2) We now propose to develop a scaled and fully functional prototype to demonstrate performance under scaled and controlled field conditions. This prototype will allow us to further test various aspects of the design, in order to further demonstrate feasibility and test reliability and catch frame effectiveness under controlled field conditions. This effort would consist of development of the catch frame mechanical framework, actuators, and controls for a mockup field condition. Once fabricated and assembled, testing will be conducted using simulated harvesting conditions to evaluate the efficiency of the catch frame concepts. Design modifications will be made along the way to improve performance.

Publications Related to Funding

Han, S., Burks, T.F. 2011. Image Processing based 3D Reconstruction of a Citrus Canopy. (ready to submit)

Han, S., Burks, T.F. 2011. Multiple Layered Hierarchical Feature Tracking for Grove Scene. (ready to submit)

Jayaraman, V., Burks, T.F., Ho, J., Bulanon, D.M. 2011. Three Dimensional Mapping of Citrus Fruits in the Canopy using Computer Vision. (ready to submit)

Mehta, S.S., T. F. Burks, W. E. Dixon. 2010. Target Reconstruction Based Visual Servo Control for Autonomous Citrus Harvesting. *Journal of Intelligent Service Robotics: Special Issue on Agricultural Robotics*, submitted.

Bulanon, D M, Burks, T F, Alchanatis, V. 2010. A Multispectral Imaging Analysis for Enhancing Citrus Fruit Detection. Environmental Control in Biology, 48(2) 45-55.

Conference Papers and Presentations

Han, Sanghoon; Burks. T.F. 2010 Multilayered Active Mesh Tracking for Grove Scene. 2010 ASABE Annual International Meeting, Pittsburg, PA, June 20-23, 2010. Conference Paper 1008886.

Thesis Written and Defended

Subbiah, Sundar. 2010, Robust Autonomous Guidance for Citrus Groves. Agricultural and Biological Engineering Dept. University of Florida.

YOU, KyuSuk. 2011, Adaptable Catching System for Continuous Citrus Harvesting. Agricultural and Biological Engineering Dept. University of Florida.

IFAS Citrus Initiative MH Annual 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: Huanglongbing (HLB) is a destructive disease caused by a fastidious, phloem-restricted bacterium, *Candidatus* Liberibacter spp. HLB affects all known citrus species and causes the devastating yield and tree losses. Symptoms of HLB are visually apparent on leaves as vein yellowing and blotchy mottle on the blade. In severe infections, leaf size is reduced and eventually leaves abscise. Infected fruit are lopsided, smaller in size, poor in color and drop prematurely. Many symptomatic fruit abscission zone located at the peduncle/fruit interface is orange in color, even the majority of the fruit surface is yellowish-green.

Our study with *Citrus sinensis* 'Valencia' trees indicated fruit drop in HLB-infected trees was initiated at breaker stage. When compared with fruit in healthy trees,10-fold higher symptomatic fruit dropped to the ground and the detachment force of attached fruit (FDF) was reduced 75%. There was no difference of % abscission and FDF between asymptomatic fruit and healthy fruit. When compared with abscission zones of healthy fruit, the expression of abscission-induced genes such as *1-aminocyclopropane-1-carboxylate synthase* (*CsACS1*), *cellulose-a1* (*CsCel-a1*), *polygalacturonase* (*CsPG*), *phospholipase$A_2\alpha$* (*CssPL$A_2\alpha$*) and *Phospholipase$A_2\beta$* (*CsPL$A_2\beta$*) were 17-, 7-, 530-, 1.5-, and 2-fold higher in symptomatic fruit abscission zones respectively. The expression of *CsACS1* and *CsCel-a1* were 5-fold increased and *CsPG* was 150-fold increased in asymptomatic fruit abscission zones. In addition, ethylene production was 6- and 2-fold higher in the areas of symptomatic and asymptomatic fruit abscission zones respectively.

Our study with leaf tissues revealed that HLB raised 7% of leaf abscission on 80 observation days. 10% reduction of the detachment froce and the increased expression of abscission-induced genes were also found in symptomatic laminar and petiole abscission zones compared to healthy controls.

