IFAS Citrus Initiative
Annual Research and Extension Progress Report 2008-09
Mechanical Harvesting and Abscission

Investigator:

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Objective(s) Pursued (Priority Topics):

This work evaluated the processor concern area of Food Safety and Sand Accumulation on mechanically harvested oranges. Specific objectives included

(1) Evaluate the microbiology of the surface and juice of citrus fruit collected by various mechanical harvesting systems including: OXBO pick up (if available), catch frames and with and without the application of CMNP.

(2) Evaluate the amount of sand present on the surface of citrus fruit collected by the OXBO pick up system (if available).

Detailed Accomplishments in 2008-09:

For Florida to effectively compete in the world citrus industry, significant reductions in harvesting costs will be necessary. Mechanical harvesting (MH) can be thought of a two-step process: (i) removal of fruit from the tree and (ii) collection of fruit (immediately, by a catch-frame device or during retrieval of fruit from the ground). Additionally, an abscission agent (CMNP for citrus) can be applied prior to mechanical harvest to decrease the force necessary to remove fruit from the tree. This study evaluates the microbiological aspects of mechanically-handled fruit with respect to fruit surface microflora (Objective 1) and corresponding fruit juice microflora (Objective 2).

six replicates of mechanical harvesting were performed through the 2008/2009 harvest season, including catch frame (OXBO 3210 pull-behind harvester), Self propelled continuous canopy shaker (OXBO 3220), and CMNP application for Hamlin and Valencia varieties. For each replicate, treatments groups included: (A) catch frame: (i) hand-harvested fruit (control); (ii) ground fruit (picked up directly from ground following canopy shaking); and (iii) mechanically-harvested (MH) fruit (collected from the goat following collection on a catch frame); (B) continuous canopy shaker:. (i) hand-harvested fruit (control); and(ii) ground fruit (picked up directly from ground following canopy shaking); and (C) CMNP application: (i) hand-harvested fruit (control); and (ii) ground fruit (picked up directly from ground following canopy shaking), with and without CMNP application. Within each group of fruit 30 oranges were collected, and 25 non-defective fruit were randomly selected from each group for analysis.

Thirty milliliters of buffer were added and the rub/shake/rub technique was sued to remove microorganisms from the fruit surface. Microbial analysis included total aerobic plate count (APC) on plate count agar (PCA), and acidophilic organisms count (AOC) on orange serum agar (OSA). Results are reported in Table 1 as colony forming units (CFU) per orange.

As an indicator of potential contamination, generic *Escherichia coli* and *Salmonella* testing was also done on pooled samples of 5 oranges (five pooled samples per test

group). Pooled samples, rather than individual oranges were used due to the expense and time required to run these tests. The VIP *Salmonella* test kit (BioControl) and E*Colite ™ (Charm Sciences) were used to test for *Salmonella* and *E. coli* respectively. *Alicyclobacillus* testing will be done by heat shocking the pooled sample for 10 min at 85° then plating onto Ali Agar (AA). Isolates will be identified by rDNA sequencing and analysis with NCI-BLAST. Results are reported as presence or absence of these organisms in Table 2.

Following testing the fruit surface, all samples were stored at 4°C for 18 ± 2 h. Oranges were then placed in 85°C water for 2 min to sterilize the surface of the fruit and juiced by hand through cheese cloth into a sterile container. Parallel microbial testing was done for juice samples and is reported as CFU/ml juice in Table 3 and as presence or absence of *E. coli* and *Salmonella* in Table 4.

In general control fruit had fewer microbes on the surface of the fruit when compared to ground and MH fruit from both harvesting methods, however no real trends can be attributed to harvest method for all runs and CMNP application did not seem to influence microbial levels (Table 1). Significantly lower APC counts for control fruits are often expected as these fruits were not in contact with the soil surface, the source of many microorganisms on agricultural products. However, this was not true for all trials in 2008/2009, and is consistent with results reported in previous years. CMNP application may have an influence on total microflora levels if the quality of water used for the application is low. This is not what was observed here. This result suggests that dropping fruit to the ground and picking it up mechanically or dropping it into a catch frame does not necessarily result in higher microbial loads. Moisture, other environmental variables, such as soil type and cover crop, or general grove maintance may ultimately be very important in total number microorganisms that adhere to the dropped fruit.

Results of the AOC analysis follow the same general trend as those for APC (Table 1). In general, there are no significant differences among the treatment groups. Many factors contribute to the surface microflora of a raw agricultural product. These include production practices, natural ecology of the fruit/microorganism system, equipment sanitation, geography and climate, and hygiene of harvest and packinghouse personnel. All of these factors may have impacted the results obtained from this study.

