FINAL PROJECT REPORT
WTFRC Project Number: AP-07-707

Project Title: A new approach to understand and control bitter pit in apple

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Other funding Sources: None in 2007, we just received a small grant for California Tomato Research Board for 2008

Total Project Funding: $10,000

Budget History:

Item	**Year 1:** 2007		
Salaries	4,445		
Benefits	580		
Wages			
Benefits			
Equipment			
Supplies	3,300		
Travel	1,600		
Miscellaneous	75		
Total	**10,000**		

INTRODUCTION

For many years, bitter pit in apple fruit has been studied; however, its mechanism remains unknown. This disorder is responsible for a reduction in quality and loss of commercial value of apple fruit, and the incidence can be quite high in some seasons (>15%). After an extensive literature review and in agreement with other scientists, we proposed a new approach to better understand and control bitter pit in apples. Our hypotheses are that the levels of growth regulators may regulate the amount of calcium that is translocated to the fruit, as well as how the fruit tissue regulates the availability of calcium for important structural functions such as membrane stabilization. High levels of gibberellins during fruit growth and development may change xylem function and calcium uptake to the fruit, as well as induce changes in membrane permeability, which is responsible for increasing weight loss and bitter pit incidence (SAURE, 2001; SAURE, 2004).

Bitter pit is believed to be similar to a disorder in tomato fruit, blossom end rot (SAURE, 2001). Based on this similarity, in the first part of the project we used greenhouse tomato plants as a faster and more controlled model to test our hypothesis. Next, we tested our hypothesis in a commercial apple orchard.

OBJECTIVES

1. Explore the relationship between growth regulators and xylem function as related to fruit susceptibility to bitter pit.
2. Explore the relationship between gibberellin levels late in the growing season and membrane permeability as related to water stress and fruit susceptibility to bitter pit.
3. Determine the cellular location of calcium in the apple fruit and the relationship to bitter pit susceptibility.
4. Develop management strategies to predictably reduce fruit susceptibility to bitter pit under commercial conditions.

METHODS

This work was done in 2007 at the University of California, Davis. Tomato plants (cv. Ace) were grown in 5 liter pots with organic substrate in a greenhouse environment. At full bloom, three to six fully opened flowers were selected on each plant, tagged and pollinated. One day after pollination, the plants were treated with 300ppm of gibberellins (GA4+7), 300ppm of a gibberellin inhibitor (Apogee), 500ppm of growth inhibitor (VBC30053) or water (control). Each treatment included four replications with one plant each. The treatments were applied by weekly spraying the plants with each solution containing also 0.05% polysorbate 20 (Tween® 20). On the day of the first treatment, 20g of slow release fertilizer (24-4-9 NPK) was added to each pot and from this point on, the plants were irrigated with deionized water only. The evaluations were conducted at 12, 24, 31, 38, 45, and 52 days after pollination for blossom-end rot incidence, membrane permeability, xylem function, fruit weight and diameter. At 32 days after full bloom, we evaluated dry matte, and calcium levels in the fruit.

Similar treatments were also applied to twenty year old Granny Smith apple trees growing on 111 rootstock in a commercial orchard in Stockton, California. The treatments were 1) water applied after full bloom (AFB) + before harvest (BH), 2) 300ppm of gibberellin (4+7) AFB, 3) 150ppm of gibberellin (4+7) BH, 4) 300ppm of gibberellin

inhibitor (Apogee) AFB + BH, 5) 500ppm of growth inhibitor (VBC30053) AFB, and 6) 100ppm of growth inhibitor (VBC30053) BH. Each treatment included four replications with two trees each. The treatments were applied weekly, starting two weeks after full bloom and/or four weeks before harvest and were applied for six and four weeks, respectively. Plants were sprayed with 3.9 liters of the respective solution containing also 0.05% of polysorbate 20 (Tween® 20). Commercial calcium sprays were not applied to trees in this experiment. Fruit were harvested at commercial maturity and stored at 0°C (32F) for two months. Seven weeks after full bloom, the fruit were evaluated for xylem function, weight, dry matter, and total levels of calcium in the blossom-end region. At harvest, the fruit were evaluated for bitter pit and water core incidence, dry matter, calcium content, flesh firmness, starch content, titratable acidity, and total soluble solids. After two months of storage, the fruit from each treatment were analyzed for bitter pit incidence and severity, then divided into groups with and without bitter pit and analyzed for,weight loss, total soluble solids, titratable acidity, dry matter content, and total calcium content (still being processed). We also selected one fruit with initial symptoms of bitter pit and another sound fruit without any symptoms to investigate calcium localization in the cells using electron microscopy analysis. Postharvest application of the growth regulators was tested by dipping fruit for 5 minutes in growth regulator solutions before storage.. All solutions included 0.05% polysorbate 20 (Tween® 20) as a surfactant except one water only control. The treatments were: 1) water, 2) 150 ppm GA (4+7), 3) 300ppm Apogee, 4) 100ppm VCB30053 and 5) water without surfactant. Fruit were allowed to dry before storage and evaluated after two months at 0C (32F).

