*, 2001) and subsequent photoinhibition (Liu *et al.*, 2001; Li *et al.*, 2003a; Li *et al.*, 2004; Zhang and Scheller, 2004). Photosynthetic enzyme activity may also be reduced under low temperatures (Kingston-Smith *et al.*, 1997).

On the other hand, low night temperatures (*i.e.*, below 10-15°C) delay the start and slow the rate of stomatal opening the following morning, as temperatures increase (Hendrickson *et al.*, 2004a; Hendrickson *et al.*, 2004b). Night temperatures below 15°C may also decrease photosynthesis by decreasing ribulose-1,5-bisphosphate regeneration and Pi availability for recycling (Hendrickson *et al.*, 2004a; Hendrickson *et al.*, 2004b), reducing activity of PSII (Sundar and Reddy, 2000; Bertamini *et al.*, 2005), and decreasing leaf chlorophyll content (Sundar and Reddy, 2000; Bertamini *et al.*, 2005). Reduction in Rubisco amount and activity, as well as other photosynthetic/carbohydrate metabolizing enzymes, such as fructose bisphosphatase and sucrose phosphate synthase, may also occur in response to low night temperature (Sundar and Reddy, 2000; Ramalho *et al.*, 2003; Hendrickson *et al.*, 2004a; Hendrickson *et al.*, 2004b; Bertamini *et al.*, 2005). Low night temperature (5°C) followed by high irradiance (1900 PPF) also decreases photosynthesis by increasing photoinhibition (Bertamini *et al.*, 2006).

Tropical crops, including pepper, are especially sensitive to low night temperature, and one cold night of 4 to 12°C may decrease photosynthetic rates 5 to 80% (Van-de-Dijk, 1985; Van-de-Dijk and Maris, 1985; Wolfe, 1991; Janssen *et al.*, 1992; Boese *et*

al., 1997; Ying *et al.*, 2000, and references therein; Li *et al.*, 2003a; Ramalho *et al.*, 2003; Wang *et al.*, 2004). Bhatt and Srinivasa-Rao (1993b) reported that net CO_2 exchange rates in sweet pepper were 6 to 30% higher at night temperatures of 22°C compared with 17°C.

Some species have the capacity to acclimate to low temperatures. In such cases, exposure to low temperatures for several days may markedly increase the maximum activities of Rubisco, stromal and cytosolic fructose-1,6-bisphosphatase, and sucrose-phosphate synthase in leaves, RuBP regeneration and carboxylation, and lead to significant increases in whole plant photosynthetic capacity (Holaday *et al.*, 1992; Hurry *et al.*, 1994; Du *et al.*, 1999; Yamori *et al.*, 2005). In pepper, plants that were cold acclimated (5 days at 14°C and 250 PPF) exhibited greater photosynthetic rates compared to non-acclimated plants upon subsequent exposure to low temperatures (4°C, 1200 PPF) (Liu *et al.*, 2001).

Respiration rates are also affected by low temperature, with rates in both the light and the dark increasing as temperature increases up to about 40°C (Lin and Markhart, 1990; Taiz and Zeiger, 2006). In the short term, respiration is highly responsive to ambient temperatures; however, in the long-term, temperature acclimation can occur. Tjoelker *et al.* (1999) reported that respiration in five species of high-temperature acclimated plants (30/24°C day/night) was lower than that of low-temperature acclimated plants (18/12°C day/night) when measured at temperatures between 12 and 30°C.

Net carbon exchange rates (CER) and ovary swelling in pepper are affected not only by temperature, but also by source:sink ratios. Net CER increased in newly

developed leaves of fruiting compared with non-fruiting pepper (Cruz-Huerta *et al.*, 2005). In bell pepper, fruiting decreased vegetative growth rates compared to non-fruiting plants (Hall and Brady, 1977; Hall and Milthorpe, 1978; Bhatt and Srinivasa-Rao, 1989), since growing fruits represent up to 90% of sink demand (Hall, 1977). Defruiting, which rapidly increases the source:sink ratio, increased the incidence of flower deformation and swollen ovaries in pepper (Aloni *et al.*, 1999).

The incidence of swollen ovaries in bell pepper due to low night temperature or high source:sink ratio has been correlated with increased ovary carbohydrate concentration in the flower bud (Aloni *et al.*, 1999). Under low night temperature, pepper plants exhibit slower growth rates, resulting in decreased shoot dry weight compared with plants grown under higher night temperatures (Mercado *et al.*, 1997a). In some species, leaves developed under low night temperature may acclimate to such conditions, resulting in carbon exchange rates as high as in plants growing under warmer temperatures (Singh *et al.*, 2005). Therefore, similar photosynthetic rates and slower growth rates under low night temperatures may cause excess carbohydrate accumulation in floral ovaries, resulting in swelling and deformed fruits. Similarly, developing fruits in bell pepper are a strong sink, and defruiting pepper plants increases starch concentration in stems (Hall and Milthorpe, 1978), which may also result in an excess carbohydrate supply to floral ovaries. The hypothesis tested in the present experiments is that sweet pepper leaves developed under low night temperatures acclimate to such conditions and have similar carbon exchange rates as leaves developed under high night temperatures. This, combined with reduced growth under low night temperatures, increases the incidence of swollen or deformed ovaries in

pepper flowers. A second hypothesis tested is that fruiting decreases the incidence of swollen ovaries, regardless of night temperature. The objectives were to 1) measure net CER, growth, and ovary swelling in two cultivars of pepper grown under low and high night temperatures and 2) determine fruiting effects on net CER, growth, and ovary swelling in plants grown under two night temperatures.

Material and Methods

Plant Material and Growing Conditions

The experiments were carried out using two types of sweet pepper with contrasting fruit size and fruit shape - 'Legionnaire' bell pepper (Experiments 1, 2, and 3) and 'Red Cherry Sweet' cherry pepper (Experiment 1). Seeds were germinated in a mix of peat moss, vermiculite, and dolomite limestone (SunGro Metro-Mix Ag-Lite Mix[2]) and grown in 1.4 m^2 growth chambers (Mod E15, Conviron, Winnipeg, Canada). Chamber temperature was set to 22/20°C (day/night), and PPF was maintained between 450-500 µmol m^{-2} s^{-1} above the canopy with a 14-hour photoperiod, as described in Chapter 3. Seedlings at about the sixth-leaf stage (~ 50 days after sowing) were transplanted to 1.5 L containers, using the same growing media for germination, and maintained in the growth chambers throughout the experiments.

Plants were fertigated as needed with the following nutrient solution (mM): 3.4 $Ca(NO_3)_2$, 1.8 KNO_3, 1.6 KH_2PO_4, 0.3 KCl, 2.7 $MgSO_4$, and (µM): 50.2 Fe-EDTA, 3.1 $CuSO_4$, 14.6 $MnSO_4$, 64.8 H_3BO_3, 4.6 $ZnSO_4$, 0.6 Na_2MoO_4 (modified from Cruz-Huerta *et al.*, 2005; and Jovicich, 2007). Pests were controlled as described in Chapter 3.

[2] Composition: Canadian Sphagnum peat moss (55-65%), horticultural grade vermiculite, dolomite limestone, starter nutrient charge, and wetting agents (www.sungro.com/products.php, 12/17/2008).

Plants were pruned to two main axes, with one lateral sympodial unit (one flower and two leaves) on every node in the main axis, as described in Chapter 3 (Figure 3-2A).

Two experiments were conducted to measure net CER and biomass accumulation under low (12°C) *vs* high (20°C) night temperatures (Experiments 1 and 2) and different fruit loads (Experiment 2).

Experiment 1

Eighteen seedlings each of ‘Legionnaire’ and ‘Red Cherry Sweet’ were selected when flowers in nodes 3 or 4 were at anthesis (~20 days after transplanting). Growth chamber temperatures were set to 22°C day, with night temperatures of either 20° (high night temperature, HNT) or 12°C (low night temperature, LNT). Each growth chamber was divided into three blocks to reduce PPF variation and three plants of each cultivar were randomly placed within each block. All the flowers were removed; therefore plants did not bear any fruit.

Gas exchange measurements were performed using a portable photosynthesis system (LI-6400, Licor, Lincoln, NE, USA) on the most recent mature leaf, which was the fourth or fifth from the apex. Measurements included net CER, stomatal conductance (g_s), intercellular CO_2 concentration (Ci), and leaf dark respiration (R_D, day and night). Net CER measurements were performed about six hours after lights were on and were done every three days during a 36-day period after treatments started, with a final measurement at 45 days. This period of time ensured that several new leaves in each axis completely developed under the temperature treatments. Based on data reported by Choi and Gerber (1992) and Cruz-Huerta (2001), sweet pepper leaves take four to five weeks from the beginning of formation until full expansion. Respiration measurements during the day were performed 60 to 90 seconds after net CER

measurements were completed, by covering the top of the assimilation chamber with an opaque card, and during the night before the dark period ended. Respiration measurements during the day were performed only twice at the beginning of the experiment (1 and 3 days after treatment, DAT) since no difference among treatments was found. Night respiration rates were measured at 13, 27 and 47 DAT. Fresh weight of flowers and flower parts (ovary, calyx, and petals) at anthesis and ovary diameter and length were recorded on flowers harvested from 30 to 37 DAT. At the end of the experiment, leaf area, dry weight per organ, plant height, and number of internodes were also recorded.

Experiment 2

Twenty-four 'Legionnaire' seedlings were selected ~30 days after transplanting and flower buds from the main-stem sympodial unit (first bifurcation) and sympodial unit 1 (node 1) were removed. Flowers on nodes 2 and 3 were hand-pollinated, allowing one fruit per axis (2 fruits per plant) to develop beyond petal fall, the indicator of fruit set. About ten days after petal fall, when the fruits were rapidly growing (Marcelis and Baan-Hofman-Eijer, 1995b), night temperature (HNT *vs* LNT) and source:sink ratio treatments began. Fruits were either allowed to grow or were removed in order to vary the source:sink ratio, resulting in a 2 x 2 factorial (two night temperatures and two source:sink ratios) with six replications.

As in Experiment 1, net CER measurements were performed using a portable LI-6400 photosynthesis system. Measurements were done every three to four days during a 41-day period on one to three of the most recently matured leaves per plant. Photosynthesis was measured about six hours after lights were on. Measurement on day 19 was not considered in the final analysis since plants were under water stress

when measured. Fresh weight of flowers and flower parts at anthesis and ovary diameter and length were recorded on flowers harvested from 30 to 36 DAT. Organ dry weight, plant leaf area, and plant height were recorded at end of the experiment.

Data Analysis

Data from all experiments were analyzed as a completely randomized block design, with repeated measures, except for the dry weight in Experiments 1 and 2. The subject for repeated measures was the individual plant. Comparisons performed were night temperatures, days after beginning of treatment (Experiments 1 and 2), cultivars (Experiment 1), and source:sink ratio (Experiment 2). Data from the two measurement dates for day respiration were pooled and analyzed as a 2x2 factorial (night temperature x cultivars). Night respiration data were analyzed as a factorial (night temperature x cultivars x measurement dates) with repeated measures over time.

Results

Experiment 1

Low night temperature increased flower, flower parts, and ovary fresh weight and ovary size, compared to HNT (Table 4-1). Flower fresh weight in the LNT was 32% greater than in the HNT. As expected, bell pepper had larger flowers and ovaries than cherry type pepper. The interaction between cultivar and night temperature was significant for total flower fresh weight, calyx fresh weight, and ovary diameter and length. After four weeks of night temperature treatments, ovaries of ‘Red Cherry Sweet’ exhibited a proportionately greater increase in size under LNT compared with HNT than did ovaries of ‘Legionnaire’. However, we selected ‘Legionnaire’ for the subsequent experiments due to the more rapid response of this cultivar to LNT compared to the slower initial response in ‘Red Cherry Sweet’ (Chapter 3).

Carbon exchange rate was significantly influenced by the cultivar, DAT, and interactions between DAT x cultivar and DAT x temperature (Table 4-2). Stomatal conductance was influenced by DAT and interactions where DAT was a factor, while Ci was influenced only by DAT and the interaction of DAT x cultivar.

Net CER was significantly higher in 'Red Cherry Sweet' than 'Legionnaire' over the course of the experiment (Table 4-3); however, night temperature had no effect on net CER, stomatal conductance, or intercellular CO_2.

Regardless of cultivar, CER decreased over time (Figure 4-1A). There were two periods where CER decreased rapidly; the first occurring 6 to 9 DAT and the second occurring 24 to 27 DAT. Stomatal conductance followed a similar pattern over time (*i.e.* DAT) as net CER, although with more pronounced decreases (Figure 4-1B). The intercellular CO_2 concentration (Ci) also decreased with time. Ci decreased rapidly from 287 to 196 µmol mol^{-1} during the first 6 DAT. After the initial decrease, Ci increased to reach a peak at 19 DAT, followed by a second peak at 30 DAT, before finally decreasing to ~220 µmol mol^{-1} after 33 DAT (Figure 4-1C).

Although the main effect of night temperature on net CER was not significant (Table 4-3); there was a significant interaction of DAT x temperature on net CER (Figure 4-2A). Three days after the beginning of the experiment, plants under LNT had significantly greater CER than those under HNT. After that, however, plants under HNT had either similar or significantly greater CER than those under LNT. Differences in net CER between cultivars were found between 15 and 21 DAT and at the end of the experiment (between 36 and 45 DAT), with 'Red Cherry Sweet' exhibiting greater CER than 'Legionnaire' (Figure 4-2B).

The interaction between DAT x night temperature on stomatal conductance (Figure 4-3A) was similar to that observed for net CER. Stomatal conductance was higher under LNT compared with HNT the first three days after treatments began, but were significantly lower between 21 and 33 DAT. Significant differences in stomatal conductance between cultivars were found only at 15 and 19 DAT (Figure 4-3B). The initial decrease in g_s, which was similar in both cultivars, was followed by a transient increase, with a greater increase observed in 'Legionnaire' compared with 'Red Cherry Sweet' (Figure 4-3B). The increase in g_s in 'Red Cherry Sweet' correlated with the increase observed in CER (Figure 4-2B); however, no such correlation was observed in 'Legionnaire'.

Internal CO_2 concentrations were significantly higher under LNT compared with HNT during the first 6 days of treatment and significantly lower from day 19 to day 24 (Table A-4, Appendix), which corresponded with patterns observed in both CER (Figure 4-2A) and g_s (Figure 4-3A). Although the interaction between DAT and cultivar was significant for Ci, differences between cultivars were observed only at 6 DAT, where Ci in 'Legionnaire' was significantly greater than in 'Red Cherry Sweet' (Table A-4, Appendix).

During the day, leaf respiration was not affected by night temperature (P=0.71), but was affected by cultivar (P≤0.04, Figure 4-4 A, B). No interaction between night temperature and cultivar was found (P=0.14). During the night, leaf respiration was significantly reduced by LNT compared to HNT (*P*≤0.001). Night leaf respiration was lower in 'Legionnaire' bell pepper compared to 'Red Cherry Sweet'. Night respiration was affected by DAT, increasing from 0.67 µmol m^2 s^{-1} at 13 DAT to 0.85 µmol m^2 s^{-1} at

47 DAT ($P \leq 0.008$). There were no significant interactions among night temperature, cultivar and DAT on night respiration rate (data not shown).

Assuming constant CER during the day and uniform respiration rates during the night, carbon balance for leaves was calculated (Table 4-4). At the end of the experiment, sweet pepper plants under LNT gained less carbon during the day, and although they respired less during the night, LNT plants gained less carbon than did HNT plants during a 24-h period. There were no significant effects of cultivar or the interaction of night temperature x cultivar.

LNT reduced leaf number and specific leaf area compared to HNT, but there was no significant difference in total leaf area (Table 4-5). No significant temperature effects were found in plant or organ dry weight. Plants growing under LNT, however, were shorter due to shorter internodes and had smaller main stem diameter compared to plants growing under HNT. There were significant differences in leaf number, leaf area, dry weight, and plant height between cultivars, but there were no significant interactions between night temperature and cultivar.

Experiment 2

Both LNT and reduced sink demand (i.e, non-fruiting plants) increased fresh weight of the flower, flower parts, and the ovary diameter compared to HNT (Table 4-6). Ovary length was affected only by night temperature.

Both LNT and the absence of fruit significantly decreased CER and g_s in ‘Legionnaire’ bell pepper (Tables 4-7 and 4-8). Intercellular CO_2 concentration was decreased under LNT, but was not affected by the presence or absence of fruit.

In general, CER, g_s, and Ci decreased over time (*i.e.*, DAT). A dramatic decrease in CER (26%), g_s (58%), and Ci (23%) was observed within 6 days after the beginning of night temperature treatments (Figure 4-5 A, B, and C). After this initial decrease, there was little statistical difference in CER, g_s, or Ci with time, although there was some variability. Initial values of CER at 3 DAT in Experiment 2 were similar to the initial rates in Experiment 1 (11-12 µmol m^{-2} s^{-1}), and to the rates previously reported by Cruz-Huerta (2005) at fruit set of the first fruit (12-14 µmol m^{-2} s^{-1}).

Gas exchange parameters were significantly influenced by the interaction between night temperature and days after treatment (Figure 4-6 A, B, and C). However, CER was significantly lower under LNT compared with HNT, except for two measurements dates (22 and 24 DAT) (Figure 4-6A). Stomatal conductance and intercellular CO_2 concentration (Figure 4-6B) responses were very similar to that of CER.

There were no significant interactions between night temperature x fruiting or DAT x fruiting on gas exchange parameters. Initially, net CER was similar between fruiting treatments and decreased with time until ~6 DAT. By 15 DAT, CER in non-fruiting plants decreased markedly, resulting in significantly lower CER compared with fruiting plants throughout the rest of the experiment (Figure 4-7).

LNT reduced the specific leaf area, but not leaf number or total leaf area (Table 4-9). No significant temperature effects were found on plant or organ dry weight. Plants growing under LNT were shorter due to fewer nodes compared to plants growing under HNT.

Fruiting plants had decreased leaf number per plant and greater specific leaf area compared to the non-fruiting plants, but leaf area was not affected (Table 4-9). Non-

fruiting plants had increased leaf, stem and root DW compared to fruiting plants. However, due to the obvious differences in fruit DW, total plant DW was not affected by the presence/absence of fruits. No interactions between night temperature and fruiting treatment were found for any of the parameters recorded.

Discussion

Low night temperatures (*i.e.* less than 15°C) and/or low sink demand (*i.e.*, presence of developing fruits) induced larger flowers and swollen ovaries in our study, as has been previously reported (Polowick and Sawhney, 1985; Aloni *et al.*, 1999; Shaked *et al.*, 2004). Previous results (Aloni *et al.*, 1999) showed that ovary swelling in bell pepper was correlated with increased concentration of reducing sugars and starch in the flower bud. Since carbohydrate concentration and content ultimately depends on plant photosynthesis, we quantified leaf carbon exchange rates, dry weight accumulation, and incidence of ovary swelling in pepper plants grown under low *vs* high night temperature and different source:sink ratios.

In the present work, sweet pepper leaves developed under LNT exhibited lower net CER compared with leaves developed under HNT. These results do not support our hypothesis that leaves fully developed under LNT conditions acclimate and maintain photosynthetic rates as high as those from leaves that developed under HNT. Our results are in contrast to previous reports on tomato (Van-de-Dijk and Maris, 1985), cotton (Koniger and Winter, 1993; Singh *et al.*), guayule (*Parthenium argentatum* Gray) (Sundar and Reddy, 2000), and common bean (*Phaseolus vulgaris* L) (Wolfe, 1991; Wolfe and Kelly, 1992). In these species, leaves that developed under low night temperatures exhibited CER similar to or greater than CER of leaves developed under warm night conditions. However, our results are in agreement with work in other tropical

and subtropical plants, where low night temperature (6 to 10°C below the control) decreased CER between 20 and 50% (Wolfe, 1991; Bruggemann *et al.*, 1992; Diczbalis and Menzel, 1998; Venema *et al.*, 1999b).

In our experiments, CER and g_s decreased over time, independently from night temperature, cultivar, or presence/absence of developing fruits. In general, CER decreased significantly in the first 6-9 days after treatments began. The decrease in CER over time was accompanied by decreases in both g_s and Ci concentrations, suggesting stomatal limitation to CER. This is in agreement with work by Hall and Milthorpe (1978), who reported that decreased leaf CER in bell pepper was correlated with decreased leaf and intracellular conductance.

Both net CER and night respiration were reduced by LNT compared to HNT. The lower respiration during the dark period partially compensated for the carbon balance during a 24-h period. However, considering only the leaf tissue, carbon gain during a 24-h period was higher under HNT compared to LNT. Whole plant respiration was not calculated since only leaf respiration data were available. However, on a whole plant basis, it is likely that differences in net carbon gain between LNT and HNT were negligible since no differences in DW between LNT and HNT were found. Frantz *et al.* (2004) reported that when varying night temperature from 17 to 32°C in rapidly growing lettuce, tomato and soybean, respiration rates during the night increased 2 to 4% per Celsius degree. However, photosynthetic rates remained steady or decreased slightly as night temperature increased and dry mass accumulation was not affected.

Although LNT decreased photosynthetic rates without decreasing total plant or organ dry weight, plant height, stem diameter and specific leaf area were reduced

compared with plants grown under HNT. Similarly, Mercado (1997a) reported that cool day/night temperatures (25/14°C) significantly decreased stem height and specific leaf area of bell pepper compared with plants grown under warm day/night temperatures (29/20°C).

Fruit removal (Expt. 2) decreased net CER compared to no fruit removal, supporting previous work in pepper (Hall and Milthorpe, 1978) and tomato (Hucklesby and Blanke, 1992) and other species (Setter *et al.*, 1980; Fujii and Kennedy, 1985; Schaffer *et al.*, 1987; Gucci *et al.*, 1991; Gucci *et al.*, 1995) indicating that increasing the source:sink ratio decreased leaf net CER. This decrease was likely due to non-stomatal rather than stomatal limitations, as fruit removal decreased CER without affecting Ci. The most likely non-stomatal limitation is end-product inhibition (Layne and Flore, 1995; Pieters *et al.*, 2001; Sawada *et al.*, 2001). Plants with low sink strength exhibit decreased sucrose synthesis due to low demand from the rest of the plant (Pieters *et al.*, 2001). Low sucrose synthesis reduces the recycling of Pi to the chloroplast, ATP synthesis and RUBP regeneration, and therefore, photosynthesis (Pieters *et al.*, 2001). Under sink-limited conditions, leaf starch concentration increases and specific leaf area decreases (Nederhoff *et al.*, 1992), as was found in our work. In addition, plant manipulations that cause sugars to accumulate can decrease the expression of photosynthetic genes and upregulate genes for improved C metabolism, increasing, among others, sucrose metabolism and starch accumulation (Koch, 1996), which also explain the decrease in CER due to fruit removal.

Although fruit removal decreased net CER overall, initially there was no effect of the presence or absence of fruit on CER, as CER in both treatments decreased during

the first 6 DAT and significant differences between fruiting treatments were not manifested until after ~15 DAT. The initial decrease in CER observed in fruiting plants in our study is in contrast to work by Cruz-Huerta *et al.* (2005), who reported that CER in pepper increased 40 to 50% between fruit set and 14 days after fruit set, and by Van-de-Dijk and Maris (1985), who reported that net CER increased over time in fruiting tomato plants. In our experiment, two developing fruits may not have resulted in sufficient sink demand initially to cause the increase in CER observed in previous work, where fruit load was greater (Bhatt and Srinivasa-Rao, 1997; Cruz-Huerta *et al.*, 2005). However, once fruit development progressed, differences in CER between fruiting and non-fruiting plants became more apparent. Additionally, the increase in root, stem, and leaf DW observed in non-fruiting compared with fruiting plants may have occurred quickly in response to the defruiting, thus establishing new sinks in the non-fruiting treatment and partially masking early effects of defruiting on net CER.

