IFAS Citrus Initiative
Research and Extension Final Progress Report 2009-10

Investigator:
PI – Jacqueline K. Burns
Co-PIs – Robert Ebel, Jim Syvertsen

Objective(s) Pursued (Abscission and Harvesting):
Objective 1: Define and implement strategies for abscission agent application timing
Objective 2: Manage abscission agent repository and screen new abscission compounds for registration

Detailed Accomplishments in 2009-10:

Objective 1: Define and implement strategies for abscission agent application timing. We focused on the role of light in controlling the abscission process, since we demonstrated in previous work that the abscission process was diurnally regulated. The low-molecular weight secretory phospholipase A_2 (*CssPLA*$_2$) and β (*CsPLA*$_2\beta$) cloned in this study exhibited diurnal rhythmicity in leaf tissue of *Citrus sinensis*. Only *CssPLA*$_2$ displayed distinct diurnal patterns in fruit tissues. *CssPLA*$_2$ and *CsPLA*$_2\beta$ diurnal expression exhibited periods of approximately 24 h; *CssPLA*$_2$ amplitude averaged 990-fold in the leaf blades from field-grown trees, whereas *CsPLA*$_2\beta$ amplitude averaged 6.4-fold. Diurnal oscillation of *CssPLA*$_2$ and *CsPLA*$_2\beta$ gene expression in the growth chamber experiments was markedly dampened 24 h after transfer to continuous light or dark conditions. *CssPLA*$_2$ and *CsPLA*$_2\beta$ expressions were redundantly mediated by blue, green, red and red/far-red light, but blue light was a major factor affecting *CssPLA*$_2$ and *CsPLA*$_2\beta$ expression. Total and low molecular weight $CsPLA_2$ enzyme activity closely followed diurnal changes in *CssPLA*$_2$ transcript expression in leaf blades of seedlings treated with low intensity blue light (24 μmol m^{-2} s^{-1}). Compared with *CssPLA*$_2$ basal expression, *CsPLA*$_2\beta$ expression was at least 10-fold higher. Diurnal fluctuation and light regulation of PLA_2 gene expression and enzyme activity in citrus leaf and fruit tissues suggests that accompanying diurnal changes in lipophilic second messengers participate in the regulation of physiological processes associated with phospholipase A_2 action.

To successfully use abscission agents for 'Valencia' sweet orange mechanical harvesting throughout the harvest season, unwanted flower, fruitlet, and leaf drop must be assessed and minimized. Ethephon (400 mg·L^{-1}), 1-methylcyclopropene (1-MCP; 5 mM), ethephon + 1-MCP, 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP; 200 mg·L^{-1}), and a kinetic adjuvant control [0.15% (v/v)] were applied to 'Valencia' branches at various times from full bloom in Mar. 2006 to the end of full bloom in Mar. 2008. Effects of these treatments on fruit detachment force (FDF) and abscission of developing and mature fruit, flowers, and leaves were recorded. Three separate response periods to abscission agent applications were observed: the first spanned the first 100 days after bloom (DAB) and was characterized by high initial response followed by decreasing sensitivity; the second spanned between 100 and 225 DAB and was characterized by little to no response; and the third spanned from 225 DAB to harvest and was characterized by a gain in sensitivity. Young fruitlets in the first response period were highly sensitive to ethephon but were less sensitive to CMNP or ethephon + 1-MCP. Mature fruit in the third response period were highly sensitive to CMNP and less sensitive to ethephon or ethephon + 1-MCP. The application of ethephon resulted in high leaf abscission and showed no clear sensitivity pattern throughout both cropping years. CMNP or ethephon + 1-MCP application caused minimal leaf abscission. The same abscission agent treatments were applied on whole tree canopies 6 and 28 DAB in Mar. 2007. Application date had no significant effect on the measured parameters. Although ethephon application induced high initial leaf drop, leaf area indices determined 7 months after any compound application were not significantly different. However, subsequent 2008 yield in trees sprayed with ethephon in 2007 was significantly less, whereas 2008 flower number was higher. The results indicate a complex interaction of fruitlet abscission and leaf loss during the first response period contributed to yield reduction and increased flower number in ethephon-treated trees.

