IFAS Citrus Initiative
Annual Research and Extension Final Progress Report 2010-11

Investigator:

PI – M.D. Danyluk
Co-PIs – T.M. Spann

Objective(s) Pursued (Priority Topics):

Objective 1: Evaluate standard fruit and juice quality and yield following the application of CMNP application trials for Hamlin and Valencia varieties and storage of up to 7 days (continuation of 2010).

Objective 2: Evaluate the fate of coliforms and *E. coli* sprayed onto Hamlin and Valencia trees in the field until populations can no longer be detected.

Detailed Accomplishments in 2011-12:

Objective 1:

This study evaluates the standard juice quality and yield of fruit harvested following the application of CMNP and storage for up to 7 days to determine if CMNP application has any effect on these parameters. For each replicate (two Hamlin and two Valencia), harvested fruit were collected. Fruit were then divided into treatment groups and stored for up to 7 days. Treatment groups included storage at: 10, 20, 30°C and ambient conditions (with temperature and humidity monitors). Within each group of fruit, 5 non-defective fruit were randomly selected from each group at each sample point for analysis. Quality analysis, still underway and not reported here include °Brix, Acid, % oil, and color. Additional measurement of puncture and crush forces, and decay were also collected and are still being analyzed.

To enumerate microorganisms, 30 ml of buffer were added and the rub/shake/rub technique was used 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 of trials completed are reported in Tables 1 (December, 2011 Hamlin), 2 (January, 2012 Hamlin), 3 (March, 2012 Valencia) and 4 (May, 2012 Valencia) as log colony forming units (CFU) per orange. In general, no differences were seen in total APC or AOC microflora on orange surfaces with or without CMPN application during storage at any temperature.

No *Alicyclobacillus* was isolated from any of the fruit.

Analysis of quality parameters, puncture and crush forces, and decay is ongoing.

Table 1. Fruit surface microflora in log colony forming units (CFU) per orange one trial of Hamlin fruit harvested in December with or without CMNP application (n = 5 oranges) during storage of up to 7 days at 10, 20, 30°C or under ambient conditions.

		Control		CMNP	
Temp (°C)	Time (day)	APC	AOC	APC	AOC
	0	4.7 ± 0.3	4.6 ± 0.1	4.6 ± 0.2	4.7 ± 0.2
10	3	4.2 ± 0.2	3.9 ± 0.3	4.3 ± 0.4	4.0 ± 0.5
	5	4.7 ± 0.3	4.4 ± 0.2	4.8 ± 0.3	4.3 ± 0.3
	7	4.7 ± 0.5	4.5 ± 0.4	5.0 ± 0.7	4.9 ± 0.6
20	3	4.5 ± 0.6	4.2 ± 0.5	4.7 ± 0.3	4.3 ± 0.4
	5	4.4 ± 0.5	4.7 ± 0.2	4.6 ± 0.1	4.4 ± 0.3
	7	4.0 ± 0.2	4.6 ± 0.5	4.6 ± 0.2	4.3 ± 0.2
30	3	4.6 ± 0.6	4.3 ± 0.6	4.7 ± 0.2	4.6 ± 0.2
	5	4.3 ± 0.1	4.8 ± 0.4	4.6 ± 0.2	4.6 ± 0.3
	7	4.0 ± 0.5	4.0 ± 0.3	4.5 ± 0.2	4.2 ± 0.4
Ambient	3	5.0 ± 0.5	4.7 ± 0.3	4.7 ± 0.4	4.5 ± 0.2
	5	4.5 ± 0.4	4.8 ± 0.8	4.6 ± 0.4	4.6 ± 0.4
	7	4.4 ± 0.3	4.4 ± 0.4	4.6 ± 0.3	4.5 ± 0.4

Table 2. Fruit surface microflora in log colony forming units (CFU) per orange one trial of Hamlin fruit harvested in January with or without CMNP application (n = 5 oranges). during storage of up to 7 days at 10, 20, 30°C or under ambient conditions