SIGNIFICANT FINDINGS

Tomato experiment

The results in the tomato experiment showed that growth regulators indeed play a role in blossom-end rot development. The plants treated with gibberellin had higher incidence of blossom-end rot (BER), whereas plants treated with the growth inhibitor VBC30053 had significantly lower incidence of this physiological disorder (Figure 1A). Membrane permeability also increased in fruit tissues treated with gibberellins and decreased in fruit treated with the growth inhibitor, VBC30053 (Figure 1B), matching the BER incidence. The application of gibberellin inhibitor (Apogee) or VBC30053 increased the number of functional xylem elements in the fruit compared to fruit treated with gibberellin and water (Figures 1C). Calcium concentration was consistently higher in fruit of plants treated with VBC30053 or Apogee (Figure 1D). The dry matter content was reduced in fruit treated with VBC30053 (data not shown). VBC30053 treatment also increased fruit diameter and weight (data not shown). The biggest change observed with VBC30053 treatment was an increase in total fruit weight and the average fruit weight per plant (data not shown).

Apple preharvest experiment

Seven weeks after harvest, our fruit evaluations showed the effects of treatments applied after full bloom. As expected, gibberellin treatment decreased the number of functional primary xylem elements, whereas the growth regulator VBC30053 increased the number of functional xylem elements (Figure 2A). Growth regulator treatments did not result in differences in fruit diameter (data not shown) or fruit weight as was observed in the tomato experiment (Figure 2B). Growth regulator treatments did not have a great influence on the dry matter content of apple

fruit early in the growing season (data not shown). However, gibberellin treatments reduced whereas Apogee and VBC30053 treatments increased the total level of calcium in apple fruits at this stage of development (Figures 2C and 2D).

At harvest, apples treated with gibberellins after full bloom showed the highest incidence of bitter pit (Figure 3A). Gibberellin treatment after full bloom or before harvest, as well as VBC30053 treatment before harvest reduced the incidence of water core in the apple fruit at harvest compared with untreated control fruit. However, fruit treated with Apogee or VBC30053 after full bloom had increased amounts of water core (Figure 3B). Fruit treated with VBC30053 after full bloom had much greater dry matter content (Figure 3C). The levels of calcium in the fruit at harvest were quite similar among the treatments with a slightly higher concentration in the fruit treated with Apogee (Figure 3D).

Fruit treated with VBC30053 after full bloom had the highest total soluble solids followed by fruit treated with gibberellins after full bloom. When VBC30053 was applied before harvest, total soluble solids were similar to untreated control fruit (data not shown). Fruit treated with VBC30053 after full bloom or before harvest or gibberellins before harvest had elevated levels of titratable acidity compared with untreated control fruit (data not shown). The growth regulator treatments did not have much effect on fruit firmness or starch content at harvest (data not shown).

After two months of air storage, bitter pit incidence was observed in all treatments. However, fruit treated with Apogee after full bloom or VBC30053 before harvest both showed a reduction of approximately 14% in bitter pit incidence (Figure 4A). Bitter pit severity was similar for all treatments (Figure 4B). During storage, fruits treated with VBC30053 after full bloom lost significantly less weight than fruit from other treatments (Figure 4C). After two months of storage, the highest levels of dry matter, total soluble solids, and titratable acidity were observed in fruit with bitter pit and fruit treated with VBC30053 after full bloom (Figures 4D, 5A and 5B). Preliminary electron microscopy analysis for calcium localization inside the apple fruit cells have shown differences in precipitates within storage organelles between fruit with bitter pit and fruit without bitter pit that may be calcium sequestered in these organelles (Figure 7). We will confirm this with additional testing in the near future.

Apple postharvest experiment

The severity of bitter pit was the same among the various dip treatments (Figure 6B); however, the incidence of fruit with bitter pit was much lower in fruit dipped in Apogee or VCB30053, although the levels of bitter pit remained very high (Figure 6A). Fruit dipped in water without surfactant had the highest incidence of bitter pit.

