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Prediction and Study of Bitter Pit on Apples Using

$Mg^{2+}$ Induced Bitter Pit-Like Symptoms

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# PREDICTION AND STUDY OF BITTER PIT ON APPLES USING $Mg^{2+}$ INDUCED BITTER PIT-LIKE SYMPTOMS

By

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# ABSTRACT

## PREDICTION AND STUDY OF BITTER PIT ON APPLES USING $Mg^{2+}$ INDUCED BITTER PIT-LIKE SYMPTOMS

By

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Bitter pit is a physiological disorder affecting apples. Apple fruit vacuum infiltrated with $Mg^{2+}$ develop bitter pit-like symptoms [$Mg^{2+}$ induced pits (MgIP)] that are similar to naturally occurring bitter pit. Addition of $Ca^{2+}$ to the infiltration media attenuates $Mg^{2+}$ induced pit and the number of bitter pit-like lesions induced by $Mg^{2+}$ is inversely related to the endogenous (native) $Ca^{2+}$ concentration of individual fruits. MgIP was studied as a predictor of bitter pit development in storage and as a system to study bitter pit initiation and development.

Induction of MgIP on Northern Spy apples (*Malus domestica* Borkh.) by infiltrating $Mg^{2+}$ salt solutions into the fruits was positively correlated with bitter pit that developed naturally during storage. The endogenous (native) fruit $Ca^{2+}$ concentration was inversely related to the MgIP and to bitter pit development following storage.

Golden Delicious apple fruit were infiltrated with various concentrations of $Ca^{2+}$ and $Mg^{2+}$ with and without $Ca^{2+}$-affecting reagents or other cations. Including Trifluoperazine with $Mg^{2+}$ increased pitting over $Mg^{2+}$ alone.

Verapamil and nefedipine had no effect on MgIP or its attenuation by $Ca^{2+}$. Cyclopiazonic acid attenuated MgIP. Ethyleneglycol-bis(B amino ethyl ether)-N,N'tetra acetic acid (EGTA), and 2,3,5-triiodobenzoic acid (TIBA) attenuated MgIP. Cycloheximide inhibited $Mg^{2+}$ induced pit. Heating at 38°C prior to infiltrating the fruits attenuated MgIP. Cations $Ba^{2+}$, $La^{3+}$, $Co^{2+}$, $Sr^{2+}$ included at 20.0mM all completely arrested MgIP induced by 0.18M $Mg^{2+}$. $K^{+}$ and $Na^{+}$ partially inhibited MgIP. We have demonstrated that treatments affecting calcium homeostasis or cellular metabolism can alter MgIP development. We conclude that MgIp may be useful in studying bitter pit initiation and development.

An experiment was conducted to determine if prestorage heat treatments could attenuate bitter pit development on Northern Spy and superficial scald on Red Delicious and Law Rome apples. Attenuation of physiological disorders by heat treatment beyond that attainable by CA and low temperature was not apparent. Heat treatments were effective in improving flesh firmness retention.

Dedicated to the memory of my Mom,

Helen C. Burmeister

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Guidance Committee:

The journal paper format was chosen for this thesis in accordance with departmental and university regulations. The thesis is devided into two chapters in which the first is accepted for publication in *Postharvest Biology and Technology*, the second has been accepted for publication in the *Journal of Agricultural and Food Chemistry*.

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### APPENDIX TWO

# LITERATURE REVIEW

**Description of Bitter Pit.**

Bitter pit is in a group of physiological disorders affecting apples and pears known as corking disorders. These include bitter pit, corky core, cork spot, and crinkle. Faust and Shear (1968) have reviewed corking disorders. Bitter pit is characterized by sunken lesions that may develop on fruits as they mature on the tree or after harvest during storage. The tissue below the skin in the pitted area becomes discolored and dehydrated, resembling cork. When the lesions develop while the fruit are on the tree, the disorder is sometimes referred to as tree pit. Bitter pit is distinguished from other corking disorders in that lesions are mostly located in the outer cortex just below the skin. The affected tissue is softer than adjacent healthy tissue and it occurs at harvest or during storage.

**Factors affecting bitter pit incidence.**

The first reports of bitter pit were over 100 years ago by Jager (1889) as cited by Faust and Shear (1968). The disorder has been of commercial concern since the early 20th century and factors affecting its occurrence have been well documented (Faust and Shear, 1968; Ferguson and Watkins, 1989). Environmental factors and cultural practices that contribute to increased bitter pit incidence include: light cropping, $Ca^{2+}$ deficiency, excessive tree vigor, excessive N nutrition, and moisture stress. Fruit harvested immature are also prone to the disorder.

Susceptibility to bitter pit varies among cultivars and geographically. A cultivar can be considered susceptible in one region of a country or state and not

in another. For example, Smock and Neubert (1950) reported that Golden Delicious when grown in the U.S. had no susceptibility. It is now known that immature Golden Delicious can be prone to develop bitter pit and this is common in the Pacific NW. Bitter pit has also been reported on Golden Delicious in Australia, New Zealand, and South Africa (Ferguson and Watkins, 1989 and references therein).

**Mineral Content of the Fruit.**

Of the factors contributing to bitter pit incidence, $Ca^{2+}$ deficiency of the fruit has emerged as the crucial factor. Delong (1936) was the first to observe that low fruit $Ca^{2+}$ levels correlated with bitter pit development in storage. Garman and Mathis (1956) determined that spraying trees with $Ca^{2+}$ salts to fruit during development reduced bitter pit incidence. Since that time, the effects of environment, cultural, and chemical treatments on the mineral content of apple fruit in relation to bitter pit have been thoroughly investigated. In addition, high levels of $Mg^{2+}$ and/or $K^{+}$ may also predispose fruit to bitter pit. Perring (1986) found that few apples were pitted in years when $Ca^{2+}$ levels were high even if their $Mg^{2+}$ and $K^{+}$ levels were high. When $Ca^{2+}$ levels were marginal, more of the bitter pit afflicted fruit had high $K^{+}$ and $Mg^{2+}$ levels. Thus, a direct relationship between $K^{+}$ or $Mg^{2+}$ alone and bitter pit is often not detected especially if native calcium levels are high, but high ratios of $K^{+}$, and/or $Mg^{2+}$, to $Ca^{2+}$ are associated with bitter pit frequently (Webster and Forsyth, 1979; Hopfinger and Pooviah, 1979; Martin *et al.* 1960).

Studies attempting to relate levels of N, P, B, Na, Zn, and Cu to bitter pit incidence have been reviewed in detail (Faust and Shear, 1968; Ferguson and Watkins, 1989). No consistent relationships have been found indicating a critical role for these elements in bitter pit.

**Distribution of minerals within fruit.**

Complicating the $Ca^{2+}$/bitter pit relationship is the fact that mineral constituents of apple fruit are not uniformly distributed (Wilkinson and Perring, 1961; Perring and Wilkinson 1965; Faust *et al.* 1967). The $Ca^{2+}$ concentration is higher in the stem than in the calyx end of the fruit, higher in the core and skin than in the cortex, and concentrations can vary around the fruit axis. Transverse distribution of $K^+$ and $Mg^{2+}$ exhibits a similar pattern of distribution but there is no longitudal gradient.

Considerable effort has been expended to relate changes in mineral distribution during storage with bitter pit development. Translocation of $Ca^{2+}$ from the core to the outer cortex of apples during storage has been reported (Ferguson and Watkins, 1983; Bramlage *et al.* 1979; Perring, 1986). Information concerning the movement of other ions is conflicting. In Spartan apples, an accumulation of $K^+$ P and $Mg^{2+}$ in the inner cortical zones was recorded (Perring, 1984), whereas an outward movement of $K^+$ and $Mg^{2+}$ has been recorded for Cox,s Orange Pippin (Ferguson and Watkins, 1983). In the latter case, the authors surmised that ions may have been concentrated in the outer cortex by water losses resulting in the appearance of migration. Perring (1989) proposed that the

pattern of redistribution of minerals may depend on variety. Perring (1986) and Perring and Pearson (1986) associated an irregular transient withdrawal of $Ca^{2+}$ from the mid- and outer-cortical regions during storage just prior to the appearance of bitter pit lesions. This withdrawal of $Ca^{2+}$ was less pronounced in samples that developed less bitter pit.

**Prediction.**

Another approach to reduce losses is to predict the potential of fruits to develop bitter pit using mineral analysis. This is a useful commercial practice (Ferguson and Watkins, 1989). Analysis of fruit $Ca^{2+}$ or the ratio of $K^+$, $Mg^{2+}$, $K^+ + Mg^{2+}$ to $Ca^{2+}$ is commonly employed in these predictive methods. Fruits are sampled from orchards prior to harvest and analyzed for $Ca^{2+}$. Lots of low $Ca^{2+}$ fruits can be identified and marketed or processed prior to development of bitter pit. Fruits from orchards with a high $Ca^{2+}$ status can normally be safely held for long durations in storage.

Although the relationship between bitter pit and $Ca^{2+}$ is highly variable, thresholds for $Ca^{2+}$ where minimal or no bitter pit may be expected have been established (Wills *et al.* 1976). In one study, the correlation coefficient (r) ranged from 0.37-0.90 for the Ca/bitter pit relationship (Wills *et al.* 1976). Thresholds vary depending on the tissue (eg. whole fruit, cortical plugs, or skin), variety, growing region and season. Some of the variation in thresholds may reflect different sampling and analysis procedures. Bitter pit incidence can vary greatly, even at $Ca^{2+}$ levels below threshold. This makes accurate prediction impossible.

Attempts to reduce variability by expressing $Ca^{2+}$ on a different basis ( eg. mg Ca per cell surface) or use of fractionating tissue into soluble and insoluble fractions have been unsuccessful (Perring, 1986). Incorporation of other orchard factors (eg. fruit size, tree age, cropping level) with mineral analysis is used to improve prediction of bitter pit occurrence in storage (Holland, 1980). The practicality of such predictive methods is limited due to the inherent variability of bitter pit incidence within and among orchards, varieties and growing seasons (Wills *et al.* 1976).

For a predictive system to be practical, results must be known well enough in advance of the harvest to allow time for management decisions. The relationship of $Ca^{2+}$ to bitter pit has been shown to be reliable up to 3 weeks before harvest (Ferguson *et al.*, 1979). Analysis of young fruitlet midway in the growing season has also proven to be useful (Marcelle, 1989). However, fruit $Ca^{2+}$ analysis is expensive, time consuming, and requires specialized equipment. Also, since there is a high variability among fruits within an orchard, large sample sizes are necessary to assess bitter pit potential. Waller, (1980) suggested a minimum of 30 fruit, preferably 80 to accurately assess calcium levels in orchards. An alternative method of assessing fruit Ca levels would be useful.

**Control.**

Generally, attempts to control bitter pit under commercial conditions by cultural practices such as soil $Ca^{2+}$ application, summer pruning (to increase fruit/leaf) ratio, and delayed harvesting, are only marginally effective (Perring,

1979; Boon, 1980). The primary methods employed in attempts to control the disorder are: applying $Ca^{2+}$ sprays to trees at intervals throughout the growing season (Jackson, 1962; Cooper and Bangerth, 1976) using post-harvest dips or drenches in $Ca^{2+}$ solutions (Mason and Drought, 1975), or infiltrating $Ca^{2+}$ solutions into fruit (Scott and Wills, 1979; Cooper and Bangerth, 1976). Control of bitter pit by $Ca^{2+}$ treatments is highly variable and not always complete (Ferguson and Watkins, 1989).

**Storage Conditions.**

Bitter pit have been reduced by low temperature (Perring, 1986) and CA storage (Hewett, 1984; Sharples and Johnson, 1987). High water loss during storage is sometimes associated with increased bitter pit (Hewett, 1984; Scott and Wills, 1979). The benefits of CA storage and postharvest $Ca^{2+}$ treatments are additive (Hewett, 1984; Webster and Forsyth, 1979; Johnson, 1979). CA storage lowered the threshold $Ca^{2+}$ level where no bitter pit was expected (Sharples and Johnson, 1987). If ripening and metabolism are suppressed for long periods by low $O_2$ storage, irreversible attenuation of bitter pit may result (Ferguson and Watkins, 1989; Perring, 1986).

**Structural and Biochemical Differences in Affected Tissue.**

The site of bitter pit is associated with the ends of the vascular bundles (Smock and Van Doren, 1937). Its structural development is distinguished from other corking disorders by absence of abnormal growth of cells such as observed in cork spot (Simons *et al.*, 1971). It is believed this difference could be in the

timing of the disorder. Bitter pit occurs just before harvest, or during storage when cells may be unable to initiate cell division (Faust and Shear, 1968). Pectin proturbances into intercellular spaces have been observed (Simons, 1962). The few structural studies of bitter pit development are of fruit that develop symptoms while still on the tree (Ferguson and Watkins, 1989). A systematic study of structural development of the anatomical aspects of bitter pit in storage has not been accomplished.

During bitter pit development, there appears to be a general migration of mineral and organic constituents into the pitted tissue. Pitted tissue is higher in starch, glucose, and fructose, but low in sucrose and higher in N and pectin than adjacent healthy tissue (Askew *et al.*, 1960; Faust, *et al.*, 1968a). Citric acid is the main organic acid in pitted tissue, while malic acid was the predominant acid in healthy tissue (Steenkamp and de Villiers, 1983; Faust and Shear, 1968). In studies of Jonathan spot, another $Ca^{2+}$ related disorder, Richmond *et al.*, (1964) demonstrated movement of minerals into the affected area which was associated with a higher level of total organic acids, mainly malic acid, in the affected tissue.

Consistently, the pitted zones of apple fruit are higher in $Ca^{2+}$ and $Mg^{2+}$ than non-pitted tissue (Steenkamp and de Villiers, 1983; Meyer *et al.* 1979). High $Mg^{2+}/Ca^{2+}$ (Hopfinger and Poovaiah, 1979; Hopfinger *et al.* 1984) have been reported. Ford (1979) demonstrated that $^{45}Ca^{2+}$ moved into the pitted area as symptoms developed.

Cork spot, unlike bitter pit has a red color formation on the skin above the

incipient lesion (Faust and Shear, 1968b). In etiological studies of cork spot, ethylene production followed by a respiration rise were the first signs of the onset of the disorder. This was followed by an increase protein synthesis, pectin synthesis, and movement of ions into afflicted tissues. Acetate was the major respiratory substrate utilized in the pitted tissue. They hypothesized the acetate and ethylene were breakdown products of membrane fatty acids.

The mineral and biochemical changes documented for bitter pit and other corking disorders are symptomatic of other diseased and mechanically injured tissue. It is hypothesized that these differences between pitted and healthy tissue are not related to the initiation, but are the result of the metabolic disturbance and subsequent tissue breakdown (Ferguson and Watkins, 1989; Faust and Shear, 1968).

**Calcium and Fruit Ripening.**

Calcium's influence on apple ripening is acknowledged, but not understood. Fruit with a high $Ca^{2+}$ content ripen at a slower rate than fruits with lower flesh $Ca^{2+}$ levels. This has been demonstrated by comparisons of respiration rates of fruits within a range of $Ca^{2+}$ contents (Faust and Shear 1972; Bramlage *et al.*, 1974), or between fruit with raised $Ca^{2+}$ content by sprays during development on the tree (Cooper and Bangerth 1976). Bramlage *et al.* (1974) suggested that $Ca^{2+}$ reduced the rate, but not the timing of the respiratory climacteric in apples.

Red Delicious apples of low $Ca^{+2}$ levels entered the ethylene climacteric earlier than fruits with high $Ca^{+2}$ (Tomala and Dilley, 1989). Avocado treated

with $Ca^{+2}$ had both a depressed and a delayed ethylene climacteric (Tingwa and Young, 1974). $Ca^{+2}$ reduces ethylene production, and delays ripening and subsequent softening of apples. This effect was mimicked by $Sr^{+2}$, but was not seen with $K^{+2}$, or $Mg^{+2}$ infiltration. Ida Red apples infiltrated with $Ca^{+2}$ had reduced ethylene, ACC content and ethylene forming enzyme (EFE) activity (Tomala and Dilley, 1990). It is not known how $Ca^{2+}$ or other cations affect respiration, ethylene production and ripening. $Ca^{+2}$ may diminish respiration by decreasing membrane permeability thereby reducing the diffusion of respiratory substrates from the vacuole to centers of metabolic activity in the cytoplasm (Faust and Klein, 1974; Bangerth *et al.* 1972; and Cooper and Bangerth 1976). Direct effects of $Ca^{2+}$ on respiration and ethylene production have not been demonstrated (Ferguson, 1984). It has been suggested that calcium may be required for some ethylene-dependent plant processes (Raz and Fluhr, 1992).

**Calcium as a second messenger.**

$Ca^{2+}$ is as an essential element in plant nutrition, cell wall structure, and membrane structure and function (Bangerth, 1979; Ferguson and Drobak, 1988; Pooviah, 1988). Also $Ca^{2+}$'s role as a second messenger mediating many plant responses to external stimuli is now well accepted (Heplar and Wayne, 1985; Poovaiah and Reddy, 1987). $Ca^{2+}$ mediates plant responses through changes in cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_{cyt}$). External stimuli (e.g. light, growth regulators, gravity) imposes an action potential that results in the release of $Ca^{2+}$ into the cytosol either by an influx through voltage-gated $Ca^{2+}$ channels in the

plasma membrane, or by releasing $Ca^{2+}$ from intracellular organelles such as the vacuole, endoplasmic reticulum, chloroplast, and mitochondria (Wayne, 1993). This increase in $[Ca^{2+}]_{cyt}$ is transient and can be very localized (Cheek, 1989). $Ca^{2+}$ then binds to calmodulin, or other $Ca^{2+}$ binding proteins which then leads to a physiological response. This may involve the phosphorylation or dephosphorylation of certain enzymes and possibly gene expression.

Low $[Ca^{2+}]_{cyt}$ is maintained against a concentration gradient (1000 fold) between the cytosol and other cell compartments (vacuole and cell exterior. Resting values for $[Ca^{2+}]_{cyt}$ are between 0.1-1.0$\mu$M for all plants in which this has been measured (Poovaiah and Reddy, 1987). Maintenance of low $[Ca^{2+}]_{cyt}$ is believed to be primarily accomplished by an active efflux of $Ca^{2+}$ from the cytosol via $Mg^{2+}$ dependent $Ca^{2+}$ activated ATPase located in the plasma membrane, and by the similar uptake ATPases located in the membranes of the intracellular organelles (Moore *et al.* 1984). The $Ca^{2+}$ ATPase of the plasma membrane is believed to play a major role in plant homeostasis (Brisken, 1990; Carfoli, 1987).

In plants, measuring $[Ca^{2+}]_{cyt}$ is problematic because of the presence of cell walls, large vacuoles, chloroplasts, and high cellular turgor pressure. Harker and Venis (1991) have measured intracellular and extracellular free calcium in apple fruit using calcium-sensitive microelectrodes. Because of the highly vacuolated nature of apple cells only one successful $[Ca^{2+}]_{cyt}$ measurement was accomplished. The $[Ca^{2+}]_{cyt}$ was verified to be 0.05$\mu$M. The apoplastic and vacuolar $[Ca^{2+}]$ ranged from 0.02-1.3mM and 0.06-1.0mM, respectively. The total calcium in the

apple tissue was positively correlated with the apoplastic free calcium. Assuming that 90% of the cell weight is vacuole, they calculated that the vacuole accounted for 40% of the total calcium in apple tissue. As much as 60% is thought to be in the cell wall and associated apoplastic space (Demarty *et al.* 1984). These results concur with other measurements (Miller and Sanders, 1987; Felle, 1988).