Conclusions

We hypothesized that LNT and/or high source:sink ratio would increase the incidence of swollen ovaries in pepper either through a combination of maintaining net CER under conditions of reduced growth rates (LNT), or by reducing collective fruit demand for assimilates (high source:sink ratio). Both of these conditions would theoretically result in excess assimilate availability and increased incidence of swollen ovaries. We found that sweet pepper leaves developed under LNT exhibited lower CER than leaves developed under HNT, and thus were unable to acclimate to night temperature conditions. Additionally, plant dry weight accumulation was not affected by night temperature treatments. Thus, although LNT did increase the incidence of swollen ovaries, the mechanism was not through maintenance of net CER in combination with

reduced growth rate. On the other hand, fruit removal (*i.e.* increasing the source:sink ratio) did decrease net CER and increase the incidence of swollen ovaries. This suggests that excess availability of current photosynthate may not be the mechanism that results in the increase in swollen ovaries observed under both LNT and high source:sink conditions.

Table 4-1. Effects of night temperature and cultivar on fresh weight of flower and flower parts, ovary diameter and ovary length in sweet pepper.

	Fresh weight (mg)				Ovary	Ovary
	Flower	Ovary	Calyx	Petals	diameter (mm)	length (mm)
Night temperature [z,y]						
12°C	287***	84**	61**	134**	5.3**	4.4***
20°C	217	57	46	107	4.6	3.7
Cultivar [x]						
'Legionnaire'	295***	87***	60***	140***	5.6***	3.9***
'Red Cherry Sweet'	209	55	48	101	4.3	4.2
Night temp x cultivar						
12°C 'Legionnaire'	323 a[w]	98 a	65 a	152a	5.8 a	4.1b
12°C 'Red Cherry Sweet'	251 b	70 b	58 b	117b	4.7c	4.6a
20°C 'Legionnaire'	267 b	76 b	55 b	129b	5.4 b	3.7c
20°C 'Red Cherry Sweet'	167 c	39 c	38 c	85c	3.8 d	3.7c
P-values	*	ns	***	ns	*	***

[z]Flowers at anthesis were harvested from 30-37 days after treatments began.
[y]Means were averaged across cultivars, n = 94 for 12°C and 103 for 20°C.
[x]Means were averaged across temperature, n = 101 for 'Legionnaire' and 96 for 'Red Cherry Sweet'.
[w]Means with the same letters were not significantly different (Tukey-Kramer, $P \leq 0.05$), n=48, 46, 53, and 50 for 12°C -'Legionnaire', 12°C -'Red Cherry Sweet', 20°C - 'Legionnaire', and 20°C -'Red Cherry Sweet', respectively. ns= non significant.
*, **, *** Asterisks indicate means are significantly different (t-test) at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.

Table 4-2. *P*-values for effects of night temperature (Temp), cultivar (CV), and days after treatment (DAT) and their interactions on leaf gas exchange parameters.

Source of variation[z]	Carbon exchange rate ($\mu mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)	Intercellular CO_2 ($\mu L\ L^{-1}$)
Temp	0.11	0.06	0.25
CV	0.01	0.99	0.61
Temp x CV	0.64	0.07	0.21
DAT	<0.001	<0.001	<0.001
DAT*CV	<0.001	<0.001	<0.001
DAT*Temp	0.01	0.05	0.31
DAT*Temp*CV	0.47	0.05	0.44
Block(Temp)[y]	0.13	0.39	0.13
Plant[x]	0.03	0.08	0.03

[z]Degrees of freedom: 1 for Temp, CV, and Temp x CV; 13 for DAT, DAT*CV, DAT*Temp, and DAT*Temp*CV.
[y]Random effect in the model.
[x]The individual plant was the subject for the repeated measures component.

Table 4-3. Main effects of night temperature and cultivar on mean carbon exchange rate, stomatal conductance and intercellular CO_2 in sweet pepper leaves measured six hours after lights were on.

	Carbon exchange rate ($\mu mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)	Intercellular CO_2 ($\mu mol\ mol^{-1}$)
Night temperature [z]			
12°C	8.3	0.14	238
20°C	9.2	0.16	243
Cultivar [y]			
'Legionnaire'	8.5*	0.15	240
'Red Cherry Sweet'	9.1	0.15	241

[z]Means were averaged across cultivars and days after treatment, n = 252.
[y]Means were averaged across temperature and days after treatment, n = 252.
*Asterisks indicate means are significantly different (Tukey-Kramer, $P \leq 0.05$).

Table 4-4. Main effects of night temperature and cultivar on daily net photosynthesis, daily night respiration, and calculated daily total CO_2 gain in sweet pepper leaves.

	Carbon budget (mmol $CO_2\ d^{-1}$ per plant)[z]		
	Daily net photosynthesis	Daily night respiration	Daily net CO_2 gain
Night temperature [y]			
12°C	59*	5**	54*
20°C	90	10	80
Cultivar [x]			
'Legionnaire'	70	8	62
'Red Cherry Sweet'	79	7	72

[z]Data were calculated for leaf tissue only, using plant total leaf area, net CER during the day, and night respiration rates at the end of the experiment (45-47 DAT).
[y]Means were averaged across cultivars, n = 18.
[x]Means were averaged across temperature, n = 18.
*, **Asterisks indicate means are significantly different (t-test) at $P \leq 0.05$) or $P \leq 0.01$, respectively.

Table 4-5. Night temperature and cultivar effects on vegetative growth of sweet pepper at the end of the 45-day experiment period.

	Leaf no.	Leaf area (cm^2)	SLA[z]	DW (g) Leaf	Stem	Root	Total	Plant height (cm)	Stem diam. (mm)	Node number	Internode length (cm)
Night temp[y]											
12°C	68	2273	149	15.2	20.0	10.8	49.3	69	12.1	10.7	4.4
20°C	74	2612	184	14.2	24.0	10.7	52.5	83	12.9	11.2	5.4
P values	0.03	0.06	0.006	0.13	0.13	0.72	0.31	0.006	0.05	0.16	0.005
Cultivar[x]											
LEG	60	2741	170	16.2	22.4	11.3	52.6	80	13.6	9.1	4.8
RCS	82	2144	163	13.2	21.6	10.2	49.1	72	11.5	12.8	5.0
P values	0.01	0.001	0.13	0.003	0.28	0.001	0.07	0.003	<0.001	<0.001	0.12

[z]SLA: specific leaf area ($cm^2\ g^{-1}$).
[y]Means were averaged across cultivars, n = 18.
[x]LEG: 'Legionnaire', RCS: 'Red Cherry Sweet'. Means were averaged across temperatures, n = 18.

Table 4-6. Main effects of night temperature and presence/absence of fruits on fresh weight of the flower, flower parts, ovary diameter, and ovary length in 'Legionnaire' bell pepper.

	Fresh weight (mg) Flower	Ovary	Petals	Calyx	Stamen	Ovary wall	Ovary diameter (mm)	Ovary length (mm)
Night temperature[z,y]								
12°C	451**	171**	148*	95*	26**	73**	7.5**	5.2**
20°C	352	121	128	71	21	51	6.5	4.6
Fruits[x]								
Non-fruiting plants	431**	156**	146*	94**	25**	66*	7.2**	4.9
Fruiting plants	372	136	130	72	22	58	6.8	4.9

[z]Flowers at anthesis were harvested from 30-36 days after treatments began.
[y]Means were averaged across fruiting treatment, n = 53 and 52 for 12°C and 20°C, respectively.
[x]Means were averaged across temperature, n = 60 and 45 for non-fruiting and fruiting plants, respectively.
*, **Asterisks indicate means are significantly different (t-test) at $P \leq 0.01$ or $P \leq 0.001$, respectively.

Table 4-7. *P*-values for effects of night temperature (Temp), presence/absence of fruits (Fruits), and days after treatment (DAT) and their interactions on leaf gas exchange parameters in 'Legionnaire' bell pepper.

Source of variation[z]	Carbon exchange rate ($\mu mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)	Intercellular CO_2 ($\mu mol\ mol^{-1}$)
Temp	0.006	<0.001	<0.001
Fruits	0.003	0.02	0.17
Temp*Fruits	0.33	0.26	0.92
DAT	<0.001	<0.001	<0.001
DAT*Temp	<0.001	<0.001	<0.001
DAT*Fruits	0.12	0.30	0.88
DAT*Temp*Fruits	0.70	0.87	0.63
Block(Temp)	0.20	0.45	0.47
Plant	0.09	0.21	0.40

[z]Degrees of freedom: 1 for Temp, Fruits, and Temp*Fruits, 11 for DAT, DAT*Temp, DAT*Fruits, DAT*Temp*Fruits; not available for Block(Temp) (random factor) or Plant (subject for the repeated measures).

Table 4-8. Main effects of night temperature and presence/absence of fruits on carbon exchange rate, stomatal conductance and intercellular CO_2 in 'Legionnaire' bell pepper leaves.

	Carbon exchange rate ($\mu mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)	Intercellular CO_2 ($\mu mol\ mol^{-1}$)
Night temperature[z,y]			
12°C	8.4*	0.10***	212***
20°C	10.2	0.15	238
Fruits[x]			
Non-fruiting plants	8.7**	0.11*	222
Fruiting plants	9.9	0.13	227

[z]Measurements were done six hours after lights were on and every three to four days during a 41-day period on one to three of the most recently matured leaves per plant.
[y]Means were averaged across fruiting treatment and days after treatment, n = 144.
[x]Zero or two developing fruits per plant. Means were averaged across temperature and days after treatments, n = 144.
*, **, ***Means are significantly different (t-test) at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 4-9. Night temperature and presence/absence of fruit effects on growth of 'Legionnaire' bell pepper at the end of the 45-day experiment period.

	Leaf no.	Leaf area (cm^2)	SLA[z]	DW (g)					Plant height (cm)	Node no.	Internode length (cm)
				Leaf	Stem	Root	Fruits[y]	Total			
Night temp[x]											
12°C	79	3187	106	30.0	35.3	17.4	18.9	108	75	10.5	4.8
20°C	83	3549	135	26.6	38.6	17.3	19.1	107	84	12.1	5.0
P values	0.60	0.31	0.001	0.21	0.29	0.95	0.95	0.93	0.01	0.03	0.30
Fruits[w]											
Non-fruiting[y]	86	3500	114	30.7	41.5	18.6	6.4	105	82.0	11.8	4.9
Fruiting	76	3236	126	25.9	32.4	16.0	31.6	110	76.9	10.8	4.8
P values	0.03	0.12	0.05	0.003	0.001	0.002	0.001	0.12	0.01	0.01	0.76

[z]SLA: specific leaf area ($cm^2\ g^{-1}$).
[y]In non-fruiting plants, the fruits were harvested at the beginning of the experiment.
[x]Data were averaged across fruiting treatments, n = 12.
[w]Data were averaged across temperatures, n = 12.

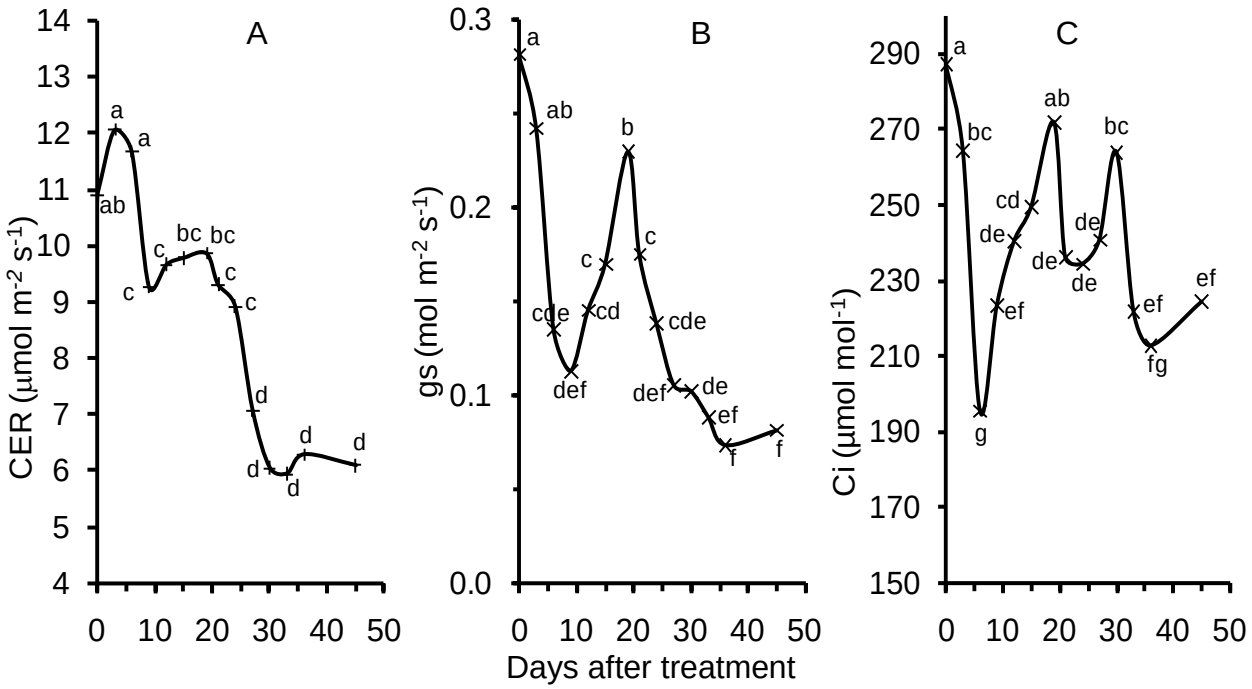

Figure 4-1. Effect of days after treatment began on (A) net carbon exchange rate (CER) and (B) stomatal conductance (g_s), and (C) intercellular CO_2 (Ci) of sweet pepper leaves. Gas exchange was measured six hours after lights were on and over a 45-day period after the beginning of night temperature treatments in two cultivars. Data were averaged across night temperatures and cultivars. Means with the same letters were not significantly different (Tukey-Kramer, $P \leq 0.05$), n=36.

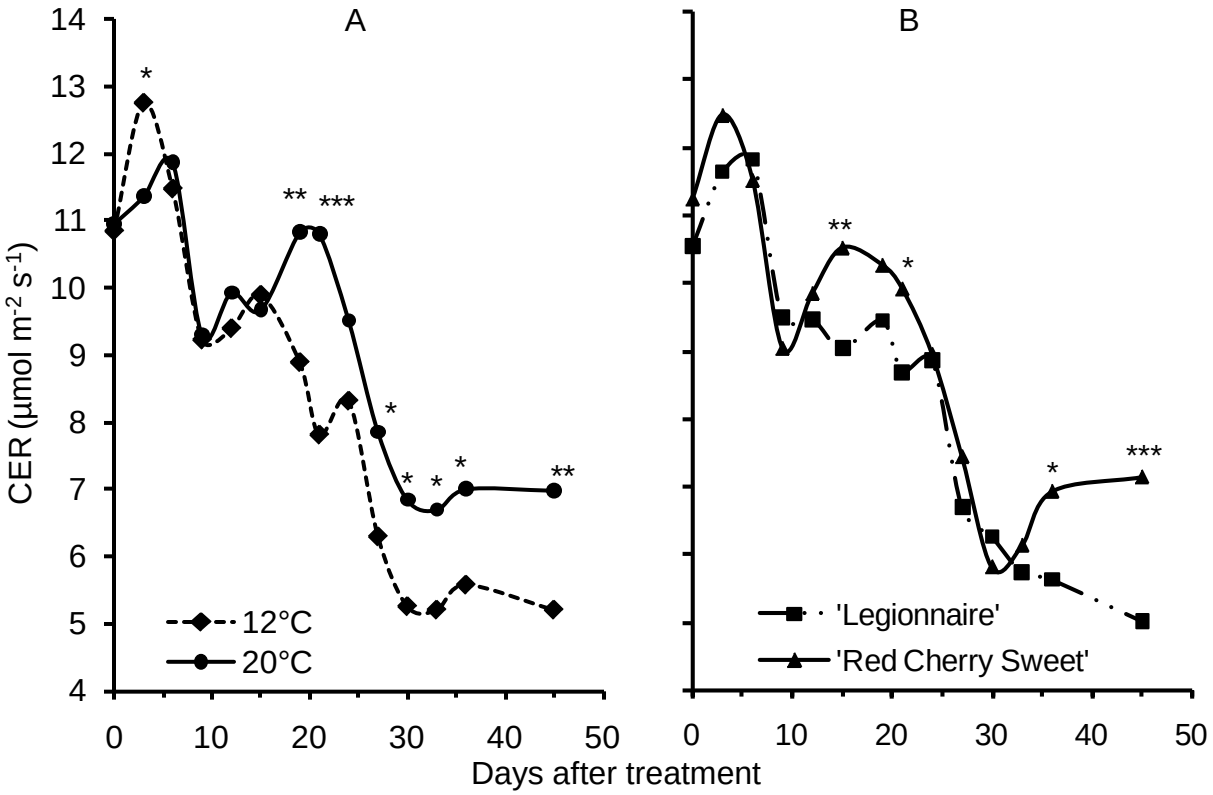

Figure 4-2. Effect of (A) night temperature and (B) cultivar on net carbon exchange rate (CER) of sweet pepper leaves over time. Gas exchange was measured six hours into the light period and over a 45-day period after the beginning of night temperature treatments. *, **, ***Means between temperature treatments (A) or cultivars (B) within each measurement time were significantly different at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively, n = 18.

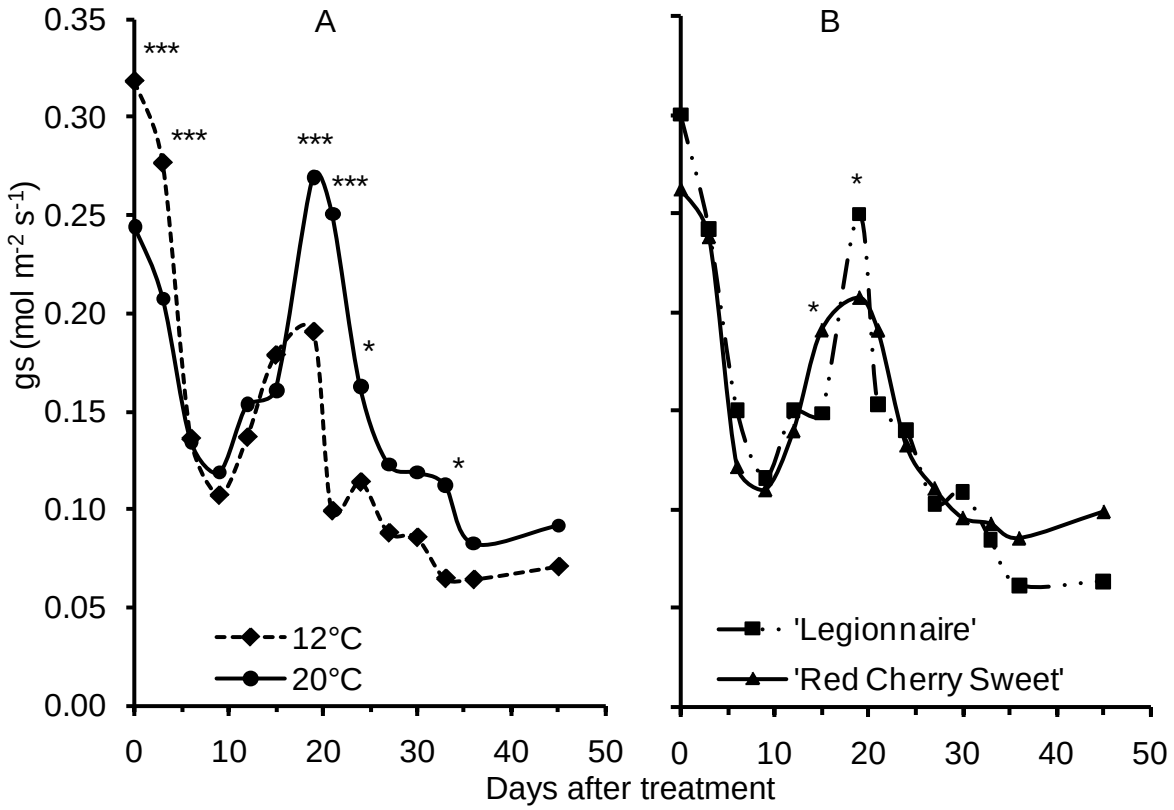

Figure 4-3. Effects of (A) night temperature and (B) cultivar on stomatal conductance (g_s) of sweet pepper leaves over time. Measurements were done six hours after lights were on and over a 45-day period after the beginning of night temperature treatments. *, **, ***Means between temperature treatments (A) or cultivars (B) within each measurement time were significantly different at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively, n = 18.

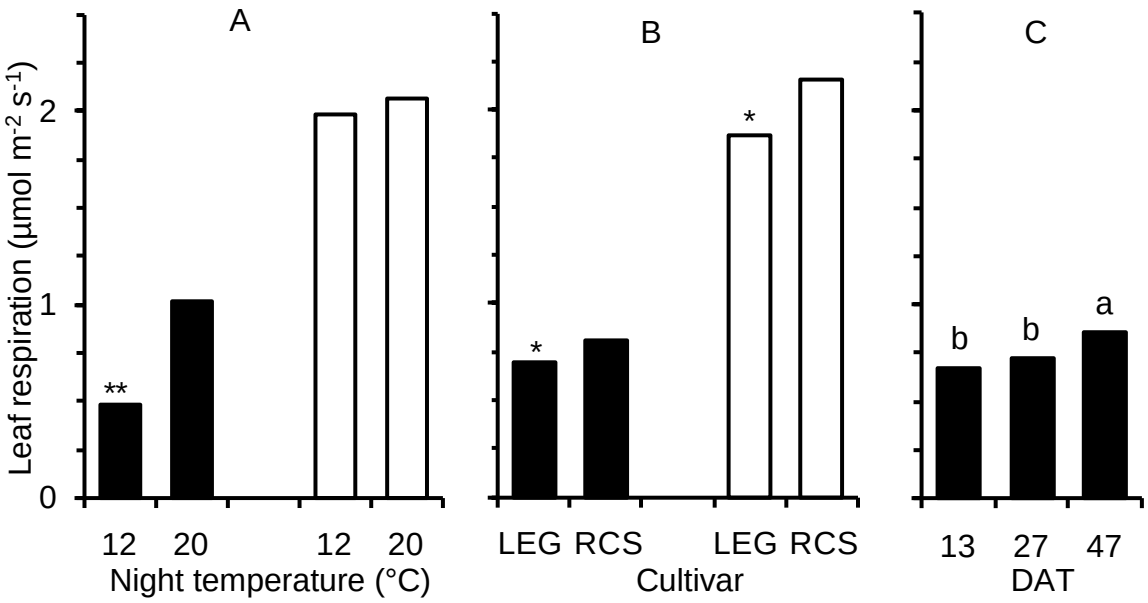

Figure 4-4. Effect of (A) night temperature, (B) cultivar, and (C) days after treatments began (DAT) on leaf respiration in sweet pepper plants during the day (□) and night (■). RCS: 'Red Cherry Sweet', LEG: 'Legionnaire'. For day respiration (A and B), n = 30; for night respiration (A and B), n=54; and for DAT (C), n = 36. *, **Means are significantly different at $P \leq 0.05$ and $P \leq 0.001$, respectively. In (C), means with the same letters were not significantly different (Tukey-Kramer, $P \leq 0.05$).