RESULTS & DISCUSSION

Many have reported that fruit with the same levels of calcium can have different incidence of bitter pit or blossom-end rot. In this case, the importance may not be the total level of calcium, but where it is located in the tissue. A number of studies have shown that growth regulators can influence calcium balance and distribution in different tissues. Our results with tomato clearly showed that gibberellins increased the incidence of blossom end rot and also decreased fruit calcium content and the number of functional xylem elements early in fruit development, and increased membrane permeability, an early indicator of susceptibility to blossom-end rot. Growth inhibitors such as

Apogee and VBC30053 had the opposite effect, controlling blossom end rot, increasing fruit calcium content, and increasing the number of functional xylem elements early in fruit development. Reductions in electrolyte leakage were mainly observed with VBC30053. In addition, VBC30053 decreased fruit dry matter content while significantly increasing fruit size and diameter.

Our results with apple had similar trends, but were not as dramatic in the ability to control bitter pit. While bitter pit incidence was reduced by treatment with Apogee after full bloom or VBC30053 at harvest, there was still incredibly high bitter pit incidence. Early in fruit development, treatment with Apogee or VBC30053 after full bloom increased fruit calcium content; however, at harvest, only fruit treated with Apogee had slightly higher calcium content. As with tomato fruit, treatment with Apogee or VBC30053 increased the number of functional xylem elements while treatment with gibberellins decreased functional xylem elements. The postharvest dip data also showed promise for control of bitter pit and should be further explored. Our preliminary data with electron microscopy indicates that a lot of calcium is stored in the vacuole of fruit with bitter pit as compared to fruit without bitter pit. This may explain why some fruit in one lot have bitter pit and others do not. We plan to increase our observations of calcium localization and learn how to manipulate this to our advantage for fruit quality.

Of course there are large differences between tomato plants and apple trees, the most obvious being one is a woody perennial. The length of time required to grow and mature a Granny Smith apple is considerably longer than for a tomato fruit. Also, bitter pit develops late in the development of apple fruit, most commonly after cold storage, while blossom end rot in tomato develops approximately 2 weeks after full bloom. We found that the timing of application of growth regulator treatments had a great influence on fruit response. Early applications of VBC30053 at the concentrations used had a profound influence on fruit drop and leaf health that may have affected fruit quality at harvest. We need to do much more work with various concentrations and timings to determine the optimum performance of growth inhibitors for reduction of bitter pit incidence. Application with calcium sprays is another option that should be explored. The postharvest dip treatments also show tremendous promise and have the advantage of no potential affects on the tree and reduced application costs. We would like to test the growth inhibitor dip treatments together with calcium solutions to see if we can influence where in the cells the applied calcium is stored to improve our ability to reduce bitter pit. Our preliminary analysis of calcium localization in fruit cells with electron microscopy appears to be a promising technique to allow a better understanding calcium deficiency disorders.

In addition, it is important to continue investigating the mode of action of growth regulators in reducing blossom end rot and bitter pit as well increase our understanding of the mechanisms involved in calcium disorder development. With this understanding, we can better develop short-term and long-term solutions to control bitter pit in apple to reduce the economic impact of producing unmarketable fruit.

REFERENCES

SAURE, M,C. Blossom-end rot of tomato (*Lycopersicon esculentum* Mill.) – a calcium – or a stress – related disorder? Scientia Horticulturae 90:193-208, 2001.

SAURE, M.C. Calcium translocation to flesh of fruit: its mechanism and endogenous control. Scientia Horticulturae 85:1-25, 2004.

