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I am submitting herewith a thesis written by Lori Denise Calhoun entitled "The influence of calcium nutrition on grey mold rot caused by *Botrytis cinerea* Pers. in three tomato cultivars". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

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**THE INFLUENCE OF CALCIUM NUTRITION ON
GREY MOLD ROT
CAUSED BY *BOTRYTIS CINEREA* PERS.
IN THREE TOMATO CULTIVARS**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Lori Denise Calhoun
May 1997

DEDICATION

This thesis is dedicated to my daughter,

Brittany Anne.

Because of her trust, support, patience and understanding

I found the determination to not only finish the race,

but also to win.

I love her and thank her from the bottom of my heart.

ACKNOWLEDGMENTS

There are many people to whom I owe a debt of gratitude for supporting and guiding me through my Master's degree here at the University of Tennessee. Thanks goes first and foremost to my major professor Carl Sams whose teaching and confidence in me contributed to my academic growth.

I thank Dr. Dennis Deyton and Dr. Bob Trigiano for serving on my committee. They made themselves available whenever I needed them and their help was invaluable.

Great appreciation goes to Dr. Foss, Dr. Allen and the Department of Plant and Soil Science for providing my assistantship, without which I would have never been able to attend graduate school.

Special thanks goes to my wonderful lab colleagues, Rebecca, John, Bob, Syamsoel, Barbara, Craig, Catherine, Renae, and Bhaskar. They all helped in so many ways and kept me laughing when the going got tough and the days got long. Each of their unique personalities contributed a great wealth to my experiences in the personal, as well as the graduate student realm. Thanks also to Cindy Stiles for running tons of samples on the ICAP.

Deep, heartfelt thanks goes to Rodney and Brittany, my husband and daughter, for an infinite number of things. But especially for putting up with the late nights, waking me up in the mornings, and fixing me coffee. Their patience, love and support never, ever wavered.

I would also like to thank my parents Michael and Jackie for their love and encouragement. Big thanks to Jawanna and Joressia, my best friends for their tireless support.

Finally, I am forever thankful to God. Without my faith in Him, I never would have been able to see the light at the end of the tunnel. He truly gave me the strength to finish what I started.

Because of everyone, I have learned more about myself, and my future will never be the same. I thank you all!

ABSTRACT

Due to the declining availability of fungicides there is a need to investigate alternative methods of disease control for use in commercial tomato production. Several theories of disease resistance are associated with an increase in plant tissue calcium (Ca) content which has been shown to increase resistance of tomato seedlings to bacterial wilt and other diseases. *Botrytis cinerea* Pers. is the major postharvest pathogen of tomatoes, causing losses of tomato fruit in storage. These experiments were conducted to determine if Ca nutrition influences resistance to fungal decay of tomato fruit caused by *B. cinerea* in commercial tomato cultivars. Two greenhouse tomato experiments containing 54 plants each were performed, one in the fall of 1995 and the other in the spring of 1996. Three tomato cultivars, 'Mountain Supreme', 'Sunrise' and 'Celebrity', were grown in a hydroponic system with perlite as a root support medium. Calcium treatments of 20 mg L⁻¹, 220 mg L⁻¹, or 1020 mg L⁻¹, were added to a commercial fertilizer solution which had an N-P-K ratio of 1.0:0.6:2.5 and contained essential micronutrients. A 3x3 factorial with three tomato cultivars and three Ca treatments in a randomized complete block design was utilized. The mineral content of leaves and fruit was determined by inductively coupled plasma emission spectrometry (ICP). Harvested fruit were inoculated with a 5x10⁵ spore ml⁻¹ conidial suspension of *B. cinerea* and the decay lesion diameter measured once daily for six (Spring 1996) or seven (Fall 1995) days.

Across cultivars, leaf Ca content significantly increased as the Ca treatments increased from low to medium ($P < 0.001$) and medium to high ($P < 0.001$; Spring only). Fruit tissue Ca content was significantly increased across cultivars as the Ca treatments

increased from low to medium ($P<0.001$) and medium to high ($P<0.07$; Spring only). In both the fall and spring experiments, the high calcium treatment significantly ($P<0.01$ and $P<0.04$, respectively) reduced the area of decay caused by *Botrytis cinerea* across all cultivars. The medium Ca treatment reduced the area of decay ($P<0.01$) in the fall. In the spring, there was a significant difference in the decay area ($P<0.01$) between the medium and high Ca treatments. There were a few significant cultivar differences for all three variables (leaf Ca, fruit Ca, and decay area) within each treatment level, but those differences were not consistent within or among the two experiments. Significant treatment x cultivar interactions were observed in fruit Ca content. Leaf and fruit tissue Ca concentrations were positively associated with resistance of greenhouse-grown tomatoes to gray mold rot in both experiments, strengthening the hypothesized role of Ca in promoting disease resistance.

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CHAPTER I

INTRODUCTION

STATEMENT OF THE PROBLEM

Greenhouse tomatoes are an economically important crop and it is the goal of growers to produce a high quality “ideal” tomato at a profit for their investment. What is the “ideal” tomato? Generally, it is one that is firm, colorful, nutritious, uniform in appearance, and without blemish. Depending upon the distance to market, the tomato should have a reasonably long shelf life and be flavorful to ensure consumer acceptance (Yoder, 1993). It should also be resistant to diseases encountered in commercial tomato production and postharvest storage. However, cultivar differences in disease resistance can make this difficult. Those cultivars genetically resistant to one disease can be susceptible to other diseases (Jarvis 1980). Resistance may also fluctuate due to varietal susceptibility to environmental factors (Dhingra, 1995; Hayward, 1991). Because of population variability, pathogenic plant fungi can usually accommodate the changing host (Agrios, 1988), requiring costly breeding programs to keep up with the demand for new and improved cultivars.

Currently, fungicides are used to counter the effects of susceptibility to diseases not controlled by genetic resistance. However, the use of fungicides in commercial production is declining due to health risk concerns by the public (Wilson *et al.*, 1993) and bans imposed by the United States Environmental Protection Agency (USEPA)

(Liu, 1993). In some cases, fungicides have been voluntarily removed because the profits are not high enough to justify the cost of re-registration with the USEPA (Wilson *et al.*, 1991). Even so, there are few effective fungicides for controlling *B. cinerea*, which is capable of quickly developing resistance to many types of fungicides (Coley-Smith, 1980; Elad, 1996; Yunis *et al.*, 1991). Alternative methods of disease control need to be investigated since fungal diseases are responsible for up to 50% of postharvest losses, (Dennis, 1983; Flaishman *et al.*, 1994; Wilson, 1991).

Botrytis cinerea Pers., which causes grey mold rot, is responsible for serious losses of tomato crops, especially in greenhouse production (Coley-Smith, 1980; Dennis, 1983; Yunis *et al.*, 1991). The fungus can infect the leaves, stems, flowers, and fruit of tomato plants. Infected flowers may either abort or provide inoculum for the developing fruit, causing decay after harvest. Postharvest losses are devastating due to the time and money invested in the crop by harvest time, making this an even more serious problem.

RATIONALE

Nutritional status can play a role in quality and susceptibility to disease of fruits and vegetables (Kader, 1986; Thompson, 1996). Calcium, a divalent cation, is an essential nutrient required by all plants. This mineral plays a role in maintaining cellular membrane integrity. In general, a balance between calcium and other major cations is essential for overall nutrition of the plant (Epstein, 1961).

Calcium is an important part of the cell wall structure since it is involved in cross-linkage of pectic molecules. An estimated 60% of the total calcium in plants is associated with the cell wall (Demarty *et al.*, 1984; Rossignol *et al.*, 1977). Calcium appears to function in the cell wall by reacting with the free carboxyl groups of polygalacturonic acid molecules forming an “egg-box” structure between the chains, increasing the intercellular bond strength, thus increasing tissue resistance to destructive forces (Burns and Pressey, 1987; Conway *et al.*, 1988; Demarty *et al.*, 1984; Fry, 1986; Grant *et al.*, 1973; Glenn and Poovaiah, 1989). Hydrolysis of pectin was promoted by calcium-chelating agents such as EDTA or citrate (Bangerth *et al.*, 1972). Jarvis (1982) extracted calcium from tomato fruit tissue and subsequent pectin extraction showed 92% of the uronic acid polymers were water-soluble in the absence of calcium ions.

Increasing calcium in plant tissue can reduce the incidence and severity of both bacterial and fungal diseases (Conway, 1982; Conway and Sams, 1984; Conway *et al.*, 1987; 1988; 1991; 1994; Elad and Volpin, 1993; Huber, 1991; Lavy-Meir *et al.*, 1989; Li and Gabelman, 1990; McGuire and Kelman, 1984; Yamazaki and Hoshina, 1995). For example, increased tissue-calcium has been shown to increase resistance of greenhouse tomato seedlings to bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith (Yamazaki and Hoshina, 1995), a serious disease in tropical and subtropical regions (Hayward, 1991). In a follow-up study by Yamazaki *et al.* (1996), twenty three tomato cultivars with disease resistance to bacterial wilt contained a significantly higher calcium concentration in leaves and stems than susceptible cultivars.

Although the exact mechanism(s) of calcium in disease resistance remains unknown, some theories include pectolytic enzyme inhibition by calcium (Corden, 1965), cell wall strengthening by calcium (giving increased resistance to pectolytic enzymes) (Bateman and Lumsden, 1965; Conway *et al.*, 1988), tolerance to pathogens (Yamazaki and Hoshina, 1995; Bowman and Sequeira, 1982; Grimault and Prior, 1993), and suppression of pathogen multiplication (Yamazaki and Hoshina, 1995).

The beneficial effects of calcium-increased disease resistance might possibly be enhanced. Selecting for disease resistance by choosing those cultivars that contain more calcium by way of greater uptake and partitioning efficiency (Li and Gabelman, 1990; Yamazaki *et al.*, 1996). Optimizing the calcium content in plant tissues and thus improving plant nutrition may be a feasible solution to decreasing postharvest crop losses.

OBJECTIVES OF THIS STUDY

The objectives of this research were to investigate the effects of different calcium levels (in fertilizer solution) and the variation in cultivars on disease resistance of three greenhouse-grown tomato cultivars to grey mold rot caused by *B. cinerea*.

CHAPTER II

LITERATURE REVIEW

PRODUCTION SYSTEMS

Tomato production systems, whether greenhouse or field, can widely vary. The choice of a particular system depends upon the cultivar grown, time of year, and size of the grower's operation (Rice, 1990; Atherton and Rudich, 1986). Distance to market, and shipping outlet availability are other factors that influence the choice of a production system. Rice (1990) found that producers view tomatoes as having high income potential. Whether field-grown or greenhouse-grown, tomatoes are an important economic crop, worldwide.

Although the climate in Tennessee is ideal for field tomato production, greenhouse tomatoes are becoming increasingly more popular because they can provide fresh fruit throughout the year. Hydroponics, growing plants without soil, is a greenhouse method used by many tomato growers (Brooks, 1973; Hale and Margham, 1991; Wittwer and Honma, 1979). Dr. W.F. Gericke developed the term "hydroponics" when he found that plants could be grown to maturity without soil, on a semi-commercial basis (Ellis *et al.*, 1974).

Hydroponic nutrient solutions are applied with either closed or open systems. In a closed system, the nutrient solution is recycled, but requires more upkeep than an open system because of leakage prevention and maintenance of continuous-use motorized pumps. Nitrogen, phosphorus, sulfur, potassium, calcium and magnesium salt usage must

be kept at lower concentrations to avoid build-up and subsequent nutrient toxicity to the plants (Ellis *et al.*, 1974). Open systems do not recycle the nutrient solution and the greatest advantage is that excess salts and nutrients are flushed out. Nutrient solutions are delivered via injection pumps connected to programmable time clocks, enabling the grower to easily specify the watering regime to suit the needs of the crop (Ellis *et al.*, 1974). Some inert plant support substrates available for use with hydroponic systems include perlite, rock wool, sand, crushed rock, and styrofoam.

There are many advantages to using hydroponics in greenhouse tomato production. The major benefit is the free-draining nature of the medium and ease of water management (Chambers, 1990). The need for suitable soil and costly labor for field cultivation is greatly reduced. Due to control over water and nutrient usage, yields can be maximized (Ellis *et al.*, 1974). When plants are grown with an inert support medium, the variability due to soil nutrient and ion interactions is practically eliminated. This enables the grower to accurately test and adjust the nutrient quantity and chemical composition of the solution to suit the changing needs of the crop (Atherton and Rudich, 1986; Brooks, 1973; Hale and Margham, 1991; Steiner, 1961). This method is especially useful in research. The disadvantages of hydroponic systems include a high initial investment, the ability of diseases and nematodes to spread rapidly, and the need for extra care in monitoring and knowledge of plant nutrition (Ellis *et al.*, 1974).

FRUIT QUALITY

Certainly yield must reach a satisfactory level in order to be profitable for the grower, but quality guidelines set by consumer demand determine the final sale. Fruit characteristics such as flavor, aroma, visual appeal, high nutritional value, and a low price seem to be the most important to the consumer (Yoder, 1993). Additional high-quality characteristics of tomato fruit include convenient size, firm consistency, balanced and uniform shape, and freedom from defects and decay.

Fruit quality is dependent upon a combination of several factors. For instance, the plant and fruit condition at harvest, the choice of cultivar, and the compositional changes and storage conditions that occur during the postharvest stage all combine and contribute to fruit quality (Kader, 1986; Thompson, 1996; Watada and Aulenbach, 1979). Ripeness stage at harvest also affects fruit quality and consumer acceptance. Tomatoes are often harvested at the mature green stage, just prior to the onset of color. Picking at this stage usually ensures the tomato will continue to ripen and mature on its own, and also gives the grower and shipper more time to store and ship the fruit before they become overripe. Those cultivars that maintain good firmness beyond the table-ripe stage permit picking at more advanced ripeness stages. Thus, they have better flavor since the tomatoes are allowed more time to accumulate higher amounts of acids, sugars and ascorbic acid as they ripen on the vine (Kader, 1986; Watada and Aulenbach, 1979).

Cultivar selection can have a positive impact on the cost of production and fruit quality. For example, costs can be reduced if an improved cultivar requires lower fertilization, fungicide and pesticide inputs, or is drought tolerant. Some improved

varieties may provide desired characteristics such as higher soluble solids, increased firmness, and more color, all of which increase consumer acceptance (Watada and Aulenbach, 1979). A particular cultivar may also have rapid growth, increased tolerance to stress and disease, and improved heat and cold fruit setting ability (Yoder, 1993). However, at the cost of being selected for certain desired horticultural characteristics, some cultivars may be unknowingly selected for increased susceptibility to diseases. For example, selection for thin cell walls will yield more tender fruit, but thicker cell walls are a characteristic associated with disease resistance in peaches (Robertson *et al.*, 1988). Therefore, cultivar selection should be approached judiciously.

BOTRYTIS TAXONOMY

Chilling injury is a common problem encountered during storage and postharvest handling and transit of tomatoes. This and other types of damage make the fruit more susceptible to decay (Dennis, 1983; Falik *et al.*, 1993). The fungal pathogen in this study, *Botrytis cinerea* Pers., is responsible for the majority of postharvest rotting of tomatoes. This fungus is the causal agent of grey mold rot, a soft rot occurring on a wide variety of crops worldwide, especially in greenhouses of temperate areas (Coley-Smith, 1980; Dennis, 1983; Eden *et al.*, 1996a; Schwinn, 1992; Yunis *et al.*, 1991). *Botrytis cinerea* is a saprophyte (Schwinn, 1992; Coley-Smith, 1980). That is, it can obtain its food source from dead or senescing plant tissue (Barkai-Golan, 1981). Once it invades weakened tissue, the fungus can become pathogenic to the remaining healthy tissue.

Botrytis cinerea infection of the plant occurs primarily via stem wounds due to lateral shoot removal or leaf removal and injury (Eden *et al.*, 1996b). Once the leaves or stems are infected, they die and are quickly colonized by saprophytic growth of the fungus (Kerssies and Frinking, 1996). Once it becomes established on the plants, the fungus is very difficult to control because it produces an abundant number of conidia that serve as a future inoculum source. *Botrytis* commonly causes “latent” infections. The spores present on senescing flowers germinate and infect young fruit. After the spores germinate, they lie dormant until the onset of fruit ripening and senescence, at which time infection resumes (Dennis, 1983; Jarvis, 1994; Lavy-Meir, 1981).

Ripening tomato fruit more readily succumb to *Botrytis* infection than green developing fruit (Lavy-Meir *et al.*, 1989). Since grey mold rot occurs in stored, ripening fruit, this makes *Botrytis* an even more economically important pathogen due to the already large financial investment put into the crop by this time. There are several hypotheses as to why this disease occurs more often in ripe fruit. The fruit tissue softening associated with maturity may increase the susceptibility of the ripe fruit to greater mechanical injury which in turn provides easy access for the fungus into the fruit. In addition, close contact with infected fruit is increased by storage conditions (Coley-Smith, 1980; Flaishman and Kolattukudy, 1994; Kader, 1986; Lavy-Meir *et al.*, 1989).

Early infection and dormancy until ripening, or latency, is commonly associated with *Botrytis* infection. The disease may be stimulated by the onset of the climacteric at ripening with the characteristic increase in ethylene production. Ethylene is suggested to

be a signal that may stimulate latent fungi to resume growth or germination (Flaishman and Kolattukudy, 1994). The onset of disease may then be dependent upon host resistance and the proper environmental conditions such as increased humidity and temperature (Lavy-Meir *et al.*, 1989). Lavy-Meir *et al.* (1989) performed *in vitro* and *in vivo* experiments that demonstrated nonripening *nor* tomato fruit extracts do not exhibit an increase in ethylene production and their F₁ hybrids suppressed elongation of *B. cinerea* germ tubes, yielding lower disease incidence.

The genus *Botrytis* was first established by Micheli in 1729 and the species *cinerea* described by Persoon in 1801 (Jarvis, 1977; Coley-Smith, 1980). *Botrytis cinerea* is heterokaryotic with multinucleate, septate hyphal cells. Conidiophores are alternately branched with straight branches, each of which have a bulbous tip called the ampulla. The ampulla supports many denticles, each of which produce the individual conidia. The multinucleate conidia are hyaline or pigmented, mostly ovate, and smooth (Coley-Smith, 1980).

Botrytis cinerea is the conidial or imperfect stage of the apothecial or perfect stage of the fungus *Botryotinia fuckeliana* (de Bary) Whetz. *Botrytis* spp. are in the family Botrytidaceae of the order Hyphales (Moniliales) in the class Hyphomycetes of the subdivision Deuteromycotina in the division Eumycota, all within the kingdom Mycota. *Botrytis cinerea* was originally placed in the Deuteromycetes before its sexual stage was identified, but some now classify it with *B. fuckeliana* in the family Sclerotiniaceae of the order Helotiales in the class Discomycetes of the subdivision Ascomycotina (Faretra and Grindle, 1992).

The life cycle of *B. cinerea* begins with the dissemination of conidia, also called spores. When a conidium makes contact with the host, it germinates, generating an appressorium that attaches to the host surface. The appressorium then produces a penetration peg and invades the tissue intercellularly. A soft rot commences as the fungal mycelium quickly colonize the surrounding cells, collapsing them as a result of secreted pectolytic enzymes. At this point the fungus gives rise to either conidiophores and conidia to repeat the cycle, or if near the end of the season, sclerotia, which are overwintering survival structures. Sclerotia then give rise to either mycelium that produce more conidiophores and conidia, or the sexual fruiting bodies of the fungus called apothecia. An apothecium is an open, cup-shaped ascocarp that yields many asci, each of which contain eight ascospores. This sexual stage of the fungus is usually produced at the end of winter or early spring and the ascospores act as the primary inoculum for the next season. The ascospores then generate mycelium which invades the host and produces spores, beginning the cycle for the new season (Coley-Smith, 1980). The conditions which determine whether or not the sclerotia produce mycelium with conidiophores or apothecia have not been determined. However, temperature and solar angle may play a role (Jarvis, 1977).

FUNGICIDE ALTERNATIVES

Tomato cultivars with a high level of disease resistance are desirable of course, but they are not always available, especially with resistance to *B. cinerea* (Gullino, 1992). Although fungicides can be effective, they must be applied frequently, especially when the

disease is severe (Elad *et al.*, 1992) and unfortunately, *B. cinerea* can quickly develop resistance to many fungicides (Elad, 1996; Stehmann and de Waard, 1996; Yunis *et al.*, 1991).

There are no registered postharvest fungicides for control of *B. cinerea* decay in tomatoes and many preharvest fungicides have been withdrawn from the market (Liu, 1993; Wilson *et al.*, 1991). These factors have prompted researchers to seek alternative, non-chemical methods of pathogen control such as use of biological antagonists (Eden *et al.*, 1996b; Elad *et al.*, 1996; Mercier, 1995; Wilson *et al.*, 1991; Wilson, *et al.*, 1993), biological antagonists in conjunction with calcium treatments in apple (Wisniewski *et al.*, 1995), calcium treatments (Conway, 1982; Conway *et al.*, 1991; Elad and Volpin, 1993; Huber, 1991; Lavy-Meir *et al.*, 1989; McGuire and Kelman, 1984; Yamazaki and Hoshina, 1995), selection of resistant cultivars (Johal and Briggs, 1991; Martin *et al.*, 1993; Li and Gabelman, 1990; Ronald *et al.*, 1992; Yamazaki *et al.*, 1996), heat treatments of tomato fruit (Fallik, 1993), application of UV light to tomato fruit (Liu *et al.*, 1993), hot water and radiation treatments of tomato fruit (Barkai-Golan *et al.*, 1993), and improved cold storage of the fruit (Reyes, 1990).

INFLUENCE OF GENETICS ON DISEASE

Depending upon the cultivar, production area, season and cultural practice, the incidence and severity of disease strongly influences the numbers of discarded tomato fruit at the packinghouse. Postharvest losses can be as high as 50% (Flaishman and

Kolattukudy, 1994; Sams, 1994), with a significant portion of this loss due to fungal diseases (Dennis, 1983; Kader, 1986; Wilson, 1991).

Pathogen and host plant interactions can be attributed in part to genetics, which may determine disease resistance or susceptibility. Resistant host plants usually contain a single locus that is specific for resistance against a pathogen having a complementary avirulence gene (Martin *et al.*, 1993; Ronald *et al.*, 1992). A mutation or a lack of either member of the gene pair usually results in a susceptible response (Bonas *et al.*, 1989; Johal *et al.*, 1991; Kearney and Staskawicz, 1990; Ronald *et al.*, 1992). This specificity, termed the “gene for gene” concept, was first described by H. H. Flor in 1942. Flor was the first plant pathologist to investigate a plant-pathogen relationship simultaneously (Schumann, 1991). His proposed theory stated that a successful pathogenic infection is controlled by genetic factors in both the host and its parasite, therefore a wide range of hosts can react differently to the same pathogen.

For example, different cultivars of tomato have been bred which have a range of resistance to the bacterial wilt disease pathogen *Pseudomonas solanacearum* (Smith) Smith. Although the *P. solanacearum* pathogen produces cell-wall degrading enzymes within the host, the absence of disease symptoms in a resistant host is determined by this host-pathogen relationship. Pathogen multiplication in tissues was suppressed in resistant cultivars, exhibiting tolerance to the pathogen (Yamazaki and Hoshina, 1995). The authors concluded that cultivar-specific host nutrition plays a role in disease level, suggesting that genetics contributed to the level of disease exhibited.

In a follow-up study twenty three-tomato cultivars were tested for degree of resistance to bacterial wilt. The plants were divided into three groups, based on their observed degree of resistance. Nutrient uptake was also observed and although nutrient concentrations were significantly different among plants, calcium was the only nutrient that was consistently and significantly correlated with a lower incidence of disease. The resistant cultivars showed a higher calcium concentration in leaves and stems than susceptible cultivars (Yamazaki *et al.*, 1996). The authors concluded that those cultivars resistant to bacterial wilt were characterized by high calcium uptake.

More specifically, calcium use efficiency is an inherited trait in tomatoes. Li and Gabelman (1990) used sixty homozygous tomato strains to study the influence of inheritance on calcium use efficiency while the plants were under low calcium stress (5 mg L⁻¹). They demonstrated genetic variation among the different cultivars and that calcium use efficiency was controlled by relatively few major genes. Progenies grown under low calcium stress indicated that genetic differences among cultivars were fairly consistent over experiments.

However, it is important to note that resistance can fluctuate to some degree due to environmental factors and pathogen mutation. Increasing the calcium availability in the nutrient solution can proportionally increase the uptake in some cultivars (English and Barker, 1987). The mechanisms that determine host response and disease resistance are still relatively unknown (Hayward, 1991).