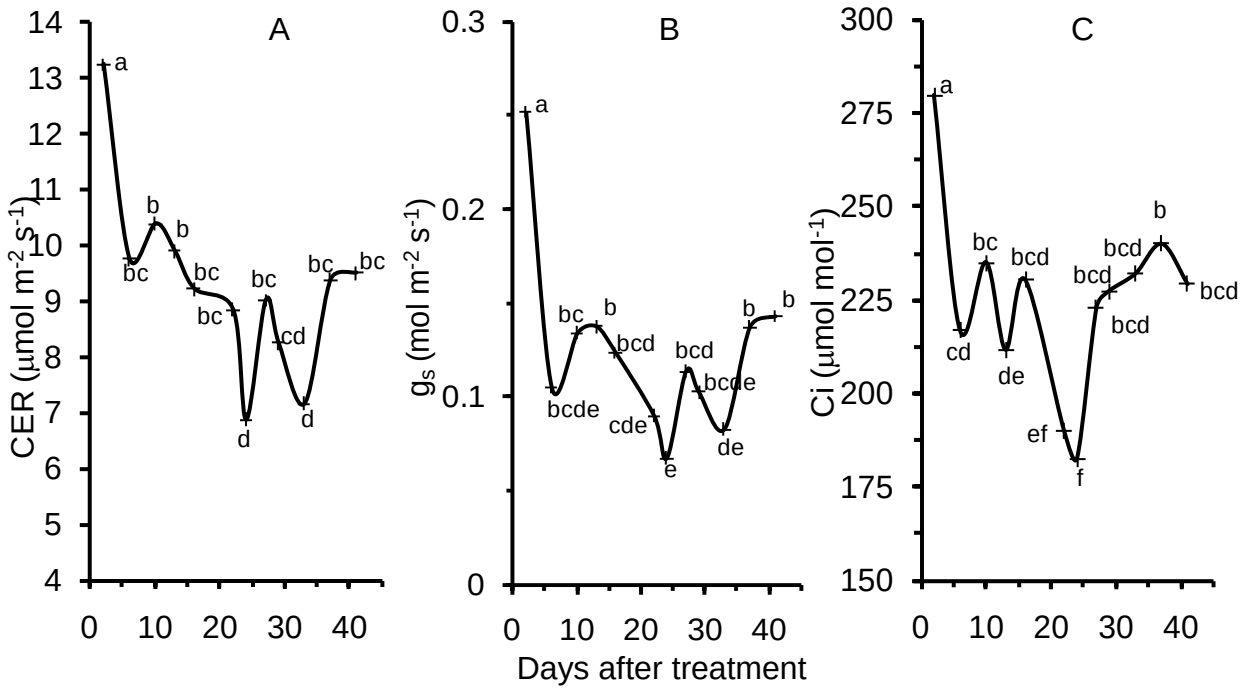

Figure 4-5. Effect of days after treatment began on (A) net carbon exchange rate (CER), (B) stomatal conductance (g_s), and (C) intercellular CO_2 (Ci) of 'Legionnaire' bell pepper leaves. Measurements were done six hours after lights were on, and over a 41-day period after the beginning of night temperature and fruiting treatments. Data were averaged across night temperatures and fruiting treatments. Means with the same letters were not significantly different (Tukey-Kramer, $P \leq 0.05$), n = 24.

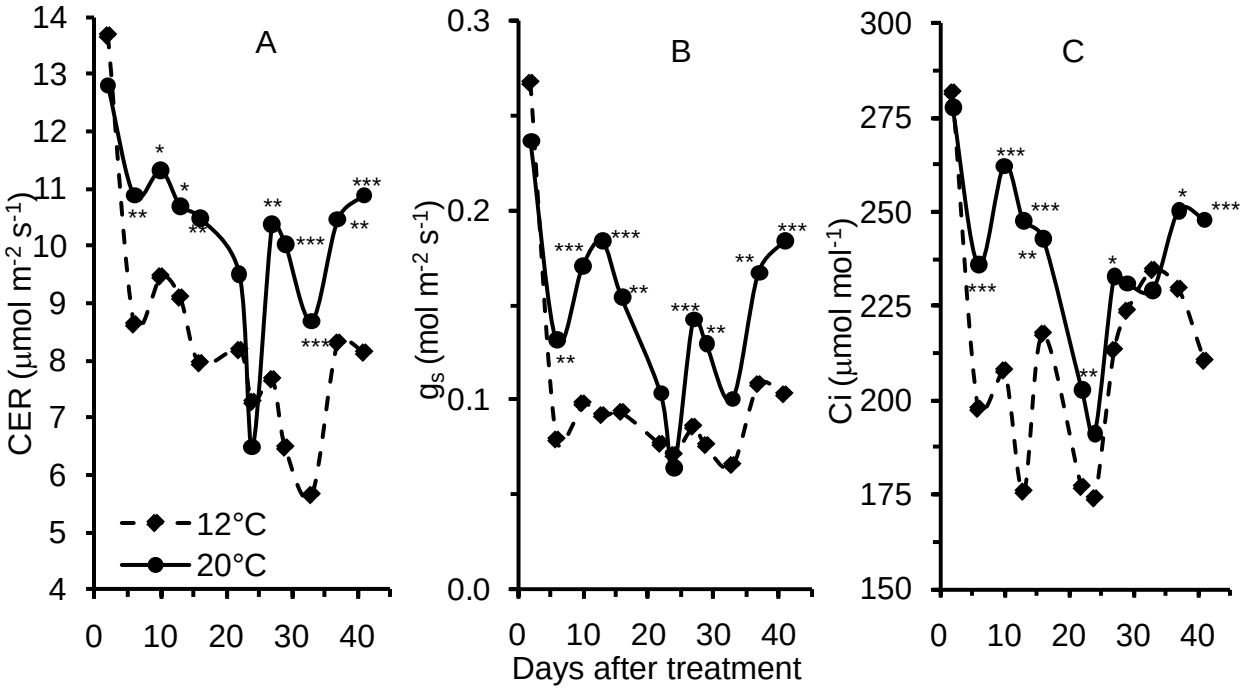

Figure 4-6. Effect of night temperature and days after treatment began on (A) net carbon exchange rate (CER), (B) stomatal conductance (g_s), and (C) intercellular CO_2 (Ci) of 'Legionnaire' bell pepper leaves. Measurements were done six hours after lights were on, and over a 41-day period after the beginning of the treatments. Data were averaged across fruiting treatments. *, **, *** Means between the two night temperature treatments within each measuring time were significantly different at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively, n=12.

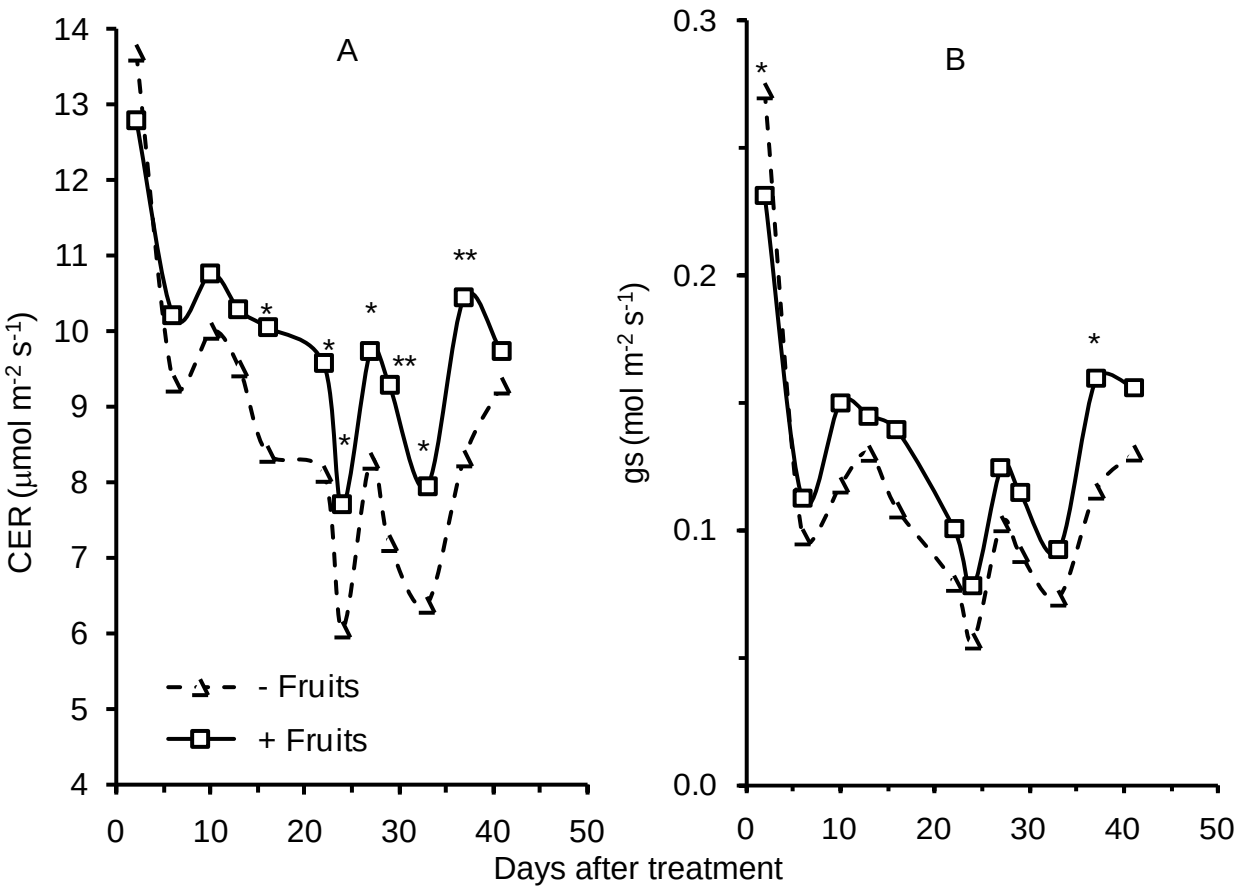

Figure 4-7. Effect of presence or absence of developing fruits and days after treatment began on (A) net carbon exchange rate (CER) and (B) stomatal conductance (g_s) of 'Legionnaire' bell pepper leaves. Measurements were done six hours after lights were on, and over a 41-day period after the beginning of the treatments. Data were averaged across night temperature treatments. *, **, *** Means between the two night temperature treatments within each measuring time were significantly different at P≤0.05, P≤0.01, P≤0.001, respectively. For each mean, n = 12.

CHAPTER 5
BELL PEPPER OVARY SWELLING DUE TO LOW NIGHT TEMPERATURE AND SOURCE SINK RATIO: OVARY WALL ANATOMY AND CARBOHYDRATES

Introduction

In pepper (*Capsicum annuum* L.), as in all horticultural crops, high quality yield is as important as total yield. Fruit weight, size, shape, pericarp thickness, and carpel number and regularity are used to evaluate pepper fruit quality parameters and to differentiate pepper types and varieties (Aloni *et al.*, 1999; Navarro *et al.*, 2002; Kissinger *et al.*, 2005; Lim *et al.*, 2007). In bell and spherical peppers, both shape and size are primarily determined at early preanthesis stage, while in elongated fruits, shape is determined in both pre and post anthesis (Munting, 1974). Early flower development, in turn, is very sensitive to several factors, including low ambient temperature and carbohydrate availability (Ali and Kelly, 1992; Tomer *et al.*, 1998; Aloni *et al.*, 1999).

Low night temperatures affect bell pepper production. In winter, lower temperatures and shorter day lengths increase the percentage of small, flattened and parthenocarpic fruits. Poor pollination is the main reason for such problems, but malformation of female organs (Polowick and Sawhney, 1985; Pressman *et al.*, 1998a; Shaked *et al.*, 2004) and other parts of the flower (Aloni *et al.*, 1999) also play a role. In tomato, low night temperature decreases ovule number in one or more locules in some cultivars, causing malformed fruits (Tomer *et al.*, 1998).

Night temperatures of 15°C or less increase ovary diameter in pepper without increasing locule number, creating “swollen” ovaries and malformed fruit (Polowick and Sawhney, 1985; Rylski, 1985; Bakker, 1989b; Aloni *et al.*, 1999; Shaked *et al.*, 2004). The longer the duration of low night temperatures, the higher the percentage of swollen flowers and ovaries (Polowick and Sawhney, 1985; Aloni *et al.*, 1999) or the greater the

swelling (Chapter 3). For instance, under night temperature of 12°C, the percentage of swollen flowers, and therefore ovaries, increased from 21% at 15 days after treatment to 78% at 49 days after treatment, compared with an increase in swollen flowers from 7 to 14% in plants growing at night temperatures of 18°C (Aloni *et al.*, 1999). Evidence indicates that flower deformation, including ovary swelling, is favored by low shoot temperature during the night, regardless of root temperature (Polowick and Sawhney, 1985; Mercado *et al.*, 1997c; Aloni *et al.*, 1999).

Increased source/sink ratio also increases the proportion of swollen ovaries. Aloni *et al.* (1999) compared fruiting with defruited pepper plants and found that defruiting increased fresh weight of flowers three to four times that of the flowers on fruiting plants, resulting in swollen ovaries and malformed fruits. In addition, the percentage of swollen flowers was inversely related to the number of growing fruits on the plant, and directly proportional to the concentration of reducing sugars and starch in the flowers developed in those plants (Aloni *et al.*, 1999). Flowers that developed on defruited plants accumulated more $^{14}CO_2$ (600 *vs* 150 µg d^{-1}) and fresh weight (180 *vs* 60 mg) than flowers that developed on fruiting plants (Aloni *et al.*, 1991b; Aloni *et al.*, 1999). These data suggest that excess assimilates are transported to flower buds on defruited plants, resulting in ovary swelling and deformation (Aloni *et al.*, 1999).

Limited work has been done on the anatomical aspects of swollen ovaries in pepper. Pressman *et al.* (1998b) reported that swollen ovaries of bell pepper that developed in response to cool night temperatures had larger cells than normal ovaries, but cell number was similar, suggesting that swollen ovaries develop after cell division ends. However, our findings (Chapter 3) suggest that in order to reach the maximum

swelling effect, the cool night temperature stimulus needs to start during the first week after flower initiation, a stage in which active cell division is taking place (Munting, 1974).

Flower and ovary swelling is favored by exposing the shoot to low night temperature or by increasing the source:sink ratio. However, the interaction between night temperature and source-sink modification on ovary swelling is unknown. For these reasons, the hypothesis tested in this study was that high night temperature (HNT) combined with high source:sink ratio or low night temperature (LNT) combined with low source:sink ratio can overcome the detrimental effects of LNT or high source: sink ratio on ovary swelling. The specific objectives of this study were to 1) investigate whether ovary size is increased by high source:sink ratio under warm night temperature, and reduced by low source:sink ratio under low night temperature, 2) investigate the combined effect of night temperature and source:sink ratio on cell number and cell size of ovary wall at flower anthesis, and 3) determine the ovary wall and placenta content and concentration of soluble sugars and starch in bell pepper ovaries developed under two night temperature regimes and two source:sink ratios.

Materials and Methods

Plant Material

‘Legionnaire’ bell pepper seedlings were grown in a growth chamber (E15 Conviron, Winnipeg, Canada) set to 22°C/20°C (day/night temperature), 400-500 µmol m^{-2} s^{-1}, and a14-h photoperiod. At the sixth-leaf stage, seedlings were transferred to 1.5 L containers using commercial growing media. Plants were trimmed to two axes, as described in Chapter 3 (Figure 3-2A). Starting in sympodial unit 1, the strongest shoot was allowed to grow and the second shoot was limited to development of one

sympodial unit (one flower and two leaves) by pruning. Flower buds from the main stem (first terminal flower) and the first node in the two axes (*i.e.*, sympodial unit 1) were removed.

Experimental Description

A 2 x 2 factorial experiment (two night temperatures regimes and two source:sink ratios) was carried out twice. Night temperatures were 20ºC (HNT) and 12ºC (LNT) with the day temperature set to 22ºC. To establish two different source:sink ratios, one fruit was allowed to set in the second or third node of each of the two axes in all plants. Five to 12 days after petal fall (depending on the experiment), fruits on the plants in one treatment were removed, while fruits on the other treatment were allowed to develop to maturity. All subsequent flowers and small fruits were removed to avoid further fruit set. These treatments resulted in low source:sink (fruiting plants, +F) or high source:sink (non-fruiting plants, -F) ratios. This resulted in four treatment combinations (HNT + F, HNT - F, LNT + F, LNT - F). The night temperature and source:sink ratio treatments started five to twelve days after petal fall, when fruits on fruiting plants exhibited maximum relative growth rate, according to Marcelis *et al.* (1995b). Each treatment combination was replicated six times in a completely randomize block design. All open flowers were removed when the treatments started. Subsequently, all flowers at anthesis were harvested for sampling, starting the day after treatments began.

Two experiments were performed. Experiment 1 was designed to determine the contribution of cell number and cell size to ovary size in the four treatment combinations. Temperature treatments began 5 to 8 days after petal fall, when one half of the plants were defruited (high source:sink ratio). Flowers from nodes 4 or 5 and the subsequent nodes were harvested daily for 42 days after treatments started (DAT).

Diameter, length and fresh weight of the ovary were recorded and ovaries were fixed in a formalin-acetic acid-ethanol (FAA) mixture for anatomical studies (see below).

Experiment 2 was designed to determine carbohydrate concentration and content in the ovary wall and placenta in the four treatment combinations. Developing fruits were between 8 and 12 days after petal fall when the defruiting was performed and the night temperature treatments started. Flowers from Experiment 2 were harvested at anthesis, starting in node 5 and 6, and flowers were harvested daily for 38 days. Fresh weight, diameter, and length of the ovary were recorded.

Anatomy

Ovaries were individually fixed in FAA. Based on the ovary size data (see below), an anatomical analysis was performed three times: at 1-2 DAT, at 18-19 DAT, and at 38-40 DAT (see Figure 5-1). A common sampling was used at the beginning of the experiment, since it was the initial control with no treatment effect. For every treatment combination and harvest time, the mean fresh weight of all flowers harvested was calculated. Then, four flowers with fresh weights closest to the mean FW for each treatment and harvest time were selected for anatomical analysis.

Samples were dehydrated and resin embedded. First, samples were transferred from the FAA to 50% ethanol to rinse the formalin and acetic acid from the FAA. In order to facilitate tissue infiltration, the bottom and top sections of the ovary were cut, and only the middle section was processed. Samples were then dehydrated by gradually increasing ethanol from 50% to 100%, before placing in 100% acetone (see Table 5-1). Resin embedding steps were done under partial vacuum. Dehydration and resin embedding were done using a microprocessor-controlled microwave oven equipped with a ColdSpot® temperature control system (PELCO BioWave® Laboratory

Tissue Processing System, Ted Pella, Inc. Redding, CA, USA), to speed the process. Microwave power output was set to 180 W, and ColdSpot® water bed temperature was 24°C. Spurr resin[3] (cycloaliphatic epoxide resin [ERL 4221, 4.10g], diglycidyl ether of polypropylene glycol [DER® 736, 1.43g], nonenyl succinic anhydride [NSA, 5.90g] [Ted Pella, Inc. and PELCO International, Redding, CA], and dimethylaminoethanol [DMAE, 0.1g] [Electron Microscopy Sciences, Hartfield, PA]) was used. After resin embedding, the samples were put in molds. Molds were kept overnight under vacuum to remove air bubbles. Samples were then polymerized in an oven at 60°C for 24 hours.

Following resin embedding, samples were cut in four pieces, and 700 nm slices were cut from one piece using a cryo-ultramicrotome (Leica Ultracut UCT, www.leica.com). Slices were stained with an aqueous solution of 1% toluidine Blue O and 1% sodium borate, and mounted with immersion oil. Slides were observed in an Olympus *BH2-RFCA* fluorescence microscope (Olympus, Melville, NY). Images were taken with a digital camera Q-Imaging Retiga 2000R and Q-Capture Pro Ver 5.1.1.14 for Win (Q-Imaging, Surrey, BC, Canada), and analyzed using ImageJ software (Rasband, 2010) after calibration. Ovary wall thickness, thickness of each ovary wall tissue layer (epidermis, hypodermis, mesocarp, and endocarp), number of cell layers in the ovary wall, and cell size of the epidermis, hypodermis, mesocarp, giant, and endocarp cells were measured (Figure 5-2). Average cell size in each tissue was measured by drawing around a set of cells, measuring the area, and determining cell number with the Cell Counter plug-in (Kurt De Vos. University of Sheffield, Academic Neurology, England). There were no differences between hypodermis and epidermis

[3] http://www.tedpella.com/technote_html/18300-4221%20TN.pdf; 3/2/2010.

cell size, therefore for the final analysis, both tissues were analyzed as epidermal tissues. No information was recorded for the vascular bundles. Transverse section area of the ovary wall and ovary wall tissue layers was calculated with the circle and the ring area formula (ring area = $\pi \cdot (r_1 - r_2)^2$, where r_1 equals diameter divided by 2, and r_2 equals r_1 minus tissue thickness). The section area was then corrected with a constant for shape irregularity. To find the constant, 23 ovaries ranging from 23 to 249 mg FW (3.5 - 9.1 mm in diameter and 2.7-5.3 mm in length) were photographed using a binocular microscope. Total area was then measured from monochromatic images using ImageJ software (Rasband, 2010), and a linear regression ($y = b \cdot x$, y = measured area, b = slope, and x = calculated area) between measured and calculated area was generated to determine the slope (Figure 5-3).

Carbohydrates

Carbohydrates were analyzed in flowers harvested in Experiment 2 during the fourth week after the beginning of treatment (26±1 DAT, nodes 8 to 10 above first terminal flower), when the maximum difference in ovary size was found. Glucose, fructose, sucrose and starch were analyzed in the ovary wall and placenta of the ovary.

Harvested flowers were maintained on ice while ovary fresh weight, length and diameter were recorded. The ovary was then separated into ovary wall and placenta and stored at -80°C (Harris. Kendro Laboratory Products, Asheville, NC, USA). Samples were lyophilized (Virtis Model 10MR-TR, The Virtis Co., Gardiner, NY, USA) for 72 hours. Samples were subsequently stored at -20°C in sealed containers until analysis.

For soluble sugar and starch analysis, samples were weighed and ground in liquid nitrogen. Samples (10-25 mg) were dissolved in 1 mL 80% ethanol, shaken for 20 min at 200 rpm in an orbital shaker (Fisher Scientific Model 361; U.S.A.), and centrifuged at

1380 x g for 10 minutes. The supernatant was removed, and the pellet was re-extracted with ethanol. The supernatants of the two extractions were combined and total volume measured. The supernatants were cleared with activated charcoal to remove pigments and centrifuged at high speed (13,250x g, 4 min). Soluble sugar recovery from samples was above 95%, as determined by a ^{14}C external standard.

Soluble sugars were analyzed with an enzymatic method (modified from Outlaw Jr. and Tarczynski, 1984). Three enzyme systems (ES) were used: 1) 'ESA' - hexokinase (E.C. 2.7.1.1, 0.0565 units/µL) and glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49, 0.0808 units/µL) to measure glucose; 2) 'ESB' - 'ESA' and phosphoglucoisomerase (EC 5.3.1.9, 0.0741 units/µL) to measure glucose and fructose; and 3) 'ESC' - 'ESB' and invertase (EC 3.2.1.26, 0.7712 units/µL) to measure glucose, fructose and sucrose. The enzymes were diluted in 1% w/v bovine serum albumin (BSA, Sigma A6003), 1 M HCl, and 40 mM tris(hydroxymethyl) aminomethane (Fisher Scientific BP152-500, pH: 10-11.5). For each ES, an aliquot of the cleared sample (30µL), the assay cocktail (1 mL), and the enzyme system (10 µL) were mixed and incubated at room temperature for 60 to 90 min, before reading absorbance at 339 nm in a spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan). The assay cocktail contained the following components: 170 mM N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES, Sigma B4554), 65 mM KOH; 15 mM $MgCl_2$, 1.5 mM EDTA, 2 mM NADP, 5 mM ATP, and 0.015% w/v BSA.