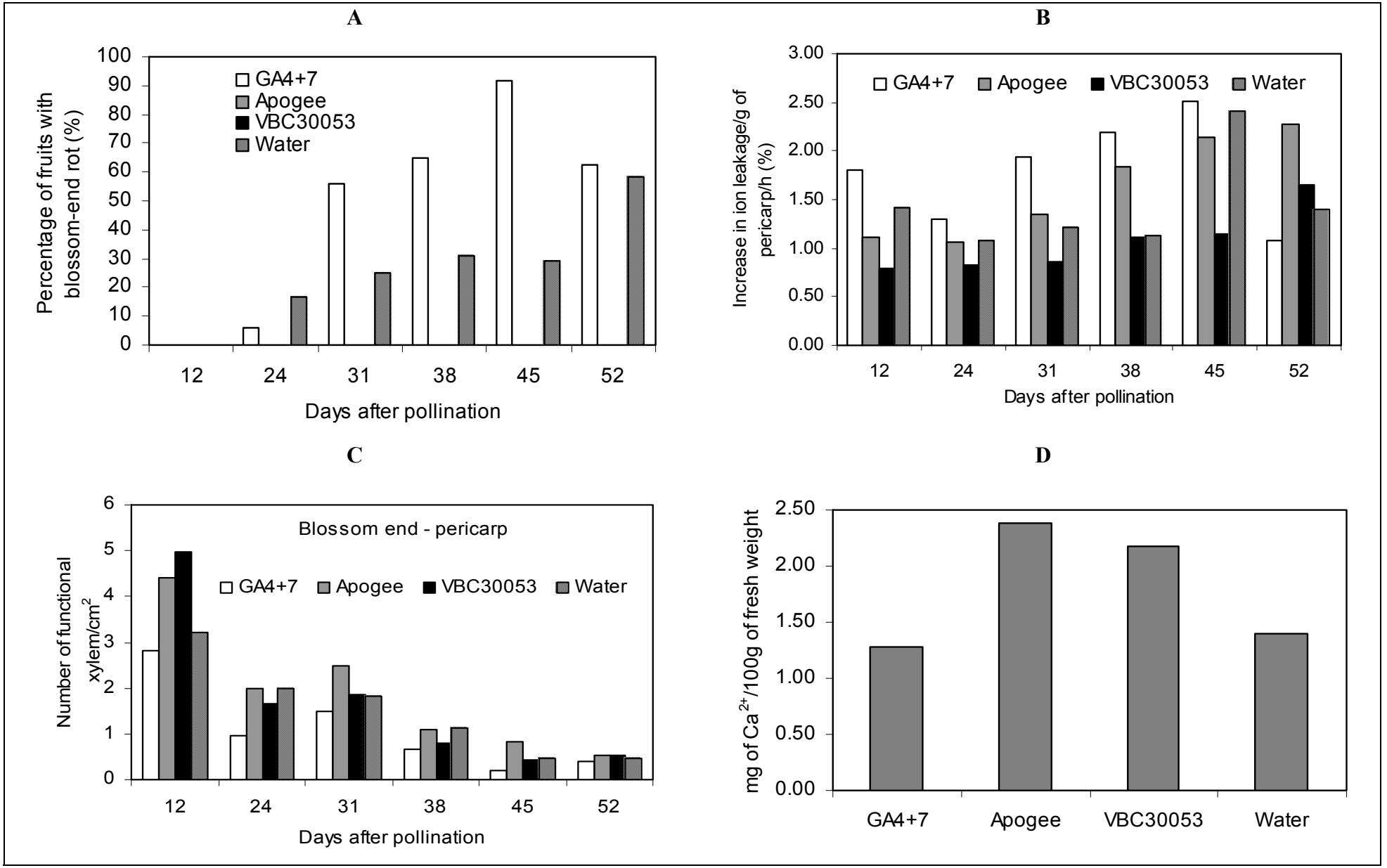

Figure 1. Blossom-end rot incidence (A), ion leakage (B), and functional xylem elements (C and D) obtained in the tomato experiment.