		Control		CMNP	
Temp (°C)	Time (day)	APC	AOC	APC	AOC
	0	5.2 ± 0.1	5.0 ± 0.1	5.4 ± 0.1	5.1 ± 0.1
10	3	4.5 ± 0.2	4.8 ± 0.2	4.4 ± 0.3	4.7 ± 0.2
	5	4.4 ± 0.3	5.1 ± 0.2	4.87± 0.1	5.2 ± 0.0
	7	4.6 ± 0.3	5.2 ± 0.1	4.9 ± 0.2	5.1 ± 0.1
20	3	5.0 ± 0.2	5.1 ± 0.1	4.5 ± 0.1	4.9 ± 0.1
	5	4.3 ± 0.4	4.8 ± 0.2	4.8 ± 0.1	4.9 ± 0.3
	7	4.8 ± 0.2	4.9 ± 0.1	5.2 ± 0.1	5.0 ± 0.1
30	3	4.4 ± 0.5	4.9 ± 0.5	4.6 ± 0.5	5.0 ± 0.2
	5	4.2 ± 0.3	4.6 ± 0.3	4.3 ± 0.4	4.9 ± 0.2
	7	4.6 ± 0.2	4.5 ± 0.1	5.0 ± 0.3	4.9 ± 0.3
Ambient	3	4.2 ± 0.5	4.6 ± 0.3	4.5 ± 0.2	4.8 ± 0.1
	5	4.1 ± 0.5	4.7 ± 0.1	4.5 ± 0.2	4.8 ± 0.1
	7	4.3 ± 0.6	4.9 ± 0.3	4.7 ± 0.2	5.0 ± 0.1

Table 3. Fruit surface microflora in log colony forming units (CFU) per orange one trial of Valencia fruit harvested in March with or without CMNP application (n = 5 oranges) during storage of up to 7 days at 10, 20, 30°C or under ambient conditions.

		Control		CMNP	
Temp (°C)	Time (day)	APC	AOC	APC	AOC
	0	5.4 ± 0.1	5.4 ± 0.2	5.5 ± 0.1	5.6 ± 0.2
10	3	5.4 ± 0.3	5.5 ± 0.2	5.0 ± 0.1	5.2 ± 0.4
	5	4.8 ± 0.2	5.1 ± 0.3	4.7± 0.2	5.0 ± 0.5
	7	5.5 ± 0.1	5.6 ± 0.0	5.0 ± 0.2	5.6 ± 0.0
20	3	5.3 ± 0.2	5.3 ± 0.4	5.1 ± 0.1	4.8 ± 0.2
	5	5.2 ± 0.4	5.7 ± 0.1	5.2 ± 0.4	4.9 ± 0.2
	7	5.5 ± 0.1	5.6 ± 0.0	5.3 ± 0.3	4.8 ± 0.4
30	3	5.2 ± 0.3	4.9 ± 0.2	5.3 ± 0.2	4.9 ± 0.3
	5	5.3 ± 0.3	5.7 ± 0.1	4.8 ± 0.4	5.4 ± 0.5
	7	5.1 ± 0.2	5.5 ± 0.0	5.0 ± 0.2	4.9 ± 0.2
Ambient	3	5.5 ± 0.2	5.5 ± 0.2	4.9 ± 0.3	5.2 ± 0.3
	5	4.9 ± 0.4	5.6 ± 0.1	4.7 ± 0.2	5.3 ± 0.2
	7	5.5 ± 0.0	5.6 ± 0.1	5.2 ± 0.2	5.1 ± 0.3

Table 4. Fruit surface microflora in log colony forming units (CFU) per orange one trial of Valencia fruit harvested in May with or without CMNP application (n = 5 oranges) during storage of up to 7 days at 10, 20, 30°C or under ambient conditions.