The application of methyl jasmonate (MeJA) to grapes (*Vitis vinifera* L.) may decrease fruit detachment force (FDF) and promote the development of dry stem scars on the berries, both of which could improve the quality of machine-harvested raisin grapes. However, treatment with MeJA also promotes preharvest fruit drop, which is undesirable. Thus, experiments were conducted to determine how the concentration of MeJA applied and time after treatment affect FDF and abscission of grapes. Mature 'Thompson Seedless' grapevines were treated with one of five different solutions containing 0, 0.2, 2, 10, or 20 mM MeJA, and FDF and fruit abscission were monitored for 2 weeks. Treatment with 2 mM or less MeJA had inconsistent effects on FDF and did not promote abscission,

whereas treatment with 10 to 20 mM MeJA reduced FDF within 2 to 3 days after treatment (DAT) and promoted abscission, which began on 3 DAT and persisted for 8 DAT. Thus, to optimize the use of MeJA as a harvest aid for 'Thompson Seedless' may require application of between 2 and 10 mM MeJA followed by harvest within 3 DAT.

Objective 2: Manage abscission agent repository and screen new abscission compounds for registration. As needed support for CMNP registration was provided. We worked closely with AgroSource, Inc., an agrochemical company contracted by the Florida Citrus industry, to manage the repository and screen new compounds. Increased requests for radio-labeled, technical and formulated CMNP shipments and receipts were experienced to support the application for CMNP EUP at the end of CY 2009. This required annual licensing for shipment and receipt of hazardous materials, approved boxing and labeling, troubleshooting and communication with vendors, and documentation and log keeping.

Impact of accomplishments towards overall goals of funding:

We are providing information identifying environmental factors that control abscission. Together with previous research, this can be used to better predict and control the abscission process. Our expertise in abscission and mechanical harvesting has been used to assist other fruit crop commodities that are seeking to control abscission and mechanize the harvesting process.

Presentations associated with 2009-10 efforts:

Karuppiah K-J, **Burns JK** (2009) Differential ethylene-related gene expression, MACC, and ACC content in fruit flavedo of 'Fallglo' and 'LeeXOrlando' citrus hybrids. 2nd International Citrus Biotechnology Symposium, November 30-December 2, 2009. Catania, Italy. Poster presentation.

Kostenyuk IA, **Burns JK** (2009) Downregulation of ACC oxidase in citrus reduces wound-induced ethylene production and leaf abscission. 2nd International Citrus Biotechnology Symposium, November 30-December 2, 2009. Catania, Italy. Poster presentation.

Rosales R., Liao H-L, **Burns JK** (2009) IAA, ABA, and ethylene content and related gene expression is altered in mature fruit fractions of Huanglongbing-infected *Citrus sinensis*. 2nd International Citrus Biotechnology Symposium, November 30-December 2, 2009. Catania, Italy. Poster presentation.

Burns JK (2009) Current state of the science related to fruit abscission. American Society for Horticultural Science Annual Conference, July 25-28, 2009. St. Louis, Missouri. Oral presentation.

Ferguson L, Rosa U, Castro S, **Burns JK**, Glozer K, Krueger WH, O'Connell N, Ortiz JM, Ladux JL, Fernandez FJ, Searles PS, Ferguson JH, Kulakow P, Rosecrance R (2009) Developing mechanical harvesting for California Black Ripe Process Table Olives *Olea europaea* cv. Manzanillo. American Society for Horticultural Science Annual Conference, July 25-28, 2009, St. Louis, MO. Poster Presentation.

Spann T, Danuluk MS, Ebel RC, **Burns JK** (2009) Debris accumulation in loads of mechanically harvested oranges. American Society for Horticultural Science Annual Conference, July 25-28, 2009, St. Louis, MO. Oral Presentation.

Publications from 2009-10 efforts:
Refereed:

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.

Liao H-L, **Burns JK** (2010) Light controls phospholipase A2α and phospholipase β gene expression. J Exp Bot doi:10.1093/jxb/erq083.

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.

Ebel RC, **Burns JK**, Morgan KT (2009) Spray volume, distribution, and efficacy of 5-chloro-3-methyl-4-nitro-1H-

pyrazole for loosening sweet orange. HortScience 44:1895-1899.