Sample pellets were analyzed for starch using amyloglucosidase (E.C. 3.2.1.3, 50 units/sample) and measuring resultant glucose equivalents. Pellets were boiled in 2 ml 0.2N KOH for 30 minutes, then acidified with 1 ml of 1M acetic acid. After cooling, 1 mL

of amyloglucosidase solution (50 units/mL in 0.2M calcium acetate buffer, pH 4.5) was added and samples were incubated for 18 hours at 37°C. Volume was recorded and the digest was centrifuged for 10 min at 1975 x g. An aliquot of the supernatant was removed and glucose was quantified. Percent recovery, as estimated using a ^{14}C external standard, was above 95%.

Data Analysis

Ovary size data from the two experiments were pooled and analyzed together using mixed models and the SAS ® program, (SAS Institute Inc., 2008). Flower harvests were grouped into 4-day intervals, resulting in 11 groups for Experiment 1 and 10 groups for Experiment 2. Data from 5-8 DAT was only from Experiment 2 and data from 41-44 DAT was only from Experiment 1. Night temperature regime, source:sink ratio (presence/absence of fruits), days after harvest (grouped in 4-day intervals), and branch (flowers from the main axis or lateral branches) were considered fixed effects. Blocks within each experiment and the experiments (1 and 2) were considered as random effects, and every plant within each experiment was the repeated measures unit. The anatomy data were analyzed in two phases. A preliminary analysis of variance performed as a 2 x 2 x 2 factorial (2 night temperatures, 2 source:sink ratios, 2 harvest times [*i.e.*, without considering the control]) indicated that the temperature x harvest time and the temperature x source:sink ratio x harvest time were not significant (Table A-6, Appendix). The final analysis was done as a randomized block design, where all combinations were handled as treatments (*i.e.*, 9 treatments: 2^3 factorial + control). Main effects and specific comparisons were performed based on the preliminary data and by using contrasts. No comparisons were performed for the non significant

interactions. Carbohydrate data were analyzed as a 2 x 2 factorial using a completely randomized block design with 6 replications.

Results

Ovary Size

Low night temperature significantly increased ovary fresh weight, diameter, and length (Table 5-2; Table A-5, Appendix). Fresh weight increased about 25%, while diameter and length increased 9 and 6%, respectively.

The presence or absence of fruits also affected ovary size parameters. In non-fruiting plants, ovary fresh weight of flowers at anthesis was 35% greater than those from fruiting plants. Both ovary diameter and length also increased in non-fruiting compared with fruiting plants.

Since flowers at anthesis were harvested over 38-42 days after temperature treatments started, ovaries were grouped into 10 (Exp. 2) or 11 (Exp. 1) 4-day harvest intervals. Harvest timing significantly influenced ovary size parameters. In general, ovary FW increased as harvesting intervals proceeded (Table 5-3). Significant increases in ovary FW were observed beginning at 13-16 DAT and maximum size was reached at 25-28 DAT. Based on ovary FW, ovaries could be classified into three groups: 1 to 12 DAT, 13 to 24 DAT, and 25 to 42 DAT. Ovary diameter increased in a similar pattern as fresh weight, with significant increases observed beginning at 9-12 DAT. Ovary length was less responsive; ovaries harvested from 1 through 20 DAT were similar in length and shorter than ovaries harvested from 25 through 44 DAT.

There was a significant interaction between night temperature and presence/absence of fruits on ovary fresh weight ($P \leq 0.02$), but not on diameter or length ($P \leq 0.08$). Ovary fresh weights in non-fruiting plants under LNT (L-) were the largest,

averaging 149 mg; while fresh weights were smallest in the HNT with fruits (H+), averaging 90 mg (Figure 5-4). A similar pattern was observed in ovary diameter and length, although the interaction was not significant.

The interaction between night temperature and harvest interval was significant for ovary FW (Figure 5-5), but not for diameter ($P \leq 0.36$) or length ($P \leq 0.76$) (Table A-5 and Figure A-2, Appendix). Ovary fresh weight increased ~42 mg (42%) between harvest intervals 1-4 DAT and 33-36 DAT in plants developed under LNT, while fresh weight increased ~25 mg (28%) in ovaries developed under HNT during the same intervals.

Ovary size was also affected by the interaction between days after treatment and fruiting treatments, which was highly significant for the three parameters (Figure 5-6). Starting at 5-8 DAT, the ovaries developed on non-fruiting plants had greater FW, diameter and length than the ovaries developed on fruiting plants. On the non-fruiting plants, ovary FW, diameter, and length increased steadily from 1-4 DAT through 25-28 DAT, then decreased between 33-36 and 41-44 DAT (t-test, $P<0.04$ for 33-36 DAT *vs* 37-40 DAT and $P<0.003$ for 33-36 DAT *vs* 41-44 DAT). In fact, pair wise comparisons revealed that ovary FW and diameter were significantly greater as early as 5-8 DAT compared to 1-4 DAT (t-test, $P \leq 0.01$). In fruiting plants; however, there was a significant reduction in ovary FW and length during the first 5 to 6 harvest intervals compared to the first harvest interval (t-test, $P \leq 0.001$), while ovary diameter remained constant. Subsequently, ovary FW, length, and diameter increased, and by 25-28 DAT (FW and length) or 29-32 DAT (diameter), were significantly larger than observed at harvest intervals 17-20 or 21-24 DAT (t-test, $P \leq 0.001$).

Anatomy

Ovaries used for the anatomical study were a subsample of the total number harvested, as described above. Ovaries developed under LNT were significantly larger compared with ovaries developed under HNT (Figure 5-7A), in accordance with data presented in Table 5-2. LNT also increased the ovary wall thickness and total transverse area in the ovary mid-section (Figure 5-7B).

Based on total transverse area of the ovary wall, mesocarp tissue accounted for 72% of the area, followed by giant cells (16%), epidermal tissue (8%) and endocarp (4%). Although there were differences between LNT and HNT in ovary FW, diameter, and ovary wall transverse area (Figure 5-7), there were no differences in cell size or cell number in mesocarp or giant cells between the two night temperature treatments (Figures 5-8 and 5-9). There was also no difference in the number of cell layers in the mesocarp (22.0 ±1.1 *vs* 20.3 ± 0.7 for LNT and HNT, respectively, data not shown). Epidermal cells were larger under LNT compared to HNT ($P \leq 0.04$; 214 *vs* 190 μm^2), but there were no differences in cell number (Figures 5-8 and 5-9). There was also no significant effect of night temperature on endocarp cell size or cell number.

Ovary FW and diameter in non-fruiting plants were significantly greater compared with ovaries developed on fruiting plants (Figure 5-10), as previously shown in Table 5-2. Ovary wall thickness was also greater in ovaries of non-fruiting compared with ovaries of fruiting plants (564 *vs* 444 µm), which in combination with the greater diameter, resulted in increased total transverse area (12.11 *vs* 7.73 mm^2 for non-fruiting and fruiting, respectively).

Increases in ovary size in non-fruiting compared with fruiting plants were due to increased cell size in mesocarp, giant cell, and epidermal tissues, which comprised 96%

of the ovary wall (Figure 5-11A, B, C; Figure 5-12). Cell number was significantly greater in ovary endocarp tissue of non-fruiting compared with fruiting plants. There was also a small, but non-significant, increase in cell number in mesocarp (P≤0.09) and epidermal tissues (P≤0.07) of ovaries in non-fruiting plants. Non-fruiting plants also had a greater number of cell layers in the mesocarp compared to the fruiting plants (23.0±1.1 *vs* 19.4±0.6 for -F and +F plants, respectively).

Ovary FW increased between 18-19 DAT and 38-40 DAT, while ovary diameter did not increase (Figure 5-13). Ovary wall thickness at 18-19 DAT (550 µm) was greater than at 38-40 DAT (458 µm), resulting in a significant increase in ovary wall area at 18-19 DAT compared with the other two harvest times. Greater ovary fresh weight at 38-40 DAT may be due to greater ovary length in that time (4.3 mm) compared to 1-2 DAT (3.9 mm) and 18-19 DAT (4.1 mm) (*P*≤0.004, data not shown).

Mesocarp and giant cell size was greater in 18-19 DAT than in 38-40 DAT (Figure 5-14A, B). However, mesocarp cell number increased from 17,100 to 22,000 per cross-section area in the ovary wall between 1-2 DAT and 38-40 DAT (*P*≤0.06). Regression analysis indicated a significant increase in giant cell number (cell number = 314.30 + 2.63 x DAT; r^2 = 0.99; P≤0.01) with days after treatment. A similar trend was observed in mesocarp cell number (cell number = 17165 + 129.4 × DAT; r^2 = 0.95, *P*≤0.10), and the increase in cell number may account for the increased ovary FW in 38-40 DAT compared with 18-19 DAT.

There was a significant interaction between night temperature and presence/absence of fruit on ovary growth and anatomy (Figures 5-15 and 5-16, Table A-5, Appendix). Flowers developed under LNT in non-fruiting plants developed the

largest ovaries (Figure 5-15), while fruiting plants under both night temperatures developed the smallest ovaries. This differs slightly from the average ovary FW determined using the entire dataset (Figure 5-4), where ovary FW in fruiting plants under HNT were significantly smaller than ovaries developed under all other conditions. Although the trend observed in Figure 5-15 is similar to that observed in Figure 5-4, the difference is likely due to the smaller sample size used in the anatomical studies, as well as the slight difference in ovary harvesting times between the anatomical study (Experiment 1) and the pooled data (Experiments 1 and 2) that was used for ovary growth measurements.

In the mesocarp, cell size was largest in non-fruiting plants under LNT and smallest in fruiting plants under HNT (Figure 5-16A). There were no differences due to temperature, but only due to presence or absence of developing fruits. Mesocarp cell number, however, was greater in non-fruiting plants under LNT than in the rest of the combinations ($P \leq 0.06$). The number of mesocarp cell layers followed the same trend, with greater number of layers in the non-fruiting plants under LNT (25.0 ± 1.6) compared with the rest of the treatment combinations (19.1±0.6, 20.7±0.7 and 19.8±1.2 for L+, F- and F+, respectively). Size of giant cells was affected only under HNT, where non-fruiting plants had larger giant cells than did fruiting plants (Figure 5-16B). Such differences in cell size were compensated for in cell number, so that the total area of giant cells was similar in the ovaries of both fruiting and non-fruiting plants under HNT (data not shown).

There were also significant interactions between harvest time and presence/absence of fruit. Ovary FW and diameter increased at both 18-19 DAT and

38-40 DAT in non-fruiting plants, compared to fruiting plants (Figure 5-17A). The smallest ovaries were harvested from fruiting plants at 18-19 DAT, in accordance with what was previously described in Figure 5-6. Although there were no differences in ovary FW or diameter between non-fruiting plants at 18-19 DAT and 38-40 DAT, ovary wall thickness was greatest in non-fruiting plants at 18-19 DAT compared with the other treatments, resulting in greater ovary wall transverse area (Figure 5-17B). Since there were no differences in ovary length between 18-19 DAT and 38-40 DAT (4.5 and 4.6 mm, respectively, $P \leq 0.50$) in non-fruiting plants, the similar ovary FW and diameter between these two harvest times suggests that the placenta in 38-40 DAT ovaries was larger than those of 18-19-DAT ovaries. In fruiting plants, there were no differences in diameter, but ovary FW was greater at 38-40 DAT due to a greater length at 38-40 DAT (4.1 mm) compared to the diameter at 18-19-DAT (3.6 mm, $P \leq 0.001$).

Differences in ovary wall transverse area are likely due to the increased cell size of the mesocarp, giant cells and epidermal tissues in non-fruiting plants at 18-20 DAT compared to the rest of the treatment combinations (Figure 5-18). There were no differences between harvest times within fruiting and non-fruiting plants for mesocarp cell number. There was, however, a trend towards increased cell number in mesocarp, epidermal tissue and endocarp of non-fruiting compared with fruiting plants.

Carbohydrates

Low night temperature (LNT) increased glucose, fructose and starch concentration and decreased sucrose concentration in the ovary wall compared to HNT (Table 5-4). In contrast, soluble sugar and starch concentrations in the placenta were not affected by night temperature. In general, there were no differences in soluble sugar or starch concentrations in the ovary wall or in placenta between fruiting and non-fruiting plants,

with the exception of sucrose in the placenta. There were no significant interactions between night temperature and presence/absence of fruits on carbohydrate concentrations (Table A-7, Appendix).

Total content of reducing sugars and starch was also greater in the ovary wall of ovaries developed under LNT compared to ovaries in the HNT (Table 5-5). Sucrose content; however, was similar, since the increased DW under LNT was compensated by the decreased sucrose concentration. In the placenta, fructose and starch content was greater under LNT compared with HNT; however, sucrose and glucose contents were not affected. Overall, total content of soluble sugars and starch was 74% greater under LNT than under HNT.

Soluble sugar content was higher in ovary wall and placenta of non-fruiting compared with fruiting plants (Table 5-5). Starch content was also higher, although the increased starch in ovary wall of non-fruiting compared with fruiting plants was not significant. Overall, carbohydrate content was 31% greater in non-fruiting plants compared with fruiting plants. No significant interaction was found between night temperature and presence/absence of fruits on carbohydrate content in the ovary wall or placenta.

Discussion

Ovary size (and therefore the incidence of swollen ovaries) of ‘Legionnaire’ bell pepper increased under LNT compared with HNT, and in non-fruiting (high source:sink ratio), compared to fruiting (low source:sink ratio) plants, confirming previous reports for bell and other sweet pepper cultivars (Chapter 3; Pressman *et al.*, 1998b; Aloni *et al.*, 1999; Shaked *et al.*, 2004). In the present study, ovary FW (which is comprised of ovary wall FW and FW of the inner placental tissues located in the cavity) increased 25% to

35% under LNT compared to HNT and non-fruiting compared with fruiting treatments, respectively. Such increases are comparatively smaller than the values reported by Aloni *et al.* (1998b; 1999), who reported increases of 100 to 300% in ovary FW of bell peppers under LNT or non-fruiting conditions. The reasons for the quantitative differences in effects of night temperature or source:sink ratio on ovary size of bell pepper between the two studies may be due to differences in cultivar, plant age, or environmental conditions used.

Ovary size under LNT increased due to a significant increase in ovary wall thickness and increased transverse area, without significant increases in cell size or cell number. However, there was a trend towards larger cells in all ovary wall tissues of floral ovaries developed under LNT compared with HNT, and a trend towards increased cell number in the mesocarp and endocarp. Taken together, these increases resulted in significant increases in ovary FW, diameter, length, and ovary wall thickness and transverse area in floral ovaries developed under LNT compared with HNT. The trend in increased cell size observed in the present study is supported by results from a previous study where LNT increased cell size in mesocarp and giant cells in bell pepper compared with HNT (Pressman *et al.*, 1998b).

In contrast to the effect of night temperature on ovary swelling, the increase in floral ovary FW in non-fruiting compared with fruiting plants observed in the present study was clearly due to significant increases in cell size of the mesocarp, giant cells and epidermal cells. Actively growing fruits are a much stronger sink for assimilates compared to flowers (Ali and Kelly, 1992; Marcelis and Baan-Hofman-Eijer, 1997), and the presence of developing fruits likely reduced the source supply available for floral

development, resulting in decreased cell size and ovary FW, and therefore the incidence of swollen ovaries compared with non-fruiting plants. Similarly, the observed decrease in the number of cell layers in floral ovary mesocarp in fruiting compared with non-fruiting plants is also a response to increased sink demand of fruiting plants, as observed by Ali and Kelly (1992).

Differences in ovary FW, diameter, and length due to night temperature were found within the first four days after treatment. Responsiveness of 'Legionnaire' bell pepper in the two experiments reported here was similar to the previous response (Chapter 3), where significant differences due to night temperature were found in the first week. In that experiment, we found that most sweet pepper cultivars required three to four weeks of LNT for induction of maximum ovary swelling and concluded that flowers must be exposed to LNT soon after floral initiation begins. 'Legionnaire' bell pepper appears more sensitive than other sweet pepper types to LNT induced ovary swelling and does not appear to require exposure during the first week after flower bud initiation. Further, although Munting (1974) reported that cell division is the main process occurring during floral development in pepper prior to anthesis, our results indicate that cell size is most affected in 'Legionnaire' pre-anthesis in response to LNT or source:sink modification. Thus, there may be genotypic differences among pepper cultivars in the intervals of cell division and cell enlargement during flower development due to the different rates of cell division between genotypes, specially elongated *vs* blocky or spherical (Munting, 1974).

The combined effects of low night temperature and source:sink ratio on ovary swelling in sweet pepper has not been studied previously. In the present research, floral

ovary FW was greatest in non-fruiting plants under LNT and smallest in fruiting plants under HNT, suggesting that night temperature and source:sink effects on ovary FW are cumulative. As hypothesized, fruiting plants under LNT and non-fruiting plants under LNT had intermediate ovary size. The increased ovary FW in non-fruiting plants under LNT was due primarily to both increased mesocarp cell number and size, with additional influences from increased epidermal cell size and increased endocarp cell number. On the other hand, the decreased ovary FW exhibited by fruiting plants under HNT compared with other treatments was due to decreased size of mesocarp and giant cells.

Although ovary FW and diameter were greater in non-fruiting compared with fruiting plants throughout the duration of the experiment, the response over time (*i.e.*, over DAT) was clearly different between fruiting and non fruiting plants. In fruiting plants, floral ovary FW decreased during the first 24 days after treatments started, after which, ovary FW increased. The decrease was likely due to the high sink demand of the growing collective fruits. During the first third to the first half of the fruit growth period, bell pepper fruit reaches the maximum absolute growth rate, gaining over 80% of its final fresh weight and volume, and over 90% of its final diameter and length (Hall, 1977; Nielsen *et al.*, 1991; Marcelis and Baan-Hofman-Eijer, 1995b). Flower buds and flowers, on the other hand, represent the weakest sink in bell pepper plant (Turner and Wien, 1994a). The sink demand of growing bell pepper fruits decreases ~ 35 days after anthesis (Nielsen *et al.*, 1991; Marcelis and Baan-Hofman-Eijer, 1995b), which coincides with the increase in floral ovary FW observed in the present study.

In non-fruiting plants, on the other hand, floral ovaries increased in size during the first seven harvest intervals (*i.e.*, 28 days after treatments started) then remained stable,

before decreasing during the last two harvest periods. Initial increase in ovary size is likely due to the lack of fruiting and therefore increased source supply to flowers. As discussed in Chapter 3 and based on previous reports (Choi and Gerber, 1992; Cruz-Huerta, 2001), the time from flower initiation to anthesis is ~ 4 weeks in bell pepper. Thus, maximum size of floral ovaries in non-fruiting plants was reached ~28 days after treatments began, since these were flowers that developed fully under the non-fruiting conditions. The decrease in floral ovary size in non-fruiting plants at the end of the experiment may be related to a decreased photosynthetic rate in the non-fruiting treatment as previously reported in pepper (Chapter 4; Hall and Milthorpe, 1978) and tomato (Hucklesby and Blanke, 1992).

Anatomical analysis of a subsample of flowers harvested at discrete intervals (*i.e.* 1-2 DAT, 18-19 DAT, and 38-40 DAT) revealed that ovary wall transverse area and cell size of mesocarp, giant cells, and epidermis were significantly greater in ovaries harvested at 18-19 DAT in non-fruiting plants compared with 38-40 DAT in non-fruiting or either harvest time in fruiting plants. This correlates well with the larger ovary FW at 18-19 DAT non-fruiting compared with either harvest time in fruiting plants. This does not explain, however, why there was no difference in ovary FW in flowers harvested at 38-40 DAT *vs* 18-19 DAT in non-fruiting plants. Since there were no differences in ovary length between 18-19 and 38-40 DAT in non-fruiting plants, the similar ovary FW and diameter between the harvest times suggests that the placenta at 38-40 DAT ovaries was larger than those of 18-19 DAT ovaries. This is supported by previous data showing that placenta FW increases and the ratio of ovary wall FW:total ovary FW decreases with harvest interval (Chapter 3).

The increase in the ovary wall transverse area in ovaries harvested at 38-40 DAT of non-fruiting plants compared with ovary wall transverse area of fruiting plants at both harvest times may be due to the sum of the non significant increases in cell numbers of the mesocarp, epidermis, and endocarp. Changes in source:sink ratios may affect fruit size by regulating cell division as early as petal and sepal differentiation, and before carpel initiation (Brukhin *et al.*, 2003; Baldet *et al.*, 2006). Our results suggest that reducing fruit load initially increased ovary wall cell size, but later, as the fruits became weaker sinks (Marcelis and Baan-Hofman-Eijer, 1995b), the effect on cell size of initiating flowers was negligible.

The increase in ovary FW in harvest at 38-40 DAT compared with harvest at 18-19 DAT in fruiting plants was not reflected in significant increases in cell size in ovary wall tissues. However, there was a trend towards increasing cell number in all ovary wall tissues in fruiting plants at 38-40 DAT compared with 18-19 DAT. This, combined with the greater ovary length, likely resulted in greater ovary FW at 38-40 DAT compared with harvested flowers at 18-19 DAT on fruiting plants.

Carbohydrate accumulation was also affected by both night temperature and source:sink ratio. As with effects on ovary cell size and number; however, the effects of night temperature and source:sink ratio on carbohydrate accumulation as related to ovary size differed. Low night temperature and high source:sink ratio both increased floral ovary size, but the increase in ovary size was associated with increased ovary carbohydrate concentration only in the low night temperature, not the high source:sink, treatment. The increased reducing sugar and starch concentration in floral ovaries

developed under LNT compared with HNT may be due to decreased night respiration at the lower temperature, as observed earlier in this work (see Chapter 4).

Non-fruiting conditions, even though they also caused ovary swelling, did not increase ovary sugar or starch concentrations. This is in contrast to work by Aloni *et al.* (1991b; 1999) who found increased carbohydrate concentrations in flower buds of non-fruiting compared with fruiting bell pepper. In their work, Aloni *et al.* (1999) used plants bearing five fruits that resulted in floral ovary weights that were one-third to one-half the weight of ovaries on non-fruiting plants. In our work, the plants had only two developing fruits, resulting in the development of floral ovaries with an average 25% decrease in FW compared with floral ovaries on non-fruiting plants. It may be that under the conditions of our experiment, the increased source:sink ratio on the non-fruiting plants was sufficient to cause increase ovary size and therefore carbohydrate content, but not sufficient to increase storage of carbohydrate and therefore increase concentration.

Our results are in accordance with previous reports that both LNT and high source:sink ratio increase ovary size, and therefore, ovary swelling. Our hypothesis that LNT combined with low source:sink ratio or HNT combined with high source:sink ratio can overcome the detrimental effects of LNT or high source:sink ratio on ovary swelling is also supported by our results. However, it appears that the mechanisms by which floral ovary size increases, resulting in increases in swollen ovaries, differs depending on whether the swollen ovaries occur in response to LNT or in response to excess source supply. Ovary swelling caused by LNT is associated with an increased concentration of reducing sugars and starch in the ovary wall, but not in the placental tissues, as well as with slight increases in both cell size and cell number in the ovary

wall. In contrast, ovary swelling caused by high source:sink ratio seems to be most clearly associated with increased cell size in the mesocarp, giant cells and epidermal cells, with negligible effects on carbohydrate concentration.

