ed pilot plant degreening rooms of this study were <1500 ppm. Using the personal computer control system, levels could be maintained at <850 ppm via energizing the muffin fans added to the degreening room creating a higher fresh air exchange rate. However, this level would be elevated with greater fruit loads. In measuring fan capacity, the differential pressure transducer yielded consistent results when under load by fruit in the degreening room. However, under free air conditions, significant positive to negative fluctuation was noted.

Conclusions

Monitoring and control of citrus degreening was implemented in a pilot plant degreening facility for temperature, relative humidity, airflow and carbon dioxide levels. Discharge temperatures were controlled ± 0.5°C and relative humidity at ± 0.9% RH. This control was accomplished through either dedicated local controllers or through a remote PC-based control system. Principal advantage of the PC approach is the capability for data logging, interfacing to remote locations, alarming and coupling monitoring or control strategies. The system could be extended to multiple rooms typically found in commercial operation. An economic sensor system for ethylene at 0 to 10 ppm was not identified. Carbon dioxide was monitored at < 2000 ppm with an infra-red detection system. For economic viability, a multi-port sampling system would be required for both ethylene and carbon dioxide sensing for packinghouse implementation. Control of such a multi-port configuration is adaptable to the digital I/O capability of PC-control.

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GRANULATION IN GRAPEFRUIT

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Additional index words. Alcohol-insoluble solids, *Citrus paradisi*, fruit size, granulation, juice vesicle.

Abstract. **Temporal studies were conducted from mid- to late-harvest season of 'Ruby Red' and Marsh grapefruit (*Citrus paradisi* Macf.) to evaluate the effect of on- and off-tree storage, fruit size, and juice vesicle position on the development of granulation. Juice vesicle fresh and dry weights were highest at the stem and stylar positions of the fruit section and did not significantly change for fruit remaining on the tree or harvested and stored. Juice vesicles isolated from each position were subjectively evaluated for the presence of granulation. No granulation was observed in juice vesicles of Marsh grapefruit of any size. Granulation was highest in stylar juice vesicles obtained from large Ruby Red fruit (~600 g) that were harvested late in the season (March and May) and stored in air at 21°C for 60 days. Stylar juice vesicles from freshly harvested large Ruby Red fruit in March and May or fruit harvested in January and stored for 60 d had low granulation scores. Thus, Ruby Red fruit remaining on the tree until May, but of the same chronological age as fruit harvested in March and held in storage until May, were less susceptible to the disorder. Alcohol-insoluble solids (AIS), largely composed of pectins and other cell wall materials, significantly increased in juice vesicles that were granulated. The results suggest that storage itself was not responsible for the marked accumulation of AIS measured in granulated juice vesicles. Rather, some interaction of fruit size with maturation, as well as other factors, likely contributed to the development of granulation.**

Florida grapefruit have a lengthy harvest season that typically begins in October and lasts through April or May. In the latter part of the grapefruit harvest season, a physiological disorder known as granulation can appear (Bartholomew et al., 1941). A symptom of granulation is the tough, dry nature of individual juice vesicles within the segment. Grapefruit contain 10-14 sections/fruit, and 154 to 319 juice vesicles are found in each section, depending on variety (Tisserat et al., 1990). Each juice vesicle is composed of large, highly vacuolate parenchyma cells surrounded by a defined hypodermis and overlying epidermis (Burns et al., 1992). Granulated juice vesicles have been reported to be enlarged, tough in texture, discolored and lower in soluble sugars and acids (Bar-

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tholomew et al., 1941; Goto and Araki, 1983; Nakajima, 1976; Sinclair and Jolliffe, 1961).

Anatomical investigations have demonstrated extensive cell wall thickening and, in severe cases, secondary wall formation in parenchyma cells of granulated juice vesicles (Burns and Achor, 1989; Shomer et al., 1989). Respiratory rates of granulated juice vesicles were elevated (Burns, 1990) and accompanied by lowered acid and sugar levels, suggesting that these substrates could be used to support cell wall synthesis. Alcohol-insoluble solids (AIS), composed primarily of pectinaceous cell wall material, accumulated in granulated juice vesicles (Hwang et al., 1990), and this characteristic has been used as an index for granulation (El-Zeftawi, 1978; Sinclair and Jolliffe, 1961).