Gonzalez-Herranz R, Cathline KA, Fidelibus MW, **Burns JK** (2009) Potential of methyl jasmonate as a harvest aid for 'Thompson Seedless' grapes: Concentration and time needed for consistent berry loosening. HortScience 44:1330-1333

Pozo L, **Burns JK** (2009) Organ loss and yield impacts of 'Valencia' sweet orange in response to fruit abscission agents. HortScience 44:83-88.

Non-refereed:

Ebel RC, **Burns JK**, Morgan K, Roka FM (2009) Interaction of CMNP concentration and canopy shaker setting on fruit removal of sweet orange. Proc. Fla. State Hort. Soc. 122: 132-135.

Next steps:

We will continue working on abscission control points for the purpose of controlling and predicting abscission. Special emphasis will be placed on utilizing this information to support the abscission product label and for outreach activities related to the anticipated Experimental Use Permit/Temporary Tolerance allowance in 2010/2011.

IFAS Citrus Initiative
Annual Research and Extension Final Progress Report 2009-10

Investigator:

PI – R. Ebel

Co-PIs – J. Burns, K. Morgan, F. Roka, and T. Spann

Objective(s) Pursued (Priority Topics):

1. *Interaction of CMNP and mechanical harvester setting on harvest efficiency.*
2. *Efficacy of CMNP on mechanical harvester efficiency over multiple harvest dates.*
3. *Modeling efficacy of CMNP based on application and environmental variables.*

Detailed Accomplishments in 2009-10:

1. *Interaction of CMNP and mechanical harvester setting on harvest efficiency.*

- This study was completed in April, 2009 and a manuscript prepared and is currently in press in HortScience.

2. *Efficacy of CMNP on mechanical harvester efficiency over multiple harvest dates.*

- We conducted three identical studies in December (Hamlin) and two in May (Valencia) with CMNP applied at 2 rates (0, 200 or 300 ppm) and with trees harvested 2, 3, 4, and 5 days after CMNP application. We obtained excellent results that we plan to publish in a refereed journal.

3. *Modeling efficacy of CMNP based on application and environmental variables.*

- We conducted a series of early season (December) and late season (May) high frequency studies to complete our data set for development of the model.
- We have determined the variability of fruit detachment force within a tree and how that variability changes over time (This work will be presented at the FSHS meetings in June, 2010).
- We have developed the model, but need to incorporate the newest data.

Areas where progress exceeded expectations:

1. *Interaction of CMNP and mechanical harvester setting on harvest efficiency.* None
2. *Efficacy of CMNP on mechanical harvester efficiency over multiple harvest dates.* None
3. *Modeling efficacy of CMNP based on application and environmental variables.* None

Areas where progress didn't meet expectations:

1. *Interaction of CMNP and mechanical harvester setting on harvest efficiency.* None
2. *Efficacy of CMNP on mechanical harvester efficiency over multiple harvest dates.* None
3. *Modeling efficacy of CMNP based on application and environmental variables.*

- We were unable to repeat the drought study due to a very cool and wet spring.

- This work is being conducted by a new PhD student who is working on development of the model. Progress has been slower than I had expected, but she her progress rate is increasing as her understanding of the complexities of modeling CMNP has developed.
- Although we had hoped to have a publication prepared from this work, in developing the model we were concerned we did not have enough data for the May period, which is of considerable interest and because of our concern of the "less responsive" period, we felt it important to conduct high frequency studies throughout this month to complete our data set for development of the model.

Impact of accomplishments towards overall goals of funding:

- This work is part of a series to develop BMPs for CMNP and mechanical harvester setting that maximize harvest efficiency of sweet oranges.
- This work is highly visible, with presentations given at grower and professional meetings multiple times during the year.
- With the advent of the EUP expected to be available the summer of 2011, this research is critical for developing recommendations by IFAS for use of CMNP as an aid to mechanical harvesting of sweet oranges.

Presentations associated with 2009-10 efforts:

1. **Ebel, R.C. 2010**. CMNP Trials. Central Florida Production Managers, Winter Garden, FL, Apr. 27th (28 participants).

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

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

4. **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).

5. **Ebel, R.C. 2009**. Mechanical Harvesting Update: Abscission. Southwest Florida Squeezer meeting, Southwest Florida Research and Education Center, Immokalee, FL, Dec. 8th. (21 participants).