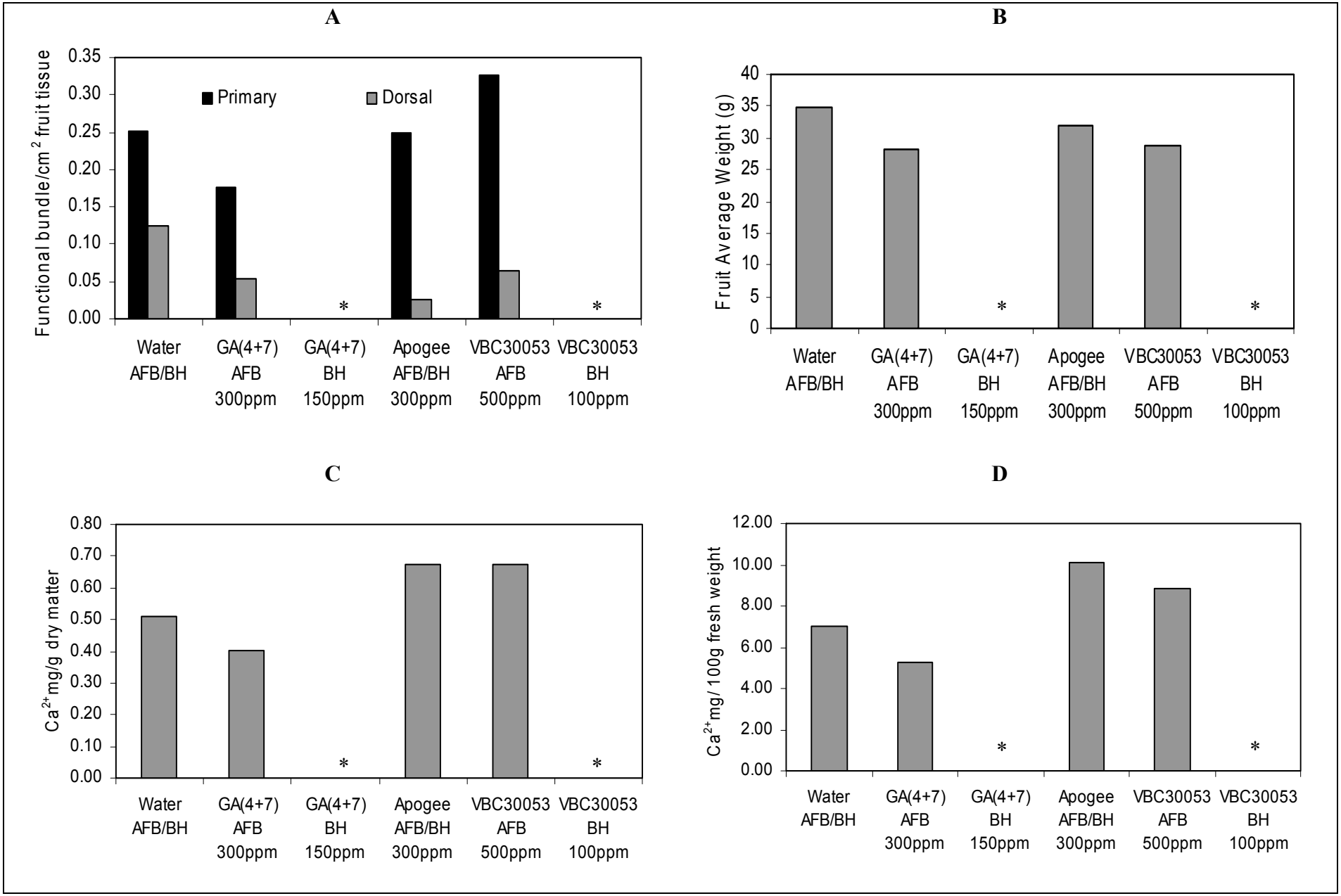

Figure 2. Functional xylem elements (A), avg. fruit weight (B), and calcium content (C and D) of apple fruits 7 wks after full bloom. * = treatments not yet applied.