E. coli was detected a significant number of pooled samples that came into contact with the catch frame (Table 2). High isolation frequencies of *E. coli* for the catch frame samples indicates a potential for cross contamination from these surfaces and highlights the need for adequate cleaning and sanitation of the machinery. No *Salmonella* was detected this year, as opposed to previous harvest seasons where at least one *Salmonella* isolate was identified. Any fruit in contact with soil has the potential to become contaminated. Soil, other organic materials present on the orchard floor, and machines are potential sources of both *E. coli* and *Salmonella* contamination of fruit surfaces. Alicyclobacillus was only isolated from the first Hamlin run, when a significant amount of moisture (rainfall and irrigation) was present, indicating the potential for transfer under wet conditions.

In all cases, juice samples contained significantly less microflora than the corresponding fruit, often times being at or below the limit of detection (Table 3). For both APC and AOC in fruit juice, no real trends can be attributed to the harvest method.

The interior of sound fruits harbor few microbes as is apparent by these results. The indicator organism *E. coli* and *Salmonella* were not detected in any of the juice samples, despite the presence of these organisms on the fruit surface in some samples (Table 4).

No indication that fruit which come in contact with the ground, or catch frame machinery are consistently and significantly higher in surface or corresponding juice microflora is indicated by the results of the six trials run during 2008/2009. While generic *E. coli* are not considered foodborne pathogens, their presence can be indicative of fecal contamination from warm-blooded animals and the high isolation frequency from CF harvest indicate a potential for cross contamination, and a need to clean this equipment during harvesting.

Areas where progress exceeded expectations:

Due to an enhanced working relationship with collaborators, a total of 6 harvest dates were evaluated for the 2008-2009 harvest year, which significantly exceeded the number of harvest dates surveyed in previous years. Additionally this year, identical samples were taken for both Valencia and Hamlin varieties, allowing for direct comparison between the two. The addition of *Alicyclobacillus* testing allowed us to respond to specific concerns from industry members.

Identification of *E. coli* from multiple catch-frame samples, was observed again this year and continues to highlight the need for developing a cleaning and sanitizing protocol for this equipment.

Areas where progress didn't meet expectations:

No OXBO pick up machine samples were collected this year, and quality testing in terms of Brix, Ratio and color were not done.

Impact of accomplishments towards overall goals of funding:

There is practical importance to the surface microflora of oranges delivered to the processor. Contamination of raw materials is listed as the second most serious food safety problem in the food processing industry, after deficiencies in employee training. However, incoming fruit to citrus processing plants is typically washed and sanitized, and the vast majority (>98%) of Florida-processed orange juice is pasteurized or similarly treated to inactivate spoilage enzymes and to microbiologically stabilize the product. Wider adoption of mechanical harvest/pick up systems will be somewhat determined by the quality of fruit delivered to the processor. This quality includes potential microbiological contamination as well as the typical measures of machine yield and efficiency, and economics. For these reasons, it is important to continue to collect fruit and juice microbiological quality information for any harvest/collection system that promises commercial viability and due to the role environmental factors may play on this quality, to include more sampling sited in widely different groves and weather conditions, and to evaluate cleaning and sanitizing protocols of Mechanical Harvesting Equipment.

Presentations associated with 2008-09 efforts:

A summary of 4 years work was presented at the Mechanical Harvest Field Day, April 22 at the SWREC. The work is set to be presented on June 8th at the Florida State Horticultural Society Annual meeting.

Publications from 2008-09 efforts:
Refereed:
Non-refereed: This work will be published in the 2009 Proceedings of the Florida State Horticultural Society's annual meeting

Next steps:

Objectives for the 2009/2010 season will involve a shift to only evaluating CMNP applications to fruit, and we will target two Hamlin and two Valencia trials, in collaboration with Dr. Spann and Dr. Ebel.

Additional work will focus on determining what current cleaning and sanitizing regimes are being used on catch-frame devices.

Tables and Figures:

Table 1. Fruit surface microflora in log colony forming units (CFU) per orange for six trials of mechanical harvesting, Hamlin (H) and Valencia (V), with catch frames (CF), continuous shaking (CS), or CMNP application (n = 25 oranges).

