Schneider, H. 1968. The anatomy of citrus, p 1-85. In: W. Reuther, L. D. Batchelor, and H. J. Webber (eds.). The Citrus Industry. Vol. II. Anatomy, Physiology, Genetics and Reproduction. Univ. California Press, Berkeley, CA.

Shomer, I., E. Chalutz, R. Vasiliver and M. Berman. 1989. Sclerification of juice sacs in pummelo (*Citrus grandis*) fruit. Can. J. Bot. 67:625-632.

Sinclair, W. B. and V. A. Jolliffe. 1961. Chemical changes in the juice vesicles of granulated Valencia oranges. J. Food Sci. 26:276-282.

Tisserat, B., D. Jones and P. D. Galletta. 1990. Juice vesicle populations in citrus fruit. Bot. Gaz. 151:64-72.

*Proc. Fla. State Hort. Soc.* 110:208-211. 1997.

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

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*Additional index words.* *Citrus paradisi* Macf., *Malus domestica* Borkh., *Prunus avium L.*

*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 ($1 \times 10^9$ 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.

The field trial was conducted in three packing houses in Washington State in January 1997. Packing house A and B had Red Delicious apples and packing house C had Golden Delicious. Three treatments were applied, 1), water control, 2), TBZ (570 ppm) and 3), BioSave 1000 ($1 \times 10^8$ cfu/ml). Each treatment had three or four replicates, varied by packing houses. Each replicate had ten fruits.

Apples were removed from CA storage. Each fruit received one artificial wound (3 x 4 mm), allowing for natural inoculation by the indigenous microbial population. Treatment suspensions were prepared with water in a pre-size drench tank which contained considerable debris. The treatment suspension was prepared in a 5 gallon pail and was applied by dipping the wounded fruits for 15 seconds. After air drying for a few minutes, ten fruits of each replicate were placed on packing trays. Three or four trays were placed in one carton. Treated apples were shipped to Orlando via UPS and were stored at 13°C for two weeks. Disease incidence was recorded and data obtained from each packing house were analyzed as single factor with Microsoft Excel. Means were separated by Duncan's multiple range test, at the 1% level.

*Cherry*

A field trial was conducted to evaluate decay control of sweet cherries by BioSave 1000. Fresh-picked sweet cherries (Rainier) were randomly taken from packing line in Washington. Each fruit received one wound (1 mm in diameter and 3 mm in depth). The wounded fruits were inoculated by dipping in an aqueous suspension of *Penicillium expansum* (3000 spore/ml) for about one minute. After inoculation, fruits were either sprayed with water or with label rate BioSave 1000 ($1 \times 10^9$ cfu/ml). Water and BioSave were applied through a full-cone pattern, non-recovery spray over the conveyor belt carrying the fruit. Contact time was about one second.

Treated fruits were packed in boxes and kept at room temperature. Two boxes of fruits (40 fruits per box) per treatment were sampled and evaluated for disease incidence after three and six days. Data were analyzed as a factorial with Microsoft Excel and means were separated by Duncan's multiple range test at 1% level.

