# Washington Tree Fruit Research Commission 2013 Technology Research Review March 7, 2013 WA Cattlemen's, Ellensburg

Dama				
No.	Time	PI	Project Title	Yrs
	9:00	McFerson	Introduction	
			Final Reports	
1	9:05	Lewis	Mechanical pruning in apple, pear and sweet cherry (Continuing Report	13-15
3	9:15	Eastwell	Evaluating a universal plant virus microarray for virus detection	11-12
9		Eastwell	Efficient strategy to diagnose important virus diseases (New Proposal)	13-15
			Continuing Reports	
13	9:30	Dekleine	Design and development of apple harvesting techniques*: Extension	12
18	9:40	Karkee	3D machine vision for improved apple crop load estimation*: <i>Extension</i>	11-12
25	9:50	Zhang	Intelligent bin-dog system for tree fruit production: <i>Extension</i>	11-12
32	10:00	Unruh	Protein-based foam for applying lacewings eggs to fruit trees: Extension	12
38	10:10	Black	New woven pesticide applicator garments with repellent: Teleconference	13
40	10:20	Salazar	Development of apple bloom phenology and fruit growth models*	12-14
48	10:30	Salazar	Effect of early spring temperature on apple and sweet cherry blooms	12-14
55	10:40	Ashmore	Technology roadmap implementation: Teleconference	13-15

#### **YEAR**: 1 of 3

#### CONTINUING PROJECT REPORT WTFRC Project Number: TR-13-101

PI: **Co-PI (2):** Karen M. Lewis Matthew Whiting **Organization:** Washington State University **Organization**: Washington State University **Telephone**: 509-754-2011 X 412 **Telephone**: 509-786-9260 Email: Email: kmlewis@wsu.edu mdwhiting@wsu.edu Address: POB 37 Courthouse Address: 24106 N. Bunn Rd. City/State/Zip: Ephrata, WA. 98837 City/State/Zip: Prosser, WA. 99350 **Co-PI(3)**: Stefano Musacchi **Organization**: TFREC, Wenatchee **Telephone**: 509 663 8181 Email: musacci@agrsci.unibo.it Address: 1100 N. Western Ave.

**Project Title**: Mechanical pruning in apple, pear and sweet cherry

Cooperators: WA tree fruit producers

City/State/Zip: Wenatchee, WA. 98801

Total Project Request: Year 1: 73,536

Year 2: 43,959

**Year 3:** 46,210

Percentage time per crop: Apple: 70% Pear: 10% Cherry: 20% Other funding sources: None

WTFRC Collaborative expenses:

Item	2013	2014	2015
Wages	3,000	3,000	3,000
Travel	1,000	1,000	1,000
Total	4,000	4,000	4,000

Footnotes: Tractor / pruner operation and data collection

Budget 1

Organization Name: WSU Contract Administrator: Carrie Johnston Telephone: 509 335-4564 Email address: carriei@wsu.edu

1 ciepiione, 309 333-4304 En	ian auuress. carriej	e wsu.cuu	
Item	2013	2014	2015
Salaries <sup>1</sup>	26,295	26,307	27,359
<b>Benefits<sup>2</sup></b>	2,183	2,271	3,135
Wages	7,214	7,503	7,803
Benefits	844	878	913
Equipment <sup>3</sup>	25,000		
Supplies	5,000	2,000	2,000
Travel	7,000	5,000	5,000
Total	73,536	43,959	46,210

Footnotes: <sup>1</sup>Salary for student. <sup>2</sup>Medical costs include increase of 4% per year. <sup>3</sup>Purchase or lease of 1 sickle-bar pruner and 1 circular saw pruner and tractor attachments.

# **OBJECTIVES**

The primary goal of this project is to establish best management practices for pruning PNW apple, pear and sweet cherry orchards mechanically. We will follow four steps to achieving this goal:

- (1) Understand equipment and orchard requirements for successful operation of both a circular saw and sickle bar mechanical pruning system
- (2) Compare pruning technologies for their effects on fruit yield and quality
- (3) Conduct a preliminary economic assessment of mechanical pruning systems
- (4) Train an M.S. student in horticulture with extensive exposure to tree fruit horticulture, agricultural engineering and applied economics

## WORK SCHEDULE

# Spring 2013

1) Identify Graduate student for fall enrollment

2) Apple: Preliminary replicated cooperator trials in Fuji, Gala, Jazz, Ambrosia and Braeburn

3) Cherry: Replicated trials at WSU Prosser

# **METHODS**

Replicated field trials and demonstration plots will be established on both WSU R & E Centers (IAREC and TFREC) and commercial orchards. Blocks will receive same treatments over the life of the project to better evaluate multiple year effects.