CALCIUM AND THE CELL WALL

Cell walls are the backbone of the plant, providing rigidity, support and protection. They are physically and chemically involved in plant biology, and contribute to many aspects of structure, growth, and development. The cell wall can be of two types: 1) primary or 2) secondary. The primary cell wall is laid down by actively growing cells. Once the cell stops growing, it is converted into a secondary wall. The major components of the cell wall are cellulose, hemicellulose, pectic polysaccharides, proteins and lignin. Lignin is a component of secondary cell walls, giving woody plants their characteristic strength, while the other components have many different properties and functions (Talmadge *et al.*, 1973). The cell wall can be divided into two fractions: 1) the inner wall and 2) the outer wall or middle lamella. It is this middle lamella which is shared in common by adjacent cells.

The plant cell wall primarily consists of protein which makes up 5-10% of the wall and three carbohydrate fractions (pectin, cellulose, and hemicellulose) which comprise 90-95% of the wall (Gross, 1990). "Cellulose, a homopolymer, consists of β -1,4-glucosyl residues and the resulting linear hydrogen bonded cellulose chains form microfibrils, the backbone for other wall polymers. Pectic polysaccharides are primarily comprised of an α -1,4-galacturonan backbone with 2- and 2,4-rhamnosyl residues that may serve as branching points for neutral sugar side chains consisting primarily of arabinosyl and galactosyl residues. The galacturonan backbone can also contain methyl esters at the C-6 carboxyl or calcium bridges between adjacent carboxyls or adjacent galacturonan chains. Hemicelluloses are polymers that contain various neutral sugar residues such as xylosyl,

glucosyl, fucosyl, and mannosyl, that may be covalently linked to pectin, and hydrogen bonded with cellulose” (Gross, 1990). *In vitro* degradation of green tomato cell walls showed that the solubilized material was primarily polyuronide (Wallner and Bloom, 1977). Pectic polysaccharides are the most abundant component of most flowering plants (Jarvis, 1984). The major sugars found in tomato fruit cell walls are glucose and fructose accompanied by very small amounts of sucrose (Davies and Kempton, 1975). Galactose is the major neutral monosaccharide present in unripe fruit and accounts for 14% of the cell walls of tomato (Gross and Sams, 1984).

Calcium, a divalent cation, is an essential nutrient required by all plants. This mineral plays a role in maintaining the membrane integrity of the cell. In general, a balance between calcium and other major cations is essential for overall nutrition of the plant (Epstein, 1961). Calcium is an important part of the cell wall structure since it is involved in cross-linkage of pectic molecules. An estimated 60% of the total calcium in plants is associated with the cell wall fraction (Demarty *et al.*, 1984; Rossignol *et al.*, 1977). Distribution of ions in the cell walls depends upon the charge density and configuration of the pectic fraction which behaves as a cation exchanger. Biddulph *et al.* (1961) showed that ascending calcium in the transpiration stream of bean plant participates in exchange and deposition reactions, by an exchange mechanism. Bell and Biddulph (1963) also confirmed this phenomenon.

Calcium appears to function by reacting with the free carboxyl groups of polygalacturonic acid molecules and increasing the intercellular bond strength which results in tissue resistance to damage. These pectic chains are suggested to form an “egg-

box” structure by combining with calcium to form “cross-bridges” between the chains, leading to strengthening and stabilizing of the middle lamella thus preventing degradation of the cell wall to destructive forces (Burns and Pressey, 1987; Demarty *et al.*, 1984; Fry, 1986; Grant *et al.*, 1973; Glenn and Poovaiah, 1989). When calcium-chelating agents such as EDTA or citrate are used, hydrolysis of the pectin molecules is promoted (Bangerth, *et al.*, 1972). Jarvis (1982) extracted calcium from tomato fruit tissue and subsequent pectin extraction showed 92% of the uronic acid polymers were water-soluble in the absence of calcium ions.

Calcium transport through the plant is apoplastic, via the xylem (Hylmo, 1953) and is dependent upon transpiration. However, once deposited, calcium is relatively immobile except under certain laboratory conditions (Loneragan and Snowball, 1969). This may explain why older leaves contain relatively more calcium whereas younger, developing leaves exhibit calcium deficiency symptoms. Therefore, a constant supply is needed by the plant. Of all plant parts sampled, tomato leaves were more active in calcium uptake than any other organ (Park and Lee, 1991). Calcium was present in the pectin fraction of the tomato leaf cell wall and was associated with pectin constituents (Yamauchi *et al.*, 1986).

Increasing amounts of calcium are deposited in the developing fruit and continue to do so until the fruit have reached approximately 80% of their development (Rigney and Wills, 1981). Sequential fractionation of fruit tissue calcium into CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ (both water soluble), Ca pectate (NaCl soluble), Ca phosphate (acetic acid soluble), and Ca oxalate (HCl soluble), has demonstrated that the concentration of calcium in different tissues of tomato fruit changed with fruit development. For example, in young fruit more

than half of newly deposited ^{45} calcium was in the flesh as and not water soluble, while in mature ripening fruit more than half of the ^{45} calcium in the flesh was water soluble (Minamide and Ho, 1993). This pattern is as expected since tomato fruit ripening is accompanied by tissue softening.

CALCIUM NUTRITION AND DISEASE

Optimizing the nutritional status of the plant and fruit tissues with minerals, specifically calcium, is a promising approach with potential to positively influence quality and tomato disease resistance. The degree of disease resistance or susceptibility of a plant can be strongly influenced by the nutritional condition of the plant (Huber, 1991). In addition, different tomato varieties accumulate or utilize calcium to differing degrees (refer to previous section entitled “Influence of genetics on disease” for references).

It may therefore be possible to enhance the natural resistance of plant tissues by increasing the amount of calcium in those tissues. This increase has been shown to increase resistance of tomato seedlings to bacterial wilt (Yamazaki and Hoshina, 1995), a widely distributed and serious disease of many crops (Hayward, 1991). A highly resistant cultivar to bacterial wilt, ‘Hawaii 7998’ contained relatively higher calcium and magnesium concentrations than less resistant cultivars. In this cultivar, seedlings with medium and high calcium treatments lacked disease symptoms, and the seedlings with the low calcium treatment showed symptoms 20 days after inoculation. This effect of the cultivar resistance was clear. In addition, the experiment showed that calcium delayed

disease symptoms on moderately resistant plants. However, at all calcium concentrations, the highly susceptible cultivar, 'Ponderosa', began to wilt only four days after inoculation.

In earlier disease experiments, growth of *Fusarium oxysporum* f. *lycopersici* in tomato stems was inversely related to the calcium concentration in the sap (Corden, 1965). Fungal polygalacturonase (PG) activity is inhibited by the presence of calcium (Conway, 1994; Corden, 1965). McGuire and Kelman (1984; 1986) had similar results with *Erwinia carotovora* pv. *atroseptica* (van Hall) Dye. In laboratory experiments, they found that the severity of the bacterial soft rot was inversely related to the concentration of vacuum-infiltrated calcium concentration in potato tissue. Similarly, in field experiments, they found that as the soil calcium increased, the percent surface area of decay by *E. carotovora* was reduced.

Increased resistance of bean hypocotyl tissue to *Rhizoctonia solani* Kuhn during tissue maturation has been associated with the conversion of cell wall pectins to calcium pectate, rendering the pectic materials resistant to fungal PG (Bateman and Lumsden, 1965). Tobias *et al.* (1993) extracted cell walls from calcium-treated infected apples and suggested that the decay-reducing role of calcium is associated with maintenance of cell wall structure and delays or modifies chemical changes in cell wall composition.

Increased calcium content has also been shown to increase resistance of fruit to postharvest decay. Pressure infiltration of apples with 8% calcium chloride prior to three months of controlled atmospheric storage reduced decay caused by *Penicillium expansum* Link ex Thom when inoculated after the storage period (Conway, 1982). In a similar experiment, Conway and Sams (1984) found that pressure-infiltration of apples with 4, 6,

or 8% calcium chloride had > 50% less decay than non-treated fruit. Since fungal growth on artificial media amended with calcium chloride was not retarded, the authors suggested that calcium's effect in reducing decay may be due to the formation of fruit cell wall components resistant to *P. expansum* rather than a direct effect on the fungus. The results were repeated with pressure infiltration with 4% calcium chloride and six months' storage time (Conway *et al.*, 1987). The authors suggested that calcium may strengthen the cell wall by binding the uronic acids and forming cross-bridges. The decay rate was therefore reduced because the cell wall was less accessible to fungal cell wall-degrading enzymes. Conway *et al.* (1988) extracted calcium-treated fruit cell walls, using them to test fungal PG enzyme activity. The high calcium cell walls yielded 60% less uronic acid than the low calcium cell walls. Since the decay area was reduced by approximately the same amount, 60%, they attributed this to a reduction in the fungal PG activity by the calcium present in the tissues due to the treatments.

Calcium treatments have the potential to provide broad-spectrum disease control that is not limited to only a few pathogens. Conway *et al.* (1991) pressure infiltrated both 'Delicious' and 'Golden Delicious' apple fruit with a range of calcium chloride solutions. The fruit were stored for 6 months at 60C. After removal to 20C the fruit were wound inoculated with *P. expansum*, *B. cinerea*, and *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, all of which were known to produce pectolytic enzymes. The infiltrated calcium concentration was negatively correlated with decay for all three pathogens. Decay by *P. expansum*, *B. cinerea*, and *G. cingulata* was reduced by 70%, 50% and 37%, respectively, by the calcium-infiltration treatments.

Many plants, including tomato, contain fungal polygalacturonase inhibitor proteins (PGIP) as a defense mechanism against fungal enzyme attack. Results from research in this area have been contradictory. Stotz *et al.* (1995) found tomato PGIP to be only slightly effective against *B. cinerea*, and pear PGIP to be more effective against *B. cinerea* in transgenic tomato fruit *in vitro*. However, Brown *et al.* (1983) found that PGIP extracted from tomato fruits did not inhibit *B. cinerea*. Yao *et al.* (1995) purified a cell wall bound PGIP from apple fruit and studied its inhibitory effect on five PG isozymes produced by *B. cinerea* in liquid culture. Apple PGIP showed differences in *in vitro* inhibitory activity against the five PG isozymes, but not against PG extracted from apple fruit inoculated with the same fungus. The authors suggested that enabling constitutive expression of PGIP or retardation of PGIP dissociation from the cell wall with calcium and heat treatments may make it possible to reduce decay caused by postharvest pathogens.

The addition of both 1 and 3 mM calcium chloride or calcium nitrate to perlite-grown tomato plants significantly increased tissue calcium and reduced grey mold severity and ethylene production in tomato leaves (Elad and Volpin, 1993). Calcium nitrate added to the nutrient solutions of greenhouse-grown rose plants increased tissue calcium and reduced severity of floral infection to differing degrees, depending upon the cultivar (Volpin and Elad, 1991). Pathogen growth and PG activity in liquid culture were inhibited by the addition of 3 mM calcium in the growth medium which also contained Na-pectate

as the sole carbon source (Volpin and Elad, 1991). In addition, ethylene production was reduced by 50-90% in those flowers grown in 2.1 and 3.5 mM calcium (Volpin and Elad, 1991). When cucumber fruit or leaves of tomato and cucumber were treated with ethephon or methionine, decay by *B. cinerea* was increased. Likewise, when the tissues were treated with AOA or AVG (aminooxyacetic acid and aminoethoxyvinylglycine) and STS (silver-thiosulphate), decay development was reduced or completely inhibited (Elad, 1990). The author concluded that ethylene increased the severity of decay which develops in response to *B. cinerea* infection. Although it was not discussed, the possibility exists that the calcium content of the cultivars used may have contributed to the results.

Vacuum infiltration of calcium has been found to increase tissue calcium and cause a delay in ripening of tomatoes (Wills *et al.*, 1977). The calcium-induced delay of ripening may somehow be related, in part, to a reduction in the ethylene production that normally occurs at ripening, although this was not included in their discussion. In support of this, Sams and Conway (1984) showed that pressure-infiltration with calcium chloride lowered ethylene production in apples.

In 1995, Wisniewski *et al.* found that in the presence of calcium chloride, *in vitro* spore germination and germ tube elongation of both *B. cinerea* and *P. expansum* was significantly reduced in apples, also a climacteric fruit. Magnesium chloride had no effect on germination and germ-tube growth. The authors suggested that calcium reduced fungal infection through direct inhibition of spore germination and growth as well as through an effect on the activity of cell-wall-macerating enzymes by decreasing the pectinolytic activity of *B. cinerea*.

Although the exact mechanism of calcium in disease resistance remains unknown, some theories have been proposed. Direct inhibition of pathogen-produced PG and other pectolytic enzymes by calcium (Corden, 1965; Wisniewski *et al.*, 1995), increased resistance to pectolytic enzymes in cell walls strengthened by calcium (Bateman and Lumsden, 1965; Conway, 1982; Conway *et al.*, 1988; Conway *et al.*, 1987; McGuire and Kelman, 1986; Tobias *et al.*, 1993) and inhibition of ethylene production by calcium (Elad and Volpin, 1993; Raz and Fluhr, 1992; Volpin and Elad, 1991). Pathogen multiplication was suppressed by calcium in resistant and moderately-resistant cultivars (Bowman and Sequeira, 1982; Grimault and Prior, 1993; Yamazaki and Hoshina, 1995) and has been expressed as an increased tolerance to pathogens.

CONCLUSION

As the literature points out, the potential is high for using calcium to reduce postharvest decay in tomatoes, especially to *B. cinerea*. However, additional research needs to be conducted on the mechanisms and other factors or possible combinations of factors which may contribute to disease resistance. The influence of environmental variables on calcium uptake and distribution in different tomato cultivars warrant further study. Since tissue calcium has been shown to reduce disease incidence and severity, screening for those cultivars that are more calcium-efficient could preclude random screening for disease resistance. The optimization of plant nutrition, reduced chemical input and implementation of improved cultural systems to maintain disease resistance

should yield more cost-efficient, high quality, disease-free tomatoes. Through nutritional and disease physiology research, knowledge and understanding may be gained which will enable us to develop effective disease management strategies.

CHAPTER III

THE INFLUENCE OF CALCIUM NUTRITION ON GREY MOLD ROT CAUSED BY *BOTRYTIS CINEREA* PERS. IN THREE TOMATO CULTIVARS

ABSTRACT

Two greenhouse experiments, one in the fall of 1995 and the other in the spring of 1996, contained three tomato cultivars, 'Mountain Supreme', 'Sunrise', and 'Celebrity'. These were grown in a hydroponic system with perlite as a root support medium to study the role of calcium in reducing decay of tomato fruit by *Botrytis cinerea* Pers. Calcium treatments of 20 mg L⁻¹, 220 mg L⁻¹, or 1020 mg L⁻¹, were added to a commercial fertilizer solution that had an N-P-K ratio of 1.0:0.6:2.5 and contained essential micronutrients. A 3x3 factorial with three tomato cultivars and three calcium treatments in a randomized complete block design was utilized. Harvested fruit were inoculated with a 5x10⁵ spore ml⁻¹ conidial suspension of *B. cinerea* and the decay lesion diameter measured once daily for up to seven days. Across cultivars, leaf and fruit calcium content significantly increased as the calcium treatments increased. The high calcium treatment significantly reduced the area of decay caused by *Botrytis cinerea* across all cultivars. Significant treatment x cultivar interactions were observed in fruit calcium content. Leaf and fruit tissue Ca concentrations were positively associated with resistance of greenhouse-grown tomatoes to gray mold rot, strengthening the hypothesized role of calcium in promoting disease resistance.

INTRODUCTION

Botrytis cinerea Pers., which causes grey mold rot, is responsible for serious losses of greenhouse tomato crops worldwide (Dennis, 1983; Coley-Smith, 1980; Yunis *et al.*, 1991). *Botrytis cinerea* is a saprophyte (Schwinn, 1992; Coley-Smith, 1980), obtaining its food source from dead or senescing plant tissue (Barkai-Golan, 1981). The fungus can infect the leaves and stems of tomato plants via lateral shoot or leaf removal and injury (Eden *et al.*, 1996b). Infected flowers may either abort or provide inoculum for the developing fruit, producing a latent infection that can resume upon ripening (Dennis, 1983; Flaishman and Kolattukudy, 1994; Lavy-Meir, 1981).

Fungicides are used to reduce disease in the greenhouse. However, the number and effectiveness of fungicides available for use in commercial tomato production is declining due to health risk concerns by the public and bans imposed by the United States Environmental Protection Agency (USEPA) (Liu, 1993; Wilson *et al.*, 1993). Even so, there are few effective fungicides for *B. cinerea*, which is capable of quickly developing resistance (Elad, 1996; Coley-Smith, 1980; Yunis *et al.*, 1991) and there are no registered postharvest fungicides for this fungus. Since fungal decay is the main cause of postharvest losses (Dennis, 1983; Flaishman *et al.*, 1994; Sams, 1994; Wilson, 1991), alternative disease control methods need to be investigated.

Use of specific tomato cultivars with various levels of disease resistance is possible, but those cultivars resistant to one disease can be susceptible to other diseases (Coley-Smith, 1980). In addition, *B. cinerea* can accommodate the host due, at least in part, to population variability resulting from the fungus' heterokaryotic nature (Jarvis,

1977), requiring costly breeding programs to keep up with the constant demand for new and improved cultivars. Disease resistance may also be influenced by other factors including but not limited to cultivar susceptibility to adverse environmental conditions, poor plant nutritional status, or physical injury and compositional changes associated with ripening during the postharvest stage (Dennis, 1983; Dhingra, 1995; Hayward, 1991).

Disease resistance has been associated with an increase in plant tissue calcium content. Resistance of tomato seedlings to bacterial wilt increased as plant tissue calcium content increased (Yamazaki and Hoshina, 1995). The mechanism by which calcium reduces disease levels is still unknown, but it is possible that it affects pectolytic enzymes produced by pathogens, and an increase in the plant cell wall calcium may have regulatory activity (Conway *et al.*, 1988). Pathogenic plant fungi, including *B. cinerea*, initiate fruit infection by producing cell wall-degrading enzymes (Coley-Smith, 1980; Collmer, 1986; Weste, 1970). It has been demonstrated that polygalacturonase activity produced by some fungi and bacteria was decreased with an increase in cell wall-associated calcium (Blais *et al.*, 1992; Cooper, 1983). This is important because the majority of plant cell calcium is found in the cell wall (Demarty *et al.*, 1984; Rossignol *et al.*, 1977). Theories about the role of calcium in disease resistance include pectolytic enzyme inhibition (Bateman and Lumsden, 1965; Conway *et al.*, 1988; Corden, 1965) and tolerance to and suppression of pathogen multiplication (Bowman and Sequeira, 1982; Grimault and Prior, 1993; Yamazaki and Hoshina, 1995). Cell wall strengthening and stabilization of the middle lamella by calcium has also been suggested to improve disease resistance. Resistance occurs because the polygalacturonic acid chains form an “egg-box” structure when they

combine with calcium (Grant *et al.*, 1973), and create “cross-bridges” between the chains, thus strengthening the cell wall to resist fungal attack (Conway *et al.*, 1987).

The objective of this research was to investigate the effects of calcium concentration and cultivar variation on disease resistance of three tomato cultivars to grey mold rot caused by *B. cinerea* Pers.

MATERIALS AND METHODS

Greenhouse experiment and plant material

This research consisted of two greenhouse tomato experiments. Experiment 1 was conducted from August 18, 1995 through March 8, 1996 and experiment 2 from January 10, 1996 through July 3 for a total of 172 and 206 days, respectively. Each experiment consisted of 54 plants divided into three blocks, with each block having two replicates of three cultivars, and three calcium treatments. Each pot was provided with 1.6 liters of fertilizer solution four times a day (with at least 20% flow through).

The three tomato cultivars, ‘Mountain Supreme’, ‘Sunrise’, and ‘Celebrity’, were selected because of reported differences in disease susceptibility. Plants were grown in a hydroponic system consisting of 16-liter pots with perlite as an inert root-support medium. Calcium treatments of either 0 mg L⁻¹ (low), 200 mg L⁻¹ (medium) or 1000 mg L⁻¹ (high) were added as CaCl₂ · 2 H₂O to a commercial hydroponic fertilizer solution containing recommended amounts of all other nutrients (Appendix A, page 69). The greenhouse water supply contained 20 mg L⁻¹, so final calcium concentrations were 20 mg L⁻¹, 220 mg L⁻¹, and 1020 mg L⁻¹, representing near-deficiency through near-toxicity levels. Solution

samples flowing through pots were collected weekly and analyzed by inductively coupled plasma emission spectrometry (ICP).

Statistical analysis

Both experiments were 3x3 factorials in a randomized complete block design having two replications per each of three blocks. Analysis of variance and preplanned treatment contrasts were performed using SAS (Statistical Analysis System, 1989, version 6.11).

Leaf and fruit calcium analysis

One mature, whole, leaf with petiole was collected from immediately below (Fall 1995) or above (Spring 1996) each of the first, third, and fifth flower clusters at 16 weeks for every plant. Fruit were collected at maturity stages two through five (USDA guidelines, see page 93 of this thesis) in the fall experiment (at least three per plant were analyzed), and only at maturity stage two in the spring (at least three per plant were analyzed). The mineral content of the fruit and leaves (on a dry weight basis from dry ashed samples) was measured by ICP.

Inoculation and decay measurements

Additional tomato fruit, at least two per plant, were harvested from the same plants, in both the fall and the spring experiments. They were wiped with sterile, distilled water, wounded to a depth and width of 3 mm, and inoculated with 50 μ l of a 5×10^5 spore ml^{-1} conidial suspension of *B. cinerea* previously isolated from apple. The fruit were then placed in a growth chamber at 20C and $\geq 95\%$ RH. Once daily, for seven days (fall) or for six days (spring) the decay lesion diameter was measured across the widest and

narrowest portion of the lesion and the average of the two measurements was used to calculate the decay area in cm².

RESULTS

Leaf calcium treatment differences

In both experiments, the average leaf calcium (Ca) concentration per plant across cultivars was significantly increased from the low (20 mg L⁻¹) Ca treatment by both the medium (220 mg L⁻¹) and the high (1020 mg L⁻¹) Ca treatments (all $P < 0.001$). In the spring only, there was a significant increase in leaf Ca between the medium and high Ca treatments ($P < 0.001$) (Table 1)*. Least square means from analysis of variance (SAS) are listed in Table 2.

Treatment differences for leaf Ca content of all three cultivars in both experiments increased significantly from the low to the medium (ranging from $P < 0.001$ to $P < 0.04$) and the low to high Ca treatments. In the spring, 'Mt. Supreme' ($P < 0.04$) and 'Celebrity' ($P < 0.001$) were the only two cultivars that had a significant increase in leaf Ca when the Ca treatments increased from medium to high (Table 1).

Leaf calcium cultivar differences

Across all treatments in both experiments, there were no significant cultivar differences in leaf Ca content. Contrasts were performed for cultivars within each treatment. Across cultivars in the fall under the low Ca treatment, there was a significant

*All tables and figures may be found in the Appendices

increase in leaf Ca ($P < 0.04$). Individually under the low Ca treatment, 'Mountain Supreme' VS 'Celebrity' and 'Celebrity' VS 'Sunrise' showed significant differences ($P < 0.02$ and $P < 0.07$) (Table 1). The Ca content was greatest in 'Mountain Supreme' > 'Sunrise' > 'Celebrity' (least square means (ls means), Table 2).

Fruit calcium treatment differences

The fruit Ca content across cultivars, in both experiments, was increased significantly from the low Ca treatment by both the medium and high Ca treatments ($P < 0.001$). A significant increase from the medium to high Ca treatments across cultivars ($P < 0.07$) was observed only in the spring (Table 3) (ls means, Table 4).

For the fall experiment, fruit Ca content was significantly increased by the medium and the high Ca treatments in 'Mountain Supreme' ($P < 0.001$) and in 'Sunrise' ($P < 0.02$ and $P < 0.05$). Fruit Ca content was increased in 'Celebrity' when the treatments changed from low to high ($P < 0.02$) and from medium to high ($P < 0.07$) (Table 3).

In the spring experiment all cultivars had a significant increase in fruit Ca content due to the medium and high Ca treatments ($P < 0.02$). 'Celebrity' and 'Mountain Supreme' had a significant increase in fruit Ca ($P < 0.02$) when the Ca treatments increased from medium to high Ca (Table 3).

Fruit calcium cultivar differences

For each cultivar in both the fall and spring, there were significant differences in fruit Ca content across treatments (from $P < 0.001$ to $P < 0.05$). In the spring, significant fruit Ca differences ($P < 0.001$) were observed across treatments between 'Celebrity' and

'Sunrise', and 'Mountain Supreme' and 'Sunrise' (Table 3), where 'Celebrity' > 'Mountain Supreme' > 'Sunrise' (ls means, Table 4).

In the fall 'Mountain Supreme' fruit contained significantly more Ca content than 'Celebrity' fruit (Table 4) under both the medium ($P<0.01$) and high ($P<0.02$) Ca treatments. Under the medium Ca treatment, 'Sunrise' fruit contained significantly more Ca than 'Celebrity' fruit ($P<0.01$).

