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EFFECTS OF PRE- AND POSTHARVEST CALCIUM SUPPLEMENTATION ON LONGEVITY OF SUNFLOWER (HELIANTHUS ANNUUS CV. SUPERIOR SUNSET)

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Horticulture

by
Sergio J. Sosa Nan
B.S., Louisiana State University, 2002
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ABSTRACT

The sunflower is one of the most important specialty cut flowers produced. Sunflowers have a short and variable postharvest longevity that is dependent upon cultivar. Research in postharvest physiology of cut flowers indicates that calcium (Ca) may be involved in delaying flower senescence by postponing cell membrane degradation. Cut flowers with intact cell membrane structure and function maintain their water balance and last longer. This study was developed to determine the effects of Ca supplementation on longevity of fresh cut sunflowers. The cultivar 'Superior Sunset' was used in this study; the sources of Ca were $Ca(NO_3)_2$, $CaCl_2$ or a chelated Ca at 125 (low), 250 (medium), or 500 (high) mg/l amounts of Ca. The chelate minus Ca and sodium nitrate ($NaNO_3$) were used as control treatments. Untreated flowers were included in all the experiments; means and standard errors were calculated for comparison with treatments. Ca was applied prior to harvest as a foliar spray or a weekly drench. Results indicated that the Ca treatments did not increase postharvest longevity of sunflower when treated preharvest; however, there was an increase in Ca concentration of stem tissue content compared to the untreated plants. Postharvest application of Ca chelate supplied as a 2-h pulse increased postharvest longevity of sunflower by up to 2 d compared to untreated flowers. Sunflowers treated with Ca also had a greater increase in fresh weight after 8 d in postharvest and improved water retention. Sunflowers treated with Ca had a greater concentration of the cation compared to the untreated flowers.

CHAPTER 1. INTRODUCTION

1.1 SPECIALTY CUT FLOWERS AND CUT SUNFLOWER

Specialty cut flowers can be defined as crops other than roses, carnations and chrysanthemums, or flowers that are present in the market only at a special time of the year (Armitage and Laushman, 2003). The type of cut flowers grown for the specialty cut market are usually field-grown flowers with poor shipping characteristics (Armitage and Laushman, 2003; Cummins, 2001). The specialty cut flower business is becoming an increasingly important part of floriculture in Louisiana (Cummins, 2001; Young, 2002). This can be attributed to the warm climatic conditions that provide a long growing season and to the adaptability of an array of many different specialty cut flower species (Cummins, 2001; Young, 2002). Local markets, such as farmers' markets or fresh produce markets, are a great niche for marketing fresh specialty cut flowers (Armitage and Laushman, 2003; Cummins, 2001; Young, 2002). Louisiana growers can produce quality specialty cut flowers for the local markets, but this product does not have the characteristics needed for shipping. Growers can secure a reasonable price if they can assure an extended postharvest longevity of the cut flower (Armitage and Laushman, 2003).

Sunflowers (*Helianthus annuus* L.) are native to North America, where they were grown by indigenous people for food and medicinal purposes (Putt, 1978). In later years, sunflowers became a very important crop around the world due to the industrial value of their oily seeds and their nutritional value as forage (Putt, 1978). In the early 1990s, sunflowers regained popularity as a cut flower (Armitage and Laushman, 2003; Fanelli et al., 2001). Several authors describe sunflowers as a highly marketable crop with an increasing economic importance in the specialty cut flower business (Devecchi, 2005; Celikel and Reid, 2002; Yañez et al., 2005). According to Devecchi (2005), sunflowers have shifted from 35th to 18th rank in the Dutch flower auction

between 1994 and 2000. The development of new cultivars offering a wide range of flower colors is one of the reasons for this revival of the sunflower (Armitage and Laushman, 2003; Fanelli et al., 2001). The availability of sunflowers year round is another reason for their popularity (Armitage and Laushman, 2003). This crop is fairly easy to grow and adapts to an array of climatic and soil conditions (Armitage and Laushman, 2003; Fanelli et al., 2001; Stevens et al., 1993; Schoellhorn et al., 2003).

1.2 IMPORTANCE OF POSTHARVEST MANAGEMENT

The final quality and postharvest longevity of cut flowers is affected by many cultural practices (Armitage and Laushman, 2003; Dole and Wilkins, 2005). One of the most important processes in specialty cut flower production is postharvest handling (Armitage and Laushman, 2003; Dole and Wilkins, 2005). Harvested flowers of excellent quality, vivid colors, strong stems, and fragrance can be lost by employing inappropriate postharvest procedures (Armitage and Laushman, 2003; Dole and Wilkins, 2005). Postharvest longevity of cut flowers will be reduced significantly if the grower does not utilize a proper postharvest protocol, and buyers may acquire a product of a substandard quality (Armitage and Laushman, 2003; Dole and Wilkins, 2005). This can result in decreased sales due to shortened postharvest life and poor quality of the cut flower.

Postharvest treatments may include: sanitized cutting utensils and buckets, temperature management, humidity management, control of gas exchange (i.e. O_2, ethylene and CO_2 during storage and/or transportation), and hydrating or preservative solution application to the holding water during storage and/or transportation (Armitage and Laushman, 2003; Stevens et al., 1993; Schoellhorn et al., 2003; Smith, 2003; Young, 2002). Combinations of these practices may

provide a longer postharvest life for the cut flower (Armitage and Laushman, 2003; Stevens et al., 1993; Schoellhorn et al., 2003; Smith, 2003; Young, 2002).

Small, local, specialty cut flower growers may not have sufficient funds to acquire the latest postharvest technology and equipment to control all of the factors affecting cut flower postharvest longevity. These specialty cut flower growers usually deliver their flowers to local markets the same day they are harvested, without the need of a cold storage facility. There is still a need, however, to find inexpensive, rapid and simple methods to increase the postharvest life of cut flowers immediately after harvest to remain competitive in the market.

Postharvest management research has focused primarily on major cut flowers, such as roses, carnations and chrysanthemums (Armitage and Laushman, 2003; Young, 2002). Specialty cut flowers, for instance sunflowers, have been a lesser subject of investigation; however, there has been some research on the optimal storage temperature of sunflowers. Gast (1995) reported that different cultivars of cut sunflower, after being stored for 24 hours at 4°C, had a longevity varying from 5 to 13 days, depending on the cultivar. Cut sunflowers have a short postharvest longevity and are considered sensitive to bacterial attack that can obstruct the vascular system inside the stem and prevent water from reaching the flower head (Devecchi, 2005; Gast, 1995; Smith, 2003). Species with fleshy stems, such as sunflower, may exude enzymes and carbohydrates into the holding water. This holding water may transform itself into an excellent growing media for bacteria (Smith, 2003). These species may need to be kept in a solution that prevents bacterial growth (Smith, 2003).

Reducing the storage temperature to 0°C during commercial handling and transport may favor a greater postharvest longevity and lower incidence of stem bending of cut flowers from the *Asteraceae* family (Celikel and Reid, 2002). Cut flowers of *Gerbera jamesonii* H. Bolus ex

Hook.f. 'Vesuvio' and sunflower were subjected to different storage temperature treatments ranging from 0 to 20°C for 5 days (Celikel and Reid, 2002). Post-storage life at 20°C of both species was extended by the 0°C treatment compared to the control (untreated flowers) (Celikel and Reid, 2002). This increase in post-storage life may be correlated to a lower respiration rate at the lower storage temperature (Celikel and Reid, 2002).

Cut sunflowers treated with a 0.01% solution of Triton X-100, a non-ionic detergent, for a 1-hour stem pulse showed an increase in water uptake (Jones et al., 1993). Fresh weight loss was significantly diminished by the treatment, and postharvest life increased by 2 d compared to the deionized (DI) water control (Jones et al., 1993).

Several research studies have tested the effects of different sources of calcium (Ca) on various cut flower species. The effects of calcium chloride ($CaCl_2$) have been studied on *Gladiolus* cv. 'Happy End' (Pruthi et al., 2001), *Gerbera jamesonii* 'Campitano', 'Dino', 'Sangria' and 'Testarossa' (Gerasopoulos and Chebli, 1999), and *Rosa hybrida* cvs 'Mercedes' and 'Baroness' (Torre et al., 1999). Other research studies have looked at the effects of calcium nitrate on *Rosa hybrida* 'Raktagandha', 'Sonia', 'Celica', 'Samantha', 'Mercedes' and 'Ilseta' (Bhattacharjee and Palanikumar, 2002; Michalczuk et al., 1989); *Chrysanthemum indicum* and *Tagetes erecta* (Patel and Mankad, 2002), and *Dianthus caryophyllus* (Mayak et al., 1978). Calcium sulfate has also been the subject of research on *Rosa hybrida* cv. 'Kiss' (De Capdeville et al., 2005).

The primary objective of this research study was to determine the effects of pre- and postharvest Ca supplementation on postharvest longevity of fresh cut sunflowers. In this study the sources of Ca were the following: calcium nitrate [$Ca(NO_3)_2$], calcium chloride ($CaCl_2$), and a sugar alcohol chelated Ca. Chelate and sodium nitrate ($NaNO_3$) were used as control

treatments. Untreated flowers were included in all the experiments. These treatment solutions applied at increasing concentrations may help identify an optimum source and rate of Ca that would improve sunflower postharvest longevity and water dynamics.

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CHAPTER 2. LITERATURE REVIEW

2.1 SUNFLOWER

Native North Americans grew sunflowers for food, dye, and medicine. They also extracted the oil from the seed for ceremonial body painting and pottery (Putt 1978; Stevens et al., 1993). The careful selection for increased quality of the sunflower has resulted in a size increment of 1000% in the last 3000 years (Putt, 1978; Stevens et al., 1993). Spanish explorers collected sunflower seeds from North America, and by 1580, sunflowers were a common garden flower in Spain. English and French explorers brought sunflowers along the trade routes to Europe, Asia, Africa and the Middle East (Putt, 1978; Stevens et al., 1993). The industrialization of the oily seed gave birth to a variety of products, ranging from snack food to soap and paper (Putt, 1978; Stevens et al., 1993). Sunflower has been an economically important crop in the USA since 1966 (Heiser, 1978; Putt, 1978; Stevens et al., 1993).

Helianthus annuus L. is the annual cultivated sunflower. It is one of 67 species in the *Helianthus* genus (Heiser, 1978). The term *Helianthus* comes from the Greek *helios* for sun and *anthos* for flower (Fletcher and Taylor, 1939). The annual sunflower belongs to the *Compositae* or *Asteraceae* family, the largest family of flowering plants (Heiser, 1978). In this family, plants usually have a composite inflorescence, referred to as a capitulum or head (Heiser, 1978). The capitulum consists of: a receptacle with involucres bracts that are modified leaves; ray-flowers on the outer whorl of the receptacle that are sterile and golden yellow, but may be pale yellow, orange yellow or reddish; disk-flowers on the inner whorl of the receptacle that are perfect flowers of yellow or brown color (Knowles, 1978).

The sunflower has been used as an ornamental plant in gardens for many years, but it has only recently regained popularity as a specialty cut flower (Armitage and Laushman, 2003; Putt,

1978; Stevens et al., 1993; Schoellhorn et al., 2003; Sloan and Harkness, 2004; Young, 2002). Around 1990, the Japanese introduced F1 hybrid sunflowers that did not shed pollen (Armitage and Laushman, 2003; Schoellhorn et al., 2003; Sloan and Harkness, 2006). This made sunflowers more attractive to department stores, mail-order sources and high-end designers, who no longer had to worry about the mess left by the pollen shedding cultivars (Schoellhorn et al., 2003; Sloan and Harkness, 2006). Many cultivars have been launched for cut flower use, with a wide variety of colors ranging from yellow to bronze, red or cream, as well as different flower shapes (Armitage and Laushman, 2003; Stevens et al., 1995; Schoellhorn et al., 2003). Louisiana growers can take advantage of the resurgence of this flower and of the long warm growing season in the South to increase their profit by cultivating sunflowers.

2.1.1 Sunflower Field Production

Sunflowers adapt to an array of soil types and climatic conditions, but perform better in full sun and well-drained soil (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). The crop requirement of soil pH is in the range of 6.5 -7.5, a near- neutral pH. Preferably, annual sunflowers should be started in plug trays of 72 cells, 2-3 weeks before planting, but seeds can be sown directly in the field after the last freeze (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003).

Seeds should be sown at 2 week intervals if started in plug trays, and at 2-4 week intervals if directly sown in the field. The spacing depends on the desired plant population, length and thickness of the stem, and the size of the inflorescence (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Sloan et al., 2003, 2004). The most popular spacing range is 15-30 cm between seeds or seedlings and 45-91 cm between rows. For intensive bed culture, 15x15 cm spacing is recommended (Stevens et al.,

1995; Schoellhorn et al., 2003; Sloan et al., 2003, 2004). This bed culture may supply flowers with a 16 to 21 cm bloom diameter, 0.72 to 0.78 cm stem diameter and a stem length of 159 to 198 cm for cultivars 'Superior Sunset' and 'Sunbright Supreme', which are utilized in cut flower bouquets (Schoellhorn et al., 2003; Sloan et al., 2003, 2004; Stevens et al., 1993).

This crop is drought tolerant, but it performs better when it is not stressed for water (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). Marc and Palmer (1976) proposed that insufficient water supply during the vegetative stages of sunflower may delay flowering date and reduce the number of flowers per head. Lack of water will result in reduced production and quality, i.e. a smaller inflorescence, lack of color and shorter postharvest life (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). Excess water may cause lack of growth and promote root rot (Stevens et al., 1993; Schoellhorn et al., 2003). Overhead irrigation may physically damage flowers by causing spotting on the petals, and it may help spread soil borne diseases onto the foliage (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). Drip irrigation is highly recommended for cut flower production to avoid flower and foliar damage (Stevens et al., 1993; Schoellhorn et al., 2003).

Sunflowers are heavy feeders, but it is highly recommended to test the soil for nutrient content before starting a fertilizer program (Armitage and Laushman, 2003; Stevens et al., 1993). A complete fertilizer, such as 20-20-20 or 20-10-20 (N-P-K), is recommended. The fertilizer rate may be set at 200 mg/l nitrogen (N), and it may be supplied through irrigation once a week or 4 days, 2 weeks and 4 weeks after transplanting (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002).

Weed control is necessary for sunflower production. Weeds will compete with the crop for water and nutrients (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). The outcome of this competition is a decrease in quantity and quality of floral production (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). Also, weeds may get in the way of harvest and increase the harvesting time, which might result in an increase for labor cost (Stevens et al., 1993; Schoellhorn et al., 2003). Controlling weeds with herbicides is a very effective method, but there are alternative methods (Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). For example, plastic or organic mulches will slow down the weed incidence in the bed (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). Hoeing and hand-weeding are other alternative practices, although they are labor-intensive activities (Stevens et al., 1993).

Insect and disease control will be minimal if good cultural practices are maintained (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). The cucumber beetle is one of the most problematic pests in sunflower production (Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). The adults will feed on developing foliage and deposit eggs (Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). They will also feed on the petals of mature plants (Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003). There are several insecticides on the market that will mitigate an insect problem, but it is recommended to monitor the field closely for insects before applying chemicals (Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002). The most common sunflower diseases are powdery mildew, sclerotinia wilt, stalk rot and downy mildew (Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003;

Young, 2002). A well spaced crop with air flow between plants will help prevent disease. Also, the use of drip irrigation, instead of overhead irrigation, will reduce the incidence and spread of soil borne disease (Dole and Wilkins, 2005; Stevens et al., 1993).

2.1.2 Flower Development in *Helianthus annuus* L.

The generative or reproductive stage of the sunflower begins with the bulging and development of bracts at the periphery of the shoot apex (Schneiter and Miller, 1981; Schuster, 1985). The time from seedling emergence to the different stages of floral development may be influenced by photoperiod and temperature (Schuster, 1985). The generative or reproductive stages of *Helianthus* have been described by Schneiter and Miller (1981). R1 (Reproductive) stage is evident because of the development of the flower bud surrounded by immature bracts. When the flower bud is observed from directly above, the immature bracts have a many pointed star-like appearance. R2 stage is manifested by the elongation of the internode directly below the capitulum by 0.5 to 2.0 cm above the nearest leaf attached to the stem. In the R3 stage, elongation of the internode below the capitulum continues, lifting the flower head above the surrounding leaves in excess of 2 cm, and in the R4 stage, bracts covering the inflorescence begin to open. When viewed from directly above, small ray flowers are visible. R5 stage is the initiation of anthesis. The mature ray flowers are fully extended, and all disks flowers are visible.

Several authors have proposed that the sunflower is a day neutral species, but there are an array of cultivars that do not follow this trend and possess either a long day or short day photoperiodic response (Schuster, 1985). Other varieties have shorter vegetative periods, depending on the temperature; warmer temperatures may accelerate flowering (Schuster, 1985). Selection of these cultivated varieties was conducted to provide sunflowers that adapt to different ecological environments (Schuster, 1985; Putt, 1978; Stevens et al., 1993).

Research with plant hormones, such as gibberellic acid, has suggested that sunflowers treated with this hormone may have an accelerated transition from the vegetative to generative stage (Lagenauer et al., 1975). More research is needed on the effects of preharvest environmental conditions on sunflower postharvest longevity.

There are three important factors that affect cut sunflower marketability: the diameter of the inflorescence, and the diameter and length of the stem (Schoellhorn et al., 2003; Sloan et al., 2003, 2004; Sloan and Harkness, 2006; Stevens et al., 1993). There are studies suggesting that plant density in the field may affect these characteristics in the sunflower. Tighter spacing may result in nicer looking flowers; however, more nutrients and water will be necessary to maintain a healthy population of plants (Schoellhorn et al., 2004; Sloan et al., 2003, 2004; Sloan and Harkness, 2006; Stevens et al., 1993).

2.1.3 Heliotropism

The term heliotropism is derived from the Greek words *helios*, meaning sun and *trope*, meaning to turn (Hart, 1990). This plant movement can be described as an environmental or exogenously stimulated response that results in turgor changes of leaves and flowers, causing movement towards sunlight (Hart, 1990). This type of movement has been observed for over a century. Schaffer (1898) observed this phenomenon in sunflowers. He reported that leaves and flower buds of sunflowers in the field moved in accordance with the position of the sun. There are two types of heliotropic movements: diaheliotropism and paraheliotropism (Ehleringer and Forseth, 1980; Hart, 1990). Diaheliotropic plants will maintain their leaf laminas at a right angle to the direction of sunlight, for example sunflower; but in paraheliotropic plants, the leaf blades are maintained parallel to the sunlight, for example *Lupinus arizonicus*. This movement is also

known as “cupping” (Darwin, 1880; Ehleringer and Forseth, 1980; Hart, 1990; Wainwright, 1977).

According to Schaffer (1898), the heliotropic movement of the sunflower consists of the bending of the stem about four or five inches from the flower bud and the bending of the leaves so the lamina forms a 90° angle with the sunlight. Environmental factors, such as strong winds and high moisture due to rain, may interfere with the movement (Schaffer, 1898). When the ground was dry and the air temperature high, there was little or no diaheliotropism visible, according to Schaffer (1898). These observations, however, were not quantified. O’Connor (1937) reported that light intensity can be reduced as low as 5% of full sunlight without any reduction in the heliotropic response of sunflower, and also proposed that a light wave length of 500 nm or lower was necessary to attain the movement response.

Removal of terminal buds does not affect heliotropic movements in sunflowers (O’Connor, 1937; Schaffer, 1898). Researchers observed that removal of the leaf blades decreased the heliotropic response of sunflowers, and whole leaf removal inhibited the early morning bending of plants towards the east, as well as the afternoon response towards the west (O’Connor, 1937; Schaffer, 1898). Diaheliotropic movement of sunflower can be observed until anthesis begins (De Fina, 1943; Schaffer, 1898). During anthesis the capitulum is tipped sidewise until the flower is almost vertical towards the northeast (De Fina, 1943; Schaffer, 1898). Schaffer (1898) hypothesized that at anthesis the stem below the inflorescence hardens and makes it impossible for the head of the flower to move; however, there have been no studies testing this hypothesis. Fresh cut sunflowers have a common quality defect termed “neck bending” or “stem bending” during storage and transport (Celikel and Reid, 2002). The degree of stem bending has been determined by estimating the angle between the main stem and the stem just below the

capitulum (Celikel and Reid, 2002). A bent stem with an angle higher than 90° exhibits a flower facing downward and is not marketable (Celikel and Reid, 2002).