Data collection will include: time required to complete task, costs to complete tasks, return bloom, standard fruit quality measurements and yield assessment. Observations will be made concerning wood damage, insect and disease presence or absence, tree balance, use of platforms, mechanical thinners and harvest assist.

Field trials In each trial we will compare the following three pruning treatments:

- 1) Mechanical pruning
- 2) Hand pruning
- 3) Mechanical pruning followed by hand pruning

## Apple:

Treatments applied at 5 stages: E2, 12 leaves, 20 leaves, post harvest (leaves on), and dormant

## Cherry:

Treatments will be applied in a UFO orchard at the WSU-Roza farm. Three replicate blocks of the treatments will be established.

## Pear:

Treatments will be applied at the dormant stage.

We will create photo journals of our trials and capture video footage. Regular project updates will be posted on the WSU Stone Fruit Physiology Facebook page. The technologies and results will be demonstrated at field days at Sunrise and Roza farms annually. In addition, we will summarize the results in articles for the Good Fruit Grower after every year.

## FINAL PROJECT REPORT

PI:	Ken Eastwell	<b>Co-PI (2):</b>	James Susaimuthu			
<b>Organization</b> :	Washington State University	<b>Organization</b> :	Washington State University			
Telephone:	509-786-9385	Telephone:	509-786-9251			
Email:	keastwell@wsu.edu	Email:	James.Susaimuthu@wsu.edu			
Address:	WSU-IAREC	Address:	WSU-IAREC			
Address 2:	24106 N Bunn Road	Address 2:	24106 N Bunn Road			
City/State/Zip:	Prosser, WA 99350	City/State/Zip:	Prosser, WA 99350			
Co-PI (3):	John Hammond					
<b>Organization:</b>	USDA-ARS					
Telephone:	301-504-5313					
Email:	John.Hammond@ars.usda.gov					
Address:	Address: USDA-ARS, USNA, FNPRU					
Address 2:	10300 Baltimore Avenue, B-010	DA				
City/State/Zip:	Beltsville, MD 20705					
<b>Total Project F</b>	<b>Request: Year 1:</b> \$35,165	Year 2: \$34,58	4 Year 3: N/A			

**Project Title**: Evaluating a universal plant virus microarray for virus detection

# Percentage time per crop: Apple: 50% Pear: 10% Cherry: 18% Stone Fruit: 22%

#### **Other funding sources**

WSU is including this information on other funding available for the support of similar research undertaken by the faculty member proposing this research. These resources are listed to identify other support granted for this research and are not included as a commitment of cost-share by the institution.

Agency Na	ame:	National Clean Plant Network (NCPN)

**Amt. requested/awarded:** \$49,902 (Sept 2011 to Sept 2012)

**Notes:** Support was provided for a Master's student working on apple green crinkle disease and a Ph.D. student investigating the etiology of cherry viruses. This is part of a larger comprehensive grant from the NCPN to the WSU Clean Plant Center - Northwest.

Agency Name:

# WTFRC Cherry Research

**Amt. requested/awarded:** \$44,522 (2011); \$46,303 (to February 2012)

**Notes:** Whereas the major focus of WTFRC Project Number CH-10-108 is the management of *Cherry leaf roll virus* and related viruses in the orchard, a small portion of the funds (ca. 10%) are directed to characterization of the complete genomes of members of the virus family *Betaflexiviridae* that infect cherry.

#### WTFRC Collaborative expenses: None

**Total Project Funding**: \$69,749 from WTFRC

#### **Budget History:**

Item	Year 1:	Year 2:	Year 3: N/A
Salaries	\$13,464 <sup>1</sup>	\$14,003 <sup>1</sup>	
Benefits	\$5,655 <sup>1</sup>	\$5,881 <sup>1</sup>	
Wages			
Benefits			
Equipment			
Supplies	\$13,250 <sup>2</sup>	\$14,700	
Travel	\$2,796 <sup>3</sup>		
Plot Fees			
Miscellaneous			
Total	\$35,165	\$34,584	

#### Footnotes:

1. Salary and benefits are requested for 0.33 FTE Postdoctoral research position to perform the molecular analysis.

2. Laboratory supplies including the printing of micro-array slides, sample RNA extraction and purification, and next generation sequencing and data analysis. Travel for one co-PI to participate in a 3-day workshop in Beltsville, MD on the application and interpretation of the

3. microarray chip data for the diagnosis of plant viruses.