Conclusions

Both fruiting plants under LNT and non-fruiting plants under HNT produced an intermediate-sized ovary, which supports our hypothesis that HNT combined with high source:sink ratio or LNT combined with low source:sink ratio can overcome the detrimental effects of low night temperature or high source:sink ratio on ovary swelling in pepper. Ovary fresh weight, diameter and length of flowers at anthesis were increased by both low night temperature (12°C) as well as by absence of fruits. The mechanisms for the increase in ovary size, however, appear to be different. LNT significantly increased ovary wall thickness and transverse area, with only slight increases in cell size and number. Low night temperature also increased floral ovary reducing sugar and starch concentration. Absence of fruits increased floral ovary size mainly through increased cell size, with no effect on increasing ovary reducing sugar or starch concentration.

Table 5-1. Dehydration and resin embedding steps for 'Legionnaire' bell pepper ovaries previously fixed in FAA containing 50% ethanol.

Step	Product	Processing time[z]	Infiltration time[y]
Dehydration	Ethanol 50%	45 seconds	1 min.
	75%	45 seconds	1 min.
	95%	45 seconds	1 min.
	100%	45 seconds	1 min.
	Acetone 100%	45 seconds	
	Acetone 100%		Overnight
Spurr resin embedding	Resin: Acetone		
	30:70	3 min.	5 min.
	50:50	3 min.	5 min.
	70:30	3 min.	5 min.
	100:0	3 min.	5 min.
	100:0	-	Overnight

[z]Time that the samples were processed in a PELCO BioWave® Laboratory Tissue Processing System with ColdSpot® (Ted Pella, Inc. Redding, CA, USA) at 180 W and waterbed at 24°C.
[y]After every step, samples were left on the bench top for various times to improve infiltration.

Table 5-2. Main effects of night temperature and presence/absence of fruits on the fresh weight, diameter, and length of the ovary in 'Legionnaire' bell pepper flowers harvested at anthesis

	Fresh weight (mg)	Diameter (mm)	Length (mm)
Night temperature[z,y]			
12°C	128*	6.6*	4.5*
20°C	102	6.0	4.3
Fruits [x]			
Non-fruiting plants	132*	6.7*	4.6*
Fruiting plants	98	5.9	4.2

[z]Pooled data of two experiments. Flowers were continuously harvested for 42 days in Experiment 1 and 38 days in Experiment 2.
[y]Means were averaged across fruiting treatments and harvest intervals; n= 597 and 492 for 20°C and 12°C, respectively.
[x]Means were averaged across temperature treatments and harvest intervals; n= 595 and 494 for non-fruiting and fruiting treatments, respectively.
*Asterisks indicate means are significantly different at $P \leq 0.001$.

Table 5-3. Main effect of days after treatment on the fresh weight, diameter, and length of the ovary in 'Legionnaire' bell pepper flowers harvested at anthesis.

Days after treatment[z,y]	Fresh weight (mg)	Diameter (mm)	Length (mm)
1-4	97 e [x, w]	5.8 g	4.3 c
5-8	89 e	5.8 fg	3.9 d
9-12	101 de	6.0 ef	4.3 c
13-16	108 cd	6.2 de	4.3 bc
17-20	104 de	6.2 cde	4.3 bc
21-24	118 bc	6.4 bcd	4.5 ab
25-28	128 ab	6.6 ab	4.6 a
29-32	132 a	6.7 a	4.6 a
33-36	130 a	6.6 ab	4.6 a
37-40	129 ab	6.6 ab	4.6 a
41-44	128 ab	6.6 abc	4.6 a

[z]Pooled data of two experiments. Flowers were continuously harvested during 42 days in Experiment 1 and 38 days in Experiment 2.
[y]Ovaries were grouped at 4-day intervals. Data from 5-8 DAT were from Experiment 2 only; data from 41-44 DAT were from Experiment 1 only.
[x]Means were averaged for each 4-day interval across night temperature and fruiting treatments; n = 136, 42, 155, 106, 93, 87, 96,121,127, 93, and 33 for intervals 1-4 DAT to 41-44 DAT, respectively.
[w]Means with the same letter in the same column are not significantly different (Tukey-Kramer, $P \leq 0.05$).

Table 5-4. Main effects of night temperature and presence/absence of fruits on soluble sugar and starch concentration in ovaries (ovary wall and placenta) of 'Legionnaire' bell pepper flowers harvested at anthesis[z].

	Ovary wall (µg/g DW)				Placenta (µg/g DW)			
	Glu[y]	Fru	Suc	Starch	Glu	Fru	Suc	Starch
Temperature[x]								
12°C	31.9**	31.9*	27.9*	56.9*	62.8	72.9	45.7	99.6
20°C	26.2	26.3	35.6	44.8	61.1	65.8	50.5	87.4
Fruits[w]								
Non-fruiting	29.0	30.1	31.4	46.6	63.4	68.6	44.2*	87.4
Fruiting	29.1	28.2	32.0	55.1	60.5	70.1	52.1	99.6

[z]Flowers were harvested 24 to 29 days after treatments started.
[y]Glu: glucose, Fru: fructose, Suc: sucrose.
*, **: Significant differences at P≤0.05 and 0.01 respectively.
[x]Means were averaged across fruiting treatments, n =12.
[w]Means were averaged across temperature treatments, n = 12.

Table 5-5. Main effects of night temperature and presence/absence of fruits on soluble sugar and starch content in ovaries (ovary wall and placenta) of 'Legionnaire' bell pepper flowers harvested at anthesis.

	Ovary wall content (µg/flower)				Placenta content (µg/flower)			
	Glu[y]	Fru	Suc	Starch	Glu	Fru	Suc	Starch
Temperature[z, x]								
12°C	540**	533***	466	986*	976	1098*	658	1489*
20°C	314	319	411	552	771	813	626	1071
Fruits[w]								
Non-fruiting	492 *	501*	498*	844	1095**	1158**	729*	1494*
Fruiting	362	350	380	694	653	753	554	1066

[z]Flowers were harvested 24 to 29 days after treatments started.
[y]Glu: glucose, Fru: fructose, Suc: sucrose.
*, **: Significant differences at $P \leq 0.05$ and 0.01, respectively.
[x]Means were averaged across fruiting treatments, n =12.
[w]Means were averaged across temperature treatments, n = 12.

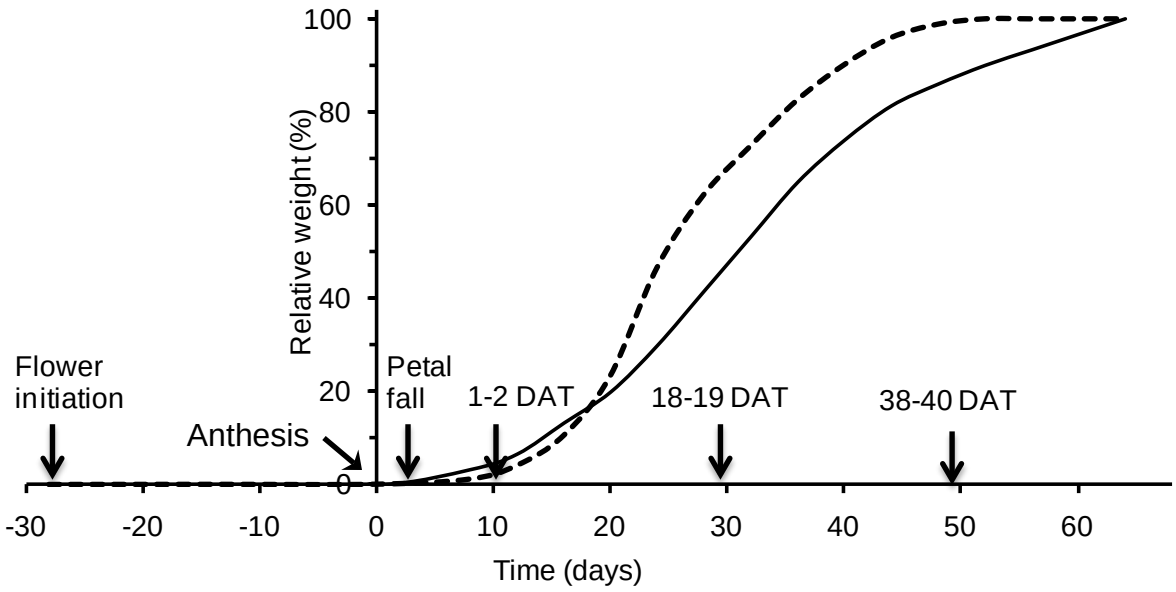

Figure 5-1. Schematic representation of harvesting times for anatomical analysis in relation to fruit growth in the fruiting plants. —— Dry weight; - - - Fresh weight. Day 0 represent anthesis. Flower initiation occurs four weeks before anthesis, and petal fall occurs ~3-4 days after anthesis. Treatments started ~6 days after petal fall. Flowers used in the anatomical analysis reached their anthesis at 1-2, 18-19 or 38-40 days after treatment (DAT).

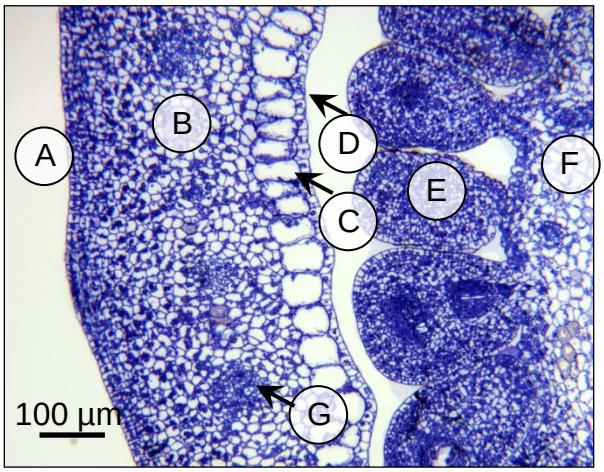

Figure 5-2. Transverse cross section of the ovary wall of ovaries at flower anthesis in 'Legionnaire' bell pepper. Tissues: A) epidermis and hypodermis, B) mesocarp, C) giant cells, D) endocarp, E) ovule, F) placenta tissue, G) vascular bundle.

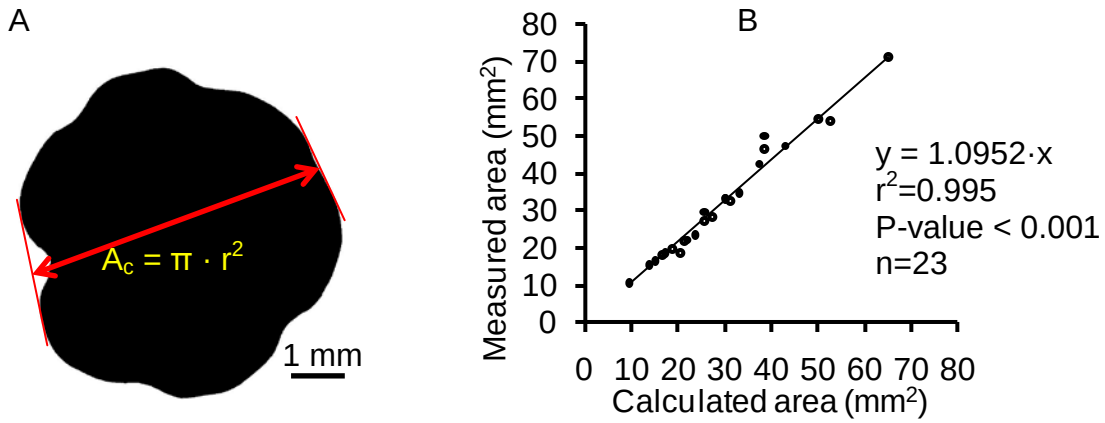

Figure 5-3. Relationship between the measured and the calculated area of a transverse section in the ovary of 'Legionnaire' bell pepper. A) Cross section area was calculated with the average diameter (arrow), then the area was measured from monochromatic images with imageJ (Rasband, 2010). B) Linear regression between calculated and measured area.

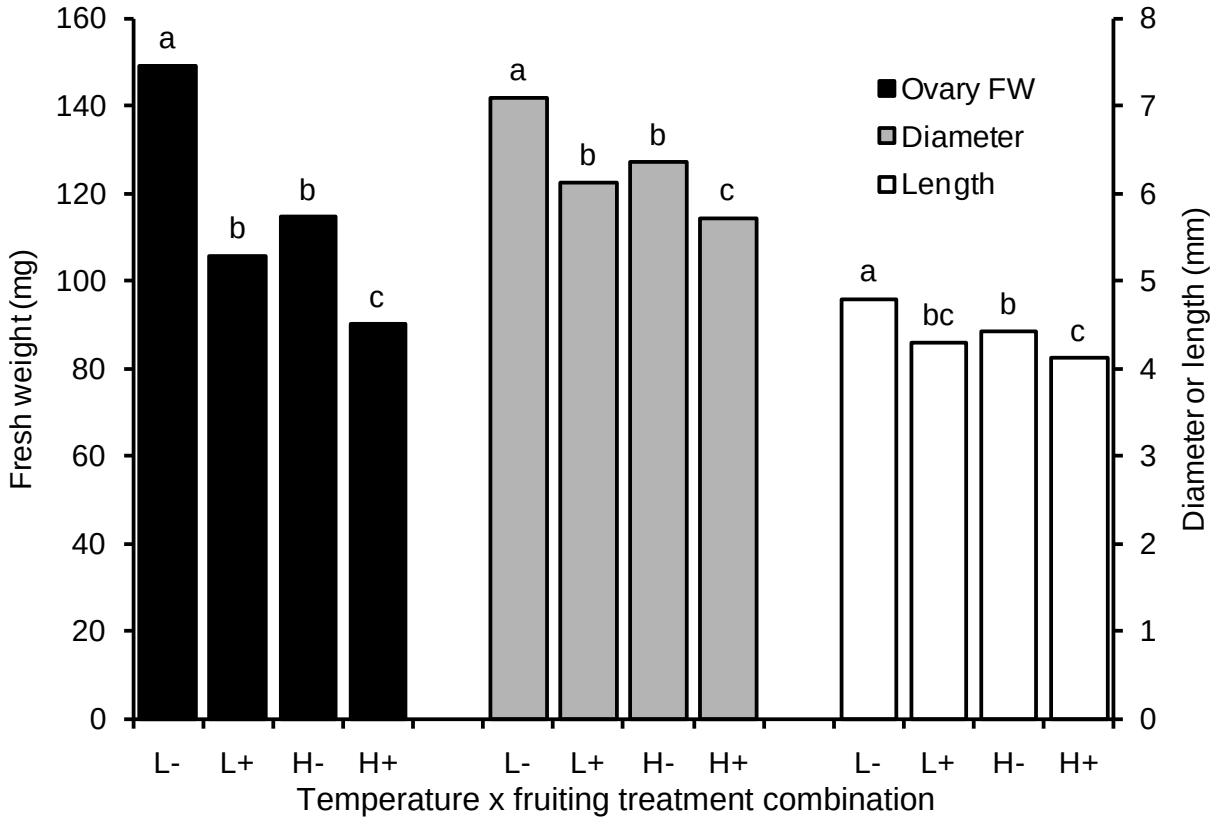

Figure 5-4. Interaction of night temperature and presence/absence of fruits on ovary characteristics of flowers harvested at anthesis in 'Legionnaire' bell pepper. Night temperature was either 12 (L) or 20°C (H), and plants had either 0 (-) or two developing fruits (+). Data for the two experiments were pooled for analysis. Flowers were continuously harvested for 42 days in Experiment 1 and 38 days in Experiment 2. Means were averaged for each combination across harvest intervals; n = 265, 227, 330, and 267 for L-, L+, H- and H+ treatments, respectively. Means with the same letter in the same parameter are not significantly different (Tukey-Kramer, $P \leq 0.05$).

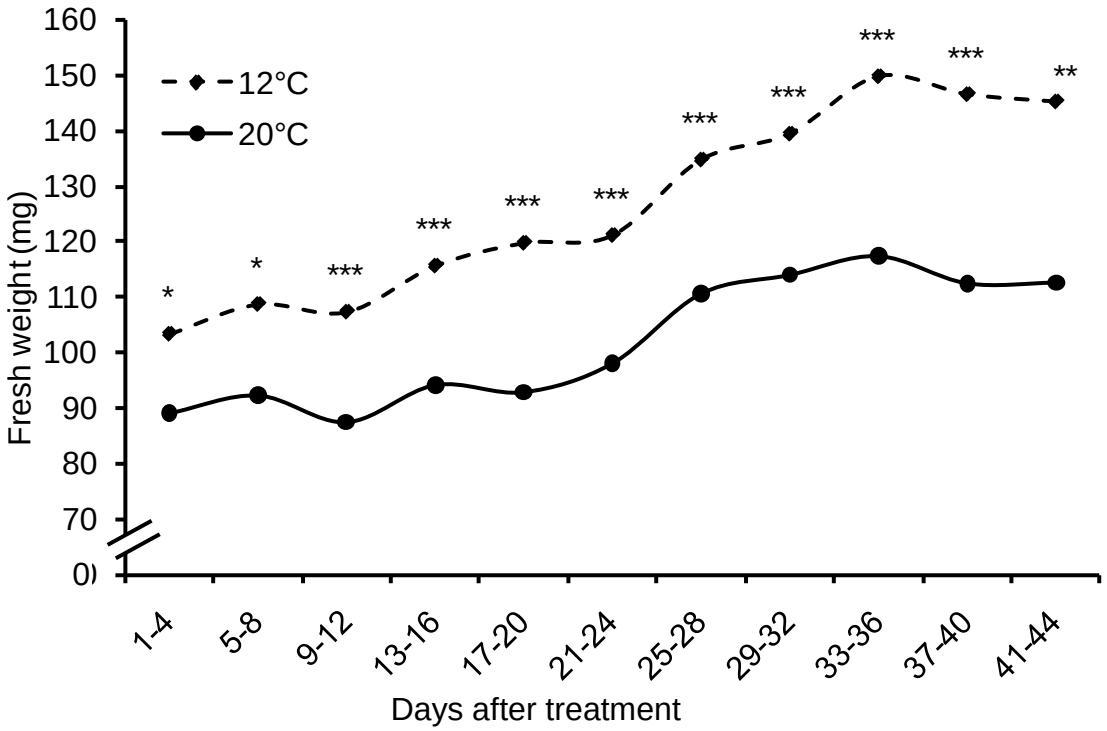

Figure 5-5. Effect of night temperature and days after treatment on ovary fresh weight of flowers at anthesis in 'Legionnaire' bell pepper. Flowers were harvested daily for 42 days in Experiment 1 and for 38 days in Experiment 2; data were grouped every 4 days. Data for the two experiments were pooled for analysis. Data from 5-8 DAT was from Exp. 2 only; data from 41-44 DAT was from Exp. 1 only. Means were averaged for each combination across fruiting treatments; n = 26 to 32 for 1-4 and 41-44 DAT, and 35 to 86 for the remaining intervals. *, **, ***: Significant differences between temperature regimes within each harvest interval at $P \leq 0.05$, 0.01 and 0.001, respectively.

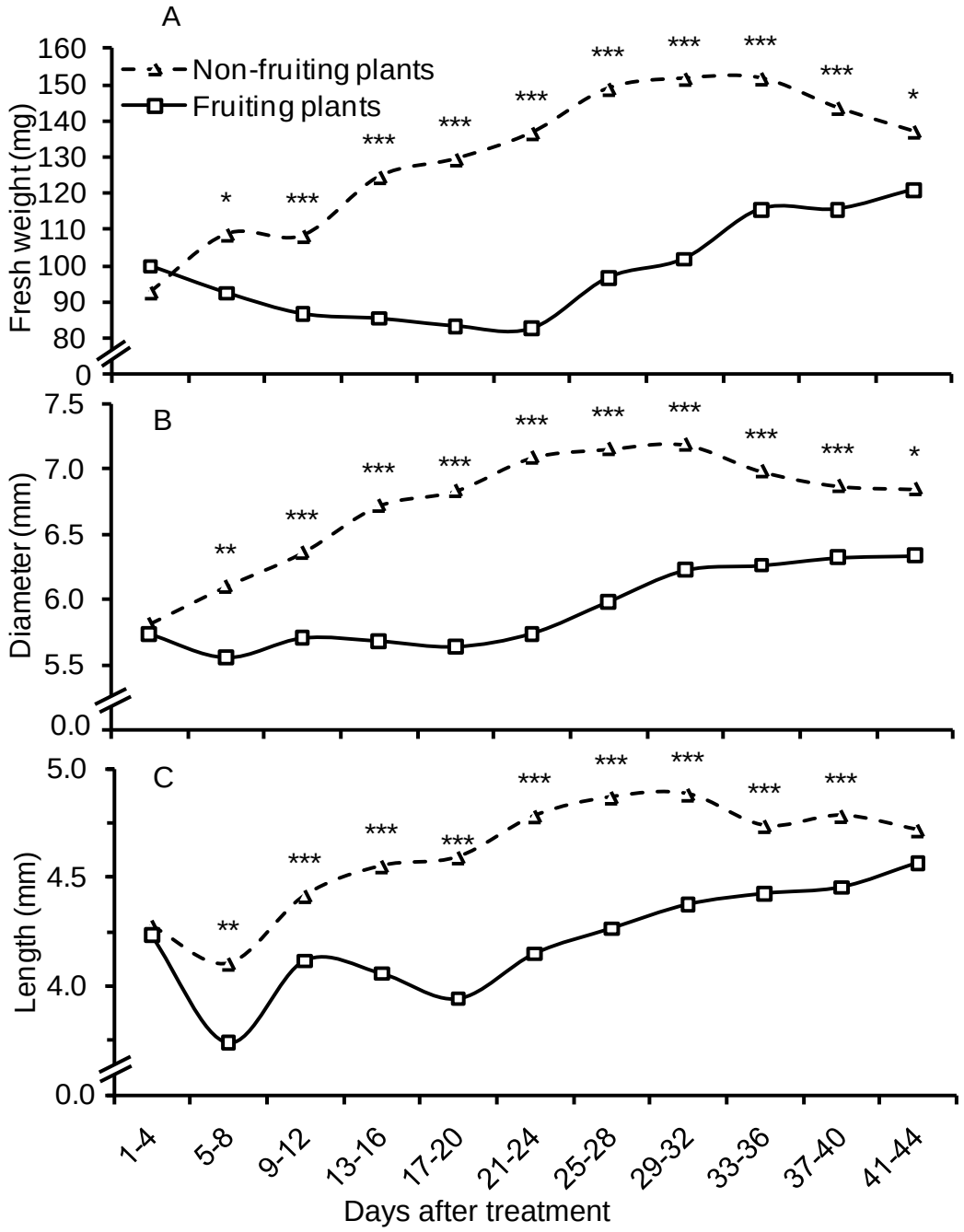

Figure 5-6. Effect of presence/absence of fruits and days after treatment on ovary size of flowers harvested at anthesis in 'Legionnaire' bell pepper. A) Ovary fresh weight; B) diameter; C) length. Flowers were harvested daily for 42 days in Experiment 1 and 38 days in Experiment 2; data were grouped every 4 days. Data for the two experiments were pooled for analysis. Data from 5-8 DAT was from Exp. 2 only; data from 41-44 DAT was from Exp. 1 only. Means were averaged for each combination across night temperature treatments; n = 22 to31 for intervals 5-8 DAT and 41-44 DAT, and 39 to 90 for the remaining intervals. *, **, ***: Significant differences within each harvest interval at $P \leq 0.05$, 0.01 and 0.001, respectively.