**Initiation and Development**

Although mineral composition is a major factor, it is not considered the cause of the bitter pit disorder. Early theories concerning the cause of bitter pit include excessive transpiration, necrosis of immature cells filled with starch, toxicity to sprays, and viral infection (Faust and Shear, 1968). The cause(s) and mechanism of initiation and development of bitter pit are not known. Currently, bitter pit is thought to result from a localized $Ca^{2+}$ deficiency or mineral imbalance, but there is no direct evidence for this (Ferguson and Watkins, 1989; Perring, 1986). Mineral analysis has only been conducted at the tissue level. Since it has not been possible to identify sites on fruit where pits might develop and measurements of the relevant pools of $Ca^{2+}$ in apple fruit are scarce, the etiology of bitter pit is unclear. Many theories have been proposed to account for development of the disorder.

Simon (1978) proposed that exogenous water in the intercellular spaces of the fruit may cause cells to swell. Because of $Ca^{2+}$ deficiency they lose permeability burst and dry out. This is not compatible with the observation that high humidity conditions attenuate bitter pit (Hewett, 1984). Ferguson and

Watkins (1989) point out that the discrete nature of the disorder would require localized changes in water relations.

Bangerth (1979) proposed that $Mg^{2+}$, $K^{+}$, $H^{+}$ and organic acid are antagonistic to membrane function. That high levels of these ions compete for $Ca^{2+}$ binding sites thereby increasing membrane permeability and leading to loss of cell function. Steenkamp and de Villiers (1983) suggested the role of $Ca^{2+}$ in preventing bitter pit was to protect membranes by chelating citric and oxalic acid which they demonstrated dissolved the middle lamellae of apple fruit cells.

The suggestion of Perring (1986) that a localized $Ca^{2+}$ deficiency resulting from withdrawal of $Ca^{2+}$ from the outer cortex accounts for development in storage needs further study (Ferguson and Watkins, 1989). Due to the high variation between samples, data are often expressed as percent of the total so quantitative relationship between redistribution of minerals and bitter pit has not been established (Fergusson and Watkins, 1989). Transverse sections used do not account for longitudal movements of $Ca^{2+}$. Storage conditions that would attenuate bitter pit such as CA storage did not alter $Ca^{2+}$ redistribution (Perring, 1984; Perring and Pearson, 1987).

Ferguson and Drobak, (1988), Ferguson and Watkins (1989), and Ferguson (1990) have considered the various cellular compartments of $Ca^{2+}$. Given: 1) reliable relationships between $Ca^{2+}$ and bitter pit are detectable; and 2) the major compartments of $Ca^{2+}$ in cells are the apoplast and vacuole (Harker and Venis, 1991), it would follow that one or both of these compartments may be critical to

bitter pit development. It is thought that the vacuole due to its low surface area and large volume may provide the rapid transient rises of $[Ca^{2+}]_{cyt}$ necessary for cellular function (Ferguson, 1990). It has been proposed (Ferguson and Drobak, 1988) that the extracellular $Ca^{2+}$ directly accessible to the plasma membrane is the critical pool involved in bitter pit development. This pool of extracellular $Ca^{2+}$ directly accessible to the plasma membrane is where exogenous $Ca^{2+}$ is suspected to exert its effect (Ferguson and Watkins, 1981). It is believed that enough $Ca^{2+}$ is usually present to maintain the structure and function of membranes. However, during certain conditions that would require transient rises in $[Ca^{2+}]_{cyt}$ for signal transduction, the $Ca^{2+}$ availability in this pool may become limiting. The cell may then be unable to respond to external stimuli, which in turn could lead to cellular disfunction (e.g. bitter pit). This could involve the failure to maintain or to prevent certain metabolic events.

In this scheme, low native $[Ca^{2+}]$ is not always detrimental unless exacerbating conditions such as drought or cold shock are present. Thus, a particular $Ca^{2+}$ level in fruit may be adequate under certain conditions, but may not be adequate when there is an increased demand for $Ca^{2+}$ for cellular activity (eg. ripening). This proposal includes the role of $Ca^{2+}$ as a second messenger and accounts for several of the observations concerning bitter pit development in apple including: 1) the variation in disorder development due to the factors previously discussed; 2) that fruits of low total (native) $Ca^{2+}$ content do not necessarily develop the disorder; 3) fruits of varying maturities with similar $Ca^{2+}$

contents differ in susceptibility; 4) irreversible attenuation can be affected by application of CA; 5) increasing $Ca^{2+}$ contents artificially in the extracellular space can attenuate bitter pit and affect intracellular events associated with ripening and senescence (Glenn *et al.*, 1988).

The apparent antagonism of bitter pit by $Mg^{2+}$ has been previously discussed. Fruit with bitter pit are often found to have high $Mg^{2+}$:$Ca^{2+}$ ratios (Perring, 1986). $Mg^{2+}$ is a necessary cofactor for many enzymatic reactions. There are examples of Ca/Mg interactions in cellular functions. The intracellular levels of $Mg^{2+}$ are thought to be maintained in the mM range. Measurements of $Mg^{2+}$ specific current ($I_{Mg}$) in *Paramecium* indicate free $Mg^{2+}$ concentrations were in the range of 0.1-0.7mM. It is suggested that most of the intracellular $Mg^{2+}$ exists in a bound form (White and Hartzell, 1989). Activation of the $I_{Mg}$ was found to be $Ca^{2+}$ dependent (Preston, 1990). Sensitivity of $Ca^{2+}$ channels to blockage by extracellular $Mg^{2+}$ has been demonstrated in N-methyl-D-Aspartate (NMDA) receptor of nerve cells in vertebrates (Burnashev *et al.*, 1992). In apple tissue, Harker *et al.* (1989) found that $Mg^{2+}$ inhibited transport of $Ca^{2+}$ in the apoplast. Thus, $Mg^{2+}$ may have a cellular role for in bitter pit development.

Bitter pit-like symptoms ($Mg^{2+}$ induced pit) have been induced on apples by $Mg^{2+}$ by infiltration treatments whereas other divalent and monovalent cations have not shown this effect (Conway and Sams, 1987; Hopfinger *et al.*, 1984; Fallahi *et al.*, 1987). Researchers disagree as to whether $Mg^{2+}$ induced pits are the same as the natural bitter pit disorder. It has been suggested that the $Mg^{2+}$

induced pits result from breakdown caused by $Mg^{2+}$ toxicity at the entry point into the fruit (Ferguson and Watkins, 1989). Also, $Mg^{2+}$ is known to stimulate polyphenol oxidase activity (an enzyme involved in the browning reactions associated with the death of cells) in fruits vacuum infiltrated with $Mg^{2+}$ (Hopfinger *et al.*, 1984). Fallahi *et al.*, 1987 compared $Mg^{2+}$ induced pits to natural bitter pit and concluded that induced pits lacked corky tissue. We have demonstrated that these $Mg^{2+}$ induced pits are similar to naturally occurring bitter pit (Burmeister and Dilley, 1991). Addition of $Ca^{2+}$ to the infiltration media attenuates $Mg^{2+}$ induced pit and the number of bitter pit-like lesions induced by $Mg^{+2}$ is inversely related to the endogenous (native) $Ca^{2+}$ concentration of individual fruits. Using these $Mg^{2+}$ induced bitter pit-like lesions, as a model for bitter pit may provide new insight into the nature of the bitter pit disorder and provide means to assess bitter pit potential.

Literature Cited:

Askew, H.O., Chittenden, E.T., Monk, R.J. and Watson, J. 1960. Chemical investigations on bitter pit of apples. III. Chemical composition of affected and neighboring healthy tissues. N.Z. J. Agr. Res. 3:169-178.

Bangerth, F., Dilley, D.R., and Dewey, D.H. 1972. Effect of postharvest calcium treatments on internal breakdown and respiration of apple fruits. J. Amer. Soc. Hort. Sci. 97:679-682.

Bangerth, F. 1979. Calcium-related physiological Disorders of plants. Ann. Rev. Phytopathol. 17:97-122.

Boon, Van der. 1980. Prediction and control of bitter pit in apples. II. Control by summer pruning, fruit thinning, delayed harvesting, and soil calcium dressings. J. Amer. Soc. Hort. Sci. 1980. 55:(3)313-21.

Bramlage, W.J., Drake, M and Haker, J.H. 1974. Relationships of calcium content to respiration and postharvest condition of apples. J. Amer. Soc. Hort. Sci. 99:376-378.

Bramlage, W.J., Drake, M., and Weis, S.A. 1979. Changes in calcium level in apple cortex tissue shortly before harvest and during postharvest storage. Commun. Soil Sci. Plant Anal. 10:417-426.

Brisken, D.P. 1990. $Ca^{2+}$-translocation ATPase of the plant plasma membrane. Plant Physiol. 94:397-400.

Burmeister, D.M. and Dilley, D.R. 1991. Induction of bitter pit-like symptoms on apples by infiltration with $Mg^{2+}$ is attenuated by $Ca^{2+}$. Postharvest Biol. Tech. 1:11-17.

Burnashev, N. Schoepfer, R. Monyer, H., Ruppersberg, J.P., Günther, W., Seeburg, P.H., and Sakman, B. 1992. Control by Asparagine Residues of Calcium Permeability and Magnesium Blockade in NMDA Receptor. Science, 257:1415-1419.

Carafoli, E. 1987. Intracellular calcium homeostasis. Annu. Rev. Biochem. 56:395-433.

Cheek, T.R. 1989. Spatial aspects of calcium signalling. J Cell Sci 93:211-216.

Conway, W.S. and Sams, C.E. 1987. The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples. J. Amer. Soc. Hort. Sci. 112:300-303.

Cooper, T. and Bangerth, F. 1976. The effect of Ca and Mg treatments on the physiology, chemical composition and bitter pit development of Cox's Orange apples. Sci. Hortic. 5:49-57.

Delong, W.A. 1936. Variations in the chief ash constituents of apples affected with blotchy cork. Plant Physiol. 11:453-456.

Demarty, M., Morvan, C., and Thellier, M. 1984. Calcium and the cell wall. Plant Cell and Environ. 7:441-448.

Fallahi, E., Fighette, T.L. and Wernz, J.G. 1987. Effects of dip and vacuum infiltrations of various inorganic chemicals on post-harvest quality of apple. Commun. Soil Sci. Plant Anal. 18:1017-1029.

Faust, M., and Klein, J.D. 1974. Levels and sites of metabolically active calcium in apple fruit. J. Am. Soc. Hort. Sci. 99:93-94.

Faust, M. and Shear, C.B. 1968. Corking disorders of apples: A physiological and biochemical review. Bot. Rev. 34:441-469.

Faust, M. and Shear, C.B. 1972. The effect of calcium on the respiration of apples. J. Amer. Soc. Hort. Sci. 97:437-439.

Faust, M. Shear, C.B., and Smith, C.B. 1967. Investigations of corking disorders of apples. I. Mineral element gradients in York Imperial apples. Proc. Amer. Soc. Hort. Sci. 91:69-72.

Faust, M., Shear, C.B. and Smith, C.B. 1968a. Investigation of corking disorders of apples. II. Chemical composition of affected tissues. Proc. Amer. Soc. Hort. Sci. 92:82-88.

Faust, M., Shear, C.B. and Smith, C.B. 1968b. Investigation of corking disorders of apples. III. Chemical composition of affected tissues. Proc. Amer. Soc. Hort. Sci. 93:746-752.

Felle, H. 1988. Cytoplasmic free calcium in Riccia fluitans L. and Zea mays L.: interaction of $Ca^{2+}$ and pH? Planta. 174:495-499.

Ferguson, I.B. 1984. Calcium in plant senescence and fruit ripening. Plant and Cell Environ. 7:477-489.

Ferguson I.B. 1990. Calcium nutrition and cellular response. In: Calcium in Plant Growth and Development. Proc. 13th Annual Riverside Symposium on Plant Physiology. January 11-13, 1990. American Society of Plant Physiologists, Rockville, MD, 20855.

Ferguson, I.B., and Drobak, B.K. 1988. Calcium and the regulation of plant growth and senescence. HortScience. 23:262-268.

Ferguson I.B., Reid, M.S. and Prasad, M. 1979. Calcium analysis and the prediction of bitter pit in apple fruit. N.Z. J. Agr. Res. 22:485-490.

Ferguson, I.B. and Watkins, C.B., 1981. Ion relations of apple fruit tissue during fruit development and ripening III. Calcium Uptake. Australian J. Plant Physiol. 8:259-266.

Ferguson, I.B. and Watkins, C.B. 1983. Cation distribution and balance in apple fruit in relation to calcium treatments for bitter pit. Scientia Hort. 19:301-310.

Ferguson, I.B. and Watkins, C.B. 1989. Bitter pit in apple fruit. Hortic. Rev. 289-353.

Ford, E.M. 1979. The distribution of calcium in mature apple fruits having bitter pit disorder. J. Amer. Soc. Hort. Sci. 54:91-92.

Garman, P. and Mathis, W.T. 1956. Studies of mineral balance as related to occurrence of Baldwin spot in Connecticut. Conn. Agric. Exp. Stn. Bull. 601:5-19.

Glenn, G.M., Reddy, A.S.N. and Poovaiah, B.W. 1988. Effect of calcium on cell wall structure, protein phosphorylation and protein profile in senescing apples. Plant and Cell Physiol. 29:565-572.

Harker, F.R., Ferguson, I.B. and Dromgoole, F.I. 1989. Calcium ion transport through tissue discs of cortical flesh of apple fruit. Physiol. Plant. 74:688-694.

Harker, F.R. and Venis, M.A., 1991. Measurement of intracellular and extracellular free calcium in apple cells using calcium sensitive electrodes. Plant Cell and Environ. 14:525-530.

Heplar, P.K., and Wayne, R.O. 1985. Calcium and plant development. Annu. Rev. Plant Physiol. 36:397-439.

Hewett, E.W. 1984. Bitter pit reduction in 'Cox's Orange Pippin' apples by controlled and modified atmosphere storage. Scientia Hort. 23:59-66.

Holland, D.A. 1980. The prediction of bitter pit. In: D. Atkinson, L.E. Jackson, R.O. Sharples and W.M. Waller (eds.) Mineral Nutrition of Fruit Trees. Butterworths, London. pp. 380-81.

Hopfinger, J.A. and Poovaiah, B.W. 1979. Calcium and magnesium gradients in apples with bitter pit. Commun. Soil Sci. Plant Anal. 10:57-65.

Hopfinger, J.A., Poovaiah, B.W. and Patterson, M.E. 1984. Calcium and magnesium interactions in browning of Golden Delicious Apples with bitter pit. Sci. Hortic. 23:345-351.

Jackson, D.I. 1962. The effects of calcium and other minerals on incidence of bitter pit in Cox's Orange apples. N.Z. J. Agric. Res. 5:302-309.

Johnson, D.S. 1979. New techniques in the post-harvest treatment of apple fruits with calcium salts. Commun. Soil Sci. Plant Anal. 10:373-382.

Marcelle, R.D., Porreye, W., Goffings, G. and Herregods, M. 1989. Relationship between fruit mineral composition and storage life of apples, cv. Jonagold. Acta Hort. 258:373-378.

Martin, D., Lewis, L. and Cerny, J. 1960. Bitter pit in the apple variety cleopatra in Tasmania in relation to calcium and magnesium. Aust. J. Agric. Res. 11:186-192.

Mason, J.L. and Drought, B.G. 1975. Penetration of calcium into Spartan apple fruits from a post-harvest calcium chloride dip. J. Amer. Soc. Hort. Sci. 100:413-439.

Meyer, B.R., Peisach, M. and Kotze, W.A.G. 1979. Analysis of sound and pitted tissue of apple fruit by proton-induced x-ray spectrometry. Scientia Hort. 10: 57-61.

Miller A.J., and Sanders, D. 1988. Depletion of cytosolic free calcium induced by photosynthesis. Nature. 326:397-400.

Moore, A.L., and Akerman, K.E.O. 1984. Calcium and plant organelles. Plant Cell and Environ. 7:423-429.

Perring, M.A. 1979. The effects of environment and cultural practices on calcium concentration in the apple fruit. Comm. Soil Sci. Plant Anal. 10:279-293.

Perring, M.A. 1984. Redistribution of minerals in apple fruit during storage. Preliminary investigations with the variety Spartan. J. Sci. Food Agr. 35:182-190.

Perring, M.A. 1986. Incidence of bitter pit in relation to calcium content of apples: problems and paradoxes, a review. J. Sci. Food Agric. 37:591-606.

Perring, M.A. 1989. Apple fruit quality in relation to fruit chemical composition. Acta Hort. 258:365-372.

Perring, M.A., and Pearson, K. 1986. Incidence of bitter pit in relation to the calcium content of apples:calcium distribution in the fruit. J. Sci. Food Agric. 37, 709-718.

Perring, M.A., and Pearson, K. 1987. Redistribution of minerals in apple fruit during storage: the effect of storage atmosphere on calcium concentration. J. Sci. Food Agric. 40:37-42.

Perring, M.A. and Wilkinson, B.G. 1965. The mineral composition of apples. IV. The radial distribution of chemical constituents in apples, and its significance in sampling for analysis. J. Sci. Food Agric. 16:535-41.

Poovaiah, B.W. 1988. Molecular and cellular aspects of calcium action in plants. HortScience, 23:267-271.

Poovaiah, B.W. and Reddy, A.S.N. 1987. Calcium messenger system in plants. CRC Crit. Rev. Plant Sci., 6:47-103.

Preston, R.R. 1990. A Magnesium Current in Paramecium. Science, 250:285-288.

Raz, V., and Fluhr, R. 1992. Calcium requirement for ethylene-dependent responses. Plant Cell. 4:1123-1130.

Richmond, A.E., Dilley, D.R. and Dewey, D.H. 1964. Cation, organic acid and pH relationships in peel tissue of apple fruits affected with Jonathan Spot. Plant Physiol. 39:1056-1060.

Scott, K.J. and Wills, R.B.H. 1979. Effects of vacuum and pressure infiltration of calcium chloride and storage temperature on the incidence of bitter pit and low temperature breakdown of apples. Aust. J. Agric. Res. 30:917-28.

Sharples, R.O., and Johnson, D.S. 1987. Influence of agronomic and climatic factors on the response of apple fruit to controlled atmosphere storage. HortScience 22:763-766.

Simon, E.W. 1978. The symptoms of calcium deficiency in plants. New Phytol. 80:1-15.

Simons, R.K. 1962. Anatomical studies of the bitter pit area of apples. Proc. Amer. Soc. Hort. Sci. 81:41-50.

Simons R.K., Hewetson, F.W., and Chih-Yu Chu Mel. 1971. Sequential Development of the 'York Imperial' Apple As Related to Tissue Variances Leading to Corking Disorders. J. Amer. Soc. Hort. Sci. 96(2):247-252.

Smock, R.M. and Neubert, A.M. 1950. Apples and apple products. Interscience Pub., New York.

Smock, R.M. and Van Doren. 1937. Histology of bitter pit in apples. Proc. Amer. Soc. Hort. Sci. 35:176-179.

Stenkamp J., and de Villiers, O.T. 1983. The role of organic acids and nutrient elements in relation to bitter pit in Golden Delicious apples. Acta Hort. 138:35-42.

Tingwa, P.D. and Young, R.E. 1974. The effect of calcium on the ripening of avocado (*Persea americana Mill.*) fruits. J. Amer. Soc. Hort. Sci. 99:540-542.