## **OBJECTIVES**

This project evaluated the effectiveness of contemporary technologies for the detection of viruses found in fruit trees. The most appropriate technology is being pursued for the detection and rapid identification of viruses associated with diseases of fruit trees, and for delivery of virus-tested fruit tree cultivars to the industry in an efficient and safe manner.

# SIGNIFICANT FINDINGS

- Both the Universal Plant Virus Microarray (UPVM) and deep sequencing technologies require careful interpretation of raw data, particularly if the presence of previously uncharacterized pathogens is suggested.
- Frequent occurrence of multiple viruses in a single fruit tree was documented.
  - Deep sequencing effectively resolved complex mixtures of viruses in tissue samples, including multiple strains of the same virus in a single sample.
  - Accurate interpretation of UPVM data from samples with multiple infections was limited.
- Deep sequencing identified virus sequences in samples without any prior knowledge of viruses that may be present, and can reveal previously uncharacterized viruses.
  - New hosts of two known viruses were identified.
  - Five potentially new virus species were identified including a DNA-containing virus.
    - Requires further investigation for confirmation of virus identification and association with disease.
    - Previously unreported viruses found in fruit trees from the U.S.A., Spain, Israel, New Zealand and Brazil.

## **RESULTS & DISCUSSION**

This investigation compares emerging technologies with existing methods for the detection and identification of viruses. Proper virus identification is crucial for proper disease management in growers' blocks. Although there are few alternatives available once an otherwise productive tree has become infected with virus, correct identification of the pathogen will allow growers to make economically sound decisions about tree removal and replanting, and about measures that can be taken to minimize further spread of the virus to adjacent plantings.

At the commencement of this project, the use of microarray technology appeared to offer an efficient path forward for rapid plant virus diagnosis. The Universal Plant Virus Microarray (UPVM) had been developed by USDA for the floriculture industry and its application to perennial crops was investigated. Preliminary trials in 2010 quickly revealed that this technology and the associated computer software were unable to reliably and correctly identify viruses present when more than one virus occurred in a sample; it is common for fruit trees to be infected with several different virus species. In the summer of 2012, a new software package was developed by the laboratories advancing the UPVM. To evaluate the potential of this new software to overcome the above limitation, a scientist from the Dr. Claude Fauquet group at the Danforth Plant Science Center worked in our facility for two weeks preparing additional samples for analysis by the UPVM. RNA was extracted from sixty fruit trees derived from 22 cherry, 20 apple, six plum, five peach, four pear, two apricot and one quince trees. Included in these samples are trees affected by diseases with unknown etiology. Among the diseases included are apple rubbery wood, apple green crinkle, Stavman blotch, green Newton, apple rough skin and Bisbee internal bark necrosis. All of these diseases are grafttransmissible suggesting a virus may be the causal agent. The UPVM of the 60 samples and related positive control samples are still being analyzed at the Danforth Center using the UPVM and the updated version of the T-predict software. Results will be compared to reverse transcription polymerase chain reaction (RT-PCR) analysis for the presence of specific viruses.

The initial difficulty experienced with the UPVM in resolving complex virus populations led the project to re-evaluate its potential for addressing the desired objective of a reliable method to identify viruses present in fruit trees. Concomitantly, during the first few months of this project, deep sequencing became much more accessible and the cost of that technology declined significantly. The project therefore investigated deep sequencing as a viable alternative to microarray methods.

Deep sequencing is a procedure that allows researchers to look at the entire genetic composition of a plant sample, including any viruses or microorganisms that might be associated with the tissue sample. Since deep sequencing looks at all genetic information in the sample simultaneously and indiscriminately, prior knowledge of the presence of specific disease agents is not required. This is the underlying power of the technology. The ability of deep sequencing to correctly detect pathogens in fruit tree tissue was evaluated by comparing results with those obtained by virus-specific RT-PCR.

The results obtained from 68 deep sequencing reactions illustrate the powerful potential of this method. In general, results from deep sequencing were in agreement with the results obtained by conventional RT-PCR. Only two samples yielded RT-PCR results that suggested that a virus was present that was not detected by deep sequencing. Apple sample 237.15 was tested twice. In both cases, RT-PCR suggested that *Apple stem grooving virus* was present whereas no *Apple stem grooving virus* sequences were detected by deep sequencing. Similarly, cherry sample 8863 yielded a band in the RT-PCR that suggested it was infected with *Prune dwarf virus* whereas no *Prune dwarf virus* sequences were detected by deep sequencing. In both of these cases, the RT-PCR amplification products will need to be sequenced to verify that the product is derived from the indicated virus and not gratuitous amplification of host or contaminating sequences.