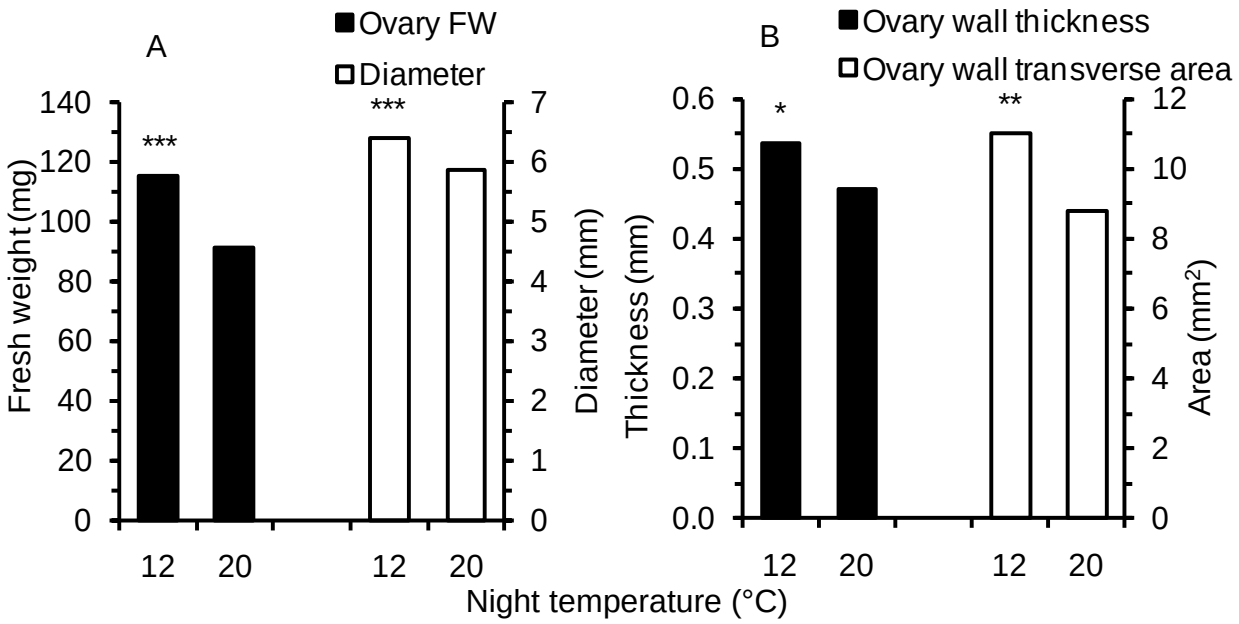

Figure 5-7. Effect of night temperature on ovary size and ovary wall thickness and area in 'Legionnaire' bell pepper flowers at anthesis. A) ovary size; B) ovary wall thickness and ovary wall transverse area. *, **, ***: Means between temperature treatments are significantly different at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; n = 16.

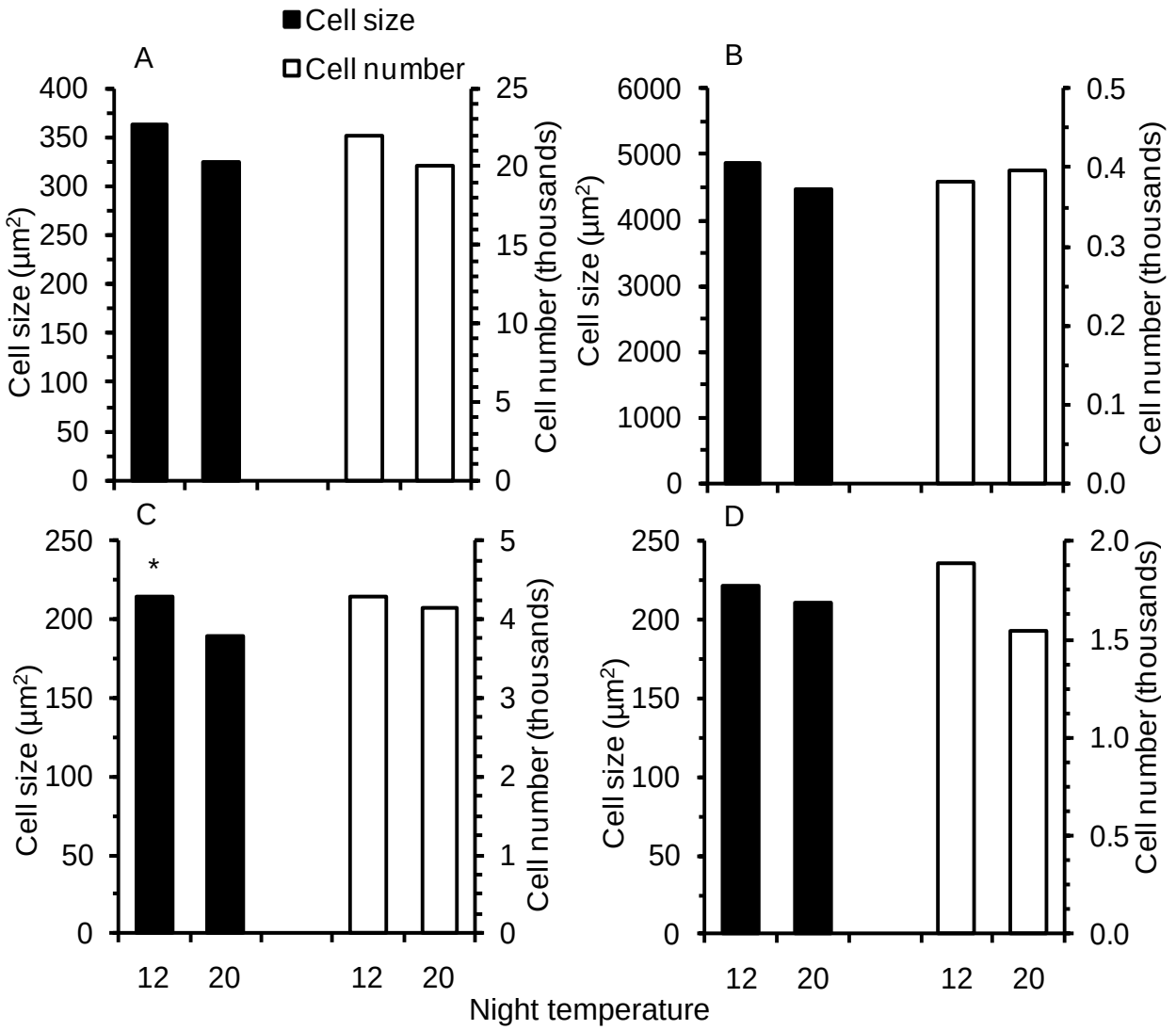

Figure 5-8. Effect of night temperature on ovary wall cell size and cell number in 'Legionnaire' bell pepper flowers harvested at anthesis. A) mesocarp; B) giant cells; C) epidermal tissues; D) endocarp. * Means between temperature treatments are significantly different at $P \leq 0.05$; n = 16.

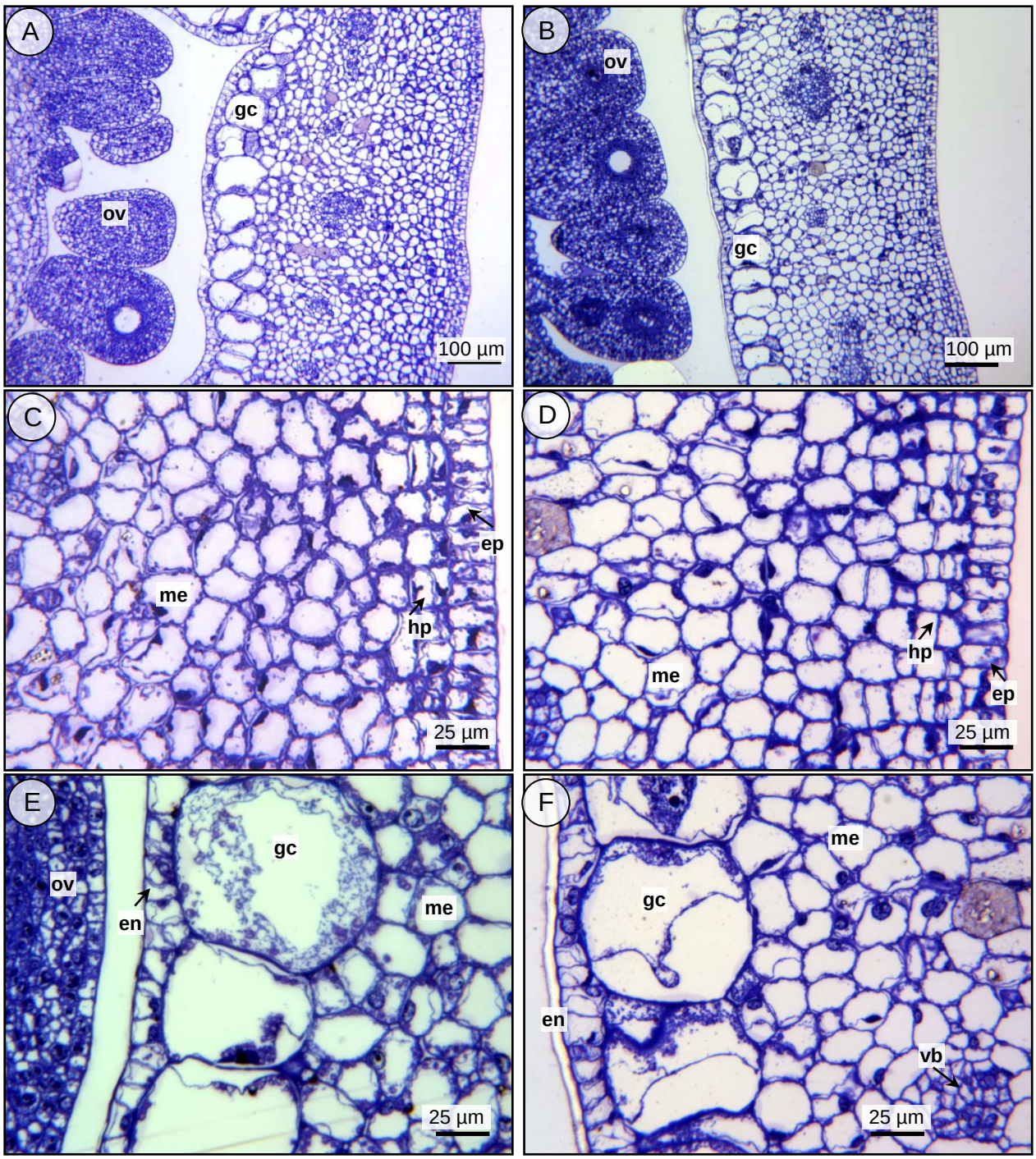

Figure 5-9. Median cross section of ovaries in ‘Legionnaire’ bell pepper flowers developed under two night temperatures. A, C, E: low night temperature, B, D, F: high night temperature; A, B: total ovary wall; C, D: outer ovary wall; E, F: inner ovary wall; ep, epidermis; hp, hypodermis; me, mesocarp; vb, vascular bundle; gc, giant cell; en, endocarp; ov, ovule. Note the thicker ovary wall under LNT compared to HNT but no difference in cell size.

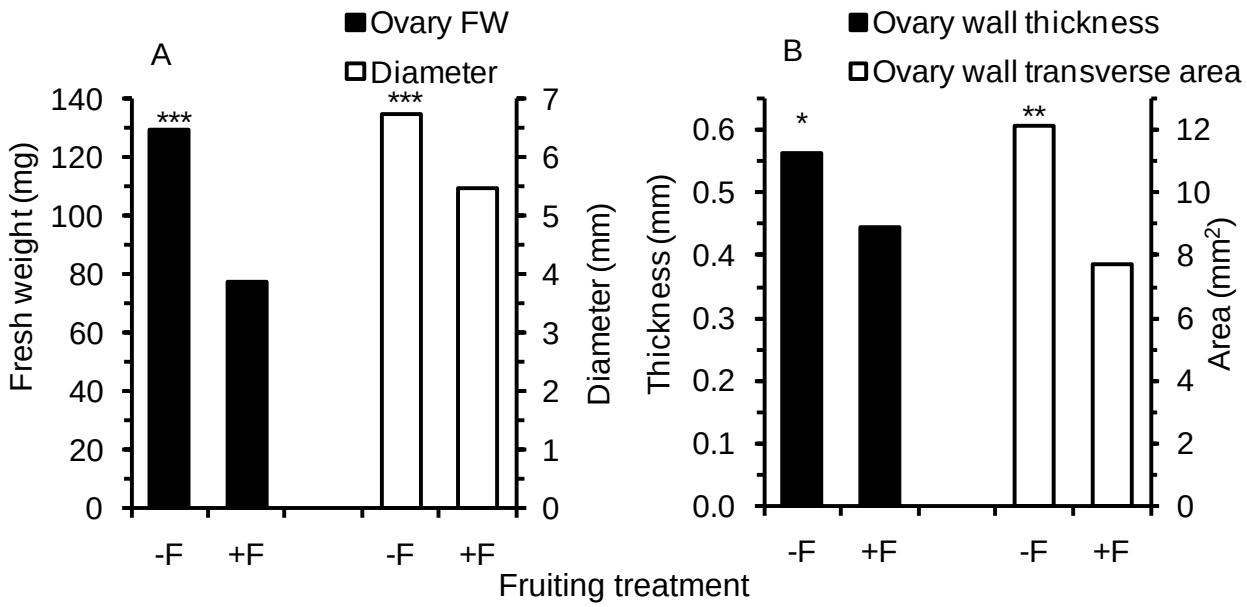

Figure 5-10. Effect of presence (+F) or absence (-F) of fruits on ovary size and ovary wall thickness and area in 'Legionnaire' bell pepper flowers harvested at anthesis. A) ovary size, B) ovary wall thickness and ovary wall transverse area. +F: 2 developing fruits per plant. *, **, ***: Means between fruiting treatments are significantly different at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; n = 16.

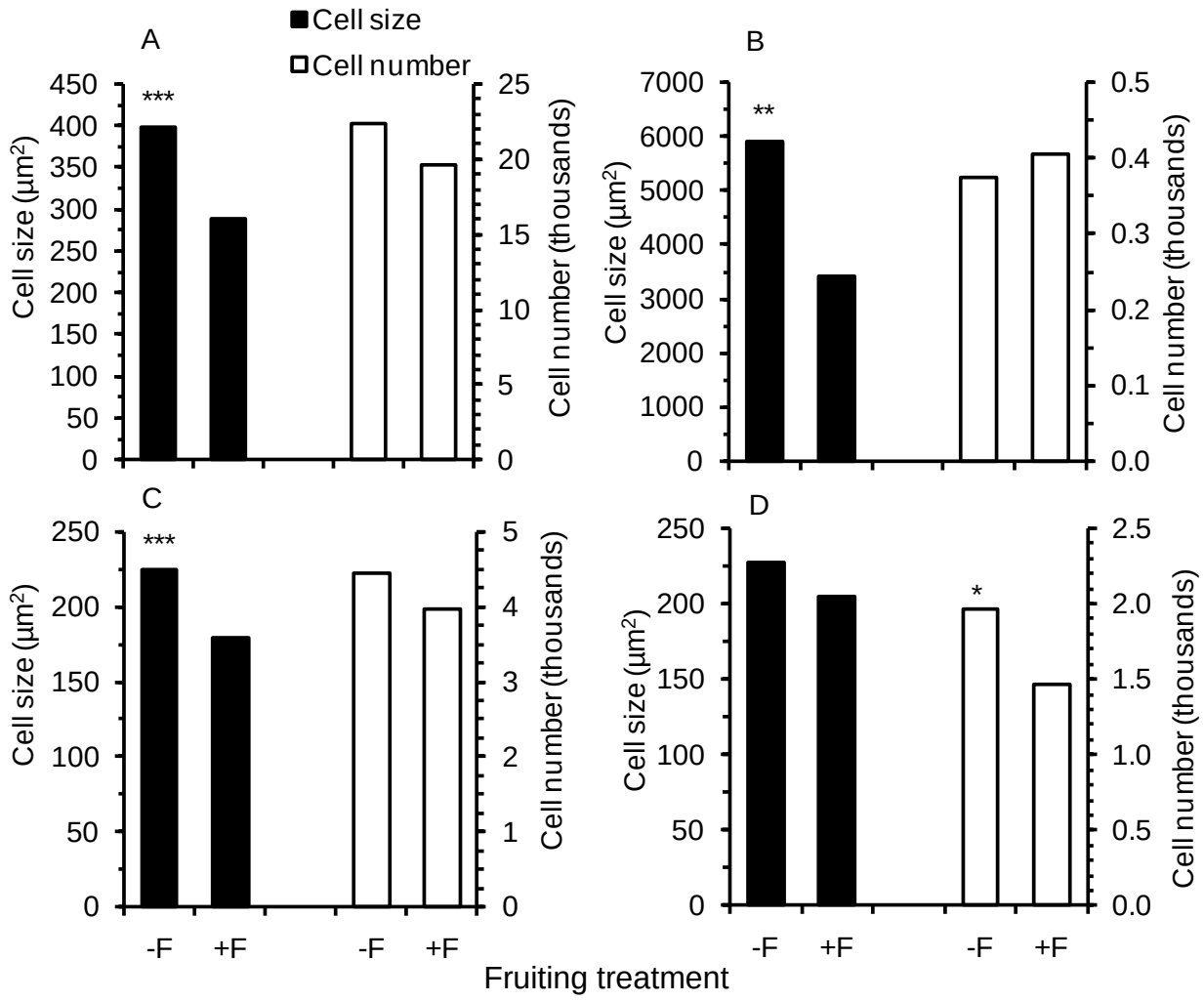

Figure 5-11. Effect of the presence (+F) or absence (-F) of developing fruits on ovary wall cell size and cell number in 'Legionnaire' bell pepper flowers harvested at anthesis. A) mesocarp; B) giant cells; C) epidermal tissues; D) endocarp. +F: 2 developing fruits per plant. *, **, ***: Means between fruiting treatments are significantly different at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; n = 16.

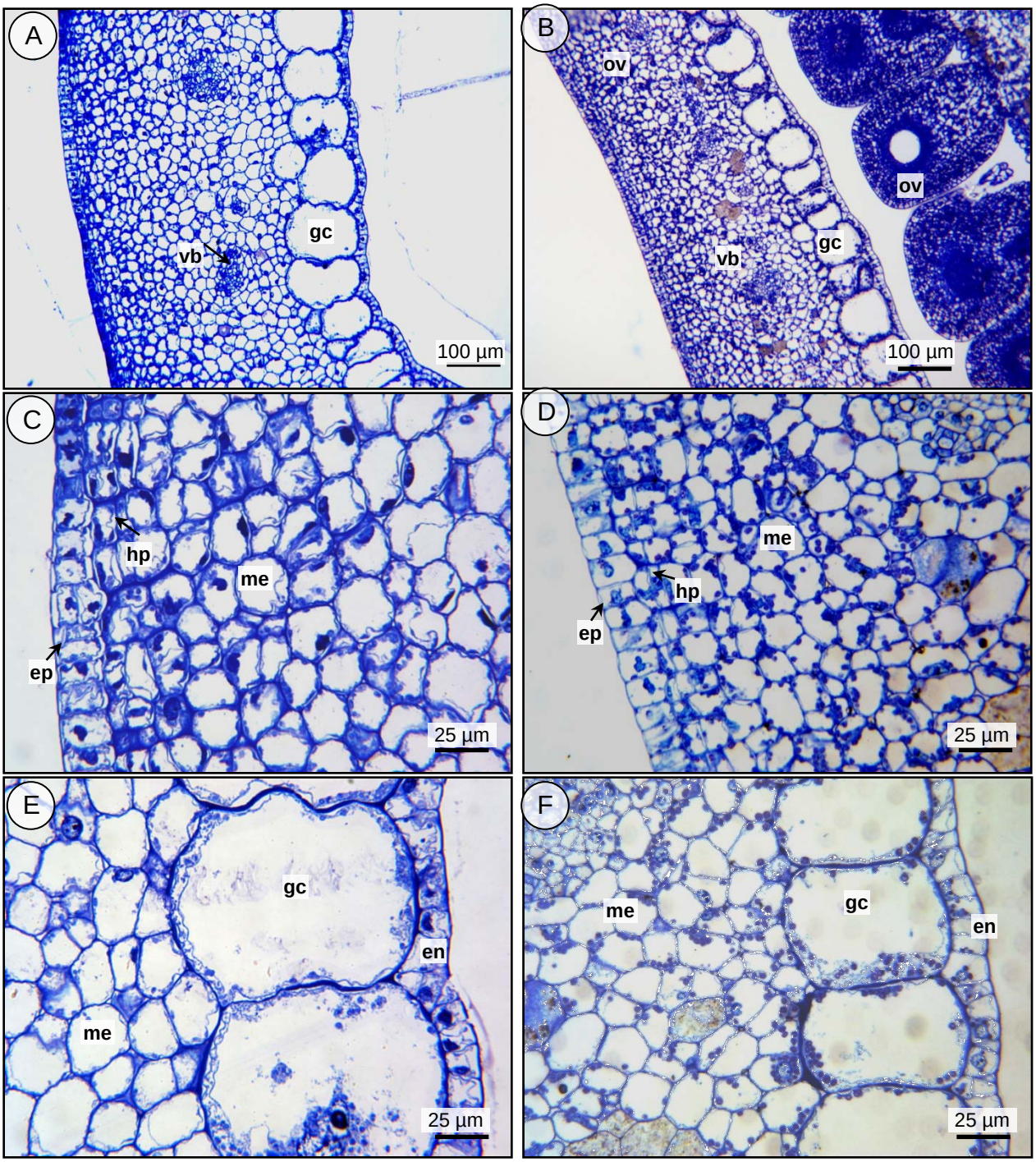

Figure 5-12. Median cross section of ovaries in ‘Legionnaire’ bell pepper flowers developed in fruiting and non-fruiting plants. A, C, E: non-fruiting plants; B, D, F: fruiting plants (2 developing fruits per plant); A, B: total ovary wall; C, D: outer ovary wall; E, F: inner ovary wall; ep, epidermis; hp, hypodermis; me, mesocarp; vb, vascular bundle; gc, giant cell; en, endocarp; ov, ovule. Note the thicker ovary wall and larger cells in the mesocarp and giant cells in the non-fruiting compared to the fruiting plants.