We analyzed girdled tissues to study the impacts of carbohydrate deprivation on abscission. A bark full ring or half ring around the twig located 10-cm above a fruit was removed at immature stage. Leaves between the girdled region and fruit were removed. Girdling was also applied on a branch which held around 50 to 100 of leaves. The study showed symptomatic and full girdled fruit shared the similar impacts on size, weight, and color. Lopsided symptom only presented in symptomatic, but not in girdled fruit. In most of the full girdled fruit, flavedo region very closed to fruit abscission zone was orange in color. Yellowish color appeared on full girdled leaves. There was no visual differences appeared on half-girdled tissues compared to ungirdled tissues. Similar to symptomatic tissues, full girdled tissues showed the shift of starch and sucrose contents, premature fruit drop, leaf drop, and the increased expression of abscission-induced genes at abscission zones. However, the impacts of girdling were greater than HLB infection especially on tissue drop and carbohydrate metabolism.

Over the past 7 years we generated and evaluated the performance of Valencia orange and Marsh grapefruit transgenic plants with a down-regulated anti-sense construct of ACO (1-aminocyclopropane-1-carboxylate oxidase). On average, application of 450 ppm ethrel to these trees in the greenhouse resulted in a six-fold increase in leaf abscission compared to non-transgenic control trees. Trees transformed with a sense construct of ACP treated in the same manner had 2.5 times lower abscission than non-transgenic trees. When trees carrying anti-sense and sense ACO constructs were treated with gaseous ethylene, leaf abscission was significantly

less than non-transgenic trees. Additional work with these trees will be necessary to fully characterize the responses and determine what value, if any, they may have to the program.

The entire inventory of CMNP samples (both non-radioactive and radioactive) has been safely stored at CREC, locked at all times, with log in entries recorded. The integrity of containers and bottles has been periodically inspected and cleanliness maintained. For the RA part of CMNP inventory: CMNP inventory is being stored in a locked freezer. Currently we are maintaining 11 mCi ^{14}C-labeled water based solution of CMNP that has been periodically inspected and swiped for vial and packaging integrity and monitored using a dosimeter to ensure proper storage conditions. Quarterly RA Inventory reports have been furnished to UF main campus upon request.

Both general and RA data base inventories have been maintained and updated when needed. Upon authorized requests, CMNP formulation has been transferred in requested amounts either to Immokalee UF REC or within CREC to be used in the mechanical harvesting program. CMNP storage facility temperature and humidity readings have been recorded every other 15 minutes by HOBO devices 24/7/365 and periodically uploaded to appropriate CMNP database subfolder. All related files are duplicated for safety reason.
Appropriate coordination and instructional support to management and personnel at AgroSource Inc., Florida Department of Citrus, SWFREC (Immokalee, FL) and within CREC (Lake Alfred, FL) has been provided when needed.

Impact: We are working towards larger scale CMNP trials so that label directions can be clarified. Our work with transgenic plants altered in their ability to respond to ethephon will provide important information on the role of ethylene in differential induction of abscission in leaves and fruit.

Publications:

1. Rosales R, Burns JK (2011) Phytohormone changes and carbohydrate status in sweet orange fruit from Huanglongbing-infected trees. J Plant Growth Regul DOI 10.1007/s00344-011-9193-0.
2. Alferez F, Alquezar B, Burns JK, Zacarias L (2010) Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. Postharvest Biol Technol 56:44-49.
3. Ebel RC, Burns JK, Morgan KT, Roka FM (2010) Abscission agent application and canopy shaker frequency effects on mechanical harvest efficiency of sweet orange. HortScience 45:1079-1083.
4. Ferguson L, Rosa UA, Castro-Garcia S, Lee SM, Guinard JX, Burns JK, Krueger WH, O'Connell NV, Glozer K (2010) Mechanical harvesting of California table and oil olives. Adv Hort Sci 24:53-63.
5. Karuppiah-John K-J, Burns JK (2010) Degreening behavior in 'Fallglo' and 'Lee x Orlando' is correlated with differential expression of ethylene signalling and biosynthesis genes. Postharvest Biol Technol 58:185-193.
6. Karuppiah-John K-J, Burns JK (2010) Expression of ethylene biosynthesis and signalling genes during differential abscission responses of citrus leaves and mature fruit. J Amer Soc Hort Sci 135:456-464.
7. Liao H-L, Burns JK (2010) Light controls phospholipase A2α and phospholipase β gene expression. J Exp Bot 61:2469-2478.