Tomala, K. and Dilley, D.R. 1989. Calcium content of McIntosh and Spartan apples is influenced by the number of seeds per fruit. In: Proc. 5th Int. Controlled Atmosphere Research Conf., J.K. Fellman (ed.), 14-16 June 1989, Wenatchee, WA, Washington State University, Vol. 1, pp. 75-82.

Tomala, K. and Dilley, D.R. 1990. The role of calcium in maturation, ripening, and physiological disorders of apple fruits. Proc. 23rd International Hort. Congress, Firenze, Italy, Aug. 27-Sept. 1, pp. 10.

Waller, W.M. 1980. Use of apple analysis. In: D. Atkinson, L.E. Jackson, R.O. Sharples and W.M. Waller (eds.) Mineral Nutrition of Fruit Trees. Butterworths, London. pp. 383-394.

Wayne, R. 1993. Excitability in plant cells. American Scientist. 83:140-151.

Webster, D.H., and Forsyth, R.F. 1979. Partial control of bitter pit in Northern Spy apples with a post-harvest dip in calcium chloride solution. Can. J. Plant Sci. 59:717-723.

White, R.E. and Hartzell, C.H. 1989. Magnesium Ions in Cardiac Function. Biochem. Pharm. 38:859-867

Wilkinson, B.G. and Perring, M.A. 1961. Variation in the mineral composition of Cox's Orange Pippin apples. J. Sci. Food Agric. 12:74-80.

Wilkinson, B.G. and Perring, M.A. 1964. Further investigations of chemical concentration gradients in apples. J. Sci. Food Agric. 15:378-84.

Wills, R.B.H., Scott, K.J. and Smale, P.J. 1976. Prediction of bitter pit with calcium content of apple fruit. N.Z. J. Agr. Res. 19:513-519.

# Chapter 1

## Correlation of Bitter Pit on Northern Spy Apples with Bitter Pit-like Symptoms Induced by $Mg^{2+}$ Salt Infiltration.

ABSTRACT:

Induction of bitter pit-like symptoms ($Mg^{2+}$ induced pits [MgIP]) on Northern Spy apples (*Malus domestica* Borkh.) by infiltrating $Mg^{2+}$ salt solutions into the fruit was positively correlated with bitter pit that developed naturally during storage. Fruit at harvest were infiltrated with 0.1M $MgCl_2$ in 0.3M sorbitol with 0.1% Tween 20 and placed at 20°C for 10 days (d) after which the number of MgIP was determined on individual fruits. A parallel sample of fruits from each orchard was stored at 5°C in air (both years) and at 3°C in air or controlled atmosphere (CA) storage in the second year. MgIP was positively correlated with bitter pit development in storage for fruits harvested 20d and 10d before and at optimal maturity for long term storage. The endogenous (native) fruit $Ca^{2+}$ concentration was inversely related to MgIP and to bitter pit development following storage.

INTRODUCTION:

Bitter pit is a physiological disorder affecting apples. It is characterized by sunken lesions that may develop on fruits as they mature on the tree or during storage. The tissue below the skin in the pitted area becomes discolored and dehydrated (Faust and Shear, 1968). Certain environmental and cultural conditions contribute to increased bitter pit incidence including: light cropping, $Ca^{2+}$ deficiency, excessive tree vigor, excessive N nutrition, and moisture stress. Of these, calcium deficiency is the major factor that has been associated with bitter pit (Delong 1936; Garman and Mathis, 1956; Ferguson and Watkins, 1989).

Low fruit Ca levels consistently correlate with high levels of bitter pit (Ferguson *et al.*, 1979; Wills *et al.*, 1976; Waller, 1980). The primary methods employed in attempts to control the disorder are: applying $Ca^{2+}$ sprays to trees at intervals throughout the growing season, using post-harvest dips or drenches in $Ca^{2+}$ solutions, or infiltrating $Ca^{2+}$ solutions into fruit (Scott and Wills, 1979; Cooper and Bangerth, 1976). Control of bitter pit by $Ca^{2+}$ treatments is highly variable and not always complete.

Another approach employed to reduce losses from bitter pit is to use predictive methods based upon mineral analysis to determine the fruits potential to be afflicted with the disorder. This has been shown to be useful and is practiced commercially (Ferguson and Watkins, 1989). Analysis of fruit $Ca^{2+}$ is commonly employed for these predictive methods. Fruit are sampled from orchards prior to harvest and analyzed for Ca. Orchards with low Ca fruits can thereby be segregated for early marketing or the fruits can be processed prior to development of the bitter pit disorder. Fruit from orchards with a high $Ca^{2+}$ status can normally be safely held for long durations in storage. Predictive systems are in practice that use fruit $Ca^{2+}$ analysis alone, or in combination with other orchard factors (eg. fruit size, tree age, cropping level), to predict bitter pit occurrence in storage (Holland, 1980). The practicality of such predictive methods is limited due to the inherent variability of bitter pit incidence within and among orchards, varieties and growing seasons (Wills *et al.*, 1976). Timeliness is important. For any predictive system to be practical, the result must be known

well enough in advance of the harvest to allow time for management decisions. The relationship of $Ca^{2+}$ to bitter pit has been shown to be reliable up to 3 weeks before harvest (Ferguson *et al.*, 1979). Analysis of young fruitlets midway in the growing season has also proven to be useful (Marcelle *et al.*, 1989). However, fruit $Ca^{2+}$ analysis is expensive, time consuming, and requires specialized equipment. Also, because of the high variability among fruits within an orchard, large sample sizes are necessary to assess bitter pit potential (Waller, 1980). An alternative method of assessing fruit Ca levels would be useful. Researchers have reported the induction of bitter pit-like symptoms (MgIP) on apples within 7d of $Mg^{2+}$ treatment by infiltration (Conway and Sams, 1987; Hopfinger *et al.*, 1984; Fallahi *et al.*, 1987). We have demonstrated that MgIP are similar to naturally occurring bitter pit (Burmeister and Dilley, 1991). Addition of $Ca^{2+}$ to the infiltration media attenuates MgIP and the number of MgIP is inversely related to the endogenous (native) $Ca^{2+}$ concentration of individual fruits. If orchards of a low $Ca^{2+}$ status have fruit more susceptible to MgIP then it may be possible to assess bitter pit potential in a relatively quick and inexpensive manner. The results reported herein are from a 2-year study correlating susceptibility to MgIP with the occurrence of bitter pit in storage.

MATERIALS AND METHODS:

Orchards were selected that exhibited a range of bitter pit susceptibilities based on orchard history. The number of orchards sampled, locations and harvest dates varied between years. Orchards from the eastern, western, and

southwestern districts in Michigan were used in this study.

1990 Studies. Northern Spy and Red Delicious cultivars were employed. Apples from 5 locations were harvested at ca. 1 week prior to, and from 11 locations at maturity (ca. 10% of fruits at > 0.2 $\mu$l $l^{-1}$ internal ethylene concentration). Samples of 10-15 fruit from each of 20 trees were randomly selected at each location and were divided into two lots. One lot was vacuum infiltrated (absolute pressure of 100mmHg for 2 min.) while submersed in 0.1M $MgCl_2$ (50 fruit) in 0.3M sorbitol as osmoticum with 0.1% Tween 20 as surfactant. The other lot was infiltrated with 0.3M sorbitol 0.1% Tween 20 which served as the control (30 fruit). The fruits were held for 10d at 20° C, and the number of MgIP lesions on individual fruits was recorded. None of the fruits infiltrated with 0.3M sorbitol with Tween 20 developed pits (data not shown). Parallel samples of fruit not infiltrated were stored in perforated polyethylene bags in air at 5° C for 5 months after which the number of naturally-occurring bitter pit lesions on each individual fruit was recorded. Individual fruits minus the core were analyzed for calcium by atomic absorption spectrometry according to Tomala and Dilley, (1989). Calcium data are expressed as ppm dwt (ug $g^{-1}$ dry wt).

1991 Studies. Only the Northern Spy cultivar was employed. Five orchards were sampled ca. 20d, and 6 orchards at 10d prior to and at optimal maturity for long term storage (Dilley and Dilley, 1985). Procedures were as previously described except calcium analysis was conducted using a bulked sample consisting of longitudinal sections (1/16 of each fruit excluding seeds) taken from 40 fruit

from each location/harvest combination. Storage treatments included air at 3°C, and controlled atmosphere (CA) of 3.0% $O_2$ + 3.0% $CO_2$ at 3°C for 6 months. Fruit were held in 3°C air for 7d before CA was applied. Following storage fruit were held for 7d at 20°C before assessment of bitter pit. These are the commercially recommended storage conditions for Northern Spy in Michigan.

Data were analyzed by regression of MgIP vs bitter pit occurring during storage. MgIP and bitter pit were assessed by determining the average number of pits/fruit and the % of fruits with bitter pit. Calcium data were analyzed by regression of endogenous (native) Ca vs mean number of MgIP, or % of fruit with bitter pit that developed during storage.

RESULTS AND DISCUSSION:

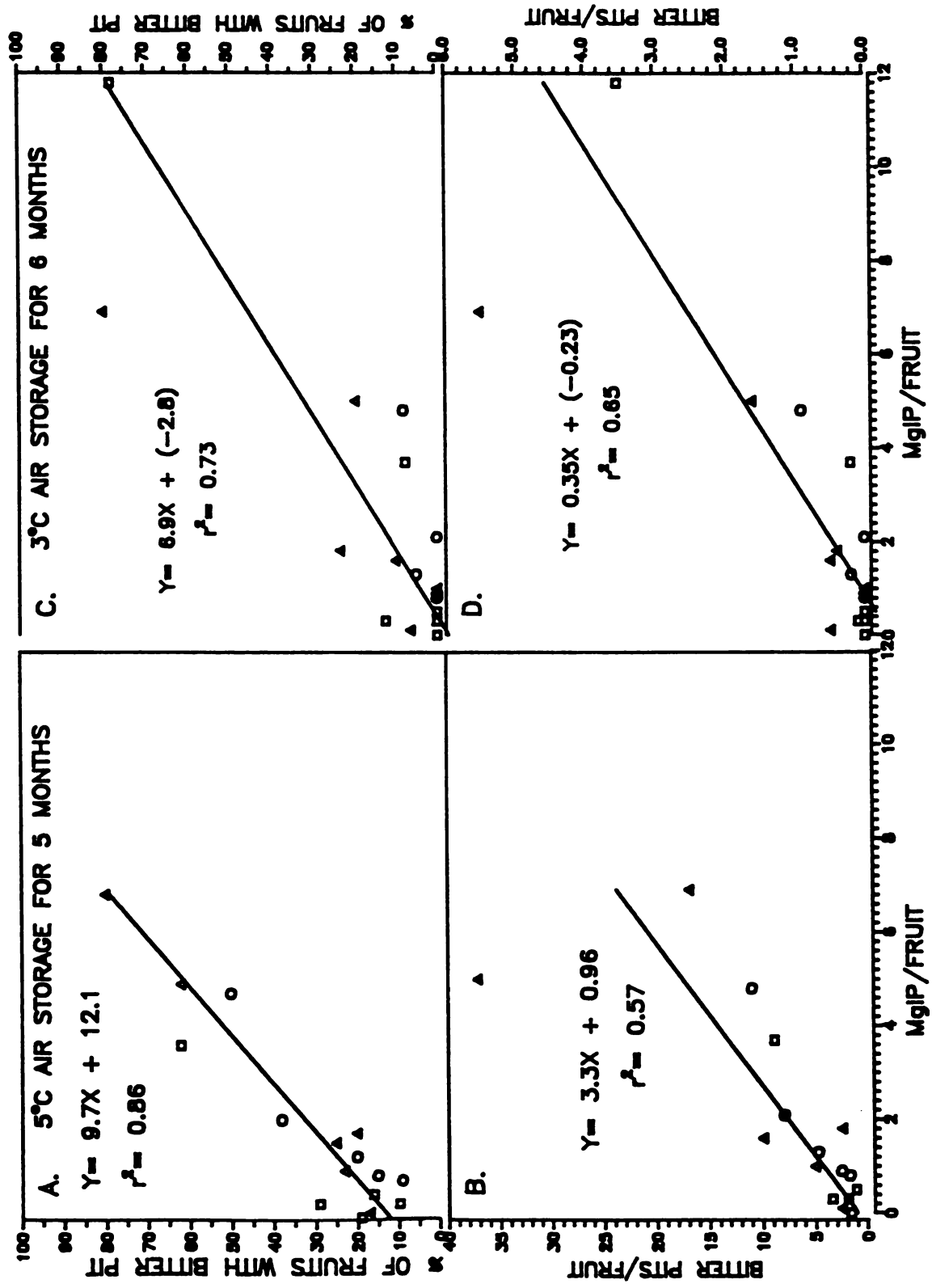
For Red Delicious, incidence of MgIP and natural bitter pit development were low for all locations sampled. This precluded development of prediction equations for this variety. This may reflect a high calcium status of these blocks, but this was not verified. Only the results for Northern Spy are presented.

1990. For the Northern Spy harvested early there was no correlation between MgIP and natural bitter pit (see appendix 2, Fig. 1). For late harvest 1990, severity (bitter pits/fruit) and incidence (% of fruits with bitter pit) was positively correlated with bitter pit after storage; $Y = 1.76X + 1.2$, $r^2 = 0.69$ and $Y = 6.7X + 20.0$, $r^2 = 0.57$, respectively. In 1991, severity and incidence was positively correlated with the severity of MgIP for all harvests for both the 5°C (Fig. 1, A and B) and 3°C (Fig. 1 A and C) storage temperatures. Maturity of

fruit at harvest can be an important factor in subsequent development of bitter pit (Ferguson and Watkins, 1989). We monitored fruit maturity in the 1991 season to determine if maturity at harvest affected the correlation of bitter pit with MgIP. An effect of harvest date on the correlation was not apparent (Fig. 1) but due to weather conditions there was not as much variation in maturity between districts as normally would be expected in a typical season (data not shown). Bitter pit levels were higher for fruits stored in air at 5°C (Fig. 1, A and B) than stored in air stored at 3°C (Fig. 1, C and D). Regressions for the 3°C CA yielded similar prediction equations; severity vs severity and incidence were; $Y = 0.32X + (-0.27)$, $r^2 = 0.69$ and $Y = 5.7X + (-5.6)$, $r^2 = 0.73$, respectively. All regressions were significant at $^{**}P < 0.01$. In most instances, severity and incidence of bitter pit were lower in the 3°C CA treatment. We attribute this to a lower rate of metabolism of fruits in CA. It has been demonstrated that postharvest conditions that slow ripening and metabolism can reduce bitter pit levels (Perring, 1986).

The endogenous (native) Ca was inversely related to the average number of MgIP/fruit and bitter pits/fruit that developed during storage (data not shown). This type of relationship is considered typical when relating bitter pit to fruit calcium levels. The exceptions to the relationship being fruit that are low in Ca do not necessarily develop bitter pit (Ferguson and Watkins, 1989). In this study, endogenous Ca levels below ca. 200 $\mu$g/g dwt., a range of bitter pit levels can be expected from 0 - >35 pits/ fruit depending on storage temperature (see appendix 2, Fig. 2 and 3, respectively).

Figure 1. Correlation between MgIP/fruit at harvest and natural bitter pit development on Northern Spy apples during storage in air. Fruit were harvested 20d (O) and 10d (□) prior to and at optimal maturity (Δ). Each data point represents the average of 50 fruits. All regressions were significant at $^{**}P<0.01$. For 5°C storage, A) MgIP/fruit vs % of fruits with bitter pit and B) MgIP/fruit vs bitter pits/fruit and for 3°C storage, C) MgIP/fruit vs % of fruits with bitter pit and D) MgIP/fruit vs bitter pits/fruit.


A. 5°C AIR STORAGE FOR 5 MONTHS
Y= 9.7X + 12.1
r²= 0.86
% OF FRUITS WITH BITTER PIT
B.
Y= 3.3X + 0.96
r²= 0.57
BITTER PITS/FRUIT
MgIP/FRUIT
C. 3°C AIR STORAGE FOR 6 MONTHS
Y= 6.9X + (−2.8)
r²= 0.73
% OF FRUITS WITH BITTER PIT
D.
Y= 0.35X + (−0.23)
r²= 0.65
BITTER PITS/FRUIT
MgIP/FRUIT

In utilizing MgIP to predict bitter pit some of the same problems encountered with the use of Ca analysis are evident. The relationship between bitter pit and Ca is highly variable. This makes accurate prediction of levels of bitter pit impossible (Perring, 1986). However, threshold levels of Ca where minimal or no bitter pit may be expected have been established (Wills *et al.*, 1976). Therefore, high variation does not necessarily preclude the use of MgIP as a method to assess bitter pit potential. The correlation coefficient ($r^2$) values presented here are similar in magnitude to others used for bitter pit prediction (Holland, 1980; Waller, 1980). Moreover, in no instance was a high level of MgIP associated with a low level of bitter pit. For fruits stored at 3°C, our data indicate when MgIP is below ca. 5 pits/fruit, bitter pit incidence was below 25% and number of bitter pits/fruit 2.0 (Fig. 1, C and D). Prediction of % incidence of bitter pit from % incidence of MgIP was highly correlated ($r^2$ = 0.81) (see appendix 2, Fig. 4).

Developing a practical prediction scheme for physiological disorders such as bitter pit is problematic. Many issues need to be considered. We sampled fruit from orchards with a wide range of tree ages, fruit maturities, and environmental, and cultural conditions. We observed a good correlation between MgIP and natural bitter pit occurrence up to 20d prior to the commercially recommended harvest date. This time frame is desirable to provide lead time to segregate fruit lots to be processed immediately from those to be processed after long-term storage. A MgIP/bitter pit relationship could also be useful to indicate

the need for additional $Ca^{2+}$ treatment by postharvest dips. In order to determine if the relationship we have found between the propensity of fruits for MgIP and occurrence of bitter pit during storage may be generally applicable, experiments need to be conducted with various cultivars under a variety of storage temperature and atmosphere conditions. Postharvest handling and storage practices can affect bitter pit occurrence. For example, a delay in establishing CA conditions of greater than 7d promotes ripening during storage and can have a detrimental effect by promoting the development of physiological disorders such as bitter pit (Fica *et al.*, 1985).

Assessment and prediction of bitter pit has been reviewed (Ferguson and Watkins, 1989). It is unclear from the published reports whether low % incidence of bitter pit represent a few fruit with light or severe symptoms or, if a high average number of pits/fruit is the result of a few fruit with severe symptoms. Any amount of pitting on an individual fruit destroys its fresh market (dessert) value. In the case of the pie slice industry in Michigan both bitter pit incidence and severity are considered important in regard to processing Northern Spy apples (Geisler, 1992, personal communication, Coloma Fruit, Inc.). Fruits visually estimated to have >15 pits after peeling are culled. Apple slices affected with bitter pit can be removed during processing, or the pitted area peeled away, but extremely high incidence of pitting slows the processing of apples destined to secondary markets.

The correlation between MgIP and development of bitter pit in storage

extends our previous observations (Burmeister and Dilley, 1991) that MgIP is similar to the bitter pit disorder. We believe that the method described has the potential to be a reliable and inexpensive means to assess fruits according to their potential for bitter pit. Can the assessment of MgIP at harvest be related to bitter pit development potential in a practical manner? Counting the number of fruit affected in a sample can be accomplished easily, rather than counting the number of pits on each individual fruit. Perhaps an index that relates incidence and severity such as used for superficial scald (Lurie *et al.*, 1989) would be useful.