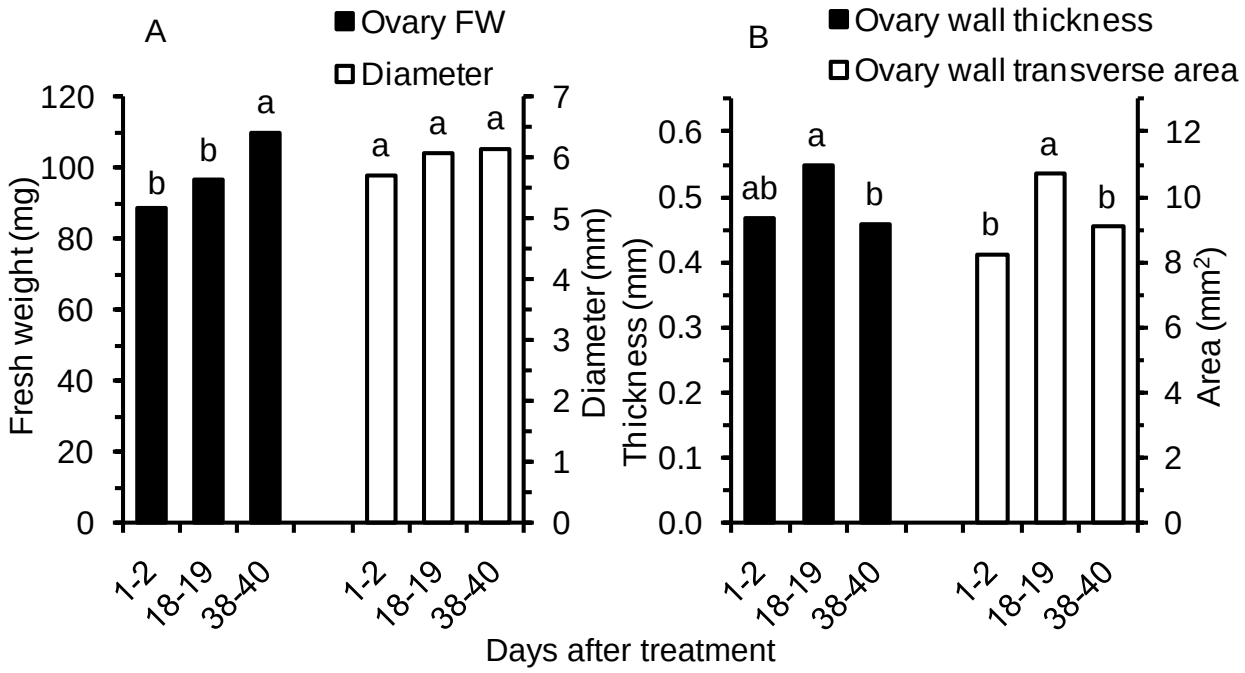

Figure 5-13. Effect of harvest time on the ovary size and ovary wall thickness and area in ‘Legionnaire’ bell pepper flowers harvested at anthesis. A) Ovary size, B) Ovary wall thickness and ovary wall transverse area. Means with the same letter are not significantly different at $P \leq 0.05$ (LSD test); n = 4 for 1-2 DAT, n = 16 for 18-19 and 38-40 DAT.

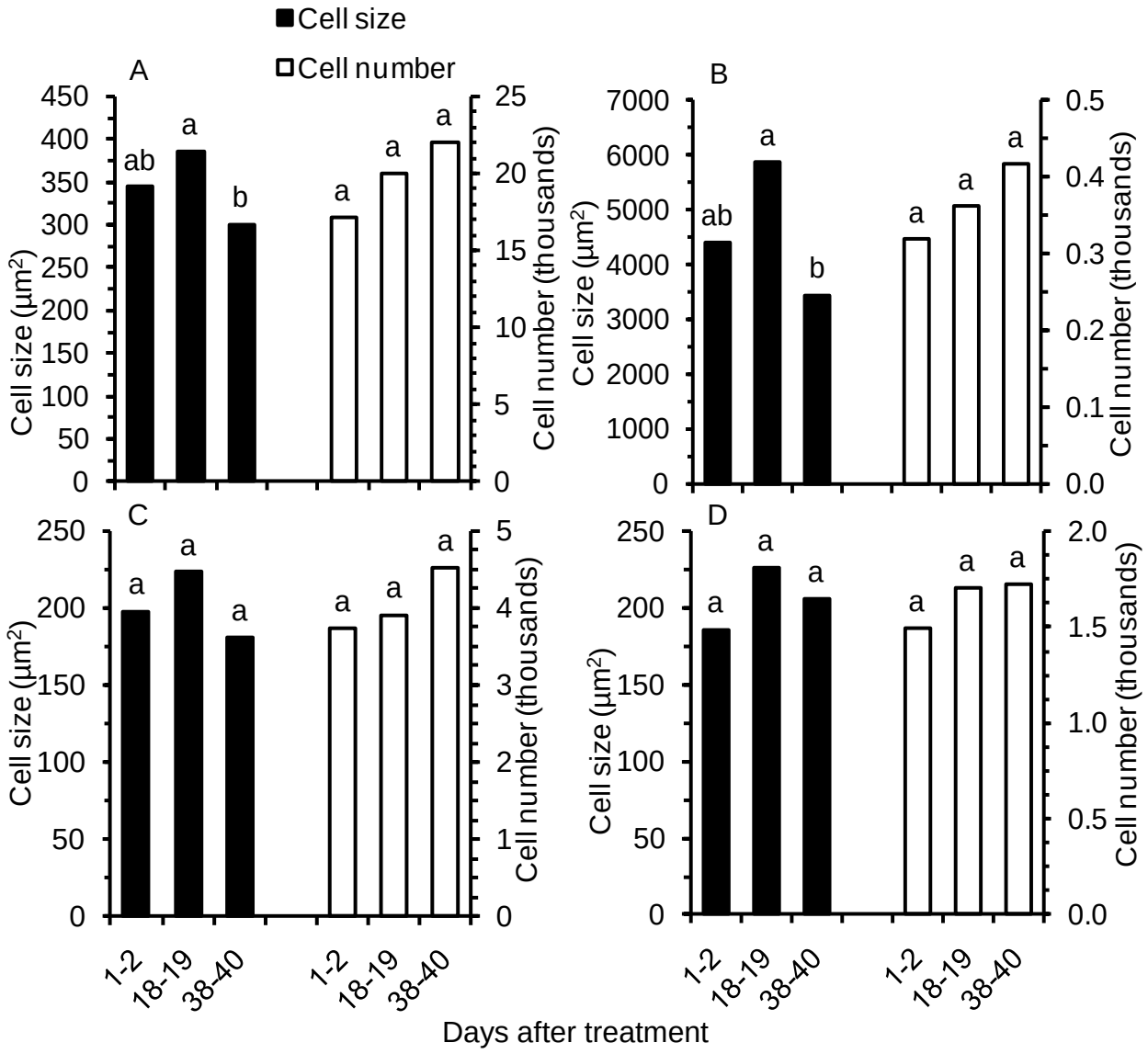

Figure 5-14. Effect of harvest time on ovary wall cell size and cell number in 'Legionnaire' bell pepper flowers harvested at anthesis. A) mesocarp; B) giant cells; C) epidermal tissues; D) endocarp. Means with the same letter are not significantly different at $P \leq 0.05$ (LSD test); n = 4 for 1-2 DAT, n = 16 for 18-19 and 38-40 DAT.

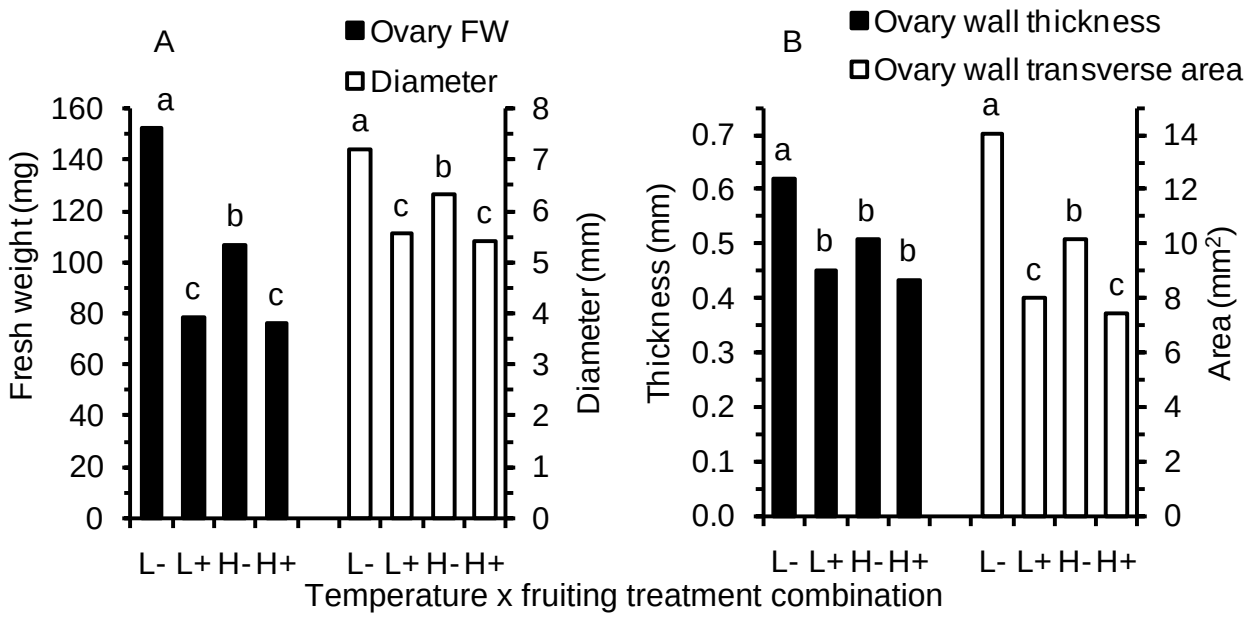

Figure 5-15. Interaction of night temperature and presence/absence of fruits on ovary size and ovary wall thickness and area in 'Legionnaire' bell pepper flowers harvested at anthesis. Night temperature was either 12 (L) or 20°C (H), and plants had either 0 (-) or 2 developing fruits (+). Means with the same letter are not significantly different at $P \leq 0.05$ (LSD test); n=8.

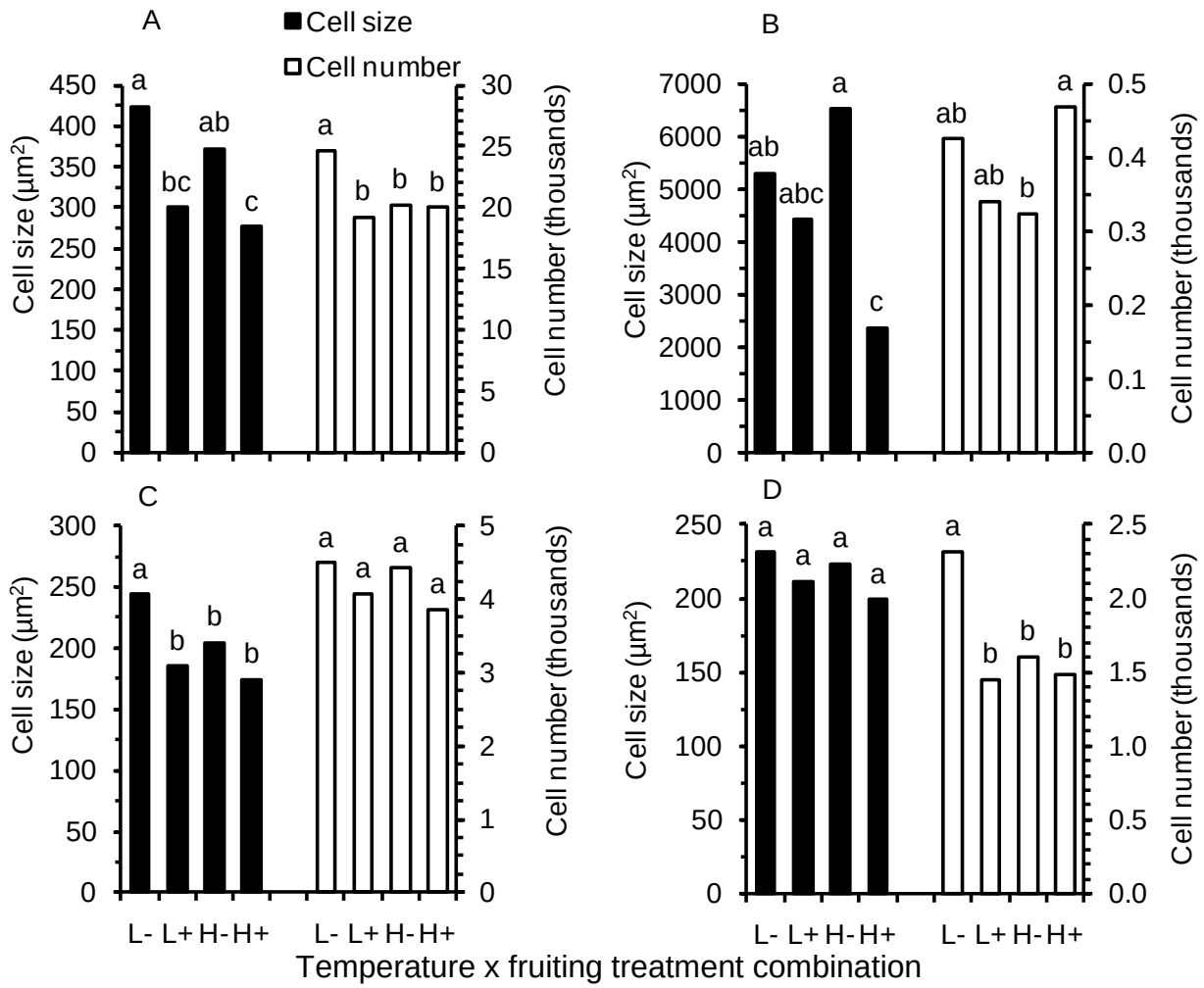

Figure 5-16. Interaction of night temperature and presence/absence of fruits on ovary wall cell size and cell number in 'Legionnaire' bell pepper flowers harvested at anthesis. A) mesocarp, B) giant cells, C) epidermal tissues, D) endocarp. Night temperature was either 12 (L) or 20°C (H), and plants had either 0 (-) or 2 developing fruits (+). Means with the same letter were not significantly different at $P \leq 0.05$ (LSD test); n=8.

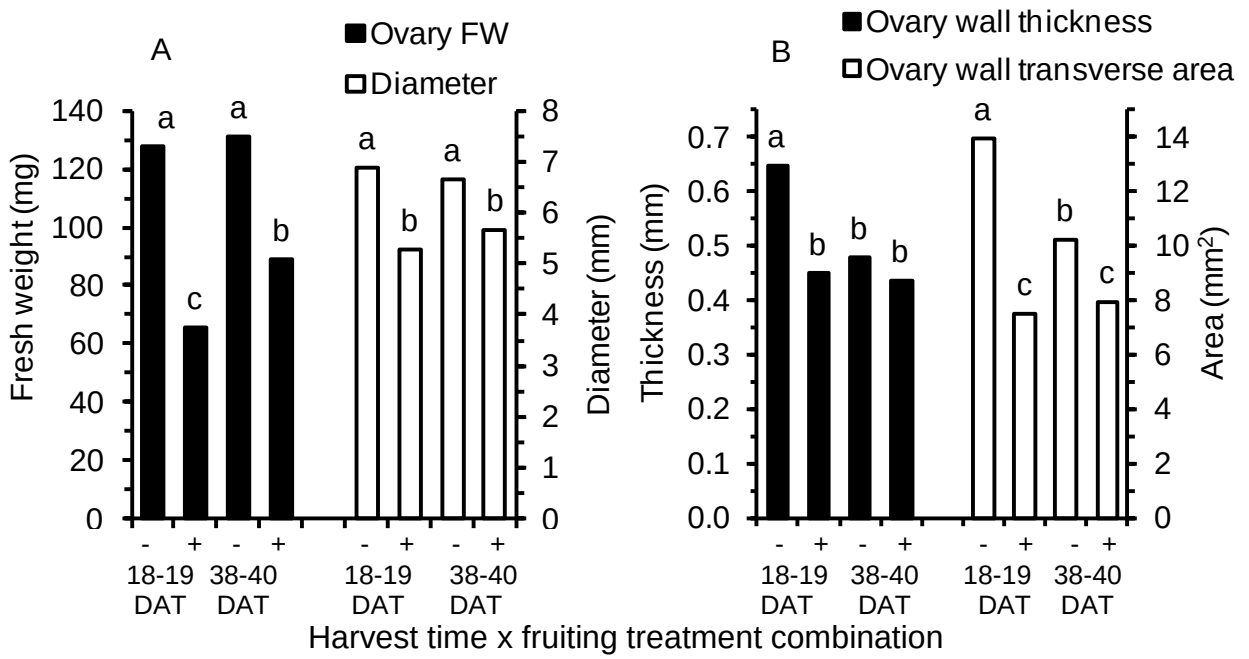

Figure 5-17. Effect of harvest time and presence/absence of developing fruits on ovary size and ovary wall thickness and area in 'Legionnaire' bell pepper flowers harvested at anthesis. Flowers were harvested at 18-19 days after treatment (DAT) or 38-40 DAT and plants had either 0 (-) or 2 developing fruits (+). Means with the same letter are not significantly different at $P \leq 0.05$ (LSD test); n=8.

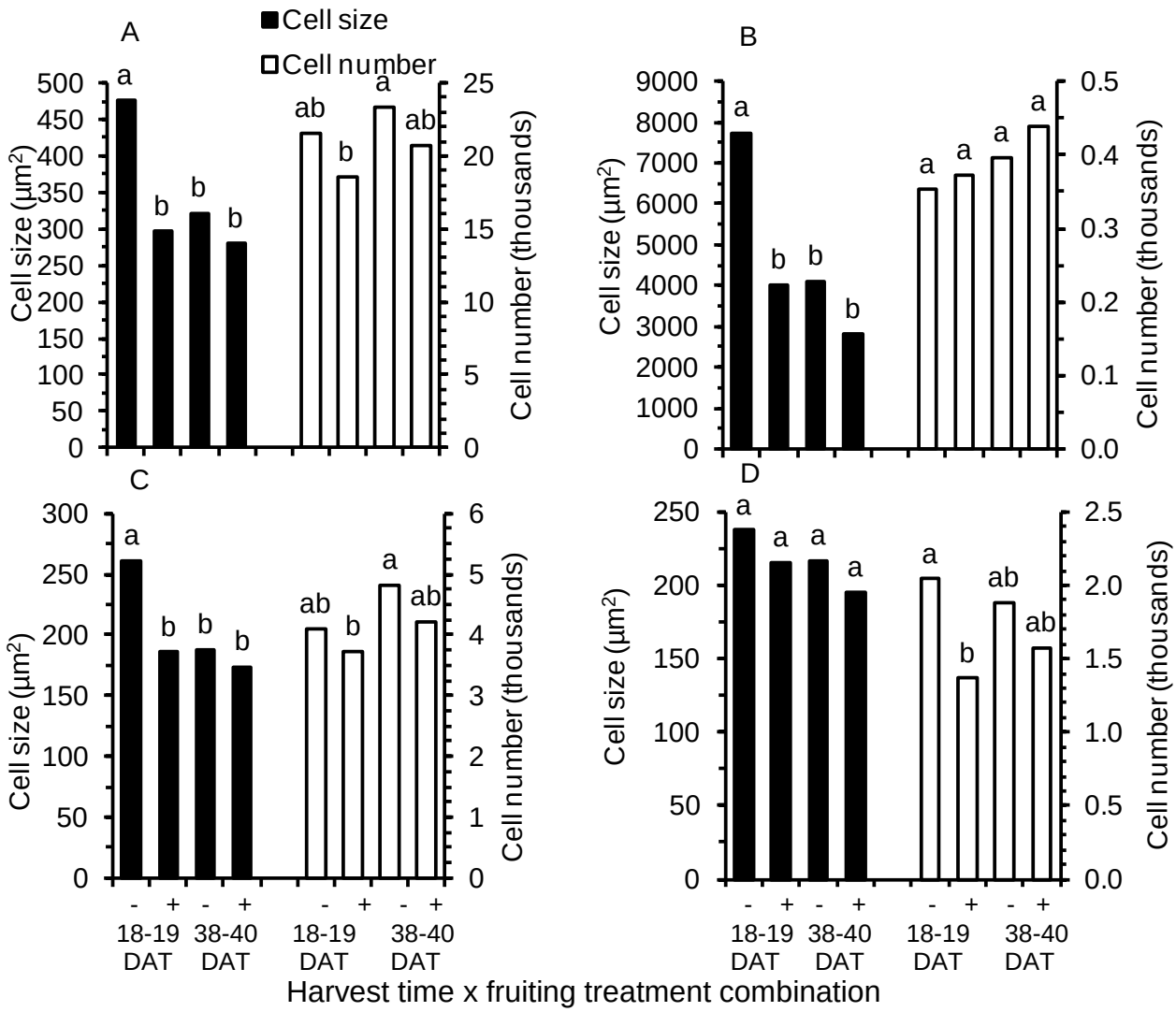

Figure 5-18. Effect of harvest time and presence/absence of developing fruits on ovary wall cell size and cell number in 'Legionnaire' bell pepper flowers harvested at anthesis. A) mesocarp, B) giant cells, C) epidermal tissues, D) endocarp. Flowers were harvested at 18-19 days after treatment (DAT) or 38-40 DAT and plants had either 0 (-) or 2 developing fruits (+).Means with the same letter are not significantly different at $P \leq 0.05$ (t-test).

CHAPTER 6
SUMMARY

Sweet peppers are an important vegetable crop whose fruits are mostly consumed as fresh produce all year. Cultivated species of peppers were domesticated in tropical and temperate areas, and are sensitive to low temperatures (Harlan, 1971; Eshbaugh, 1993). Pepper plants grown under cool night temperatures (<15°C) develop deformed flowers, including swollen flowers and ovaries (*i.e.*, flowers and ovaries that are larger than the ones developed under optimum temperature), resulting in a high percentage of malformed fruits that are not marketable (Mercado *et al.*, 1997c; Pressman *et al.*, 1998a; Pressman *et al.*, 1998b; Aloni *et al.*, 1999). Swollen ovaries also develop under high source:sink ratios in pepper (Aloni *et al.*, 1999). Under both conditions, *i.e.* low night temperatures and high source:sink ratios, swollen flowers are reported to have increased reducing sugars and starch concentration compared to normal (not swollen) flowers (Aloni *et al.*, 1999). Although several studies have been done, the relation between assimilate availability and ovary swelling in pepper remains unclear. Furthermore, the anatomical basis for ovary swelling and deformation under these conditions is unknown. In this study, the central hypothesis was that both low night temperature and high source:sink ratio increase ovary size and therefore ovary swelling via effects on assimilate availability/accumulation and/or cell size/number.

The series of experiments were carried out under growth chamber conditions by controlling night temperature at either 12°C (low night temperature, LNT) or 20°C (high night temperature, HNT). The experiments also implied continuous leaf pruning to keep plants at two main axis and continuous flower removal. In the first three experiments (*i.e.,* Experiments 1 and 2 in Chapter 3, and Experiment 1 in Chapter 4) plants did not

bear any developing fruits before and during the duration of the experiment. In two experiments (Experiment 1 and 2 [same as Experiment 2 in Chapter 4] in Chapter 5), 2 fruits were allowed to set and ~6-10 days after fruit setting (petal fall), fruits were removed in one treatment, while fruits on the other treatment were allowed to develop to maturity.

The discussion of the results was addressed from a source-sink point of view. We are aware that plant growth regulators might be masking or enhancing some responses, since the experiments implied continuous leaf and flower pruning, and in some cases, presence or absence of fruits. However, since we did not have any data on hormones, such effects were considered as an integral component of the treatment effect.

It has been previously suggested that other factors such as hormones may also influence the development of swollen ovaries (Polowick and Sawhney, 1985; Aloni *et al.*, 1995; Pressman *et al.*, 1998b). one hypothesis is that low night temperature inhibits the basal auxin transport from the flower bud to other parts of the plant, and then the increased auxin concentration in the flower favors ovary growth (Aloni *et al.*, 1995; Pressman *et al.*, 1998b). Presence/absence of fruits may also alter the hormonal balance in the plant. It has been suggested that growth inhibition of a second flower, and therefore a fruit, is controlled by both competition for limited assimilates (Ali and Kelly, 1992; Marcelis and Baan-Hofman-Eijer, 1997) and by dominance due to the production of plant growth regulators by the earlier developed fruit (Marcelis and Baan-Hofman-Eijer, 1997).

