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

Title of the subproject: Abscission management and managing abscission agent repository.

Participants: Jackie Burns (PI), Sunny Liao, Igor Kostenyuk, Bob Ebel, Fritz Roka, Jim Syvertsen

Progress: Distribution of viable Candidatus Liberibacter asiaticus (CaLas) in sweet orange fruit and leaves ('Hamlin' and 'Valencia') and transcriptomic changes associated with huanglongbing (HLB) infection in fruit tissues are reported. Viable CaLas was present in most fruit tissues tested in HLB trees, *with the highest titer detected in vascular tissue near the calyx abscission zone*. This likely contributes to premature fruit drop measured in HLB-infected fruit. Transcriptomic changes associated with HLB infection were analyzed in flavedo (FF), vascular tissue (VT), and juice vesicles (JV) from symptomatic (SY), asymptomatic (AS), and healthy (H) fruit. In SY 'Hamlin', HLB altered the expression of more genes in FF and VT than in JV, whereas in SY 'Valencia', the number of genes whose expression was changed by HLB was similar in these tissues. The expression of more genes was altered in SY 'Valencia' JV than in SY 'Hamlin' JV. More genes were also affected in AS 'Valencia' FF and VT than in AS 'Valencia' JV. Most genes whose expression was changed by HLB were classified as transporters or involved in carbohydrate metabolism. Physiological characteristics of HLB-infected and girdled fruit were compared to differentiate between HLB-specific and carbohydrate metabolism-related symptoms. SY and girdled fruit were smaller than H and ungirdled fruit, respectively, with poor juice quality. However, girdling did not cause misshapen fruit or differential peel coloration. Quantitative PCR analysis indicated that many elected genes changed their expression significantly in SY flavedo but not in girdled flavedo. Mechanisms regulating development of HLB symptoms may lie in the host disease response rather than being a direct consequence of carbohydrate starvation.

Dr. Igor Kostenyuk resigned his position, and with this, the management of the CMNP repository was handed over to the FDOC under Dr. Dan King's supervision. Included in the transfer was the technical and formulated CMNP materials managed by us for over 6 years. Before Dr. Kostenyuk resigned, we returned the radiolabelled CMNP materials to vendors as directed by AgroSource, Inc., our private partner in registration of the CMNP material.

Impact: We showed that although reduced carbohydrate accumulation in HLB fruit could be a consequence of dysfunctional sieve elements in the phloem, disruption of cellular carbohydrate metabolic regulation and transport could be another contributing factor to carbohydrate imbalance. Carbohydrate imbalance and the presence of CLas in the fruit abscission zone contribute to premature fruit abscission. Numerous genes involved in carbohydrate transport and metabolism changed expression in 'Hamlin' and 'Valencia'. In fact, several genes involved in starch metabolism that changed expression after girdling were altered in HLB and girdled FF, supporting the hypothesis that in addition to disrupted carbohydrate transport in phloem, HLB and fruit stem girdling also induce major changes in the cellular metabolism of carbohydrates. Such changes can disrupt fruit growth and lead to small fruit size typical of HLB-affected fruit and fruit from girdled stems. Since girdling did not result in misshapen fruit or irregular peel color typical of HLB-affected fruit and several representative genes for specific pathways were affected by HLB but not girdling, the mechanisms regulating the development of these symptoms may lie in the host disease response rather than being a direct consequence of carbohydrate starvation.

Publications:

1. Liao H-L, Burns JK (2012) Gene expression in *Citrus sinensis* fruit tissues harvested from huanglongbing-infected trees: comparison with girdled fruit. J Exp Bot 63: 3307-3319.
2. Rosales R, Burns JK (2011) Phytohormone changes and carbohydrate status in sweet orange fruit from Huanglongbing-infected trees. J Plant Growth Regul 30:312-321.
3. Spann TM, Pozo LV, Kostenyuk I, Burns JK (2011) Application of the abscission agent 5-chloro-3-metyl-4-nitro-1H-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 46:1-4.

