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

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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 with Mg^{2+} increased pitting over that with Mg^{2+} alone. Verapamil and nefedipine had no effect on MgIP or its attenuation by Ca^{2+}. Cyclopiazonic acid attenuated MgIP. Ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid and 2,3,5-triiodobenzoic acid 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+}, and Sr^{2+} included at 20.0 mM prevented MgIP (induced by 0.18 M Mg^{2+}). Ca^{2+} (3.0 mM) included with 0.18 M 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.

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 that 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 treatment 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, of our laboratory, unpublished data). Mg^{2+}-induced pits were synonymous to bitter pit as indicated by the following: 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, 1993). Here we have used MgIP as a model for investigating the physiology and biochemistry of bitter pit initiation and development.

EXPERIMENTAL PROCEDURES

Experiments were conducted with Golden Delicious apples (*Malus domestica* borkh.), harvested in 1991 from the Michigan State University Clarksville Horticultural Experiment Station, Clarksville, MI. Fruits (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 100 mmHg for 2 min. All solutions contained 0.3 M 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 was 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: ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), a chelator; 2,3,5-triiodo-

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Table I. Effects of Ca^{2+} and Ca^{2+} Channel Blockers on Mean Number of Mg^{2+}-Induced Pits per Fruit

Mg^{2+} concn, M	Ca^{2+} concn, 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
100 μM 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

benzoic acid (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 and Nefedipine and Calmodulin Antagonist Trifluoperazine. Treatments were factorially arranged with three levels of Ca^{2+} (0.0, 0.01, 0.02 M) and three levels of Mg^{2+} (0.0, 0.04, 0.18 M), alone or with Vp (100 μM) or TFP (100 μM). Fruits were infiltrated with 100 μM Nf alone or with 0.18 M Mg^{2+}. Nf was dissolved in dimethyl sulfoxide (DMSO) and added to the solutions, resulting in a final concentration of 0.1% DMSO. All controls contained 0.1% DMSO.

Cyclopiazonic Acid. Fruits were infiltrated with CPA (40 μM) or 0.18 M Mg^{2+} or CPA (40 μM) with Mg^{2+}.

2,3,5-Triiodobenzoic Acid and Ethylene Glycol Bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic Acid. Fruits were treated with EGTA (100 μM) and TIBA (100 μM) alone or with 0.18 M Mg^{2+}.

Protein Synthesis Inhibitors and Antibiotics. Fruits were infiltrated with 0.18 M Mg^{2+} alone or with cycloheximide (25 μg mL^{-1}), puromycin (6.25 μg mL^{-1}), or actinomycin D (25 μg mL^{-1}).

Heat Treatments. *Experiment 1.* Fruits were heated for 0, 1, 2, or 3 days at 38 °C and then infiltrated with 0.18 M Mg^{2+}. During the heat treatments the fruits were in perforated polyethylene bags to prevent desiccation.

Experiment 2. Fruits were infiltrated with 0.18 M Mg^{2+} and placed at 20 °C for 7 days, at 38 °C for 3 days followed by 4 days at 20 °C, or at 20 °C for 3 days followed by 3 days at 38 °C and then 7 days at 20 °C. Fruits were enclosed in polyethylene bags as in experiment 1.

Divalent and Monovalent Cations. Fruits were infiltrated with 0.18 M Mg^{2+} alone or with 1.25, 2.5, 5.0, 10, or 20 mM Ca^{2+}. Fruits were infiltrated with either 0.18 M Mg^{2+} or 0.18 M Mg^{2+} + 20 mM 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 0.18 M Mg^{2+} alone or including K^+ or Na^+ at 40 mM.

RESULTS AND DISCUSSION

Including TFP with Mg^{2+} increased pitting over that with Mg^{2+} alone, and Ca^{2+} attenuated MgIP in the treatments that included TFP (Table I). All main effects and interactions were significant ($p \leq 0.05$) except for $Mg^{2+} \times$ TFP and $Mg^{2+} \times Ca^{2+} \times$ 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, nonspecific detergent properties (Dr. Ian Ferguson, DSIR Auckland, NZ, (personal communication).

Table II. Effect of Cyclopiazonic Acid (CPA) on Mg^{2+}-Induced Pitting

treatment	mean no. of pits per fruit	sig[a]
0.18 M Mg^{2+}	14.5	
0.18 M Mg^{2+} + CPA (40 μM)	6.4 vs 0.18 M Mg^{2+}	***
controls		
0.3 M sorbitol alone	0.0	
0.3 M sorbitol + CPA (40 μM)	0.0	

[a] Treatments were partitioned into single degree of freedom contrasts as indicated. * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

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 references cited 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 affect 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 μM 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 $p \leq 0.1$. 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 μM, also did not significantly reduce pitting induced by 0.18 M Mg^{2+} (data not shown). Similar experiments with Vp and Nf included at 500 μM 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+}.