Ultimately, from the results of this research, it is concluded that LNT induced ovary swelling in different kinds of sweet pepper (cherry, elongated and blocky bell). Three to

four weeks of continuous low night temperature were required for maximum response, suggesting that flowers must be exposed to LNT soon after flower initiation in order for this response to occur. However, the minimum duration of exposure to LNT required to induce this response is unknown, as the present research maintained LNT throughout the course of flower development. Fruit removal (*i.e.* increasing the source:sink ratio) also increased the incidence of swollen ovaries. Both LNT and fruit removal decreased net CER without reducing plant growth, suggesting that excess availability of current photosynthate may not be the mechanism that results in the increase in swollen ovaries observed under both LNT and high source:sink conditions. HNT combined with high source:sink ratio or LNT combined with low source:sink ratio can overcome the detrimental effects of low night temperature or high source:sink ratio on ovary swelling in pepper. Finally, although ovary swelling is induced by both LNT and by absence of fruits, the mechanisms for the increase in ovary size, however, appear to be different. LNT significantly increased pericarp thickness and transverse area, with only slight increases in cell size and number. LNT also increased ovary wall reducing sugar and starch concentration. Absence of fruits increased floral ovary size mainly through increased cell size, with no effect on increasing ovary reducing sugar or starch concentration.

APPENDIX
ADDITIONAL TABLES AND FIGURES

A) Tables

Table A-1. P-values for temperature (Temp), cultivar (CV), and days after treatment (harvest) main effects and their interactions. Branch within each cultivar was also considered. Number of days after anthesis (DAA) was used as covariate, and the subject for repeated measures was branch within each plant. (Chapter 3)

Source of variation	DF	Fresh weight (mg)	Volume (mm^3)	Diameter (mm)	Length (mm)	Ovary wall thickness (mm)	Length: diam. ratio	FW : Vol. ratio
Temp	1	0.008	0.002	0.003	<0.001	0.004	0.59	<0.001
CV	5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temp x CV	5	0.03	0.03	0.03	0.007	0.19	<0.001	0.96
Harvest	4	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temp x Harvest	4	<0.001	<0.001	<0.001	<0.001	0.009	0.29	0.84
CV x Harvest	20	<0.001	<0.001	<0.001	0.19	<0.001	0.01	0.006
Temp x CV x Harvest	20	0.003	0.004	0.07	0.008	0.43	0.05	0.49
Branch(CV)	6	0.03	0.03	0.05	0.07	0.21	0.66	0.39
DAA	1	<0.001	<0.001	<0.001	<0.001	<0.001	0.17	<0.001
Branch(Plant)		<0.001	<0.001	<0.001	0.004	0.02	0.02	0.88

Table A-2. *P* values for ovary, ovary wall (OW) and placenta fresh weight, ovary wall fresh weight: ovary fresh weight ratio, ovary diameter and length, ovary length/diameter ratio, and ovary wall thickness.[z]

	FW (mg)			OW FW:	Diameter	Length	Length:	OW
Source of variation	Ovary	OW	Placenta	ovary FW	(mm)	(mm)	diameter ratio	thickness (mm)
Temperature	0.01	0.01	0.01	0.36	0.010	0.007	0.31	0.02
Cultivar (CV)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temp*CV	0.001	0.01	<0.001	0.04	0.004	0.34	<0.001	0.29
Branch	<0.001	<0.001	<0.001	0.03	<0.001	<0.001	0.17	0.005
Node	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.88	<0.001
Node*Branch	0.002	0.02	0.001	0.99	0.01	0.10	0.28	0.21
Temp*Node	<0.001	<0.001	<0.001	0.92	<0.001	<0.001	0.62	<0.001
CV*Branch	<0.001	<0.001	<0.001	0.32	<0.001	0.06	0.05	0.009
CV*Node	<0.001	0.01	<0.001	<0.001	<0.001	0.007	0.01	0.37
CV*Node*Branch	0.03	0.01	0.05	0.07	0.07	0.11	0.37	0.41
Temp*CV*Branch	0.04	0.05	0.05	0.07	0.11	0.56	0.24	0.46
Temp*CV*Node	<0.001	<0.001	0.003	0.04	<0.001	0.05	<0.001	0.28

[z] Factorial experiment with two night temperatures and four cultivars. Flowers were harvested at anthesis from nodes 2 to 7.

Table A-3. Number of days after beginning of temperature treatments at which flowers from nodes 2 to 7 were harvested in four cultivars of sweet pepper. Means ± S.E. (Chapter 3)

	Night	Node number[z]					
Cultivar	temp (°C)	2	3	4	5	6	7
'Ariane'	12	4.5±0.5	11.5±0.8	20.7±1.5	28.6±1.0	35.1±0.9	40.4±0.8
	20	4.9±0.5	11.3±0.8	18.4±1.6	25.9±1.4	31.9±1.1	36.7±1.6
'Banana	12	6.3±0.7	13.7±0.6	20.6±1.0	26.3±0.7	30.8±0.6	35.6±0.7
Supreme'	20	5.2±0.8	11.3±1.5	14.6±1.1	21.7±1.9	26.9±2.0	39.0±5.0
'Legionnaire'	12	8.5±0.8	16.9±0.9	24.6±1.1	31.6±1.1	36.6±0.8	42.4±0.7
	20	5.4±0.3	12.7±0.9	25.8±2.0	31.2±1.1	37.7±1.0	41.4±1.4
'Red Cherry	12	1.5±0.2	7.4±0.6	14.3±1.1	19.9±1.1	27.0±1.1	32.1±1.0
Sweet'	20	2.2±0.3	6.1±0.5	11.1±1.0	15.7±0.7	22.7±0.9	27.6±0.7

[z]Nodes counted above the first branching node, *i.e.*, flower from sympodial unit 1 was node 1.

Table A-4. Effect of the interaction between days after treatment began (DAT) with night temperature and cultivar on the intercellular CO_2 (Ci, µmol mol^{-1}) of sweet pepper. Gas exchange was measured over a 45-day period after the beginning of night temperature treatments[z]. (Chapter 4)

	Temperature[y]			Cultivar[x]		
DAT	12°C	20°C	P values	LEG	RSC	P values
0	296	278	0.05	291	283	0.35
3	274	255	0.02	267	262	0.50
6	206	185	0.02	205	186	0.03
9	217	230	0.12	222	225	0.71
12	238	242	0.67	245	236	0.28
15	249	249	0.99	244	254	0.24
19	263	280	0.05	277	267	0.23
21	208	265	<0.001	238	235	0.74
24	222	247	0.004	238	231	0.45
27	233	248	0.09	239	242	0.67
30	262	266	0.71	264	264	0.94
33	222	222	0.99	220	224	0.64
36	208	217	0.31	206	219	0.12
45	229	220	0.28	221	227	0.45

[z]Gas exchange was measured over a 45-day period after the beginning of night temperature treatments.
[y]Data were averaged across cultivars. n = 18.
[x]Data were averaged across temperature treatments. n = 18.

Table A-5. P-values for ovary fresh weight, diameter and length, ovary length/diameter ratio, and ovary wall thickness for a 2 factor experiment (2 night temperature regimes, 2 levels of fruits) repeated over time (11 harvest intervals of 4 days each). (Chapter 5)

Effect	Fresh weight	Diameter	Length
Temperature (Temp)	<0.001	<0.001	<0.001
Fruits	<0.001	<0.001	<0.001
Temp*Fruits	0.02	0.08	0.08
Harvest interval (HI)	<0.001	<0.001	<0.001
Temp*HI	0.04	0.36	0.76
Fruits*HI	<0.001	<0.001	<0.001
Temp*Fruits*HI	0.57	0.16	0.20
Branch	<0.001	<0.001	<0.001
DAA	<0.001	<0.001	<0.001

[z] Pooled data from two experiments. Experiments were conducted with a factorial design (two night temperatures and presence/absence of developing fruits). Flowers were harvested at anthesis for 42 days in Experiment 1 and 38 days in experiment 2, and grouped in 11 harvest intervals of 4 days.

Table A-6. *P* values for ovary characteristics, ovary wall thickness and ovary wall transverse area, and thickness, area, cell number and cell size for mesocarp, giant cells, epidermal tissues and endocarp for a 3 factor experiment (2 night temperature regimes, 2 levels of fruits and 2 harvest times) (Chapter 5)

		Sources of variation [z]							
Tissues and parameters		T	F	T x F	HT	HT x F	HT x T	HT x T x F	Block
Ovary	FW	0.001	0.001	0.001	0.02	0.09	0.83	0.73	0.58
	Diameter	0.01	0.001	0.02	0.58	0.05	0.63	0.43	0.23
	Length	0.05	0.001	0.001	0.01	0.05	0.90	0.80	0.93
Ovary wall	Thickness	0.05	0.001	0.14	0.01	0.02	0.06	0.74	0.60
	Total area	0.01	0.001	0.03	0.03	0.01	0.08	0.77	0.56
Mesocarp	Thickness	0.04	0.01	0.04	0.05	0.04	0.01	0.40	0.62
	Total area	0.01	0.001	0.01	0.11	0.02	0.03	0.48	0.65
	Cell number	0.28	0.11	0.14	0.24	0.90	0.13	0.32	0.84
	Cell size	0.23	0.01	0.65	0.01	0.04	0.63	0.43	0.74
Giant cells	Thickness	0.35	0.01	0.55	0.01	0.06	0.95	0.62	0.56
	Total area	0.14	0.001	0.98	0.01	0.02	0.98	0.62	0.38
	Cell number	0.79	0.55	0.03	0.29	0.83	0.26	0.75	0.70
	Cell size	0.64	0.01	0.07	0.01	0.18	0.97	0.23	0.60
Epidermal	Thickness	0.28	0.04	0.83	0.23	0.23	0.73	0.41	0.93
tissues	Total area	0.04	0.001	0.40	0.37	0.09	0.91	0.38	0.73
	Cell number	0.61	0.09	0.81	0.04	0.69	0.28	0.83	0.96
	Cell size	0.05	0.001	0.26	0.01	0.02	0.29	0.68	0.53
Endocarp	Thickness	0.29	0.14	0.20	0.31	0.53	0.74	0.26	0.97
	Total area	0.06	0.01	0.06	0.64	0.33	0.95	0.20	0.67
	Cell number	0.12	0.03	0.09	0.92	0.39	0.41	0.89	0.34
	Cell size	0.60	0.25	0.89	0.28	1.00	0.15	0.12	0.77

[z] T, temperature; F, non-fruiting/fruiting treatment; HT, harvest time.

Table A-7. P-values for carbohydrate concentration and content.[z] (Chapter 5)

	Ovary wall			Placenta		
Parameter	Temp	Fruits	T x F	Temp	Fruits	T x F
Dry weight	0.06	0.001	0.16	0.06	0.001	0.16
Concentration						
Glucose	0.01	0.95	0.09	0.73	0.57	0.24
Fructose	0.02	0.39	0.22	0.19	0.77	0.30
Sucrose	0.04	0.86	0.84	0.19	0.04	0.12
Starch	0.17	0.33	0.69	0.29	0.29	0.65
Reducing sugars	0.01	0.64	0.11	0.36	0.89	0.24
Red. Sugars + starch	0.03	0.50	0.76	0.25	0.55	0.72
Content						
Glucose	0.03	0.06	0.10	0.11	0.00	0.05
Fructose	0.001	0.01	0.09	0.02	0.00	0.02
Sucrose	0.30	0.04	0.57	0.66	0.03	0.85
Starch	0.02	0.40	0.69	0.05	0.04	0.38
Reducing sugars	0.002	0.03	0.09	0.05	0.00	0.03
Red. Sugars + starch	0.005	0.13	0.31	0.03	0.01	0.09

[z]Flowers were harvested 24 to 29 days after treatments started.

B) Figures

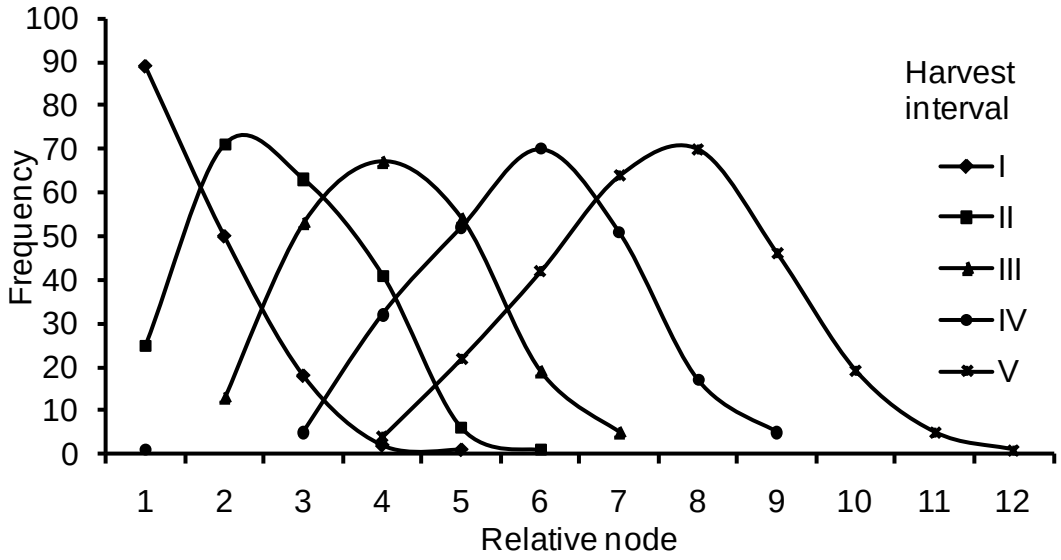

Figure A-1. Distribution of harvested nodes per harvest interval. Nodes were counted from the first harvested node on every stem. n = 160, 207, 211, 233, 273 for harvest intervals I, II, III, IV, and V, respectively. (Chapter 3)

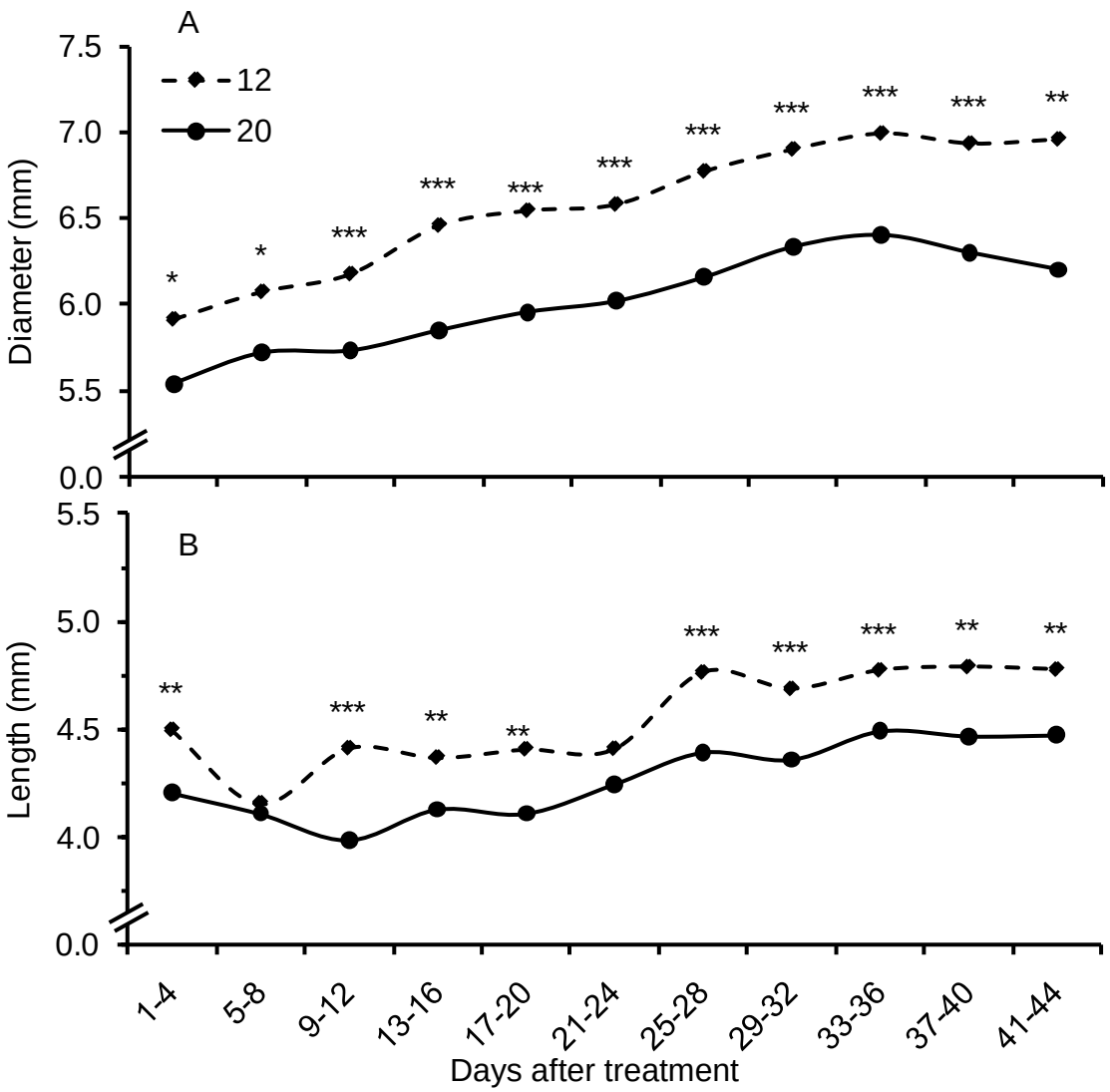

Figure A-2 Effect of night temperature and days after treatment on ovary diameter (A) and length (B) of flowers at anthesis in sweet pepper 'Legionnaire'. Day temperature was set to 22°C. Pooled data of two experiments. Flowers were continuously harvested during 42 days in Experiment 1, and 38 days in Experiment 2. Means were averaged for each combination across presence/absence of fruits treatments; n = 26 to 32 for 1-4 and 41-44 DAT, and 35 to 86 for the remaining intervals. *, **, ***: Significant differences between temperature regimes within each harvest interval at $P \leq 0.05$, 0.01 and 0.001 respectively. (Chapter 5).

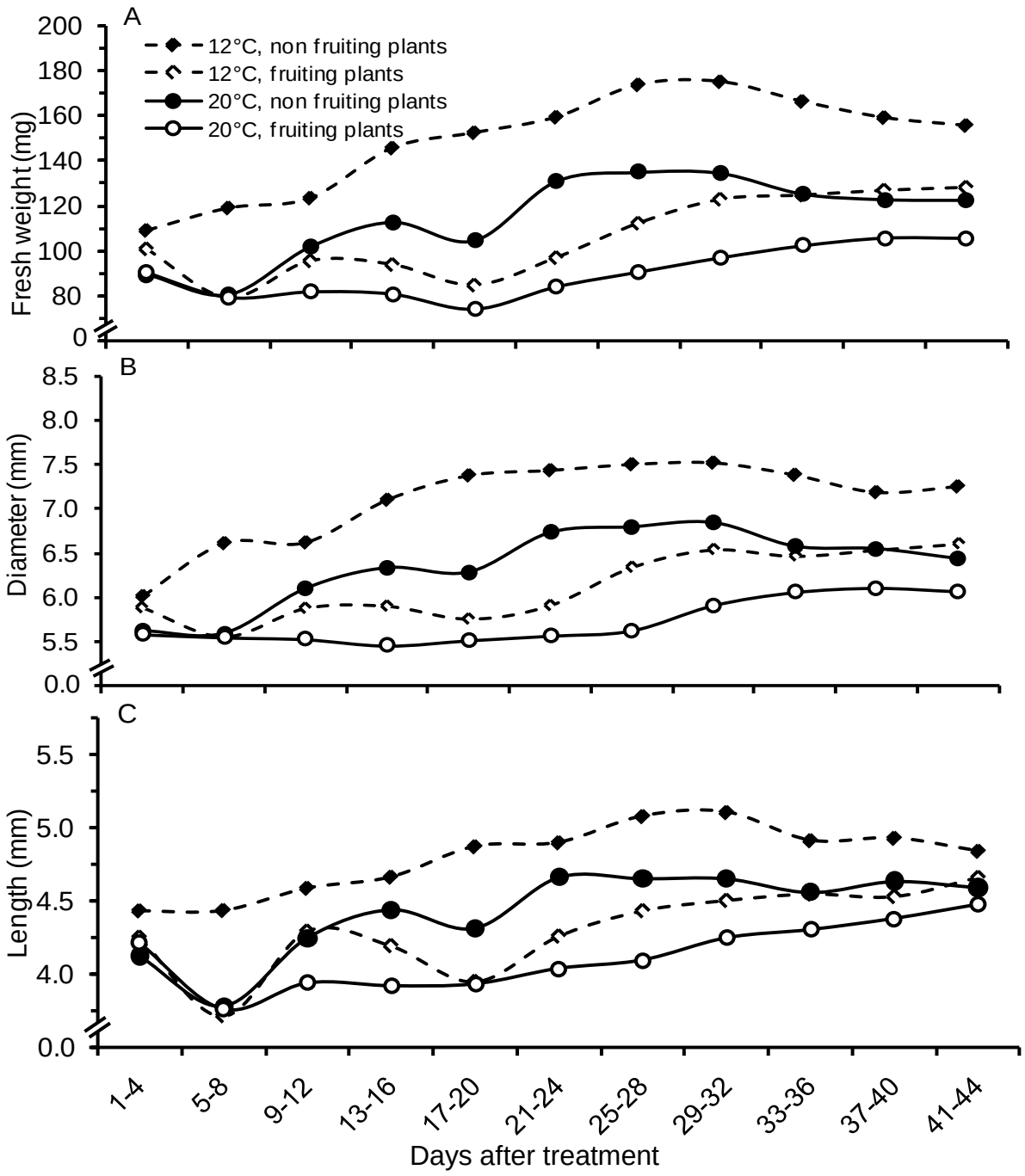

Figure A-3. Effect of night temperature, presence/absence of fruits and days after treatment on ovary fresh weight (A) diameter (B) and length (C) of flowers at anthesis in sweet pepper 'Legionnaire'. Day temperature was set to 22°C. Pooled data of two experiments. Flowers were continuously harvested during 42 days in Experiment 1, and 38 days in Experiment 2. Means were averaged for each combination across presence/absence of fruits treatments. n = 5 to15 for intervals 2 and 11, and from 14 to 48 for the remaining intervals. *, **, ***: Significant differences between temperature regimes within each harvest interval at $P \leq 0.05$, 0.01 and 0.001 respectively. (Chapter 5)

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BIOGRAPHICAL SKETCH

Nicacio Cruz-Huerta was born in Mexico. He is married and has a daughter. Being raised in a family of farmers, his interest in plants started in his childhood. His formal training on agriculture began in the middle and high school. He received his BS degree in plant sciences from the Universidad Autónoma Chapingo (Chapingo Autonomous University), and his M Sc degree in plant physiology from the Colegio de Postgraduados (Postgraduate College in Agricultural Sciences). He worked in plant physiology, with special focus on gas exchange, source sink relationships, plant growth analysis, and plant growth modeling. He was also the teaching assistant during several years in the graduate course “Crop Physiology”. Later, he obtained a scholarship from the Mexican National Council for Science and Technology to start his PhD program in the Horticultural Sciences Department at the University of Florida. His research focused on studying how low night temperatures and source and sink ratios affect flower development in bell pepper. After graduation, he plans to continue working on physiology, production techniques, and modeling of horticultural crops.