In direct contrast to this observation, deep sequencing revealed many more viruses than were detected by current standard RT-PCR protocols. Deep sequencing identified viruses in 16 samples that were not detected by RT-PCR. Important observations from the deep sequencing project are summarized below:

- 1. Grower sample number 8863 was taken from an orchard that exhibited rapid decline in sweet cherry production over the past four years. The symptoms observed in the orchard resembled those typical of little cherry disease, but repeated attempts to detect *Little cherry virus 2* by RT-PCR in samples taken from that orchard yielded negative results. In contrast, deep sequencing revealed the presence of a virus that appears to be a unique variant of *Little cherry virus 2*. Since deviation of this sequence from published *Little cherry virus 2* sequences is significant, further research is being conducted to determine if it is a variant of *Little cherry virus 2* or a new virus species related to *Little cherry virus 2*.
- 2. American plum line pattern virus sequences were detected in five pome fruits originating from the U.S.A., Israel and New Zealand. American plum line pattern virus has a wide host range, but has not been reported in Malus or Pyrus species. The contiguous sequences indicative of this virus were fairly short, so additional testing is necessary to confirm the association of this virus with these new hosts.
- 3. *Cherry virus A* was recently discovered in *Prunus* species and is causing some concern in those fruit tree species where it is suspected of increasing the severity of disease caused by other viruses. In this project, *Cherry virus A* sequences were detected in six apple trees from the U.S.A., Israel and New Zealand. In parallel to the observation of *American plum line pattern virus* sequences in pome fruits, further analyses are required to confirm the association between the deep sequencing results and the presence of the virus in host tissue.
- 4. Nine samples were tested by RT-PCR using the TriFoCap primer set (Foissac *et al.*, 2005). This test is a broad spectrum test that will detect the presence of viruses that are members of the genera *Trichovirus, Foveavirus* or *Capillovirus*. In all nine samples where the TriFoCap assay was

positive, at least one virus expected to react with this assay was detected. This provided support for the use of the broad spectrum TriFoCap assay. However, the TriFoCap assay will not identify the specific virus species present; that additional information would need to be obtained by further RT-PCR analyses or by sequencing.

- 5. Apple green crinkle disease can be a serious disease that can render a crop unmarketable. The disease is difficult to diagnose since the etiological agent is unknown and the disease only appears under certain environmental conditions. Adding to the uncertainty in diagnosing apple green crinkle disease is that feeding on young apple shoots by rosy apple aphids (*Dysaphis plantaginea*) can lead to fruit deformation that resembles apple green crinkle disease. The saliva injected into the plant by the aphid is translocated to the fruit where the fruit symptoms develop. Deep sequencing was being explored as a tool to confirm the association of a specific virus to the disease. In a single tree sample number 119.65 that exhibited apple green crinkle-like symptoms, the suspected plant virus could not be detected by RT-PCR or by deep sequencing. However, in leaf samples from this tree, deep sequencing did reveal the presence of *Rosy apple aphid virus* (genus: *Densovirus*), a virus that replicates in aphids and is present in the salivary material secreted by aphids. Although this virus is not thought to replicate in plants, its presence in this tissue sample confirms that rosy apple aphids had infested the tree and that the symptoms were likely the result of that feeding rather than by apple green crinkle disease. Thus, deep sequencing resolved an incongruity obtained from RT-PCR analysis.
- 6. A virus sequence that is similar to *Citrus leaf blotch virus* (genus: *Citrivirus*) was detected in two plum samples from Israel. This virus is known to induce stem pitting in citrus hosts, and no insect vector is known. The complete genome of this virus was obtained by both deep sequencing and RT-PCR so there is no doubt that the sequences are viral in nature. However, neither the incidents of this virus in stone fruits nor the impact of the virus on fruit quality and yield are known.
- 7. Partial genomic sequences of four additional new viruses were revealed by deep sequencing. Although the complete genomes were not obtained in this project, the segments of virus-specific sequence were quite large, and thus provide convincing evidence that novel viruses are present. The virus sequences are related to four different virus genera, and members of each genus are known to be insect transmitted. Additional sequencing will be needed to confirm that all of these virus-like sequences are associated with viruses and to confirm the identity of the viruses.