## Results and Discussion

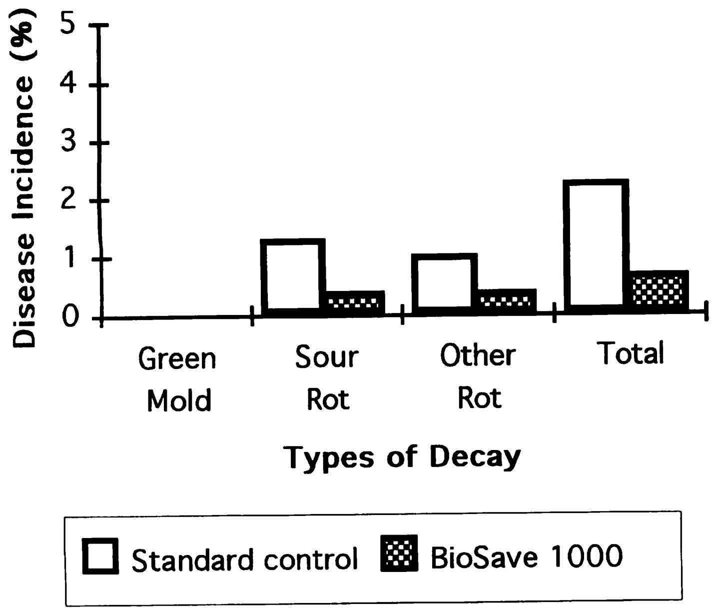
*Grapefruit*

Decay rate of grapefruits stored for six weeks was very low in both standard control and in the BioSave 1000 treatment. Standard control resulted in 0.31% and 0% green mold decay when the fruits were stored at 13°C for six weeks continuously or one week at ambient condition before cold storage, respectively. Replacing 1000 ppm imazalil in water with BioSave 1000 completely controlled green mold development in both storage conditions (Fig. 1 and 2). This complete control of green mold in the BioSave 1000 treatment might have been achieved by the combination of chemical and biological agents because BioSave 1000 is effective for the control of resistant strains. In laboratory tests, BioSave 1000 showed 80-100% protection against a TBZ and OPP resistant strain of *Penicillium digitatum* on grapefruits. Sour rot and other rot caused slightly more decay than green mold, however, the difference in cumulative total disease incidence between the two treatments was not statistically significant.

This field trial result indicates that the 1000 ppm imazalil in water treatment can be replaced by BioSave 1000.

*Apple*

After two weeks of storage at 13°C and 95% RH, disease incidence of apples in the water control was different among the three packing houses. It was 17.8%, 42.5% or 100% in packing house A, B and C, respectively. This variation is more likely caused by pathogen population in different packing houses. The majority of decay was blue mold caused by *Penicillium expansum.* BioSave 100 and TBZ treatments significantly reduced disease incidence at all of the three packing



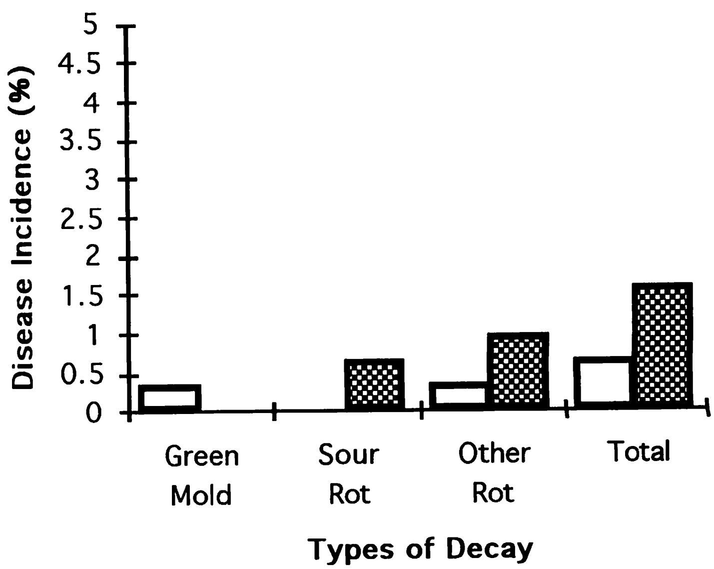




Figure 1 (left) and Figure 2 (right). Cumulative disease incidence of grapefruits treated with the standard packing house method of imazalil in water or BioSave 1000. The fruits were kept at ambient conditions for one week and then at 13°C, 95% RH for five weeks.