In the spring 'Mountain Supreme' fruit contained significantly more Ca than 'Sunrise' fruit ($P<0.02$) for all three Ca treatments. Under the medium and high Ca treatments, 'Celebrity' contained significantly more fruit Ca than 'Sunrise' ($P<0.03$) (Table 3).

Fruit decay treatment differences

Across cultivars in the fall, the medium and high Ca treatments significantly reduced the decay area on the fruit by 58% ($P<0.001$) and 47% ($P<0.01$), respectively (Table 5). In the spring there was a significant reduction ($P<0.01$) in decay area from the low to high ($P<0.01$) and the medium to high ($P<0.04$) Ca treatments (ls means, Table 6).

The medium Ca treatment significantly ($P<0.03$) reduced decay by 72% in 'Celebrity' in the fall. Decay was significantly reduced ($P<0.04$) by 57% in 'Sunrise' by the high Ca treatment. In the spring experiment the high Ca treatment significantly ($P<0.03$) reduced decay by 17% in 'Mountain Supreme' while the high significantly ($P<0.001$) reduced decay from the medium treatment by 29%. The high Ca treatment also reduced decay in 'Celebrity' by 36% ($P<0.05$) and by 34% ($P<0.02$) from the medium to the high Ca treatments (Table 5).

Fruit decay cultivar differences

In the spring, both 'Celebrity' and 'Sunrise' fruit had significantly less decay than 'Mountain Supreme' ($P < 0.02$) across treatments. In the fall under the low Ca treatment, there were significant cultivar differences ($P < 0.04$) between 'Mountain Supreme' and 'Celebrity' fruit, where 'Mountain Supreme' and 'Sunrise' had 40% and 42% less fruit decay than 'Celebrity' ($P < 0.05$) (Table 5) (ls means, Table 6).

Under low Ca, 'Sunrise' fruit had 23% less decay than 'Mountain Supreme' fruit ($P < 0.001$) in the spring. In the same experiment under the medium Ca treatment, 'Sunrise' had 23% less fruit decay than 'Mountain Supreme' and 33% less fruit decay than 'Celebrity' ($P < 0.001$ and $P < 0.02$, respectively) (Table 5).

DISCUSSION

The medium and high Ca treatments provided enough additional Ca to cause an increase in the tomato leaf and fruit tissue Ca content. In most cases, the Ca content in both the leaves and the fruit was consistently and significantly doubled by the medium Ca treatments, regardless of the cultivar. However, increasing the Ca treatments from medium to high did not result in significant tissue Ca increases. The exceptions were 'Mountain Supreme' leaf and fruit Ca in the spring, and 'Celebrity' leaf Ca in the spring and fruit Ca in both the fall and spring.

When the treatments increased from medium to high Ca, 'Sunrise' exhibited a decrease in leaf Ca in the fall experiment (Table 7) and a decrease in fruit Ca content in both the fall and the spring (Table 8). It is possible that the high Ca levels in the form of a

salt such as CaCl_2 were detrimental to the plants. All of the high Ca treatment plants in these experiments exhibited some degree of leaf burn, indicating borderline toxicity. It is likely that 1020 mg L^{-1} Ca in the nutrient supply is excessive and unnecessary for economic reasons.

Plant nutritional response, e.g. Ca uptake, can be limited to some degree by the growth environment. Salinity, light intensity, temperature and relative humidity are some of the limiting factors (Adams, 1994). Relationships between the ionic concentration in the leaf and those in the nutrient solution have been shown to affect plant mineral uptake (Satti and Al-Yahyai, 1995). Osmotic stress reduces Ca uptake by roots and can cause a local deficiency to distal fruit tissue (Bradfield and Guttridge, 1984; Ehret and Ho, 1986). At high cation concentrations, excess salinity has shown a pattern of mineral uptake that restricts water and thus Ca uptake (Hohjo *et al.*, 1995). The osmotic potential of solution samples taken on 7 June, 1996 was determined for each Ca treatment at the 180 mg L^{-1} nitrogen level. Values (averaged over three blocks per treatment) were: -0.098 , -0.1197 , and -0.248 MPa , for the low, medium, and high Ca treatments, respectively. A normal value for a Hoagland's solution is -0.05 MPa . This large difference even at the low Ca level shows that the solutions at the highest fertilizer concentration ($180 \text{ mg L}^{-1} \text{ N}$) imparted high water stress on the plants, probably resulting in reduced Ca to the leaves and fruit.

The differences in the Ca content of leaves and especially fruit between the two experiments may be partially explained by seasonal differences between the two cropping seasons. The effect of seasonal on calcium uptake and translocation plays a role due to

humidity and temperature differences. Vegetative and reproductive plant growth can be manipulated by several factors, including light level (Heuvelink and Marcelis, 1996), temperature, and humidity (Atherton and Rudich, 1986). Light intensity is greater in the spring than in the fall and affects photosynthesis during fruit development.

Temperatures of 17C to 28C and RH of 60-70 % are optimum for greenhouse tomatoes (Snyder, 1994). High temperature and low humidity encourages higher leaf transpiration rates. Ca moves in the xylem and if the humidity is high, transpiration and thus Ca movement is reduced (Adams, 1994). Although there was some temperatures variation (Table 17) in these experiments due to the inadequate greenhouse cooling system, the temperatures were typical of the seasons. With greater light levels in the spring, more transpiration would have occurred, aiding Ca movement into the leaves. Spring temperatures were warmer and the leaf Ca concentrations under the high Ca treatment reflected that. However, most of the cultivars had higher leaf and fruit Ca contents in the fall, especially under the low Ca treatment (Tables 2 and 4). Increased humidity increases Ca to the fruit since leaf transpiration is reduced (Adams and Holder, 1992). The humidity levels in the fall experiment were higher than optimum which the higher fruit Ca levels reflect, but the spring humidity was even higher. Thus, fruit Ca should have been higher in the spring. However, tomato fruit are more affected by higher salinity than leaves (Adams, 1994) and although the Ca treatments remained the same, the fertilizer concentration was higher in the spring (Tables 9-14). Temperature and RH were less extreme in the fall and may have contributed to more Ca being imported into the fruit.

Different cultivars respond differently to various environmental conditions and this is likely to affect Ca uptake. Some cultivars are more heat tolerant and thus more efficient in absorbing water and nutrients. They may have different fertilizer and management needs compared to non heat-tolerant cultivars (Nkansah and Ito, 1995). Unfortunately, it is not known how heat tolerant the cultivars used in this experiment were.

Of the three cultivars, 'Mountain Supreme' contained the highest leaf and fruit Ca under the low Ca treatment in both the fall and the spring. Since the low Ca treatment does not impart a large amount of Ca to the tissues, 'Mountain Supreme' may be the most Ca-efficient cultivar at that treatment level under those specific conditions. Subjected to the medium Ca treatment, 'Sunrise' contained the most leaf and fruit Ca in the fall and 'Celebrity' contained the most leaf and fruit Ca in the spring. While under the high Ca treatment, 'Mountain Supreme' contained the highest amounts of Ca in both tissues in the fall and 'Celebrity' contained the most in both tissues in the spring. The inconsistency among cultivars indicated that each cultivar responds differently to season and possibly the ionic concentration of the nutrient solutions.

The choice of cultivar for production can also somewhat influence the effect of Ca on disease. Differences in decay resistance (Table 6) may be partially due to the differences in the leaf and fruit Ca content observed among the cultivars (Tables 1 and 3). Other research supports this (English and Barker, 1987; Li and Gabelman, 1990; Yamazaki *et al.*, 1996). More research on those tomato cultivars with a known high Ca partitioning efficiency is recommended to see if decay resistance in the already efficient cultivars could be improved.

For plant disease to become established, inoculation, incubation and appearance of symptoms must occur (Dhingra, 1995). In this experiment, disease was artificially established in order to evaluate the resistance of three tomato cultivars to grey mold rot caused by *B. cinerea* and to test the hypothesis that increased tissue calcium reduces the severity of that disease. In some cases, the contrast analysis results in these experiments indicate a low (non-significant) reduction in decay severity, possibly owing to the conditions used in this experiment. In disease assessment, methods used to induce symptoms require ideal inoculation and incubation conditions (Dhingra, 1995). However, if the artificial conditions are “too ideal”, disease may develop regardless of plant or fruit condition. False or exaggerated symptoms may also occur on non-host plants under these conditions (Dhingra, 1995). *Botrytis cinerea* grows best under high relative humidity (RH) >93% and a temperature of 21C (Hawker, 1950). The environmental conditions in these experiments (95% RH and 20C) were optimum for disease expression. However, the incubation chamber environment was higher than is found in typical postharvest storage conditions of >10C and 85-90% RH (Kader, 1986). This may not be a good indicator of what happens with these cultivars under storage conditions. In an actual postharvest storage situation, the environment is controlled to reduce respiration and transpiration. Temperatures are cooler than 20C and the RH not usually quite as high as 95%, as were the conditions in the experiments. Under normal storage situations, the medium Ca treatment may be helpful in reducing decay more consistently than the data show. The use of Ca might be enhanced by a controlled environment to reduce decay even further.

It is also important to note that high salinity not only decreases Ca transport, it also affects the distribution of Ca within the fruit. Ehret and Ho (1986) found that at high salinity, ^{45}Ca was restricted into fruit and especially to the distal end of the fruit. That the fruit were inoculated at the distal end may partially explain why the high Ca treatments, thus the highest treatment salinity, did not consistently yield the least amount of decay than the other treatments.

CONCLUSION

This experiment showed that the uptake of additional available Ca from the nutrient solution, and the subsequent movement of Ca to the leaves and fruit generally reduces decay severity in inoculated tomato fruit. Although both the medium and high Ca treatments significantly reduced decay, the high Ca treatment did not significantly further reduce decay beyond that of the medium Ca treatment. Thus, it is not possible to determine which Ca treatment is best for reducing grey mold rot in tomato fruit. The high Ca treatment, 1020 mg L^{-1} in the fertilizer solution, was possibly near toxicity level and likely to be above the sufficiency range needed for optimal fruit decay reduction. In order to determine optimum Ca levels, future research in this area should investigate nutrient solutions with Ca in the 200 mg L^{-1} range. These results strongly suggest that leaf and fruit Ca content is positively associated with resistance of greenhouse-grown tomatoes to grey mold rot, and strengthen the hypothesized role of calcium in promoting disease resistance.

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APPENDICES

APPENDIX A

TABLES

TABLE 1: Leaf calcium content significance probabilities of analysis of variance and preplanned treatment contrasts, fall 1995 and spring 1996

SOURCE OF VARIATION	Fall 1995	Spring 1996
Treatment	0.01	0.01
Cultivar	0.24	0.50
Treatment x cultivar	0.63	0.58
Low calcium Vs medium calcium	0.01	0.01
Med Ca Vs high calcium	0.65	0.01
Low Ca Vs high calcium	0.01	0.01
'Mountain Supreme' Vs 'Celebrity'	0.09	0.34
'Celebrity' Vs 'Sunrise'	0.37	0.31
'Mountain Supreme' Vs 'Sunrise'	0.54	0.88
CONTRASTS		
'Mountain Supreme'		
Across treatments	0.01	0.01
Low calcium Vs medium calcium	0.01	0.01
Medium calcium Vs high calcium	0.40	0.04
Low calcium Vs high calcium	0.01	0.01
'Celebrity'		
Across treatments	0.01	0.01
Low calcium Vs medium calcium	0.01	0.01
Medium calcium Vs high calcium	0.41	0.01
Low calcium Vs high calcium	0.01	0.01
'Sunrise'		
Across treatments	0.06	0.01
Low calcium Vs medium calcium	0.03	0.01
Medium calcium Vs high calcium	0.55	0.31
Low calcium Vs high calcium	0.04	0.01
Low calcium		
Across cultivars	0.04	0.51
'Mountain Supreme' Vs 'Celebrity'	0.02	0.27
'Celebrity' Vs 'Sunrise'	0.07	0.83
'Mountain Supreme' Vs 'Sunrise'	0.10	0.45
Medium calcium		
Across cultivars	0.75	0.63
'Mountain Supreme' Vs 'Celebrity'	0.49	0.36
'Celebrity' Vs 'Sunrise'	0.55	0.85
'Mountain Supreme' Vs 'Sunrise'	0.94	0.68
High calcium		
Across cultivars	0.87	0.24
'Mountain Supreme' Vs 'Celebrity'	0.63	0.60
'Celebrity' Vs 'Sunrise'	0.98	0.11
'Mountain Supreme' Vs 'Sunrise'	0.70	0.36

TABLE 2: LS means from analysis of variance of tomato leaf calcium content ($\mu\text{g g}^{-1}$ DW)

SOURCE OF VARIATION	Fall 1995	Spring 1996
Low calcium	36585.30	24569.69
Medium calcium	76816.71	75326.34
High calcium	79117.23	95037.77
'Mountain Supreme'	68414.58	64025.16
'Celebrity'	59279.20	67502.83
'Sunrise'	64825.45	63405.81
Low calcium, 'Mountain Supreme'	47178.97	26339.51
Low calcium, 'Celebrity'	26245.14	23570.53
Low calcium, 'Sunrise'	36331.78	23799.03
Medium calcium, 'Mountain Supreme'	76095.30	71207.99
Medium calcium, 'Celebrity'	73923.07	78550.28
Medium calcium, 'Sunrise'	80431.74	76220.75
High calcium, 'Mountain Supreme'	81969.45	94527.99
High calcium, 'Celebrity'	77669.39	100387.68
High calcium, 'Sunrise'	77712.84	90197.64

TABLE 3: Fruit calcium content significance probabilities of analysis of variance and preplanned treatment contrasts, fall 1995 and spring 1996

SOURCE OF VARIATION	Fall 1995	Spring 1996
Treatment	0.01	0.01
Cultivar	0.01	0.01
Treatment x cultivar	0.05	0.01
Low calcium Vs medium calcium	0.01	0.01
Med Ca Vs high calcium	0.55	0.07
Low Ca Vs high calcium	0.01	0.01
'Mountain Supreme' Vs 'Celebrity'	0.01	0.92
'Celebrity' Vs 'Sunrise'	0.02	0.01
'Mountain Supreme' Vs 'Sunrise'	0.08	0.01
CONTRASTS		
'Mountain Supreme'		
Across treatments	0.01	0.01
Low calcium Vs medium calcium	0.01	0.01
Medium calcium Vs high calcium	0.54	0.01
Low calcium Vs high calcium	0.01	0.01
'Celebrity'		
Across treatments	0.05	0.01
Low calcium Vs medium calcium	0.32	0.01
Medium calcium Vs high calcium	0.07	0.05
Low calcium Vs high calcium	0.02	0.01
'Sunrise'		
Across treatments	0.05	0.01
Low calcium Vs medium calcium	0.02	0.01
Medium calcium Vs high calcium	0.35	0.33
Low calcium Vs high calcium	0.05	0.02
Low calcium		
Across cultivars	0.42	0.05
'Mountain Supreme' Vs 'Celebrity'	0.77	0.09
'Celebrity' Vs 'Sunrise'	0.38	0.39
'Mountain Supreme' Vs 'Sunrise'	0.22	0.02
Medium calcium		
Across cultivars	0.01	0.01
'Mountain Supreme' Vs 'Celebrity'	0.01	0.64
'Celebrity' Vs 'Sunrise'	0.01	0.01
'Mountain Supreme' Vs 'Sunrise'	0.80	0.01
High calcium		
Across cultivars	0.05	0.01
'Mountain Supreme' Vs 'Celebrity'	0.02	0.75
'Celebrity' Vs 'Sunrise'	0.22	0.03
'Mountain Supreme' Vs 'Sunrise'	0.11	0.01

TABLE 4: LS means from analysis of variance of tomato fruit calcium content ($\mu\text{g g}^{-1}$ DW)

SOURCE OF VARIATION	Fall 1995	Spring 1996
Low calcium	1168.62	539.32
Medium calcium	1902.99	1595.17
High calcium	1823.79	1781.98
'Mountain Supreme'	1899.75	1493.72
'Celebrity'	1330.73	1584.58
'Sunrise'	1664.92	938.16
Low calcium, 'Mountain Supreme'	1269.91	660.93
Low calcium, 'Celebrity'	1196.08	481.70
Low calcium, 'Sunrise'	1039.85	475.33
Medium calcium, 'Mountain Supreme'	2171.17	1715.26
Medium calcium, 'Celebrity'	1351.28	1769.03
Medium calcium, 'Sunrise'	2186.52	1301.22
High calcium, 'Mountain Supreme'	2258.16	2104.99
High calcium, 'Celebrity'	1444.82	2203.02
High calcium, 'Sunrise'	1768.39	1037.94

TABLE 5: Area of decay significance probabilities of analysis of variance and preplanned treatment contrasts, fall 1995 and spring 1996

SOURCE OF VARIATION	Fall 1995	Spring 1996
Treatment	0.01	0.02
Cultivar	0.37	0.01
Treatment x cultivar	0.46	0.49
Low calcium Vs medium calcium	0.00	0.28
Med Ca Vs high calcium	0.43	0.01
Low Ca Vs high calcium	0.01	0.04
'Mountain Supreme' Vs 'Celebrity'	0.73	0.02
'Celebrity' Vs 'Sunrise'	0.18	0.16
'Mountain Supreme' Vs 'Sunrise'	0.29	0.01
CONTRASTS		
'Mountain Supreme'		
Across treatments	0.31	0.01
Low calcium Vs medium calcium	0.14	0.07
Medium calcium Vs high calcium	0.41	0.01
Low calcium Vs high calcium	0.46	0.03
'Celebrity'		
Across treatments	0.08	0.05
Low calcium Vs medium calcium	0.03	0.64
Medium calcium Vs high calcium	0.43	0.02
Low calcium Vs high calcium	0.10	0.05
'Sunrise'		
Across treatments	0.07	0.59
Low calcium Vs medium calcium	0.07	0.55
Medium calcium Vs high calcium	0.31	0.52
Low calcium Vs high calcium	0.04	0.33
Low calcium		
Across cultivars	0.07	0.01
'Mountain Supreme' Vs 'Celebrity'	0.04	0.15
'Celebrity' Vs 'Sunrise'	0.05	0.13
'Mountain Supreme' Vs 'Sunrise'	0.89	0.01
Medium calcium		
Across cultivars	0.81	0.01
'Mountain Supreme' Vs 'Celebrity'	0.52	0.22
'Celebrity' Vs 'Sunrise'	0.73	0.02
'Mountain Supreme' Vs 'Sunrise'	0.77	0.01
High calcium		
Across cultivars	0.67	0.53
'Mountain Supreme' Vs 'Celebrity'	0.73	0.30
'Celebrity' Vs 'Sunrise'	0.53	0.76
'Mountain Supreme' Vs 'Sunrise'	0.39	0.61

TABLE 6: LS means from analysis of variance of tomato fruit area of decay (cm²) five days after inoculation with *Botrytis cinerea*

SOURCE OF VARIATION	Fall 1995	Spring 1996
Low calcium	8.84	8.42
Medium calcium	3.79	9.31
High calcium	4.68	6.90
'Mountain Supreme'	5.91	9.64
'Celebrity'	6.35	8.32
'Sunrise'	4.55	6.71
Low calcium, 'Mountain Supreme'	7.71	9.68
Low calcium, 'Celebrity'	11.94	8.46
Low calcium, 'Sunrise'	7.22	7.21
Medium calcium, 'Mountain Supreme'	4.29	11.33
Medium calcium, 'Celebrity'	3.29	10.07
Medium calcium, 'Sunrise'	3.81	6.83
High calcium, 'Mountain Supreme'	5.97	8.06
High calcium, 'Celebrity'	5.25	6.61
High calcium, 'Sunrise'	3.08	6.11

TABLE 7: Effect of calcium-fertilizer solution on mineral composition of tomato leaves ($\mu\text{g g}^{-1}$ DW), LS means by treatment and cultivar, fall 1995 and spring 1996

Treatment-Cultivar Combination	Calcium	Boron	Sulfur	Magnesium	Potassium	Phosphorus
<i>Fall 1995</i>						
Low, 'Mountain Supreme'	47178.97	193.25	19055.45	21328.18	28187.71	14794.72
Low, 'Celebrity'	26245.14	153.92	13135.84	15864.73	28445.54	8193.50
Low, 'Sunrise'	36331.78	181.00	16460.35	18701.95	33001.68	9255.47
Medium, 'Mountain Supreme'	76095.31	146.34	20232.21	6744.22	26669.57	8417.67
Medium, 'Celebrity'	73923.07	137.79	13273.46	5303.56	29730.74	6520.30
Medium, 'Sunrise'	80431.74	151.18	15968.84	6545.14	29764.39	8220.19
High, 'Mountain Supreme'	81969.45	143.73	16123.49	2778.54	31816.42	5487.83
High, 'Celebrity'	77669.39	110.77	11313.07	2570.57	32669.70	4607.19
High, 'Sunrise'	77712.84	132.42	13835.54	2863.90	36996.30	5576.00
<i>Spring 1996</i>						
Low, 'Mountain Supreme'	26339.51	376.36	14218.91	25506.46	39563.29	8749.97
Low, 'Celebrity'	23570.53	350.94	10438.61	22196.91	42229.53	7186.24
Low, 'Sunrise'	23799.03	377.66	13628.89	22371.56	42087.50	9056.53
Medium, 'Mountain Supreme'	71207.99	303.17	11511.66	9062.61	23834.57	7133.56
Medium, 'Celebrity'	78550.28	284.02	11141.82	8809.17	23138.15	6319.28
Medium, 'Sunrise'	76220.75	302.85	12521.10	9066.73	29608.84	7161.13
High, 'Mountain Supreme'	94527.99	251.26	7893.54	5032.10	24925.06	5412.81
High, 'Celebrity'	100387.68	266.84	7426.07	4844.82	24401.49	5035.74
High, 'Sunrise'	90197.64	252.97	7325.90	4370.06	26435.31	5003.07

TABLE 8: Effect of calcium-fertilizer solution on mineral composition of tomato fruit ($\mu\text{g g}^{-1}$ DW), LS means by treatment and cultivar, fall 1995 and spring 1996

Treatment-Cultivar Combination	Calcium	Boron	Sulfur	Magnesium	Potassium	Phosphorus
<i>Fall 1995</i>						
Low, 'Mountain Supreme'	1269.91	90.99	141.18	2320.54	45673.98	7812.42
Low, 'Celebrity'	1196.09	80.62	174.91	1991.14	35767.65	4969.99
Low, 'Sunrise'	1039.85	78.03	174.98	2547.63	43082.14	7413.07
Medium, 'Mountain Supreme'	2171.17	97.88	154.68	1499.05	39757.26	6081.95
Medium, 'Celebrity'	1351.28	81.92	151.92	1531.56	36938.07	4382.21
Medium, 'Sunrise'	2186.52	105.16	151.62	2034.58	49931.22	7260.37
High, 'Mountain Supreme'	2258.16	94.08	120.28	1541.41	44106.28	5632.06
High, 'Celebrity'	1444.82	84.05	137.68	1436.71	43740.31	4434.51
High, 'Sunrise'	1769.39	97.18	160.69	2020.38	55213.57	6777.31
<i>Spring 1996</i>						
Low, 'Mountain Supreme'	660.93	112.30	1142.59	1664.78	31354.03	4326.73
Low, 'Celebrity'	481.70	95.94	863.54	1759.64	33467.77	3733.20
Low, 'Sunrise'	475.33	92.40	920.91	1585.14	31485.96	3903.93
Medium, 'Mountain Supreme'	1715.26	106.73	700.86	965.75	29719.72	3375.75
Medium, 'Celebrity'	1769.03	105.48	538.56	1044.91	33499.85	3038.32
Medium, 'Sunrise'	1301.22	109.65	796.96	1134.80	34009.82	3784.92
High, 'Mountain Supreme'	2104.99	102.18	610.07	758.28	29017.39	3073.52
High, 'Celebrity'	2203.02	90.66	393.47	616.69	30549.92	2518.44
High, 'Sunrise'	1037.94	83.59	651.38	1057.64	35598.58	3775.02

TABLE 9: Average^y nutrient composition (mg L⁻¹) of inflowing and outflowing low calcium-fertilizer solution as determined by ICP analysis, fall 1995