Floral heliotropism has been studied in other species, such as *Ranunculus adoneus* (Sherry and Galen, 1998; Stanton and Galen, 1993). Similar findings have been reported, such as the requirement of blue light (400 to 500 nm) for heliotropic movement, similar to the sunflower (Stanton and Galen, 1993). Removal of the flower head from the *Ranunculus adoneus* plant had no effect on the phenomenon (Sherry and Galen, 1998).

Wainwright (1977) proposed that heliotropic movements of *Lupinus arizonicus* leaves may comprise the same mechanism as the seismonastic movements of mimosa (*Mimosa pudica)* leaves. The seismonastic movement of mimosa is the response of the leaves to a non-directional stimulus (touch) by changes in cell turgor (Hart, 1990). Mimosa leaf movement may be caused by changes in turgor of motor cells in the pulvinal region, which is located at the base of the leaf petiole (Allen, 1969; Toriyama, 1955). Migration of potassium (K) and Ca ions in and out of motor cells may be involved in the turgor adjustment of such cells (Allen, 1969; Toriyama, 1955; Toriyama and Jaffe, 1972).

At the beginning of movement, ion efflux may increase the solute concentration outside the cell; this imbalance in osmotic concentration may be resolved by permeation of water out of the cell, thus a loss of cell turgidity (Allen, 1969; Toriyama, 1955; Toriyama and Jaffe, 1972). An ion influx may occur during the second part of the movement, which increases the solute concentration inside the cell, resulting in water permeating to the inside of the cell; thus, the cell regains turgor (Allen, 1969; Toriyama, 1955; Toriyama and Jaffe, 1972). The hypothesis of a similar movement mechanism in *L. arizonicus* has been tested by applying a K pump inhibitor, lanthanum nitrate [$La(NO_3)_3$], to the leaves. Stems of *L. arizonicus* were cut 1 h before sunrise

and re-cut in distilled water or in solutions of 0.25, 2.5, or 25 mM $La(NO_3)_3$. Inhibition of sun-tracking leaf movement with the highest concentration was attained at 12 h after treatment. Although these studies relate to the possible mechanism of heliotropism of sunflower, they do not help to explain the quality defect of stem bending that occurs during postharvest. Further research should focus on the anatomical changes that may occur in the neck of cut sunflower during postharvest.

2.1.4 Sunflower Harvest and Postharvest Management

Consumer demand for sunflowers has increased in recent years, but there is little information about the postharvest management of this cut flower (Mensuali-Sodi and Ferrane, 2005). The stage at which sunflowers are harvested is directly related to postharvest management practices after the stems are cut (Armitage and Laushman, 2003; Dole and Wilkins, 2005). If the stems are to be sold directly from the field at a farmers' market or local grocery store, they should be harvested with a completely open flower (Armitage and Laushman, 2003; Dole and Wilkins, 2005). Cut stems should be placed in sanitized buckets with clean tap water and, if possible, a hydrating solution should be added to the water (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002). Sunflower stems that are to be stored for an extended period of time, prior to sale, should be harvested in the cup stage, when ray flowers begin to unfold from the center of the inflorescence, and the flower head viewed from the side looks like a cup (Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002).

Harvest should occur in the cooler hours of the day, preferably in the morning, when the plants and the flowers are free from dew and moisture (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002). Harvest containers

and cutting utensils should be cleaned and disinfected prior to harvest (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002). The sunflower stems should be cut as long as possible from the field (Armitage and Laushman, 2003). Stems can be re-cut in the postharvest area to a desired length, and all foliage must be removed to decrease transpiration, as well as to avoid disease proliferation in the postharvest area (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002).

Sunflowers do not benefit as much as other cut flowers from floral preservatives (Armitage and Laushman, 2003). It is recommended to use a hydrating solution or an antibacterial solution to prevent proliferation of bacteria in the holding water and inside the stem, stimulate water uptake and avoid the clogging of the vascular system in the stem (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002). In the postharvest area, stems can be graded by length and thickness of the stem and the size of the flower (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002). Sunflower stems may be bunched, depending on buyer specifications, and stored at 2-4 °C and 85-95% relative humidity for up to a week (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003; Young, 2002). As mentioned previously, postharvest longevity of sunflowers may vary depending on the cultivar, and a cut sunflower may last from 5-13 days with an average of 8 days (Gast, 1995).

2.2 POSTHARVEST PHYSIOLOGY AND CALCIUM

Imbalance in the water status of the cut flower is detrimental to postharvest longevity (Halevy, 1976). The most common symptoms of imbalance in the water status are wilting, stem

or neck bending, and lack of full opening of the flower (Halevy, 1976; Halevy and Mayak, 1981). There are four physiological components to water balance that are strongly interrelated in the cell: water uptake and transport, water loss and the capacity of water retention by the tissue. In fresh cut roses, experimental evidence has suggested that there is a steady decline in water uptake combined with continuous water transport and water loss (Burdett, 1970; Halevy and Mayak, 1981; Mayak et al., 1974). This relationship increases the water deficit and results in decreased water potential of the flower (Halevy and Mayak, 1981; Eze et al., 1986).

Water potential is maintained by the water content inside the cell and the solute concentration of this intracellular water (Halevy and Mayak, 1981; Eze et al., 1986). It has been proposed that cell membrane deterioration, which results in solute leakage out of the cell, could contribute negatively to the components of water balance inside and outside the cell (Halevy and Mayak, 1981; Eze et al., 1986; Borochov and Woodson, 1989; Torre et al., 1999).

Cell membranes are responsible for the regulation of the content of nutrient ions and other metabolites inside the cell by selective transport in and out of the cell, for the preservation of compartments in the cell and for water retention (Rubinstein, 2000). The survival of an organism is bound to the conservation of the structure and function of cellular membranes (Rubinstein, 2000). According to literature on flower senescence, cell membrane deterioration plays an important role in this process (Adam et al., 1983; Eze et al., 1986; Borochov and Woodson, 1989; Itzhaki et al., 1990). Cell membrane disruption has been suggested to precede several physiological alterations that lead to petal and flower senescence, i.e. increased ethylene production and abscisic acid content, decreased sucrose uptake, and decreased ATPase activity (Adam et al., 1983; Eze et al., 1986; Borochov and Woodson 1989; Itzhaki et al., 1990). The deterioration of the cell membranes may be expressed as a loss of membrane permeability, which

in turn can be measured as increments in solute leakage from the cell. There are several biochemical and molecular modifications in cell membranes that will lead to the loss of its structure and function (Paliyath and Droilldard, 1992; Rubinstein, 2000). Membrane permeability is controlled by changes in membrane fluidity and microviscosity (Borochov and Woodson, 1989; Itzhaki et al., 1990; Marangoni et al., 1996; Torre et al., 1999; Rubinstein, 2000). Alterations in cell membrane fluidity and microviscosity are the results of an imbalance in phospholipid synthesis and degradation processes, membrane protein degradation, increase of the sterol/phospholipid ratio, and changes in the ratio of unsaturated/saturated fatty acids (Borochov and Woodson, 1989; Itzhaki et al., 1990; Marangoni et al., 1996; Torre et al., 1999; Rubinstein, 2000). Research has suggested that the increase of enzyme activity related to lipid catabolism is faster than that related to lipid synthesis during membrane deterioration (Borochov and Woodson, 1989; Marangoni et al., 1996; Rubinstein, 2000).

There is experimental evidence suggesting that the metabolites resulting from phospholipid degradation may be involved in up-regulating ethylene synthesis (Borochov et al., 1997). Research in postharvest physiology suggests that Ca may be involved in control of membrane stability and senescence of plant cells (Leshem, 1992; Paliyath and Droilldard, 1992; Torre et al., 1999; Rubinstein, 2000). Alterations of the intercellular and/or cytosolic concentrations of Ca may trigger either catabolism or remodeling and the turnover process of the cell membrane components. Calcium can be transported in and out of cytosol through proton pumps, depending on the electrical gradient across membranes (Ferguson, 1984; Leshem, 1992). In the cytosol, Ca is maintained at very low concentrations by intracellular binding or uptake into organelles (Ferguson, 1984; Leshem, 1992). It has been hypothesized that extracellular concentrations of Ca may have inhibitory effects on senescence (Ferguson, 1984; Leshem,

1992). In contrast, increasing concentrations of cytosolic Ca may activate a sensor protein named calmodulin, which in turn may activate catabolic enzymes, accelerating the senescence process (Ferguson, 1984; Leshem, 1992). Therefore, close regulation of Ca concentration in the cytosol, the external surface of the plasma membrane and cell wall may be required to delay senescence (Ferguson, 1984).

Several studies have been conducted to determine the effect of Ca on postharvest longevity of fresh cut flowers as related to delay of the degradation processes that affect cell membrane integrity (Borochov and Woodson, 1989; Itzhaki et al., 1990; Marangoni et al., 1996; Torre et al., 1999; Rubinstein, 2000). Other studies have shown that supplemental calcium applied as calcium nitrate [$Ca(NO_3)_2$], calcium chloride ($CaCl_2$), and calcium sulfate ($CaSO_4$) (Bhattacharjee and Palanikumar, 2002; De Capdeville et al., 2005; Michalczuk et al., 1989; Torre et al., 1999) may decrease the rate of senescence or increase postproduction longevity. Calcium can be supplied as a preharvest fertilizer supplement or as a postharvest pulse. Data from several studies with different cut flower species, *Rosa hybrida* (Bhattacharjee and Palanikumar, 2002; De Capdeville et al., 2005; Michalczuk et al., 1989; Torre et al., 1999), *Dianthus caryophyllus* (Mayak et al., 1978), *Gerbera jamesonii* (Gerasopoulos and Chebli, 1999), *Gladiolus* (Pruthi et al., 2001), *Chrysanthemum indicum* and *Tagetes erecta* (Patel and Mankad, 2002), indicate that Ca may increase postharvest longevity of cut flowers. This increased postharvest longevity may be due to a delay of physiological events related to senescence, such as a decrease in water uptake, increased water transpiration loss, decreased fresh weight, stem bending, or prevention of disease during propagation.

2.3 MANNITOL AND CALCIUM CHELATE

Calcium absorption from the soil solution has been suggested to occur by young root tips in which cell walls of the endodermis are still unsuberized (Clarkson and Saunderson, 1968; Mengel and Kirby, 1987). Mengel and Kirby (1987) also indicated that Ca uptake is primarily the result of high concentrations of Ca in soil, rather than from the efficiency of Ca uptake.

Mannitol is a sugar alcohol synthesized from the reduction of mannose-6-phosphate (Everard et al., 1993). In celery plants, mannitol is produced from photosynthesis in mature leaves; it can not be synthesized in sink tissue (Everard et al., 1993; Loescher et al., 1992). According to Noiraud et al. (2001), mannitol may be loaded into the phloem via a mannitol-specific transporter. Mannitol may support the translocation of a relatively immobile micronutrient, such as boron, in celery plants (Hu et al., 1997). Previously, mannitol was used to estimate the free space of algal cells and as an osmotic agent because it was thought that the plasma membrane was impermeable to mannitol (Heath, 1977). Heath (1977) reported from an experiment using ^{14}C-mannitol, that the penetration rate of mannitol into the protoplasts is relatively slow, but it has normal passive-like kinetics, however; it leads to the accumulation of the compound in the cell.

Chelated forms of Ca are very stable and highly soluble compounds that have been used to delay senescence or to alleviate Ca deficiency disorders in fruit (Lester and Grusak, 1999, 2001, 2004; Mengel and Kirby, 1987). Postharvest applications of different Ca chelate solutions, such as mannitol complexed Ca, amino acid chelated Ca or EDTA-chelated Ca on honeydew melons have been shown to delay senescence of the fruit and to increase tissue firmness, without affecting the sugar content and palatability (Lester and Grusak, 2004). Most field grown melons are packaged in the field at harvest, and postharvest treatment with Ca chelate is not possible.

Preharvest applications of Ca have been tested with good results in postharvest attributes of honeydew melon. In this study, mannitol was used as a chelating agent of Ca (ClawEl, Brandt Consolidated, Pleasant Plains, IL). Chelated Ca was compared to non-chelated calcium forms to determine if there were any changes in Ca uptake and translocation. Lester and Grusak (2004) reported that honeydew melons treated with mannitol chelated Ca preharvest had equally beneficial results as those melons treated with non-chelated Ca sources. The concentration of Ca in the fruit tissue of plants treated with the mannitol chelated calcium, however, was higher than the concentration of Ca in control fruit.

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CHAPTER 3. EFFECTS OF PREHARVEST CALCIUM SUPLEMENTATION ON SUNFLOWER LONGEVITY

3.1 INTRODUCTION

Several studies that have shown that supplemental calcium (Ca) may increase longevity and control diseases of cut flowers. Calcium can be made available to the plant as a preharvest supplement to recommended fertilization or to the cut flower, as a postharvest pulse, and can be supplied from different chemical sources. Research that has investigated the use of different Ca sources on cut flower species has included: calcium nitrate [$Ca(NO_3)_2$], calcium chloride ($CaCl_2$), and calcium sulfate ($CaSO_4$) (Bhattacharjee and Palanikumar, 2002; De Capdeville et al., 2005; Michalczuk et al., 1989; Torre et al., 1999). The effect of these Ca sources on postharvest longevity of several cut flower species has been investigated. They include: *Rosa hybrida* (Bhattacharjee and Palanikumar, 2002; De Capdeville et al., 2005; Michalczuk et al., 1989; Torre et al., 1999), *Dianthus caryophyllus* (Mayak et al., 1978), *Gerbera jamesonii* (Gerasopoulos and Chebli, 1999), *Gladiolus* spp. (Pruthi et al., 2001), *Chrysanthemum indicum* and *Tagetes erecta* (Patel and Mankad, 2002). The data from these studies indicate that Ca may increase longevity of cut flowers by delaying physiological events related to senescence, such as decreased water uptake, increased water transpiration loss, decreased fresh weight, stem bending, or prevention of disease during propagation.

Solutions of $CaSO_4$ at 0, 2.5, 5, 10 or 20 mM Ca, containing 0.01% Tween 20, were sprayed on *Rosa* 'Kiss' until run-off. Applications were made 1 day before harvest; 1 and 3 days before harvest or 1, 3 and 5 days before harvest. These preharvest applications reduced the progress and severity of gray mold, *Botrytis cinerea*, and increased longevity (De Capdeville et al., 2005). In both assays, with and without inoculation of the pathogen, the disease progress and severity were reduced. A similar trend occurred for postharvest longevity where increasing

concentrations of $CaSO_4$ to 20 mM Ca increased postharvest longevity by 20% from the control, with an average of 8 d (De Capdeville et al., 2005).

Gerasopoulos and Chebli (1999) studied the effect of pre- and postharvest applications of 0.0, 0.5, 1.0, or 1.5% $CaCl_2$ on *Gerbera jamesonii,* a species in the *Asteraceae* family. The preharvest application consisted of four sprays on four cultivars with a scape length of 10, 20, 30 or 40 cm. The postharvest applications included a 1-h pulse or injecting treatment solution into the scape 3-5 cm below the capitulum until run-off at the cut edge. Injecting the stems with a 1.0% $CaCl_2$ extended postharvest life of cultivars 'Campitano', 'Dino', and 'Testarossa' by 5.6, 4 and 5.9 days, respectively. Bending incidence in the 'Campitano', 'Dino', and 'Testarossa' cultivars was delayed by 2 to 3 days with 1% $CaCl_2$. 'Sangria' had the greatest increase in flower longevity by 4 days and lowest bending incidence at day 8 of 30% with the preharvest scape spray of 1% $CaCl_2$. Results from these studies suggest that supplemental, preharvest Ca application may increase postharvest longevity and decrease stem bending. Similar treatments may have positive physiological effects in sunflower.

Sunflowers have been described by many authors as a highly marketable crop with an increasing economic importance in the specialty cut flower business (Devecchi, 2005; Celikel et al., 2002; Yañez et al., 2005). Sunflower longevity varies from 5 to 13 d, depending on the cultivar (Gast, 1995). Postharvest trials of cut sunflowers showed that commercial holding solutions containing soluble carbohydrates may have positive effects in postharvest longevity, extending it 1 to 4 d depending on the cultivar (Fanelli et al., 2003). Young (2002) reported postharvest longevity of up to 11 d for sunflower cultivars 'Valentina', 'Full Sun' and 'Sunbright' treated with a commercial holding solution containing dextrose as a sugar source.

Sunflowers have a common quality defect termed bent neck or stem bending during storage or transport (Celikel and Reid, 2002).

Based on the previously mentioned research, application of supplemental Ca might provide a method of reducing the high variability in postharvest longevity and a high incidence of stem bending of sunflower. Chelated forms of Ca are very stable and highly soluble compounds (Mengel and Kirby, 1987). Ca has been chelated with different organic compounds such as amino acids, EDTA (ethylene-diamine-tetraacetic-acid) or EGTA (ethylene-glycol-tetraacetic acid). Various forms of chelated Ca have been used to delay senescence or to alleviate Ca deficiency disorders in fruits and vegetables (Lester and Grusak, 1999, 2001, 2004; Mengel and Kirby, 1987).

Mannitol is a sugar alcohol metabolized from photosynthesis in mature leaves (Everard et al., 1993; Loescher et al., 1992). Mannitol may permeate through the plasma membrane, and there is evidence indicating that mannitol may support translocation of micronutrients such as boron (Heath et al., 1977; Hu et al., 1997). These two characteristics may be useful to Ca uptake by roots or leaves and translocation inside the plant. A sugar alcohol chelated Ca (ClawEl, Brandt Consolidated, Pleasant Plains, IL) applied preharvest to honeydew melons provided for longer shelf life and greater tissue firmness without affecting palatability of the produce compared to untreated fruit (Lester and Grusak, 1999, 2001, 2004). The objective of this experiment was to determine the effects of preharvest Ca supplementation as a spray or drench in the form of $Ca(NO_3)_2$, $CaCl_2$ or a Ca chelate at 125, 250, or 500 mg/l of Ca on the longevity of fresh cut sunflowers.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material

Helianthus annuus L. 'Superior Sunset' (Fred C. Gloeckner & Company, Inc., Harrison, NY) was used for these experiments. 'Superior Sunset' has been described as tolerant to cold temperatures and vigorous, with uniform flowering. Ray flowers have long, rich yellow orange petals. Disc flowers are dark brown and do not produce pollen. Plants grow 150 to 195 cm tall with straight and thick stems. The capitulum ranges from 15 to 20 cm in diameter. Most cut sunflower cultivars are considered day-neutral (Armitage and Laushman, 2003; Arnosky and Arnosky, 2000; Sloan et al., 2003, 2004; Sloan and Harkness, 2006).

Sunflowers were cultivated in a greenhouse located at the Burden Center, 30° N 91° W, Baton Rouge, Louisiana. 'Superior Sunset' seeds were sown on three dates: 20 February 2006 for the foliar spray experiment, 8 April and 28 August 2006 for the two drench experiments. Seeds were planted directly in trade gallon containers (3785.4 cm^3) with a 5:3:2 peat: pine bark: perlite substrate, amended with 4.75 kg/m^3 of dolomitic limestone, 0.89 kg/m^3 of triple superphosphate, and 0.6 kg/m^3 of Micromax™. Plants were grown inside a polycarbonate covered greenhouse. Temperature (mean = 24.9± 3.2) and relative humidity (mean = 70.7± 9.7) inside the greenhouse were recorded throughout the growing season (Fig 3.1A & B). Containers were set on inverted trays on the floor of the greenhouse and grouped in rows of 32 pots on 15 cm centers to mimic production in ground beds. Plants were fertilized with a complete liquid fertilizer 20-20-20 (20N-4.4P-16.6K) (The Scotts Co., Maryville, OH) at 200 mg/l nitrogen (N). Plants were fertilized at each watering through drip irrigation on an as needed basis. The plants were grown with net support until harvest (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003).

