



Influence of Mechanical Harvesting System and Abscission Agent on Microflora of Citrus Fruit

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For Florida to continue to compete effectively in the citrus industry, significant reductions in harvesting costs will be necessary. Mechanical harvesting (MH), a possible means of cost reduction, can be thought of as a two-step process: 1) removal of fruit from the tree, and 2) collection of fruit (immediately by a catch-frame device, or by retrieval of fruit from the ground). This study evaluated the microbiology of the surface and juice of citrus fruit collected by various mechanical harvesting systems (OXBO 3220 or OXBO 3210) and with or without the application of the abscission compound 5-chloro-3-methyl-4-nitro-pyrazole (CMNP). Samples included fruit harvested by the following methods: 1) hand-harvested fruit (control); 2) ground fruit (picked up directly from ground following canopy shaking); and 3) mechanically-harvested (MH) fruit (collected from a catch frame), or any of these groups sprayed with CMNP. Microbial analysis included a total plate count (TPC), an acidophilic organisms count (OSA), a thermophillic aciduric bacillus count (TAB) and generic *Escherichia coli* and *Salmonella* testing on pooled samples of five oranges. Juice samples were subjected to the same tests, with the exception of TAB. Control fruit generally had fewer microbes on the surface of the fruit and in the juice than either ground or MH fruit on both TPC and OSA. TAB were rarely detected. Application of CMNP did not significantly alter microflora. However, no real trends can be attributed to harvest method for all runs. Generic *E. coli* was detected in ground, MH and control pooled fruit, and *Salmonella* spp. was not detected in any of the pooled fruit or juice samples. These results suggest that fruit which come in contact with the ground, catch-frame or CMNP are not consistently or significantly higher in surface or corresponding juice microflora than the hand harvested control.

Development of various mechanical harvesters and pick-up machines for citrus has been explored since the 1970s (Whitney, 1995; Whitney and Summer, 1977). Two general systems for fruit collection are commonly seen, including 1) using a catch frame (CF) to collect fruit as it is falling from the tree, or 2) dropping the fruit directly to the grove floor for future collection. To detach the fruit from the tree, a canopy shaker is often used; however, trunk shakers and blowers have also been used. If fruit is not caught in a catch frame, collection by either hand crews or pick-up (PU) machines can occur. Due to the large amounts of fruit and potential for hand labor shortages in the future, efficient CF or PU machines are desired by the industry. Recent interest has also focused on the application of the abscission compound 5-chloro-3-methyl-4-nitro-pyrazole (CMNP) to ease the removal of fruit from the tree.

Within the citrus production and processing industries, sensitivity to food safety risks exists as a result of *Salmonella* outbreaks associated with fresh orange juice that occurred in the mid-1990s (Vojdani et al., 2008), and has increased due to recent outbreaks associated with produce. The impact of a new harvesting system, that may place fruit in contact with the grove floor, needs to be

explored to quell food safety fears that may prevent mechanical harvesting from moving forward. Much of the data collection on mechanical harvesting systems currently consists of yield, performance, and efficiency studies, as well as the effect of tree shaping and grove design (Roka and Rouse, 2004; Whitney et al., 1986). Data are available on the overall prevalence of pathogens such as *Salmonella* on the surface of oranges destined for processing (Parish et al., 2001), and some information about the microbiological effects of mechanical harvesting is available from the three previous years of this study (Danyluk et al., 2008; Goodrich-Schneider et al., 2007; Goodrich et al., 2006).

The objective of this work is to summarize the 2008–09 research results from this ongoing study (begun in 2005–06) evaluating the microbiological surface and juice microflora of citrus fruit collected by various mechanical harvesting systems.

Materials and Methods

FRUIT SAMPLING. Six samples of mechanical harvesting systems were collected through the 2008–09 harvest season, including self propelled continuous canopy shaker with catch frame (Oxbo 3220), tractor-drawn pull-behind canopy shaker (Oxbo 3210) and CMNP application (harvested with the Oxbo 3210 canopy shaker) for Hamlin and Valencia varieties. Samples collected

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from the Oxbo 3220 included fruit from 1) the ground (i.e., fruit that missed the catch frame), 2) the goat (i.e., fruit captured by the catch frame, hereafter referred to as mechanically harvested or MH), and 3) hand-harvested fruit (control). Samples collected from the Oxbo 3210 were the same, but did not include the goat since this system does not utilize a catch frame. All samples collected from CMNP application trials were harvested using the Oxbo 3210; thus, samples included 1) hand-harvested fruit, and 2) ground fruit with and without CMNP application. Within each sample group 30 oranges were collected, and 25 non-defective fruit were randomly selected from each sample for analysis. All samples were collected using latex gloves (changed between sample groups), and placed directly into sterile collection bags.

MICROBIOLOGICAL METHODS AND REPORTING. All microbiological media were purchased from Becton Dickinson (Sparks, MD) unless otherwise noted. Fruit were stored at 4 °C for no longer than 24 h prior to analysis. Each orange was transferred to an individual, sterile whirl-pak bag using latex gloves. Thirty milliliters of sterile 0.1% peptone buffer was poured over the orange in each plastic bag and was manipulated using the rub-shake-rub technique to remove surface microorganisms (Parish et al., 2001).