Conditions that lead to the appearance of granulation are not well-defined. Rootstock, crop load, tree age, rainfall, and grove location have been reported to affect the appearance and development of granulation (Awasthi and Nauriyal, 1972a; 1972b; Bartholomew et al., 1941; El-Zeftawi, 1973; 1978; Noort, 1969), but considerable yearly variation in granulation exists (Hwang et al., 1988), making prediction of the disorder difficult. Fruit size has been reported to be a factor in the development of granulation. Larger fruit are more frequently affected by the disorder, whereas smaller fruit either display less-severe symptoms or show no symptoms of granulation (Bartholomew et al., 1941; Noort, 1969; Sinclair and Jolliffe, 1961). Juice vesicle positional effects have also been reported, where juice vesicles from the stem and/or stylar ends of the fruit section are more frequently affected (Awasthi and Nauriyal, 1972b; Hwang et al., 1988). As the disorder progresses, the entire fruit section can become affected. (Awasthi and Nauriyal, 1972b; Bartholomew et al., 1941; Noort, 1969; Sinclair and Jolliffe, 1961).

Granulation can occur in late-season fruit on the tree, but often fruit free of the disorder at harvest appear to become affected in storage (Hwang et al., 1990). It is unclear whether granulation of grapefruit is a consequence of on-tree fruit age or whether storage causes or in some way enhances the disorder. Under commercial conditions, most fruit are removed from trees in a once-over harvest. This prevents comparisons of affected fruit in storage with fruit remaining on the tree at a specific grove location. The objective of this study was to compare the progression of granulation in Ruby Red and Marsh grapefruit as affected by fruit size, juice vesicle location, and storage on and off the tree. We have used the accumulation of AIS in juice vesicles as a means to compare the progression of granulation.

Materials and Methods

Fruit of 'Ruby Red' and 'Marsh' grapefruit (*Citrus paradisi*) were harvested from trees in the Indian River district of Florida. Trees were between 15 and 25 years of age and were grown on sour orange (*Citrus aurantium* L.) rootstocks. Marsh trees were located in a grove approximately 5 miles west of the Vero Beach city limits, whereas Ruby Red trees were located in a grove approximately 15 miles south of the Marsh grove. Three representative trees of each variety were identified and used throughout the duration of the study. Fruit were harvested approximately 60 d apart in January, March, and May. Fruit were transported to the Citrus Research and Education Center in Lake Alfred, FL, where they were washed and waxed with FMC 360 water-based wax that contained 2000 ppm thiabendazole for decay control. At each harvest date, fruit were either evaluated 24 h after harvest or evaluated after storage for 60 d at 21°C, 95% RH.

Fruit were weighed and three size categories were selected for removal of juice vesicles: small (~350 g), medium (~450 g), and large (~600 g). Six fruit of each size category (two fruit/tree) were chosen at each harvest date and from each storage period. Each fruit was dissected and 25 juice vesicles were removed from the stem, stylar and mid sections of four separate fruit sections. Juice vesicles from two fruit sections were pooled and weighed, dried at 70°C for 24 h, then reweighed to obtain dry weight. Juice vesicles from each of the remaining two fruit sections were individually weighed, homogenized at 4°C, and stored at -15°C until needed.

Each population of juice vesicles was visually evaluated for the presence and severity of granulation after removal from the fruit segment. Granulation was scored as 0, no granulation (juice vesicles turgid); 1, slight granulation (slight loss of color, light-colored granulated area in the center of the juice vesicle); 2, moderate granulation (juice vesicle hardened, moderate loss of color); and 3, severe granulation (hardened juice vesicle, complete loss of color, discolored lignified areas present (Burns and Achor, 1989).

Juice vesicle homogenates (representing 2 sections and 50 juice vesicles) of each fruit were pooled for AIS extraction. Juice vesicle homogenates were thawed and homogenized again with two volumes of 100% EtOH. Precipitated AIS were collected by centrifugation. Solids were resuspended in 10 mL 100% EtOH and then recovered by vacuum filtration. AIS were washed with 100 mL EtOH followed by an acetone wash of equal volume. The resulting solids were dried overnight at room temperature under vacuum and desiccant and then weighed.

Juice vesicle weight data were statistically analyzed using LSD, 5% level. AIS changes were analyzed using Student's t-test, 5% level. Mean granulation scores are presented with their standard deviations.