Trial	Tree	Ground	CMNP Tree or CF	CMNP Ground
	Log CFU/orange on PCA			
H1 CMNP	4.2 ± 0.5	5.4 ± 0.2	4.8 ± 0.3	5.5 ± 0.1
H2 CF	3.9 ± 0.7	4.2 ± 0.4	4.2 ± 0.3	
H3 CS	4.9 ± 0.3	4.8 ± 0.2		
V1 CMNP	3.7 ± 0.6	4.3 ± 0.6	4.3 ± 0.5	4.6 ± 0.5
V2 CF	4.7 ± 0.4	5.0 ± 0.4	5.9 ± 0.4	
V3 CS	5.6 ± 0.4	5.7 ± 0.3		
	Log CFU/orange on OSA			
H1 CMNP	3.8 ± 0.4	5.0 ± 0.2	4.2 ± 0.4	5.2 ± 0.1
H2 CF	4.0 ± 0.5	4.2 ± 0.4	4.6 ± 0.3	
H3 CS	0 ± 0	0 ± 0		
V1 CMNP	4.2 ± 0.6	4.2 ± 0.3	4.5 ± 0.4	5.0 ± 0.4
V2 CF	4.7 ± 0.4	5.9 ± 0.4	5.0 ± 0.3	
V3 CS	5.5 ± 0.3	5.5 ± 0.2		

Table 2. Summary of fruit surface indicator (*E. coli*), pathogenic organisms (*Salmonella*), and juice spoilage organism (*Alicyclobacillus*) for six trials of mechanical harvesting, Hamlin (H) and Valencia (V), with catch frames (CF), continuous shaking (CS), or CMNP application (n = 5 enrichments).

Trial	Tree	Ground	CMNP Tree or CF	CMNP Ground
	E. coli enrichments			
H1 CMNP	0[z]	5	**1**	**1**
H2 CF	0	0	**4**	
H3 CS	0	0		
V1 CMNP	0	0	0	0
V2 CF	0	0	**5**	
V3 CS	0	0		
	Salmonella enrichments			
H1 CMNP	0	0	0	0
H2 CF	0	0	0	
H3 CS	0	0		
V1 CMNP	0	0	0	0
V2 CF	0	0	0	
V3 CS	0	0		
	Alicyclobacillus counts (CFU/orange)			
H1 CMNP	>0.04	**32 ± 22**	> 0.04	**32 ± 39**
H2 CF	> 0.04	> 0.04	> 0.04	
H3 CS	> 0.04	> 0.04		
V1 CMNP	> 0.04	> 0.04	> 0.04	> 0.04
V2 CF	> 0.04	> 0.04	> 0.04	
V3 CS	> 0.04	> 0.04		

Table 3. Juice microflora in colony forming units (CFU) per ml for six trials of mechanical harvesting, Hamlin (H) and Valencia (V), with catch frames (CF), continuous shaking (CS), or CMNP application (n = 25 juice samples).

Trial	Tree	Ground	CMNP Tree or CF	CMNP Ground
	CFU/ml on PCA			
H1 CMNP	0.4 ± 0.4	3.9 ± 2.9	1.5 ± 1.4	4.0 ± 1.8
H2 CF	0.5 ± 0	0.8 ± 0	0.2 ± 0	

H3 CS	4.9 ± 0.3	4.8 ± 0.2		
V1 CMNP	0	0	0	0
V2 CF	0	0	0	
V3 CS	0	0		
	CFU/ml on OSA			
H1 CMNP	0.06 ± 0.2	1.0 ± 1.6	0.3 ± 1.6	0.7 ± 1.6
H2 CF	0.7 ± 0	0.3 ± 0	0.7 ± 0	
H3 CS	5.7 ± 0.1	5.7 ± 0.2		
V1 CMNP	0	0	0	0
V2 CF	0	0	0	
V3 CS	0	0		

Table 4. Summary of juice fecal indicator (*E. coli*), and pathogenic organisms (*Salmonella*), for six trials of mechanical harvesting, Hamlin (H) and Valencia (V), with catch frames (CF), continuous shaking (CS), or CMNP application (n = 5 enrichments).

Trial	Tree	Ground	CMNP Tree or CF	CMNP Ground
	E. coli enrichments			
H1 CMNP	0[z]	0	0	0
H2 CF	0	0	0	
H3 CS	0	0		
V1 CMNP	0	0	0	0
V2 CF	0	0	0	
V3 CS	0	0		
	Salmonella enrichments			
H1 CMNP	0	0	0	0
H2 CF	0	0	0	
H3 CS	0	0		
V1 CMNP	0	0	0	0
V2 CF	0	0	0	
V3 CS	0	0		