8. Castro-Garcia S, Rosa UA, Gliever CJ, Smith D, Burns JK, Krueger WH, Ferguson L, Glozer K (2009) Evaluation of table olive damage during harvest with a canopy shake-and-catch system. HortTech 19:260-266.

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IFAS Citrus Initiative MH Annual Progress Report 2010-11

Investigator:

PI – M.D. Danyluk
Co-PIs – T.M. Spann, J.K. Burns

Objective(s) Pursued (Priority Topics):

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

Detailed Accomplishments in 2010-11:

This study evaluates the standard juice quality and yield of fruit harvested following the application of CMNP and stored for up to 7 days to determine if CMNP application has any effect on these parameters. For each replicate (one Hamlin and two Valencia), harvested fruit will be collected. Fruit were then be divided into treatment groups and stored for up to 7 days. Treatments groups will included storage at: 10, 20, 30°C and ambient conditions (with temperature and humidity monitors). Within each group of fruit, 5 non-defective fruit will be 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.

To enumerate microorganisms, 30 ml 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 of trials completed are reported in Tables 1 (January, 2011 Hamlin), 2 (April, 2011 Valencia) and 3 (May 2011 Valencia) as 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.

Alicyclobacillus testing was be done by heat shocking the remaining fruit wash described above for 10 min at 85° then plating onto Ali Agar (AA). No *Alicyclobacillus* was identified from any of the samples.

Table 1. 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	4.6 ± 0.3	4.9 ± 0.2	5.0 ± 0.6	5.1 ± 0.3
10	3	4.5 ± 0.2	5.0 ± 0.2	4.6 ± 0.5	4.6 ± 0.4
	5	5.1 ± 0.2	4.9 ± 0.2	5.5 ± 0.2	5.2 ± 0.4
	7	5.1 ± 0.2	5.3 ± 0.1	5.0 ± 0.3	5.3 ± 0.2
20	3	4.7 ± 0.2	4.7 ± 0.2	4.7 ± 0.4	4.7 ± 0.3
	5	5.2 ± 0.4	5.0 ± 0.3	5.4 ± 0.5	4.9 ± 0.3
	7	5.1 ± 0.2	5.3 ± 0.1	5.1 ± 0.4	5.3 ± 0.1

In addition to simply testing for *Alicyclobacillus presence*, APC of all microorganisms and AOC counts were obtained.

Areas where progress didn't meet expectations:

Originally we had planned to collect fruit from large CMNP harvest trials run by Eble. Unfortunately for this project, these harvest trials were all pushed into May and April of 2011. Thus, small scale trials run on trees at CREC were used instead.

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 2010-11 efforts:

Results will be presented at the 2011 Florida State Horticultural Society.

Publications from 2010-11 efforts:

Refereed:

Spann, T.M. and Danyluk, M.D. 2010. Mechanical harvesting increases leaf and stem debris in loads of mechanically harvested citrus fruit. HortSci. 45:1297-1300.

Non-refereed:

A paper will appear in the 2011 FSHS Proceedings

Next steps:

Objectives for the 2010/2011 season will involve continuing to evaluate CMNP applications to fruit, and we will target two Hamlin and two Valencia trials. Additionally, experiments to determine the fate of indicator organisms on fruit will begin.

IFAS Citrus Initiative
Research and/or Extension progress report 2010-11

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 a model to predict decline in fruit detachment force (FDF) and pre harvest fruit drop for sweet orange by CMNP. We are in the process of developing manuscripts of these models. We will also be presenting the models 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. Achievement of this objective has been conducted over the last 3 years. Before the current year, we had determined how best to apply CMNP via a multiple fan sprayer, 2) determined how CMNP interacted with canopy shaker frequency, and 3) determined the interaction of CMNP x harvest date. The goal for the current year was to evaluate how tractor speed of the canopy shakers interacted with CMNP application. We conducted a CMNP x tractor speed (0.5, 1.5, and 2.5 mph) study on Hamlin and found that at slower tractor speed, more fruit was removed with CMNP compared to the controls. However, temperatures were low for this test and removal rates were low.