Results and Discussion

The mean fruit weights were 352, 450, and 605 g for small, medium, and large fruit of both varieties, respectively, with standard deviations less than 5% of the means. Variety, harvest date and storage had no effect on juice vesicle fresh or dry weight at each position and fruit size (data not shown), so data were pooled. Both fresh (Table 1) and dry weight (data not shown) were affected by juice vesicle position and fruit size. Juice vesicles from large fruit had greater weight than

Table 1. Fresh weight of juice vesicles located in the stem, stylar or mid positions of grapefruit sections of three fruit sizes. Fresh weights of juice vesicles isolated from fruit at harvest and storage of both varieties were pooled.

	Juice vesicle position			
	Stem	Mid	Stylar	
Fruit size class	mg FW/jv	mg FW/jv	mg FW/jv	LSD
Small	65.6 a[z]	46.4 b	70.0 a	8.5
Medium	86.0 a	64.2 b	86.6 a	8.4
Large	106.4 a	82.6 b	102.1 a	9.0

[z]Means followed by the same letter within rows are not significantly different, as indicated by LSD, $P = 0.05$.

Table 2. Subjective granulation scores of juice vesicle populations isolated from large Ruby Red grapefruit. Juice vesicles were evaluated at harvest or after 60 days storage at 21°C, 95% RH. Granulation scores range from 0 (no granulation) to 3 (severe granulation). Granulation scores for January harvest and storage in Ruby Red and all scores for Marsh were 0 and therefore not shown. Data are the means ± s.d.

	Harvest Date			
	March		May	
	at harvest	storage	at harvest	storage
Stem	0.2 ± 0.1	0.8 ± 0.4	0.2 ± 0.2	0.5 ± 0.2
Mid	0	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
Stylar	0	2.3 ± 0.3	0.2 ± 0.1	2.5 ± 0.5

those of medium and small fruit sizes. Juice vesicle dry weight was also greater in larger fruit (data not shown). More importantly, juice vesicle fresh weight was affected by position within the segment (Table 1). Juice vesicles isolated from either the stem or stylar positions of fruit sections had similar fresh and dry weights, but those juice vesicles from the mid position had lower fresh and dry weights.

Fruit size and vesicle position affected the development of granulation in Ruby Red grapefruit (Table 2). Juice vesicles of larger fruit were more frequently affected by the disorder, whereas juice vesicles of smaller fruit were devoid of symptoms (data not shown). Marsh grapefruit juice vesicles did not show visual signs of granulation. Total AIS from juice vesicles at each position of on-tree large fruit was not affected by harvest date (Fig. 1). However, a trend of increasing AIS was seen in stylar juice vesicles of on-tree Ruby Red grapefruit from the March to May harvest dates. A slight increase in AIS was observed in stylar juice vesicles between fruit stored for 60 d from the January harvest and those held on the tree and harvested in March. AIS markedly increased in stylar juice vesicles of stored large Ruby Red fruit of March and May harvests when compared to AIS obtained from on-tree fruit. AIS for stylar juice vesicles for fruit harvested in March and stored until May were higher than for those held on the tree until May. AIS levels of granulated and freshly-harvested grapefruit juice vesicles in this study were similar to those previously reported (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961). Because we worked in a commercial grove where fruit were removed by the end of May, fruit were not harvested in July; therefore, AIS from stylar juice vesicles of May-harvested stored fruit could not be compared to on-tree samples.

The high severity of granulation in off-tree-stored large grapefruit harvested late in the season relative to stored fruit from earlier harvests suggests that storage may accelerate processes that lead to granulation. Since storage resulted in only a slight increase of juice vesicle AIS in Ruby Red fruit harvested in January, storage was not the only factor that caused the marked accumulation of AIS associated with granulation. Juice vesicles from Marsh grapefruit did not significantly accumulate AIS, and had low granulation scores, even when stored. In other studies, however, juice vesicles of Marsh grapefruit readily granulated (Hwang, et al., 1988; 1990). This fact demonstrates the unpredictable nature of granulation, and suggests that other factors, such as grove location, soil type, crop load, or environmental conditions may have a more prominent role in the development of the disorder (Awasthi and Nauriyal, 1972a; 1972b; Bartholomew et al., 1941; El-Zeftawi, 1973; 1978; Noort, 1969).