These results provide a measure of the power of deep sequencing to reveal the presence of virus sequences with no prior knowledge of the pathogens present at the initiation of the test. Because several of these viruses were previously undescribed, no virus-specific assay system existed to detect them. Even within a virus species, considerable sequence variation can occur. If sequence differences occur at specific nucleotides, they could render the RT-PCR assay unreliable. This is exemplified by the detection of a virus sequence related to *Little cherry virus 2* in a symptomatic tree. The detailed analysis provided by deep sequencing suggests that a distinct variant of *Little cherry virus 2* could be responsible for the decline of the orchard. This relationship had remained hidden for three seasons because of the inability of the available RT-PCR assays to detect the virus.

The presence of a virus sequence in a particular plant does not necessarily mean that the virus is pathogenic. Although most viruses rob vital metabolites from the tree and thus reduce growth to some degree, information about more serious effects cannot be predicted. Obtaining virus sequences via deep sequencing is just the first step from which we can determine the biology of the associated viruses including its mode of transmission and its impact on production. Fortunately, the results of deep sequencing provide the sequence information that is necessary to build other testing formats that allow such investigations to proceed. The utility of this technology is clearly demonstrated by this preliminary assessment.

#### **EXECUTIVE SUMMARY**

#### Project Title: Evaluating a Universal Plant Virus Microarray for virus detection

The object of this project was to investigate the utility of contemporary laboratory tools for the reliable detection of plant viruses. The two technologies investigated were the use of microarrays and deep sequencing. Although both strategies have the potential to detect pathogens without prior detailed knowledge of the viruses present, deep sequencing emerged as the preferable method. The use of a previously developed microarray would detect and identify previously uncharacterized viruses to the genus level; however, the technique was unable to unravel the individual viruses that could exist as complexes in fruit trees. Moreover, characterization of the virus(es) required reliance on additional sequencing reactions. For these reasons, deep sequencing provided much more precise information about the virus(es) present in a given sample. In this assessment of the application of deep sequencing to 68 samples, several new viruses and virus variants were identified. The deep sequencing results provided an important foundation for further investigation of the viruses detected. The sequence data can be used directly for development of virus-specific assays that could be used in studies of virus host range and vectors. The economic impact of each virus must be determined. This is a combination of the virus impact on fruit quality and quantity, and the ability to move quickly to adjacent fruit trees. These important questions of epidemiology are beyond the immediate goal of virus detection.

# NEW PROJECT PROPOSAL

## **PROPOSED DURATION: 3 YEARS**

**Project Title:** Efficient strategy to diagnose important virus disease of fruit trees

PI:	Ken Eastwell	<b>Co-PI(2):</b>	Dr. Tefera Mekuria
<b>Organization</b> :	Washington State University	<b>Organization</b> :	Washington State University
Telephone:	509-786-9385	Telephone:	509-786-9206
Email:	keastwell@wsu.edu	Email:	tmekuria@wsu.edu
Address:	24106 N Bunn Rd	Address:	24106 N Bunn Rd
City/State/Zip:	Prosser, WA 99350	City/State/Zip:	Prosser, WA 99350

<b>Co-PI(3):</b>	Dr. Dan Villamor			
Organization:	Washington State University			
Telephone:	509-786-2226			
Email:	dvillamor@wsu.edu			
Address:	24106 N Bunn Rd			
City/State/Zip: Prosser, WA 99350				

Cooperators: Dr. Shulu Zhang, Senior Research Scientist, Research & Development, Agdia, Inc.

Total Project Request: \$109,256	Year 1:	\$35,000 Ye	ear 2: \$36,400	<b>Year 3</b> : \$37,856
Percentage time per crop: Apple	: 33%	Pear: 0%	Cherry: 67%	Stone Fruit: 0%

#### Other funding sources: None

#### WTFRC Collaborative expenses: None

Budget 1				
<b>Organization Name:</b>	Washington	n State University	<b>Contract Adminis</b>	strator: Carrie Johnston
Telephone:	(509) 335-4	4563	Email address:	carriej@wsu.edu
Item		2013	2014	2015
Salaries		$$17,717^{1}$	\$18,426 <sup>1</sup>	\$19,163 <sup>1</sup>
Benefits		\$7,025 <sup>2</sup>	\$7,306 <sup>2</sup>	\$7,598 <sup>2</sup>
Wages		\$0	\$0	\$0
Benefits		\$0	\$0	\$0
Equipment		\$0	\$0	\$0
Supplies		\$10,258 <sup>3</sup>	\$10,668 <sup>3</sup>	\$11,095 <sup>3</sup>
Travel		\$0	\$0	\$0
Miscellaneous		\$0	\$0	\$0
Plot Fees		\$0	\$0	\$0
Total		\$35,000	\$36,400	\$37,856

Footnotes:

A Post Doctoral Research Associate and a Research Associate at 20% of full time, each.
 Benefits calculated at the state standard rate.