Note: JK Burns will no longer be conducting active research in abscission and harvesting, but remains committed to successful implementation of the citrus mechanical harvesting system through administrative duties. It has been a pleasure working with our IFAS research and outreach group, and with the citrus industry growers and cooperators.

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IFAS Citrus Initiative
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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.

IFAS Citrus Initiative
Research and/or Extension progress report 2011-12

Investigator(s):
PI – Robert C. Ebel
Co-PIs – Fritz Roka, Kelly Morgan

Priority area addressed: (Abscission management and harvester efficiency)
Objective 1: Develop best management practices for application of the abscission agent CMNP.
Objective 2: Develop best management practices for harvester settings utilizing the abscission agent CMNP to maximize harvest efficiency.
Objective 3: Enhance understanding of the mode of action of CMNP in promoting abscission.

1) Why this work is critical and needed

Labor shortages and cost have compelled the sweet orange industry to seek an alternative way to harvest the crop. Mechanical harvesters are currently being used, but must stop before the end of the season due to the newly developing crop. The abscission agent CMNP has proven effective at increasing fruit removal and as a result the industry is proceeding with its registration for use on citrus. Economic analysis estimate that CMNP will lower cost for mechanical harvesting. An Experimental Use Permit has been applied for with the EPA to spray 9,000 acres of sweet oranges. In preparation for commercial availability of CMNP, this research program is designed to understand the factors that interact with CMNP with the longterm goal of developing recommendations for its use on sweet oranges.

2) Objectives and accomplishments

Objective 1: Develop best management practices for application of the abscission agent CMNP. We have developed an empirical model to predict decline in fruit detachment force (FDF) and pre harvest fruit drop for sweet orange by CMNP based on air temperature. This data was presented at the ASHS meetings in September 2011.

Objective 2: Develop best management practices for harvester settings utilizing the abscission agent CMNP to maximize harvest efficiency. A major field study was initiated in 2011 to evaluate the use of CMNP on the late season Valencia harvest by self-propelled canopy shakers. The specific objectives are: 1) to determine the interaction of CMNP application and 2 canopy shaker frequency settings, the setting used by commercial operators, and a lower setting that was 40 cpm less than the higher setting, on fruit removal, fruit recovery, deck loss, and gleaning, 2) to determine the carry over effect on yield for each treatment in comparison to the controls.

This study was replicated 4 times beginning in early May, 2011 and conducted every 2 weeks. CMNP was applied at the maximum label rate (300 ppm and 300 gal/acre) and the trees harvested 4 days later. Approximately 130 trees per treatment were used. The machine settings varied for each harvest in consultation with the commercial harvest managers, but the lower setting was always 40 cpm less than the higher setting. The tractors were run at 1.0 mph for all trials. There was also a hand-harvested control that was not treated with CMNP. Data collected included diameter of newly developing fruit and weights of preharvest fruit drop, fruit removed, fruit not captured by the deck during the harvesting process, and fruit left in the tree that had to be gleaned. This study is currently being repeated on the same set of trees.

2011 results: The immature fruit were slightly under 1 inch in diameter, the diameter commercial mechanical harvesting normally ends, for the first trial and have grown to 1.5 inch by the early June trial. A final trial is planned for June 11th (spray) and June 14th (harvest). Fruit drop in these trials has been small (less than 3% of the total yield). Fruit removal has ranged from 55-81% for unsprayed trees and 76-90% for trees treated with CMNP. Deck loss, that is fruit not captured by the catch frame of the harvesters, has been less than 8% in all trials, with no consistent results with respect to CMNP application. Removal of immature fruit was slightly higher for the higher canopy shaker frequencies, as would be expected, but was not affected by CMNP application. These results indicate that CMNP at lower canopy shaker frequency provides good removal that will allow later mechanical harvesting of Valencia, although the effect on yield loss will have to be determined next year.