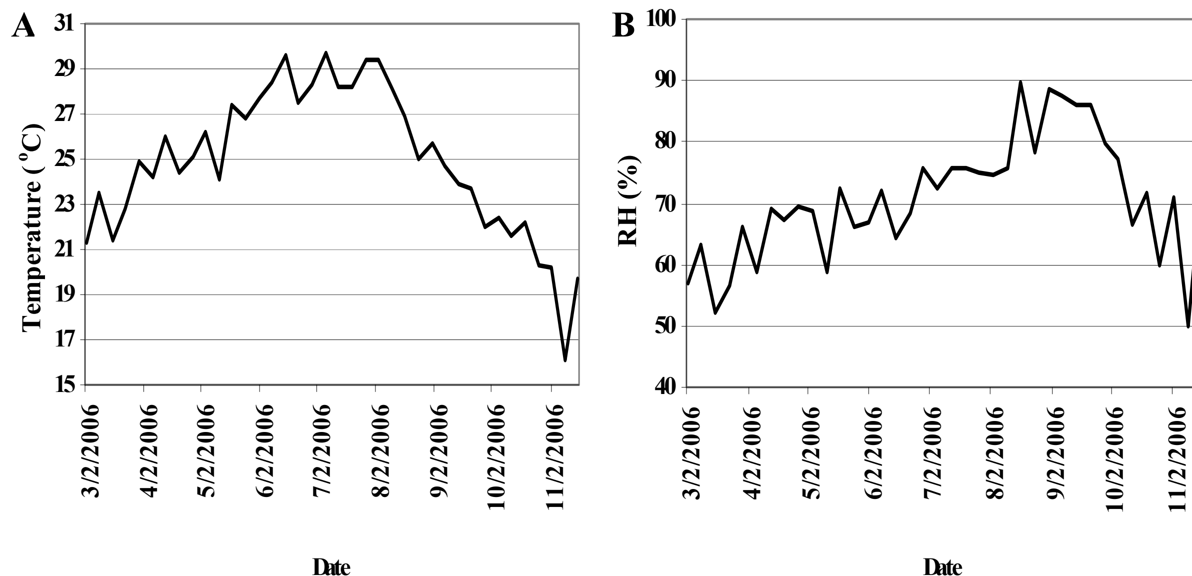

Fig 3.1. Weekly average (A) air temperature and (B) relative humidity (RH) during 2006 growing season inside the greenhouse at the Burden Center, Baton Rouge, Louisiana.

3.2.2 Preharvest Treatment

To determine the effects of preharvest supplemental Ca on growth and postharvest longevity of sunflower, separate studies included two different methods of application: a foliar spray at 1 and 2 weeks prior to harvest, or a weekly drench initiated after the seedlings presented 4-6 true leaves and continued until harvest.

The sources and rates of Ca were:

- Ca chelate [10 % $Ca(NO_3)_2$ + 37 % proprietary blend of alcohol sugars](ClawEl, Brandt Consolidated, Pleasant Plains, IL) at 125 (low), 250 (medium) or 500 (high) mg/l of Ca.
- Calcium nitrate [$Ca(NO_3)_2 \cdot 4H_20$] (Fisher Scientific International, Fair Lawn, NJ) at 125 (low), 250 (medium) or 500 (high) mg/l of Ca.
- Calcium chloride [$CaCl_2 \cdot 2H_2O$] (Mallinckrodt Baker Inc., Paris, KY) at 125 (low), 250 (medium) or 500 (high) mg/l of Ca.

- Sodium nitrate [$NaNO_3$] (Fisher Scientific International, Fair Lawn, NJ) at 100 (low), 200 (medium) or 400 (high) mg/l of N simulating the concentrations of nitrogen in calcium nitrate.
- Chelate without Ca [37 % proprietary blend of alcohol sugars] (Brandt Consolidated, Pleasant Plains, IL) diluted at the same concentrations as the Ca chelate.

For experiment one, plants were sprayed with each treatment to runoff, approximately 700 ml per plant. For experiment two, the substrate of each container was saturated with 300 ml of treatment solution through drip irrigation. In both experiments, the control solution was deionized (DI) water from combination of 4 deionizer columns, cation-bed deionizer, anion-bed deionizer, mixed-bed deionizer and ultra-bed deionizer; these have a high capacity for removing positively and negatively charged ions (U.S. Filters, New Orleans, LA).

3.2.3 Harvest

Sunflowers were harvested at 0800 H, and were cut at soil surface with sanitized utensils. The flowers were at the same physiological stage at the time of harvest; when ray petals begin to lift from the central disk or "cup stage", the capitulum viewed from the side looked like a cup. The harvested flowers were placed in sanitized buckets with DI water and immediately transported to the postharvest area. The stem ends of the cut flowers were dipped in a 10% Clorox™ solution for 20 minutes to decrease proliferation of bacteria. Cut sunflowers were placed in a postharvest room under fluorescent light (900 ± 20 lumen/m^2), and a 12-h photoperiod for the duration of the postharvest trials. The postharvest room temperature (mean = 22 ± 2 °C) and relative humidity (RH) (mean = 46 ± 6 %) were recorded (Fig 3.3A & B). In the postharvest room, all foliage was removed, except for the leaf just below the capitulum. The cut

sunflowers were selected through visual rating for uniformity in stem thickness and capitulum size, the cuts that had thicker stems or bigger capitula were discarded. After selection, the stems were re-cut at 50 cm below the capitulum, and each cut flower was placed in its respective 900 ml container filled with DI water. (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Sloan and Harkness, 2004). To evaluate the normal development of the sunflower plant due to treatment solution supplementation preharvest, the following the growth parameters of sunflower were recorded: days from transplant to flower, plant height, capitulum diameter, number of leaves, basal and apical stem diameter (Kamenidou, 2005) .

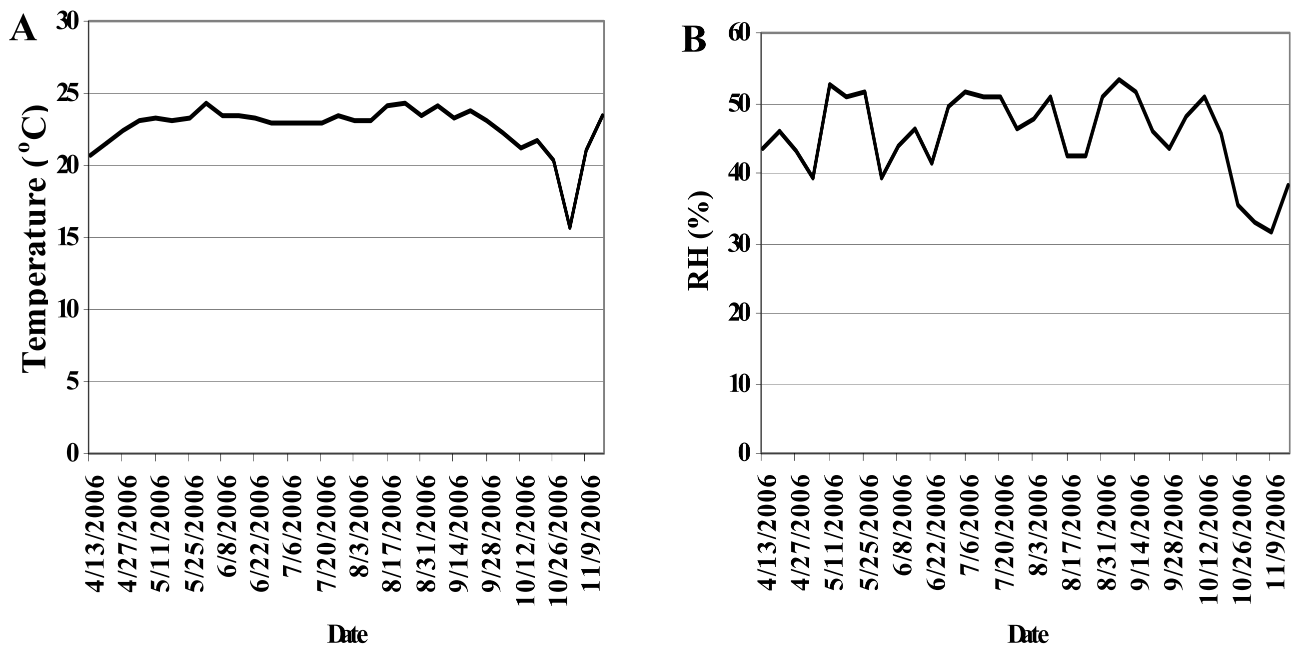

Fig 3.2. Weekly average (A) air temperature and (B) relative humidity (RH) during 2006 postharvest trials inside the postharvest room at the Burden Center, Baton Rouge, Louisiana.

3.2.4 Determination of Postharvest Longevity

Cut flowers were examined daily, and postharvest longevity was recorded as the time to occurrence of symptoms that indicate senescence. Symptoms of senescence included: petal wilting or curling, petal abscission, leaf yellowing or blackening and stem bending (Jones et al.,

1993). The weight of the containers with and without flowers was recorded daily in order to calculate total water uptake, total water loss and change in flower fresh weight (Van Meeteren, 1978). The change in weight between two consecutive measurements of the container + DI water (without the flower) corresponded to the water uptake by the cut flower for that day. The difference between consecutive measurements of the container + DI water + flower represented the water loss. The fresh weight (FW) of the flower was calculated by subtracting the weight of the container + DI water from the weight of the container +DI water + flower on that particular day (Van Meeteren, 1978; Venkatarayappa et al., 1980). There were four containers filled with DI water without flowers; the weight of these containers was recorded each day to monitor the rate of evaporation from the container. The average rate of evaporation was subtracted from water uptake and water loss.

3.2.5 Determination of Stem Bending

Stem bending in sunflowers during postharvest was measured at day of harvest, 3 and 7 days after harvest, and at senescence. The estimation of the angle between the main stem and the stem below the capitulum was recorded as a measurement of stem bending. A rating system for determining the degree of stem bending of cut sunflower has been defined by Celikel and Reid (2002): 1 = slight bending up to 45°; 2 = moderate bending between 45° and 90°; and 3 = advance (downward) bending more than 90°.

3.2.6 Calcium Extraction

Calcium was extracted from the plant tissue by wet acid digestion (Mills and Jones, 1991). Harvested plants were divided into three tissue samples: leaves, stem and capitulum. Tissue samples were dried at 80°C for 24-h and ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass an 850 μm (20-mesh) screen. A ground tissue sample of 0.5 grams was

placed in a digestion tube with 4 ml of concentrated nitric acid (HNO_3) and let stand overnight inside a fume hood at room temperature (25°C).

The digestion tubes were placed in a BD40 digestion block (Bran+Luebe, Germany) set at 120°C. The tubes were removed from the block after 1 h and allowed to cool; 4 ml of hydrogen peroxide (H_2O_2) was added to the digestion tubes. After addition of H_2O_2, the tubes were returned to the digestion block until the digest solution became colorless, approximaltely1.5 h. The solution was transferred into a 10 ml volumetric flask, brought to volume, and filtered (Whatman #2 slow flow rate filter paper) into a 45 ml plastic vial. Nitric acid and hydrogen peroxide were added to six digestion tubes without ground tissue as blank samples. The blanks were used as control for Ca contamination of the glassware.

3.2.7 Calcium Analysis

The concentration of Ca in the tissue of sunflowers was obtained by a colorimetric assay, Calcium L3K® Assay (Diagnostic Chemical Limited (DCL), Oxford,CT). This procedure uses a Ca complexing dye, Phosphonazo III, which forms a blue-purple color with a maximum absorption at 660 nm (Onishi, 1986). A 10 µl aliquot of tissue extract was added to a polycarbonate centrifuge tube plus 1 ml of the Phosphonazo III solution. The solution was mixed and left for 3 min at room temperature. This volume was transferred into disposable cuvettes, and the readings were recorded at an absorbance of 660 nm using a Perkin Elmer (Lamda-35) UV/VIS Spectrometer. A standard curve was prepared with 0, 50, 70, 90, 120, 150 mg/l of Ca using $CaCO_3$ for each set of samples measured. The stock solution was prepared following the procedure of Moorehead and Biggs (1974). There was no absorbance reading from the blank samples. To ensure that there was no Ca residue during Ca extraction and analysis, a set of six

extractions were conducted without plant tissue. A zero absorbance was recorded for all six samples.

3.2.8 Statistical Analysis

The experimental design was a 5 by 3 factorial design (5 chemicals at 3 levels) and a control (DI water) with 2 blocks and 6 experimental units per treatment combination within each block. Untreated control sunflowers were included in all of the experiments. The means and standard errors were assessed for the control with the univariate procedure for comparison with the treatments. There were no significant differences in treatments between the blocks and the data were pooled. The significance of treatment effects on growth parameters was tested in a multivariate analysis by the GLM procedure in SAS. The postharvest parameters were tested with multiple regression and analysis of variance with MIXED procedure in SAS. Stem bending was not visible in this cultivar, with the number 2 rating being the common characteristic for treated and untreated flowers.

3.3 RESULTS

3.3.1 Foliar Spray Experiment

There was no treatment effect in this experiment for cut sunflower postharvest attributes: postharvest longevity (Table 3.1), total fresh weight increase, total water uptake, total water loss, or water loss/water uptake ratio (Table 3.2). There were also no treatment effects on the growth parameters of sunflower: days to flower, plant height, capitulum diameter, apical stem diameter, basal stem diameter or number of leaves (Table 3.3). The tissue analysis revealed that Ca concentration was influenced by the tissue sampled (leaf, capitulum or stem) and an interaction effect between the chemical and the tissue (Table 3.4). The leaf tissue had the highest concentration of Ca in treated and untreated plants, followed by the stem (Fig. 3.3A). The

concentration of Ca in the stem tissue of treated plants was higher in relation to the stem tissue of untreated plants (Fig 3.3A). There were no significant differences in Ca concentration of leaf and capitulum tissue of treated plants compared to untreated control plants (Fig 3.3B). Stem tissue of sunflower plants sprayed with $Ca(NO_3)_2$ and $CaCl_2$ showed higher Ca concentrations than the stem tissue of plants treated with any of the other sources or untreated plants (Table 3.4; Fig 3.3B).

Table 3.1. Effect of supplemental, preharvest, spray applications of chemical treatments on postharvest longevity of *Helianthus annuus* L. 'Superior Sunset'.

Chemical Treatment	**Ca or N Rate (mg/l)**	**Longevity (Days)**
Untreated (Control)[y]	0	10
$Ca(NO_3)_2$	125	12
	250	11
	500	11
$CaCl_2$	125	11
	250	11
	500	12
Calcium Chelate[z]	125	11
	250	12
	500	12
Chelate	125	11
	250	13
	500	11
$NaNO_3$	100	12
	200	11
	400	12
Chemical Treatment		NS
Rate		NS
Interaction		NS

Values not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n = 6).
[Z] Chelate = 37 % blend of alcohol sugars.

Table 3.2. Effect of supplemental, preharvest, spray applications of chemical treatments on postharvest total fresh weight (FW) increase and postharvest water dynamics of *Helianthus annuus* L. 'Superior Sunset'.

Chemical Treatment	Ca or N Rate (mg/l)	Total FW Increase (%)	Total Water Uptake[w] (g/flower)	Total Water Loss[w] (g/flower)	Ratio[x]
Untreated (Control)[y]	0	27.3	171.9	149.2	0.86
$Ca(NO_3)_2$	125	26.1	158.6	140.3	0.88
	250	24.9	164.5	146.1	0.86
	500	29.2	162.1	138.8	0.85
$CaCl_2$	125	24.2	162.7	146.1	0.89
	250	32.7	162.4	137.8	0.85
	500	28.7	169.7	149.8	0.88
Calcium Chelate[z]	125	31.9	166.1	144.6	0.87
	250	28.6	180.1	153.9	0.85
	500	29.2	170.1	149.1	0.87
Chelate	125	28.4	168.8	147.9	0.87
	250	29.7	181.5	159.4	0.87
	500	27.6	182.1	157.3	0.86
$NaNO_3$	100	31.9	182.9	158.7	0.86
	200	29.9	162.9	140.4	0.86
	400	27.6	172.0	150.6	0.87
Chemical Treatment		NS	NS	NS	NS
Rate		NS	NS	NS	NS
Interaction		NS	NS	NS	NS

Values not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[W] Total water uptake and total water loss were recorded at the end of 8 days in postharvest.
[X] Water loss/water uptake ratio.
[Y] Values in table are averages (n = 6).
[Z] Chelate = 37 % blend of alcohol sugars.

Table 3.3. Effect of supplemental, preharvest, spray applications of chemical treatments on growth parameters of *Helianthus annuus* L. 'Superior Sunset'.

Chemical Treatment	Ca or N Rate (mg/l)	Days to Flower	Plant Height (cm)	Flower Diameter (cm)	Apical Stem Diameter (mm)	Basal Stem Diameter (mm)	No. of Leaves
Untreated (Control)[y]	0	58	149.3	9.0	7.7	11.3	24
$Ca(NO_3)_2$	125	58	140.0	7.4	7.0	11.3	24
	250	58	148.0	8.4	6.6	11.7	24
	500	58	142.3	8.8	7.3	11.7	24
$CaCl_2$	125	57	140.5	7.7	6.3	11.8	23
	250	58	137.8	7.1	7.1	11.8	22
	500	56	135.1	8.5	6.9	11.2	22
Calcium Chelate[z]	125	57	139.3	8.2	7.2	11.0	23
	250	60	151.7	8.7	7.0	12.0	26
	500	58	142.9	8.5	6.5	11.1	24
Chelate	125	57	147.9	8.3	7.4	11.4	23
	250	56	137.4	8.3	7.3	11.3	21
	500	59	155.3	7.3	7.2	11.8	25
$NaNO_3$	100	58	142.9	7.7	6.7	11.6	24
	200	58	150.3	7.2	7.1	11.6	25
	400	58	151.3	8.2	7.0	11.9	24
Chemical Treatment		NS	NS	NS	NS	NS	NS
Rate		NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS	NS

Values not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n = 6).
[Z] Chelate = 37 % blend of alcohol sugars.

Table 3.4. Effect of supplemental, preharvest, spray applications of chemical treatments on Ca concentration in leaf, stem and capitulum tissue of *Helianthus annuus* L. 'Superior Sunset'.

Chemical Treatment	Ca or N Rate (mg/l)	Ca Concentration (µg/g)		
		Leaf	**Stem**	**Capitulum**
Untreated (Control)y	0	18356	4909	6982
$Ca(NO_3)_2$	125	19138	8034	6596
	250	17379	17471	7597
	500	18653	10690	6157
$CaCl_2$	125	17735	12067	7086
	250	18211	9415	6050
	500	18864	10257	6577
Calcium Chelate[z]	125	21509	8953	7242
	250	19068	8118	6000
	500	18403	5451	5312
Chelate	125	18247	5327	5736
	250	19658	5447	6427
	500	20883	5307	5307
$NaNO_3$	100	21679	5370	5867
	200	22077	9319	6922
	400	20775	5171	7055
Chemical Treatment (C)		NS	NS	NS
Rate (R)		NS	NS	NS
Sample (Sa)		*	*	*
Interaction (C)*Sa		*	*	*
Interaction R*Sa		NS	NS	NS
Interaction C*R*Sa		NS	NS	NS

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n=6).
[Z] Chelate = 37 % blend of alcohol sugars.