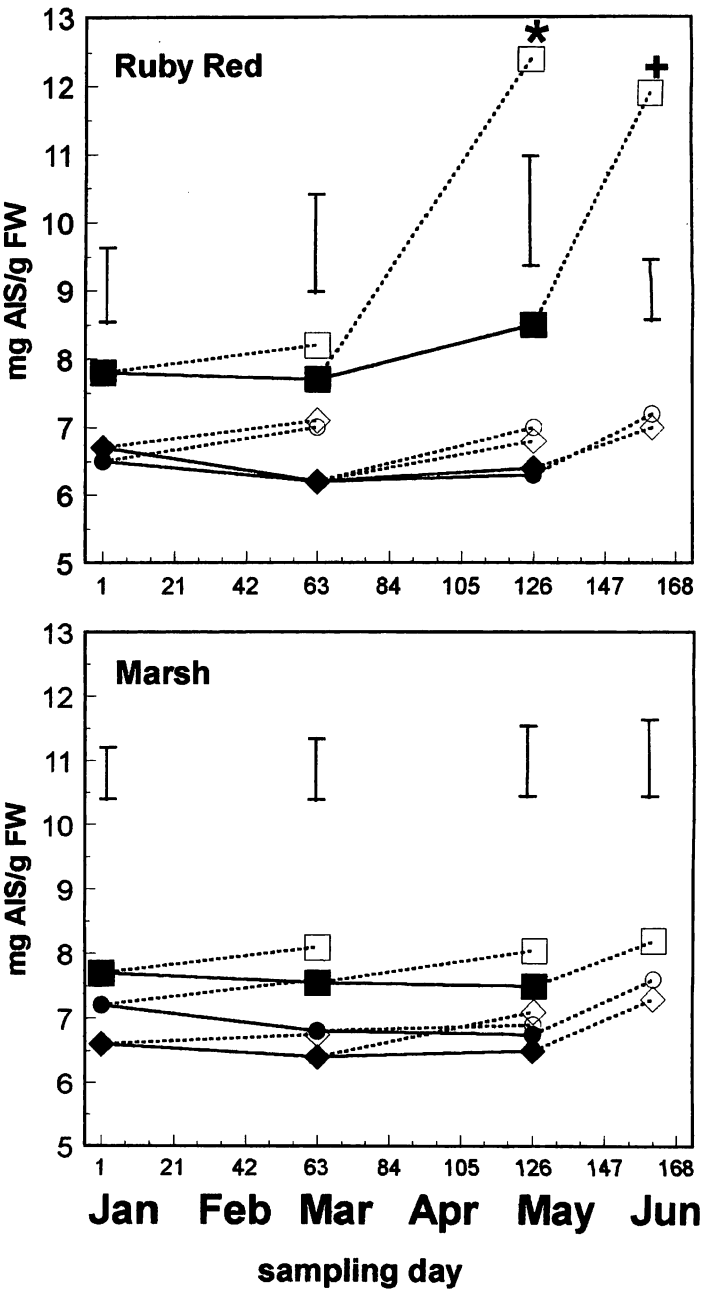

Figure 1. Temporal changes in juice vesicle AIS isolated from large 'Ruby Red' and 'Marsh' grapefruit. Data are the means of stem (◆), mid (●), and stylar (■) juice vesicle positions. Solid lines and closed symbols represent values of juice vesicles isolated from freshly harvested fruit; dashed lines and open symbols represent values of juice vesicles isolated from stored fruit. Bars represent standard error at each sampling day. *, denotes significant difference between stylar juice vesicle AIS from March stored fruit and stylar juice vesicle AIS from March or May harvest; +, denotes significant difference between stylar juice vesicle AIS from May stored fruit and stylar juice vesicle AIS from May harvest; Student's t-test, P = 0.05.

As reported by Bartholomew et al. (1941), Noort (1969) and Sinclair and Jolliffe (1961), AIS in our study did not significantly change in juice vesicles of medium- or small-sized fruit throughout the harvest season or during storage (data not shown). Granulation only occurred in late-season and increased in severity when late-season Ruby Red fruit were stored. In citrus species other than grapefruit, severe on-tree granulation was frequently observed late in the season and

was correlated with AIS accumulation (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961).

Accumulation of AIS was only associated with granulated stylar juice vesicles of stored Ruby Red fruit harvested late in the season (March and May). AIS remained low and granulation was absent in juice vesicles of grapefruit left on the tree. A slight increase was noted at the last harvest date, suggesting that grapefruit left on the tree would continue the process of granulation and AIS accumulation. Subjective granulation scores for large grapefruit closely followed the increased accumulation of alcohol-insoluble solids in juice vesicles (Table 2, Fig. 2). No granulation was found in juice vesicles of small or medium fruit sizes.