2012 results: The immature fruit were much smaller than 1 inch in diameter for the first trial but were close to 1 inch in diameter by the second trial. The third trial has been completed but data not yet analyzed. The fourth trial will be conducted in less than two weeks. FDF was reduced in CMNP treated trees by almost 50% in the first trial and over 80% in the second trial. Fruit drop was low in the first trial but high in the second. Fruit removal of CMNP treated trees was higher than non-treated trees in both trials. Yields of mechanically harvested trees compared to hand harvested trees were variable. There appeared to be a slight yield reduction in the first trial, but no yield reduction by mechanical harvesting in the second trial.

Objective 3: Enhance understanding of the mode of action of CMNP in promoting abscission. This is a new project and is designed to increase our understanding of the mode of action of CMNP, which we believe may help in development of best management practices. In preliminary work we determined that CMNP treated fruit produce nitrous oxide (NO) and that exogenously applied NO to sweet oranges at concentrations similar to CMNP applied in the field produced similar reductions in FDF. It is our hypothesis that ADH in the flavedo tissues catabolizes CMNP to produce nitric oxide (NO), a known signal that induces an increase in production of jasmonic acid, which is transported to the abscission zone causing abscission. The PhD student working on this project at the FSHS meetings in June will present the results of this work.

We have also determined the effects of CMNP on oxidative metabolism and reactive oxygen species (ROS). CMNP promotes production of H_2O_2 and alters enzymes involved its metabolism. This work is continuing, especially with special reference to how temperature affects this important pathway that is known to produce signals that promote abscission.

3) Research gaps

Objective 1: The models we have developed to predict the decrease in FDF and fruit drop by CMNP and air temperature have been determined empirically and with data that does not span the entire temperature range that could be encountered commercially. We need to verify the models based on a mechanistic understanding of how CMNP promotes abscission as discussed in Objective 3. This work is continuing, however, in light of the probability that CMNP will be registered next Feb., we will publish the empirical model if we are not able to determine the mechanistic disruption of loosening by CMNP when air temperatures are low.

Objective 2: We need to continue the CMNP x canopy shaker frequency study of the late season Valencia's to evaluate the carry over effect on yield, as well as to continue to build a database that allows us to determine how late in the Valencia season trees can be mechanically harvested using CMNP without affecting yield.

Objective 3: Understanding the mechanism of CMNP and especially how air temperature affects efficacy is vital to developing best management practices. We need to continue to determine if ADH produces NO with CMNP as a substrate, whether this reaction is temperature sensitive and if the temperature profile is similar to that of CMNP, and whether temperature affects uptake of CMNP. We also need to determine how temperature interacts with oxidative metabolism, especially in regards to the production of JA, and to determine if JA is the signal that stimulates formation of the abscission layer.

4) Employees being supported on CI funds and their roles in meeting objectives.

a) PhD student: her responsibility is to work on the aspects of the mechanism of CMNP, especially those related to NO and ADH activity (Objective 3)
b) Postdoc: his responsibility is to work on the aspects of the mechanism of CMNP, especially those related to oxidative metabolism (Objective 3)
c) Biological Scientist: This position is only partly funded by CI. His responsibilities include preparing equipment and materials and assisting in the field studies. Funding for his Teams position has been requested. He is vital support to the late season trials due to his organizing and leadership abilities in preparing and executing the study.

Publications and presentations related to this work

Ebel, R.C., K.T. Morgan, F. Roka, and Peter Newman. **2012**. Mechanical harvester setting and abscision agent application on harvest efficiency and long-term productivity of late season 'Valencia'. HortScience, accepted.

S. Sharma, **R.C. Ebel, and N. Kumar. **2012**. Production of nitrous oxide by the abscission agent CMNP and its impact on citrus fruit loosening. Florida State Horticulture Society, Delray Beach, FL, June 3-5.

R.C. Ebel, F. Roka, and K. Morgan. **2012**. Field research with CMNP for loosening sweet oranges as an aid to mechanical harvesting. International Mechanical Harvesting Symposium, Lake Alfred, FL, April 2nd - 4th.

R.C. Ebel, F. Roka, and K. Morgan. **2012**. Field research with CMNP for loosening sweet oranges as an aid to mechanical harvesting. Acta Hort., in press.

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