Aerobic plate counts (APC) and acidophilic counts (AOC) were performed by making appropriate dilutions of the wash buffer in sterile 0.1% peptone and spiral plating onto plate count agar (PCA) and orange serum agar (OSA), respectively. When necessary, to increase the limit of detection, four spread plates of 0.25 mL each of the lowest dilution were prepared. The PCA plates were incubated 24 h at 35 °C (Morton, 2001) while the OSA plates were incubated 48 h at 30 °C (Hatcher et al., 2001). After the appropriate incubation, numbers of colonies were counted and reported as colony forming units (CFU) per fruit. Data were statistically evaluated using Excel software (Microsoft, Redmond, WA).

Due to time and expense constraints and the low expected frequency of isolation, assays of *E. coli* and *Salmonella* were performed on separate, pooled samples. Each pooled sample resulted from 5-mL buffer aliquots from each orange sample which were mixed to yield one 25-mL sample for every five fruit. This resulted in five 25-mL samples for control, ground, and MH for each replicate trial, and a total of 60 samples analyzed over the entire study.

E. coli and *Salmonella* detection was performed by adding each 25-mL composite samples to appropriate media according to Parish et al. (2001). The VIP *Salmonella* test kit (BioControl, Bellevue, Wash.) was used as specified by the manufacturer for the *Salmonella* assay, while the E*Colite™ test kit (Charm Sciences, Lawrence, MA) was used to detect the presence of generic *E. coli*. Appropriate negative and positive controls were run to ensure performance of test kits. Results were reported as the number of positive composite samples.

Alicyclobacillus testing (TAB) was done by heat shocking the pooled sample for 10 min at 85 °C then plating onto Ali Agar (AA). AA plates were incubated up to 7 d at 45 °C. Isolates were identified by rDNA sequencing and analysis with NCI-BLAST.

Following testing of 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 cheesecloth into a sterile container. Parallel microbial testing was done for juice samples and is reported as CFU/mL juice and as presence or absence of *E. coli* and *Salmonella*.

Results and Discussion

Microbial populations were enumerated using APC media. This test is also described as “Total plate count” or “Standard plate count” and, in this case, represents the number of microorganisms on the surface of the orange that are capable of growing into viable colonies aerobically and at warm temperatures. The APC gives a general indication of the overall microbial load on or in a food product. Similarly, the AOC count represents the number of microorganisms on the surface of the orange that are capable of growing into viable colonies under more acidic conditions than PCA (acidophiles; Hatcher et al., 2001). OSA is the typical media used in citrus processing quality control laboratories in order to enumerate the acidophilic organisms in the environment or in products that are capable of surviving and growing in juice-like conditions.

In general, control fruit had fewer microbes on their surface 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 2008–09, 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 maintenance may ultimately be very important in total number of 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 in 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 (data not shown). 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

Table 1. Fruit surface microflora in log colony forming units (CFU) per orange for six harvesting trials of Hamlin (H) and Valencia (V) varieties. Trials included fruit harvested with the Oxbo 3220 self-propelled continuous canopy shaker with catch frame (3220), fruit harvested with the Oxbo 3210 tractor-drawn pull-behind canopy shaker (3210), and the application of the abscission compound CMNP (harvested with the Oxbo 3210) (n = 25 oranges).

Trial	No CMNP			+ CMNP	
	Control	Ground	MH	Control	Ground
<i>Log CFU/orange on PCA</i>					
H1 CMNP	4.2 ± 0.5	5.4 ± 0.2		4.8 ± 0.3	5.5 ± 0.1
H2 3220	3.9 ± 0.7	4.2 ± 0.4	4.2 ± 0.3		
H3 3210	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 3220	4.7 ± 0.4	5.0 ± 0.4	5.9 ± 0.4		
V3 3210	5.6 ± 0.4	5.7 ± 0.3			
<i>Log CFU/orange on OSA</i>					
H1 CMNP	3.8 ± 0.4	5.0 ± 0.2		4.2 ± 0.4	5.2 ± 0.1
H2 3220	4.0 ± 0.5	4.2 ± 0.4	4.6 ± 0.3		
H3 3210	0 ± 0	0 ± 0			
V1 CMNP	4.2 ± 0.6	4.2 ± 0.3		4.5 ± 0.4	5.0 ± 0.4
V2 3220	4.7 ± 0.4	5.9 ± 0.4	5.0 ± 0.3		
V3 3210	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) varieties. Trials included fruit harvested with the Oxbo 3220 self-propelled continuous canopy shaker with catch frame (3220), fruit harvested with the Oxbo 3210 tractor-drawn pull-behind canopy shaker (3210), and the application of the abscission compound CMNP (harvested with the Oxbo 3210) (n = 5 enrichments).

Trial	No CMNP			+ CMNP	
	Control	Ground	MH	Control	Ground
<i>E. coli</i> enrichments					
H1 CMNP	0	5		1	1
H2 3220	0	0	4		
H3 3210	0	0			
V1 CMNP	0	0		0	0
V2 3220	0	0	5		
V3 3210	0	0			
<i>Salmonella</i> enrichments					
H1 CMNP	0	0		0	0
H2 3220	0	0	0		
H3 3210	0	0			
V1 CMNP	0	0		0	0
V2 3220	0	0	0		
V3 3210	0	0			
<i>Alicyclobacillus</i> counts (CFU/orange)					
H1 CMNP	>0.04	32 ± 22		> 0.04	32 ± 39
H2 3220	> 0.04	> 0.04	> 0.04		
H3 3210	> 0.04	> 0.04			
V1 CMNP	> 0.04	> 0.04		> 0.04	> 0.04
V2 3220	> 0.04	> 0.04	> 0.04		
V3 3210	> 0.04	> 0.04			

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 (data not shown).

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 2008–09. 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 MH harvest indicate a potential for cross-contamination, and a need to clean this equipment during harvesting.

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