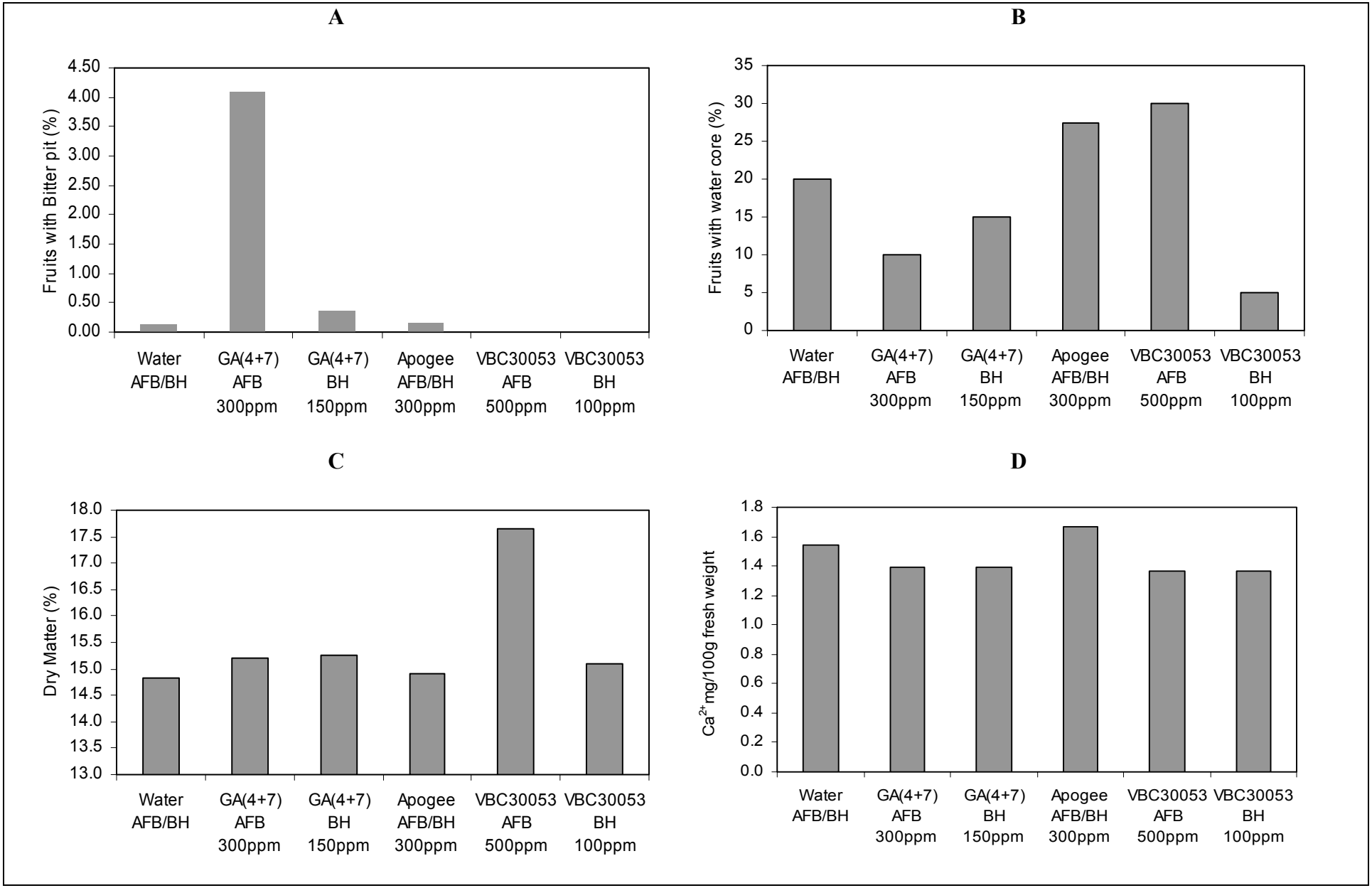

Figure 3. Bitter pit (A) and water core (B) incidence, fruit dry matter (C), and calcium content (D) in apple fruit at harvest.