We have recently demonstrated that treatments affecting calcium homeostasis (eg. calmodulin antagonists, $Ca^{2+}$ chelators) can alter MgIP development (Burmeister and Dilley, 1993). We speculate that the MgIP development and the bitter pit disorder may share common mechanisms. Calcium is recognized to be a second messenger in plant cells in mediating cellular metabolism (eg. calmodulin, and Ca-ATPase) (Heplar and Wayne, 1985). Models have been proposed to explain how calcium might regulate cell metabolism (Ferguson and Drobak, 1988; Poovaiah, 1988). The cytosolic concentrations of free $Ca^{2+}$ and $Mg^{2+}$ are known to be maintained at the submicromolar and millimolar range, respectively. Ferguson (1990) has suggested that depletion of the extracellular pool of $Ca^{2+}$ results in loss of the cells' capacity to respond to external stimulus resulting in cellular dysfunctions (eg. bitter pit).

Apple tissue susceptible to bitter pit has been shown to be low in $Ca^{2+}$, especially in relation to the $Mg^{2+}$ level (Garman and Mathis, 1956). Harker *et al.*,

(1989) found that $Mg^{2+}$ inhibits $^{45}Ca^{2+}$ transport across discs of cortical flesh of apple fruit. Gilroy *et al.*, (1989) reported that high extracellular $Mg^{2+}$ and $K^{+}$ concentrations resulted in rapid breakdown of $Ca^{2+}$ homeostasis of carrot protoplasts. Sensitivity of $Ca^{2+}$ channels to blockage by extracellular $Mg^{2+}$ has been demonstrated in animal systems (Burnashev *et al.*, 1992). Infiltration of $Ca^{2+}$ in the extracellular space of apple fruit can attenuate bitter pit and affect intracellular events associated with ripening and senescence (Glenn *et al.*, 1988). Combined with these observations, our data suggest a more specific role for $Ca^{2+}$ and $Mg^{2+}$ in bitter pit development. We speculate that extracellular $Mg^{2+}$ supplied by infiltration may affect the supply of $Ca^{2+}$ in the apoplast of apple fruit influencing the cells ability to regulate cytosolic $Ca^{2+}$; either by perturbation of a voltage-regulated $Ca^{2+}$ channel or displacing $Ca^{2+}$ from ionic binding sites in the apoplast. This could interfere with the role of $Ca^{2+}$ as a second messenger involving a $Ca^{2+}$-ATPase, or $Ca^{2+}$/calmodulin linked phosphorylation of an enzyme, or a regulatory protein involved in cellular homeostasis, or metabolism. We believe $Mg^{2+}$ exacerbates the potential for apple fruit to initiate the chain of reactions involved in expressing this $Ca^{2+}$ related disorder.

The Relationship Between the Number of Bitter Pits per Fruit and % Incidence of the Bitter Pit Disorder.

Interpretation of research data on bitter pit and how this may relate to the practical significance of bitter pit in the commercial sector may yield different opinions. Assessment of bitter pit and other fruit storage disorders is in question. It is sometimes not clear from the published reports whether low % incidence of bitter pit represent a few fruit with light or severe symptoms or, if a high number of pits/fruit is the result of a few fruit with severe symptoms. Any amount of pitting on an individual fruit destroys its fresh market (dessert) value. In the case of processing apples as fresh slices for the bakery industry both bitter pit incidence and severity are considered important (Geisler, personal communication, Coloma Fruit, Inc. 1992). Fruits visually estimated to have >15 pits after peeling are culled. Apple slices affected with bitter pit can be removed during processing, or the pitted area pared away, but extremely high incidence of pitting slows the processing of apples destined to the bakery industry. It is important to have a basis to measure the bitter pit disorder for both commercial and research purposes.

Meaningful assessment of bitter pit is important in terms of any prediction scheme that might be employed. Counting the number of fruit affected in a sample can be accomplished easily, rather than counting the number of pits on each individual fruit. An numerical index that relates incidence and severity such

as used for superficial scald may be useful.

The relationship between number of pits per fruit and % incidence of bitter pit for Northern Spy stored at 3°C appears to be linear (Fig. 2). If this relationship is proven to be consistent between seasons severity could be predicted on the basis of incidence.

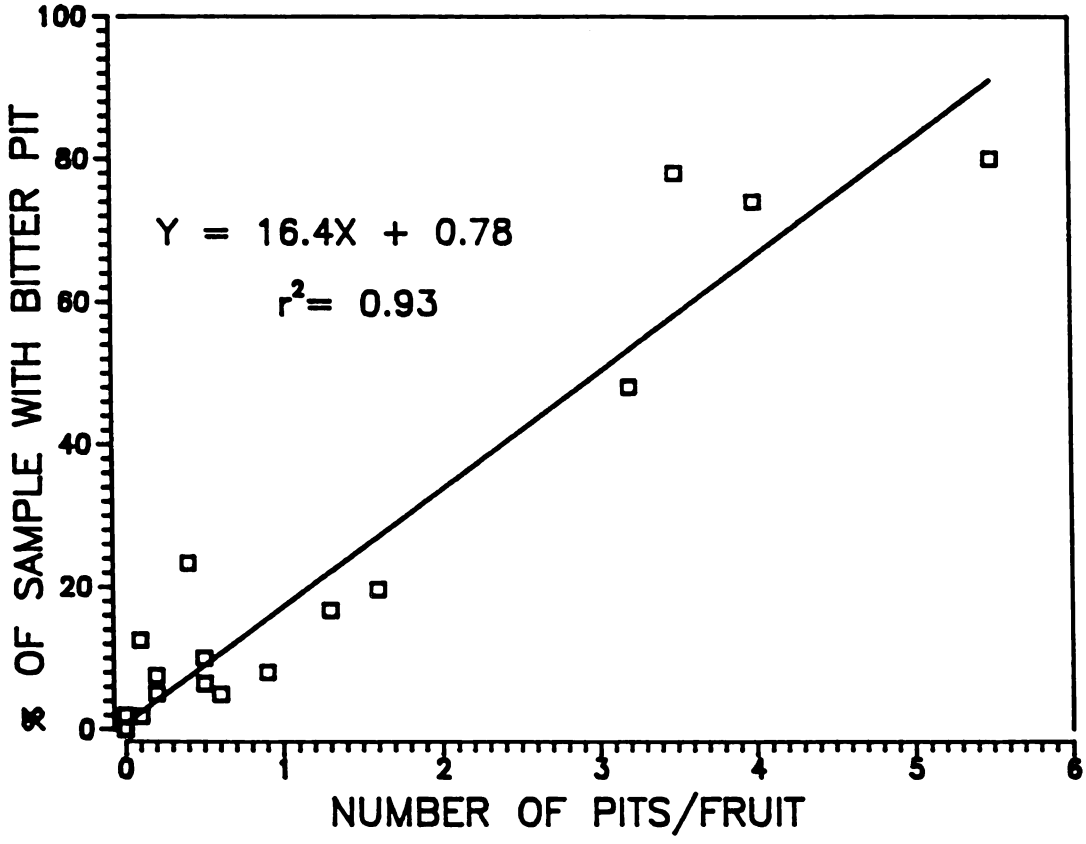



Figure 2. The relationship between the number of bitter pits per fruit and % incidence of bitter pit for Northern Spy stored at 3°C.

REFERENCES:

Burmeister, D.M. and Dilley, D.R. 1991. Induction of bitter pit-like symptoms on apples by infiltration with $Mg^{+2}$ is attenuated by $Ca^{+2}$. Postharvest Biol. Tech. 1:11-17.

Burmeister, D.M. and Dilley, D.R. 1993. Characterization of $Mg^{2+}$ induced bitter pit-like symptoms on apples: A model system to study bitter pit initiation and development. J. Agr. Food Chem. in press.

Burnashev, N., Schoepfer, R., Monyer, H., Ruppersberg, J.P., Günther, W., Seeburg, P.H., and Sakman, B. 1992. Control by asparagine residues of calcium permeability and magnesium blockade in NMDA receptor. Science. 257:1415-1419.

Conway, W.S. and Sams, C.E. 1987. The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples. J. Amer. Soc. Hort. Sci. 112:300-303.

Cooper, T. and Bangerth, F. 1976. The effect of Ca and Mg treatments on the physiology, chemical composition and bitter pit development of Cox's Orange apples. Sci. Hort. 5:49-57.

Delong, W.A. 1936. Variations in the chief ash constituents of apples affected with blotchy cork. Plant Physiol. 11:453-456.

Dilley, D.R. and Dilley, C.L. 1985. New technology for analyzing ethylene and determining the onset of the ethylene climacteric of apples. Proc. of 4th Natl. Controlled Atmosphere Res. Conf. 4:353-362.

Fallahi, E., Fighette, T.L. and Wernz, J.G. 1987. Effects of dip and vacuum infiltrations of various inorganic chemicals on postharvest quality of apple. Commun. Soil Sci. Plant Anal. 18:1017-1029.

Faust, M. and Shear, C.B. 1968. Corking disorders of apples: A physiological and biochemical review. Bot. Rev. 34:441-469.

Ferguson I.B. 1990. Calcium nutrition and cellular response. In: Calcium in Plant Growth and Development. Proc. 13th Annual Riverside Symposium on Plant Physiology. January 11-13, 1990. American Society of Plant Physiologists, Rockville, MD, 20855.

Ferguson I.B. and Drobak, B.K., 1988. Calcium and the regulation of plant growth and senescence. HortScience 23: 262-268.

Ferguson I.B., Reid, M.S. and Prasad, M. 1979. Calcium analysis and the prediction of bitter pit in apple fruit. N.Z. J. Agr. Res. 22:485-490.

Ferguson, I.B. and Watkins, C.B. 1989. Bitter pit in apple fruit. Hort. Rev. 289-353.

Fica, J., Skrzynski, J., and Dilley, D.R. 1985. The effect of delayed cooling and delayed application of CA storage of McIntosh apples under low and high ethylene levels. Proc. 4th Natl. CA Res. Conf., Hort. Rept. No. 126, North Carolina State Univ., Raleigh, N.C. pp. 82-94.

Garman, P. and Mathis, W.T. 1956. Studies of mineral balance as related to occurrence of Baldwin spot in Connecticut. Conn. Agr. Expt. Sta. Bull. 60l:5-19.

Gilroy, S., Fricker, M., Blowers, D., Harvey, H., Collinge, M. and Trewavas, A.J. 1989. Calcium channels, cytosol calcium and plasma membrane phospohorylation: an integrated calcium stat system. In: Plant Membrane Transport: The Current Position. J Dainty (ed.) Department of Botany University of Edinburgh. The King's Buildings Mayfield Road, Edinburgh. EH9 3JH, U.K., pp.215-224.

Glenn, G.M., Reddy, A.S.N. and Poovaiah, B.W. 1988. Effect of calcium on cell wall structure, protein phosphorylation and protein profile in senescing apples. Plant and Cell Physiology. 29:565-572.

Harker, F.R., Ferguson, I.B. and Dromgoole, F.I. 1989. Calcium ion transport through tissue discs of cortical flesh of apple fruit. Physiol. Plant. 74:688-694.

Heplar P.K. and Wayne, R.O. 1985. Calcium and plant development. Annu. Rev. Plant Physiol. 36:397-439.

Holland, D.A. 1980. The prediction of bitter pit. In: D. Atkinson, L.E. Jackson, R.O. Sharples and W.M. Waller (eds.) Mineral Nutrition of Fruit Trees. Butterworths, London. pp. 380-81.

Hopfinger, J.A., Poovaiah, B.W. and Patterson, M.E. 1984. Calcium and magnesium interactions in browning of Golden Delicious Apples with bitter pit. Sci. Hort. 23:345-351.

Lurie, S., Maier, S. and Ben Arie, R. 1989. Preharvest ethephon sprays reduce superficial scald of "Granny Smith" apples. HortScience 24:104-106.

Marcelle, R.D., Porreye, W., Goffings, G. and Herregods, M. 1989. Relationship between fruit mineral composition and storage life of apples, cv. Jonagold. Acta Hort. 258:373-378.

Perring, M.A. 1986. Incidence of bitter pit in relation to the calcium content of apples: problems and paradoxes, a review. J. Sci. Food Agr. 37:591-606.

Pooviah B.W. 1988. Molecular and cellular aspects of calcium action in plants. HortScience 23: 267-271.

Scott, K.J. and Wills, R.B.H. 1979. Effects of vacuum and pressure infiltration of calcium chloride and storage temperature on the incidence of bitter pit and low temperature breakdown of apples. Aust. J. Agric. Res. 30:917-28.

Tomala, K. and Dilley, D.R. 1989. Calcium content of McIntosh and Spartan apples is influenced by the number of seeds per fruit. In: J.K. Fellman (ed.). Proc. 5th Int. Controlled Atmosphere Research Conf., 14-16 June, 1989, Wenatchee, WA, Washington State University, Vol. 1, pp. 75-82.

Waller, W.M., 1980. Use of apple analysis. In: D. Atkinson, L.E. Jackson, R.O. Sharples and W.M. Waller (eds.) Mineral Nutrition of Fruit Trees. Butterworths, London. pp. 383-394.

Wills, R.B.H., Scott, K.J. and Smale, P.J. 1976. Prediction of bitter pit with calcium content of apple fruit. N.Z. J. Agr. Res. 19:513-519.

# Chapter 2

## Characterization of $Mg^{2+}$ Induced Bitter Pit-like Symptoms on Apples: A Model System to Study Bitter Pit Initiation and Development.

## ABSTRACT

Vacuum infiltration of $MgCl_2$ solutions into intact apple fruits induces bitter pit-like symptoms [$Mg^{2+}$ induced pits, (MgIP)]. Including $Ca^{2+}$ in the infiltration media prevents MgIP. Golden Delicious apple fruit were infiltrated with various concentrations of $Ca^{2+}$ and $Mg^{2+}$ with and without $Ca^{2+}$-affecting reagents or other cations. Including trifluoperazine (TFP) with $Mg^{2+}$ increased pitting over $Mg^{2+}$ alone. Verapamil and nefedipine had no effect on MgIP or its attenuation by $Ca^{2+}$. Cyclopiazonic acid (CPA) attenuated MgIP. Ethyleneglycol-bis(B-amino-ethyl-ether)-N,N'tetra acetic acid (EGTA), and 2,3,5-triiodobenzoic acid (TIBA), attenuated MgIP. Cycloheximide and actinomycin D inhibited MgIP, while puromycin had no effect. Heating fruits at 38°C prior to infiltrating the fruits with $MgCl_2$ attenuated MgIP. Cations $Ba^{2+}$, $La^{3+}$, $Co^{2+}$, $Sr^{2+}$ included at 20.0mM prevented MgIP (induced by 0.18M $Mg^{2+}$). $Ca^{2+}$ (3.0mM) included with 0.18M $Mg^{2+}$ inhibited MgIP 50%. $K^+$ and $Na^+$ partially inhibited MgIP. We have demonstrated that treatments affecting calcium homeostasis or cellular metabolism can alter MgIP development. We conclude that MgIP may be a useful tool to understanding natural bitter pit development.


*Key words*: apple, bitter pit, calcium, channel blocker, homeostasis

# INTRODUCTION

Bitter pit is a corking disorder in apples characterized by sunken lesions that develop just prior to harvest or during storage. The tissue below the skin in the pitted area becomes discolored and dehydrated (Faust and Shear, 1968). Susceptibility to bitter pit varies among cultivars and geographic regions. Disorder incidence has been associated with environmental and cultural conditions. Excessive tree vigor, light cropping, calcium deficiency, and moisture stress are among the factors that predispose the fruits to bitter pit (Faust and Shear, 1968; Perring, 1986; Ferguson and Watkins, 1989). Fruits which are immature at harvest are also prone to develop bitter pit. The relationship between elemental nutrition and bitter pit development has been studied extensively (Garman and Mathis, 1956; Martin *et al.*, 1960; Jackson, 1962; Cooper and Bangerth, 1976). Fruits with bitter pit are generally low in Ca, especially in relation to high Mg levels. Treating fruits with $Ca^{2+}$ reduces pitting, while treatments with $Mg^{2+}$ increases the incidence of pitting.

Pitted tissue contains high concentrations of $Ca^{2+}$ and $Mg^{2+}$ (Garman and Mathis, 1956; Hopfinger and Poovaiah, 1979; Askew *et al.*, 1960; Meyer *et al.*, 1979). Ford (1979) demonstrated that $^{45}Ca^{2+}$ moved into the pitted area as the tissue symptoms developed. Pitted tissue and normal tissue differ in many organic and mineral constituents (Faust and Shear, 1968). Studies of Jonathan spot, Richmond *et al.* (1964), another $Ca^{2+}$ related disorder, demonstrated movement of minerals into the affected area and this was associated with a higher level of total

organic acids, mainly malic acid, in the affected tissue. It is hypothesized that these differences between pitted and healthy tissue are not related to the initiation, but are the result of the metabolic disturbance and subsequent tissue breakdown (Ferguson and Watkins, 1989).

The cause and mechanism of initiation and development of bitter pit are not known. Bitter pit is thought to result from a localized $Ca^{2+}$ deficiency or mineral imbalance, but there is no direct evidence for this (Ferguson and Watkins, 1989; Perring, 1986). Since it has not been possible to identify sites on fruit where pits might develop, studies of bitter pit are normally conducted on fruit tissue showing visible symptoms of the disorder.

Bitter pit-like lesions were induced on apples after $Mg^{2+}$ treatment (Hopfinger *et al.*, 1984; Conway and Sams, 1987; Fallahi *et al.*, 1987; K. Tomala, 1988 in our laboratory, unpublished data). $Mg^{2+}$ induced pits were synonymous to bitter pit as indicated by: MgIP is counteracted by including $Ca^{2+}$ in the infiltration media, and pitting incidence was inversely related to the native fruit $Ca^{2+}$ level (Burmeister and Dilley, 1991). Further, we have correlated susceptibility to $Mg^{2+}$ induced pitting with bitter pit occurring in storage (Burmeister and Dilley, in press). Here we have used MgIP as a model for investigating the physiology and biochemistry of bitter pit initiation and development.

## EXPERIMENTAL

Experiments were conducted with Golden Delicious apples (*Malus domestica* borkh.), harvested in 1991 from the Michigan State University Clarksville Horticultural Experiment Station, Clarksville, MI. Fruit (preclimacteric at harvest) were held in controlled atmosphere storage (3% $CO_2$ + 1.5% $O_2$ at 0°C) for approximately 7 months. Randomly selected blemish-free fruits (6-9 cm diameter) were vacuum infiltrated with various solutions by submersing them at an absolute pressure of 100mm Hg for 2 min. All solutions contained 0.3M sorbitol as an isotonic osmoticum, and 0.1% Tween 20 as a surfactant. Sorbitol/surfactant solutions were included as controls. Thirty-six fruits were employed in each treatment. After infiltration, fruits were stored for 10 days in air at 20°C and the number of bitter pit-like lesions MgIP on individual fruits were then recorded. Analysis of variance was used to test for main effects and interactions, or treatment sum of squares was partitioned into single degree of freedom contrasts as appropriate for each experiment (Little, 1981).

Various chemical agents known to affect $Ca^{2+}$ availability, transport, binding, or action were investigated to learn how perturbations of [$Ca^{2+}$] might be involved in bitter pit development. These included: EGTA, a chelator; TIBA, an auxin/$Ca^{2+}$ transport inhibitor; verapamil (Vp) and nefedipine (Nf), $Ca^{2+}$ channel blockers; trifluoperazine (TFP), a calmodulin antagonist; cyclopiazonic acid (CPA), a $Ca^{2+}$-ATPase inhibitor; cycloheximide and puromycin, protein synthesis inhibitors; actinomycin-D, an inhibitor of RNA synthesis and several cations.