CPA, an inhibitor of Ca^{2+}-ATPase, significantly attenuated MgIP (Table II). Including CPA (10 μM) 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+} efflux (Riley and Goeger, 1990).

EGTA at 100 μM significantly attenuated pitting induced by 0.18 M 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 μM also significantly reduced the amount of pitting

Table III. Effect of EGTA and TIBA on Mg^{2+}-Induced Pit

treatment	mean no. of pits per fruit	sig[a]
0.18 M Mg^{2+}	21.5	
0.18 M Mg^{2+} + EGTA (100 μM)	6.8 vs 0.18 M Mg^{2+}	***
0.18 M Mg^{2+} + TIBA (100 μM)	4.1 vs 0.18 M Mg^{2+}	***
controls		
EGTA (10^{-4} M) alone	0.0	
TIBA (10^{-4} M)	0.5	
0.3 M sorbitol	0.0	

[a] See footnote to Table II.

Table IV. Effect of Protein Synthesis Inhibitors and Antibiotics on Mg^{2+}-Induced Pitting

treatment	mean no. of pits per fruit	sig[a]
0.18 M Mg^{2+}	25.8	
0.18 M Mg^{2+} + puromycin (6.25 μg mL^{-1})	21.7 vs Mg^{2+} alone	ns[b]
0.18 M Mg^{2+} + actinomycin D (25 μg mL^{-1})	9.4 vs Mg^{2+} alone	***
0.18 M Mg^{2+} + cycloheximide (25 μg mL^{-1})	0.0 vs Mg^{2+} alone	***
controls		
puromycin (6.25 μg mL^{-1})	0.0	
actinomycin D (25 μg mL^{-1})	0.0	
cycloheximide (25 μg mL^{-1})	0.0	
0.3 M sorbitol alone	0.0	

[a] See footnote to Table II. [b] Not significant.

induced by 0.18 M 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 0.18 M Mg^{2+} treatment.

Cycloheximide at 25 μM totally inhibited MgIP development (Table IV). This was also found in another experiment with cycloheximide at 10 μg mL^{-1} (data not shown). Cycloheximide blocks protein synthesis by inhibiting aminoacyl transferase in peptide bond formation in the ribosome. Puromycin at 6.25 μg 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 μg 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.

Heating apples at 38 °C for 1–3 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 the number of pits that developed (Table VI), whereas fruits subjected to 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 35–40 °C can profoundly affect physiological and

Table V. Effect of Heat Treatment at 38 °C prior to Mg^{2+} Infiltration

heat treatment, days	$[Mg^{2+}]$, M	mean no. of pits per fruit	sig[a]
0	0.18	8.4	
1	0.18	1.9 vs days, 0.18 M Mg^{2+}	***
2	0.18	3.7 vs 0 days, 0.18 M Mg^{2+}	***
3	0.18	2.9 vs 0 days, 0.18 M Mg^{2+}	***
controls			
0	0.00	0.0	
1	0.00	0.0	
2	0.00	0.0	
3	0.00	0.0	

[a] See footnote to Table II.

Table VI. Effect of Heat Treatment at 38 °C following Mg^{2+} Infiltration

heat treatment	$[Mg^{2+}]$, M	mean no. of pits per fruit	sig[a]
20 °C continuous	0.18	18.3	
3 days heat times 20 °C	0.18	46.2 vs 20 °C continuous	***
3 days 20 °C times 3 days heat	0.18	21.0 vs 20 °C continuous	ns[b]
controls			
20 °C continuous	0.00	0.0	
3 days heat times 20 °C	0.00	1.6	
3 days 20 °C times 3 days heat	0.00	0.0	

[a] See footnote to Table II. [b] Not significant.

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 35–40 °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 10 000 to nearly 100 000 (Kimpel and Key, 1985). Prestorage heat treatments of apples have been found to inhibit ripening (although not irreversibly), to reduce the rate of subsequent softening of apples, and to 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.

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 ≈3.0 mM (data not shown). La^{3+}, Co^{2+}, Sr^{2+}, and Ba^{2+} at 20.0 mM all completely attenuated Mg^{2+} pitting induced by 0.18 M 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^+ (40.0 mM) and Na^+ (40.0 mM) included with 0.18 M 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 0.18 M Mg^{2+}. The

Table VII. Effect of Na^+ and K^+ on Mg^{2+}-Induced Pitting

treatment	mean no. of pits per fruit	sig[a]
0.18 M Mg^{2+} alone	17.4	
0.18 M Mg^{2+} + 0.04 M K^+	10.7 vs 0.18 M Mg^{2+}	*
0.18 M Mg^{2+} + 0.04 M Na^+	5.8 vs 0.18 M Mg^{2+}	***

[a] See footnote to Table II.

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).

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 the 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 dysfunction (e.g., bitter pit). This explanation may account for the fact that fruits of low Ca may not develop bitter pit unless conditions (e.g., 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 disks 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 enzymes 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.

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