Sample Date	Nitrogen (mg L ⁻¹)	Calcium		Boron		Potassium		Magnesium		Phosphorus		Sulfur	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
10/20/95	30	17.85	16.22	1.13	1.05	56.69	45.80	11.58	11.08	13.36	13.01	17.17	17.30
10/27/95	30	23.23	17.35	1.15	0.99	65.11	48.82	13.32	11.26	15.05	12.70	18.13	16.98
11/3/95	60	20.77	14.14	1.41	1.17	120.63	55.57	20.37	10.33	28.83	13.71	27.92	17.59
11/10/95	60	19.85	14.92	1.65	1.49	140.70	102.10	19.88	15.63	24.34	24.40	30.26	27.38
11/17/95	90	16.95	13.68	1.96	1.93	183.86	153.47	26.69	19.90	42.87	35.94	35.93	34.50
11/24/95	90	20.15	13.43	2.07	2.14	180.42	153.84	28.76	20.71	42.31	37.00	38.51	38.67
12/1/95	90	20.22	16.82	1.71	2.16	169.49	180.34	27.14	26.50	35.55	39.23	37.54	38.74
12/8/95	90	17.71	15.09	1.27	1.44	168.10	154.37	27.26	25.83	37.74	37.45	36.46	38.09
12/15/95	90	24.98	18.58	1.18	1.26	168.48	151.93	29.99	27.32	36.95	37.36	36.45	37.12
12/22/95	90	27.27	23.45	2.70	3.09	163.02	159.05	30.55	31.83	39.49	41.12	38.97	39.71
1/4/96	90	26.03	25.87	3.32	4.38	165.36	164.29	31.13	34.79	39.75	41.68	44.75	45.65
1/12/96	10	28.88	23.36	1.16	3.13	12.08	68.59	8.12	20.89	2.49	20.77	14.70	31.27
1/19/96	10	28.33	20.32	1.49	2.08	27.85	22.18	10.34	13.63	6.62	8.63	17.91	20.22
1/26/96	10	27.64	17.84	1.39	1.49	22.74	9.11	9.35	10.09	5.44	3.98	19.21	16.53
2/9/96	30	23.44	14.90	2.09	1.35	68.58	9.54	14.28	9.44	14.75	4.14	21.27	18.01
2/16/96	40	24.30	11.73	2.51	1.54	79.75	13.82	17.19	8.43	18.42	4.03	24.25	21.79
3/1/96	40	22.71	11.28	2.55	1.84	84.71	25.69	17.34	6.64	19.99	3.53	24.14	25.52
3/7/96	80	22.35	13.86	4.37	2.03	158.38	25.31	26.11	6.84	38.46	5.40	34.04	31.01
mean	NA	22.90	16.80	1.95	1.92	113.10	85.77	20.52	17.29	25.69	21.34	28.76	28.67
standard deviation	NA	03.76	04.13	0.87	0.87	60.36	64.80	8.06	8.95	13.90	15.23	9.35	9.70
range	NA	16.95-28.88	11.28-25.87	1.13-4.37	0.99-4.38	12.08-183.86	9.11-180.34	8.12-31.13	6.64-34.79	2.49-42.87	3.53-41.68	14.70-44.75	16.53-45.65

^yAverage across three blocks

TABLE 10: Average^y nutrient composition (mg L⁻¹) of inflowing and outflowing medium calcium-fertilizer solution as determined by ICP analysis, fall 1995

Sample Date	Nitrogen (mg L ⁻¹)	Calcium		Boron		Potassium		Magnesium		Phosphorus		Sulfur	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
10/20/95	30	209.46	193.89	1.11	1.15	63.23	52.72	11.54	11.42	11.58	4.25	17.26	17.69
10/27/95	30	201.95	198.34	1.10	1.14	68.06	50.50	12.96	11.59	8.70	4.19	18.31	17.55
11/3/95	60	209.94	197.50	1.73	1.12	126.68	58.51	19.94	11.77	26.43	5.52	28.02	17.71
11/10/95	60	207.99	194.58	1.60	1.62	131.01	103.38	19.15	17.75	27.35	14.14	29.50	27.07
11/17/95	90	207.74	202.14	2.09	2.01	192.78	157.44	25.92	23.17	42.49	26.93	35.83	34.37
11/24/95	90	213.13	216.68	2.02	1.90	172.90	165.87	26.83	26.96	40.54	29.59	37.50	38.46
12/1/95	90	208.71	216.28	1.73	1.94	157.01	163.15	25.54	28.62	33.95	31.35	36.46	38.15
12/8/95	90	200.45	399.99	1.78	2.17	148.55	152.16	24.45	31.34	34.20	29.41	33.22	37.95
12/15/95	90	184.91	253.21	2.81	3.12	163.71	155.16	29.43	32.93	21.99	31.88	37.83	39.50
12/22/95	90	190.60	247.60	2.91	3.29	167.94	159.09	29.53	35.04	24.23	32.64	39.01	40.92
1/4/96	90	192.34	247.57	4.55	5.00	175.91	172.28	30.70	36.09	24.87	29.38	46.07	45.20
1/12/96	10	30.50	124.87	1.18	3.17	13.36	68.05	8.47	22.77	2.94	13.44	15.81	29.51
1/19/96	10	154.09	96.91	1.69	2.42	30.23	19.69	10.34	18.76	5.70	6.71	18.55	21.89
1/26/96	10	108.27	122.40	1.51	1.79	24.34	7.57	9.36	15.00	5.33	2.40	19.72	17.22
2/9/96	30	136.06	109.94	2.35	1.44	65.43	6.64	13.71	12.39	7.02	1.79	21.31	17.45
2/16/96	40	104.98	151.28	2.47	1.52	79.44	12.90	16.62	13.24	10.63	0.71	24.36	22.33
3/1/96	40	204.86	241.00	2.69	1.76	85.37	40.20	16.64	13.92	4.84	0.47	23.49	25.21
3/7/96	80	205.72	305.75	4.60	2.07	162.28	35.54	25.40	16.73	24.52	2.01	34.40	30.67
mean	NA	176.21	206.66	2.22	2.15	112.68	87.83	19.81	21.08	19.85	14.82	28.70	28.83
standard deviation	NA	50.4	73.89	1.02	0.98	58.88	64.13	7.59	8.73	13.02	13.16	9.15	09.61
range	NA	30.50-213.13	96.91-399.99	1.10-4.60	1.12-5.00	13.36-192.78	6.64-172.28	8.47-30.70	11.42-36.09	2.94-42.49	0.47-32.64	15.81-46.07	17.22-45.20

^yAverage across three blocks

TABLE 11: Average^y nutrient composition (mg L⁻¹) of inflowing and outflowing high calcium-fertilizer solution as determined by ICP analysis, fall 1995

Sample Date	Nitrogen (mg L ⁻¹)	Calcium		Boron		Potassium		Magnesium		Phosphorus		Sulfur	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
10/20/95	30	900.18	951.53	9.66	8.73	76.95	73.49	10.88	11.73	6.52	2.57	16.57	18.14
10/27/95	30	978.46	989.53	10.32	9.95	98.70	78.10	14.03	12.52	7.38	2.25	19.04	18.01
11/3/95	60	969.15	1011.28	10.36	9.50	158.68	85.26	20.46	12.79	20.07	3.58	27.84	17.77
11/10/95	60	1011.38	1054.44	10.76	10.99	133.99	121.61	19.46	19.27	22.64	11.78	29.18	26.50
11/17/95	90	981.60	1057.58	12.05	12.01	192.97	167.25	25.67	24.42	32.00	24.31	35.33	34.11
11/24/95	90	967.33	1129.45	13.93	12.49	194.50	183.12	28.15	29.72	27.17	27.80	39.44	40.76
12/1/95	90	1010.37	1126.02	10.70	11.28	172.44	188.20	27.10	30.67	35.89	30.38	39.10	41.08
12/8/95	90	1020.01	1190.57	9.68	10.32	190.61	179.32	28.39	32.84	39.61	30.00	38.07	40.80
12/15/95	90	997.18	1179.80	11.05	10.76	192.06	186.24	30.41	34.00	23.64	29.12	38.97	40.44
12/22/95	90	956.40	1144.17	9.78	10.09	208.08	186.97	31.54	34.97	16.81	27.98	40.90	42.03
1/4/96	90	1009.99	1175.56	9.59	11.01	204.44	205.53	31.51	36.92	34.49	25.40	46.01	44.57
1/12/96	10	1031.23	1282.18	8.36	9.86	30.95	61.35	7.95	19.14	1.98	6.86	14.23	22.70
1/19/96	10	997.44	1320.71	6.55	9.61	48.43	25.67	9.91	14.89	0.23	2.47	16.49	13.76
1/26/96	10	1010.99	1362.71	3.68	2.91	41.93	14.40	8.97	12.81	0.98	1.45	18.25	13.07
2/9/96	30	988.11	1363.27	1.22	0.94	82.72	27.78	13.90	15.05	10.74	2.15	24.26	20.86
2/16/96	40	959.10	1357.30	1.32	1.05	95.92	38.21	16.02	15.50	1.12	0.49	26.34	21.33
3/1/96	40	997.94	1695.60	1.18	1.09	103.44	69.06	16.91	20.74	15.44	0.74	27.20	30.10
3/7/96	80	982.29	1815.91	1.49	1.21	179.40	70.29	25.63	21.56	35.74	0.96	38.81	33.39
mean	NA	987.18	1233.76	7.87	7.99	133.68	108.99	20.38	22.20	18.47	12.79	29.78	28.86
standard deviation	NA	30.4	230.27	4.19	4.29	61.47	67.42	8.31	8.80	13.63	12.70	10.08	10.94
range	NA	900.18-1031.23	951.53-1815.91	1.18-13.93	0.94-12.49	30.95-208.08	14.40-205.53	7.95-31.54	11.73-36.92	0.23-39.61	0.49-30.38	14.23-46.01	13.07-44.57

^yAverage across three blocks

TABLE 12: Average^y nutrient composition (mg L⁻¹) of inflowing and outflowing low calcium-fertilizer solution as determined by ICP analysis, spring 1996

Sample Date	Nitrogen (mg L ⁻¹)	Calcium		Boron		Potassium		Magnesium		Phosphorus		Sulfur	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
1/26/96	10	31.36	---	0.15	---	31.57	---	11.01	---	5.51	---	19.90	---
2/9/96	30	25.96	---	0.25	---	82.16	---	16.56	---	15.79	---	25.28	---
2/16/96	40	25.45	---	0.28	---	102.75	---	18.64	---	18.31	---	26.92	---
3/1/96	40	25.16	---	0.31	---	114.05	---	20.45	---	22.02	---	29.14	---
3/8/96	80	24.67	---	0.61	---	226.21	---	30.43	---	42.46	---	41.17	---
3/15/96	80	26.95	---	0.58	---	218.63	---	31.69	---	38.86	---	41.85	---
3/25/96	80	27.69	27.67	0.57	0.49	185.35	103.37	29.58	24.11	40.35	31.00	38.82	34.84
3/29/96	80	22.29	28.80	0.49	0.58	179.84	152.67	29.09	30.26	38.84	38.98	48.87	44.69
4/5/96	80	22.47	28.62	0.53	0.58	163.99	160.24	26.30	30.16	35.93	39.33	33.93	38.81
4/12/96	80	30.46	28.20	0.55	0.56	211.77	183.05	32.04	30.87	42.33	41.42	40.74	40.27
4/19/96	80	30.10	27.06	0.55	0.57	203.49	176.91	31.65	29.01	40.63	41.00	40.08	39.62
4/26/96	80	26.97	20.39	0.53	0.57	205.08	160.60	31.71	22.83	40.94	39.02	42.40	41.38
5/3/96	100	28.77	16.81	0.75	0.66	258.50	126.82	39.04	18.66	48.79	32.07	50.88	38.44
5/10/96	140	23.50	16.35	1.01	0.97	332.17	231.07	49.23	33.38	68.09	59.80	67.18	62.26
5/17/96	180	20.63	16.86	1.35	1.44	326.91	251.46	48.32	38.43	72.36	73.23	65.27	67.43
5/24/96	180	21.17	18.39	1.43	1.56	356.04	305.99	53.92	49.99	81.67	78.70	72.55	77.15
6/7/96	180	30.52	28.98	1.43	1.71	374.17	392.96	60.95	74.15	85.23	96.09	78.62	97.14
6/21/96	180	27.32	32.51	1.46	1.73	365.94	436.08	66.18	85.85	85.20	103.40	71.05	98.90
6/28/96	90	29.64	36.66	0.97	1.63	176.29	410.09	35.50	81.13	42.38	102.38	39.15	95.77
mean	NA	26.37	25.18	0.73	1.00	216.57	237.79	34.86	42.22	45.56	59.73	45.99	59.75
standard deviation	NA	3.32	6.65	0.43	0.52	99.10	113.38	14.90	23.17	23.28	27.60	17.27	24.93
range	NA	20.63-31.36	16.35-36.66	0.15-1.46	0.49-1.73	31.57-374.17	103.37-436.08	11.01-66.18	18.66-85.85	5.51-85.23	31.00-103.40	19.90-78.62	34.84-98.90

^yAverage across three blocks

^zNo outflowing samples were taken from 1-26-96 through 3-15-96

TABLE 13: Average^y nutrient composition (mg L⁻¹) of inflowing and outflowing medium calcium-fertilizer solution as determined by ICP analysis, spring 1996

Sample Date	Nitrogen (mg L ⁻¹)	Calcium		Boron		Potassium		Magnesium		Phosphorus		Sulfur	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
1/26/96	10	66.61	---	0.18	---	32.68	---	11.25	---	5.53	---	19.40	---
2/9/96	30	138.80	---	0.38	---	84.77	---	16.48	---	0.85	---	22.86	---
2/16/96	40	112.68	---	0.34	---	105.95	---	19.80	---	3.26	---	25.07	---
3/1/96	40	254.34	---	0.43	---	119.28	---	21.17	---	4.36	---	26.27	---
3/8/96	80	263.74	---	0.63	---	238.10	---	33.19	---	24.06	---	38.05	---
3/15/96	80	267.99	---	0.64	---	226.27	---	34.80	---	20.37	---	38.95	---
3/25/96	80	233.62	176.81	0.58	0.46	207.75	123.66	32.90	27.29	21.56	14.37	49.97	43.19
3/29/96	80	215.55	182.78	0.58	0.50	182.78	143.65	29.82	28.04	23.81	17.70	37.99	34.84
4/5/96	80	200.16	209.08	0.52	0.51	203.13	170.36	31.22	32.24	24.00	20.43	37.37	40.00
4/12/96	80	210.97	214.37	0.57	0.56	219.51	188.45	33.71	32.22	20.50	23.70	38.28	39.78
4/19/96	80	198.91	213.01	0.55	0.60	206.62	184.77	32.93	32.34	18.05	23.71	37.82	42.15
4/26/96	80	205.58	227.65	0.59	0.62	219.21	169.15	34.32	31.91	22.01	22.96	41.00	43.71
5/3/96	100	217.97	256.07	0.87	0.79	273.76	173.26	42.22	35.09	27.47	21.09	48.25	44.04
5/10/96	140	148.80	283.93	0.75	1.10	235.71	262.20	34.10	51.58	38.13	48.54	43.29	68.00
5/17/96	180	200.83	257.19	1.36	1.60	320.82	274.00	47.67	53.74	68.10	67.52	64.05	72.50
5/24/96	180	213.67	293.10	1.47	1.67	372.29	360.54	56.02	67.23	77.85	80.97	77.76	92.22
6/7/96	180	170.31	296.95	1.46	1.84	386.05	438.49	71.08	97.25	75.06	95.05	74.38	99.36
6/21/96	180	212.22	324.35	1.44	1.90	369.72	512.06	66.23	109.83	82.81	108.87	70.86	111.24
6/28/96	90	39.82	295.22	1.00	1.78	178.73	479.09	35.45	100.90	44.35	106.13	40.60	104.75
mean	NA	188.03	248.5	0.76	1.07	220.17	267.67	36.02	53.82	31.69	50.08	43.80	64.29
standard deviation	NA	62.10	47.90	0.40	0.59	096.34	135.30	15.45	30.30	26.01	36.57	16.99	28.52
range	NA	39.82- 267.99	176.8- 324.35	0.18- 1.47	0.46- 1.90	32.68- 386.05	123.66- 512.06	11.25- 71.08	27.29- 109.83	0.85- 82.81	14.37- 108.87	19.40- 77.76	34.84- 111.24

^yAverage across three blocks

^zNo outflowing samples were taken from 1-26-96 through 3-15-96

TABLE 14: Average^y nutrient composition (mg L⁻¹) of inflowing and outflowing high calcium-fertilizer solution as determined by ICP analysis, spring 1996

Sample Date	Nitrogen (mg L ⁻¹)	Calcium		Boron		Potassium		Magnesium		Phosphorus		Sulfur	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
1/26/96	10	1287.27	--- ^z	2.37	--- ^z	51.20	--- ^z	12.76	--- ^z	1.72	--- ^z	22.65	--- ^z
2/9/96	30	1150.46	---	2.48	---	92.80	---	16.72	---	11.15	---	24.42	---
2/16/96	40	1289.81	---	2.68	---	120.80	---	22.49	---	3.38	---	32.79	---
3/1/96	40	1213.59	---	2.48	---	114.13	---	20.99	---	16.23	---	28.38	---
3/8/96	80	1143.04	---	2.79	---	210.13	---	32.04	---	32.94	---	42.49	---
3/15/96	80	3216.40	---	2.71	---	269.33	---	34.80	---	22.38	---	41.88	---
3/25/96	80	3774.64	968.59	7.36	8.17	258.64	146.25	18.54	19.89	15.78	10.92	42.57	40.94
3/29/96	80	2013.57	1467.29	6.88	6.90	197.02	201.99	18.09	24.14	18.64	18.47	37.18	42.56
4/5/96	80	966.32	1154.97	8.57	7.55	188.74	174.39	21.42	21.93	18.20	15.53	41.56	40.41
4/12/96	80	999.16	983.08	8.44	7.16	195.36	174.39	25.86	21.15	18.76	16.63	47.14	40.18
4/19/96	80	1167.01	1156.55	8.22	7.68	200.88	171.08	23.01	24.62	29.02	16.54	43.04	46.06
4/26/96	80	1007.56	1260.78	7.15	7.50	196.47	163.36	21.33	23.96	23.30	13.91	43.17	44.26
5/3/96	100	3548.77	1299.97	8.33	7.07	279.25	132.45	35.79	21.88	28.30	8.31	64.98	41.17
5/10/96	140	1781.25	1517.08	8.13	7.37	321.19	229.58	45.84	40.45	49.50	27.63	82.96	65.86
5/17/96	180	574.76	1688.33	7.38	7.56	324.50	274.28	42.42	54.47	59.39	51.52	72.52	82.62
5/24/96	180	900.09	2356.19	8.27	8.55	381.90	402.87	53.79	92.51	70.70	96.49	88.39	137.44
6/7/96	180	1059.67	1916.38	6.70	8.25	400.66	471.85	54.37	92.37	54.71	90.65	84.13	129.69
6/21/96	180	1151.87	2357.30	7.64	9.26	363.13	560.15	48.15	110.02	67.31	108.57	78.97	158.33
6/28/96	90	561.88	1817.53	6.81	8.39	201.43	532.56	24.98	91.16	41.32	91.69	47.46	130.00
mean	NA	1516.16	1534.16	6.07	7.80	229.87	279.63	30.18	49.12	30.67	43.61	50.88	76.89
standard deviation	NA	0953.84	469.73	2.50	0.68	98.68	155.53	13.08	34.53	20.80	38.66	21.04	45.19
range	NA	561.88-3548.77	968.59-2357.30	2.37-8.57	6.90-9.26	51.20-400.66	132.45-560.15	12.76-54.37	19.89-110.02	1.72-70.70	8.31-108.57	22.65-88.30	40.18-158.33

^xBased on full strength of fertilizer solution of 180 mg L⁻¹ Nitrogen

^yAverage across three blocks

^zNo outflowing samples were taken from 1-26-96 through 3-15-96

TABLE 15: Cultivars exhibiting the highest^y tissue calcium content ($\mu\text{g g}^{-1}$ DW) and lowest area of decay (cm^2)^y, as determined by contrast analysis for each treatment

Treatment	Season	Highest Leaf Ca	Highest Fruit Ca	Highest Ca Partitioning	Lowest Area of Decay
20 mg L ⁻¹ Ca	Fall	*Mountain Supreme	Mountain Supreme	Celebrity	***Sunrise
	Spring	Mountain Supreme	*Mountain Supreme	Mountain Supreme	*Sunrise
220 mg L ⁻¹ Ca	Fall	Sunrise	**Sunrise	Mountain Supreme	Celebrity
	Spring	Celebrity	**Celebrity	Mountain Supreme	***Sunrise
1020 mg L ⁻¹ Ca	Fall	Mountain Supreme	*Mountain Supreme	Mountain Supreme	Sunrise
	Spring	Celebrity	*Celebrity	Mountain Supreme	Sunrise

^yAs observed from lsmeans

* Denotes significance at the 0.05 level; **0.01; ***0.001

**TABLE 16: Peters Professional 5-11-26
Hydro-Sol Nutrient Composition
(per 100 mg L⁻¹ N)**

Nutrient	Concentration (mg L^{-1})
Nitrate-N	100.00
Phosphorus (P)	96.00
Potassium (K)	431.70
Calcium (Ca)	0.00
Magnesium (Mg)	62.20
Boron (B)	1.00
Copper (Cu)	0.30
Iron (Fe)	6.00
Manganese (Mn)	1.00
Molybdenum (Mo)	0.21
Zinc (Zn)	0.30

TABLE 17: Monthly greenhouse temperature and relative humidity during Fall 1995 and Spring 1996

Month	Temperature (°C) ^z		Percent Relative Humidity ^z	
	Minimum	Maximum	Minimum	Maximum
FALL 1995				
September 1995	18.4 ± 1.5	35.1 ± 2.2	33.1 ± 6.50	76.9 ± 1.90
October 1995	18.1 ± 2.5	32.9 ± 4.6	31.7 ± 16.1	73.4 ± 11.8
November 1995	18.1 ± 1.1	30.3 ± 5.7	36.4 ± 16.6	71.4 ± 7.80
December 1995	15.6 ± 3.5	27.2 ± 4.8	40.7 ± 13.0	72.1 ± 7.50
January 1996	16.1 ± 2.9	27.1 ± 4.4	42.3 ± 11.7	70.5 ± 5.80
February 1996	17.1 ± 4.4	31.3 ± 6.0	35.5 ± 12.4	69.0 ± 7.50
March 1996	18.4 ± 2.8	33.8 ± 7.1	28.2 ± 18.3	64.2 ± 11.4
Range over experiment	15.6 -18.4	27.1-35.1	28.2 - 42.3	64.2 - 76.9
SPRING 1996				
January 1996	16.1 ± 2.9	27.1 ± 4.4	42.3 ± 11.7	70.5 ± 5.80
February 1996	17.1 ± 4.4	31.3 ± 6.0	35.5 ± 12.4	69.0 ± 7.50
March 1996	18.4 ± 2.8	33.8 ± 7.1	28.2 ± 18.3	64.2 ± 11.4
April 1996	17.8 ± 3.9	33.0 ± 4.2	39.5 ± 4.90	80.8 ± 7.40
May 1996	17.5 ± 3.2	34.0 ± 3.1	45.0 ± 9.40	89.2 ± 4.40
June 1996	19.4 ± 2.0	35.6 ± 3.9	48.2 ± 9.20	92.3 ± 1.70
July 1996	22.2 ± 1.0	38.3 ± 1.0	46.5 ± 2.20	93.0 ± 0.50
Range over experiment	16.1-22.2	27.1-38.3	28.2 - 48.2	64.2 - 93.0

^zValues are the mean ± standard deviation

TABLE 18: Timetable of events

Event	Fall 1995	No. Days	Spring 1996	No. Days
Planting	8/18/95	---	1/10/96	---
Harvest	12/27/95	100	5/23/96	165
Termination	03/8/96	72	7/03/96	41
Total Days	172	---	206	---

APPENDIX B

FIGURES

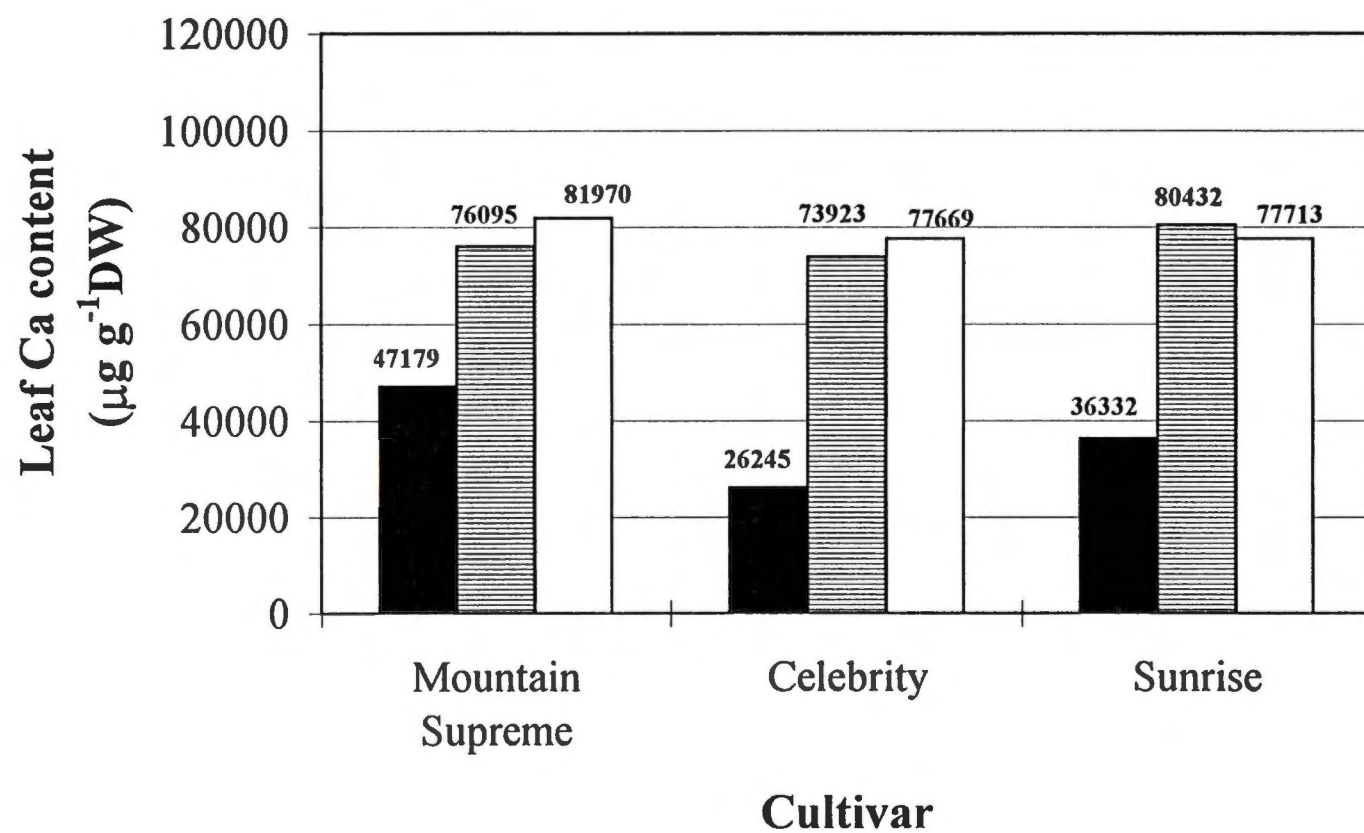


FIGURE 1: Leaf calcium (Ca) content of three tomato cultivars subjected to three Ca treatments in fall 1995. Ca treatments are: black bars=20 mg L⁻¹, lined bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above bars are the means.