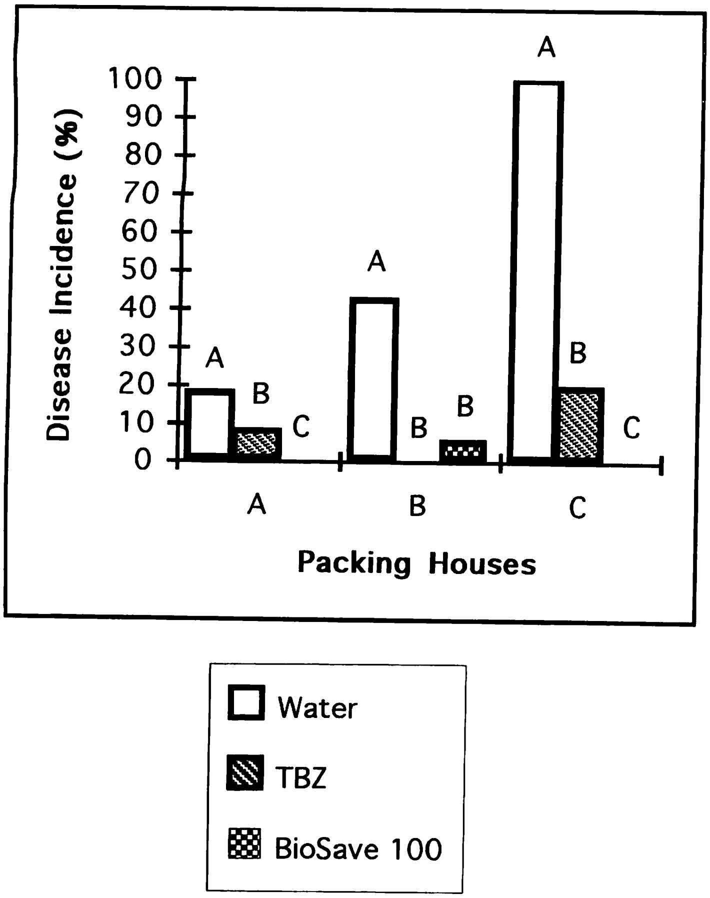



Figure 3. Disease incidence of apples treated with TBZ, BioSave 100 or water in three packing houses in Washington. Mean separation within packing houses by Duncan's multiple range test, 1% level.

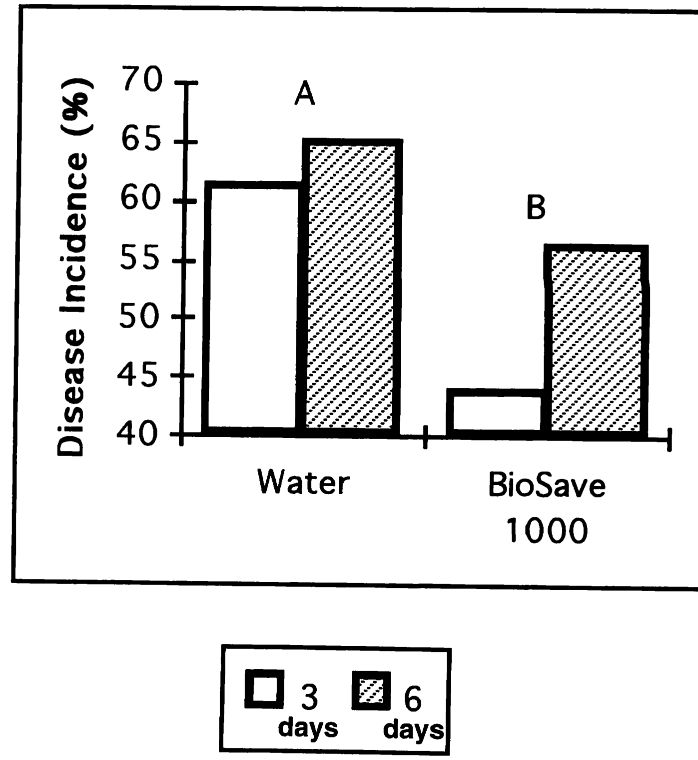



Figure 4. Effect of BioSave 1000 on disease control of inoculated cherry fruits kept at room temperature for 3 and 6 days. The main effect means of water and BioSave 1000 treatment were statistically different (Duncan's multiple range test, 1% level).

houses (Fig. 3). BioSave 100 resulted in a lower disease incidence than that of label rate TBZ treatment in packing houses A and C. In packing house B, BioSave 100 and TBZ were equally effective.