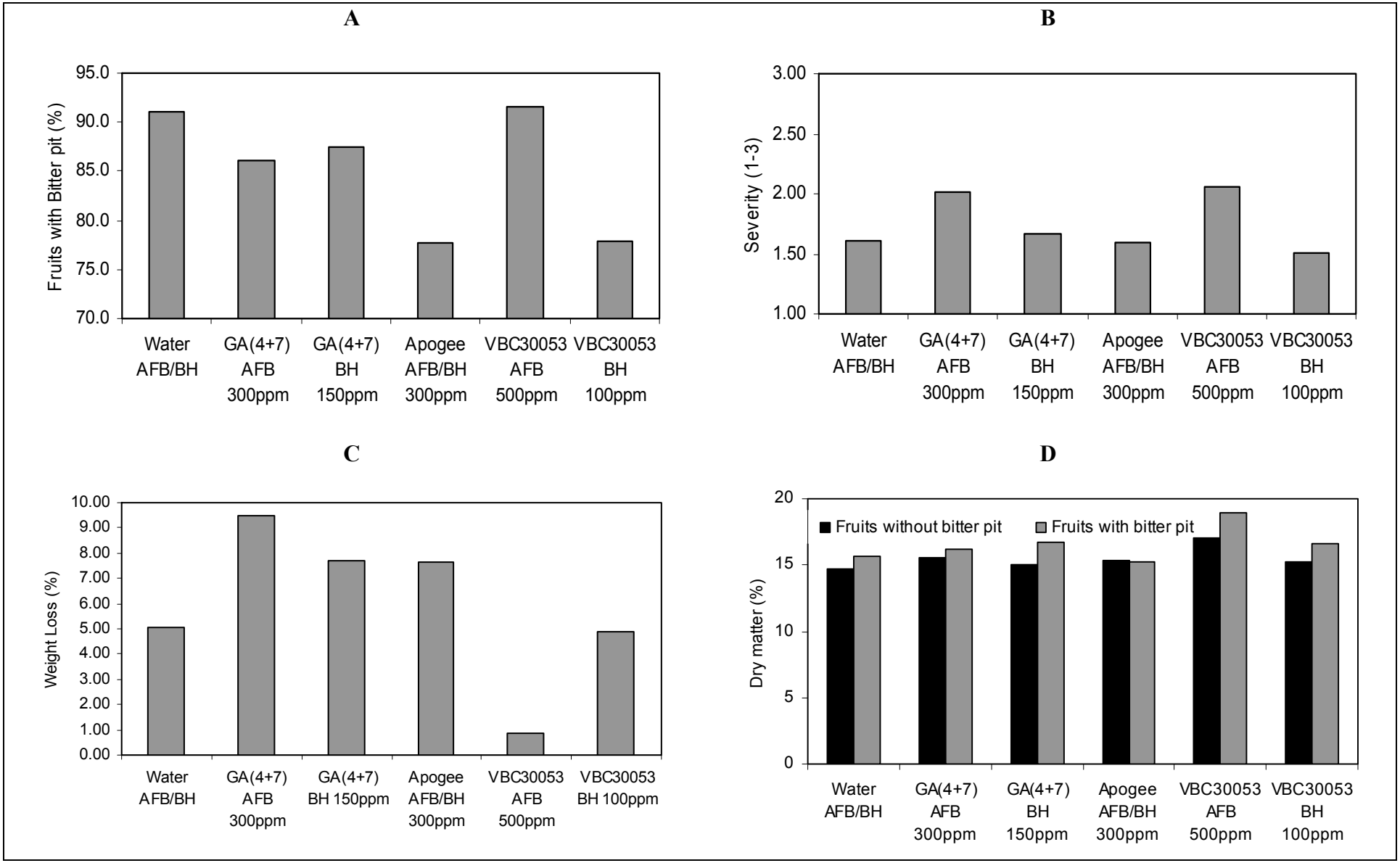

Figure 4. Bitter pit incidence (A) and severity (B), weight loss (C), and dry matter (D) of apple fruits stored for two months at 0ºC (32ºF).

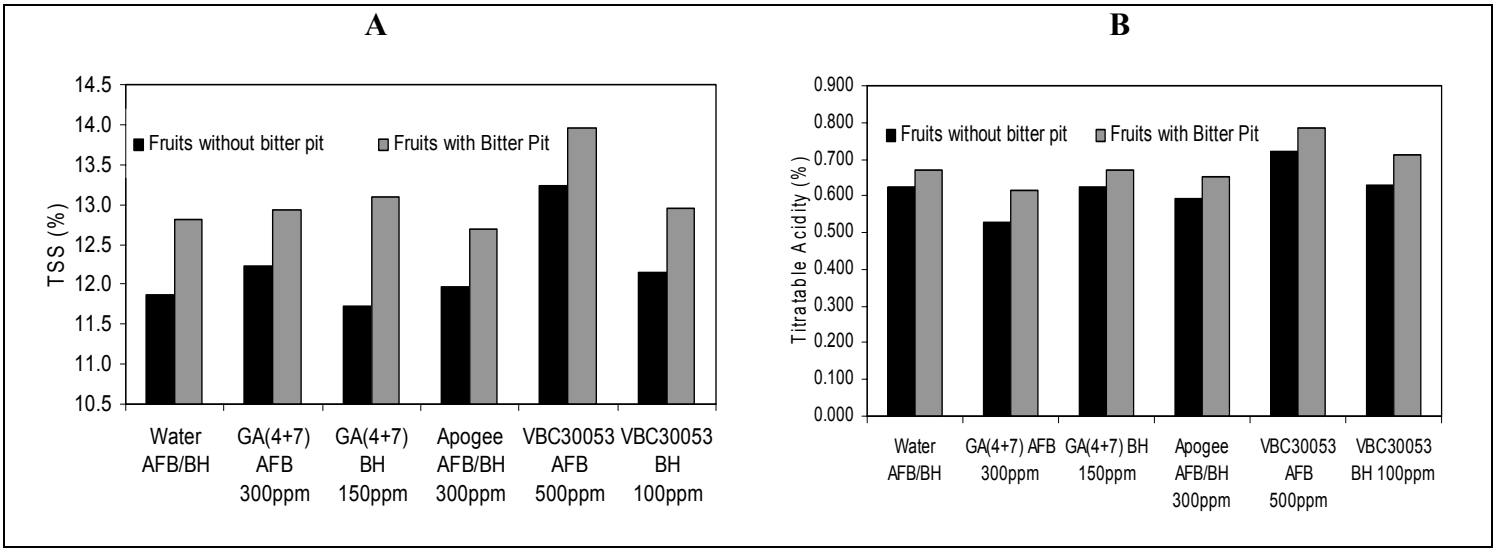

Figure 5. Apple experiment - analysis of apple fruits after two months of cold storage at 0ºC (32ºF).