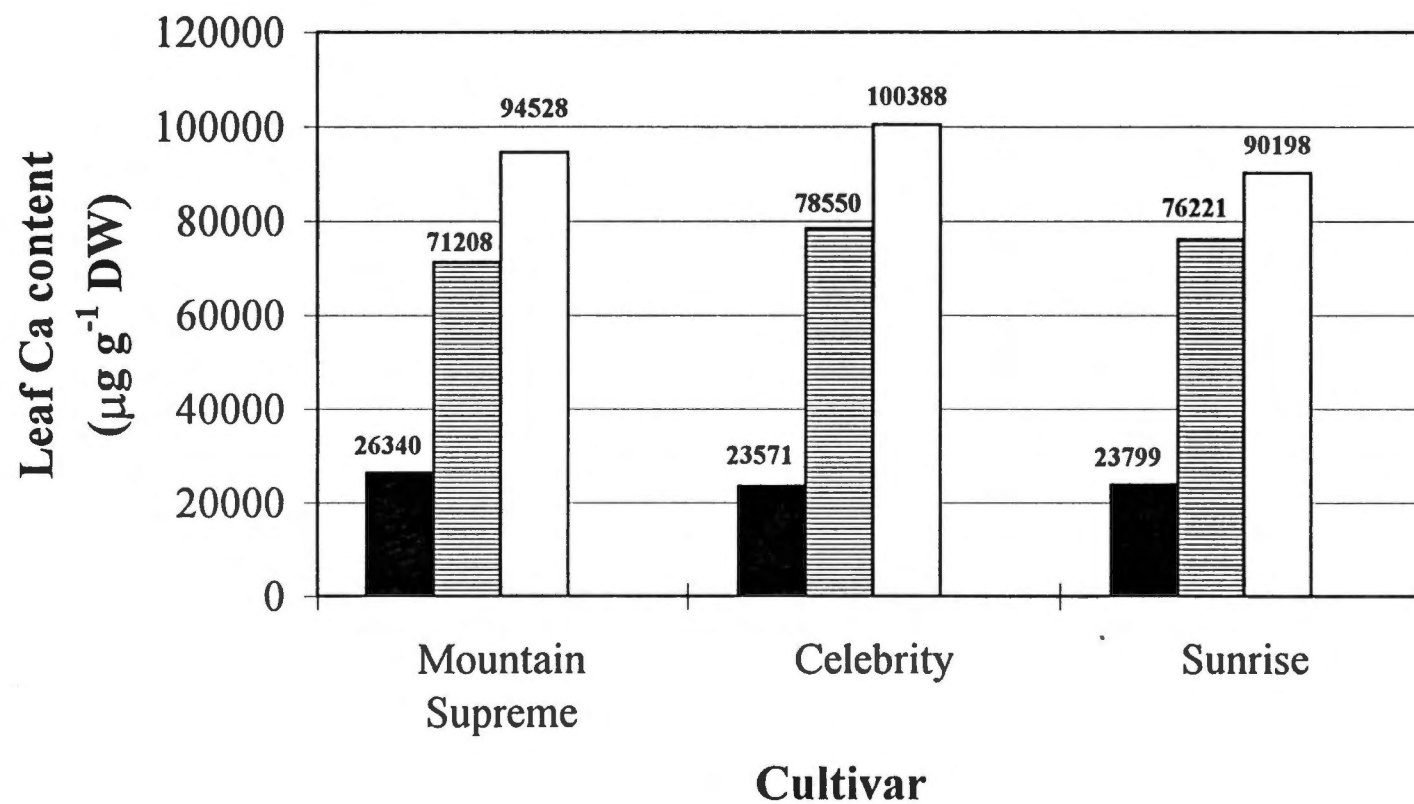


FIGURE 2: Leaf calcium (Ca) content of three tomato cultivars subjected to three Ca treatments in spring 1996. Ca treatments are: black bars=20 mg L⁻¹, lined bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above bars are the means.

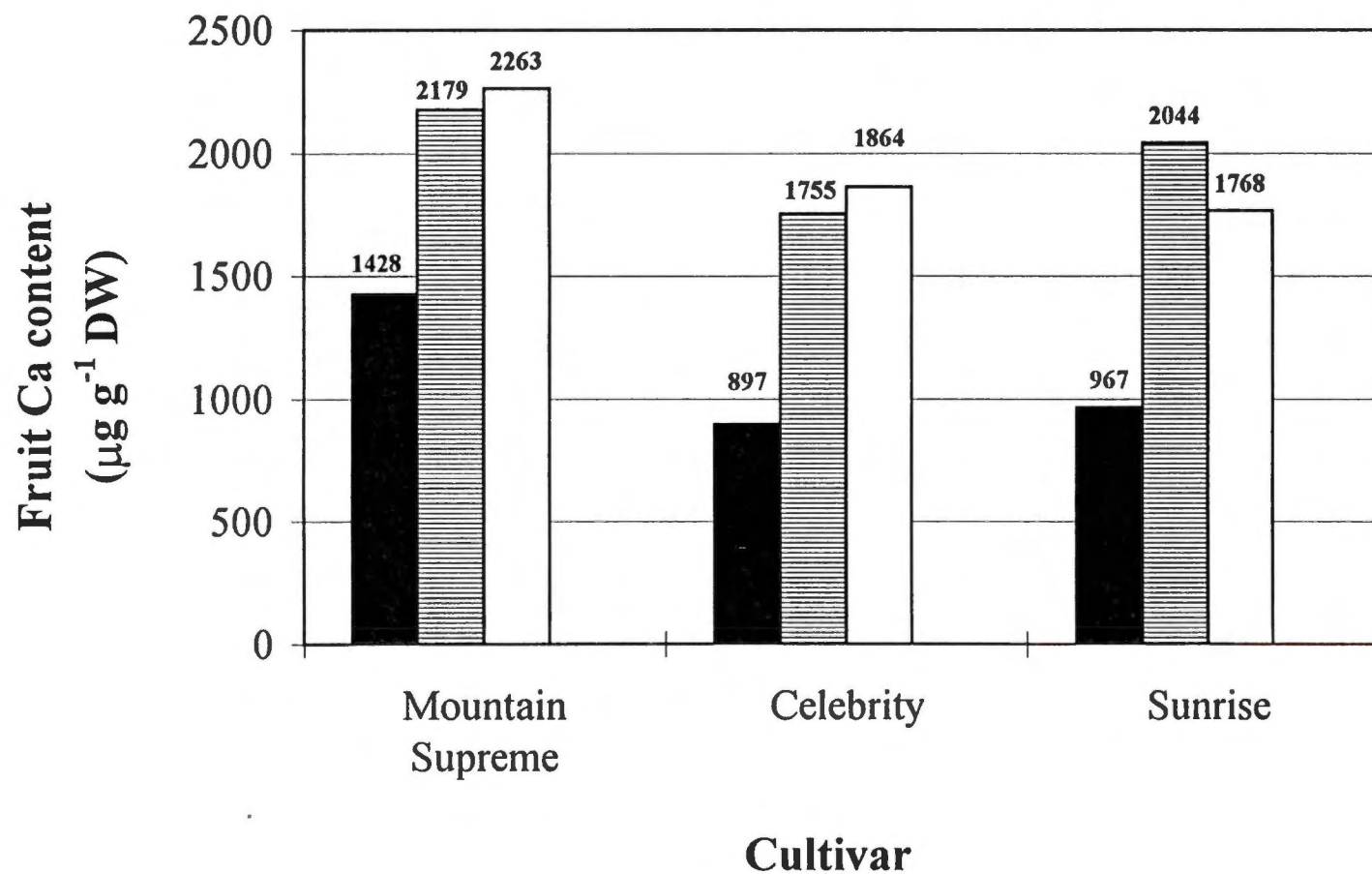


FIGURE 3: Fruit calcium (Ca) content of three tomato cultivars subjected to three Ca treatments in fall 1995. Ca treatments are: black bars=20 mg L⁻¹, lined bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above the bars are the means.

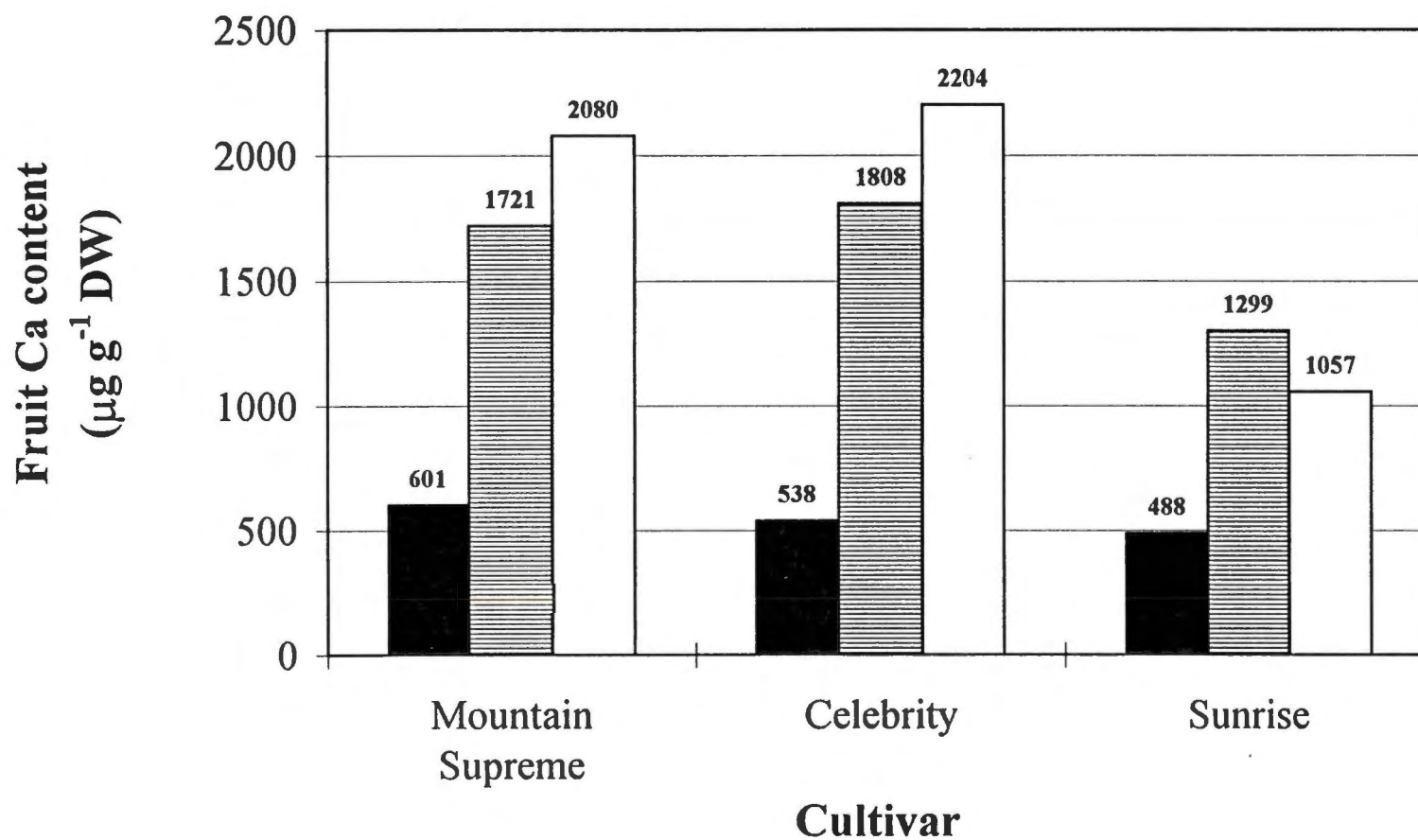


FIGURE 4: Fruit calcium (Ca) content of three tomato cultivars subjected to three Ca treatments in spring 1996. Ca treatments are: black bars=20 mg L⁻¹, horizontal bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above the bars are the means.

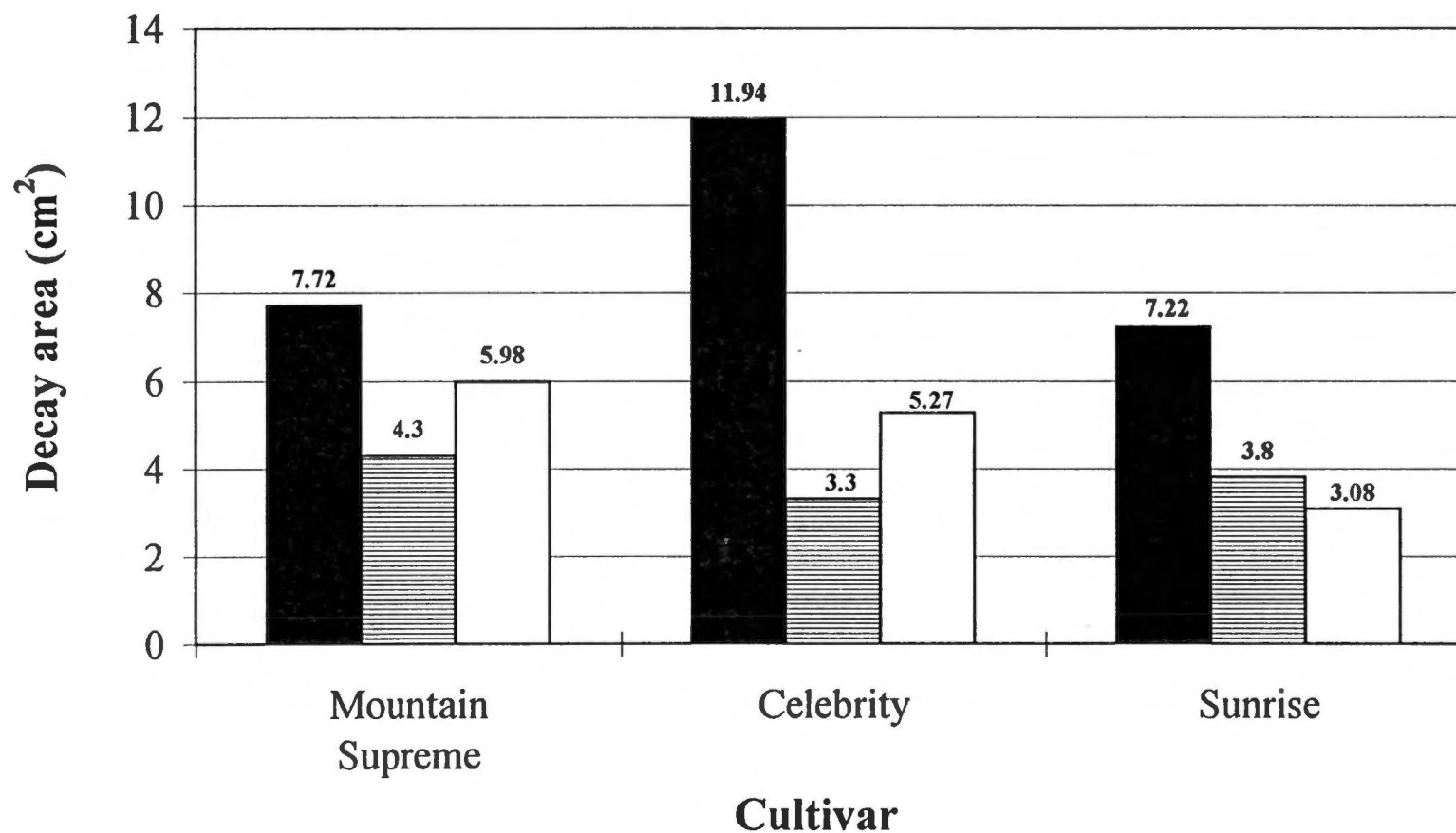


FIGURE 5: Fruit decay area of three tomato cultivars five days after inoculation with *Botrytis cinerea* in fall 1995. Calcium treatments are: black bars=20 mg L⁻¹, lined bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above bars are the means.

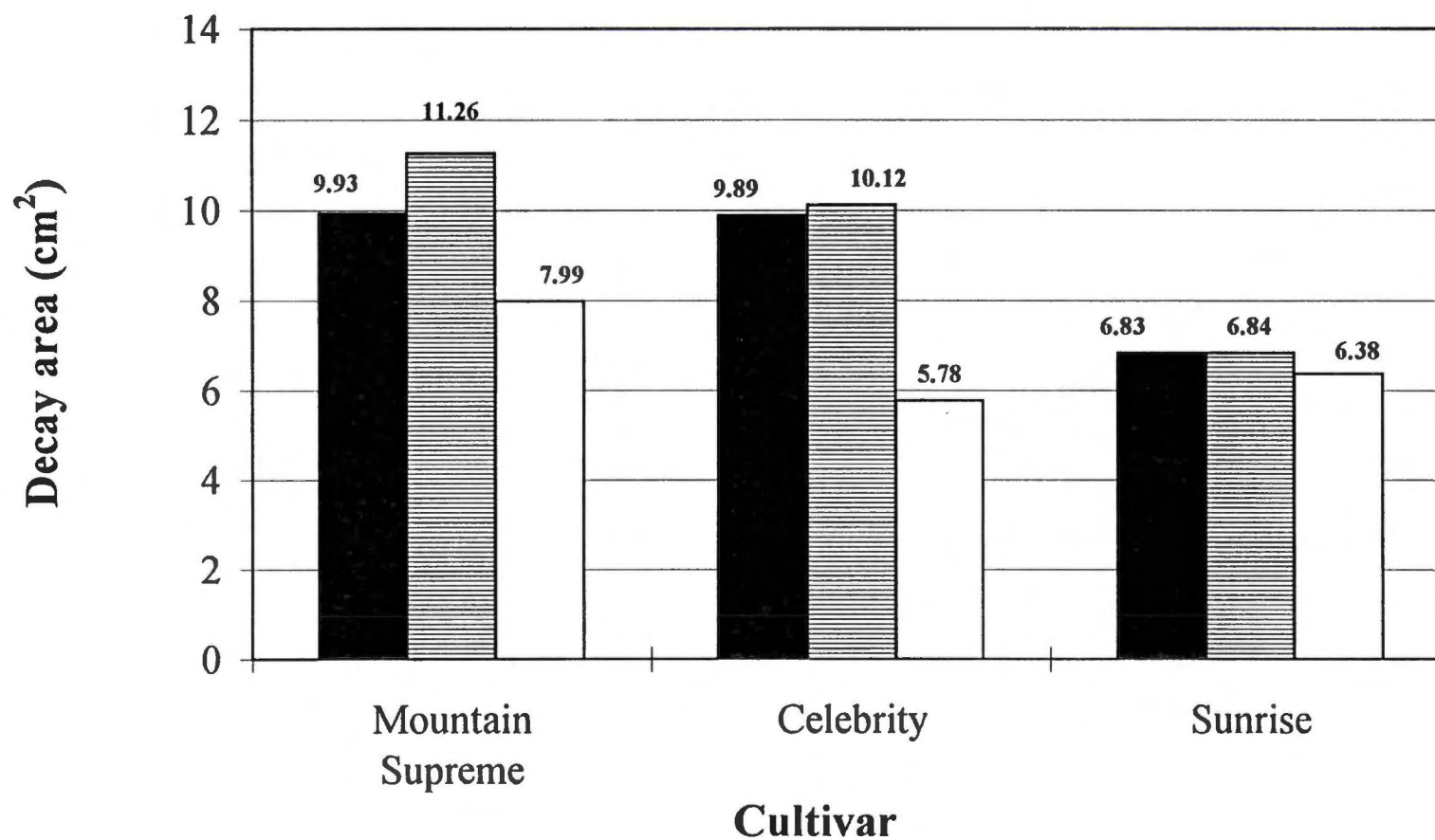


FIGURE 6: Fruit decay area of three tomato cultivars five days after inoculation with *Botrytis cinerea* in spring 1996. Calcium treatments are: black bars=20 mg L⁻¹, lined bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above bars are the means.