3. Purchase of enzymes and primers; deep sequencing of virus isolates.

#### JUSTIFICATION

Virus diseases can rob orchards of the profit margin necessary for their economic sustainability. Although there are no remedies that can effectively cure a tree once it has become infected with virus, implementation of aggressive virus management is required to maximize economic returns from a fruit tree orchard. Generally, viruses cause the reduction in fruit quality and quantity, and the resulting loss in economic return will be seen each year after infection occurs. Moreover, valuable resources may be squandered on attempts to improve the yield of an infected tree. It is believed that tree-to-tree spread of viruses in the orchard can occur through root grafting. Additionally, the transmission of many viruses is facilitated by nematodes, insects and other arthropods. Therefore, it is important for the economic well being of an orchard operation to quickly and correctly diagnose the presence of virus infections. While the cost effective enzyme-linked immunosorbent assay (ELISA) is practical for many viruses, it is not sensitive enough to detect many viruses that occur in low concentrations. Furthermore, the development of usable serological assays for many viruses is a formidable task. Molecular techniques such as polymerase chain reaction (PCR) have become increasingly available, but the cost for routine testing is prohibitive in many cases. PCR is a valuable tool but its success depends on the careful preparation of target nucleic acids from the sample. Current preparation procedures account for approximately one-half of the cost of analysis. Additionally, specialized equipment is required to perform the analysis by PCR. We wish to develop new diagnostic technologies that reduce this cost substantially, and that could be implemented in field offices for more rapid response to diseases in question.

#### **OBJECTIVES**

Previous projects funded by the Washington Tree Fruit Research Commission (WTFRC) led to the development of a library of virus sequences representing some of the most economically important viruses in the Pacific Northwest. We are seeking funds to translate this information into the development of detection strategies. One format currently being explored is recombinase polymerase amplification (RPA) assay. Preliminary results obtained for *Little cherry virus 2* (LChV2) suggests that this technique offers a cost-effective diagnostic method. We wish to further develop this method for other fruit tree viruses. Initially, we are focusing on three critical diseases of cherry and apple. When the best strategy for developing RPA systems is established, the use of RPA could be expanded to include the detection of many diseases associated with other pome and stone fruit crops.

Specific objectives of this project are:

- 1. Validate use of RPA for the detection of LChV2. This is the highest priority because of the recent escalation in disease in Washington State;
- 2. Develop RPA for the rusty mottle group of cherry viruses, a complex group of viruses that moves into cherry orchards from surrounding native vegetation;
- 3. Develop RPA for the identification of apple stem pitting virus associated with apple green crinkle disease. This will aid in confirming the etiology of the disease and provide a diagnostic tool to growers.

#### **METHODS**

1. With previous funding from the WTFRC, we constructed a significant database of virus sequences from different isolates of LChV2 in Washington State. The database was used to identify relatively short conserved regions within the highly variable virus genome. This was a critical first step in building a useful assay system, and allowed for a preliminary favorable assessment of sample preparation methods for RPA. With the requested funding, the test will be subjected to complete validation in samples derived directly from grower orchards to verify the accuracy of the test, and to verify that the test will detect LChV2 from a wide range of isolates. The results will be compared to those obtained with traditional reverse transcription polymerase

chain reaction (RT-PCR) assays. This assessment should be completed within the first growing season of the project.

Once validity of the test is confirmed, other test formats will be evaluated. The RPA format used in the preliminary trials required analysis by gel electrophoresis as a final step. RPA is adaptable to several different test formats that can be applied in field station settings including a simple real-time fluorescence detection system and a lateral flow detection system to replace the electrophoresis step. These will be evaluated for their accuracy and sensitivity relative to current RT-PCR methods.