In consultation with the FDOC harvest council, the research focus was switched to determine the effects of CMNP application and harvester setting (shaker frequency) on harvest components and carry over effect on yield of late season Valencia. These trials consisted of using self-propelled machines and whole tree rows as replications with about 65 trees/row. Three trials have been conducted in early and late May and early June. 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.

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. The

mechanism in which CMNP promotes loosening is incompletely understood. We have studied the effects of CMNP application on oxidative metabolism and have found that it promotes oxidative stress through an increase in reactive oxygen species (ROS). Enzymes involved in promoting and dissipating ROS changed to initially promote ROS and decreased again to diminish ROS. Furthermore, in previous work it had been shown that alcohol dehydrogenase (ADH) activity was affected by CMNP. 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. We have found that NO applied as nitroprusside at concentrations similar to what CMNP would provide and found a decrease in fruit detachment force similar to that of CMNP alone. ADH is affected by air temperature and we are currently determining if the relationship of temperature on CMNP activity is similar to the temperature effect on ADH activity.

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.

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.

Publications and presentations related to this work

- **Ebel, R.C.**, J.K. Burns, K. Morgan, and F. Roka. **2010.** Abscission agent application and canopy shaker frequency on mechanical harvest efficiency of sweet orange. HortScience, 45:1079-1083.

- Sharma, S., **R.C. Ebel**, and K.M. Morgan. **2010**. Modeling loosening of sweet orange with CMNP: Variation in fruit detachment force. Proceedings of the Florida State Horticulture Society, in press.

- **Ebel, R.C.**, J.K. Burns, K.M. Morgan, and F. Roka. **2010**. Interaction of CMNP application and harvest date of 'Hamlin' sweet orange. Proceedings of the Florida State Horticulture Society, in press.

- **Ebel, R.C. 2010.** Late Season Mechanical Harvesting Research on 'Valencia' and Research Plans for the Upcoming Harvest Season. Citrus Harvesting Research and Advisory Council, Department of Citrus, Lake Wales, FL, Nov. 2nd. (25 participants).

- **Ebel, R.C.**, **2010.** An abscission agent for facilitating mechanical harvesting of sweet oranges in Florida. International Society of Horticultural Science, Lisbon, Portugal, Aug. 20th-Aug. 28th. (abstr).

- **Ebel, R.C. 2010.** Potential improvement of mechanical harvesting with the abscission agent CMNP. Cooperative Producers Incorporated Board of Directors, Lake Wales, FL, June 16th.

- Sharma, S., **R.C. Ebel**, and K.M. Morgan. **2010**. Modeling loosening of sweet orange with CMNP: Variation in fruit detachment force. Florida State Horticulture Society annual conference, Crystal River, FL, June 6-8th. (abstr).

- **Ebel, R.C.**, J.K. Burns, K.M. Morgan, and F. Roka. **2010**. Interaction of CNP application and harvest date of 'Hamlin' sweet orange. Florida State Horticulture Society annual conference, Crystal River, FL, June 6-8th. (abstr).

- **Ebel, R.C. 2010.** CMNP trials for 2009/2010. Southwest Florida mechanical Harvesting Advisory group, Southwest Florida Research and Education Center. May 26th. (15 participants).

- **Ebel, R.C. 2010**. CMNP Trials. Hillbilly Production School, Winter Garden, FL, Apr. 27th (28 participants).

- **Ebel, R.C. 2010**. CMNP Trials. Mechanical Harvesting Field Day, Immokalee, FL, April 21st. (60 participants).

- **Ebel, R.C. 2010**. Abscission material update: CMNP as an aid to mechanical harvesting. DeSoto Citrus Production School, Turner Agri-Civi Center's Exhibition Hall, Feb. 23rd. (45 participants).

- **Ebel, R.C. 2010.** Update on current mechanical harvesting trials. Citrus Harvesting Research and Advisory Council, Department of Citrus, Lake Wales, FL, Feb. 2nd. (15 participants).