**Calcium channel blockers verapamil (Vp), nefedipine (Nf) and calmodulin antagonist trifluoperazine (TFP).** Treatments were factorially arranged with 3 levels of $Ca^{2+}$ (0.0M, 0.01M, 0.02M), and 3 levels of $Mg^{2+}$ (0.0M, 0.04M, 0.18M), alone or with Vp (100$\mu$M) or TFP (100$\mu$M). Fruits were infiltrated with 100$\mu$M Nf alone, or with 0.18M $Mg^{2+}$. Nf was dissolved in dimethylsulfoxide (DMSO) and added to the solutions resulting in a final concentration of 0.1% DMSO. All controls contained 0.1% DMSO.

**Cyclopiazonic Acid (CPA).** Fruits were infiltrated with CPA (40$\mu$M) or 0.18M $Mg^{2+}$, or CPA (40$\mu$M) with $Mg^{2+}$.

**2,3,5-triiodobenzoic acid (TIBA) and ethyleneglycol-bis(B amino ethyl ether)-N,N,N',N'-tetra acetic acid (EGTA).** Fruits were treated with EGTA (100 $\mu$M), and TIBA (100 $\mu$M) alone or with 0.18M $Mg^{2+}$.

**Protein synthesis inhibitors and antibiotics.** Fruits were infiltrated with 0.18M $Mg^{2+}$ alone, or with cycloheximide (25 $\mu$g $ml^{-1}$), or puromycin (6.25 $\mu$g $ml^{-1}$), or actinomycin D (25 $\mu$g $ml^{-1}$).

**Heat treatments.** Experiment 1. Fruits were heated for 0, 1, 2 or 3 days at 38°C then infiltrated with 0.18M $Mg^{2+}$. During the heat treatments the fruits were in perforated polyethylene bags to prevent desiccation. Experiment 2. Fruits were infiltrated with 0.18M $Mg^{2+}$ and placed at 20°C for 7 days, or 3 days at 38°C followed by 4 days at 20°C, or 3 days at 20°C then 3 days at 38°C and then to 20°C for 7 days. Fruits were enclosed in polyethylene bags as in experiment 1.

**Divalent and monovalent cations.** Fruits were infiltrated with 0.18M $Mg^{2+}$ alone or with 1.25mM, 2.5mM, 5.0mM, 10mM, or 20mM $Ca^{2+}$. Fruits were infiltrated with either 0.18M $Mg^{2+}$, or 0.18M $Mg^{2+}$ + 20mM $Ca^{2+}$ alone, or with $La^{3+}$, $Sr^{2+}$, $Co^{2+}$ or $Ba^{2+}$ at 20 mM as chlorides. Fruits were also infiltrated with these cations alone as control. Fruits were infiltrated with 0.18M $Mg^{2+}$ alone or including $K^{+}$ or $Na^{+}$ at 40 mM.

## RESULTS AND DISCUSSION

Including TFP with $Mg^{2+}$ increased pitting over $Mg^{2+}$ alone, and $Ca^{2+}$ attenuated MgIP in the treatments that included TFP (Table I). All main effects and interactions were significant (P = 0.05) except for $Mg^{2+}$*TFP, and $Mg^{2+}$*$Ca^{2+}$*TFP interactions. TFP is a calmodulin antagonist of the phenothiazine series. Cytosolic [$Ca^{2+}$] increased in carrot protoplasts treated with TFP (Gilroy *et al.*, 1987). Inhibitors of this class have induced bitter pit-like symptoms (Fukumoto and Nagai, 1983). Results of experiments using TFP must be interpreted with caution because these drugs are suspected to have general, non-specific detergent properties (Personal communication, Dr. Ian Ferguson, DSIR Auckland, NZ). However, in more recent studies of $Ca^{2+}$ fluxes across the plasma membrane of *Commelina commuis L.*, Siebers *et al.* (1990) concluded that the effect of TFP was to mobilize membrane associated $Ca^{2+}$ and trigger release of $Ca^{2+}$ from vesicles. They suggest that TFP induces $Ca^{2+}$ influx and/or inhibits $Ca^{2+}$ efflux across the plasma membrane. No evidence of a detergent effect of TFP was found. TFP treated plasma membrane-rich vesicles were still able to

import $^{45}Ca^{2+}$ after being washed of excess TFP. Since $Ca^{2+}$ attenuates pitting induced by TFP, we believe that TFP may act through specific binding rather than by a detergent effect. However, binding of TFP is not specific to calmodulin. There are many examples of TFP binding to other $Ca^{2+}$ related proteins such as troponin C, S-100, and $Ca^{2+}$ activated phospholipid-dependent protein kinase (Hartshorne, 1985 and ref. therein). Roufogalis *et al.* (1983) suggested that TFP binds to the activated state of the $Ca^{2+}$ and $Mg^{2+}$ stimulated ATPase of erythrocytes. Therefore, TFP may cause several effects on calcium-linked metabolism rather than affecting a single event by binding to one specific site.

Vp and Nf are members of the dihydropyridine series of $Ca^{2+}$ channel blockers and are believed to block voltage gated $Ca^{2+}$ channels from the inner side of the plasma membrane; they enter the $Ca^{2+}$ channel while it is in the open state (Carfoli, 1987). These drugs affect many plant systems (Heplar and Wayne, 1985). Vp at 100 $\mu$M reduced pitting induced by 0.18 M $Mg^{2+}$ (Table I). However, the main effects of Vp and Vp*$Mg^{2+}$ interaction were only significant at P = 0.1. Analysis of variance showed no other significant effects. Vp did not affect $Ca^{2+}$ attenuation of MgIP. Another $Ca^{2+}$ channel blocker Nf at 100 $\mu$M also did not significantly reduce pitting induced by 0.18M $Mg^{2+}$ (data not shown). Similar experiments with Vp and Nf included at 500 $\mu$M showed no consistent reduction in MgIP (data not shown). These results suggest that $Ca^{2+}$ entry into the cell is not required for induction of pitting by $Mg^{2+}$.

Table I

The effects of $Ca^{2+}$ and $Ca^{2+}$ channel blockers on mean number of $Mg^{2+}$ induced pits per fruit.

| $Mg^{2+}$ Concn | $Ca^{2+}$ concn. M | | |
|---|---|---|---|
| (M) | 0.0 | 0.01 | 0.2 |
| | Average number of MgIP per fruit | | |
| 0.0 | 0.0 | 0.0 | 0.0 |
| 0.04 | 1.0 | 0.0 | 0.0 |
| 0.18 | 18.7 | 0.6 | 0.0 |
| | 100μM TFP | | |
| 0.0 | 4.6 | 1.0 | 0.0 |
| 0.04 | 3.8 | 0.2 | 1.0 |
| 0.18 | 22.7 | 3.5 | 1.0 |
| | 100 μM Verapamil | | |
| 0.0 | 0.0 | 0.0 | 0.0 |
| 0.04 | 1.7 | 0.2 | 0.2 |
| 0.18 | 12.0 | 0.1 | 0.4 |

CPA, an inhibitor of $Ca^{2+}$-ATPase significantly attenuated MgIP (Table II). Including CPA (10$\mu$M) (a $Ca^{2+}$-ATPase inhibitor) with $Ca^{2+}$ did not affect the $Ca^{2+}$ attenuation of MgIP (data not shown). CPA is a specific inhibitor of $Ca^{2+}$-ATPase and is believed to act by preventing the conformational change (E1 to E2) of the enzyme that is necessary for $Ca^{2+}$ transport (Seidler *et al.* 1989). In animal systems, CPA inhibits P-type calcium dependent ATPases of the endoplasmic and sarcoplasmic reticulum that do not require calmodulin for activation. CPA treated vesicles have a reduced rate of $Ca^{2+}$

Table II

The effect of cyclopiazonic acid (CPA) on $Mg^{2+}$ induced pitting.

| **Treatment** | **Mean number of pits per fruit** | **Sig.[1]** |
|---|---|---|
| 0.18M $Mg^{2+}$ | 14.5 | |
| 0.18M $Mg^{2+}$ + CPA (40$\mu$M) | 6.4 vs 0.18M $Mg^{+2}$ | *** |
| **CONTROLS:** | | |
| 0.3M Sorbitol Alone | 0.0 | |
| 0.3M Sorbitol + CPA (40$\mu$M) | 0.0 | |

[1]Treatments were partitioned into single degree of freedom contrasts as indicated.

* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

efflux (Riley and Goeger, 1990).

EGTA at 100 $\mu$M significantly attenuated pitting induced by 0.18M $Mg^{2+}$ (Table III). EGTA is a specific chelator of $Ca^{2+}$ (Heplar and Wayne, 1985). Its effect may have been exerted by sequestering $Ca^{2+}$ in the apoplast thereby reducing the amount of $Ca^{2+}$ available to be transported across the plasmalemma. TIBA at 100 $\mu$M also significantly reduced the amount of pitting induced by 0.18M $Mg^{2+}$ (Table III). TIBA has been demonstrated to decrease calcium accumulation in apple fruits (Tomala and Dilley, 1989). $Ca^{2+}$ transport in plants has been linked to the polar transport of auxin and the latter is known to be inhibited by TIBA (dela Fuente and Leopold, 1973; Banelos *et al.*, 1987). TIBA, by blocking auxin efflux from the cell could prevent $Ca^{2+}$ entry into the cell enhanced by 0.18M $Mg^{2+}$ treatment.

Table III

The effect of EGTA and TIBA on $Mg^{2+}$ induced pit.

| **Treatment** | **Mean number of pits per fruit** | **Sig.[1]** |
|---|---|---|
| 0.18M $Mg^{2+}$ | 21.5 | |
| 0.18M $Mg^{2+}$ + EGTA (100$\mu$M) | 6.8 vs 0.18M $Mg^{2+}$ | *** |
| 0.18M $Mg^{2+}$ + TIBA (100$\mu$M) | 4.1 vs 0.18M $Mg^{2+}$ | *** |
| **CONTROLS:** | | |
| EGTA ($10^{-4}$M) Alone | 0.0 | |
| TIBA ($10^{-4}$M) | 0.5 | |
| 0.3M Sorbitol | 0.0 | |

[1]see footnote to table 2.

Cycloheximide at 25$\mu$M totally inhibited MgIP development (Table IV). This was also found in another experiment with cycloheximide at 10$\mu$g ml$^{-1}$ (data not shown). Cycloheximide blocks protein synthesis by inhibiting aminoacyl transferase in peptide bond formation in the ribosome. Puromycin at 6.25$\mu$g ml$^{-1}$ did not significantly reduce MgIP development. This antibiotic is also an inhibitor of normal protein synthesis by causing the cell to produce an abnormal polypeptide. The reason puromycin was less inhibitory than cycloheximide in reducing MgIP may be because the concentration employed was too low. Actinomycin-D at 25$\mu$g ml$^{-1}$ significantly inhibited MgIP development (Table IV). This antibiotic inhibits RNA synthesis by binding to DNA. Fruit of treatments that included antibiotics had decay symptoms that were distinguishable from MgIP. Collectively, the results with the antibiotics suggest that the induction of pitting by $Mg^{2+}$ may involve mRNA and proteins synthesized *de novo* subsequent to $Mg^{2+}$ treatment.

Table IV

The effect of protein synthesis inhibitors and antibiotics on $Mg^{2+}$ induced pitting.

| **Treatment** | **Mean number of pits per fruit** | | **Sig.[1]** |
|---|---|---|---|
| 0.18M $Mg^{2+}$ | 25.8 | | |
| 0.18M $Mg^{2+}$ + Puromycin (6.25$\mu$g $ml^{-1}$) | 21.7 | vs $Mg^{2+}$ Alone | n.s. |
| 0.18M $Mg^{2+}$ + Actinomycin D (25$\mu$g $ml^{-1}$) | 9.4 | vs $Mg^{2+}$ Alone | *** |
| 0.18M $Mg^{2+}$ + Cycloheximide (25$\mu$g $ml^{-1}$) | 0.0 | vs $Mg^{2+}$ Alone | *** |
| **CONTROLS:** | | | |
| Puromycin (6.25$\mu$g $ml^{-1}$) | 0.0 | | |
| Actinomycin D (25$\mu$g $ml^{-1}$) | 0.0 | | |
| Cycloheximide (25$\mu$g$ml^{-1}$) | 0.0 | | |
| 0.3m Sorbitol alone | 0.0 | | |

[1]see footnote to table 2.

Heating apples at 38°C for 1 to 3 days prior to infiltrating them with $Mg^{2+}$ markedly reduced the amount of MgIP (Table V). Heating fruits immediately after $Mg^{2+}$ infiltration more than doubled in number of pits that developed (Table VI), whereas heat treatment applied 3 days following infiltration with $Mg^{2+}$ pitted to the same degree as fruits not heated. Collectively, these data indicate that heating fruits prior to subjecting them to the stress of $Mg^{2+}$ infiltration lessens their susceptibility to MgIP but exacerbates pitting when applied immediately after $Mg^{2+}$ infiltration. Exposure of plants and harvested plant organs to temperatures in the range of 35 to 40°C can profoundly affect physiological and biochemical processes during and subsequent to the heat stress. Alteration of transcription and translation is a response common to all plants heated in the range of 35 to 40°C and this is known as the heat shock (HS) response (Nagao *et al.*, 1986). Heating induces the formation of a complex family of heat shock proteins (HSP) ranging in molecular weight from about 10 kD to nearly 100 kD (Kimpel and Key, 1985). Prestorage heat treatments of apples have been found to inhibit ripening (although not irreversibly), reduce the rate of subsequent softening of apples, and attenuate storage disorders (Porritt and Lidster, 1978; Klein and Lurie, 1992). We hypothesize that heating ameliorated MgIP as a consequence of evoking the heat shock response.

Table V

The effect of heat treatment at 38°C prior to $Mg^{2+}$ infiltration.

| **Heat Treatment** | $[Mg^{2+}]$ | **Mean number of pits per fruit** | **Sig.**[1] |
|---|---|---|---|
| 0d | 0.18M | 8.4 | |
| 1d | 0.18M | 1.9 vs 0d, 0.18M $Mg^{2+}$ | *** |
| 2d | 0.18M | 3.7 vs 0d, 0.18M $Mg^{2+}$ | *** |
| 3d | 0.18M | 2.9 vs 0d, 0.18M $Mg^{2+}$ | *** |
| **CONTROLS:** | | | |
| 0d | 0.00M | 0.0 | |
| 1d | 0.00M | 0.0 | |
| 2d | 0.00M | 0.0 | |
| 3d | 0.00M | 0.0 | |

[1]see footnote to table 2.

Table VI

The effect of heat treatment at 38°C following $Mg^{2+}$ infiltration.

| **Heat Treatment** | **[$Mg^{2+}$]** | **Mean number of pits per fruit** | **Sig.[1]** |
|---|---|---|---|
| 20°C Continuous | 0.18M | 18.3 | |
| 3d Heat 20°C | 0.18M | 46.2 vs 20°C Continuous | *** |
| 3d 20°C 3d Heat | 0.18M | 21.0 vs 20°C Continuous | n.s. |
| **CONTROLS:** | | | |
| 20°C Continuous | 0.00M | 0.0 | |
| 3d Heat 20°C | 0.00M | 1.6 | |
| 3d 20°C 3d Heat | 0.00M | 0.0 | |

[1]see footnote to table 2.

A relatively low $Ca^{2+}$ concentration was found to counteract pitting induced by $Mg^{2+}$ (Burmeister and Dilley, 1991). The concentration of $Ca^{2+}$ necessary to attenuate pitting induced by 0.18 M $Mg^{2+}$ by 50% was ≈3.0mM (data not shown). $La^{3+}$, $Co^{2+}$, $Sr^{2+}$ and $Ba^{2+}$ at 20.0mM all completely attenuated $Mg^{2+}$ pitting induced by 0.18M $Mg^{2+}$ (data not shown). $La^{3+}$ is a $Ca^{2+}$ channel blocker (Heplar and Wayne, 1985). $La^{3+}$ may not be able to cross plant membranes (Thompson *et al.*, 1973). It has been demonstrated to block turnover of the phosphorylated intermediate of the Ca-ATPase in the microsomal fraction of maize coleoptiles (Briars and Evans, 1989). $Co^{2+}$ is known to block $Ca^{2+}$ induced seed germination (Wayne and Heplar, 1984) and also to inhibit ethylene production by inhibiting ACC oxidase (Kuai and Dilley, 1992). $Ba^{2+}$ and $Sr^{2+}$ ions can often substitute for $Ca^{2+}$ in the binding of ligands such as membrane proteins (Heplar and Wayne, 1985). $Ca^{2+}$ channels of charophytes do not transport $Ba^{2+}$ and only slowly transport $Sr^{2+}$. Our results suggest that these cations may act on $Ca^{2+}$ binding sites in the extracellular space.

$K^+$ (40.0mM) and $Na^+$ (40.mM) included with 0.18M $Mg^{2+}$ only partially attenuated MgIP (Table VII). This is about twice the concentration of $Ca^{2+}$ that completely inhibited the induction of MgIP by 0.18M $Mg^{2+}$. The effect of $Na^+$ could be on the $Ca^{2+}$-$Na^+$ antiport (Darnell *et al.*, 1990). $K^+$ has been shown to stimulate ATPase activity in microsomal preparations of apple fruit (Lurie and Ben-Arie, 1983).

Table VII

The effect of $Na^+$ and $K^+$ on $Mg^{2+}$ induced pitting.

| **Treatment** | **Mean number of pits per fruit** | **Sig.**[1] |
|---|---|---|
| 0.18M $Mg^{2+}$ Alone | 17.4 | |
| 0.18M $Mg^{2+}$ + 0.04M $K^+$ | 10.7 vs 0.18M $Mg^{2+}$ | * |
| 0.18M $Mg^{2+}$ + 0.04M $Na^+$ | 5.8 vs 0.18M $Mg^{2+}$ | *** |

[1]see footnote to table 2.

We have demonstrated that we can alter the development of MgIP with treatments that are known to affect $Ca^{2+}$ homeostasis and cellular metabolism. Given the similarities between MgIP and bitter pit, our results may imply specific roles for $Ca^{2+}$ and $Mg^{2+}$ in bitter pit initiation and development. $Ca^{2+}$ mediates many responses in plants and animals (Carafoli, 1987; Heplar and Wayne, 1985). The concentration of $Ca^{2+}$ in the cytosol ($[Ca^{2+}]_{cyt}$) is maintained in the submicromolar range (Ferguson and Drobak, 1988; Poovaiah and Reddy, 1987; Poovaiah, 1988) by the sequestering of calcium into organelles and export of $Ca^{2+}$ across the plasmalemma by ATPases. Extracellular signals give rise to transient increases in $[Ca^{2+}]_{cyt}$ either by release from cellular organelles, or by the opening of specific $Ca^{2+}$ channels in the plasma membrane. $[Ca^{2+}]_{cyt}$ affects cellular processes by binding to enzymes, or to $Ca^{2+}$ binding proteins such as calmodulin. Ferguson (1990) suggested that the critical pool of $Ca^{2+}$ involved in bitter pit initiation and development is the extracellular compartment directly accessible to the plasma membrane. Sufficient extracellular $Ca^{2+}$ would be needed for cells to respond to environmental signals. Insufficient $Ca^{2+}$ would prevent cells from responding and cause cell disfunction (e.g. bitter pit). This explanation may account for the fact that fruits of low Ca may not develop bitter pit unless conditions (eg. drought, excessive tree vigor, immaturity of fruit at harvest) trigger the cellular response.