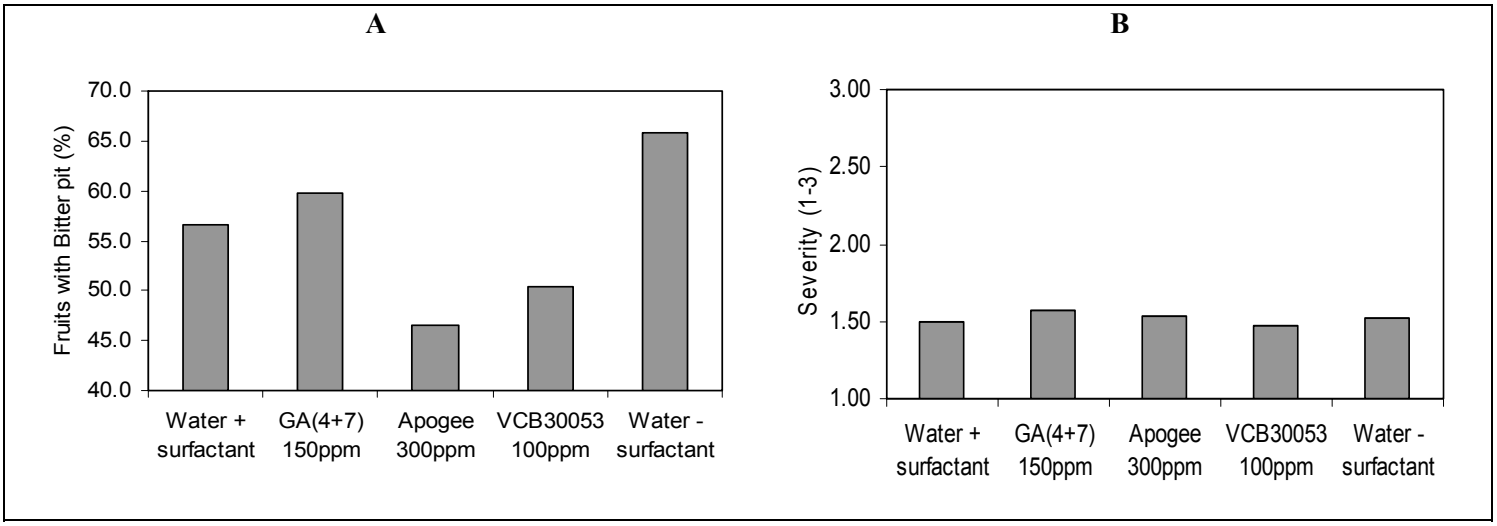

Figure 6. Bitter pit incidence (A) and severity (B) in apple fruits treated after harvest with growth regulators and stored at 0ºC (32ºF) for two months.

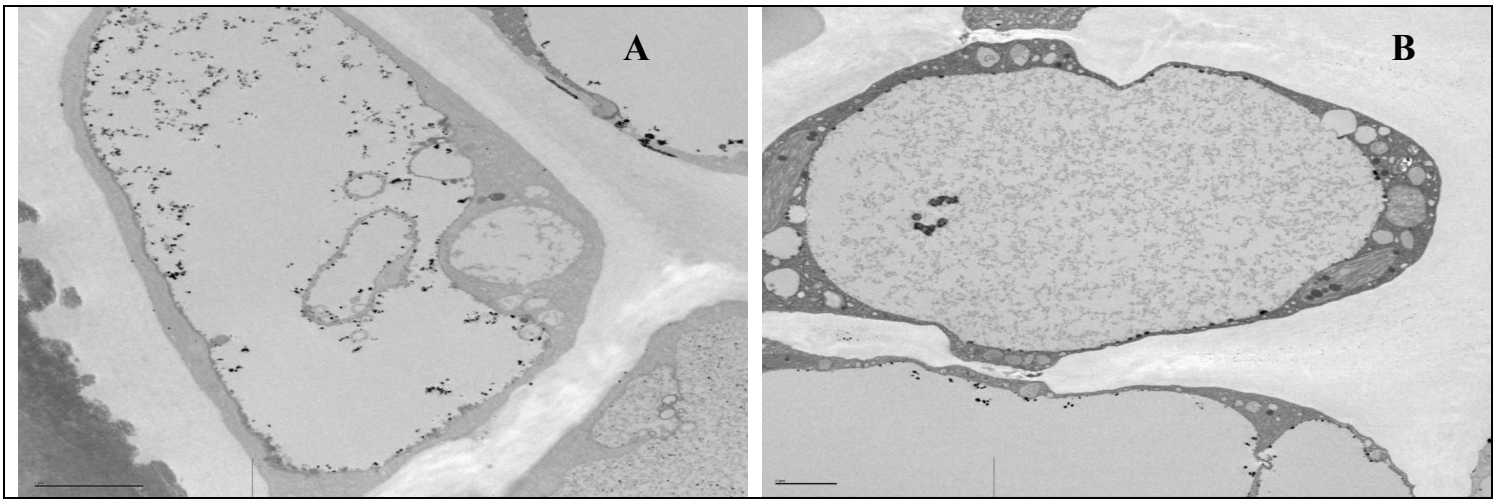

Figure 7. Comparison of bitter pitted fruit (A) and sound fruit (B) cells, looking for calcium localization inside storage organelles (vacuole).