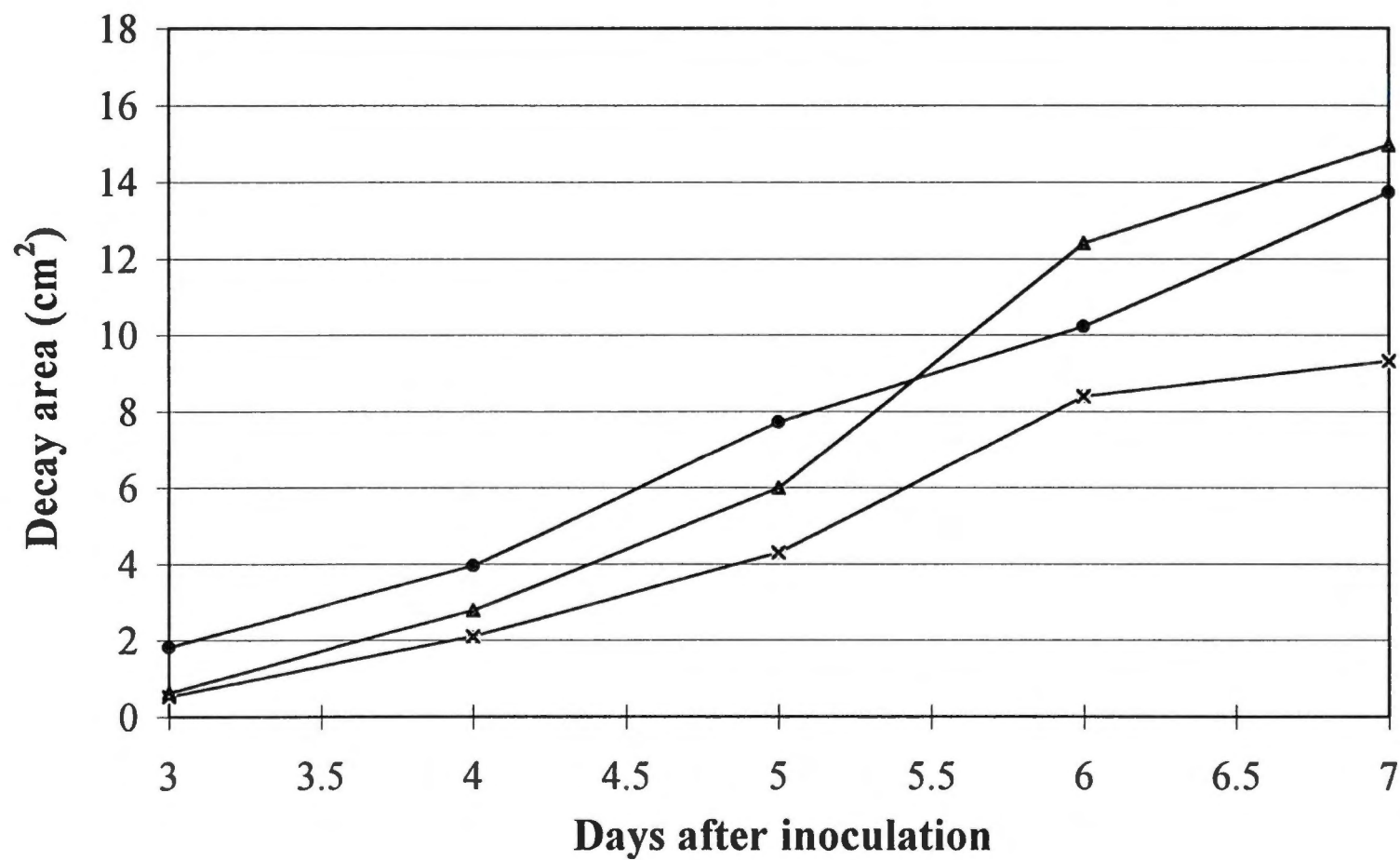


FIGURE 7: Tomato fruit decay area over time in 'Mountain Supreme' after inoculation with *Botrytis cinerea* in fall 1995. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

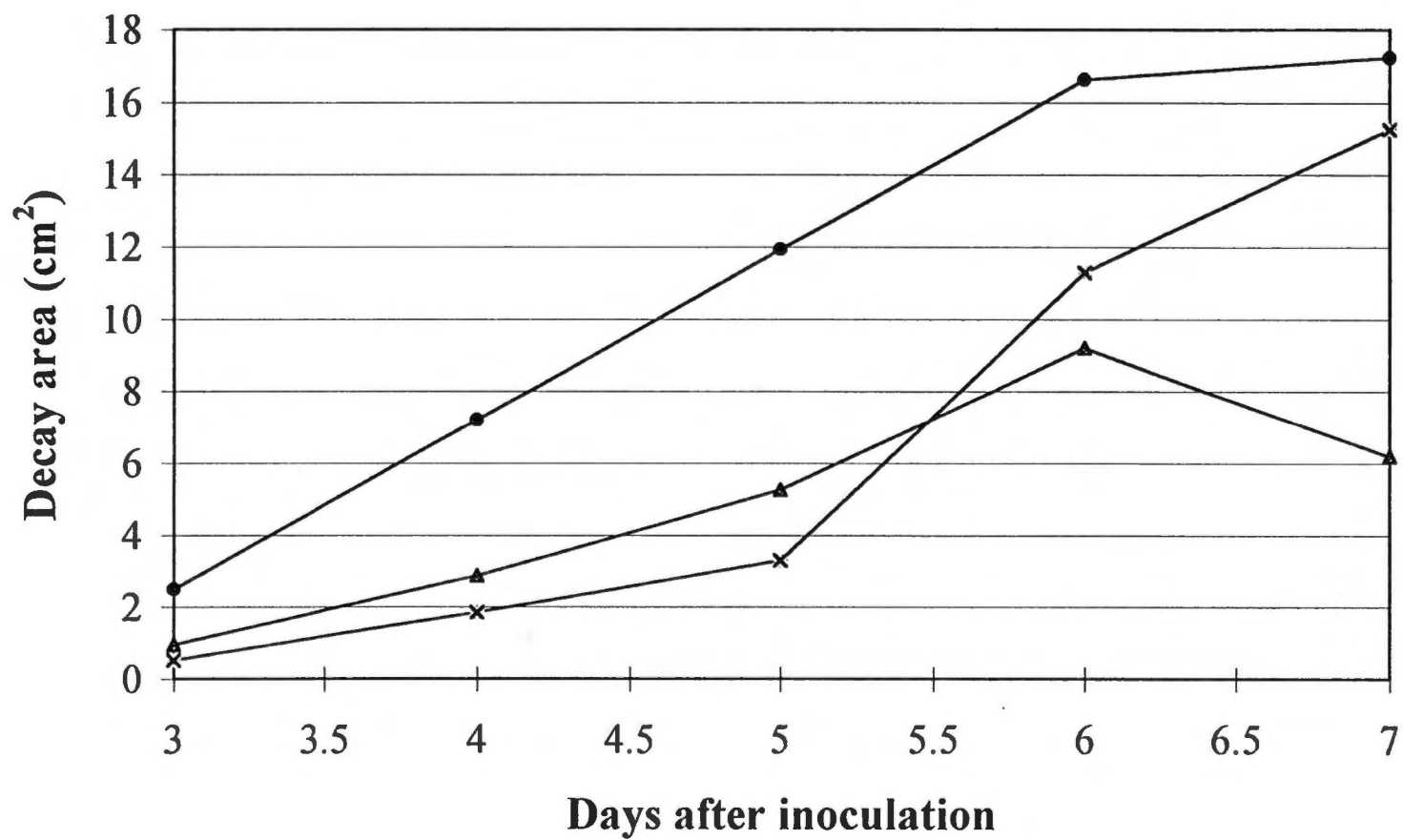


FIGURE 8: Tomato fruit decay area over time in 'Celebrity' after inoculation with *Botrytis cinerea* in fall 1995. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

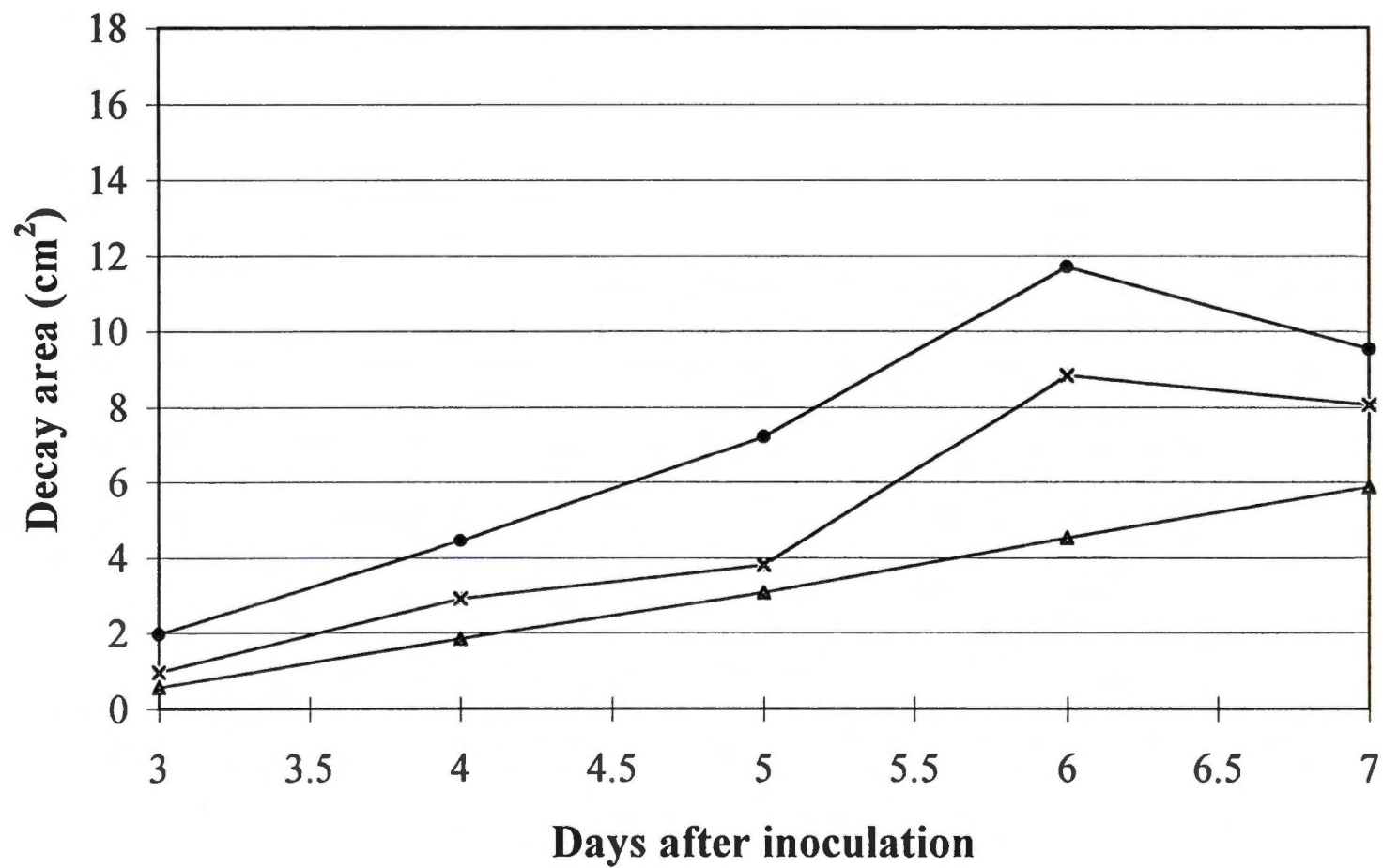


FIGURE 9: Tomato fruit decay area over time in 'Sunrise' after inoculation with *Botrytis cinerea* in fall 1995. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

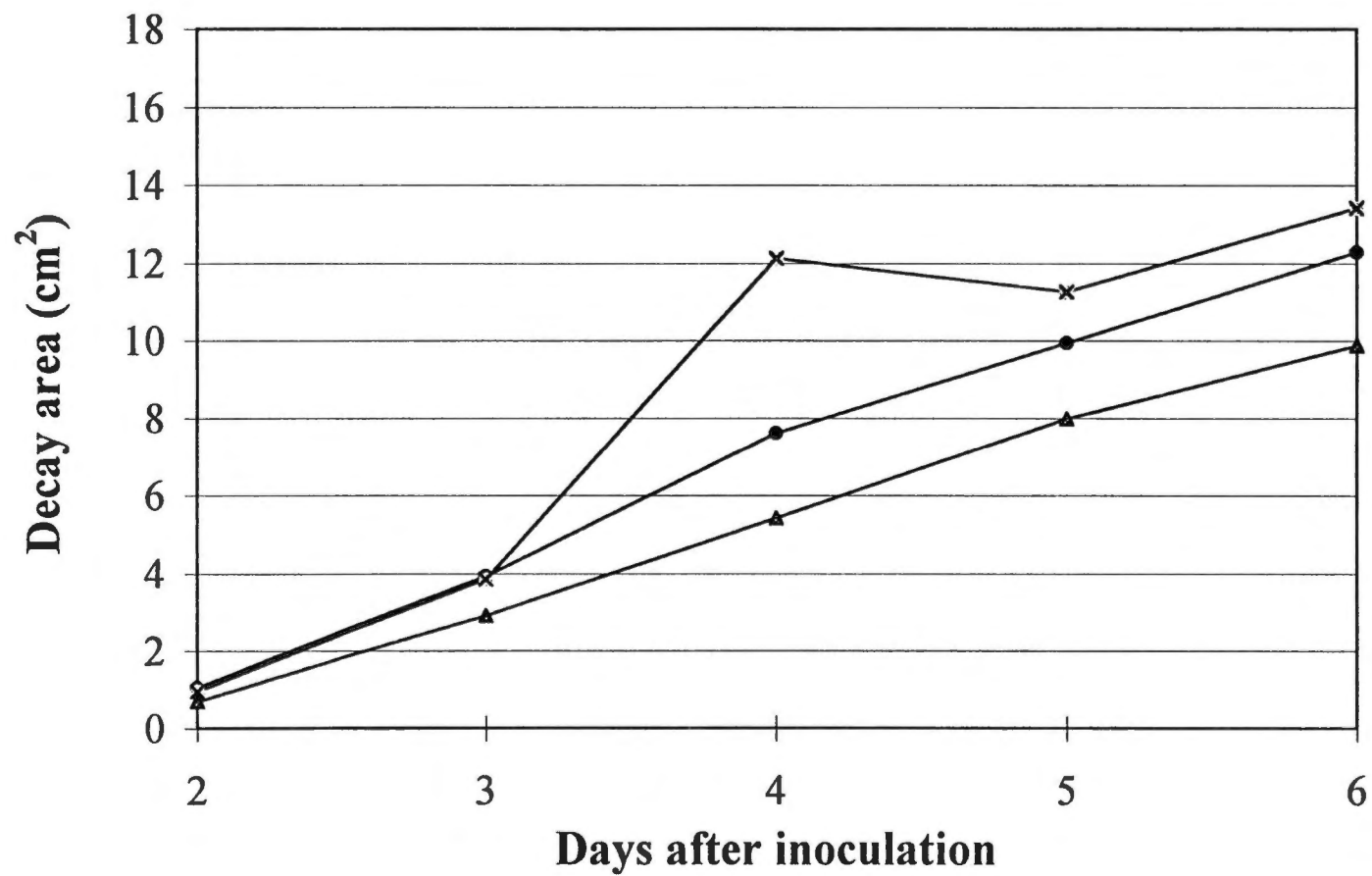


FIGURE 10: Tomato fruit decay area over time in 'Mountain Supreme' after inoculation with *Botrytis cinerea* in spring 1996. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

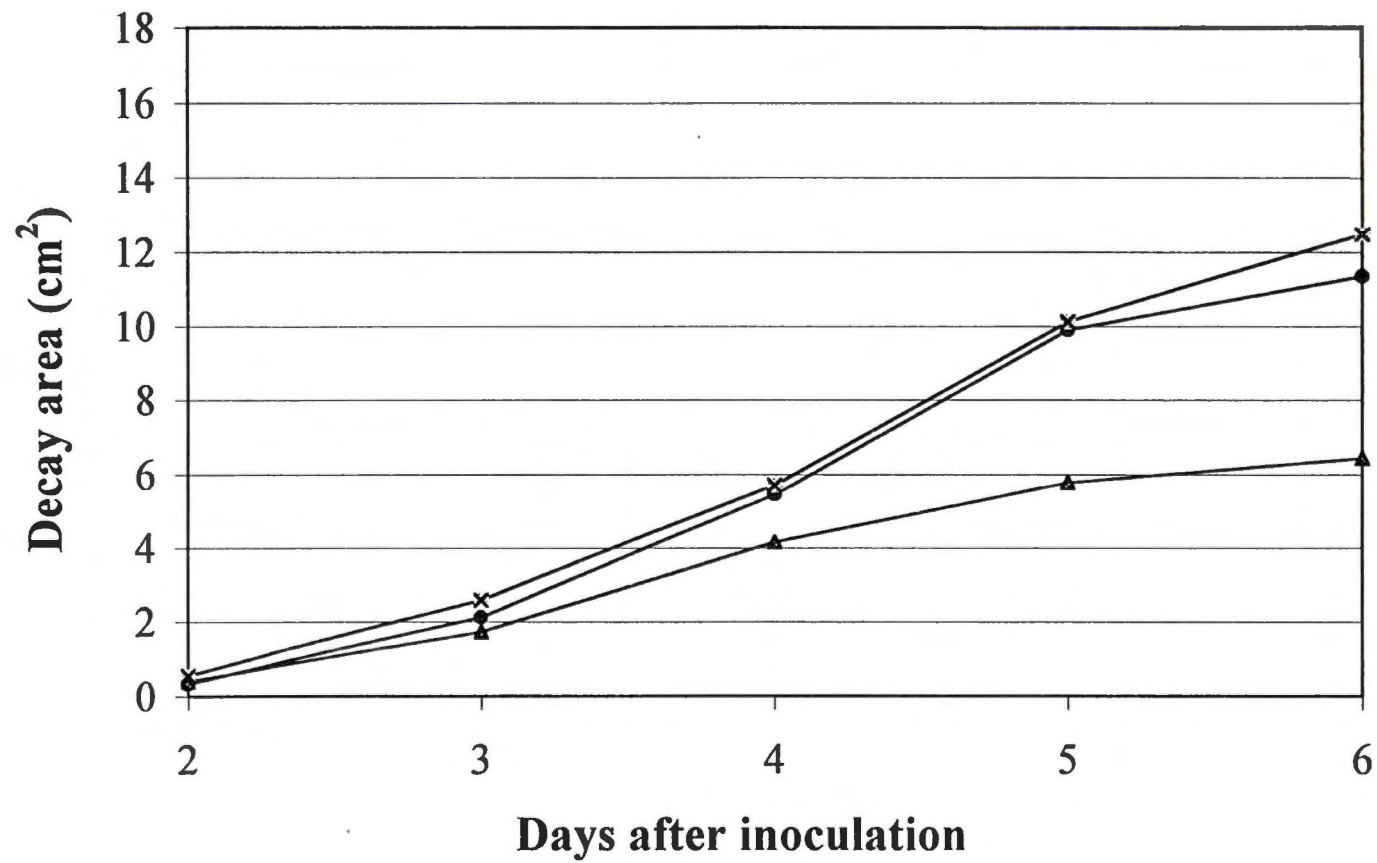


FIGURE 11: Tomato fruit decay area over time in 'Celebrity' after inoculation with *Botrytis cinerea* in spring 1996. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

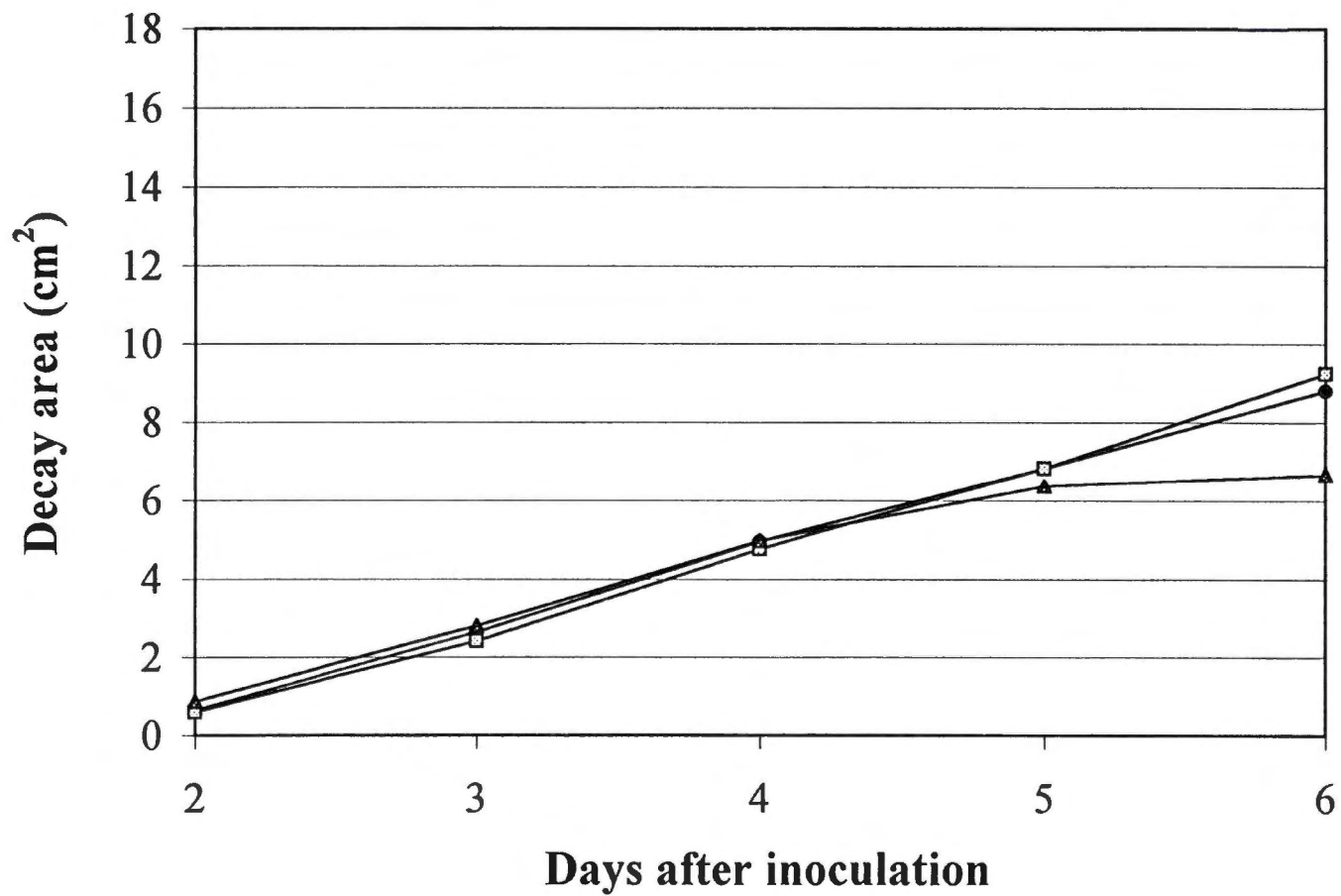


FIGURE 12: Tomato fruit decay area over time in 'Sunrise' after inoculation with *Botrytis cinerea* in spring 1996. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

APPENDIX C
MATERIALS AND METHODS

APPENDIX C

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

This research comprised three greenhouse experiments conducted in Greenhouse 6 located on the Agricultural Campus at The University of Tennessee, Knoxville.

Experiment 1 was conducted in room 3 of that greenhouse in the Fall of 1995 from August 18, 1995 through March 8, 1996, for a total of 172 days. Experiments 2 and 3 were conducted in rooms 2 and 3, respectively, in the Spring of 1996 both from January 10, 1996 through July 3, 1996, for a total of 206 days. Hereafter, the experiments will be referred to as “Experiment 1”, “Experiment 2”, and “Experiment 3”.

Experiments 1 and 3 contained 54 plants each and were 3x3 factorials in randomized complete block designs with three calcium treatments and three tomato cultivars. There were two replications in each of three blocks. Experiment 2 was the same design except that it contained subreps, and as a result, the number of plants were doubled to 108. SAS (1989, version 6.11) was used to perform analysis of variance and preplanned treatment comparisons.

PLANT MATERIAL

For all experiments, three tomato (*Lycopersicon esculentum* Mill.) cultivars, ‘Mountain Supreme’, ‘Sunrise’, and ‘Celebrity’, were used. Seed source was ‘Asgrow’ purchased from Knox Seed and Greenhouse, Knoxville, TN. All of the plants were

cultivated in either 15 L - 11x11.5" (Experiment 2) or 16 L - 10x13.25" (Experiments 1 and 3) black plastic pots with perlite (horticultural grade, Carolina Perlite Co., Gold Hill, NC). Spacing arrangements were as follows: adjacent plants measured between base of stems were 25" (Experiments 1 and 3) or 12.5" (Experiment 2) and facing rows measured from base of stems were 27" (all experiments). Each of the three benches in both rooms were 43" x 222". Since all of the experiments were conducted in the same greenhouse, further references will be made only to the specific room used in that greenhouse.

GREENHOUSE CULTIVATION

Experiment 1

Seeds were sown in trays of perlite and kept moist with distilled water. Once they produced their first true leaf, a 10% solution concentration (one tablespoon/gal water) of Peter's fertilizer solution was applied. Eighteen seedlings of each cultivar were transplanted, one per pot, when they reached the third true leaf stage. The irrigation system was then turned on for the remainder of the experiment.

Experiment 2

Five seeds of each cultivar were sown in each of 36 pots in room 2. The irrigation system was turned on and a 10 mg L⁻¹ N fertilizer solution and the respective calcium treatments applied. When the seedlings reached the seventh true leaf stage they were thinned to one per pot and Experiment 3 seedlings were moved to room 3.

Experiment 3

After the seedlings were thinned from the original pots in room 2, 54 seedlings, one per pot, were transferred to room 3. They were placed in the same layout plan as was in room 2, except that there were no subreps, duplicating the layout of Experiment 1.

Environment

In the winter, greenhouse temperature controls were set at 65°F-80°F by use of an overhead heater and two window exhaust fans per room. Summer temperatures were maintained at 70°F-90°F with the window exhaust fans and an evaporative water-cooling system. Minimum and maximum temperature and humidity data were recorded daily by means of two thermohygrometers (Baxter, Control Co., Friendswood, TX) placed at both ends of each room 152 cm high (Tables 14 and 15).

Cultivation

All plants were pruned to a single main stem by removing all of the lateral shoots, thus allowing the plants to produce larger fruit. Pollination was carried out with a hand-held battery-powered pollinator by vibrating each cluster for one second every other day when open flowers were present.

The growth-support system consisted of pre-existing overhead pipes running parallel to the length of the greenhouse and in the direction of the benches (east-west). The pipes themselves were supported by the greenhouse cross beams. Plastic twine was loosely tied at the base of the plant and wrapped in an upward spiral around the stem and tied to the supporting pipes above. As the plants grew, plastic clips (purchased from Hummert International) were attached to the twine and around the stem for continued

support. This was done as needed, usually every other day when there were twelve to fourteen inches of new growth above the last clip. This method ensured straight plants and kept the stems from breaking when the plants became too heavy with fruit.

Growth Data

Stem diameter (Experiment 1 only), plant height, leaf count, and cluster counts for each plant were recorded once weekly for the first half of the experiment. Additional observations include noting occurrence of lateral growth (which was pruned) on flower clusters, and the overall appearance of each plant. Plants produced four to six clusters of fruit. Plants that did not terminate by the sixth cluster had their terminal growing point removed to stop growth.