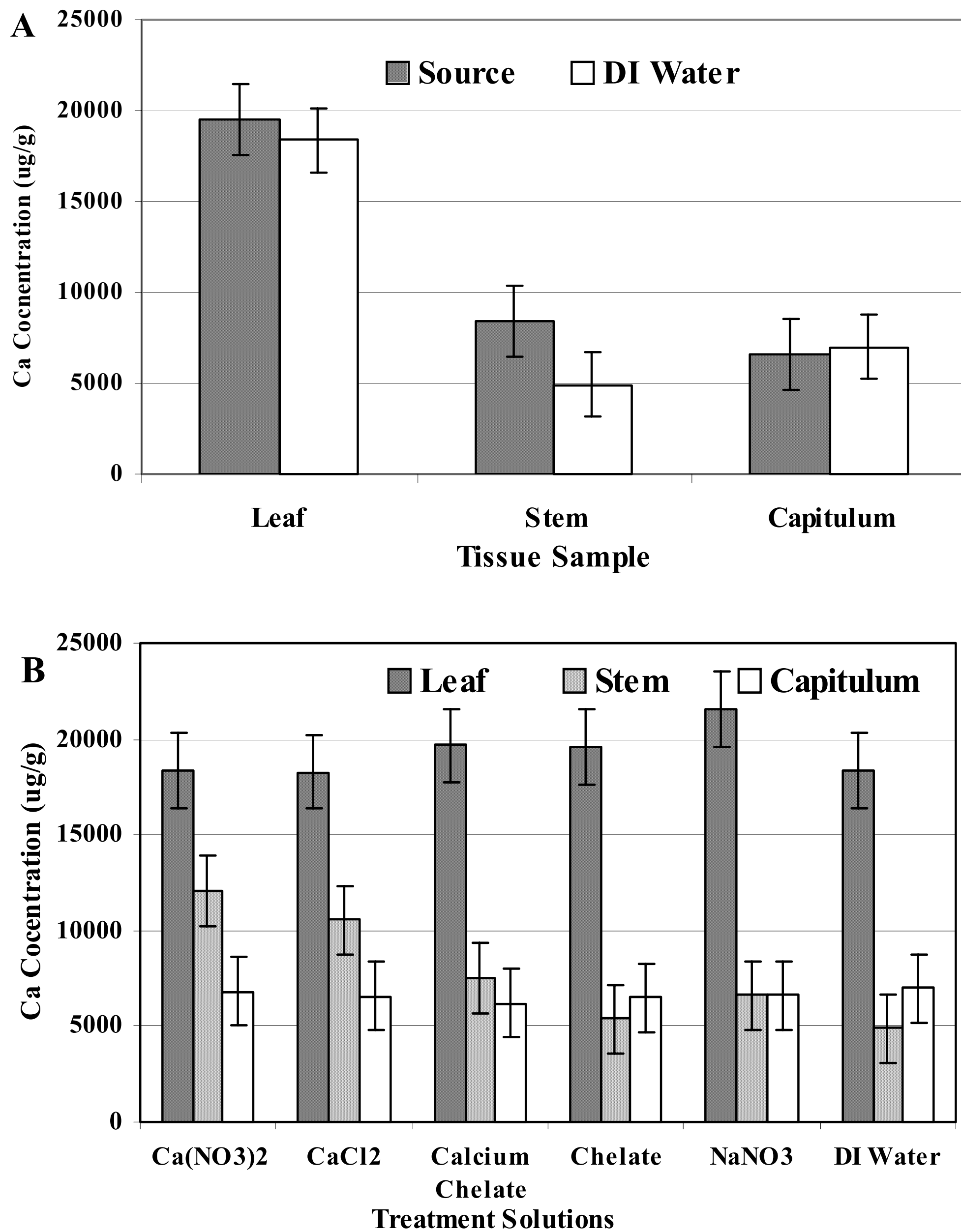

Fig 3.3. Effect of supplemental, preharvest, spray applications of calcium chemical treatments on Ca concentration in the leaf, stem and capitulum tissue of *Helianthus annuus* L. 'Superior Sunset'. A) Ca concentration by tissue sample and B) Ca concentration by treatment and tissue sample. Vertical bars show standard error for six replicates

3.3.2 Drench Experiments

This study was replicated twice. Experiment I was planted 8 April 2006 and experiment II was planted 28 August 2006. Experiment I treatments had no effect on sunflower postharvest longevity (Table 3.5). Among all treatments, total water uptake, total water loss and water

loss/water uptake ratio were influenced by chemical and concentration (Table 3.6). The increase in total water uptake and total water loss appeared to be related to an increase in concentration. For the water loss/water uptake ratio, the response decreased with increasing concentration, except for the $NaNO_3$ treated sunflowers (Table 3.6).

Table 3.5. Effect of weekly, preharvest, drench applications of chemical treatments on postharvest longevity of *Helianthus annuus* L. 'Superior Sunset'. Experiment I planted 8 April 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Longevity (Days)
Untreated (Control)[y]	0	11
$Ca(NO_3)_2$	125	11
	250	11
	500	11
$CaCl_2$	125	12
	250	11
	500	11
Calcium Chelate[z]	125	11
	250	12
	500	11
Chelate	125	12
	250	11
	500	10
$NaNO_3$	100	11
	200	10
	400	11
Chemical Treatment		NS
Rate		NS
Interaction		NS

Values not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n = 6).
[Z] Chelate = 37 % blend of alcohol sugars.

Table 3.6. Effect of weekly, preharvest, drench applications of chemical treatments on postharvest total fresh weight (FW) increase and postharvest water dynamics of *Helianthus annuus* L. 'Superior Sunset'. Experiment I planted 8 April 2006.

Chemical Treatment	**Ca or N Rate (mg/l)**	**Total FW Increase (%)**	**Total Water Uptake[w] (g/flower)**	**Total Water Loss[w] (g/flower)**	**Ratio[x]**
Untreated (Control)[y]	0	25.9	195.3	175.4	0.89
$Ca(NO_3)_2$	125	25.3	198.4	178.9	0.90
	250	24.9	210.6	189.3	0.89
	500	27.9	213.3	187.2	0.87
$CaCl_2$	125	25.1	192.2	172.5	0.89
	250	32.1	190.9	168.4	0.88
	500	31.6	200.5	177.1	0.88
Calcium Chelate[z]	125	25.4	194.1	174.8	0.90
	250	33.3	209.3	179.2	0.85
	500	30.9	219.3	192.9	0.88
Chelate	125	26.9	209.7	188.2	0.89
	250	31.2	208.8	181.9	0.87
	500	27.9	232.3	204.3	0.87
$NaNO_3$	100	27.2	173.1	155.1	0.89
	200	22.9	188.9	172.0	0.91
	400	26.6	211.4	179.3	0.90
Chemical Treatment		NS	*	*	*
Rate		NS	*	*	*
Interaction		NS	NS	NS	NS

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[W] Total water uptake and total water loss were recorded after 8 days in postharvest.
[X] Water loss/water uptake ratio.
[Y] Values in table are averages (n = 6).
[Z] Chelate = 37 % blend of alcohol sugars.

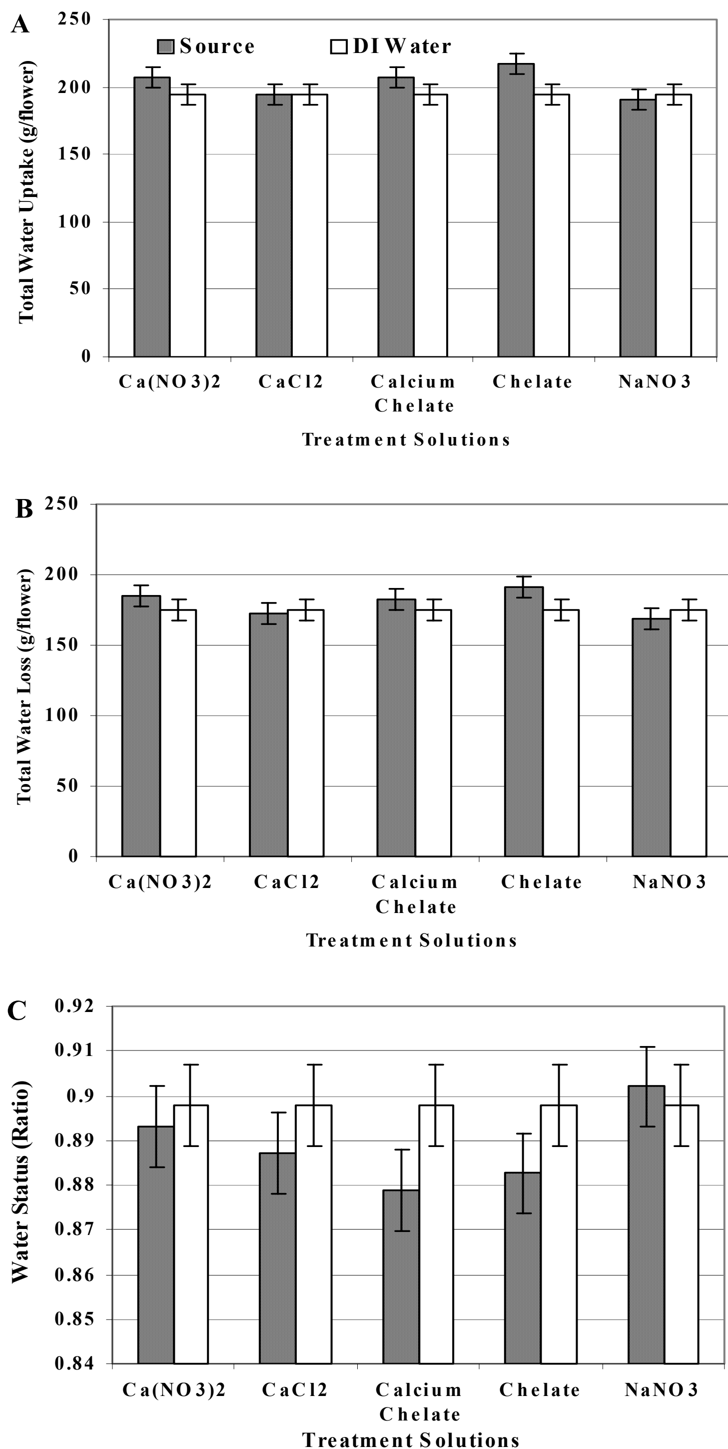

Fig 3.4. Effect of weekly, preharvest, drench applications of chemical treatments on postharvest water dynamics of *Helianthus annuus* L. 'Superior Sunset'. (A) Total water uptake; (B) total water loss; (C) water loss/water uptake ratio. Experiment I planted 8 April 2006. Vertical bars show standard error of six replicates.

Total water uptake was higher in sunflowers treated with the chelate solution compared to untreated control flowers, $CaCl_2$ and $NaNO_3$ treated flowers; however chelate treated sunflowers also showed the highest total water loss in relation to flowers in the previously mention treatments (Fig3.4A & B; Table 3.6). Sunflowers treated with $Ca(NO_3)_2$ and Ca chelate showed a higher water uptake than untreated control sunflowers, $CaCl_2$ and $NaNO_3$ treated sunflowers (Fig3.4A; Table 3.6). Total water uptake and total water loss were not significantly different among flowers treated with $Ca(NO_3)_2$, Ca chelate and the chelate (Fig3.4A & B; Table 3.6). The water loss/water uptake ratio was higher in sunflowers treated with $NaNO_3$ solution when contrasted with flowers in the calcium chelate treatments, but this was not true for untreated sunflowers (Table 3.6; Fig 3.2C). The water loss/water uptake ratio was lower in sunflowers treated with Ca chelate at 250 mg/l Ca in relation to other treatments and the untreated control sunflowers (Table 3.6).

The results for the growth parameters indicated that the days to flower in sunflower plants was influenced by a chemical effect and an interaction effect between chemical and concentration, whereas plant height was only affected by the interaction (Table 3.7). The response in apical stem diameter of sunflower plants was influenced by chemical, concentration and interaction effects (Table 3.7). Diameters may increase or decrease depending on concentration; however, there was no specific effect of chemical treatment. For example, apical stem diameter increased with an increase in concentration for sunflowers treated with the chelate and $NaNO_3$, whereas the same response decreased with an increase in concentration of $CaCl_2$ (Table 3.7). For the basal stem diameter response, only the chemical effect was found influential (Table 3.7). There were no treatment effects on sunflower growth parameters, such as capitulum diameter or number of leaves (Table 3.7).

Table 3.7. Effect of weekly, preharvest, drench applications of chemical treatments on growth parameters of *Helianthus annuus* L. 'Superior Sunset'. Experiment I planted 8 April 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Days to Flower	Plant Height (cm)	Flower Diameter (cm)	Apical Stem Diameter (mm)	Basal Stem Diameter (mm)	No. of Leaves
Untreated (Control)[y]	0	61	207.7	9.3	6.9	11.3	33
$Ca(NO_3)_2$	125	59	201.3	9.3	6.7	12.9	33
	250	60	212.9	9.9	6.4	12.0	32
	500	61	207.6	9.4	7.8	12.9	33
$CaCl_2$	125	60	204.9	10.2	7.7	11.3	32
	250	59	217.3	9.3	6.1	11. 7	32
	500	59	200.6	10.0	7.0	11.4	32
Calcium Chelate[z]	125	61	211.8	9.9	6.8	12.3	33
	250	58	192.0	8.6	7.8	12.9	33
	500	58	187.9	9.2	7.2	12.7	33
Chelate	125	60	206.3	10.2	7.1	12.4	34
	250	59	207.8	9.8	7.5	12. 7	33
	500	60	200.6	11.6	7.8	13.4	34
$NaNO_3$	100	59	210.3	9.0	6.0	11.6	33
	200	59	190.5	9.1	6.4	11.6	31
	400	62	213.0	9.8	6.9	12.0	34
Chemical Treatment		*	NS	NS	*	*	NS
Rate		NS	NS	NS	*	NS	NS
Interaction		*	*	NS	*	NS	NS

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n = 6).
[Z] Chelate = 37 % blend of alcohol sugars.

Sunflowers treated with $NaNO_3$ at 400 mg/l of N showed greater number of days to flower compared to untreated control sunflower plants and compared to the other treatments (Fig 3.5A). Plants treated with Ca chelate at 500 mg/l of Ca had the shorter number of days to flower than untreated plants and the other treatments, except for the plants treated with Ca chelate at 250 mg/l of Ca (Fig 3.5A). Sunflower plants treated with Ca chelate at 250 or 500 mg/l of Ca were shorter than the plants treated the non-chelated Ca sources, the chelate and untreated plants, but not shorter than sunflowers treated with $NaNO_3$ at 200 mg/l of N (Fig 3.5B).

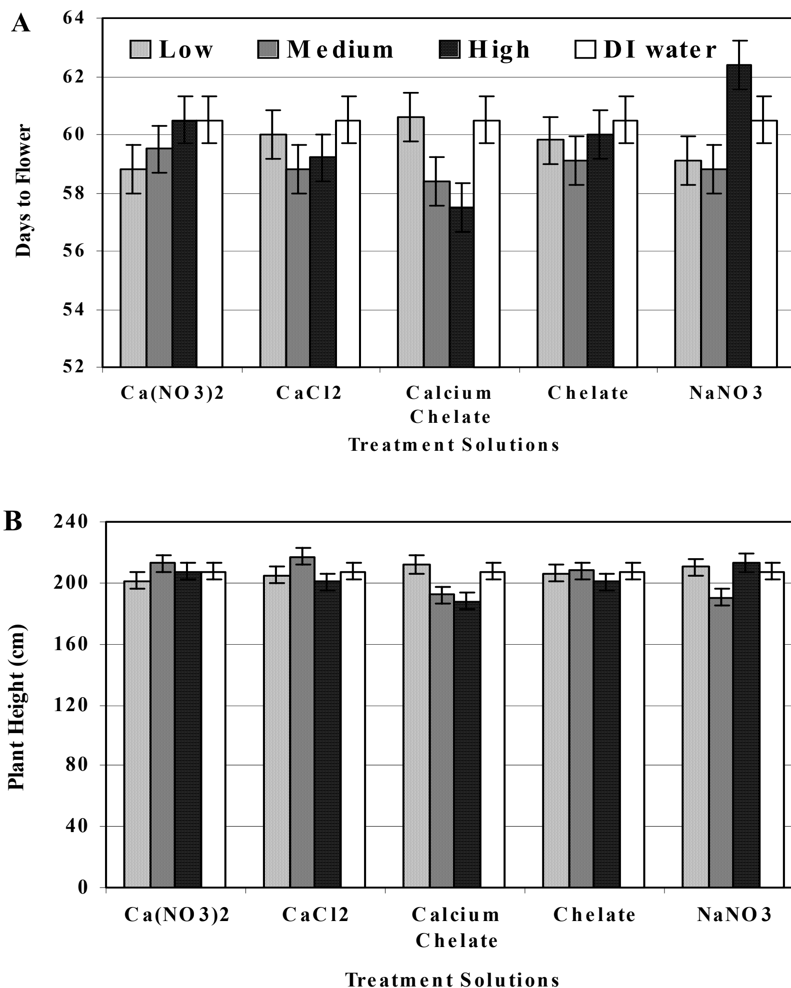

Fig 3.5. Effect of weekly, preharvest, drench applications of low, medium or high rates of chemical treatments on growth parameters of *Helianthus annuus* L. 'Superior Sunset'. (A) Days to flower and (B) plant height. Experiment I planted 8 April 2006. Vertical bars show the standard error of six replicates.

Apical stems of sunflower plants treated with $NaNO_3$ were thinner than the apical stem of sunflower plants in the other treatments, but not thinner than untreated plants (Fig 3.6A). Sunflower plants treated with $Ca(NO_3)_2$, Ca chelate and the chelate showed an increase in basal stem diameter compared to sunflower plants treated with $CaCl_2$, $NaNO_3$ and untreated plants (Fig 3.6B).

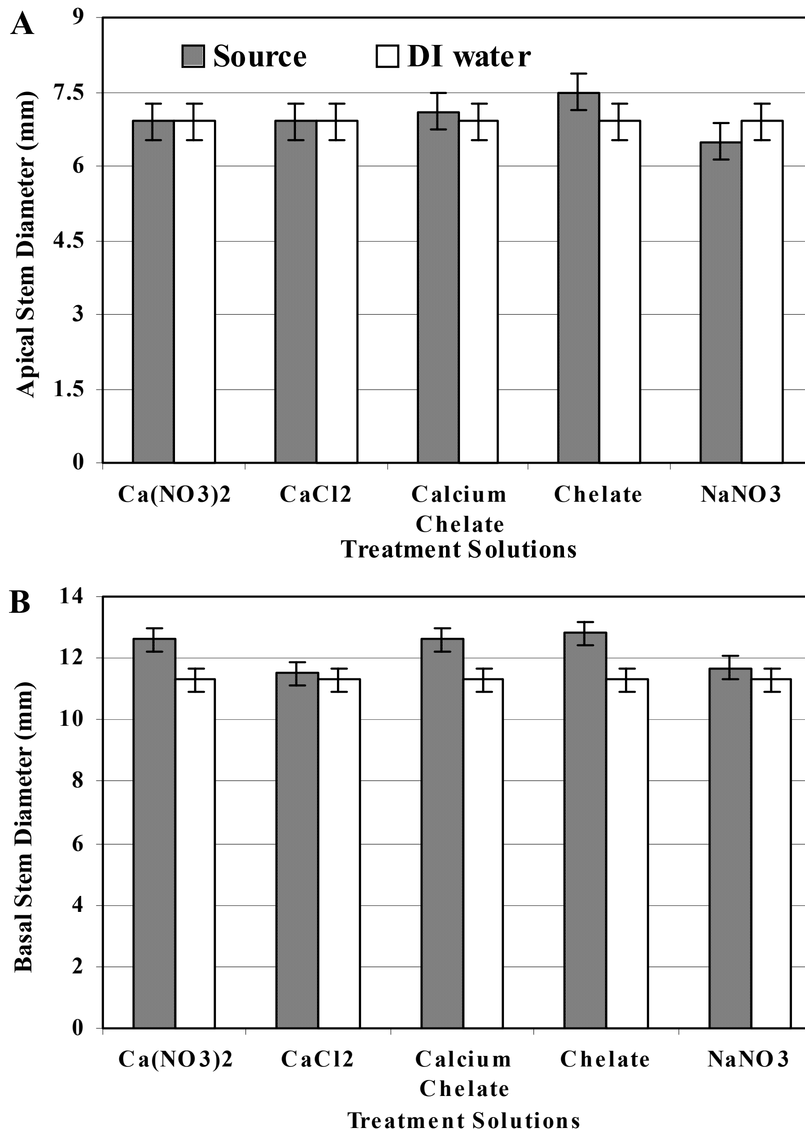

Fig 3.6. Effect of weekly, preharvest, drench applications of chemical treatments on growth parameters of *Helianthus annuus* L. 'Superior Sunset'. (A) Apical stem diameter and (B) basal stem diameter. Experiment I planted 8 April 2006. Vertical bars show the standard error of six replicates.

For experiment I, a set of six sunflower plants was collected from the greenhouse at harvest to measure the concentration of calcium in the tissue. A set of six sunflowers was harvested and placed in the postharvest room to record postharvest attributes, and after senescence, the cut sunflowers were collected to record the concentration of Ca in the tissue.

The tissue analysis for the set of six sunflower plants harvested prior postharvest showed that treatment had an effect on the concentration of Ca, and the response was also influenced by the nature of the tissue (Table 3.8). The leaf tissue presented the highest concentration of Ca, followed by the capitulum and the stem tissue; however, there was no significant difference in Ca concentration between leaf and stem tissue of treated and untreated sunflower plants (Fig 3.7A). Sunflower plants treated with Ca chelate had low Ca concentrations in leaf and capitulum tissue compared to sunflowers treated with $CaCl_2$, $NaNO_3$ and untreated plants (Fig 3.7B).