The role of $Mg^{2+}$ in bitter pit initiation is not understood. The concentrations of $Mg^{2+}$ and $K^{+}$ are generally high in relationship to calcium in

fruits with bitter pit (Perring, 1986). Harker *et al.*, (1989) found that $Mg^{2+}$ inhibits $^{45}Ca^{2+}$ transport across discs of cortical flesh of apple fruit. Our data suggest that high levels of $Mg^{2+}$ in the extracellular space may be an important factor in bitter pit initiation perhaps by preventing the influx of extracellular $Ca^{2+}$ to the cytoplasm via specific $Ca^{2+}$ channels.

Gilroy *et al.*, (1989) reported that high extracellular $Mg^{2+}$ and $K^{+}$ concentrations resulted in rapid breakdown of $Ca^{2+}$ homeostasis of carrot protoplasts. There is evidence for a $Ca^{2+}/Mg^{2+}$ antagonistic relationship in the activation and inhibition of the $Mg^{2+}$ dependent $Ca^{2+}$ ATPases (Kawaski *et al.*, 1979; Kylin and Kähr, 1973; Vianna, 1975) in the microsomal fractions of plants and animals. This seems to vary among species and tissues. In apple fruit, Lurie and Ben-Arie (1983) found both $Mg^{2+}$ and, to a lesser degree, $Ca^{2+}$ inhibited ATPase activity of the plasma membrane. We speculate that extracellular $Mg^{2+}$ supplied by infiltration could disrupt cellular homeostasis via the key enzyme(s) that regulate intracellular $Ca^{2+}$. This in turn results in the chain of reactions that result in MgIP. The high levels of $Mg^{2+}$ infiltrated into the extracellular space presumably are akin to the high $Mg^{2+}$ levels often found in fruits with bitter pit. We believe that the pitting symptoms induced by $Mg^{2+}$ infiltration are physiologically synonymous with the bitter pit disorder.

Additional Experiment.

**$O_2$ Dependency of MgIP.** Controlled atmosphere storage is known to reduce the incidence and severity of bitter pit in apple fruit (Perring, 1986). This may be attributed to retardation of ripening and senescence development and/or the lower rate of metabolism at the lowered $O_2$ level. If the effect of $Mg^{2+}$ infiltration in MgIP is a consequence of reducing the rate of $O_2$-dependent metabolism, MgIP should be lowered as the $O_2$ supply to the fruit becomes limiting. The $O_2$ dependency was determined for Golden Delicious apple fruit.

Golden Delicious apple fruit were harvested preclimacteric (ca. 10% of fruits at $>0.2\mu l\ l^{-1}$ internal ethylene concentration) from the Clarksville Michigan State University and held in air at 1°C for 4 weeks. A preliminary experiment was conducted in which fruits were infiltrated with 0.0M, 0.09M, 0.18M, and 0.36M $Mg^{2+}$ in 0.4M sorbitol: 0.1% Tween 20 and stored at 20°C in air or 3.0% $CO_2$ with 1.5% or 3.0% $O_2$ for 1 month. More MgIP was found in air than at the reduced $O_2$ atmospheres but symptoms of fermentation damage were evident at the reduced $O_2$ levels (data not presented).

In a second experiment, fruits were infiltrated with 0.0M, 0.18M as before and stored at 20°C ventilating with 2.5, 5.0, 7.5, 10.0, or 21.0% $O_2$ for 12d. MgIP increased for fruits infiltrated with 0.18M $Mg^{2+}$ as the $O_2$ concentration increased to 7.5% with no further increase (Fig 1). There was no apparent fermentation damage evident at any of the $O_2$ concentration employed. Since ripening changes were not at issue in this experiment it appears that MgIP is an $O_2$ dependent

metabolic process showing a similar $O_2$ saturation to respiration.

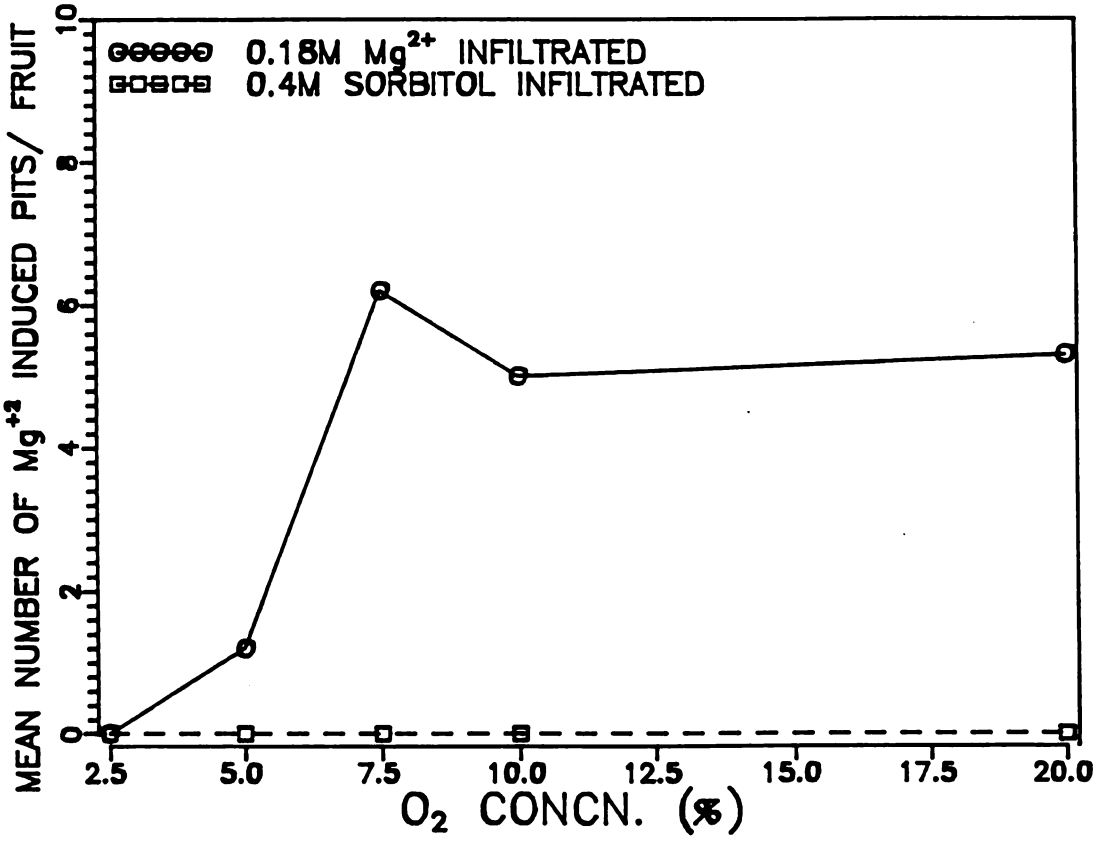



Fig 1. Dependency of MgIP on $O_2$ concentration (%).

## REFERENCES

Askew, H.O.; Chittenden, E.T.; Monk, R.J.; Watson, J. "Chemical investigations on bitter pit of apples. III. Chemical composition of affected and neighboring healthy tissues". *N.Z. J. Agr. Res.* 1960, 3, 169-178.

Banuelos, G.S.; Bangerth, F.; Marschner, H. "Relationship between polar basipetal auxin transport and acropetal $Ca^{2+}$ transport into tomato fruits". *Physiol. Plant.* 1987, 71, 321-327.

Briars, S.A.; Evans, D.E. "The calmodulin-stimulated ATPase of maize caloptiles in a 140,000-Mr polypeptide." *Planta* 1989, 176, 283-5.

Burmeister, D.M.; Dilley, D.R. "Induction of bitter pit-like symptoms on apples by infiltration with $Mg^{2+}$ is attenuated by $Ca^{2+}$". *PostHarvest Biol. Tech.* 1991, 1, 11-17.

Burmeister, D.M.; Dilley, D.R. "Prediction of bitter pit on Northern Spy Apples Using $Mg^{2+}$ salt Infiltration to Induced bitter pit-like symptoms." *PostHarvest Biol. Tech.*, in press.

Carafoli, E. "Intracellular calcium homeostasis." *Annu. Rev. Biochem.* 1987, 56, 395-433.

Conway, W.S.; Sams, C.E. "The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples." *J. Amer. Soc. Hort. Sci.*" 1987, 112, 300-303.

Cooper, T.; Bangerth, F. "The effect of Ca and Mg treatments on the physiology, chemical composition and bitter pit development of Cox's Orange apples." *Sci. Hortic.* 1976, 5, 49-57.

Darnell, J. "Transport across cell membranes." In: *Molecular Cell Biology.*" Darnell, J., Lodish, H., Baltimore, D., Eds.: Scientific New York, 1990, Chapter 14, 545-547.

dela Fuente, R.K.; Leopold, A.C. "A role of calcium in auxin transport." *Plant Physiol.* 1973, 51, 845.

Fallahi, E.; Fighette, T.L.; Wernz, J.G. "Effects of dip and vacuum infiltrations of various inorganic chemicals on post-harvest quality of apple." *Commun. Soil Sci. Plant Anal.* 1987, 18, 1017-1029.

Faust, M.; Shear, C.B.; "Corking disorder of apples: a physiological and biochemical review." *Bot. Rev.* 1968, 34, 441-469.

Ferguson, I.B. "Calcium nutrition and cellular response." In: *Calcium in Plant Growth and Development.* Proc. 13th Annual Riverside Symposium in Plant Physiology. January 11-13, 1990, pp. 1-8.

Ferguson, I.B., and Drobak, B.K. "Calcium and the regulation of plant growth and senescence." *HortScience.* 1988, 23, 262-268.

Ferguson, I.B. and Watkins, C.B. "Bitter pit in apple fruit." *Hortic. Rev.* 1989, 289-353.

Ford, E.M. "The distribution of calcium in mature apple fruits having bitter pit disorder." *J. Hort. Sci.* 1979, 54, 91-92.

Fukumoto, M.; Nagai, K. "Possible roles of calcium and ammonium in the development of bitter pit in apple." *Physiol. Plant.* 1983, 59, 171-176.

Garman, P. and Mathis, W.T. "Studies of mineral balance as related to occurrence of Baldwin spot in Connecticut." *Conn. Agric. Exp. Stn. Bull.* 1956, 601 5-19.

Gilroy, S.; Hughes, W.A.; Trewavas, A.J. "Calmodulin antagonists increase free cytosolic calcium levels in plant protoplasts *in vivo*." *FEBS Lett.*, 1987, 212, 133-137.

Gilroy, S., Fricker, M., Blowers, D., Harvey, H., Collinge, M. and Trewavas, A.J. "Calcium channels, cytosol calcium and plasma membrane phosphorylation: an integrated calcium stat system." In: *Plant Membrane Transport: The Current Position.* J. Dainty (ed.). Department of Botany, University of Edinburgh. The King's Buildings, Mayfield Road, Edinburgh, EH9 3JH, U.K., 1989; pp. 215-224.

Harker, F.R.; Ferguson, I.B.; Dromgoole, F.I. "Calcium ion transport through tissue discs of cortical flesh of apple fruit." *Physiol. Plant.* 1989, 74, 688-694.

Hartshorne, D.J. "Calmodulin: An introduction to biochemical aspects." In: *Calmodulin Antagonists and Cellular Physiology*; Hiroyoshi, H., Hartshorne, D.J.,; Eds.; Academic Press: Orlando, 1985, Vol 1, 3-11.

Heplar, P.K.; Wayne, R.O. "Calcium and plant development." *Annu. Rev. Plant Physiol.* 1985, 36, 397-439.

Hopfinger, J.A.; Poovaiah, B.W. "Calcium and magnesium gradients in apples with bitter pit." *Commun. Soil Sci. Plant Anal.* 1979, 10, 57-65.

Hopfinger, J.A.; Poovaiah, B.W.; Patterson, M.E. "Calcium and magnesium interactions in browning of Golden Delicious Apples with bitter pit." *Sci. Hortic.* 1984, 23 345-351.

Jackson, D.I. "The effects of calcium and other minerals on incidence of bitter pit in Cox's Orange apples." *N.Z. J. Agric. Res.* 1962, 5, 302-309.

Kawasaki, I.; Kähr, M.; Kylin, A. "Interactions of divalent cations in their action on ATPases from cucumber roots." *Physiol. Plant.* 1979, 45, 437-439.

Kimpel, J.A.; Key, J.L. "Heat shock in plants." *Trends Biochem. Sci.* 1985, 10, 53-357.

Kuai J.,; Dilley D.R. "Extraction, partial purification and characterization of 1-aminocyclopropane-1-carboxylic acid oxidase from apple (Malus domestica Borkh.) fruit." *Postharvest Biol. Tech.* 1992, 1, 203-211.

Klein, J.D.; Lurie, S. "Prestorage heating of apple fruit for enhancement of post-harvest quality: interaction of time and temperature." *HortScience.* 1992; 3 27 326-328.

Kylin, A.; Kähr, M. "The effects of magnesium and calcium ions on adenosine triphosphatases from wheat and oat roots at different pH." *Physiol. Plant.* 1973, 28, 452-457.

Little, T.M. "Interpretation and presentation of results." Proceedings of the Symposium Statistics: A tool for the horticultural scientist. *HortScience* 1981, 16 262-266.

Lurie, S.; Ben-Arie, R. "Characterization of plasmalemma ATPase from apple fruit." *Phytochemistry.* 1983, 22, 49-52.

Martin, D.; Lewis, L.; Cerny, J. "Bitter pit in the apple variety Cleopatra in Tasmania in relation to calcium and magnesium." *Aust. J. Agric. Res.* 1960, 11, 186-192.

Meyer, B.R.; Peisach, M.; Kotze, W.A.G. "Analysis of sound and pitted tissue of apple fruit by proton-induced x-ray spectrometry." *Scientia Hort.* 1979, 10, 57-61.

Nagao, R.T.; Kimpel, J.A.; Vierling, E.; Key, J.L. "The heat shock response: a comparative analysis." In: *Oxford Surveys of Plant Molecular and Cell Biology*, Vol. 3. B.J. Miflin (ed.), Oxford Univ. Press, Oxford, 1986; pp. 384-438.

Perring, M.A. 1986. "Incidence of bitter pit in relation to calcium content of apples: Problems and paradoxes, a review." *J. Sci. Food Agric.* 1986, 37, 591-606.

Poovaiah, B.W. "Molecular and cellular aspects of calcium action in plants." *HortScience* 1988, 23, 267-271.

Poovaiah, B.W.; Reddy, A.S.N. "Calcium messenger system in plants." *CRC Crit. Rev. Plant Sci.* 1987, 6, 47-103.

Porritt, S.W.; Lidster, P.D. "The effect of prestorage heating on ripening and senescence of apples during cold storage." *J. Amer. Soc. Hort. Sci.* 1978, 103, 584-587.

Richmond, A.E.; Dilley, D.R.; Dewey, D.H. "Cation, organic acid and pH relationships in peel tissue of apple fruits affected with Jonathan Spot." *Plant Physiol.* 1964, 39, 1056-1060.

Riley, R.T., and Goeger, D. "Cyclopiazoic acid: speculation on its function in fungi". In: *Handbook of Applied Mycology, Vol. V: Role of Mycotoxins in Ecological Systems*; Bhatnagar, D., Lillchoj, E.B., and Arora, D.K., Eds. Marcel Dekker, Inc., NY., 1992; 385-402.

Roufogalis, B.D.; Minocherhowjee, Arda-E-Veraf M.; Al-Jobore, A. "Pharmalogical antagonism of calmodulin." *Can. J. Biochem. Cell Biol.* 1983, 61, 927-933.

Siebers, B.; Gräf, P.; Weiler, E.W. "Calcium fluxes across the plasma membrane of *Commelina communis* L. assayed in a cell-free system." *Plant Physiol.* 1990, 93 940-947.

Seidler, N.W.; Jona, I.; Vegh, M.; Martonosi, A. "Cyclopiazonic acid is a specific inhibitor of the $Ca^{2+}$-ATPase of the sarcoplasmic reticulum." *J. Biol. Chem.* 1989, 264 17816-17823.

Thompson, W.W.; Platt, K.A.; Campbell, N. "The use of lanthanum to delineate the apoplastic continuum in plants." *Cytobios* 1973, 8, 57-62.

Tomala, K.; Dilley, D.R. "Calcium content of McIntosh and Spartan apples is influenced by the number of seeds per fruit." In: *Proc. 5th Int. Controlled Atmosphere Research Conf.*, J.K. Fellman (ed.), Washington State University, 1989; Vol. 1, pp. 75-82.

Vianna, A.L. "Interaction of calcium and magnesium in activating and inhibiting the nucleoside triphosphatase of sarcoplasmic reticulum vesicles." *Biochem. Biophys. Acta* 1975, 410, 389-406.

Wayne, R.; Heplar, P.K. "The role of calcium ions in phytochrome-mediated germination of spores of *Onoclea sensibilis* L." *Planta* 1984, 160, 12-20.

# SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS:

Bitter pit is a commercially important physiological disorder of apples. The factors affecting its occurrence are well characterized . However, the causes and mechanisms of bitter pit initiation and development, respectively are not known. The disorder has been mainly associated with low fruit Ca and often appears to be exacerbated by high Mg levels (Ferguson and Watkins, 1989). Apple fruit vacuum infiltrated with isotonic sorbitol solutions containing $Mg^{2+}$ develop bitter pit-like symptoms [$Mg^{2+}$ induced pits (MgIP)]. We have demonstrated that MgIP is similar to naturally occurring bitter pit. Addition of $Ca^{2+}$ to the infiltration media attenuates MgIP and the number of lesions induced by $Mg^{2+}$ is inversely related to the endogenous (native) $Ca^{2+}$ concentration of individual fruits (Burmeister and Dilley, 1991). Studies were conducted using MgIP as a means to assess bitter pit potential and as a model for bitter pit initiation and development. Induction of MgIP on Northern Spy apples 10 days after infiltrating 0.1M $MgCl_2$ salt solutions into the fruits was positively correlated with bitter pit that developed naturally in nontreated fruits during five and seven months in storage at 5°C and 3°C , respectively. The endogenous (native) fruit $Ca^{2+}$ concentration was inversely related to the number of pits induced by $Mg^{2+}$ and to bitter pit development following storage.

Golden Delicious apple fruit were infiltrated with various concentrations of $Ca^{2+}$ and $Mg^{2+}$ with and without $Ca^{2+}$-affecting reagents or other cations.

Including Trifluoperazine (TFP), a calmodulin antagonist with $Mg^{2+}$ increased pitting over $Mg^{2+}$ alone. Verapamil and nefedipine (calcium channel blockers) had no effect on MgIP or its attenuation by $Ca^{2+}$. Cyclopiazonic acid ($Ca^{2+}$-ATPase inhibitor) attenuated MgIP. Ethyleneglycol-bis(B amino ethyl ether)-N,N ,N',N'-tetra acetic acid (EGTA), and 2,3,5-triiodobenzoic acid (TIBA) attenuated MgIP. Cycloheximide (a protein synthesis inhibitor) inhibited $Mg^{2+}$ induced pit. Heating at 38°C prior to infiltrating the fruits attenuated MgIP. Cations $Ba^{2+}$, $La^{3+}$, $Co^{2+}$, $Sr^{2+}$ included at 0.02M all completely arrested MgIP induced by 0.18M $Mg^{2+}$. $K^+$ and $Na^+$ partially inhibited MgIP.