When granulation was found in 'Ruby Red' grapefruit, granulated juice vesicles were frequently found next to normal juice vesicles. This pattern has led to the premise that, during granulation, juice vesicles actually swell and increase in fresh and dry weight. In our study, large juice vesicles were the first to accumulate AIS and show the visual symptoms of granulation. The increase in juice vesicle size reported to occur in granulated tissue (Bartholomew et al., 1941; Goto and Araki, 1983; Hwang et al., 1990) most likely reflects the tendency of larger juice vesicles in a heterogeneous population to first undergo granulation as the harvest and storage season progresses.

AIS comprised a larger percentage of stylar juice vesicle dry weight in granulated juice vesicles of March and May Ruby Red harvests (Fig. 2). During granulation, new cell wall material is deposited in juice vesicle cells as indicated by increased amounts of pectin (Bartholomew et al., 1941; El-Zeftawi, 1973; Hwang et al., 1990; Sinclair and Jolliffe, 1961), cellulose, hemicellulose, and lignin (Burns and Achor, 1989; Hwang et al., 1990 Shomer et al., 1989). The reduced soluble solids and acids reported in granulated juice vesicles (Bartholomew et al., 1941; Sinclair and Jolliffe, 1961) suggests that these substrates are being utilized to synthesize new cell wall materials. This new cell wall synthesis occurs despite unchanged total fresh and dry weights throughout the harvest season and storage period.

We have demonstrated that stylar juice vesicles of large Ruby Red grapefruit undergo granulation, but juice vesicles located in the stem region can also granulate (Bartholomew et al., 1941; Hwang et al., 1990). Larger fruit on average may be of a more advanced age, since they typically arise from the terminal portion of the cymose inflorescence and development is initiated up to 2 weeks in advance of the lateral florets (Lord and Eckard, 1985; Schneider, 1968). However, it is unlikely that this age difference significantly contributed to appearance of section drying in large fruit since medium-sized fruit failed to show symptoms of granulation throughout the duration of this study. In addition, we have observed synchronous juice vesicle initiation and growth along the carpel wall of grapefruit sections (Burns et al., 1992), suggesting that significant age differences do not occur and cannot account for the presence of granulation. Rather, granulation in Ruby Red grapefruit is a complex interaction between storage, fruit size and maturation. Furthermore, in Ruby Red grapefruit of advanced maturity, storage off-tree favors development of granulation compared to on-tree storage during the harvest season.

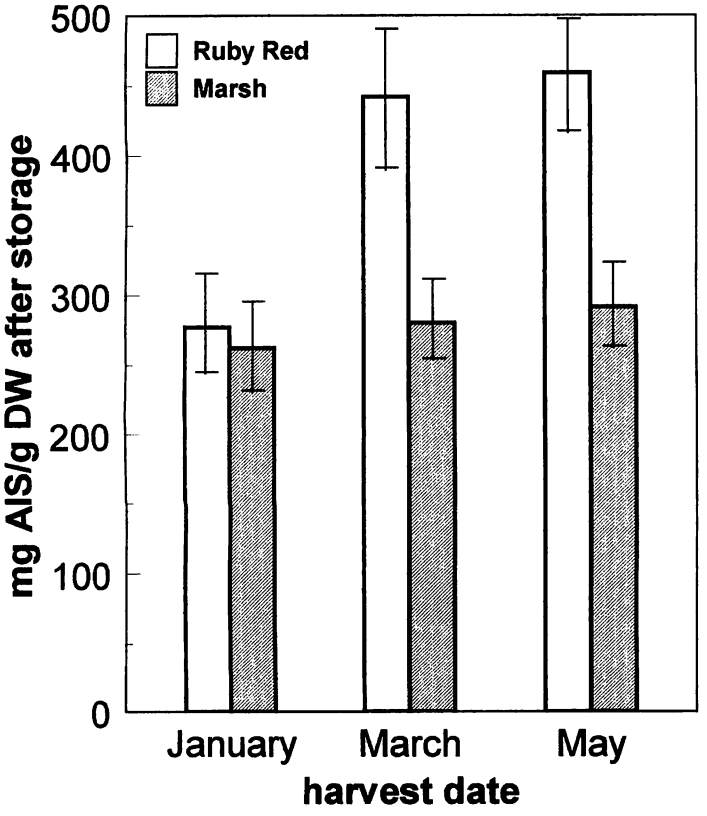

Figure 2. Changes in mg AIS/g DW in stylar juice vesicles of large Ruby Red and Marsh grapefruit after storage at 21°C, 95% RH for 60 d. SE are shown.