The results for the set of cut sunflowers showed that chemical and concentration effects were significant (Table 3.9). The response was also affected by the type of tissue sampled (Table 3.9). Because these flowers were in postharvest, they did not have any leaves; the tissue analyzed was from the stem and capitulum. Capitulum tissue presented a higher concentration of Ca than the stems in treated sunflowers, but this difference was not significant for untreated sunflowers (Fig 3.8A). There was not a substantial increase in Ca concentration in the capitulum tissue of treated flowers compared to untreated flowers. Sunflowers treated with $CaCl_2$ showed a higher Ca concentration in capitulum tissue than sunflowers treated with Ca chelate or chelate (Fig 3.8B). The differences in Ca concentration of stem tissue among the treatments, including untreated flowers, are not significant (Fig 3.8B)

Table 3.8. Effect of supplemental, preharvest, drench applications of chemical treatments on Ca concentration in the tissue of *Helianthus annuus* L. 'Superior Sunset'. Sunflowers from greenhouse. Experiment I planted 8 April 2006.

Source	Ca or N Rate (mg/l)	Ca Concentration (μg/g)		
		Leaf	Stem	Capitulum
Untreated (Control)[y]	0	29326	5547	10444
$Ca(NO_3)_2$	125	24856	3456	8280
	250	28918	4078	6387
	500	30015	3408	5863
$CaCl_2$	125	28954	4661	7473
	250	28941	6420	8473
	500	29876	4580	10842
Calcium Chelate[z]	125	27407	3297	8087
	250	22103	2450	6619
	500	30523	5206	5664
Chelate	125	27502	3148	6616
	250	28263	7698	7291
	500	27363	3256	6416
$NaNO_3$	100	27608	4026	7833
	200	29265	2971	8478
	400	33311	4730	10081
Source (So)		*	*	*
Rate (R)		NS	NS	NS
Sample (Sa)		*	*	*
Interaction So*Sa		NS	NS	NS
Interaction R*Sa		NS	NS	NS
Interaction So*R*Sa		NS	NS	NS

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n=6).
[Z] Chelate = 37 % blend of alcohol sugars.

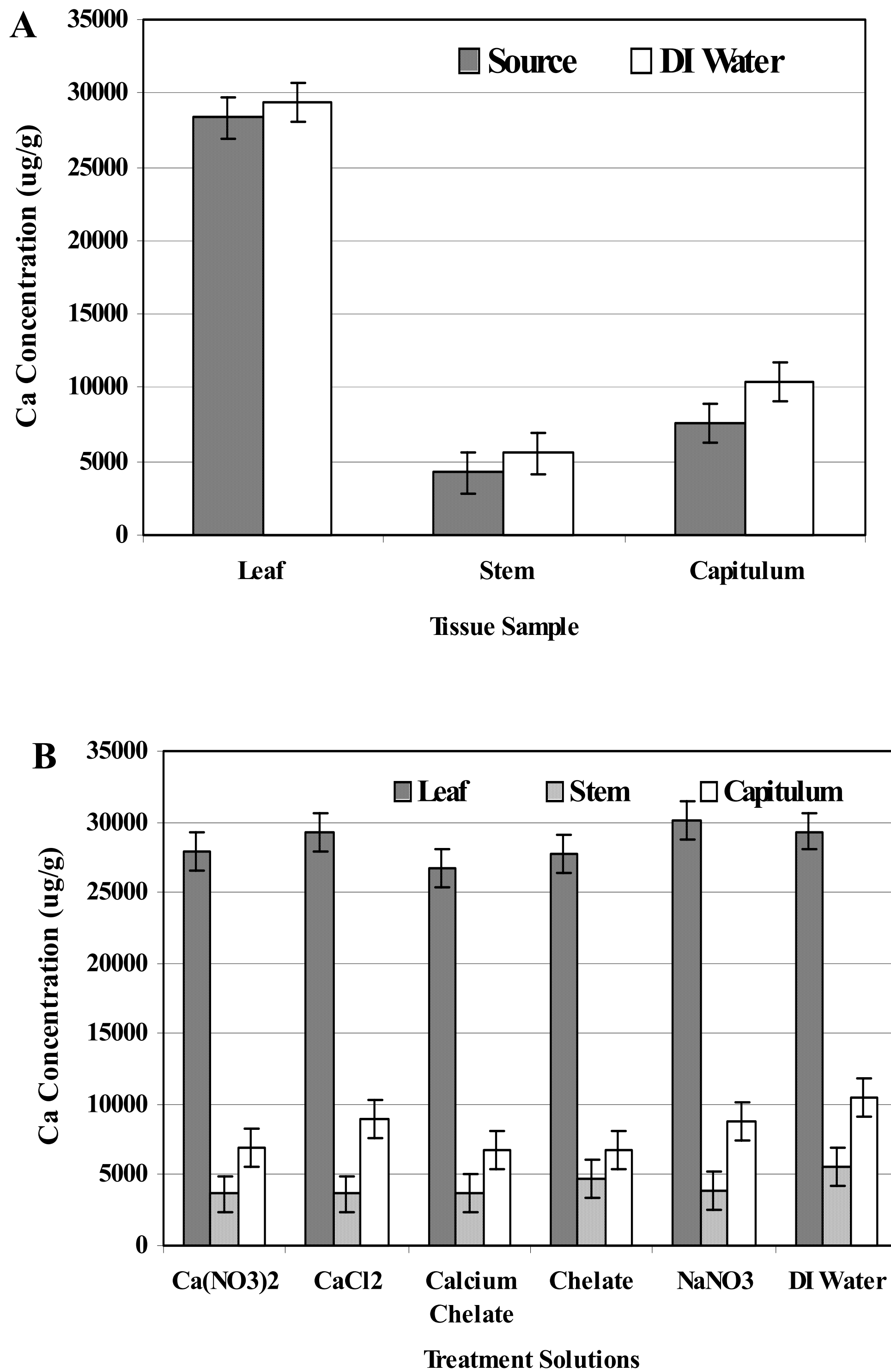

Fig 3.7. Effect of supplemental, preharvest, drench applications of chemical treatments on Ca concentration in the leaf, stem and capitulum tissue of *Helianthus annuus* L. 'Superior Sunset'. (A) Ca concentration by tissue sample; (B) Ca concentration by chemical and tissue sample. Sunflowers from greenhouse. Experiment I planted 8 April 2006. Vertical bars show the standard error for six replicates.

Table 3.9. Effect of supplemental, preharvest, drench applications of chemical treatments on Ca concentration in the tissue of *Helianthus annuus* L. 'Superior Sunset'. Sunflowers from postharvest room. Experiment I planted 8 April 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Ca Concentration (mg/l)	
		Stem	Flower
Untreated (Control)[y]	0	7532	9432
$Ca(NO_3)_2$	125	6787	11215
	250	3332	8956
	500	7466	9171
$CaCl_2$	125	5837	12171
	250	7538	12231
	500	6977	12273
Calcium Chelate[z]	125	4503	10824
	250	3721	5527
	500	5998	9597
Chelate	125	9115	7659
	250	4686	6630
	500	6405	9898
$NaNO_3$	100	4895	11591
	200	5849	9589
	400	7333	11313
Chemical Treatment (C)		*	*
Rate (R)		*	*
Sample (Sa)		*	*
Interaction C*Sa		NS	NS
Interaction R*Sa		NS	NS
Interaction C*R*Sa		NS	NS

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[Y] Values in table are averages (n=6).

[Z] Chelate = 37 % blend of alcohol sugars.

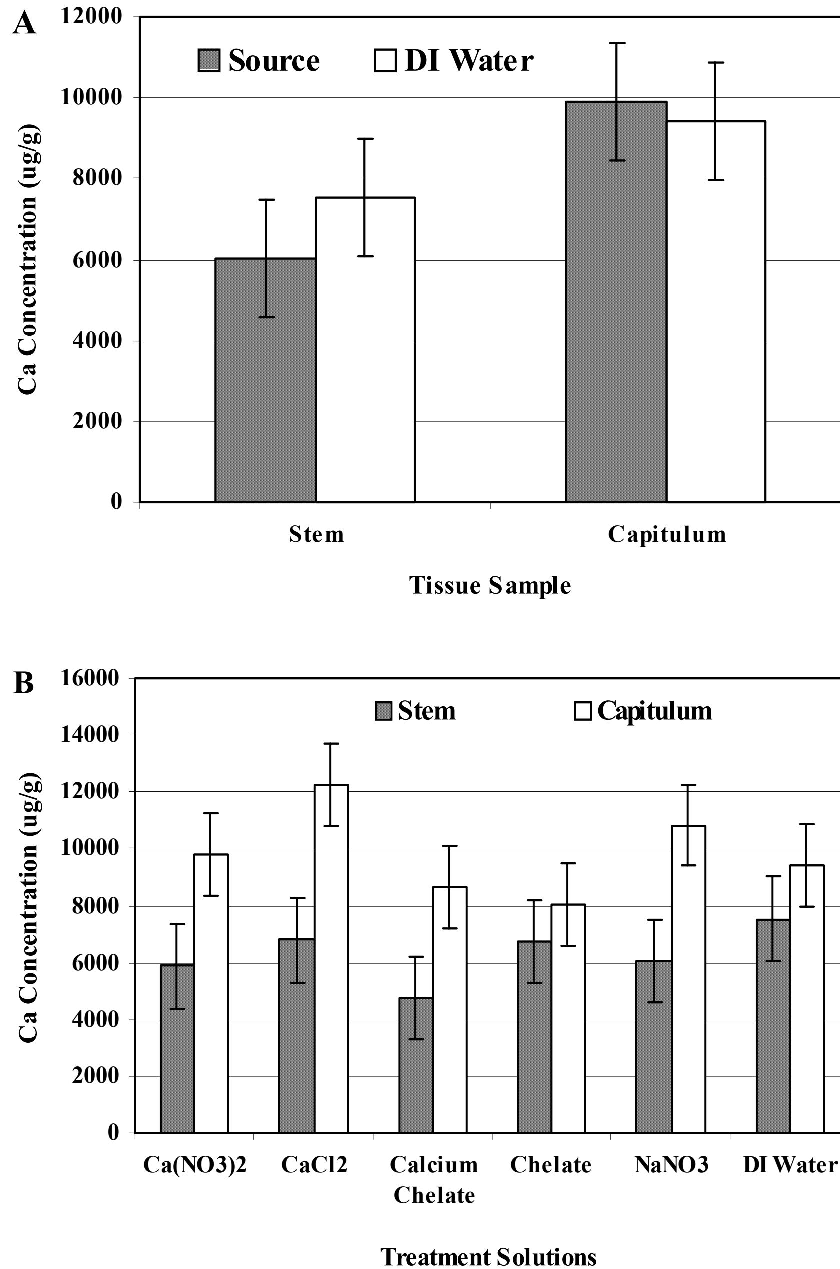

Fig 3.8. Effect of weekly, preharvest, drench applications of chemical treatments on Ca concentration in the stem and capitulum tissue of *Helianthus annuus* L. 'Superior Sunset'. (A) Ca concentration by tissue sample; (B) Ca concentration by chemical and tissue sample. Cut Sunflowers. Experiment I planted 8 April 2006. Vertical bars show standard error for six replicates.

In experiment II, postharvest attributes of cut sunflowers, postharvest longevity, total fresh weight increase and water loss/water uptake ratio were not influenced by the treatment effects (Table 3.10; Table 3.11). The effect of treatments on sunflower growth parameters were

also not significantly different (Table 3.12). Concentration and interaction effects influenced total water uptake and water loss response in treated sunflower plants (Table 3.11). The differences in total water uptake and total water loss were not significant among treatments or when comparing treated and untreated sunflowers (Fig 3.9A & B).

Table 3.10. Effect of weekly, preharvest, drench applications of chemical treatments on postharvest longevity of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 28 August 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Longevity (Days)
Untreated (Control)[y]	0	13
$Ca(NO_3)_2$	125	15
	250	14
	500	13
Calcium Chelate[z]	125	15
	250	15
	500	16
Chelate	125	15
	250	14
	500	15
Chemical Treatment		NS
Rate		NS
Interaction		NS

Values not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n=6).
[Z] Chelate = 37 % blend of alcohol sugars.

Table 3.11. Effect of weekly, preharvest, drench applications of chemical treatments on total fresh weight (FW) increase and postharvest water dynamics in cut stems of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 28 August 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Total FW Increase (%)	Total Water Uptake[w] (g/flower)	Total Water Loss[w] (g/flower)	Ratio[x]
Untreated (Control)[y]	0	28.3	140.5	107.1	0.78
$Ca(NO_3)_2$	125	31.9	132.6	99.4	0.75
	250	31.1	126.5	97.3	0.78
	500	27.1	131.1	102.6	0.80
Calcium Chelate[z]	125	33.8	146.7	106.9	0.73
	250	32.4	114.9	89.7	0.78
	500	29.9	157.9	118.6	0.75
Chelate	125	30.8	137.9	102.4	0.75
	250	30.9	128.3	96.4	0.75
	500	27.1	116.7	94.4	0.81
Chemical Treatment		NS	NS	NS	NS
Rate		NS	*	*	NS
Interaction		NS	*	*	NS

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[W] Total water uptake and total water loss were recorded at the end of 8 days in postharvest.
[X] Water loss/water uptake ratio.
[Y] Values in table are averages (n=6).
[Z] Chelate = 37 % blend of alcohol sugars.

Table 3.12. Effect of weekly, preharvest, drench applications of chemical treatments on growth parameters of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 28 August 2006.

Chemical Treatment	**Ca or N Rate (mg/l)**	**Days to Flower**	**Plant Height (cm)**	**Flower Diameter (cm)**	**Apical Stem Diameter (mm)**	**Basal Stem Diameter (mm)**	**No. of Leaves**
Untreated (Control)[y]	0	68	208.3	9.1	8	15.1	30
$Ca(NO_3)_2$	125	70	213.8	12.3	7.5	15.3	29
	250	68	221.5	11.5	7.7	14.7	30
	500	66	208.3	11.4	7.7	13.7	30
Calcium Chelate[z]	125	69	211.4	12.4	7.8	15	30
	250	65	209.5	10.9	7.5	14.3	29
	500	69	213.2	12.8	8.7	14.8	30
Chelate	125	68	211	10.9	8	14.3	29
	250	69	216.9	11.9	7.7	15.3	29
	500	69	211.5	11.1	7.1	13.9	28
Chemical Treatment		NS	NS	NS	NS	NS	NS
Rate		NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS	NS

Values not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n=6).
[Z] Chelate = 37 % blend of alcohol sugars.

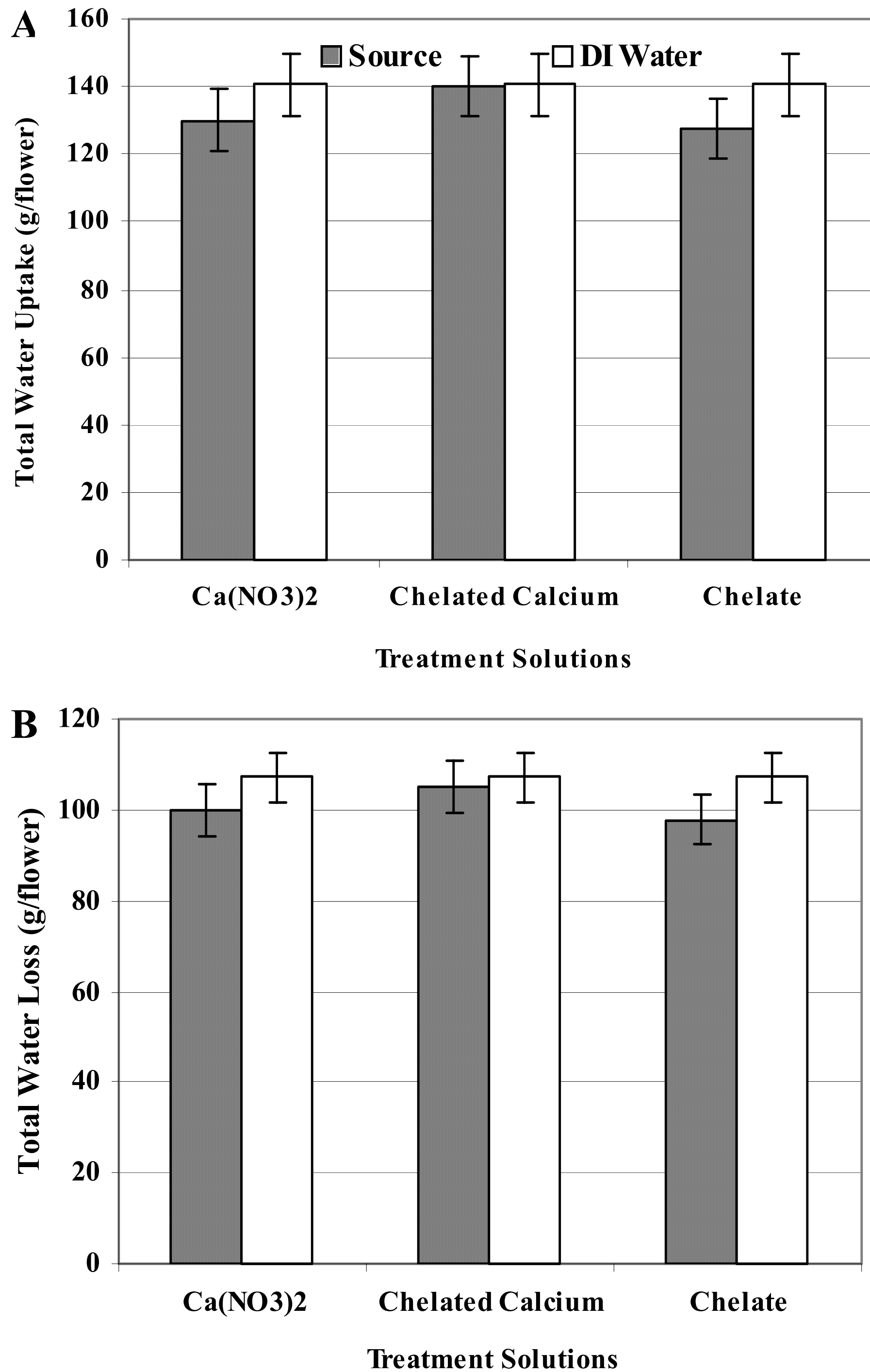

Fig 3.9. Effect of weekly, preharvest, drench applications of chemical treatments on postharvest water dynamics of *Helianthus annuus* L. 'Superior Sunset'. (A) Total water uptake; (B) total water loss; (C) water loss/water uptake ratio. Experiment II planted 28 August 2006. Vertical bars show standard error for six replicates.

3.4 DISCUSSION

Preharvest Ca application via spray or drench had no significant effect on postharvest longevity of fresh cut sunflower regardless of Ca source or rate. Preharvest spray application of Ca resulted in an increased postharvest longevity of fresh cut gerbera (Gerasopoulos and Chebli, 1999). Sunflower and gerbera belong to the same family of plants, *Asteraceae*. Their physiological and anatomical differences, however, may have played an important role in how these species absorb nutrients, such as Ca, through the aerial parts of the plant.

Gerasopoulos and Chebli (1999) also reported that preharvest Ca application increased Ca concentration in scape tissue of gerbera. Calcium concentration in the capitulum or leaf tissue of treated sunflower plants compared to untreated sunflowers, however, was not increased when applied as a foliar spray. Tissue analysis did reveal that $Ca(NO_3)_2$ and $CaCl_2$ treatments increased the Ca concentration of stem tissue in sunflowers, compared to untreated plants or to chelate and $NaNO_3$ treated plants. Leaf tissue of treated and untreated sunflower plants had the highest concentration of Ca; this response may be related to the involvement of Ca in stomatal closure in leaves (Buchanan et al., 2000).

The uptake of nutrients by foliar applications can be limited by environmental and phenotypic factors (Mengel and Kirby, 1987). The plants in the foliar spray experiment, however, were grown inside a greenhouse, reducing the influence of environmental factors such as changes in temperature or the effect of precipitation. Calcium deficiency, which causes ‘bitter pit’ in apples, can be alleviated by spraying the fruit with Ca solutions, but more than two applications were needed to obtain a response (Mengel and Kirby, 1987). In this experiment only two spray applications were made before anthesis, which might explain the low response of sunflower to Ca treatments.