		Control		CMNP	
Temp (°C)	Time (day)	APC	AOC	APC	AOC
	0	5.5 ± 0.1	5.2 ± 0.1	5.3 ± 0.4	4.9 ± 0.2
10	3	5.2 ± 0.4	4.6 ± 0.5	4.8 ± 0.4	4.8 ± 0.3
	5	4.5 ± 0.4	4.9 ± 0.3	4.6 ± 0.2	4.9 ± 0.2
	7	4.7 ± 0.3	5.2 ± 0.1	4.7 ± 0.4	5.1 ± 0.1
20	3	4.9 ± 0.5	4.7 ± 0.1	4.9 ± 0.2	4.7 ± 0.2
	5	4.8 ± 0.2	5.1 ± 0.2	4.8 ± 0.3	5.0 ± 0.3
	7	5.0 ± 0.3	5.1 ± 0.2	5.0 ± 0.2	5.1 ± 0.1
30	3	5.0 ± 0.4	4.8 ± 0.5	4.4 ± 0.1	4.4 ± 0.1
	5	4.7 ± 0.3	4.9 ± 0.3	4.9 ± 0.4	5.1 ± 0.3
	7	5.0 ± 0.3	5.2 ± 0.1	4.8 ± 0.2	5.1 ± 0.1
Ambient	3	5.1 ± 0.4	4.9 ± 0.5	4.9 ± 0.3	4.9 ± 0.1
	5	4.8 ± 0.5	5.2 ± 0.4	4.8 ± 0.4	5.1 ± 0.2
	7	4.8 ± 0.3	4.9 ± 0.2	5.0 ± 0.1	5.0 ± 0.1

Objective 2:

To evaluate the fate of coliforms and *E. coli* that may be sprayed onto oranges close to harvest if low microbial quality water is used to apply the abscission agent, low microbial quality water (ca. 6 log CFU/ml coliforms) was applied to Valencia trees, in March, April, May, and June. Three replicates of 10 oranges each, from each of three trees (n =

90 fruit) were be removed immediately prior to and following spraying, and at 2 and 6 h following application, and approximately every other day until *E. coli* was no longer detectable by enrichment, or equivalent to control trees.

Results of trials completed are reported in Tables 5 (March, 2012), and 6 (April, 2012) as colony forming units (CFU) or most probable number (MPN) per orange. For the final two experimental dates (May and June, 2012) data collection and analysis is ongoing. Based on current data it appears *E. coli* populations can be detected on fruits between 12 and 17 days after application.

Table 5. Fruit surface coliform and *E. coli* populations in log colony forming units (CFU) or Most Probable Number per orange. Trial of Valencia fruit harvested in March with or without low microbial quality water application (n = 90 oranges; 3 x 10 fruit from each of 3 trees).

	Sprayed		Control	
Time (hour)	Coliforms	*E. coli*	Coliforms	*E. coli*
Pre Spray	ND	ND	ND	ND
0	5.1 ± 0.0	5.1 ± 0.1	3.2 ± 0.6	0.3 ± 0.6
2	3.1 ± 0.3	2.6 ± 0.2	3.3 ± 0.9	0.1 ± 0.2
6	3.1 ± 0.1	2.4 ± 0.2	2.6 ± 0.5	0.7 ± 0.6
24	2.5 ± 0.5	2.4 ± 0.1	1.9 ± 0.8	< -0.1
48	2.8 ± 0.3	2.8 ± 0.2	3.1 ± 0.3	< -0.1
72	3.5 ± 0.1	2.6 ± 0.2	3.8 ± 0.4	1.0 ± 0.0
144 (6 d)	3.4 ± 0.2	1.4 ± 1.4	3.4 ± 0.2	< -0.1

Table 6. Fruit surface coliform and *E. coli* populations in colony forming units (CFU) or Most Probable Number per orange. Trial of Valencia fruit harvested in April with or without low microbial quality water application (n = 90 oranges; 3 x 10 fruit from each of 3 trees).

	Sprayed		Control	
Time (day)	Coliforms	*E. coli*	Coliforms	*E. coli*
Pre Spray	1.7 ± 0.4	< -0.1	1.5 ± 0.8	< -0.1
0	4.7 ± 0.5	4.7 ± 0.4	2.8 ± 0.9	< -0.1
2	2.8 ± 0.7	0.73 ± 1.1	2.1 ± 0.2	< -0.1
6	2.3 ± 0.2	0.6 ± 0.2	2.1 ± 0.9	< -0.1
24	2.7 ± 0.2	0.5 ± 0.4	2.7 ± 0.4	< -0.1
48	2.2 ± 0.2	− 0.5 ± 0.4	2.1 ± 0.2	< -0.1
72	2.1 ± 0.2	− 0.4 ± 0.4	2.0 ± 0.0	< -0.1
168 (7 d)	2.0 ± 0.1	− 0.9 ± 0.1	2.0 ± 0.1	< -0.1
192 (8 d)	2.0 ± 0.1	− 0.8 ± 0.3	1.5 ± 0.7	< -0.1
288 (12 d)	2.0 ± 0.5	− 0.9 ± 0.6	1.8 ± 0.2	< -0.1
408 (17 d)	1.9 ± 0.1	< − 0.1 ± 0.0	1.9 ± 0.2	< -0.1
480 (20 d)	2.0 ± 0.5	< − 0.1 ± 0.0	1.8 ± 0.2	< -0.1
528 (22 d)	1.9 ± 0.1	< − 0.1 ± 0.0	1.5 ± 0.6	< -0.1
552 (23 d)	1.7 ± 0.1	< − 0.1 ± 0.0	1.3 ± 0.6	< -0.1