6. **Ebel, R.C. 2009.** Challenges of the Florida Citrus Industry. Delegation of agricultural producers from Germany, Southwest Florida Research and Education Center, Immokalee, FL., Nov. 16th. (35 participants).

7. **Bob Ebel. 2009**. Mechanical harvesting and CMNP abscission update. Southwest Florida IFAS Citrus Advisory Committee, Southwest Florida Research and Education Center, Immokalee, FL, Oct. 30th. (23 participants).

8. Friedrick, L.M., T.M. Spann, **R.C. Ebel**, and M.D. Danyluk. **2009**. Microbiological evaluation of mechanically harvested citrus fruit. Citrus Processing and Technology Conference, Citrus Research and Education Center, Lake Alfred, FL, Oct. 22nd. (25 participants). Abstr.

9. **R.C. Ebel. 2009**. Additional update on the 2008-2009 harvest season and research plans for the 2009-2010 harvest season. Citrus Harvesting Research and Advisory Council, Department of Citrus, Lakeland, FL, Oct. 6th. (15 participants).

10. **R.C. Ebel. 2009**. Abscission management. Mechanical Harvesting Workshop, Indian River Research and Education Center, Sept. 24th. (50 participants).

11. **R.C. Ebel. 2009**. Abscission and mechanical harvesting. 2009 Citrus Expo, Fort Myers, FL, Aug. 19-20th. (100 participants).

Publications from 2009-10 efforts:

Refereed:

1. **Ebel, R.E.**, J.K. Burns, K. Morgan, and F. Roka. **2010.** Abscission agent application and canopy shaker frequency on mechanical harvest efficiency of sweet orange. HortScience, in press.

2. **Ebel, R.C.,** J.K. Burns, and K. Morgan. **2009**. Distribution and efficacy of CMNP for loosening sweet oranges using a vertical, multiple fan. HortScience, 44:1895-1899.

Non-refereed:

1. **Ebel, Robert C**., Jackie Burns, Kelly Morgan, and Fritz Roka. **2009.** Interaction of CMNP concentration and canopy shaker setting on fruit removal of sweet orange. Fla. State Hort. Soc. Proc., 122:132-135.

2. L.M. Friedrich, T.M. Spann, R.McEgan, **R.C. Ebel**, and M.D. Danyluk. **2009**. Influence of mechanical harvesting system and abscission agent on microflora of citrus fruit. Proc. Fla. State Hort. Soc., 122:343-346.

3. **Bob Ebel. 2009**. Late season trial update. Citrus Industry magazine, What's Shakin' column, Oct. issue.

4. **Bob Ebel. 2009**. Facilitating mechanical harvesting with the abscission agent CMNP. Citrus Industry, August issue, pages 19-21, 31.

5. Fritz Roka, Jackie Burns, Jim Syvertsen, and **Robert Ebel. 2009.** Economic Advantages from Registration of an Abscission Product. Citrus Industry Magazine, March Issue.

6. Barbara Hyman, Jacqueline Burns, **Robert Ebel. 2009**. Abscission Compound and Mechanical Harvesting Research Update. Florida Grower, Feb. Issue.

Next steps:

New objectives:

1. *Efficacy of CMNP on mechanical harvester efficiency over multiple harvest dates.* Publish these results in HortScience.
2. *Modeling efficacy of CMNP based on application and environmental variables.* Publish results of variation in fruit detachment force by CMNP over time in the Proc. of FSHS, publish model to predict fruit detachment force by CMNP in either the J. ASHS or HortScience. Continue conducting research as needed to fill in variables identified as affecting CMNP efficacy. Write EDIS documents that explain the model, and incorporate the model into FAWN.
3. *Evaluate CMNP application and variable tractor speeds on harvest efficiency.* The tractor speeds we've used to date are below that used by the commercial industry. We need to conduct research on speeds used by the industry in order to develop robust CMNP and mechanical harvesting BMPs.
4. *Develop a series of EDIS documents for CMNP and mechanical harvester setting.* With the EUP expect for the summer of 2011, we need EDIS documents as educational tools that can be used by county extension faculty and industry personnel for maximizing efficacy of CMNP and harvest efficiency.