Young (2002) reported differences in the time between developmental stages and postharvest longevity of sunflowers among different planting schedules and growing seasons. In the drench experiments, the average of postharvest longevity of untreated sunflowers from experiment II (planted 28 August, 2006) increased by 2 d, compared to untreated sunflowers in experiment I (planted 8 April, 2006). Furthermore, the average postharvest longevity of sunflower treated with $Ca(NO_3)_2$, Ca chelate or chelate was increased by 3 to 4 d in experiment II compared to experiment I. The average of days to flower for untreated and treated sunflowers was longer in experiment II by 7 to 8 d, respectively, compared to experiment I. This indicates that further research should be conducted on the effects of the growing environment on sunflower postharvest longevity.

For the drench experiments in this study, Ca concentration in the tissue of treated sunflower was not significantly higher than the Ca concentration in the tissue of untreated plants. The rates of Ca used in these experiments were based on recommendations stated on the label of the sugar alcohol chelated Ca (ClawEl, Brandt Consolidated, Pleasant Plains, IL) for ornamental crops. Mengel and Kirby (1987) suggested that the uptake of Ca by the roots was related to the levels of Ca in the soil. Ca concentration in plants varies between 0.1 to >5 % depending on growing conditions, plant species and plant organ (Marschner, 1995). The sufficiency requirement of Ca for sunflower leaf tissue ranges from 1.5 to 3 % (Mills and Jones, 1991). The tissue analysis revealed that the concentration of Ca in the leaves of sunflowers in the preharvest experiments was in the sufficiency range. Thus, the treatment applications were adequate for optimum growth of sunflowers; however, positive responses in postharvest attributes may require higher concentrations of this cation. Increased postharvest response of honeydew melon resulted when ClawEl was applied at the manufacturer's recommendation rate of 2.3 l/ha in 75.7

to 113.6 l of water sprayed at 345 kPa. Rates of application may need to be increased to obtain a positive response in fresh cut sunflowers.

Sunflowers treated with the highest rates of $NaNO_3$ had longer days to flower (1 to 2 d) than the other treatments and untreated sunflowers. Plant height in sunflowers treated with the medium rate of $NaNO_3$ was reduced by 4 cm compared to untreated sunflower, but this was not true when compared to Ca chelated treated sunflower plants. Sunflower is not listed as tolerant species to Na (Marschner, 1971; Mengel and Kirby, 1987), and many non-salt-tolerant herbaceous crops, grapevines and fruit trees may suffer growth inhibition and foliage injury (marginal chlorosis, and necrosis on mature leaves) even at low levels of NaCl (Marschner, 1995). None of these symptoms, however, were observed on plants grown in the greenhouse for these experiments. According to Bhatt and Indirakutty (1972), sunflower may be able to remove Cl and Na from red sandy loam soils and heavy clay soils. The sufficiency requirement of Na in sunflower leaves ranges from 200 to 5000 μg/g (Mills and Jones, 1991). The substrate utilized in the greenhouse experiments was amended with 4.75 kg/m^3 of dolomitic limestone. Calcium function in membrane integrity and control of selectivity in ion uptake and transport has been related to increasing salt tolerance of plants (Marschner, 1995). The presence of Ca in the substrate may have interfered with Na or Cl uptake by roots.

Although postharvest research studies have indicated increased Ca concentration and its relation to increased postharvet longevity (Mayak et al., 1978; Gerasopoulos and Chebli, 1999; Michalczuck et al., 1989; Torre et al., 1999), the results of these experiments suggest that Ca concentration of treated sunflowers was not significantly higher than the concentration in untreated sunflowers. Hence, there was a lack of response in postharvest attributes of sunflower

to supplemental Ca. This research indicates that application of supplemental Ca as a spray or a drench to increase the postharvest longevity of sunflower should not be recommended.

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CHAPTER 4. EFFECTS OF POSTHARVEST CALCIUM SUPPLEMENTATION ON SUNFLOWER LONGEVITY

4.1 INTRODUCTION

Several studies have shown that postharvest application of calcium (Ca) may increase the longevity of fresh cut flowers. These studies indicated that the effects of Ca on postharvest longevity are related to a delay of the degradation processes that may affect cell membrane integrity. Cell membranes of senescing rose petals undergo various physiological and biochemical alterations that cause cell membrane deterioration, resulting in a decrease of cell water content and increase of electrolyte leakage; thus, the cell will loose its turgidity (Borochov and Woodson, 1989; Itzhaki et al., 1990; Marangoni et al., 1996; Torre et al., 1999; Rubinstein, 2000).

Postharvest treatment with 5 mM calcium chloride ($CaCl_2$) delayed the speed of membrane deterioration in detached petals of 'Mercedes' and 'Baroness' rose flowers (Torre et al., 1999). Calcium chloride applied as a vase solution treatment not only delayed senescence, but promoted bud opening of cut rose cultivars. A concentration of 5 mM $CaCl_2$ was optimal for improving vase life of 'Mercedes' flowers by 4 days, compared to the DI water control, while a concentration of 1 mM $CaCl_2$ increased postharvest longevity of 'Baroness' flowers by 2 d.

Calcium nitrate [$Ca(NO_3)_2$], used as a vase solution at 0.25 % Ca, extended vase life and promoted bud opening of cut roses 'Sonia', 'Celica', 'Samantha', and 'Mercedes' (Michalczuk et al., 1989). Continuous treatment with either a basic preservative solution of 2 % sucrose and 8-hydroxyquinoline (HQC+S), or with 0.25% $Ca(NO_3)_2$ alone, extended the vase life of 'Sonia' roses by almost 3 d compared to DI water. Both treatment solutions eliminated the "bent neck" phenomena exhibited by the DI water treatment. When $Ca(NO_3)_2$ was combined with the basic preservative solution, the flower longevity was extended significantly by 1 to 3 d, compared to

'Sonia' flowers treated with the preservative solution alone, both as a pulse and as a continuous treatment. For 'Celica', 'Samantha', and 'Mercedes', a concentration of 0.25% $Ca(NO_3)_2$ added to the preservative solution extended vase life of the flowers by 2 to 3 d, both as a pulse and as a continuous treatment (Michalczuk et al., 1989).

Pruthi et al. (2001) showed that *Gadiolus* 'Happy End' pulsed with 4 mM $CaCl_2$ exhibited a higher percentage of opened florets in comparison to a DI water control. Pulsing duration had no effect on the postharvest performance of the flowers. Postharvest longevity of spikes treated with 2 mM $CaCl_2$ solution displayed a 1.5 d increase over the DI water control.

The postharvest longevity of *Dianthus caryophyllus* L. 'Improved White Sim' increased 4.2 d compared to a DI water control with postharvest supplementation of 180 mM potassium nitrate (KNO_3) in combination with 5 mM $Ca(NO_3)_2$ (Mayak et al., 1978). Development of stem softening was retarded with the addition of 5 mM of $Ca(NO_3)_2$ to the KNO_3 solution (Mayak et al., 1978).

Demand for fresh cut sunflowers has increased in the specialty cut flower market and so has the need for more information about its postharvest management (Devecchi, 2005; Celikel and Reid, 2002; Yañez et al., 2005). Longevity of cut sunflowers can be short and varies from 5 to 13 days, depending on the cultivar (Gast, 1995). Sunflowers often suffer from "neck" or "stem bending" during transport (Celikel and Reid, 2002). Postharvest trials of cut sunflowers showed that commercial holding solutions containing soluble carbohydrates may have a positive effect in postharvest longevity, extending it 1 to 4 d, depending on the cultivar (Fanelli et al., 2003). Young (2002) reported postharvest longevity of up to 11 d for sunflower cultivars 'Valentina', 'Full Sun' and 'Sunbright' treated with a commercial holding solution containing dextrose as a sugar source.

Mannitol is a sugar alcohol metabolized from photosynthesis in mature leaves (Everard et al., 1993; Loescher et al., 1992). Mannitol may permeate the plasma membrane, and there is evidence indicating that mannitol may support translocation of micronutrients such as boron (Heath et al., 1977; Hu et al., 1997). These two characteristics may be useful to Ca translocation inside the plant. Chelated forms of Ca are very stable and highly soluble compounds (Mengel and Kirby, 1987).Ca has been chelated with different organic compounds such as amino acids, EDTA (ethylene-diamine-tetraacetic-acid) or EGTA (ethylene-glycol-tetraacetic acid). Ca chelates have been used to delay senescence or to alleviate Ca deficiency disorders in fruits and vegetables (Lester and Grusak, 1999, 2001, 2004; Mengel and Kirby, 1987).

Mannitol chelated Ca (ClawEl, Brandt Consolidated, Pleasant Plains, IL), supplied as a postharvest dip, may delay senescence in fruit and increase firmness, without affecting the sugar content and palatability in honeydew melons (Lester and Grusak, 2004). The objective of these experiments was to determine the effects of postharvest Ca supplementation in the form of a 2-h pulse of $Ca(NO_3)_2$, $CaCl_2$ or a Ca chelate at 125, 250, or 500 mg/l of Ca on the longevity of fresh cut sunflowers.

4.2 MATERIAL AND METHODS

4.2.1 Plant Material

Helianthus annuus L. 'Superior Sunset' (Fred C. Gloeckner & Company, Inc., Harrison, NY) was used for these experiments. 'Superior Sunset' has been described as tolerant to cold temperatures and vigorous, with uniform flowering. Ray flowers have long, rich yellow orange petals. Disc flowers are dark brown and do not produce pollen. Plants grow 150 to 195 cm tall with straight and thick stems. The capitulum ranges from 15 to 20 cm in diameter. Cut sunflower

cultivars are considered day-neutral (Armitage and Laushman, 2003; Arnosky and Arnosky, 2000; Sloan et al., 2003, 2004; Sloan and Harkness, 2006).

Planting dates were 9 May and 27 July 2006 for two pulse experiments. 'Superior Sunset' seeds were sown in growing media (Scotts Metro Mix 366, The Scotts Co., Maryville, OH) in 1204 cell packs (3.81 x 6.2 x 6.2) inside a greenhouse located at the Burden Center, 30° N 91° W, Baton Rouge, Louisiana. Seedlings were grown in the greenhouse for 3 weeks prior to planting and fertilized with a complete liquid fertilizer 20-10-20 (20N-4.4P-16.6K) (The Scotts Co., Maryville, OH) at 200 mg/l nitrogen (N) once a week before transplanting to the field. The field consisted of an Olivier silt loam soil. Soil samples were collected to assess the nutrient content and pH (Table 4.1). Air temperature for the 2006 growing season was obtained from the Louisiana Office of State Climatology, Ben Hur, Baton Rouge (Fig 4.1). The field was prepared with raised beds (45 cm wide by 43 m long) covered with black plastic mulch, and drip tape was buried in the middle of the bed for irrigation and fertilization. The 3-week-old seedlings were spaced 15 x 15 cm apart and fertilized once a week with (20N-4.4P-16.6K) at 200 mg/l N (The Scotts Co., Maryville, OH). The plants were grown without net support (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Stevens et al., 1993; Schoellhorn et al., 2003).

Table 4.1. Soil analysis from growing plots at Burden Center, Baton Rouge, Louisiana prior to planting 2006.

Growing Plot	**Ca (mg/l)**	**Cu (mg/l)**	**Mg (mg/l)**	**P (mg/l)**	**K (mg/l)**	**Na (mg/l)**	**S (mg/l)**	**Zn (mg/l)**	**pH**
1	2491.7	1.4	372.9	194.1	313.2	110.3	57.4	7.3	6.8
2	3143.5	1.5	481.2	321.5	405.7	115.2	56.4	13.6	7.0
3	2996.6	1.5	457.7	242.5	345.9	117.8	50.6	11.4	7.3
4	3549.9	1.7	558.7	348.5	472.0	119.1	55.6	16.5	7.3
5	3510.1	1.7	615.9	419.7	573.8	148.2	85.7	18.9	7.1

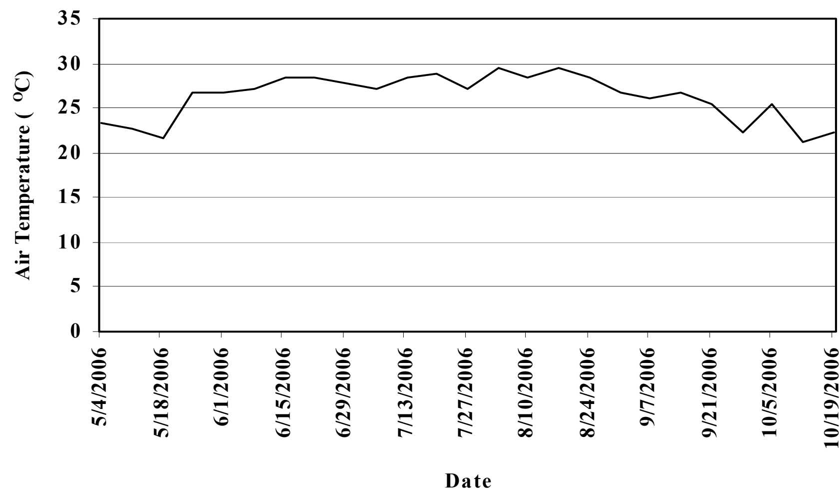

Fig 4.1. Weekly average air temperature during 2006 growing season at the Burden Center, Baton Rouge, Louisiana.

4.2.2 Harvest

Sunflowers were harvested at 0800 H and were cut at the soil surface with sanitized utensils. The flowers were at the same physiological stage at the time of harvest; when ray petals begin to lift from the central disk or "cup stage", the capitulum viewed from the side looks like a cup. The harvested flowers were placed in sanitized buckets with DI water and immediately transported to the postharvest area. The stem ends of the cut flowers were dipped in a 10% Clorox™ solution for 20 min to decrease proliferation of bacteria. In the postharvest room, all foliage was removed, except for the leaf just below the capitulum. The cut sunflowers were selected through visual rating for uniformity in stem thickness and capitulum size; the cuts that had thicker stems or bigger capitula were discarded. After selection, the stems were re-cut at 50 cm below the capitulum and placed in the treatment bucket for a 2-h pulse. (Armitage and Laushman, 2003; Dole and Wilkins, 2005; Sloan and Harkness, 2006).

4.2.3 Postharvest Treatment

To determine the effects of postharvest Ca application on the longevity of sunflowers, the study included a 2-h pulse of treatment solutions. The sources and rates of calcium were:

- Ca chelate [10 % $Ca(NO_3)_2$ + 37 % proprietary blend of alcohol sugars](ClawEl, Brandt Consolidated, Pleasant Plains, IL), at 125 (low), 250 (medium) or 500 (high) mg/l of Ca.
- Calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$] (Fisher Scientific International, Fair Lawn, NJ) at 125 (low), 250 (medium) or 500 (high) mg/l of Ca.
- Calcium chloride [$CaCl_2 \cdot 2H_2O$] (Mallinckrodt Baker Inc., Paris, KY) at 125 (low), 250 (medium) or 500 (high) mg/l of Ca.
- Sodium nitrate [$NaNO_3$] (Fisher Scientific International, Fair Lawn, NJ) at 100 (low), 200 (medium) or 400 (high) mg/l of N simulating the concentrations of nitrogen in calcium nitrate.
- Chelate without Ca [37 % proprietary blend of alcohol sugars] (Brandt Consolidated, Pleasant Plains, IL) diluted at the same concentrations as the Ca chelate.

The flowers were placed in 18.9 l buckets with 7.6 l of treatment solution for a 2-hour pulse. The control solution was deionized (DI) water from a Mega-Pure 12A Water Still deionizer (Barnstead International, Dubuque, IA). After treatment, each flower was placed in its respective 900 ml container filled with DI water in a postharvest room under fluorescent light (900 ± 20 lumen/m^2) and a 12-h photoperiod for the duration of the postharvest evaluation. The postharvest room temperature (mean = 22 ± 2 °C) and relative humidity (RH) (mean = 46 ± 6 %) were recorded (Fig 4.2A & B).

4.2.4 Determination of Postharvest Longevity

Stems were examined daily, and postharvest longevity was recorded as the time to occurrence of symptoms that indicate senescence. Symptoms of senescence included: petal

wilting or curling, petal abscission, leaf yellowing or blackening and stem bending (Jones et al., 1993). The weight of the containers with and without flowers was recorded daily to calculate total water uptake, total water loss and change in flower fresh weight (Van Meeteren, 1978). The change in weight between two consecutive measurements of the container + DI water (without the flower) corresponded to the water uptake by the cut flower for that day. The difference between consecutive measurements of the container + DI water + flower represented the water loss. The fresh weight (FW) of the flower was calculated by subtracting the weight of the container + DI water from the weight of the container +DI water + flower on that particular day (Van Meeteren, 1978; Venkatarayappa et al., 1980). There were four containers filled with DI water without flowers; the weight of these containers was recorded each day to monitor the rate of evaporation from the container. The average rate of evaporation was subtracted from water uptake and water loss.

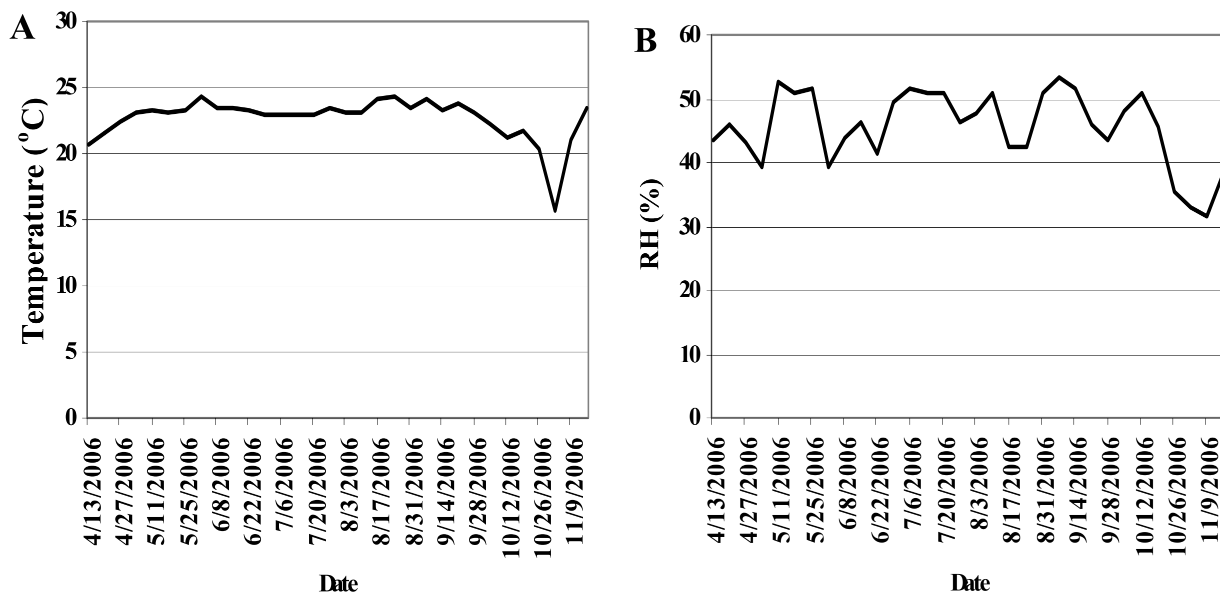

Fig 4.2. Weekly average (A) air temperature and (B) of relative humidity (RH) during 2006 postharvest evaluation inside the postharvest room at Burden Center, Baton Rouge, Louisiana.

4.2.5 Determination of Stem Bending

Stem bending in sunflowers during postharvest was measured at day of harvest, 3 and 7 days after harvest, and at senescence. The estimation of the angle between the main stem and the stem below the capitulum was recorded as a measurement of stem bending. A rating system for determining the degree of stem bending of cut sunflowers has been defined by Celikel and Reid (2002): 1 = slight bending up to 45°; 2 = moderate bending between 45° and 90°; and 3 = advance (downward) bending more than 90°.

4.2.6 Calcium Extraction

Calcium was extracted from the plant tissue by wet acid digestion (Mills and Jones, 1991). Harvested plants were divided into three tissue samples: leaves, stem and capitulum. Tissue samples were dried at 80°C for 24-h and ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass an 850 μm (20-mesh) screen. A ground tissue sample of 0.5 grams was placed in a digestion tube with 4 ml of concentrated nitric acid (HNO_3) and let stand overnight inside a fume hood at room temperature (25°C).

The digestion tubes were placed in a BD40 digestion block (Bran+Luebe, Germany) set at 120°C. The tubes were removed from the block after 1 h and allowed to cool; 4 ml of hydrogen peroxide (H_2O_2) was added to the digestion tubes. After addition of H_2O_2, the tubes were returned to the digestion block until the digest solution became colorless after 1 h and 30 min, approximately. The solution was transferred into a 10 ml volumetric flask, brought to volume, and filtered (Whatman #2 slow flow rate filter paper) into a 45 ml plastic vial. Nitric acid and hydrogen peroxide were added to six digestion tubes without ground tissue as blank samples. The blanks were used as controls for Ca contamination of the glassware.