Areas where progress exceeded expectations:

In addition to the work discussed above, a hand harvesting CMNP trial was done at Lykes Camp Mack grove in late April. We sprayed 3 rows (~150 trees) at 300 ppm and 3 rows at 200 ppm on April 27 (1-2 pm). Three untreated rows served as controls. We used a standard air blast sprayer for the application.

Fruit detachment forces were:

Control - 110.7 N
300 ppm - 69.9 N
200 ppm - 54.2 N.

This equals a 37% and 51% reduction in fruit detachment force for the 300 and 200 ppm treatments, respectively. We sampled 20 fruit from each treatment from 5-6 trees from the middle row of each plot.

We had a 15 person crew. The control plot was harvested first followed by the 300 ppm plot and then the 200 ppm plot. Fatigue was definitely an issue on the 200 ppm plot. The control and 300 ppm plots were complete by 1:30 pm. It took until nearly 5:30 pm to finish the 200 ppm plot.

The numbers work out to: Control - 2780 man minutes to harvest 340 boxes = 8.17 min/box; 300 ppm - 2328 man minutes to harvest 333 boxes = 6.99 min/box (a 14.5% increase in harvesting rate; 200 ppm - 2872 man minutes to harvest 330 boxes = 8.70 min/box.

Initial assessment of this trial is that it was a success. We demonstrated that CMNP can improve the efficiency of a harvest crew. However, there are issues to figure out for future trials, chief among them how to design the trial to NOT disrupt the natural work patterns of the crew and how to account for fatigue. The very modest (30 second) increase in time per box on the 200 ppm plot demonstrates that CMNP was effective. We estimate that the crew would have been at least 1 minute or more slower per box by the late afternoon if the fruit had not been sprayed.

Areas where progress didn't meet expectations:

Initial plans included low quality water trials in the fall and winter. Due to timing issues these trials were not conducted. Additionally, due to extended survival of organisms on fruit surfaces, times between samplings needed to be expanded due to not enough fruit present on the trees.

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 collect fruit and juice

microbiological quality information for any harvest/collection system that promises commercial viability.

Presentations associated with 2011-12 efforts:
Results will be presented at the 2011 Florida State Horticultural Society.

Publications from 2011-12 efforts:
Spann, T. M., L. V. Pozo, I. Kostenyuk and J. K. Burns. 2011. Application of the abscission agent 5-chloro-3-methyl-4-nitro-1*H*-pyrazole does not affect peel integrity or postharvest decay of mechanically harvested late-season 'Valencia' orange fruit during the normal commercial harvest-to-processing period. HortScience 46(7): 1-4.

Danyluk, M.D., L.M. Friedrich, and T.M. Spann. 2011. Effect of abscission agent on citrus juice quality. Abstracts of the 2011 Meeting of the Florida State Horticultural Society, HP-4.

Friedrich, L.M., R.M. Goodrich-Schneider, R. Ehsani, R.C. Ebel, T.M. Spann and M.D. Danyluk. 2012. Microbiological evaluation of mechanically harvested citrus fruit. International Symposium on Mechanical Harvesting & Handling Systems of Fruits and Nuts Abstracts, p. 23.

Next steps:
Objectives for the 2010/2011 season will involve continuing to evaluate fate of indicator organisms on fruit following the application of low microbial quality water at different levels over 9 months. A separate set of experiments led by Spann will be established to continue hand harvesting work.