Many models could be proposed to explain MgIP development. From the data presented it is difficult to draw specific conclusions. In general reagents, or cations that would ameliorate influx of $Ca^{2+}$ across the plasma membrane or sequester $Ca^{2+}$ attenuate MgIP. Cyclopiazonic acid would be expected to increase MgIP by preventing Ca-ATPase from maintaining low cytosolic [$Ca^{2+}$]. Its attenuation of MgIP could be the result of reducing membrane permeability. In any case factors that would decrease [$Ca^{2+}$] attenuated MgIP. The multivalent cations $Ba^{2+}$ and $Sr^{2+}$ and channel blockers $Co^{2+}$ and $La^{3+}$ substitute for $Ca^{2+}$ in attenuation of MgIP. $La^{3+}$ is not believed to cross the plasma membrane. Therefore $Ca^{2+}$ may be acting at the plasma membrane in preventing MgIP development. $Na^+$ and $K^+$ may attenuate of MgIP by displacing $Ca^{2+}$ from ion exchange sites in the cell wall. This would increase [$Ca^{2+}$] in the apoplastic solution adjacent to the plasma membrane. Trifluoperazine may act directly on

calmodulin or calcium-modulated proteins interfering with cell function resulting in MgIP. Cycloheximide may inhibit protein synthesis necessary for MgIP development.

There is a paucity of information available concerning the roles of extracellular $Ca^{2+}$ and $Mg^{2+}$ in the function of plant cells, especially bulky storage organs such as apple fruit. In animal systems, recent evidence suggests that both the extracellular and intracellular concentrations of $Ca^{2+}$ and $Mg^{2+}$ are important in maintenance of cell $Ca^{2+}$ homeostasis. Intracellular $Ca^{2+}$ is suspected to be modulated by both extra and intracellular [$Mg^{2+}$] in cardiac cells (White and Hartzell, 1989). We speculate that high extracellular $Mg^{2+}$ may interfere with a $Ca^{2+}$ channel in the plasma membrane. The presence of extracellular $Ca^{2+}$ may alter membrane permeability to $Mg^{2+}$ thereby allowing the channel to be inactivated. Little is known about $Mg^{2+}$ transport acrose cell membranes and a regulatory role for $Mg^{2+}$ in cells. Our data indicate that an effect akin to this may be occurring in development of MgIP. Thus, high [$Mg^{2+}$] in apple fruit may result in inability of a $Ca^{2+}$ channel to close efficiently resulting in impairment of the cells ability to regulate cytosolic [$Ca^{2+}$]. High levels of $Mg^{2+}$ and low levels of $Ca^{2+}$ may in themselves not be detrimental, but when such a fruit is confronted with a stress such as cold storage then the regulation of [$Ca^{2+}$] becomes critical. This would lead to the event(s) (eg. protein synthesis) that result in bitter pit.

We have demonstrated that susceptibility to MgIP at harvest is correlated with bitter pit development in storage and that treatments affecting calcium

homeostasis or cellular metabolism can alter MgIP development. These studies indicate that extracellular $Ca^{2+}$ and $Mg^{2+}$ concentrations are important in bitter pit development. This may be due to the influence of apoplastic $Ca^{2+}$ and $Mg^{2+}$ concentrations on maintenance of the cytosolic $Ca^{2+}$ concentration.

MgIP may be a useful to study bitter pit. Bitter pit is usually studied after the disorder has already developed. MgIP can be induced in a matter of days after $Mg^{2+}$ infiltration and this is attenuated with $Ca^{2+}$. MgIP may be useful as model system to study bitter pit at the molecular level. Further research on the effects of exogenous [$Ca^{2+}$] and [$Mg^{2+}$] on intracellular events such as protein synthesis and phosphorylation following infiltration is warranted. Changes in specific proteins could then be correlated with natural bitter pit development. If changes in specific proteins occurred were identified then *in vitro* translation of mRNA extracted at various times could be employed to detect mRNAs novel to MgIP and bitter pit development.

The use of MgIP to study bitter pit is limited by the necessity of using whole fruit. It would be desirable to use a tissue disc or cell culture to study the effects of $Ca^{2+}$ and $Mg^{2+}$. Ideally, measurement of cytosolic $Ca^{2+}$ with fluorescent dyes or Ca-selective electrodes would be measured. However, apple tissue is not amiable to these techniques due to highly vacuolated cells and sensitivity to wounding. Future effort should focus on development of an isolated system to study the effects of $Ca^{2+}$ and $Mg^{2+}$ on apple fruit cells.

It is apparent that MgIP may be useful in understanding of the etiology of

bitter pit initiation and development as well as a practical means of assessing bitter pit potential.

APPENDIX ONE: Table of means for Chapter 1.

Table 1. Parameters measured for 1990, early harvest. Each number represents an average of a minimum of 30 fruit.

| Location | DIAM. (cm) | WT. (grams) | SEED COUNT | MgIP (Avg) | (%) | Ca ($\mu g\ g^{-1}\ dwt^{-1}$) | BITTER PIT AFTER STORAGE (Pits/fruit) | (%) |
|---|---|---|---|---|---|---|---|---|
| Epple Orchards | 7.6 | 172 | 7.2 | 3.6 | 50 | 208 | 24.8 | 81 |
| Molter Farms | 8.5 | 230 | 9.2 | 3.7 | 36 | 237 | 17.2 | 59 |
| Rosenburg Farms | 7.8 | 192 | 5.0 | 1.3 | 29 | 264 | 1.5 | 44 |
| WmB. Inc. A. | 8.0 | 209 | 8.6 | 1.3 | 17 | 193 | 2.6 | 37 |
| Wittenbach Orch. | 8.0 | 289 | 4.0 | 5.8 | 17 | 289 | 22.9 | 88 |

Table 2. Parameters measured for 1990, late harvest. Each number represents an average of a minimum of 30 fruit.

| Location | DIAM. (cm) | WT. (grams) | SEED COUNT | MgIP (Avg) | (%) | Ca ($\mu g\ g^{-1}\ dwt^{-1}$) | BITTER PIT AFTER STORAGE (Avg) | (%) |
|---|---|---|---|---|---|---|---|---|
| Epple Orchards | 7.5 | 169 | 6.7 | 7.7 | 58 | 219 | 9.6 | 70 |
| Molter Farms | 8.7 | 260 | 7.2 | 9.6 | 51 | 195 | 22.2 | 76 |
| Rosenburg Farms | 7.8 | 189 | 3.4 | 2.2 | 16 | 238 | 9.1 | 54 |
| WmB. Inc. A. | 8.4 | 236 | 9.8 | 6.6 | 60 | 201 | 11.9 | 81 |
| WmB. Inc. B. | 8.1 | 208 | 13.0 | 5.7 | 30 | 201 | 9.1 | 40 |
| Hort. Res. Cent. | 8.7 | 257 | 6.0 | 2.4 | 18 | 182 | 9.2 | 64 |
| Clarksville Expt. | 8.6 | 258 | 9.0 | 2.4 | 26 | 198 | 9.9 | 52 |
| Mueller Farms A. | 8.3 | 230 | 11.0 | 2.1 | 20 | 256 | 2.9 | 24 |
| Mueller Farms B. | 7.5 | 181 | 6.8 | 1.9 | 20 | 321 | 1.7 | 14 |
| Mueller Farms C. | 7.8 | 182 | 10.0 | 2.2 | 16 | 256 | 2.8 | 32 |
| Peabody Orchards. | 7.2 | 155 | 8.7 | 0.0 | 0 | 357 | 0.0 | 0 |

Table 3. Parameters measured for fruit harvested August 22, 1991. Each number is the average of 50 fruit.

| Location | WT. (grams) | SEED COUNT | MgIP (Avg) | MgIP (%) | Ca ($\mu g\ g^{-1}\ dwt^{-1}$) | BITTER 5°C (Avg) | BITTER 5°C (%) | PIT AFTER 3°C Air (Avg) | PIT AFTER 3°C Air (%) | STORAGE 3°C CA (Avg) | STORAGE 3°C CA (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Clarksville Expt. | 170 | 10.0 | 0.8 | 14 | 355 | 1.6 | 9.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Peabody Orchards. | 146 | 11.2 | 2.1 | 27 | 260 | 7.9 | 38 | 0.0 | 0.0 | 0.0 | 0.0 |
| Hort. Res. Cent. | 154 | 9.0 | 4.8 | 58 | 221 | 10.9 | 50 | 0.9 | 8.0 | 0.0 | 0.0 |
| Blossum Orchard. | 144 | 9.0 | 0.9 | 20 | 240 | 11.9 | 15 | 81 | 0.0 | 0.0 | 0.0 |
| Mueller Farms. | 141 | 11.5 | 1.3 | 28 | 366 | 9.1 | 20 | 40 | 5.0 | 0.0 | 0.0 |

Table 4. Parameters measured for fruit harvest Sept. 3, 1991. Each number represents the mean of 50 fruit.

| Location | WT. (grams) | SEED COUNT | MgIP (Avg) | MgIP (%) | Ca ($\mu g\ g^{-1}\ dwt^{-1}$) | BITTER PIT AFTER STORAGE 5°C (Avg) | 5°C (%) | 3°C Air (Avg) | 3°C Air (%) | 3°C CA (Avg) | 3°C CA (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Clarksville Expt. | 168 | 9.7 | 0.3 | 12 | 342 | 3.4 | 29 | 0.0 | 0.0 | 0.0 | 0.0 |
| Peabody Orchards. | 173 | 11.6 | 0.0 | 0.0 | 251 | 1.6 | 19 | 0.0 | 0.0 | 0.0 | 0.0 |
| Hort. Res. Cent. | 165 | 9.3 | 3.7 | 48 | 208 | 8.8 | 62 | 0.2 | 7.5 | 0.0 | 0.0 |
| Blossum Orchard. | 148 | 8.9 | 0.5 | 22 | 231 | 1.1 | 16 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mueller Farms. | 166 | 9.5 | 0.3 | 10 | 236 | 1.9 | 9.7 | 0.1 | 12.5 | 0.0 | 0.0 |
| WmB. Inc. | 186 | 11.2 | 11.8 | 79 | 195 | - | - | 3.5 | 78.0 | 4.0 | 74.0 |

Table 5. Parameters measured for fruit harvested Sept. 11, 1991. Each number represents an average of 50 fruit.

| Location | WT. (grams) | SEED COUNT | MgIP (Avg) | (%) | Ca ($\mu g\ g^{-1}\ dwt^{-1}$) | BITTER PIT AFTER STORAGE 5°C (Avg) | (%) | 3°C Air (Avg) | (%) | 3°C CA (Avg) | (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Clarksville Expt. | 172 | 9.6 | 1.0 | 16 | 286 | 5.0 | 23 | 0.0 | 0.0 | 0.0 | 0.0 |
| Peabody Orchards. | 177 | 11.2 | 1.6 | 10 | 194 | 9.9 | 25 | 0.5 | 10.0 | 0.6 | 4.9 |
| Hort. Res. Cent. | 178 | 8.0 | 5.0 | 58 | 180 | 37.1 | 62 | 1.6 | 19.6 | 0.1 | 1.8 |
| Blossum Orchard. | 163 | 9.4 | 1.8 | 20 | 210 | 2.4 | 20 | 0.4 | 23.3 | 1.3 | 16.7 |
| Mueller Farms. | 142 | 9.6 | 0.1 | 2 | 295 | 2.5 | 17 | 0.5 | 6.4 | 0.0 | 1.9 |
| WmB. Inc. | 194 | 11.2 | 6.9 | 70 | 167 | 16.8 | 80 | 5.5 | 80.0 | 3.2 | 48 |

APPENDIX TWO: Additional figures for Chapter 1.

Fig. 1. Plot of MgIP/Fruit vs % of fruit with bitter pit that developed during storage at 5°C for 5 months. Data are cumulative for both the 1990 and 1991 seasons.

For MgIP/fruit vs bitter pits/fruit that developed in storage, $Y = 1.9X + 3.3$, $r^2 = 0.31$. For % of fruit with MgIP vs % of fruit with bitter pit after storage, $Y = 0.92X + 14.5$, $r^2 = 0.51$.

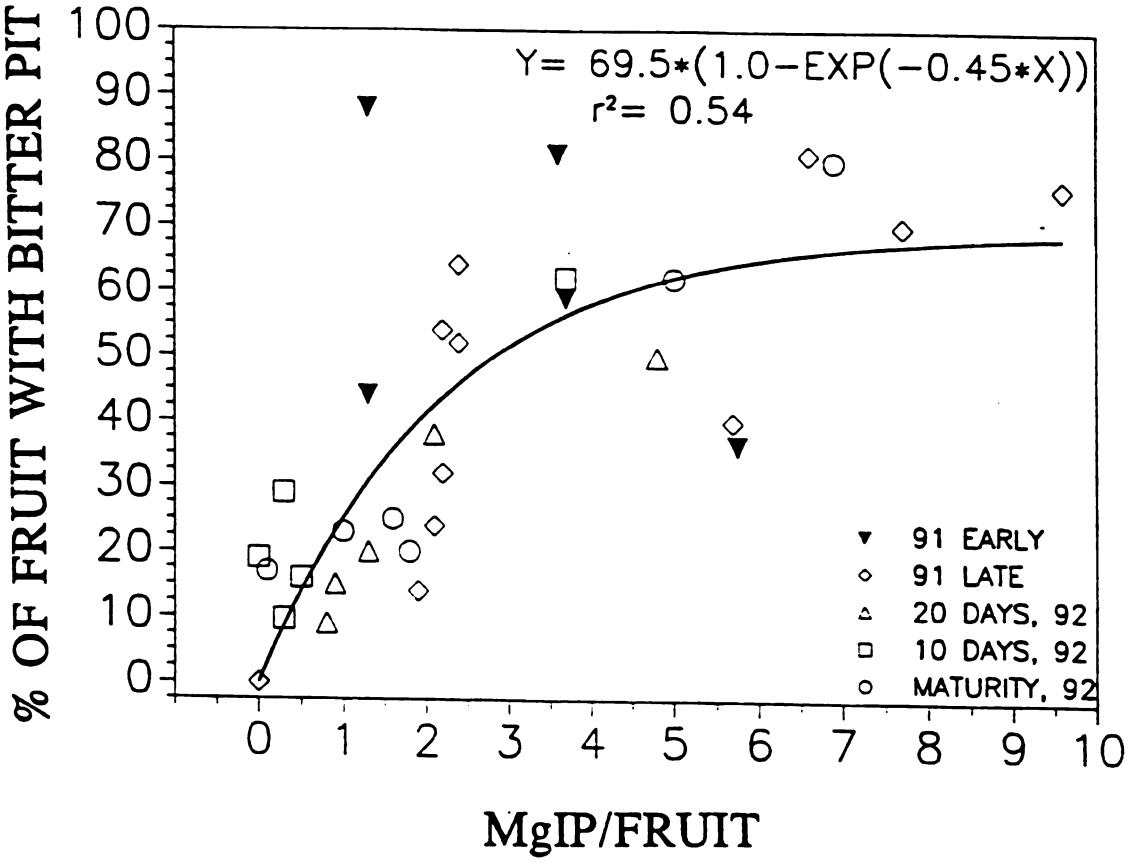

% OF FRUIT WITH BITTER PIT
100
90
80
70
60
50
40
30
20
10
0
Y= 69.5*(1.0-EXP(-0.45*X))
r²= 0.54
91 EARLY
91 LATE
20 DAYS, 92
10 DAYS, 92
MATURITY, 92
0 1 2 3 4 5 6 7 8 9 10
MgIP/FRUIT

Fig. 2. Plot of endogenous (native) calcium ($\mu g\ g^{-1}$ dwt.) vs MgIP/fruit.

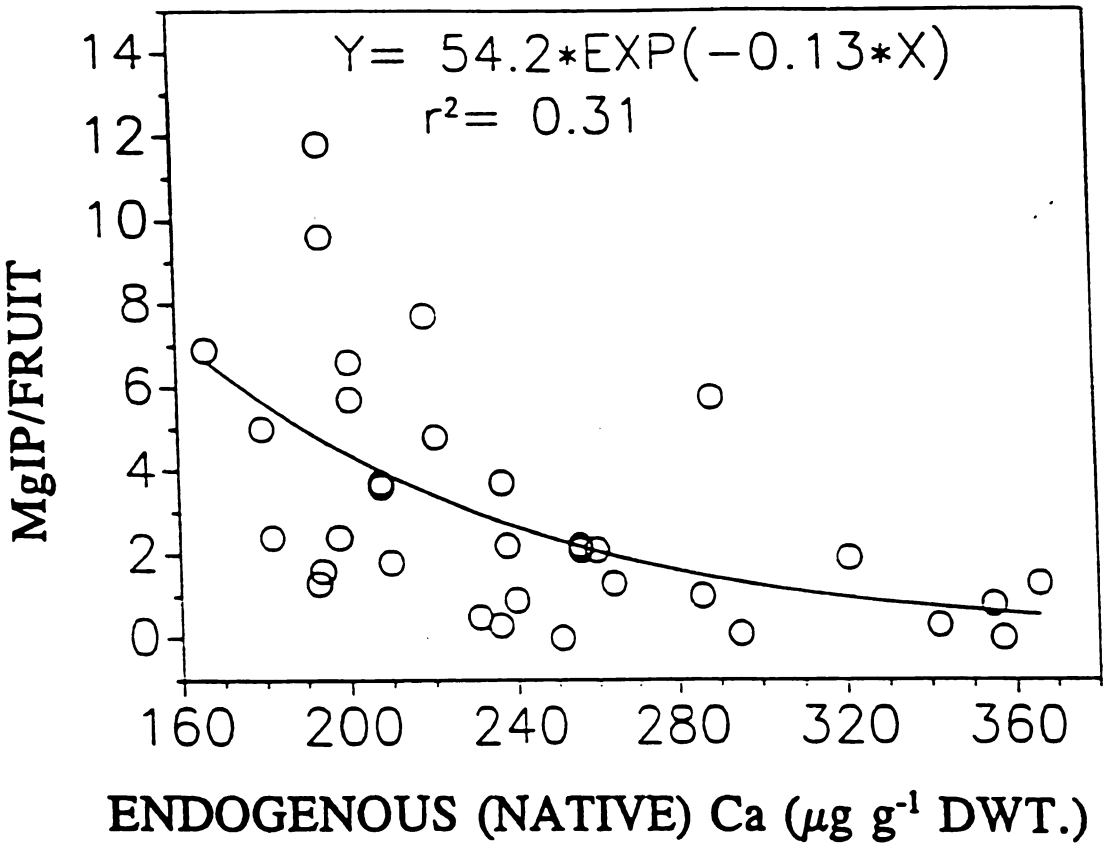

Y= 54.2*EXP(-0.13*X)
r²= 0.31
MgIP/FRUIT
0
2
4
6
8
10
12
14
160
200
240
280
320
360
ENDOGENOUS (NATIVE) Ca (μg g⁻¹ DWT.)

Fig. 3. Plot of endogenous (native) calcium ($\mu g\ g^{-1}$ dwt.) vs bitter pits/fruit that developed in storage. For 5°C and 3°C storage Y = 473.6*EXP(-0.02*X), $r^2$ = 0.49, and Y = 502.0*EXP(-0.043), $r^2$ = 0.59, respectively.