TREATMENTS

The calcium treatments were prepared with $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Mallinckrodt Chemical, Inc., St. Louis, MO) and greenhouse tap water which contained ca $20 \text{ mg}\cdot\text{L}^{-1}$ calcium. Including background calcium, the calcium treatments were as follows: low, $20 \text{ mg}\cdot\text{L}^{-1}$; medium, 220; high, $1020 \text{ mg}\cdot\text{L}^{-1}$. The treatments were added to the fertilizer, via injector pumps as it was delivered to the system. All of the calcium and fertilizer solutions were prepared and contained separately in 20-liter plastic reservoir buckets, allowing ease in making fertilizer concentration changes. The water source was greenhouse tap, unless specified otherwise.

FERTILIZER

Peter's Professional 5-11-26 Hydrosol (Grace-Sierra Horticultural Products Co., Milpitas, CA) and analytical grade ammonium nitrate (Johnson City Chemical Co., Johnson City, TN) provided all of the macro- and micro-nutrients (Table 15). Solutions were mixed and added to their respective reservoirs approximately once every five days or completely removed when the fertilizer concentration was changed. The solution concentrations were increased when the plants began to pale, when they began to flower and when fruit began developing.

IRRIGATION SYSTEM

The irrigation-fertilization system consisted of a total of four injector pumps: one Dosmatic (model 30-2S, Lewisville, TX) injector which diluted the fertilizer 1:100 and delivered equal amounts to each of three Dosatron (DI16-11GPM, Clearwater, FL) injector pumps. The three final injectors diluted the calcium solutions 1:100 and delivered each treatment to the fertilizer passing through the system and then through a series of ¾" PVC pipes leading to each of the three benches in both rooms.

Attached to the PVC irrigation system on each block were two-foot lengths of ¼" tubing coming out from each of the three main treatment lines. In Experiments 1 and 3, each individual tubing supported two pressure-compensating emitters (Netafim), each of which delivered the solutions at a rate of 4 liters hour⁻¹ plant⁻¹ to each pot. In Experiment 2 in order to support double the number of plants previously used, the tubing had a T-fitting to divert the solution to two adjacent pots which then had two emitters per pot.

The system was electronically controlled by a time clock (Rainmatic 2000 Electronic Water Timer, Omaha, NE) which activated the system to deliver a total of 1.6 liters of the properly diluted fertilizer-calcium treatment solutions four times per day for five minutes each time at 6, 10, 2, and 6 o'clock.

One extra tubing + emitter assembly was connected to each treatment line on each block. Once a week, a ten-ml solution sample was taken from each extra emitter, representing the solution that the plants were receiving. Experiments 1 and 3 were in room 3 which was designed to collect the excess drainage from the pots. These nine extra samples represented, by block and treatment, any nutrients unused by the plants. All nutrient samples were analyzed by inductively coupled argon plasma atom emission spectrometry (ICP) (Thermo Jarrell Ash, Franklin MA, model ICAP 61) for calcium, magnesium, manganese, zinc, phosphorus, boron, copper, aluminum, potassium, sodium, molybdenum, sulfur, and iron.

HARVEST

Experiment 1

The harvest period for Experiment 1 began December 27, 1995, 100 days after planting, and continued for 72 days, ending March 8, 1996. Plants were harvested once weekly for fruit with any detectable color (breaker stage, described in the following section).

Experiments 2 and 3

In Experiments 2 and 3, harvest was conducted two to three times per week from May 23, 165 days after planting, and continued for 41 days until July 3, 1996. In order to have uniformly mature fruit for firmness, tissue-calcium analysis, and disease inoculations, the number of harvests per week was increased so that more fruit were available at the breaker stage.

GRADING AND PROCESSING

Data for individual fruit from each block, treatment, cultivar, replication, subrep and cluster were collected. Characteristics such as weight (Sartorius, model 1413 MP8-1), diameter, maturity (Experiment 1 only), and firmness were recorded. Culls were categorized by size (less than five cm), the presence of physiological disorders and/or physical defects such as cracking, blossom-end rot, and catfacing. All harvested fruit were from the main stem since the lateral shoots had been removed. Firmness was measured by placing the fruit on a firmness tester for thirty seconds under the applied pressure of a one-kilogram weight. Fruit were frozen in a Coldspot 30 Thin Wall freezer and freeze-dried in a Cascade Freeze Dry System (Labconco, Lyph-Lock 12, Kansas City, MO).

Experiment 1

After collecting all initial harvest data, one portion of the fruit was tested for firmness and another portion set aside for disease inoculations to be performed on the same day. After testing the fruit for firmness, they were then cut into quarters. One quarter was immediately used to measure soluble solids with a refractometer (ATAGO,

Tokyo, No. 49347). The second quarter was frozen for later freeze-drying and tissue analysis of mineral content by ICP. The remaining samples were frozen as extra samples.

Maturity (by percent surface color) was rated using the following scale: 1=mature green; 2=breaker; 3=turning; 4=pink; 5=light red; 6=red (United States Department of Agriculture maturity grading chart for tomatoes).

Experiments 2 and 3

After collecting the initial data for each fruit, firmness measurements were taken only on fruit at maturity stage two. Those same fruit were then used for disease inoculations on the same day. Six days after harvest and after the final inoculation data were collected, the fruit were cut and a non-diseased quarter frozen for subsequent freeze-drying and tissue analysis of mineral content by ICP.

Final Harvest

Once most of the “marketable” main-stem fruit were harvested, the experiment was terminated. All of the plants were stripped of the remaining fruit and the data recorded as previously described.

PLANT TISSUE ANALYSIS

Leaf tissue

Two weeks prior to final harvest, the leaf immediately below (Experiment 1) or above (Experiments 2 and 3) the first, third, and fifth flower clusters were removed from each plant, placed in brown paper bags, and dried in the greenhouse for three weeks. They were then placed in a drying oven (Fisher Isotemp Oven, model 338F) at 70C for

two to three days until dried to a constant weight and then stored in plastic bags. Later, the leaves were individually ground to 18 mesh in a cyclone sample mill (UDY Corp., Fort Collins, CO, model 3010-030) which was cleaned between samples, and the ground leaves stored in individual 'Whirl Pak' bags (Nasco).

Approximately 0.5g of each ground leaf sample was weighed in borosilicate tubes (Baxter) on an analytical balance (Sartorius, model A200S) and ashed in a 500C furnace (Thermolyne, model 30400) for 96 hours. The ash was dissolved in 2N HCl (prepared from concentrated analytical grade HCl; Baxter) and the samples diluted to eight ml. One ml was then removed and added to nine ml 2N HCl for a final dilution of 1:80. Samples were vortexed (Glas-col, Model PV-6, Terre Haute, IN) after every dilution and nutrients were analyzed by ICP.

Element concentration was determined by the following formula (Kennedy, 1994):

$$\mu\text{g.g}^{-1} \text{ element} = (\text{ICP value for nutrient} * \text{dilution factor}) / \text{sample tissue weight}$$

Fruit tissue

The fruit were prepared and frozen as previously described. Sixty frozen samples at a time were placed in the freeze dryer in open whirlpaks. The samples were checked periodically for dryness and were removed when the tissues powdered when crushed, usually in 3-4 weeks. Dried samples were re-sealed and placed inside plastic bags containing Dri-Rite desiccant gel (Baxter) wrapped in cheesecloth (Baxter) and placed inside a sealed plastic container. Samples were placed into 15 ml borosilicate tubes,

weighed to approximately 1.0 gram, and placed in a 500C furnace to be ashed for 5-6 days. The remainder of the fruit sample preparation was the same as the previously described leaf analysis.

FUNGAL CULTURE

The *Botrytis cinerea* Pers. culture used to inoculate all tomato fruit was isolated from apple tissue and obtained from Dr. W. S. Conway, USDA, Beltsville, MD. All cultures were prepared and handled under a laminar flow hood (Bellco Glass Co., Vineland, NJ) which was surface sterilized by wiping with either 70% alcohol or 5% Clorox. Sporulation and maintenance of cultures was conducted in a Calumet Scientific incubator, while inoculated fruit were maintained in a Kelvinator 316438 incubator, both at 20^o C. For use in spore collection and counts, all utensils and glassware and water were covered and autoclaved. Pipettes and funnels with cheesecloth were wrapped first in wax paper, then aluminum foil; stir bars inside flasks containing water were plugged tightly with cotton, then covered with aluminum foil, and all were autoclaved (Marsh Instrument Co., Skokie, IL) for 15 minutes. All of the water used in the inoculation experiments was double deionized and autoclaved.

Culture and spore collection began with the transfer of stock culture mycelium to slants of potato-carrot media (PCM; Recipe: 300 g. potatoes sliced with skin; 25 g. carrots peeled and sliced; 15 g. agar; 1L water. Boil potatoes and carrots in water, simmer for 20 minutes, then filter. Return volume to 1 L with water and add agar. Melt, dispense, and sterilize for 20 minutes at 121°C). The slants were incubated at 20C under

constant fluorescent light for 7-14 days until the culture was sporulating profusely. Eight ml of sterile double deionized water was then poured into the slant and allowed to sit for four hours without shaking. Two ml of this water was poured from the slant into each of six 100 x 15 mm petri plates (Becton Dickinson Labware, Lincoln Park, NJ) containing ten ml of potato dextrose agar (PDA, Sigma) and the plates swirled to distribute the suspension over the surface of the plate. The plates were then incubated at 20C under constant fluorescent light for seven to ten days until they were sporulating profusely and used for the inoculations as needed.

INOCULATIONS

Inoculum Preparation

Once the plates were sporulating profusely, the agar was cut into half-inch strips and placed into a 250-ml Erlenmeyer flask containing 100 ml water and a stir bar. The flask was then placed on a stir plate (Corning, PC-351) and the suspension agitated for 15 minutes. The resulting spore suspension was filtered through a funnel with two layers of cheesecloth to remove mycelium and agar. Spore counts were made with a bright-line hemacytometer (Reichert-Jung, Horsham, PA) under a dissecting scope and the spore concentration adjusted with water to an average of 5×10^5 spores ml⁻¹ for immediate fruit inoculations.

Inoculation Procedure

Fruit inoculations were performed after each harvest. Eight sets of inoculations (Experiment 1, one set per week) and fifteen (Experiments 2 and 3, ca three sets per week) were performed. The number of inoculation sets were dependent upon the number of harvests for each particular crop. The inoculum (spore suspension) was prepared as described above and the fruit were wiped with sterile distilled water and paper towels. For the first three weeks during Experiment 1, each fruit was injured on the shoulders 3 mm deep and wide with a sterilized nail, on two sides of the fruit. An electronic pipette (Rainin, model EDP2) was used to dispense 50 µl of inoculum over one injury site and 50 µl water over the other served as a control. Since no fungal growth was observed on the control site, it was not used during the remaining five weeks of the experiment, nor in Experiments 2 and 3.

Inoculated fruit were placed on plastic-wrapped apple trays and the trays placed in a growth chamber at 20°C. The chamber maintained only 85% relative humidity (as measured by the same brand thermohygrometers used in the greenhouse), yet 90-95% was desired. To increase humidity, the fruit trays were placed in large, clear plastic trash bags which were held open and above the fruit by seven-inch wooden skewers pierced through the tray. Two water-soaked paper towels were placed under the bag on the corner of each tray. During Experiments 2 and 3, a humidifier (Emerson Electric Co., St. Louis, MO, model HD092) was added to the chamber and kept the humidity at 95%, omitting the need for the plastic “tents”.

DECAY RATINGS

The decay lesion consisted of an area of rot under the skin having a water-soaked appearance. Two or three days after inoculation, the skin would split with two to four cracks radiating out from the injury site. Mycelial growth over the injury site and skin split was always associated with the decay.

Decay diameter was determined by averaging the widest and narrowest portion of the circular decay lesion in centimeters and converting the value to area ($\text{area} = \pi r^2$) in cm^2 . Decay measurements were taken every 24 hours for seven days (Experiment 1) or for six days (Experiments 2 and 3).

Once the final measurements had been taken for a particular batch of fruit, they were either wrapped in a plastic bag and thrown away (Experiment 1) or quartered and one disease-free quarter was frozen for subsequent freeze drying and tissue mineral content analysis by ICP as previously described. Measurement data were entered into the computer and analyzed by SAS.

APPENDIX D

ADDITIONAL EXPERIMENTS

TABLE 19: Tomato leaf and fruit calcium content and area of decay significance probabilities of analysis of variance and preplanned treatment contrasts, Experiment 3

SOURCE OF VARIATION	Leaf Calcium	Fruit Calcium	Disease Area
Treatment	0.01	0.01	0.59
Cultivar	0.61	0.01	0.01
Treatment x cultivar	0.35	0.36	0.61
Low calcium Vs medium calcium	0.01	0.01	0.92
Med Ca Vs high calcium	0.01	0.05	0.32
Low Ca Vs high calcium	0.01	0.01	0.37
'Mountain Supreme' Vs 'Celebrity'	0.38	0.81	0.60
'Celebrity' Vs 'Sunrise'	0.97	0.01	0.01
'Mountain Supreme' Vs 'Sunrise'	0.40	0.01	0.01
CONTRASTS			
'Mountain Supreme'			
Across treatments	0.01	0.01	0.91
Low calcium Vs medium calcium	0.01	0.01	0.77
Medium calcium Vs high calcium	0.01	0.88	0.86
Low calcium Vs high calcium	0.01	0.01	0.69
'Celebrity'			
Across treatments	0.01	0.01	0.14
Low calcium Vs medium calcium	0.01	0.01	0.11
Medium calcium Vs high calcium	0.01	0.02	0.12
Low calcium Vs high calcium	0.01	0.01	0.65
'Sunrise'			
Across treatments	0.01	0.01	0.65
Low calcium Vs medium calcium	0.01	0.01	0.37
Medium calcium Vs high calcium	0.01	0.51	0.89
Low calcium Vs high calcium	0.01	0.01	0.55
Low calcium			
Across cultivars	0.67	0.05	0.01
'Mountain Supreme' Vs 'Celebrity'	0.62	0.84	0.79
'Celebrity' Vs 'Sunrise'	0.37	0.03	0.01
'Mountain Supreme' Vs 'Sunrise'	0.71	0.05	0.01
Medium calcium			
Across cultivars	0.97	0.01	0.01
'Mountain Supreme' Vs 'Celebrity'	0.84	0.22	0.25
'Celebrity' Vs 'Sunrise'	0.83	0.08	0.00
'Mountain Supreme' Vs 'Sunrise'	0.99	0.01	0.02
High calcium			
Across cultivars	0.20	0.02	0.74
'Mountain Supreme' Vs 'Celebrity'	0.08	0.33	0.81
'Celebrity' Vs 'Sunrise'	0.31	0.02	0.78
'Mountain Supreme' Vs 'Sunrise'	0.42	0.04	0.44

TABLE 20: LS means from analysis of variance of tomato leaf and fruit calcium content ($\mu\text{g g}^{-1}$ DW) and area of decay (cm^2) five days after inoculation with *Botrytis cinerea*, Experiment 3

SOURCE OF VARIATION	Leaf Calcium	Fruit Calcium	Decay Area
Low calcium	25418.13	695.96	8.87
Medium calcium	65133.14	1716.55	8.93
High calcium	80931.12	1881.64	8.21
'Mountain Supreme'	56183.35	1511.42	9.19
'Celebrity'	57677.44	1532.03	9.59
'Sunrise'	57621.59	1250.70	7.31
Low calcium, 'Mountain Supreme'	25595.70	724.73	9.47
Low calcium, 'Celebrity'	24317.00	740.34	9.58
Low calcium, 'Sunrise'	26341.70	622.80	7.64
Medium calcium, 'Mountain Supreme'	65329.67	1861.49	9.14
Medium calcium, 'Celebrity'	64603.21	1766.77	10.81
Medium calcium, 'Sunrise'	65466.53	1521.40	7.03
High calcium, 'Mountain Supreme'	77624.69	1948.03	8.95
High calcium, 'Celebrity'	84112.12	2088.97	8.45
High calcium, 'Sunrise'	81056.55	1607.91	7.27

TABLE 21: Effect of calcium-fertilizer solution on mineral composition ($\mu\text{g g}^{-1}$ DW) of tomato leaves and fruit, LS means by treatment and cultivar, Experiment 3

Treatment-Cultivar Combination	Calcium	Boron	Sulfur	Magnesium	Potassium	Phosphorus
LEAVES						
Low, 'Mountain Supreme'	25595.70	253.95	17089.81	20058.47	46104.12	10919.08
Low, 'Celebrity'	24317.00	241.91	13469.93	17871.14	44203.54	9231.29
Low, 'Sunrise'	26341.70	249.30	15675.58	19290.28	43920.68	10742.79
Medium, 'Mountain Supreme'	65329.67	209.31	15976.49	7298.47	36205.50	8761.69
Medium, 'Celebrity'	64603.21	209.15	11896.99	5898.56	31895.12	7459.44
Medium, 'Sunrise'	65466.53	214.24	14585.93	6661.10	39684.92	10013.60
High, 'Mountain Supreme'	77624.69	181.00	13585.98	4012.75	35699.72	7849.85
High, 'Celebrity'	84112.12	161.83	10521.54	3585.61	30750.10	6150.49
High, 'Sunrise'	81056.55	168.61	12086.20	3915.96	32690.64	7826.93
FRUIT						
Low, 'Mountain Supreme'	724.73	114.23	1419.40	1638.38	34864.80	4716.65
Low, 'Celebrity'	740.34	117.36	1408.20	1922.66	39692.36	4610.46
Low, 'Sunrise'	622.80	118.68	1523.97	1953.32	40041.77	4938.66
Medium, 'Mountain Supreme'	1861.49	125.43	1424.83	1210.23	38071.02	4512.98
Medium, 'Celebrity'	1766.77	124.71	941.86	1131.25	40037.29	3749.57
Medium, 'Sunrise'	1521.40	124.83	1219.97	1299.99	41885.53	4542.31
High, 'Mountain Supreme'	1948.03	102.84	1291.15	1017.05	40123.03	4326.55
High, 'Celebrity'	2088.97	105.12	815.89	931.61	39489.60	3662.24
High, 'Sunrise'	1607.91	117.43	890.46	944.05	38400.76	3889.31

TABLE 22: Average^y nutrient composition (mg L⁻¹) of low calcium-fertilizer solution as determined by ICP analysis, Experiment 3

Sample Date	Nitrogen (mg L ⁻¹)	Calcium	Boron	Potassium	Magnesium	Phosphorus	Sulfur
1/26/96	10	31.36	0.15	31.57	11.01	5.51	19.90
2/9/96	30	25.96	0.25	82.16	16.56	15.79	25.28
2/16/96	40	25.45	0.28	102.75	18.64	18.31	26.92
3/1/96	40	25.16	0.31	114.05	20.45	22.02	29.14
3/8/96	80	24.67	0.61	226.21	30.43	42.46	41.17
3/15/96	80	26.95	0.58	218.63	31.69	38.86	41.85
3/25/96	80	26.78	0.54	210.00	31.10	39.27	41.13
3/29/96	80	22.40	0.54	193.01	29.69	37.48	39.62
4/5/96	80	26.93	0.54	213.59	31.46	41.39	41.14
4/12/96	80	29.24	0.59	208.95	32.23	41.41	39.99
4/19/96	80	29.92	0.61	207.58	32.98	41.56	40.69
4/26/96	80	26.29	0.60	203.53	31.79	41.22	42.43
5/3/96	100	28.92	0.73	255.26	38.18	48.05	48.60
5/10/96	140	25.05	0.92	354.98	50.85	70.92	66.52
5/17/96	180	17.32	0.68	277.59	37.76	55.08	50.31
5/24/96	180	26.07	1.17	485.23	66.79	92.34	87.73
6/7/96	180	30.86	1.46	367.15	67.75	83.86	73.33
6/21/96	180	28.23	1.37	363.25	67.00	86.82	72.35
6/28/96	90	29.01	0.97	179.97	36.06	42.89	39.29
mean	NA	26.66	0.68	226.08	35.92	45.54	45.65
standard deviation	NA	3.22	0.36	109.83	16.44	23.71	17.70
range	NA	17.3-31.4	0.15-1.5	31.6-485.2	11.01-67.8	5.51-92.3	19.9-87.7

^yAverage calculated across 3 blocks

TABLE 23: Average^y nutrient composition (mg L⁻¹) of medium calcium-fertilizer solution as determined by ICP analysis, Experiment 3

Sample Date	Nitrogen (mg L ⁻¹)	Calcium	Boron	Potassium	Magnesium	Phosphorus	Sulfur
1/26/96	10	66.61	0.18	32.68	11.25	5.53	19.40
2/9/96	30	138.80	0.38	84.77	16.48	0.85	22.86
2/16/96	40	112.68	0.34	105.95	19.80	3.26	25.07
3/1/96	40	254.34	0.43	119.28	21.17	4.36	26.27
3/8/96	80	263.74	0.63	238.10	33.19	24.06	38.05
3/15/96	80	267.99	0.64	226.27	34.80	20.37	38.95
3/25/96	80	244.16	0.63	226.34	34.76	21.71	38.75
3/29/96	80	242.86	0.60	204.58	32.35	23.08	36.63
4/5/96	80	204.72	0.62	207.58	32.54	21.80	36.68
4/12/96	80	214.87	0.68	224.77	35.40	21.20	37.31
4/19/96	80	202.87	0.68	210.85	34.46	17.86	37.02
4/26/96	80	207.35	0.65	215.69	34.47	21.09	38.73
5/3/96	100	202.31	0.68	236.45	36.52	25.80	41.16
5/10/96	140	206.06	0.90	328.79	47.74	44.44	57.30
5/17/96	180	189.67	0.87	330.12	47.40	50.44	55.02
5/24/96	180	236.64	1.19	446.51	69.77	68.82	82.27
6/7/96	180	175.84	1.47	382.79	70.75	71.58	74.14
6/21/96	180	214.05	1.38	359.72	65.79	82.10	70.30
6/28/96	90	34.81	0.98	182.25	36.32	43.71	39.84
mean	NA	193.70	0.73	229.66	37.63	30.11	42.93
standard deviation	NA	63.99	0.34	105.06	16.62	23.83	17.34
range	NA	34.8- 268.00	0.2- 1.47	32.7- 446.5	11.3- 70.8	0.85- 82.1	19.4- 82.27

^yAverage calculated across 3 blocks

TABLE 24: Average^y nutrient composition (mg L⁻¹) of high calcium-fertilizer solution as determined by ICP analysis, Experiment 3

Sample Date	Nitrogen (mg L ⁻¹)	Calcium	Boron	Potassium	Magnesium	Phosphorus	Sulfur
1/26/96	10	1287.27	2.37	51.20	12.76	1.72	22.65
2/9/96	30	1150.46	2.48	92.80	16.72	11.15	24.42
2/16/96	40	1289.81	2.68	120.80	22.49	3.38	32.79
3/1/96	40	1213.59	2.48	114.13	20.99	16.23	28.38
3/8/96	80	1143.04	2.79	210.13	32.04	32.94	42.49
3/15/96	80	3216.40	2.71	269.33	34.80	22.38	41.88
3/25/96	80	2358.39	2.83	265.07	37.18	25.53	46.22
3/29/96	80	1695.59	2.67	226.13	34.82	31.81	44.68
4/5/96	80	1126.14	2.65	224.53	33.83	37.25	43.15
4/12/96	80	1205.40	2.65	234.13	36.13	29.87	44.91
4/19/96	80	1220.41	2.90	228.80	35.61	28.08	43.54
4/26/96	80	1242.46	2.88	240.12	36.02	30.11	46.59
5/3/96	100	1098.17	2.04	282.29	42.70	37.24	47.50
5/10/96	140	1402.26	1.98	371.11	52.40	61.12	64.19
5/17/96	180	414.57	1.95	366.51	53.63	73.68	68.02
5/24/96	180	1019.64	2.07	445.64	64.19	82.24	76.86
6/7/96	180	1305.47	1.87	448.97	67.08	68.24	97.37
6/21/96	180	1137.53	1.62	387.62	50.81	75.76	79.24
6/28/96	90	459.49	1.00	201.95	19.70	46.23	46.44
mean	NA	1315.06	2.35	251.65	37.05	37.63	49.54
standard deviation	NA	609.88	0.50	113.52	15.26	24.23	19.46
range	NA	415.0-3216.0	1.00-2.9	41.0-449.0	12.8-67.1	1.72-82.2	23.0-97.4

^yAverage calculated across 3 blocks

TABLE 25: Monthly greenhouse temperature and relative humidity during Experiment 3

Month	Temperature (°C) ^z		% Relative Humidity ^z	
	Minimum	Maximum	Minimum	Maximum
January 1996	16.1 ± 2.9	27.1 ± 4.4	42.3 ± 11.7	70.5 ± 5.80
February 1996	17.1 ± 4.4	31.3 ± 6.0	35.5 ± 12.4	69.0 ± 7.50
March 1996	18.4 ± 2.8	33.8 ± 7.1	28.2 ± 18.3	64.2 ± 11.4
April 1996	17.8 ± 3.9	33.0 ± 4.2	39.5 ± 4.90	80.8 ± 7.40
May 1996	17.5 ± 3.2	34.0 ± 3.1	45.0 ± 9.40	89.2 ± 4.40
June 1996	19.4 ± 2.0	35.6 ± 3.9	48.2 ± 9.20	92.3 ± 1.70
July 1996	22.2 ± 1.0	38.3 ± 1.0	46.5 ± 2.20	93.0 ± 0.50
Range over experiment	16.1-22.2	27.1-38.3	28.2-48.2	64.2-93.0