4.2.7 Calcium Analysis

The concentration of Ca in the tissue of sunflowers was obtained by a colorimetric assay, Calcium L3K® Assay (Diagnostic Chemical Limited (DCL), Oxford,CT). This procedure uses a Ca complexing dye, Phosphonazo III, which forms a blue-purple color with a maximum absorption at 660 nm (Onishi, 1986). A 10 µl aliquot of tissue extract was added to a polycarbonate centrifuge tube plus 1 ml of the Phosphonazo III solution. The solution was mixed and left for 3 min at room temperature. This volume was transferred into disposable cuvettes, and the readings were recorded at an absorbance of 660 nm using a Perkin Elmer (Lamda-35) UV/VIS Spectrometer. A standard curve was prepared with 0, 50, 70, 90, 120, 150 mg/l of Ca using $CaCO_3$ for each set of samples measured. The stock solution was prepared following the procedure of Moorehead and Biggs (1974). To ensure that there was no Ca residue during Ca extraction and analysis, a set of six extractions were conducted without plant tissue. A zero absorbance was recorded for all six samples.

4.2.8 Statistical Analysis

The experimental design was a 5 by 3 factorial design (5 chemicals at 3 levels) and a control (DI water) with 10 experimental units per treatment combination. Untreated control sunflowers were included in all of the experiments. The means and standard errors were assessed for the control with the univariate procedure for comparison with the treatments. The postharvest parameters were tested with multiple regression and analysis of variance with MIXED procedure in SAS. Stem bending was not observed in this cultivar, with the number 2 rating being the common characteristic for treated and untreated flowers.

4.3 RESULTS

4.3.1 Pulse Experiments

This study was replicated twice. Experiment one was planted 9 May 2006 and experiment two was planted 27 July 2006. In experiment I, the source of Ca influenced sunflower postharvest longevity, whereas the rate of Ca had no significant effect on this postharvest attribute (Table 4.2). An interaction effect between the chemical and the concentration of the solution was significant. This interaction relates to the difference in postharvest longevity trend by treatment: in some treatments postharvest life increased with increased concentration i.e., $Ca(NO_3)_2$ and Ca chelate, whereas the highest chelate concentration decreased longevity by 1 d (Table 4.2).

Postharvest longevity was extended by 1 d in sunflowers treated with Ca chelate and $CaCl_2$ compared to sunflowers treated with $NaNO_3$ and compared to untreated sunflowers (Table 4.2; Fig 4.3). There were no significant differences, however, in postharvest longevity among sunflowers treated with pulse solutions containing Ca (Fig 4.3).

The total fresh weight increase, total water uptake, total water loss and the water loss/water uptake ratio after 8 days in postharvest were influenced by chemical treatment. The ratio was also affected by the concentration of the chemical (Table 4.3). Increased water loss/water uptake ratio was related to an increased concentration of each treatment, except for sunflowers treated with Ca chelate, where the response decreased with increased concentration. There were no significant differences in total fresh weight increase after 8 d in postharvest comparing treated sunflowers to untreated sunflowers, or when comparing sunflowers treated with Ca containing solution to sunflowers treated with the chelate or $NaNO_3$ (Fig. 4.4).

Table 4.2. Effect of a 2-h pulse application of chemical treatments on postharvest longevity of cut stems of *Helianthus annuus* L. 'Superior Sunset'. Experiment 1 planted 9 May 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Vase Life (Days)
Untreated (Control)[y]	0	11
$Ca(NO_3)_2$	125	11
	250	11
	500	12
$CaCl_2$	125	12
	250	11
	500	12
Calcium Chelate[z]	125	12
	250	12
	500	13
Chelate	125	12
	250	12
	500	10
$NaNO_3$	100	11
	200	11
	400	12
Chemical Treatment		*
Rate		NS
Interaction		*

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n = 10).
[Z] Chelate = 37 % blend of alcohol sugars.

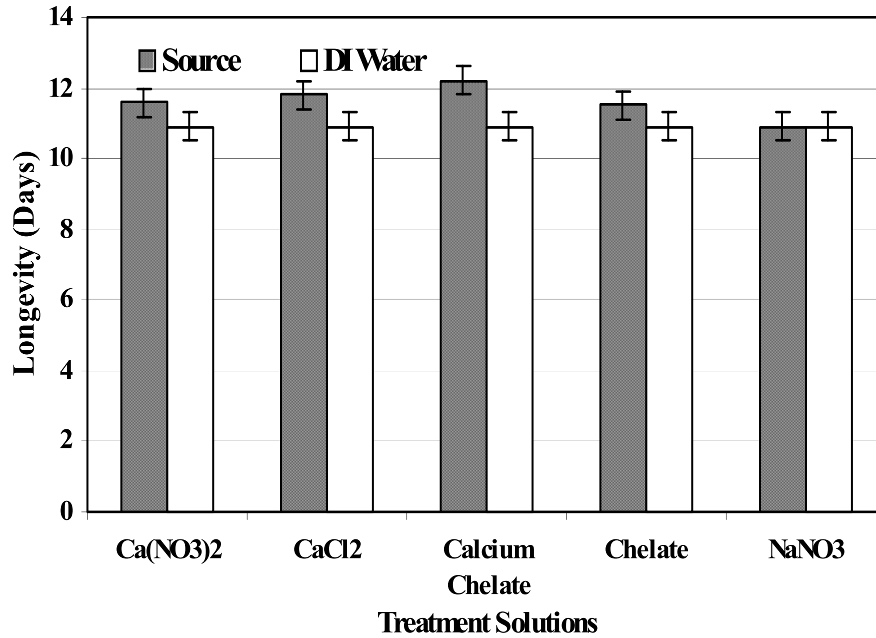

Fig 4.3. Effect of a 2-h pulse application of chemical treatments on postharvest longevity of cut stems of *Helianthus annuus* L. 'Superior Sunset'. Experiment I planted 9 May 2006. Vertical bars show the standard error for 10 replicates.

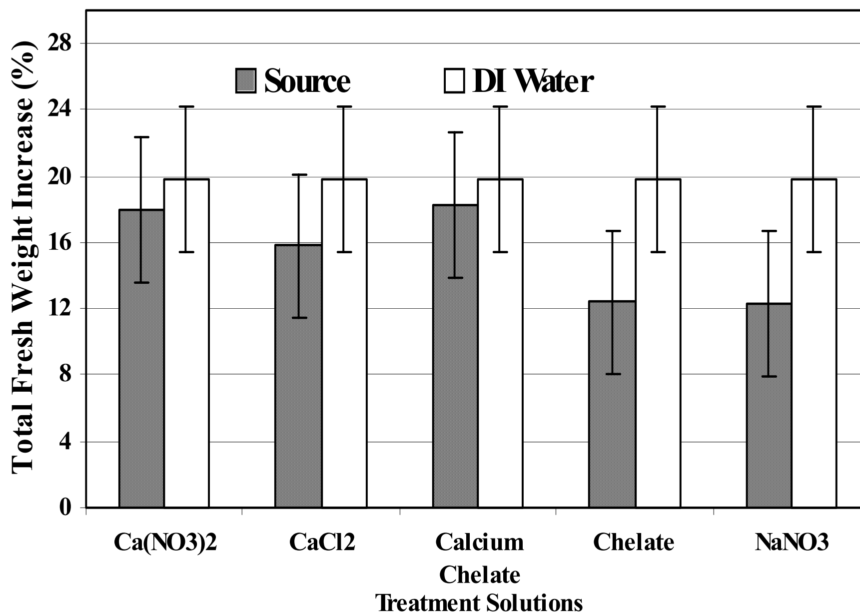

Fig 4.4. Effect of a 2-h pulse application of chemical treatments on postharvest fresh weight of cut stem of *Helianthus annuus* L. 'Superior Sunset'. Experiment I planted 9 May 2006. Vertical bars show the standard error for 10 replicates.

Table 4.3. Effect of a 2-h pulse application of calcium chemical treatments on postharvest total fresh weight (FW) increase and postharvest water dynamics of cut stem of *Helianthus annuus* L. ‘Superior Sunset’. Experiment I planted 9 May 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Total FW Increase (%)	Total Water Uptake[w] (g/flower)	Total Water Loss[w] (g/flower)	Ratio[x]
Untreated (Control)[y]	0	20.4	212.4	181.7	0.86
$Ca(NO_3)_2$	125	19.8	190.5	155.2	0.82
	250	16.9	189.4	158.4	0.83
	500	17.4	183.3	155.2	0.84
$CaCl_2$	125	17.6	196.2	165.3	0.84
	250	15.3	176.9	150.9	0.85
	500	14.6	181.9	157.7	0.87
Calcium Chelate[z]	125	17.5	205.4	175.5	0.85
	250	18.4	214.5	182.5	0.85
	500	18.9	214.6	181	0.84
Chelate	125	11.3	218.5	194.1	0.89
	250	13.7	224.4	198.1	0.88
	500	12.1	204.6	184.5	0.90
$NaNO_3$	100	13.2	197.0	164.3	0.87
	200	13.4	207.7	180.7	0.87
	400	10.2	199.7	164.2	0.88
Chemical Treatment		*	*	*	*
Rate		NS	NS	NS	*
Interaction		NS	NS	NS	NS

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey’s correction.
[W] Total water uptake and total water loss were recorded after 8 days in postharvest.
[X] Water loss/water uptake ratio.
[Y] Values in table are averages (n = 10).
[Z] Chelate = 37 % blend of alcohol sugars.

Sunflowers treated with Ca chelate (average 211 g/flower) and chelate (average 215.8 g/flower) showed greater total water uptake than sunflowers treated with $Ca(NO_3)_2$ (average

187.7g/flower) and $CaCl_2$ (average 185 g/flower), but not greater than sunflowers treated with $NaNO_3$ (average 201.5 g/flower) or untreated sunflowers (Fig 4.5A). Sunflowers treated with $Ca(NO_3)_2$ and $CaCl_2$ also had a lower water uptake than sunflower treated with $NaNO_3$ and untreated sunflowers (Fig 4.5A). The total water loss of sunflowers treated with $Ca(NO_3)_2$ and $CaCl_2$ was significantly lower than the water loss of sunflowers treated with Ca chelate, chelate, $NaNO_3$ and untreated sunflowers (Fig 4.5B). The water loss/water uptake ratio of sunflowers treated with Ca containing solutions (average 0.84) was lower than the ratio in sunflowers treated with the chelate (average 0.89) and $NaNO_3$ (average 0.87) (Fig.4.5C). Sunflowers treated with chelate and $NaNO_3$ had a greater water loss/water uptake ratio than untreated sunflowers, whereas the sunflowers treated with $Ca(NO_3)_2$ had a lower ratio than untreated sunflowers (Fig 4.5C).

For experiment I, the tissue analysis indicated that the chemical treatment and the type of tissue sampled had an effect on the concentration of Ca in the cut sunflowers (Table 4.4). There was an interaction effect between chemical treatment and tissue; and there was also an interaction between chemical treatment, rate and tissue (Table 4.4). Capitulum and stem tissue of treated sunflowers had a higher concentration of Ca than untreated sunflower tissue (Fig 4.6A). Sunflowers treated with $Ca(NO_3)_2$ had the highest concentration of Ca in capitulum tissue compared to the other treatments and to untreated sunflowers (Fig 4.6B). The concentration of Ca in the capitulum tissue of sunflowers treated with Ca chelate was lower than the other treatments, but not lower than untreated sunflowers (Fig 4.6B).The concentration of Ca in stem tissue of sunflowers treated with Ca containing solution was not significantly different than chelate and $NaNO_3$ treatments, but it was significantly higher than the concentration of Ca in untreated sunflowers.

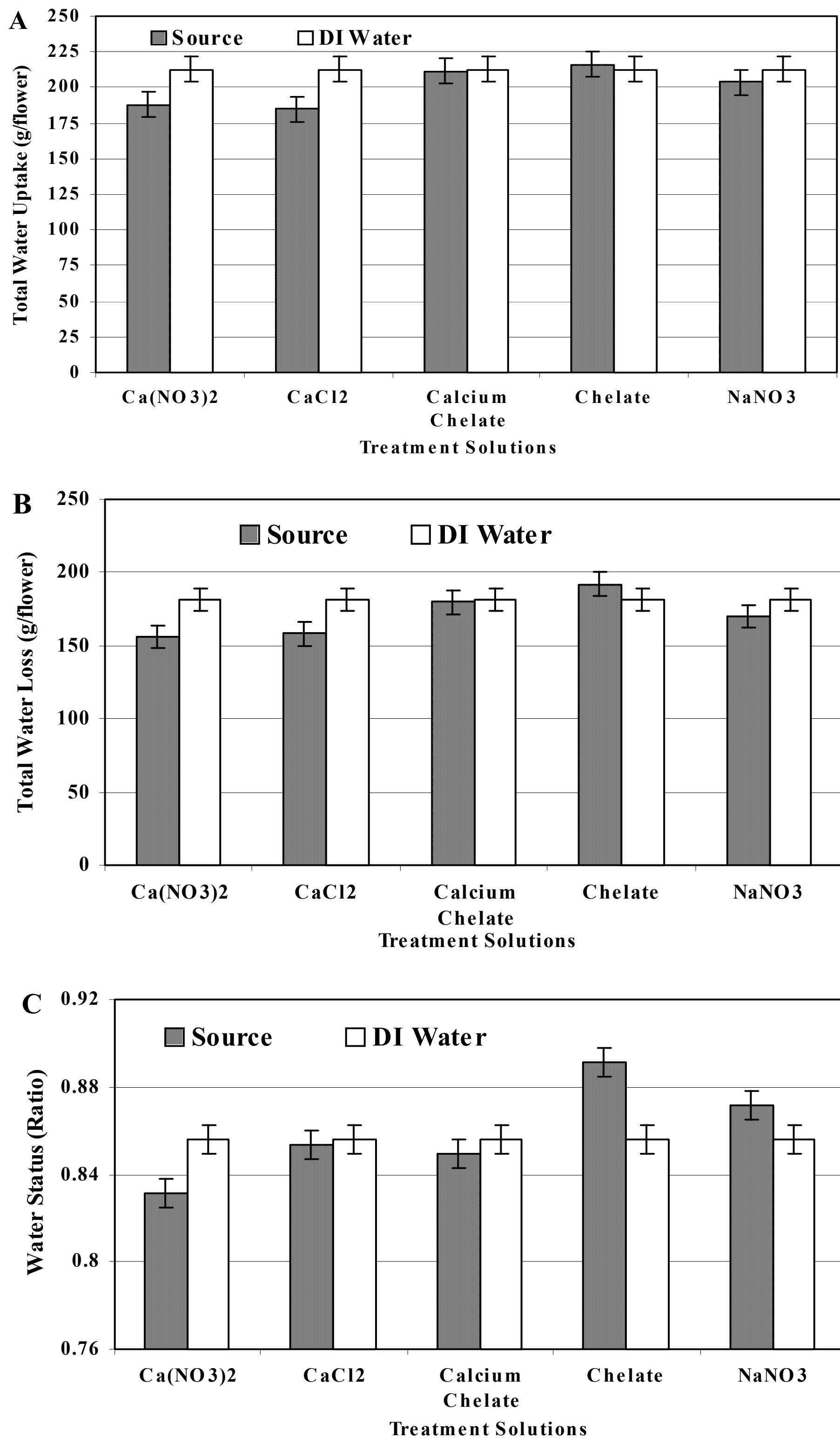

Fig 4.5. Effect of a 2-h pulse application of chemical treatments on postharvest water dynamics of *Helianthus annuus* L. 'Superior Sunset'. (A) Total water uptake; (B) total water loss; (C) water loss/water uptake ratio. Experiment I planted 9 May 2006. Vertical bars show the standard error for 10 replicates.

Table 4.4. Effect of a 2-h pulse application of chemical treatments on Ca concentration of Capitulum and Stem tissue of cut stem of *Helianthus annuus* L. 'Superior Sunset'. Experiment I planted 9 May 2006.

Chemical Treatment	Ca or N Rate	Ca Concentration (µg/g)	
	(mg/l)	Stem	Capitulum
Untreated (Control)[y]	0	8720	12174
$Ca(NO_3)_2$	125	11533	18283
	250	10326	23814
	500	10283	19219
$CaCl_2$	125	10258	18318
	250	9565	11054
	500	10841	15489
Calcium Chelate[z]	125	9641	13230
	250	10047	14011
	500	10419	13503
Chelate	125	10026	12944
	250	8687	15127
	500	9508	16604
$NaNO_3$	100	9351	16219
	200	10178	17370
	400	9552	13833
Chemical Treatment (C)		*	*
Rate (R)		NS	NS
Sample (Sa)		*	*
Interaction C*Sa		*	*
Interaction R*Sa		NS	NS
Interaction C*R*Sa		*	*

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n =10).
[Z] Chelate = 37 % blend of alcohol sugars.

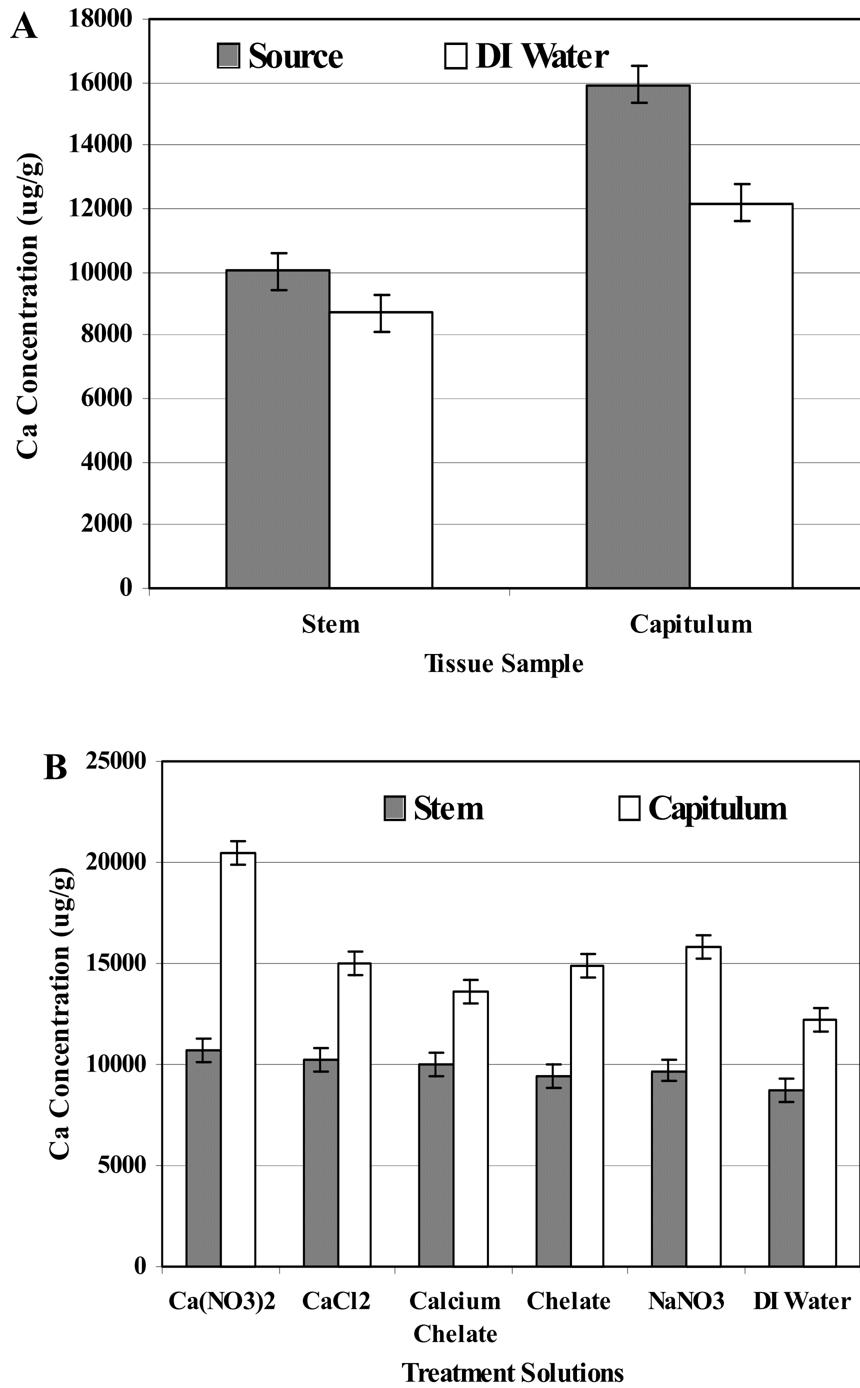

Fig 4.6. Effect of a 2-h pulse application of chemical treatments on Ca concentration of capitulum and stem tissue of cut stems of *Helianthus annuus* L. 'Superior Sunset'. (A) Ca concentration by tissue sample; (B) Ca concentration by chemical and tissue sample. Experiment I planted 9 May 2006. Vertical bars show the standard error for 10 replicates.

