

**Citrus Bio-Assay**  
Pink Grapefruit

Date Started: 11-05-01  
Date Completed: 11-27-01

Pink Grapefruits purchased from Publix on 11/05/01

Treatments

Bio-Save 10 LP Only  
0.5% Sodium Bicarbonate Only  
Bio-Save 10 LP and 0.5% bicarbonate  
Pathogen; italicum/digitatum mix (  $5 \times 10^5$  cfu/ml) pathogen used TBZ sensitive

13 fruit per treatment, 2 wounds per fruit  
Grapefruit were wounded at 3 pm on 11-05-01  
Treatments were applied at 11 am on 11-06-01 by a 10-second dip (20 hours)  
Fruit stored in plastic boxes at 13° C. Paper towels are wet with sterile water and placed in the boxes to keep the environment humid.

**Original Suspensions (11-05-06)**

Bio-Save 10 LP only	$1.90 \times 10^8$
Bio-Save with 0.5 % bicarbonate	$1.40 \times 10^8$
[ Suspensions recommended at $1 \times 10^9$ ]	

**Bio-Save Viability in Wounds (11-19-01)**

Bio-Save 10 LP Only	$2.90 \times 10^7$
Bio-Save 10 LP with 0.5% bicarbonate	$1.56 \times 10^7$
[Recommended Level $1 \times 10^5$ ]	

## PROCEDURES

All the grapefruit were washed, rinsed and dried.

The boxes and wounding tool were washed, wiped with ethanol and dried.

### Bio-Save 10 LP only suspension (200ml)

$\frac{(1 \times 10^9) \times 200 \text{ ml}}{\text{viability of 10 LP used}} = \text{grams 10 LP} / 200 \text{ ml sterile H}_2\text{O}$

(200 ml minus the amount of Bio-Save added to leave total of 200ml of suspension)

### 0.5% Sodium Bicarbonate (200ml)

Add 1 gram of baking soda to 200 ml (199 ml) of sterile water

### 10 LP + 0.5% Bicarbonate (200ml)

$\frac{(1 \times 10^9) \times 200 \text{ ml}}{\text{viability of 10 LP used}} = \text{grams 10 LP} + 1 \text{ gram of Sodium Bicarbonate} / 200 \text{ ml sterile water}$

### Pathogen

The pathogen was grown on a PDA plate at 28° C for about one week. The conidia were scraped off the plate with 0.1% Triton solution and diluted in sterile water. The suspension was set to 0.1 A at 425 nm using a spectrophotometer. Then a 1:3 dilution was performed to leave approximately a  $5 \times 10^5$  cfu / ml suspension.

The wounds were made with a 1mm wide by 3mm deep wounding tool that was dipped into the pathogen suspension before each wound was made. The grapefruit were left out at room temperature to dry for 20 hours before being treated with the Bio-Save and the sodium bicarbonate treatments. The fruit were dipped one at a time in the treatment suspensions for about 10 seconds each. They were left in the boxes to dry and were placed into the 13° C refrigerator for 1 week. After 1 week two wounds were removed from both the Bio-Save 10 LP only and the Bio-Save with 0.5% bicarbonate treatments to test the cfu / wound. The test was ended after 22 days and each lesion was counted and measured.

## Results and Discussion

Taken 11-27-01 (22 days storage)

Treatment	#lesions/wound	Avg. Size (mm)	% decay
Bio-Save 10 LP	3 / 25	70	12
Bio-Save w/ 0.5%	6 / 25	80	24
0.5% bicarbonate	7 / 25	80	28
Pathogen Only	16 / 25	85	64

Although the original suspensions were lower than the recommended level the cfu per wound was adequate to provide good decay protection from the blue/green mold pathogen. The Bio-Save only treatment performed the best in this experiment with the Bio-Save with 0.5% bicarbonate also providing good protection. The Bio-Save only treatment not only reduced the decay by 52% when compared to the pathogen only sample but also reduced the severity ( size measured in mm ) of the decay. With the bicarbonate added to the Bio-Save the decay was reduced by 40% and again the severity was also reduced. The paper towels in one container of fruit with the pathogen only treatment dried out, the low humidity could account for the lower percentage of decay in that sample.