In a pre-sort drench, the water appears to harbor pathogens and apples can be inoculated in the tank. In this field trial, disease incidence of water control was significantly higher than using a decay control agent. In wounded fruits, the incidence was as high as 100% in Golden Delicious apples. In addition, when TBZ is regularly used, resistant strains can be readily found in the drench water and throughout the packing house. The results may be that the packing houses can not obtain the expected decay control. In the laboratory tests, BioSave provided protection against resistant strains on apples and citrus (unpublished data). In this field trial, BioSave treatment had lower decay than that of the TBZ treatment in two of the three packing houses. This might be because BioSave 100 effectively controlled resistant strains. In addition, disposing of TBZ treated water may become an environmental problem, especially during winter when the ground is frozen. Therefore, BioSave is a good alternative to TBZ for apples.

*Cherry*

Cherries were inoculated with *Penicillium expansum* and then treated with BioSave 1000 or water. Disease incidence was recorded after the fruits were kept at room temperature for three and six days. Disease incidence of the water control was 61% on day 3 and 65% on day 6 (Fig. 4). Disease incidence was 44% on day 3 and 56% on day 6 for BioSave 1000-treated fruits. The difference between the two treatments was statistically significant (P = 1%). Interaction of storage time and treatment was not significant.

Until 1996, Rovral, a chemical fungicide, was approved and commonly used for postharvest decay control of cherries. The postharvest use for stone fruits was discontinued to reduce the average daily intake of Rovral residue. In response to customers request, BioSave 1000 was tested during 1996 cherry season in several experiments. BioSave 1000 showed moderate activity against postharvest decay in cherries. Further research work is required to determine the decay control efficacy of BioSave 1000 on cherries.

## Literature Cited

Brown, G. E. and M. Chambers. 1996. Evaluation of biological products for the control of postharvest disease of Florida citrus. Proc. Fla. State Hort. Soc. 109:278-282.

Bull, C.T., J. P. Stack and J. L. Smilanick. 1996. *Pseudomonas syringae PV. syringae* ESC-10 and ESC-11 survive in wounds on citrus and control green mold of citrus. Biological Control: In preparation.

Eckert, J. W., J. R. Sievert and M. Ratnayake. 1994. Reduction of imazalil effectiveness against citrus green mold in California packing houses by resistant biotypes of *Penicillium digitatum.* Plant Dis. 78:971-974.

Janisiewicz, W. J. 1987. Postharvest biological control of blue mold on apples. Phytopathology. 77:481-485.

Janisiewicz, W. J. 1988. Biological control of postharvest diseases of apples with antagonist mixture. Phytopathology. 78:194-198.

Janisiewicz, W. J. and A. Marchi. 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae.* Plant Dis. 76:555-560.

Jeffers, S. N. and T. S. Wright. 1994. Biological control of postharvest diseases of apples: progress and commercial potential. New England Fruit Meeting. 100:100-106.
McDonald, R. E., L. A. Risse and B. M. Hillebrand. 1979. Resistance to Thiabendazole and benomyl of *Penicillium digitatum* and *P. italicum* isolated from citrus fruit from several countries. J. Amer. Soc. Hort. Sci. 104:333-335.
Pusey, P. L., M. W. Hotchkiss, H. T. Dullmage, R. A. Baumgardner, E. I. Zehr, C. C. Reilly and C. L. Wilson. 1988. Pilot tests for commercial production and application of *Bacillus subtilis* (B-3) for postharvest control of peach brown rot. Plant Dis. 72:622-626.
Smilanick, J. L., C. C. Gouin-Behe, D. A. Margosan, C. T. Bull and B. E. Mackey. 1996. Virulence on citrus of *Pseudomonas syringae* strains that control postharvest green mold of citrus fruit. Plant Dis. 80:1123-1128.
Smilanick, J. L., D. A., Margosan, D. McDowell and M. Wadsworth. 1995. Biocontrol agents for postharvest decay of citrus. Citrograph 80:12-14.
Spotts, R. A. and L. A. Cervantes. 1986. Populations, pathogenicity, and benomyl resistance of *Botrytis spp.*, *Penicillium spp.*, and *Mucor piriformis* in packinghouses. Plant Dis. 70:106-108.
Stack, J. P., T. S. Wright, S. N. Jeffers, D. Cooley and W. Brameledge. 1992. Biocontrol of blue mold of apples under common and controlled atmosphere storage. Phytopathology 82:1063.
Wilson, C. L. and E. Chalutz. 1989. Potential for biological control of Penicillium rots of citrus with antagonistic yeasts and bacteria. Sci. Hortic. Amsterdam 40:105-112.
Yourman, L. F. and S. N. Jeffers. 1994. Biological control of postharvest disease of citrus with ESC-10 and ESC-11. (Abstr.). Phytopathology 84:1152.