- 2. Although little cherry disease is a major cause of concern in Washington cherry production, several other diseases that can be devastating to cherry production persist in the production areas. Members of the rusty mottle group of viruses are thought to reside in native vegetation from which they can migrate into sweet cherry orchards. Previous studies by our team have characterized several of the viruses of this group. The sequence information that we obtained will be the base from which additional RPA will be developed. Preliminary testing will be conducted in the first growing season with final test validation in the second and third seasons.
- 3. The agent that causes apple green crinkle disease is one of the most difficult pathogens to detect in fruit trees. The biological assay requires three cropping years and the validity of the test is weather dependent. Funding will be used in the first two growing seasons of the project to confirm the identity of the agent(s) associated with apple green crinkle disease. The knowledge gained from the first two years of experience with the cherry viruses will allow the development of a workable RPA for the agent, and this can be completed in a single growing season.

This project is centered on the benefits of the RPA for detection of fruit tree viruses. Preliminary test results have been very favorable for LChV2 and the desire is to expand the repertoire of RPA to include several other viruses of fruit trees that have been recalcitrant to current diagnostic methods. Should the RPA fail to live up to expectations, the project will be terminated or a new direction sought.

#### LITERATURE REVIEW

Reverse transcription polymerase chain reaction (RT-PCR) has become a standard diagnostic tool for the identification of viruses associated with diseases of fruit trees. It is a very powerful analytical technique. However, the enzyme systems used in this system are notoriously sensitive to many sample products that contaminate nucleic acid isolations (Rådström et al. 2004; Demeke & Jenkins, 2010). The presence of these inhibitors can result in the failure of the RT-PCR and a false negative interpretation of the result. Techniques to eliminate or reduce these inhibitors can be costly and technically complicated (Demeke & Jenkins, 2010). In the current diagnostic program at Washington State University, 47% of the costs of performing RT-PCR are associated with sample preparation. There have been many efforts to identify enzyme systems that are more tolerant of the contaminants that reduce the reliability of RT-PCR (Bekkaoui et al., 1996; Piepenburg et al., 2006; Kim & Easley, 2011). Methods are also being sought to reduce the need for instrumentation capable of rapid temperature cycling (Bekkaoui et al., 1996; Kim & Easley, 2011). One method has emerged with great promise: the recombinase polymerase assay (RPA) (Piepenburg et al., 2006; Kim & Easley, 2011). This method is adaptable to several formats that can be used reliably in field or remote station applications (Piepenburg et al., 2006; Lutz et al., 2020), and has found to be robust in the detection of viruses in many systems that are plagued by inhibitors of traditional RT-PCR (Euler et al., 2012a, 2012b, 2013, and Zhang et al., 2012). Specifically, RPA has been demonstrated to perform reliably with the detection of viruses in crude extracts from stone fruit trees without any preparatory sample processing (unpublished observations; and Zhang et al., 2012). Therefore, RPA could provide

substantial savings in the cost and time necessary to provide growers with important information needed in their efforts to manage diseases of orchards. The portability of the RPA technology also provides greater accessibility to advanced diagnostic technology (www.twistdx.co.uk).

- Bekkaoui F, Poisson I, Crosby W, Cloney L, Duck P (1996) Cycling probe technology with RNase H attached to an oligonucleotide. Biotechniques 20:240-248.
- Demeke T, Jenkins GR (2010) Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. Analytical and Bioanalytical Chemistry 396:1977-1990.
- Euler M, Wang Y, Nentwich O, Piepenburg O, Hufert FT, Weidmann M (2012a) Recombinase polymerase amplification assay for rapid detection of Rift Valley fever virus. Journal of Clinical Virology 54:308-312.
- Euler M, Wang Y, Otto P, Tomaso H, Escudero R, Anda P, Hufert FT, Weidmann M (2012b) Recombinase polymerase amplification assay for rapid detection of *Francisella tularensis*. Journal of clinical microbiology 50:2234-2238.
- Euler M, Wang Y, Heidenreich D, Patel P, Strohmeier O, Hakenberg S, Niedrig M, Hufert FT, Weidmann M (2013) Development of a panel of recombinase polymerase amplification assays for the detection of biothreat agents. Journal of clinical microbiology doi: 10.1128/JCM.02704-12.
- Kim J, Eadley CJ (2011) Isothermal DNA amplification in bioanalysis: strategies and applications. Bioanalysis 3:227-239.
- Lutz S, Weber P, Focke M, Faltin B, Hoffman J, Müller C, Mark D, Roth G, Munday P, Armes N, Piepenburg O, Zengerle R von Stetten F (2010) Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermal recombinase polymerase amplification (RPA). Lab Chip 10:887-893.
- Piepenburg1 O, Williams CH, Stemple DL, Armes NA (2006) DNA detection using recombination proteins. PLOS Biology 4:1115-1121.
- Rådström P, Knutsson R, Wolffs P, Lövenklev M, Lofstrom C (2004) Pre-PCR processing: Strategies to generate PCR-compatible samples. Molecular Biotechnology 26:133–146.
- Zhang S, Bohannon R, Russell P, McOwen N, Bohannon S, Vrient A, Sutula C (2012) Rapid and simple detection of Plum pox virus by recombinase polymerase amplification. Phytopathology 102:S4.143.