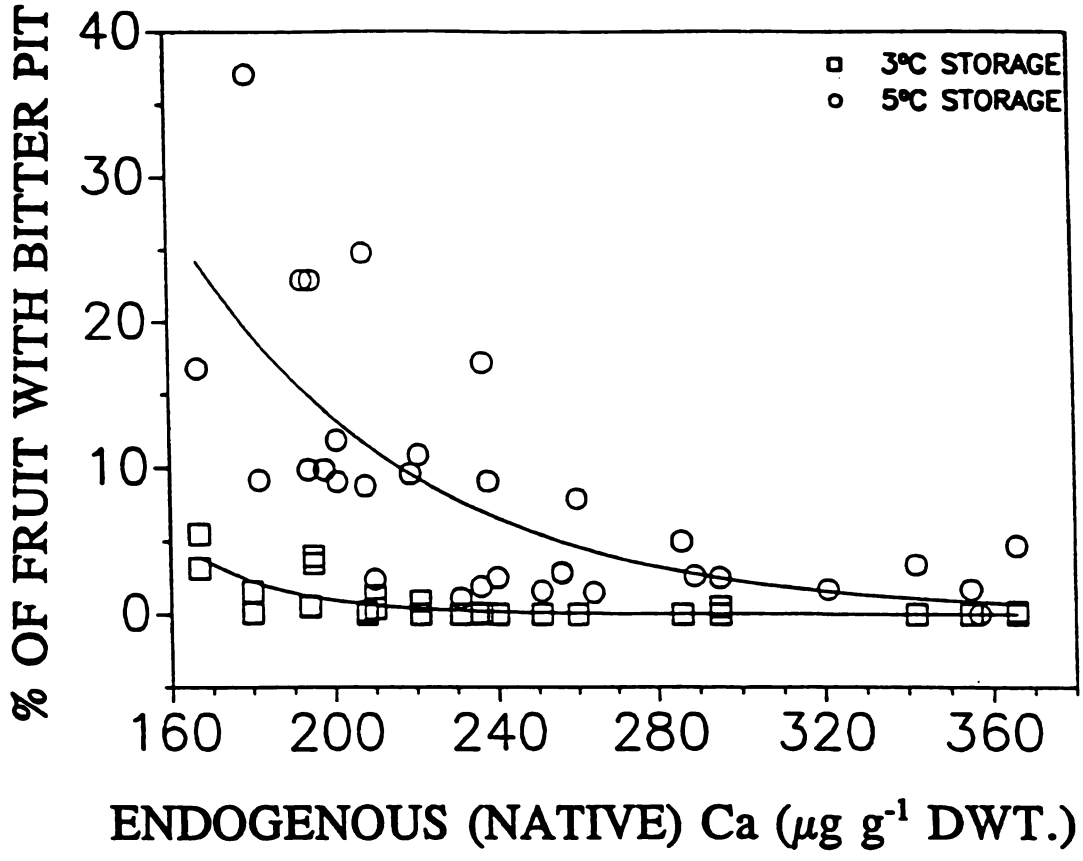
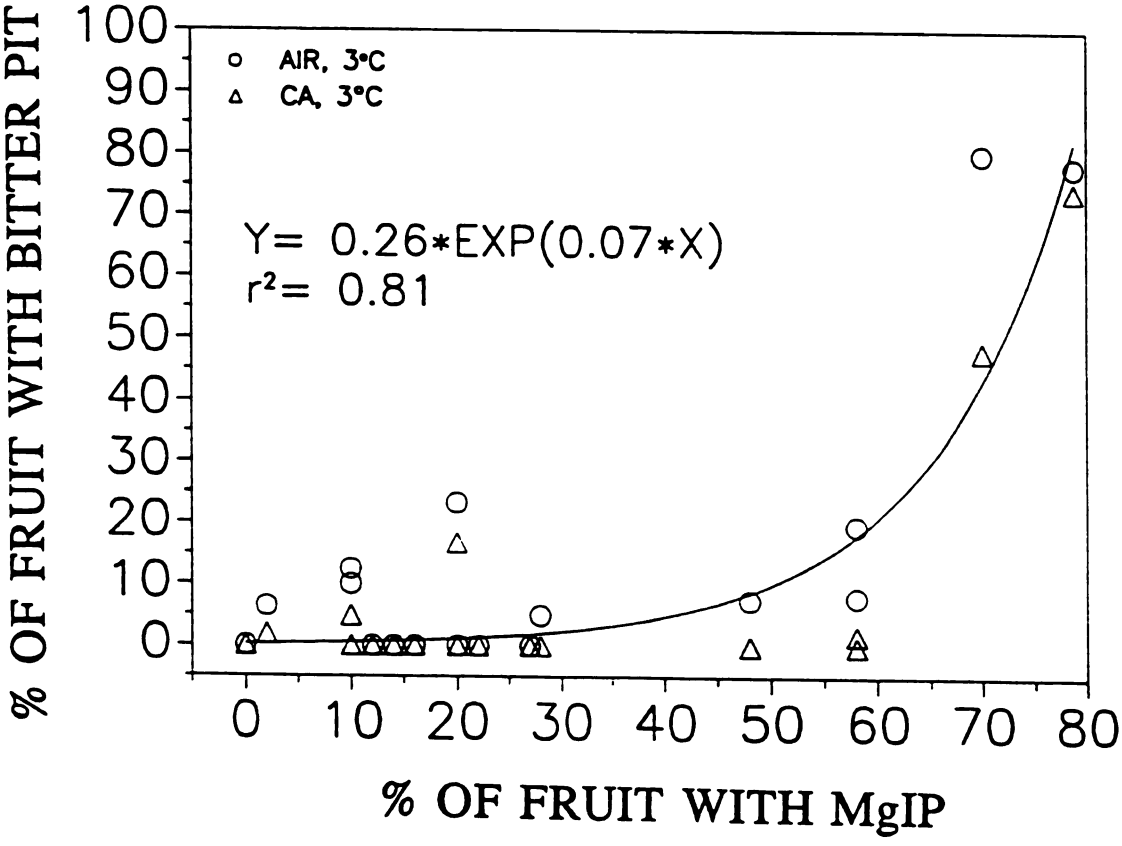

% OF FRUIT WITH BITTER PIT
40
30
20
10
0
3°C STORAGE
5°C STORAGE
160
200
240
280
320
360
ENDOGENOUS (NATIVE) Ca (μg g-1 DWT.)

Fig. 4. Plot of % of fruit with MgIP vs % of fruit with bitter pit after 3°C air and CA storage for 7 months.


% OF FRUIT WITH BITTER PIT
100
90
80
70
60
50
40
30
20
10
0
AIR, 3°C
CA, 3°C
Y= 0.26*EXP(0.07*X)
r²= 0.81
0
10
20
30
40
50
60
70
80
% OF FRUIT WITH MgIP

# APPENDIX THREE: The Effects of Prestorage Heat Treatments on Physiological Disorder Development and Softening of Northern Spy, Red Delicious and Law Rome Apples.

## INTRODUCTION

Exposure of plants and harvested plant organs to 35 to 40°C temperatures profoundly affects physiological and biochemical processes during and subsequent to the heat stress. Work in our laboratory as well as others has shown heat treated tomatoes to be less sensitive to chilling injury (Picton and Grierson, 1988; Lurie and Klein, 1990 and 1991; Burmeister *et al.*, in preparation). Other work with apples has shown an inhibition of ripening and a decrease in softening rate of heat treated fruit (Maxie *et al.* 1974; Porritt and Lidster, 1978). In addition, superficial scald on apples has been shown to be attenuated by prestorage heat treatments, although not completely (Lurie *et al.*, 1991; Klein and Lurie, 1992a). Interest in use of physical rather than chemical means to control postharvest disorders has been prompted by concern of the public over the safety of these chemicals (Klein and Lurie, 1992b). Environmental Protection Agency registration of some fungicides, and antioxidants may be withdrawn and the cost of re-registration of existing, or registration of new chemicals is prohibitive. In addition, the storage and disposal of the waste solution from drenching facilities is a problem. The objective of this study was to determine if prestorage heat treatments in combination with

subsequent controlled atmosphere (CA) storage would attenuate physiological disorder development on apples without sacrificing quality.

## MATERIALS AND METHODS

Preclimacteric Northern Spy, Red Delicious, and Law Rome apples were obtained from the Michigan State University Clarksville Experiment Station. Purge CA system: Fruits (40-60) were randomized to fill into 20L CA chambers by cultivar and held in air at 38°C for 0, 2, 3, or 4d and then placed in air at 1 or 3°C for the balance of 7d before establishing CA conditions. Chambers were ventilated with 3.0% $CO_2$:1.5% $O_2$, or 3.0% $CO_2$:3.0% $O_2$ at 75ml $min^{-1}$. Chambers left in air with loosely fitting lids served as controls. Each atmosphere/heat treatment was done in triplicate. Quasi-static system: Law Rome were randomized and loaded into CA chambers as before and held at 38°C for 0, 1, 2, 3, or 4d then placed in air at 1 or 3°C for the balance of 7d before establishing CA. CA was maintained employing a David Bishop Instrument Oxystat II CA system. Fruit were stored for 8 months and examined for disorders after a subsequent 7d period in air at 20°C. Northern Spy were evaluated for bitter pit while, those of Red Delicious and Law Rome were examined for superficial scald. Flesh firmness was determined with Effegi penetrometer (11mm dia.) for 10 fruits upon removal from storage and after 7 days at 20°C in air.

## RESULTS AND DISCUSSION

Purge system (3°C). Heating delayed softening of Northern Spy fruits stored in air and with 3:3 atmosphere. The effect of heat was evident for fruits on removal from storage and after holding them in air at 20°C for 7d (Table 1). Attenuation of softening was significantly greater ($P < 0.01$) as the duration of heating increased. Fruits stored at 1.5 $O_2$ retained the greatest firmness during storage. Heating diminished the percentage of apples stored at 3:1.5 which developed bitter pit but increased bitter pit on fruits stored at 3:3.

Red Delicious. Retention of flesh firmness and scald attenuation were greatest in the 2d heat treatment at the 3.0:1.5 atmosphere (Table 2). However, the effect of heat treatments on retention of flesh firmness and scald attenuation for Red Delicious was not significant.

Law Rome. The effect of heat treatments on firmness upon removal from storage was significant ($P < 0.01$), but not for fruits held for 7d at 20°C. Heat treatments significantly increased scald for this cultivar.

Law Rome, quasi static system 1°and 3°C. Attenuation of softening for heated fruit upon removal and after 7d at 20°C was significant for both 1° and 3°C storage temperatures ($P<0.01$). The effects of heat treatment on scald were significant in both the 1°C and 3°C storage temperatures ($P < 0.05$ and $P < 0.01$, respectively). Scald appeared to increase with duration of heating for fruit stored at 1°C while decreasing for the 3°C storage temperature (Table 4 and 5).

These results indicate that heat treatments to these varieties do attenuate softening but may not be a viable alternative to control physiological storage disorders in long term storage beyond appropriate application of low temperature and CA. These results concur with those of Klein and Lurie (1992a) who found no benefit on storage firmness following the heat treatment of Granny Smith apples held in CA for 8 months. They did not evaluate these long term CA fruit for physiological disorder development. In previous studies, storage durations have been relatively short (Lurie *et al.*, 1991). It may be that positive effects of heat treatment on scald prevention may only be realized following short storage durations or when application of appropriate CA conditions is not feasible. Further investigations will be necessary to determine conditions where prestorage heat treatment may be beneficial.

Table 1. Effect of prestorage heat treatments on flesh firmness and bitter pit on Northern Spy apples stored at 3°C for 8 months in purge mode.

| | Flesh firmness (N) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0d Days of heat | | | | 7d at 20°C Days of heat | | | |
| Atmosphere ($CO_2$:$O_2$) | 0d | 2d | 3d | 4d | 0d | 2d | 3d | 4d |
| Air | 51.2 | 61.8 | 61.7 | 64.2 | 58.7 | 61.8 | 64.9 | 65.8 |
| 3.0:3.0 | 62.8 | 68.7 | 70.8 | 69.8 | 64.1 | 71.2 | 70.9 | 73.9 |
| 3.0:1.5 | 74.6 | 75.9 | 74.7 | 72.7 | 75.4 | 81.3 | 79.4 | 81.4 |
| | $LSD_{0.05}$ = 1.7 | | | | $LSD_{0.05}$ = 1.6 | | | |
| | | | | | Bitter pit (%) | | | |
| Air | | | | | 17.6 | 10.7 | 12.8 | 12.8 |
| 3.0:3.0 | | | | | 0.7 | 0.0 | 9.0 | 6.8 |
| 3.0:1.5 | | | | | 11.1 | 1.5 | 2.6 | 0.0 |
| | | | | | $LSD_{0.05}$ = 2.3 | | | |

Table 2. Effect of prestorage heat treatments on flesh firmness and superficial scald on Red Delicious apples stored at 3°C for 8 months in purge mode.

| Atmosphere ($CO_2$:$O_2$) | Flesh firmness (N) 0d Days of heat 0d | 2d | 3d | 4d | 7d at 20°C Days of heat 0d | 2d | 3d | 4d |
|---|---|---|---|---|---|---|---|---|
| Air | 54.6 | 49.5 | 47.9 | 52.8 | 55.0 | 46.3 | 45.8 | 51.5 |
| 3.0:3.0 | 53.2 | 50.6 | 55.8 | 54.5 | 52.2 | 45.1 | 53.1 | 46.0 |
| 3.0:1.5 | 61.2 | 64.7 | 60.1 | 57.0 | 60.4 | 63.5 | 57.5 | 58.2 |
| | $LSD_{0.05}$ = 1.5 | | | | $LSD_{0.05}$ = 1.8 | | | |
| | | | | | Scald (% of surface area) | | | |
| Air | | | | | 51.7 | 57.4 | 55.8 | 52.8 |
| 3.0:3.0 | | | | | 47.2 | 60.7 | 60.1 | 49.2 |
| 3.0:1.5 | | | | | 39.4 | 18.2 | 32.1 | 32.1 |
| | | | | | $LSD_{0.05}$ = 4.3 | | | |

Table 3. Effect of prestorage heat treatments on flesh firmness and superficial scald on Law Rome apples stored at 3°C for 8 months in purge mode.

| Atmosphere ($CO_2$:$O_2$) | Flesh firmness (N) 0d Days of heat 0d | 2d | 3d | 4d | 7d at 20°C Days of heat 0d | 2d | 3d | 4d |
|---|---|---|---|---|---|---|---|---|
| Air | 56.9 | 67.8 | 73.7 | 70.1 | 52.0 | 59.7 | 71.6 | 66.1 |
| 3.0:3.0 | 65.6 | 71.7 | 73.5 | 73.0 | 65.5 | 67.8 | 64.6 | 60.5 |
| 3.0:1.5 | 75.4 | 74.6 | 73.3 | 72.4 | 67.2 | 73.3 | 67.2 | 67.7 |
| | $LSD_{0.05}$= 2.1 | | | | $LSD_{0.05}$= 2.6 | | | |
| | | | | | Scald (% of surface area) | | | |
| AIR | | | | | 75.7 | 69.9 | 74.4 | 80.1 |
| 3.0:3.0 | | | | | 16.2 | 25.6 | 37.0 | 34.0 |
| 3.0:1.5 | | | | | 10.7 | 21.3 | 16.4 | 27.4 |
| | | | | | $LSD_{0.05}$= 3.8 | | | |

Table 4. Effect of prestorage heat treatments on flesh firmness and superficial scald on Law Rome apples stored at 1°C for 8 months in quasi-static CA system.

| Atmosphere ($CO_2$:$O_2$) | Flesh firmness (N) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0d | | | | | 7d at 20°C | | | | |
| | Days of heat | | | | | Days of heat | | | | |
| | 0d | 1d | 2d | 3d | 4d | 0d | 1d | 2d | 3d | 4d |
| Air | 52.9 | 57.2 | 61.9 | 66.7 | 66.5 | 51.5 | 52.9 | 60.4 | 64.5 | 65.1 |
| 3.0:3.0 | 59.1 | 58.2 | 73.5 | 73.0 | 66.8 | 54.2 | 54.3 | 60.9 | 62.3 | 63.4 |
| 3.0:1.5 | 59.7 | 60.4 | 73.3 | 72.4 | 63.2 | 57.5 | 57.1 | 59.2 | 59.8 | 61.8 |
| | $LSD_{0.05}$ = 1.3 | | | | | $LSD_{0.05}$ = 1.7 | | | | |
| | | | | | | Scald (% of surface area) | | | | |
| Air | | | | | | 72.0 | 72.6 | 75.1 | 71.2 | 80.0 |
| 3.0:3.0 | | | | | | 34.3 | 45.4 | 47.6 | 58.1 | 54.7 |
| 3.0:1.5 | | | | | | 20.9 | 41.4 | 49.3 | 41.3 | 43.8 |
| | | | | | | $LSD_{0.05}$ = 5.3 | | | | |

Table 5. Effect of prestorage heat treatments on flesh firmness and superficial scald on Law Rome apples stored at 3°C for 8 months in quasi-static CA system.

| Atmosphere ($CO_2$:$O_2$) | Flesh firmness (N) 0d Days of heat 0d | 1d | 2d | 3d | 4d | 7d at 20°C Days of heat 0d | 1d | 2d | 3d | 4d |
|---|---|---|---|---|---|---|---|---|---|---|
| AIR | 44.0 | 47.9 | 53.6 | 57.3 | 56.4 | 42.3 | 47.7 | 46.6 | 55.3 | 50.9 |
| 3.0:3.0 | 49.5 | 53.2 | 53.1 | 54.2 | 55.8 | 48.1 | 48.3 | 51.2 | 52.3 | 51.5 |
| 3.0:1.5 | 53.5 | 53.4 | 53.8 | 55.5 | 57.5 | 50.7 | 50.8 | 50.8 | 52.6 | 54.1 |
| | $LSD_{0.05}$ = 1.5 | | | | | $LSD_{0.05}$ = 1.2 | | | | |
| | | | | | | Scald (% of surface area) | | | | |
| Air | | | | | | 78.5 | 66.1 | 68.3 | 81.6 | 73.5 |
| 3.0:3.0 | | | | | | 81.0 | 81.8 | 63.3 | 76.0 | 82.7 |
| 3.0:1.5 | | | | | | 77.7 | 72.6 | 65.0 | 64.4 | 74.8 |
| | | | | | | $LSD_{0.05}$ = 2.8 | | | | |

REFERENCES:

Burmeister, D.M., Dilley, D.R., Jianping, K., McNulty, D., Poneleitt, L.S., and Walker, J. 1993 Postharvest heat treatments attenuate chilling-injury and increase uniformity of ripening of tomatoes. Post Harvest Biol. and Tech. in preparation.

Klein, J.D., and Lurie, S. 1992a. Prestorage heating of apple fruit for enhanced post-harvest quality: Interaction of time and temperature. HortScience 27:326-328.

Klein, J.D., and Lurie, S. 1992b. Heat treatments for improved postharvest quality of horticultural crops. HortTechnology, 2(3):316-320.

Lurie, S., and Klein, J.D. 1990. Heat treatment of apples: Differential effects on physiology and biochemistry. Physiol Plant. 78:181-186.

Lurie, S., and Klein, J.D. 1991. Acquisition if low-temperature tolerance in tomatoes by exposure to high-temperature stress. J. Amer. Soc. Hort. Sci. 116:1007-1012.

Lurie, S., Klein, J.D., and Ben-Arie, R. 1991. Prestorage heat treatment delays development of superficial scald on 'Granny Smith' apples. HortScience 26:166-167.

Maxie, E., Mitchell, G., Sommer, N., Snyder, G. and Rae, H. 1974. Effects of elevated temperature on ripening of 'bartlett' pear. *Pyrus communis* L. J. Amer. Soc. Hort. Sci. 99:344-349.

Picton, S., and Grierson, D. 1988. Inhibition of expression of tomato ripening genes at high temperature. Plant Cell and Environment. 11:265-272.

Porritt, S.W., and Lidster, P.D. 1978. The effect of prestorage heating on ripening and senescence of apples during cold storage. J. Amer. Soc. Hort. Sci. 103:584-587.