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USING BIOSAVE TO REPLACE CHEMICAL FUNGICIDES FOR POSTHARVEST DISEASE CONTROL OF FRUITS

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Abstract. **Three field trials (on grapefruits, apples and sweat cherries) were conducted to compare the efficacy of BioSave products to chemical fungicides for postharvest decay control. BioSave 1000 was tested as a replacement for the 1000 ppm imazalil, normally used as an aqueous treatment for grapefruit (*Citrus paradisi* Macf.) prior to a wax treatment that includes 2000 ppm Thiabendazole (TBZ). Treatment of grapefruit with BioSave 1000 resulted in the same amount of decay rate as did imazalil after six weeks of cold storage or one week at ambient temperature before cold storage. Dip treatment of wounded apples (*Malus domestica* Borkh.) with BioSave 100 provided complete or partial control of blue mold (*Penicillium expansum*) that was as good as or better than label rate (570 ppm) of TBZ. BioSave 1000 also reduced disease incidence of wounded and inoculated sweet cherry fruits (*Prunus avium L.*) at ambient conditions for 6 days.**

The development of fruit pathogen's resistance to fungicides and public concerns over synthetic pesticides in foods and the environment have created an interest in alternative methods of disease control (Eckert et al., 1994; Spotts and Cervantes, 1986; McDonald et al., 1979). In recent years, a proliferation of research has been conducted in an attempt to develop biological products with potential to replace or reduce the use of fungicides for citrus (Brown and Chambers, 1996; Wilson and Chalutz, 1989; Smilanick et al., 1995, 1996), stone fruits (Pusey et al., 1988) and pome fruits (Stack, et al., 1992; Jeffers and Wright, 1994; Janisiewicz and Marchi, 1992; Bull et al., 1996; Janisiewicz, 1987, 1988). BioSave products (EcoScience Corp. Orlando, FL) are registered and in commercial postharvest application for fresh citrus, pome fruits and cherries.

EcoScience Corporation has conducted research and field trials on BioSave isolates (*Pseudomonas syringae)* for about seven years. According to laboratory and field trial results, use of these antagonistic bacteria resulted in decay control equal to the chemical fungicide TBZ for apples and pears in controlled atmosphere storage (Jeffers and Wright, 1994, Stack et. al 1992). The bacteria were also effective for control of postharvest decay caused by *P. digitatum* (green mold) and *P. italicum* (blue mold) on citrus (Yourman and Jeffers, 1994). Some packing houses have requested testing the feasibility of reducing or replacing chemical fungicide for decay control. Therefore, three field trials were conducted on grapefruits, apples, and cherries during the 1996-1997 packing season.

Materials and Methods

Grapefruit

Packing houses requested testing the feasibility of reducing imazalil level for grapefruits. Thus, a field trial was conducted on white grapefruits in a packing house in Florida. The packing house normally treated fruits with 2% sodium ortho-phenylphenate (SOPP), 1000 ppm imazalil in water and 2000 ppm TBZ plus 1000 ppm imazalil in wax. This procedure and their operation method served as standard control for the test. To reduce imazalil dosage, BioSave 1000 (1×10^9 cfu/ml) was applied in place of imazalil in water treatment. Other fungicides used were the same as for the control. Both imazalil and BioSave 1000 were applied by dripping over brushes. Several hours after treatment, ten replicate cartons of fruits (32 fruits/carton) per treatment were randomly taken from packing area. The test was repeated for two days. Samples taken on the first day were kept at ambient conditions for one week, and then, stored at 13°C, 95% RH for five weeks. Samples taken on the second day were continuously stored at 13°C and 95% RH for six weeks. All of the samples were shipped to the laboratory in Orlando for evaluation. Fruits were inspected weekly for decay and decayed fruits were removed from the cartons. Data were analyzed as a two factor factorial using Microsoft Excel.

Apple

The objective of this field trial was to compare the relative efficacy of BioSave 100 to TBZ for decay control of apples.