^zValues are the mean ± standard deviation

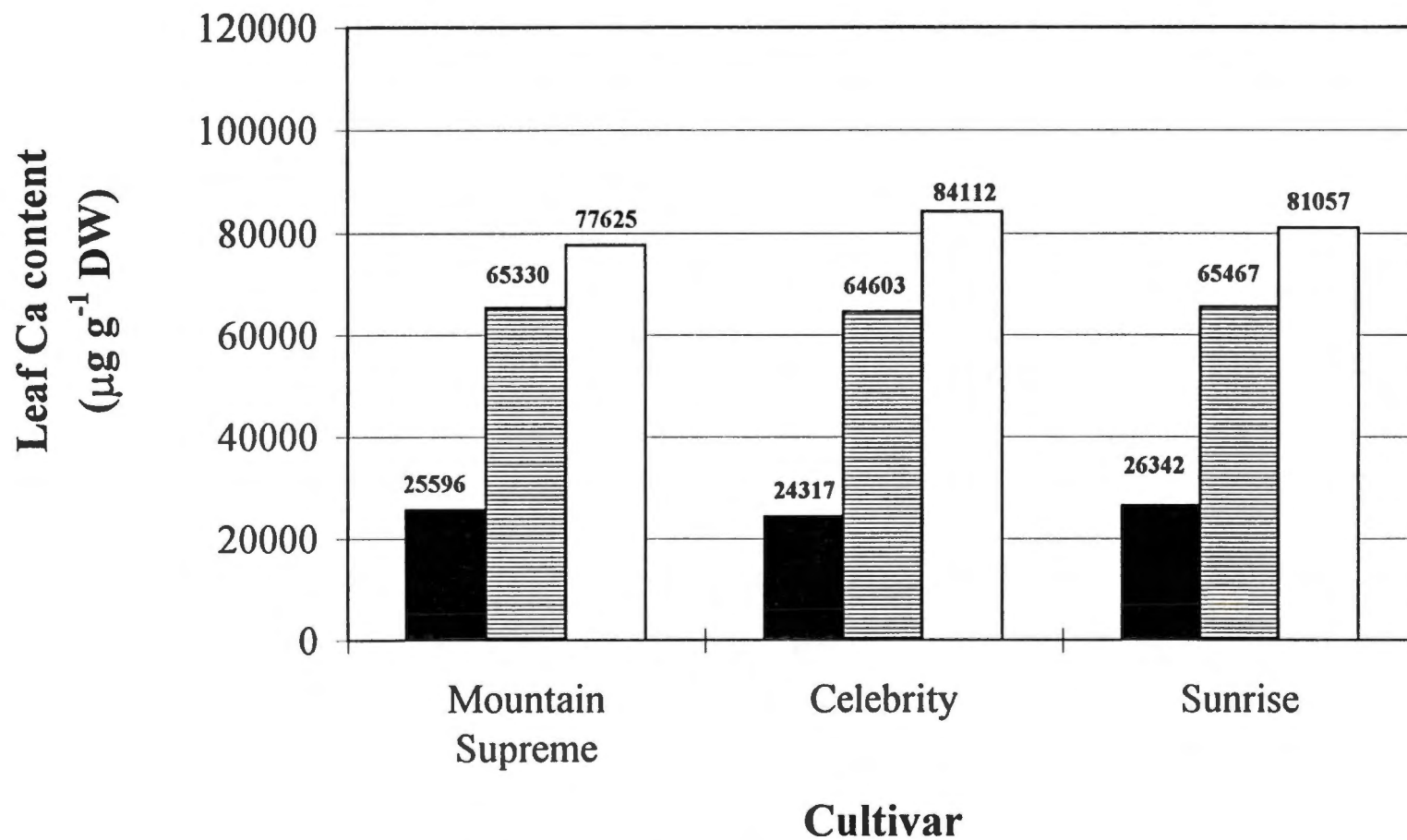


FIGURE 13: Leaf calcium (Ca) content of three tomato cultivars subjected to three Ca treatments in Experiment 3. Ca treatments are: black bars=20 mg L⁻¹, lined bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above bars are the means.

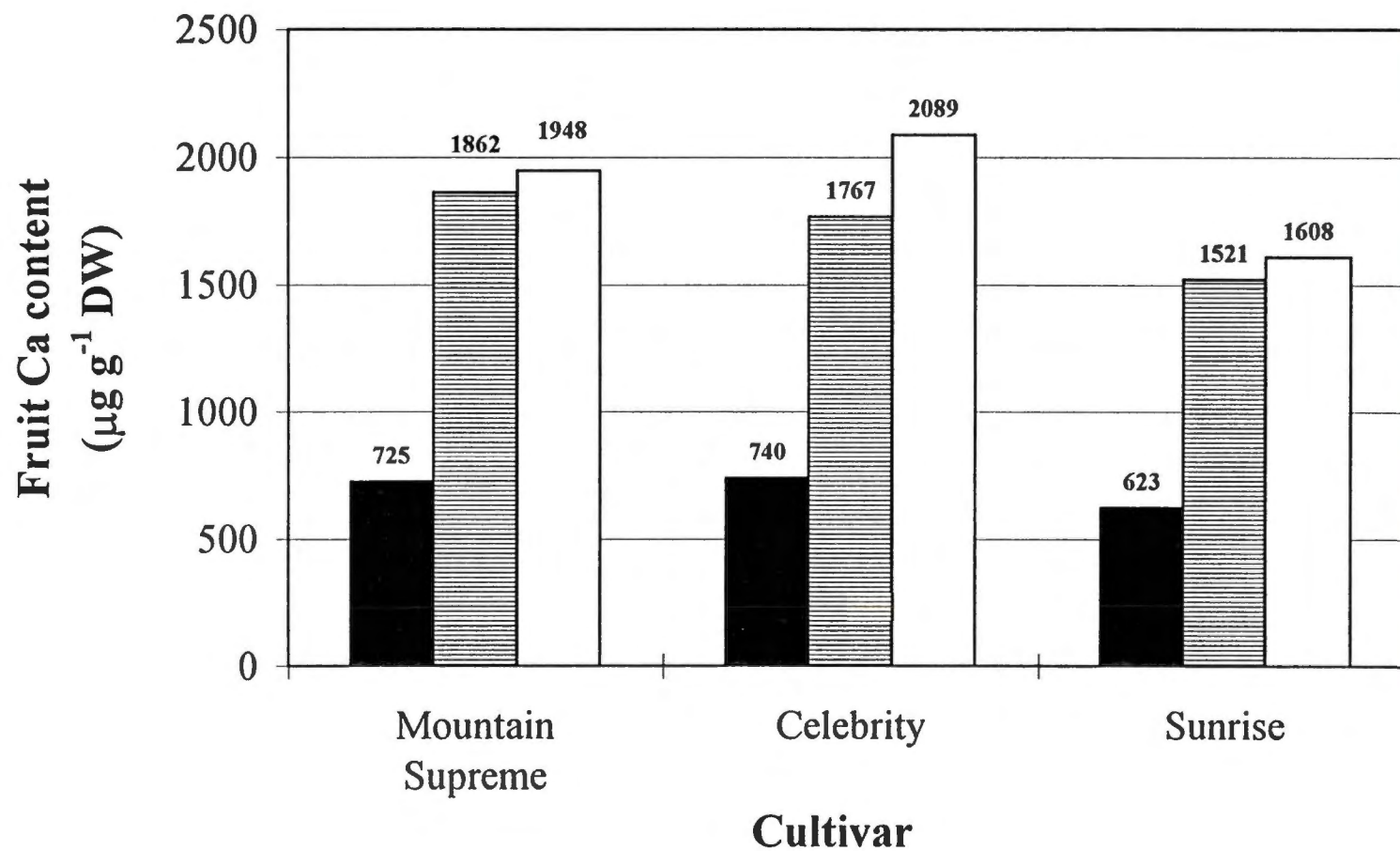


FIGURE 14: Fruit calcium (Ca) content of three tomato cultivars subjected to three Ca treatments in Experiment 3. Ca treatments are: black bars=20 mg L⁻¹, horizontal bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above the bars are the means.

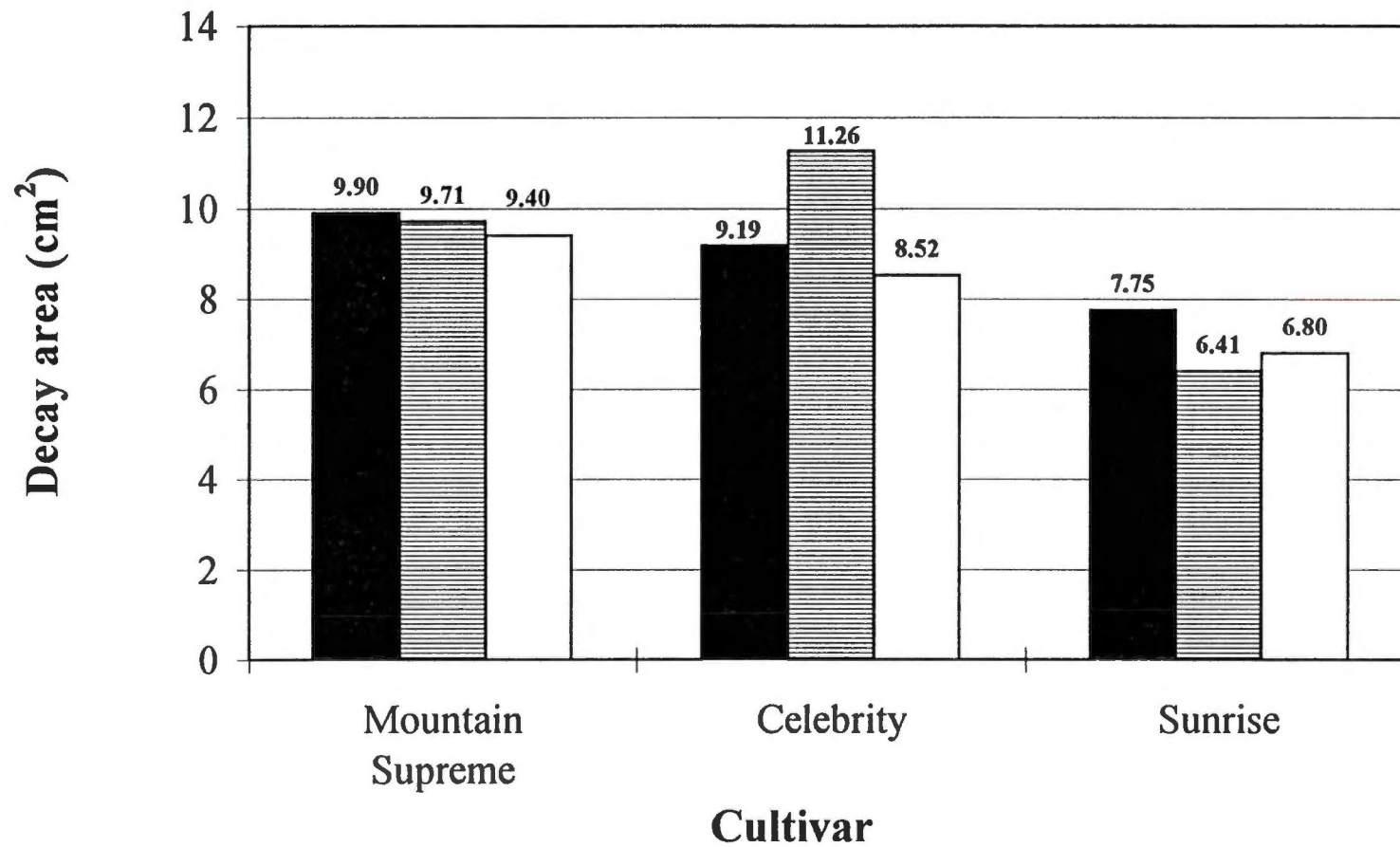


FIGURE 15: Fruit decay area of three tomato cultivars five days after inoculation with *Botrytis cinerea* in Experiment 3. Calcium treatments are: black bars=20 mg L⁻¹, lined bars=220 mg L⁻¹, and white bars=1020 mg L⁻¹. Numbers above bars are the means.

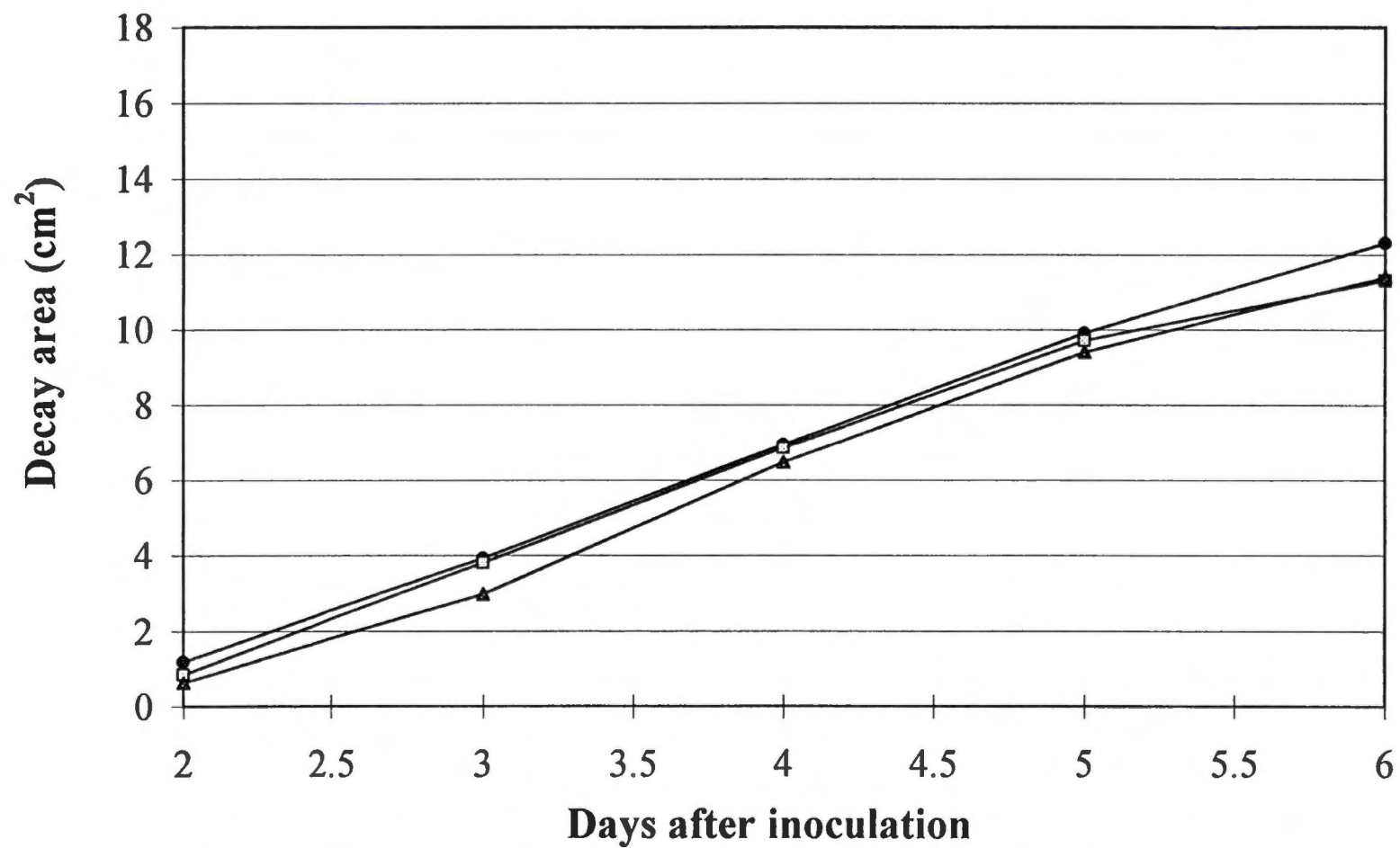


FIGURE 16: Fruit decay area over time in 'Mountain Supreme' after inoculation with *Botrytis cinerea* in Experiment 3. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

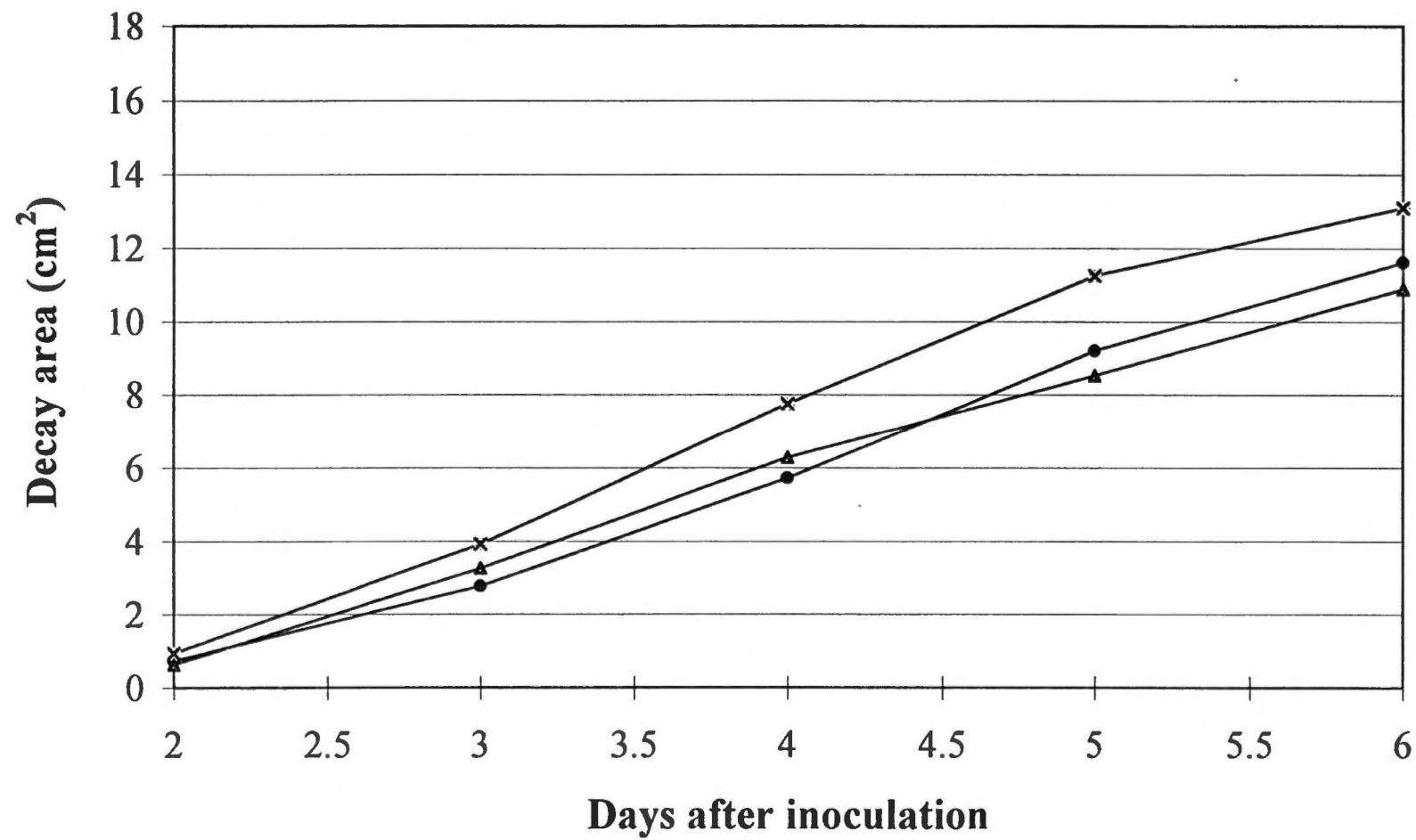


FIGURE 17: Fruit decay area over time in 'Celebrity' after inoculation with *Botrytis cinerea* in Experiment 3. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

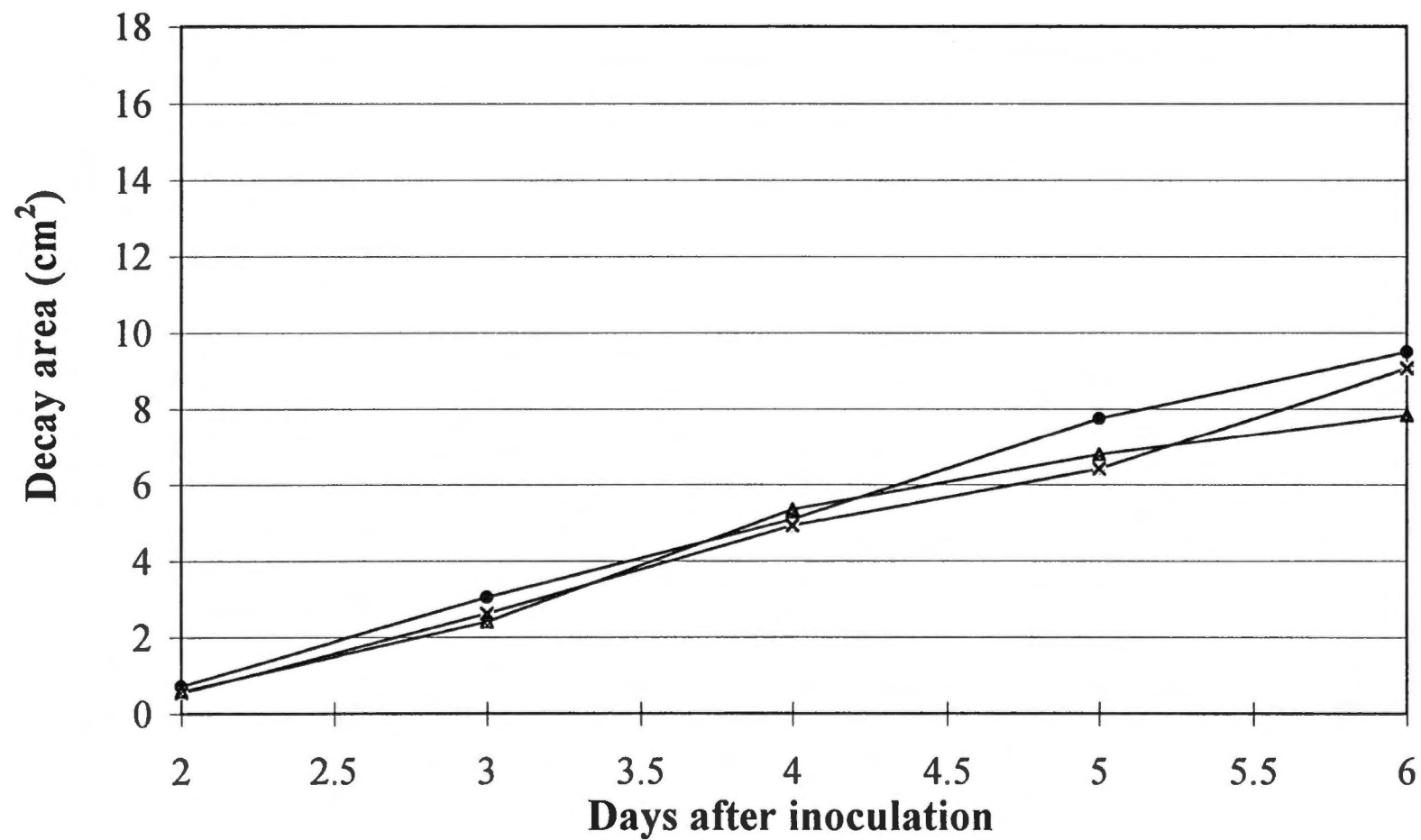


FIGURE 18: Fruit decay area over time in 'Sunrise' after inoculation with *Botrytis cinerea* in Experiment 3. Calcium treatments are: circle=20 mg L⁻¹, square=220 mg L⁻¹, and triangle=1020 mg L⁻¹.

VITA

Lori Denise Calhoun was born in Hawthorne, California on June 24, 1963. At the age of 17 she moved to Konawa, Oklahoma. Lori graduated from Konawa High School in May 1981. After one year at East Central University in Ada, Oklahoma, she joined the work force. Eight years later, in August 1990, she returned to East Central University and graduated in May 1994 with a Bachelor of Science degree in Biology and a minor in Chemistry. In July 1994 Lori moved to Knoxville with her daughter and entered the Master's program in Plant and Soil Science at the University of Tennessee with a concentration in Crop Physiology. Lori married Rodney Osburn on December 1, 1996 and graduated with a MS degree in May 1997. Lori is a member of the American Society for Horticultural Science and enjoys mountain biking, birding, and gardening. Her post-graduate plans are to teach science at the junior college level.