In experiment two postharvest attributes, longevity, total fresh weight increase, water loss/water uptake ration were influenced by the chemical treatment (Table 4.5; Table 4.7) Treatment effects on total water uptake and total water loss were not significant (Table 4.7). Sunflower postharvest longevity was extended by 1 d in sunflowers treated with Ca chelate compared to flowers treated with chelate and by up to 2 days compared to sunflowers treated with $Ca(NO_3)_2$ and untreated sunflowers (Table 4.5; Fig 4.7).

Table 4.5. Effect of a 2-h pulse application of chemical treatments on postharvest longevity of cut stems of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 27 July 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Longevity (Days)
Untreated (Control)[y]	0	10
$Ca(NO_3)_2$	125	11
	250	10
	500	10
Calcium Chelate[z]	125	12
	250	12
	500	12
Chelate	125	11
	250	11
	500	11
Chemical Treatment		*
Rate		NS
Interaction		NS

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n =10)
[Z] Chelate = 37 % blend of alcohol sugars.

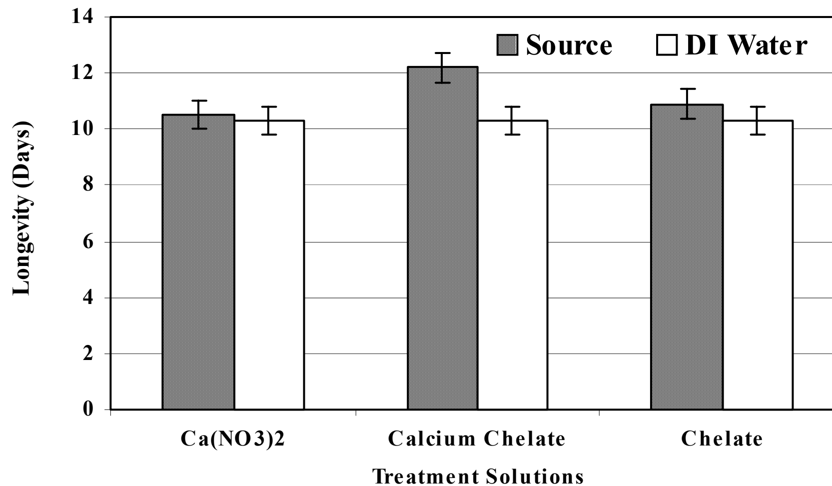

Fig 4.7. Effect of a 2-h pulse application of chemical treatments on postharvest longevity of cut stems of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 27 July 2006. Vertical bars show the standard error for 10 replicates.

Table 4.6. Effect of a 2-h pulse application of chemical treatments on postharvest total fresh weight (FW) increase and postharvest water dynamics of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 27 July 2006.

Chemical Treatment	Ca or N Rate (mg/l)	Total FW Increase (%)	Total Water Uptake[w] (g/flower)	Total Water Loss[w] (g/flower)	Ratio[x]
Untreated (Control)[y]	0	10.6	211.3	193.3	0.92
	125	18	214.9	185.5	0.86
	250	17.8	226.9	194	0.86
$Ca(NO_3)_2$	500	17.4	234.5	202.7	0.86
	125	22.1	221.6	184.2	0.83
Calcium	250	21.3	227	188.6	0.83
Chelate[z]	500	17.9	216	184.6	0.86
	125	16.7	221.8	187.6	0.85
	250	18.9	210	176.7	0.84
Chelate	500	18.4	207.5	177.8	0.86
Chemical Treatment		*	*	*	*
Rate		NS	NS	NS	*
Interaction		NS	NS	NS	NS

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[W] Total water uptake and total water loss were recorded after 8 days in postharvest
[X] Water loss/water uptake ratio
[Y] Values in table are averages (n =10)
[Z] Chelate = 37 % blend of alcohol sugars.

After 8 days in postharvest, sunflowers treated with the Ca chelate showed a greater fresh weight increase over sunflowers treated with the chelate, $Ca(NO_3)_2$ or untreated control (Fig 4.8A). Water loss/water uptake ratio was lower in sunflowers treated with Ca chelate (average 0.84) compared to those treated with $Ca(NO_3)_2$ (average 0.86) or untreated control sunflowers (average 0.92) (Fig 4.8B). The ratio and the total fresh weight increase were not affected by concentration (Table 4.6).

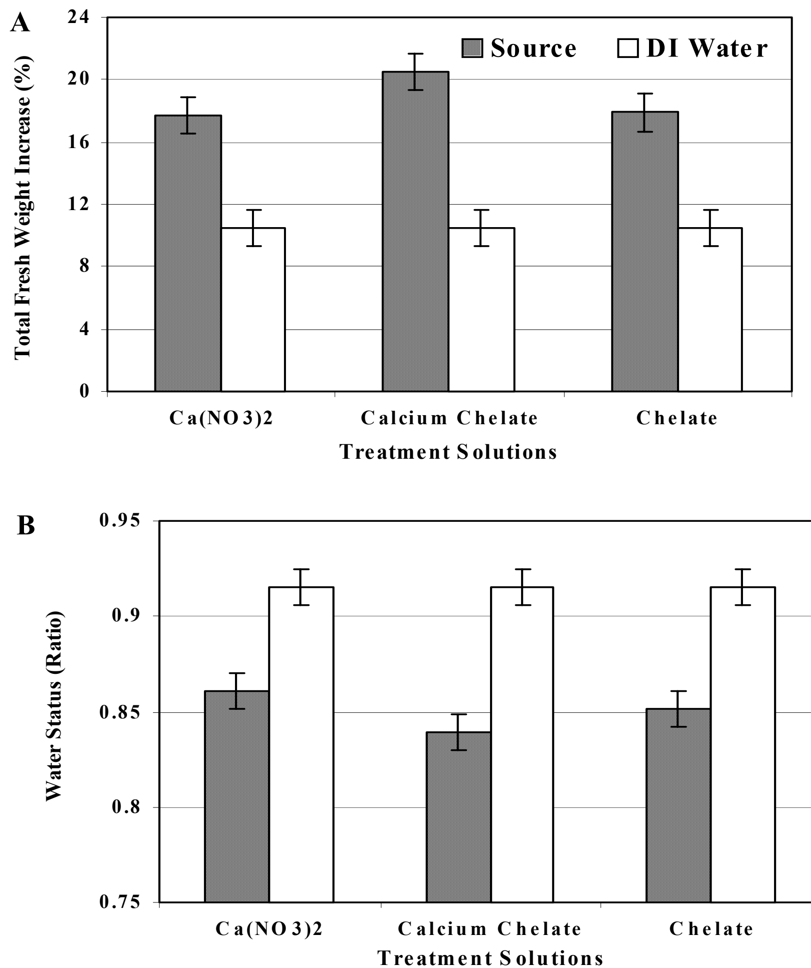

Fig 4.8. Effect of a 2-h pulse application of chemical treatments on: (A) total fresh weight increase and (B) water loss/water uptake ratio of cut stems of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 27 July 2006. Vertical bars show the standard error for 10 replicates.

Tissue analysis for experiment two showed that the treatment did not influence Ca concentration in the capitulum, stem or neck (Table 4.7). Calcium concentration was affected by

the type of tissue sampled; the capitulum and the neck of the cut flower had the highest concentration of Ca in the cut sunflower (Fig 4.9A).There was a significant increase in Ca concentration in the tissue of treated plants compared to untreated plants, but this increase was not significant among chemical treatments (Fig 4.9B; Table 4.7).

Table 4.7. Effect of a 2-h pulse application of chemical treatments on concentration of Ca in cut stem of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 27 July 2006.

Chemical Treatment	Ca or N	Ca Concentration (µg/g)		
	Rate (mg/l)	Neck	Stem	Capitulum
Untreated (Control)[y]	0	9787	7134	10610
$Ca(NO_3)_2$	125	12688	8618	14068
	250	12188	9565	14751
	500	11860	10497	15077
Calcium Chelate[z]	125	9606	10059	12795
	250	11890	10806	13593
	500	12382	9576	15644
Chelate	125	12884	12106	14781
	250	11132	9689	14303
	500	13319	9764	15003
Chemical Treatment (So)		NS	NS	NS
Rate (R)		NS	NS	NS
Sample (Sa)		*	*	*
Interaction So*Sa		NS	NS	NS
Interaction R*Sa		NS	NS	NS
Interaction So*R*Sa		NS	NS	NS

Values significant (*) or not significant (NS) at 5% by lsmean procedure in SAS with Tukey's correction.
[Y] Values in table are averages (n =10)
[Z] Chelate = 37 % blend of alcohol sugars.

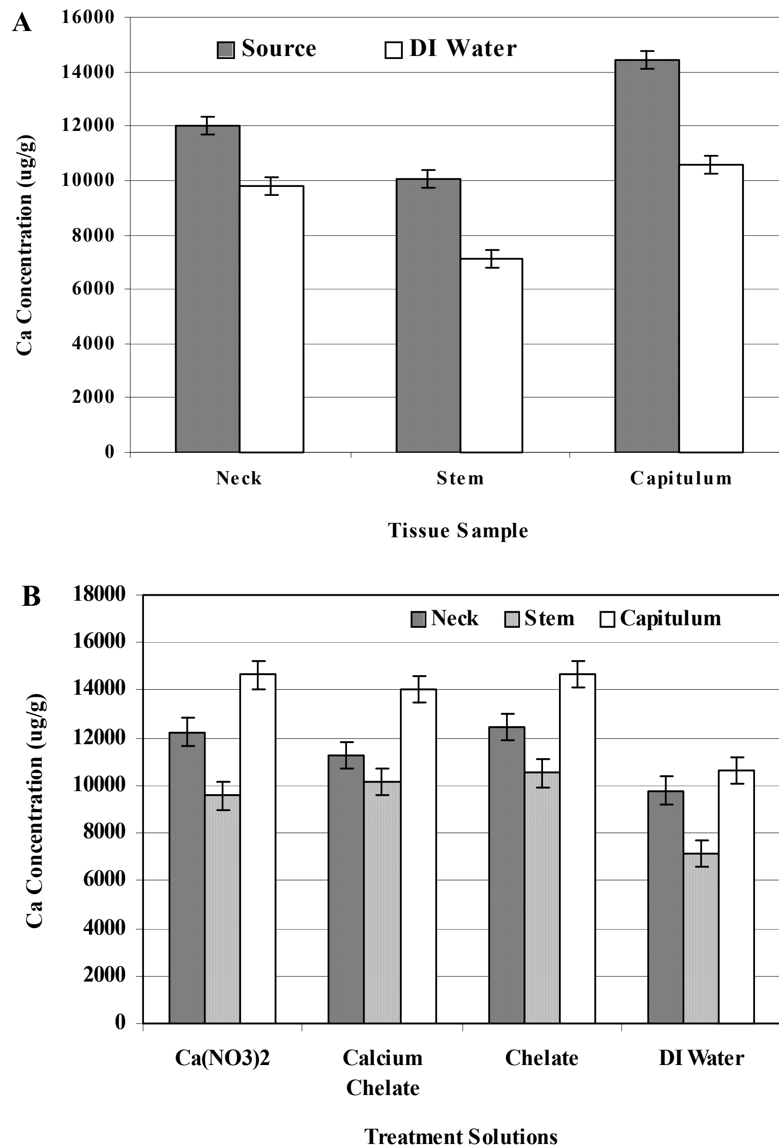

Fig 4.9. Effect of a 2-h pulse application chemical treatments on concentration of Ca of cut stems of *Helianthus annuus* L. 'Superior Sunset'. Experiment II planted 27 July 2006. Vertical bars show the standard error for 10 replicates.

4.4 DISCUSSION

The use of supplemental Ca in a pulse solution to extend postharvest longevity of sunflower concurs with similar results obtained in other plant species: *Dianthus* (Mayak et al., 1978), gerbera (Gerasopoulos and Chebli, 1999), and rose (Michalczuck et al., 1989; Torre et al., 1999). The results of this research showed that sunflowers treated with Ca chelated or $CaCl_2$

extended postharvest longevity by 1 d compared to untreated sunflowers in experiment I. In experiment II, fresh cut sunflowers treated with Ca chelate had a 2 d increase in postharvest longevity compared to sunflowers treated with $Ca(NO_3)_2$ and untreated sunflowers. The concentration of Ca in the treatment solution, however, did not significantly affect longevity of cut sunflower.

Water balance is defined by the relationship between water uptake and transport, and water loss and retention (Halevy and Mayak, 1981). Enhanced water balance is coupled to longer lasting flowers (Mayak et al., 1978). Inorganic salts and sugars have been shown to improve water absorption of cut flowers by positively affecting water balance (Mayak et al., 1974; Halevy, 1976). Water deficit in plant tissue has been found to be caused by increased water loss over water uptake or by a decreased water uptake even though water loss may remain constant (Burdett, 1970; Mayak et al., 1974). Water deficit in fresh cut flowers has direct effects on loss of turgor and accelerates senescence (Halevy et al., 1974; Mayak et al., 1974). Torre et al. (1999) suggested that calcium-delayed senescence may be related to protection of membrane proteins and phospholipids, thus preserving cell membrane integrity and maintaining a better water balance in fresh cut flowers. The total quantity of water uptake and water loss was greater in sunflowers treated with Ca chelate than in sunflowers treated with $Ca(NO_3)_2$ or $CaCl_2$; however, a significant decrease in water loss/water uptake ratio indicated improved water movement and retention in sunflowers treated with Ca containing solutions in experiment I. Chelate and $NaNO_3$ treated sunflowers had higher water uptake and water loss than Ca containing treatment solutions; conversely, the ratio of sunflower treated with chelate and $NaNO_3$ was significantly higher, which may imply an imbalance in water uptake and water loss. Water loss/water uptake ratio also decreased in sunflowers treated with Ca in experiment II. Water deficit may also be

correlated to decreased fresh weight of fresh cut flowers (Halevy et al., 1974; Mayak et al., 1974). Although in experiment I there was not a significant increase in fresh weight in sunflowers treated with Ca, there was a fresh weight increase in sunflowers pulse treated with Ca in experiment II.

Results from the tissue analysis in experiment I showed that Ca concentration was affected by chemical treatment and by the tissue type. Capitulum tissue had a greater Ca concentration in flowers treated with $Ca(NO_3)_2$ than those from other treatments or untreated flowers. Sunflowers treated with Ca chelate had lower Ca concentrations than the other treatments, but not lower than untreated sunflowers. Sunflowers treated with Ca containing solutions showed an increased Ca concentration in stem tissue significantly higher than untreated sunflowers; however, this was not true when compared to treatments without Ca. For experiment II, the results indicated that treatments did not have an effect on concentration, but that the response was influenced by the tissue type. The upper part of the flower, comprised of the neck and capitulum, had a greater concentration of Ca than the stem. These results may suggest that Ca was concentrated in the senescing organs of the cut flower. Although sunflower tissue pulse treated with Ca chelate did not have the highest concentration of Ca compared to treatments with $Ca(NO_3)_2$ or $NaNO_3$, these flowers had more Ca than untreated flowers, an improved water balance and a longer postharvest longevity.

Postharvest application of Ca is a viable option for cut sunflowers, because they are not packaged in the field. Cut sunflowers need to be taken to a postharvest area where they are classified, foliage is removed and they are re-cut. During or after these processes, cut sunflowers may also be treated with Ca. This treatment may easily be applied by small specialty cut flower growers; however, the cost of the treatment should be taken into consideration before integrating

the practice into the specialty cut flower production. Further research may be focused the postharvest physiological changes in the cell membrane of cut sunflowers. Measurements of respiration, ethylene production, electrolyte leakage from cell may help determine the mode of action of Ca in the sunflower cells.

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CHAPTER 5. SUMMARY AND CONCLUSIONS

The sunflower is one of the most important specialty cut flowers produced. Sunflowers may have a short and variable postharvest longevity which is cultivar dependent. Research in postharvest physiology of fresh cut flowers indicates that calcium (Ca) may be involved in delaying flower senescence by postponing cell membrane degradation. Cut flowers with intact cell membranes, structure and function, maintain their water balance and last longer. This research was developed to determine the effects of pre- and postharvest Ca supplementation on longevity of fresh cut sunflower. The cultivar 'Superior Sunset' was used in this study; the sources of Ca were $Ca(NO_3)_2$, $CaCl_2$ or a chelated Ca. The chelate minus Ca and sodium nitrate ($NaNO_3$) were used as control treatments. Preharvest treatments included two foliar spray or weekly drench, whereas the postharvest treatment was a 2-h pulse. Untreated flowers were included in all the experiments, means and standard errors were calculated for comparison with treatments.

Preharvest supplementation of Ca with chemical sources and rates utilized in these experiments did not increase postharvest longevity of cut sunflower. In support of this result tissue analysis indicated that Ca concentrations in leaves, stems and capitulum were unaffected by the spray treatment when compared to untreated plants. Thus supplemental Ca in the forms and rates applied was not absorbed by sunflower and therefore postharvest longevity was unaffected. More than two spray applications may be needed for Ca to be absorbed by sunflower leaves and translocated to the capitulum. Calcium concentrations in the drench experiment increased in the stem tissue but Ca did not increase in the capitulum which is the senescing organ in the cut sunflower. Thus, although Ca absorption was increased when Ca was applied as a

drench, it was not translocated in sufficient amounts to help delay senescence and increase postharvest longevity.

The Ca sufficiency concentration for sunflower in leaf tissue ranges from 1.5 to 3 % (1,500 to 3,000 ug/g). Tissue analysis revealed that the concentration of Ca in the leaf of sunflowers in the preharvest experiments was in that range, average18707 µg/g for the foliar spray experiment and 27955 µg/g for the drench experiment.

The extension of sunflower longevity using supplemental Ca in a pulse solution for both pulse experiments concurs with results obtained in other plant species, such as *Dianthus,* gerbera and rose. The results of the pulse experiments showed that sunflowers treated with Ca chelate or $CaCl_2$ extended postharvest longevity by 1 d compared to untreated sunflowers in experiment I. In experiment II, sunflowers treated with Ca chelate had an extended postharvest longevity of 2 d compared to sunflowers treated with $Ca(NO_3)_2$ and untreated sunflowers. The concentration of Ca in treatment solutions was not found to have a significant effect on longevity of cut sunflower.

Although Ca chelate extended postharvest longevity the tissue of sunflowers treated with Ca chelate did not have the highest concentration of Ca compared to $Ca(NO_3)_2$ or even $NaNO_3$; Sunflowers treated with Ca chelate, however, had more Ca than untreated flowers. Sunflowers treated with Ca chelate did show an improved water balance which has been correlated with extended postharvest longevity of other fresh cut flowers.

Postharvest application of Ca may a viable option for cut sunflowers as many cut flowers are treated with a pulse solution soon after harvest. Current recommendations suggest that fresh cut sunflowers be taken to a postharvest area where they are graded, the foliage is removed and stems re-cut. After this processes cut sunflowers may also be pulse treated with Ca. This

treatment may easily be applied by small specialty cut flower growers or larger wholesale operations; however the cost of the treatment should be taken into consideration before integrating the practice into specialty cut flower production.

Further research should focus on the effects of preharvest environmental conditions on sunflower postharvest longevity. The lack of Ca uptake or movement into the capitulum in the drench experiments suggest the higher rates of Ca in the solution could also be tested. Increased number of spray applications of Ca treatments utilized in the foliar spray experiment should be looked into, as well as the duration of the pulse for the postharvest treatment. Postharvest physiological changes in the cell membrane of cut sunflowers may offer more information on the effects obtained in the pulse experiments with Ca chelate. Measurements of respiration, ethylene production, electrolyte leakage from cells may help determine the mode of action of Ca in the sunflower cells.

VITA

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