*Proc. Fla. State Hort. Soc.* 110:211-214. 1997.

# A POSTHARVEST PITTING OF TEMPLE ORANGES STIMULATED BY HIGH TEMPERATURE STORAGE AND WAX APPLICATION

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*Additional index words.* Citrus peel disorders, gas analysis, anaerobic respiration, *Citrus sinensis, Citrus reticulata.*

*Abstract.* **The morphology and etiology of a postharvest peel disorder of Temple oranges resulting from an apparent collapse of oil glands were determined. The disorder was stimulated by high temperature storage (21°C) and wax application. Symptoms were less pronounced in fruit coated with waxes that resulted in higher internal $O_2$ levels or lower internal $CO_2$, ethanol, or acetaldehyde levels. This disorder is similar to one observed in white grapefruit, Fallglo tangerine, and navel orange, but the severity is less pronounced in Temples.**

Temple oranges (*Citrus reticulata* Blanco × *C. sinensis* L.) are probable tangors that are marketed as oranges. They were introduced from Jamaica to Florida in 1896 after the freezes of the 1895-96 season and were publicly released in 1919 (Jackson, 1991). While Temples are noted for their high internal quality, production and handling problems have, in part, limited their acceptance by the citrus industry. Among postharvest handling problems is their hypersensitivity to ethylene at degreening, resulting in a blackening of the peel (Grierson and Newhall, 1953) and stem end rot (Grierson and Newhall, 1955). Delayed handling and degreening under low humidity have been shown to aggravate the effect of ethylene (McCornack, 1972). While the effects of controlled or modified atmospheres on peel disorders have not been fully documented, Chace (1969) noted that controlled atmosphere storage improved the flavor of Temples. Moreover, the influence of the currently-used water wax formulations on the storage of Temples has not been previously examined.

"Postharvest pitting" of citrus peel is a disorder characterized by the collapse of oil glands that occurs during the early weeks of storage (Petracek et al., 1995; Petracek and Davis, 1996; Petracek et al., 1997; Petracek et al., 1998). Wax application and high temperature storage trigger pitting, reduce internal $O_2$ levels, and increase internal $CO_2$ levels. Fruit stored in low $O_2$ (4%) develop pits thus suggesting that pitting is a symptom of hypoxia (Petracek, unpublished).

Postharvest pitting has been studied in white grapefruit, California navel oranges, and Fallglo tangerines (Petracek et al., 1997). Our interest in examining postharvest pitting of Temples came from the observation that Fallglo, a variety with Temple parentage (Bower citrus hybrid [*Citrus reticulata* Blanco × (*C. reticulata* Blanco × *C. paradisi* Macf.)] × Temple [*C. reticulata* Blanco × *C. sinensis* L.]), are exceedingly susceptible to pitting with up to 96% of waxed fruit stored three weeks at 21°C showing symptoms of the disorder (Petracek et al., 1998). Thus, we decided to determine if (1) the reportedly poor handling characteristics of Temples are expressed as a susceptibility to postharvest pitting and (2) Temples would be a suitable model for further study of pitting.

## Materials and Methods

*Plant material.* Mature Temples for the first and second experiments were harvested from commercial groves in Polk County, Fla. on 13 Jan. and 3 Feb. 1997, respectively, and were transported to the Citrus Research and Education Center (CREC), Lake Alfred, Fla, stored overnight at 21 ± 1°C and 93 ± 2% R.H., and packed the next day. Mature fruit for the third experiment were harvested from the teaching block at the