# **YEAR**: 1 of 1

#### **CONTINUING PROJECT REPORT WTFRC Project Number:** TR-12-103

**Project Title**: Design and development of apple harvesting techniques

PI:	Manoj Karkee	<b>Co-PI (2):</b>	Qin Zhang
<b>Organization</b> :	WSU CPAAS, Prosser	<b>Organization</b> :	WSU CPAAS, Prosser
Telephone:	509-786-9208	Telephone:	509-786-9360
Email:	manoj.karkee@wsu.edu	Email:	qinzhang@wsu.edu
Address:	24106 N. Bunn Rd	Address:	24106 N Bunn Rd
City/State/Zip	Prosser, WA 99350	City/State/Zip	Prosser, WA 99350
<b>Co-PI(3):</b>	Karen Lewis	<b>Co-PI</b> (4):	Mark De Kleine
<b>Organization:</b>	WSU Extension and CPAAS	<b>Organization:</b>	WSU CPAAS, Prosser
Telephone:	509-760-2263	Telephone:	509-786-9257
Email:	kmlewis@wsu.edu	Email:	m.dekleine@wsu.edu

City/State/Zip: Prosser, WA 99350

City/State/Zip: Prosser, WA 99350

24106 N Bunn Rd.

Cooperators: None

Address:

**Total Project Request:** Year 1: \$53,395

24106 N. Bunn Rd

Percentage time per crop: Apple: 100%

# Other funding sources NONE

Address:

Budget 1 Organization Name: WSU Telephone: 509.335.4564

Contract Administrator: Carrie Johnston Email address: carriej@wsu.edu

<b>Telephone:</b> 509.335.4564				
Item	2012-2013			
Salaries <sup>1</sup>	\$30,534			
Benefits <sup>1</sup>	\$1,997			
Wages <sup>2</sup>	\$6,240			
Benefits	\$624			
Equipment				
Supplies <sup>3</sup>	\$10,000			
Travel <sup>4</sup>	\$4,000			
Plot Fees				
Miscellaneous				
Total	\$53,395			

Footnotes:

<sup>1</sup> Salary and benefit for a graduate student

<sup>2</sup> Wages and benefits for hourly help to fabricate sensor platform and collect field data

<sup>3</sup> Cost to purchase materials and build sensor platforms

<sup>4</sup>Travel cost for field data collection and testing

# Objectives

Our long term goal is to improve the sustainability and productivity of tree fruit production through reduced labor use and associated risks and costs. Originally, this project was proposed for three years with the following specific objectives.

- 1. Design and develop two prototypes for semi-automated apple harvesting techniques.
- 2. Characterize the efficiencies of harvesting in two variations of fruiting wall architectures.

However, the project was funded for only the first year to demonstrate the feasibility of the concept. The scope for the first year for the project involves prototype development and preliminary evaluation in lab and field environment.

# **Significant Findings**

- Vertical twisting with compressive pressure can remove apples from a spur.
- Damage can occur if the apple is rolled against the limb.
- Fruit removal classified as 'stem intact- no spur', is significantly dependent on rotational direction and cultivar.

# Methods

Two methods for apple removal are being investigated and evaluated based on fruit removal effectiveness and fruit and spur damage. The preliminary results have been used to modify the apple harvesting system design. The two methods focus on twisting apples in vertical and horizontal directions.

Fabrication and initial testing with the first proof-of-concept prototype was completed in fall 2012. The prototype consisted of two six inch rubber tires mounted to two Drillmaster 18V, 3/8" electric hand drills, as shown in Fig. 1.



Fig. 1: First apple twisting prototype (hand-held) built with rubber tires mounted to electric motors.

Two input variables were defined as speed and rotational direction. Speed was divided into three levels: 240RPM, 420RPM, and 890RPM. Wheel rotational direction was either counter-clockwise

(CCW) or clockwise (CW). Harvested apples were classified into three different categories: stem intact– no spur; stem not intact– no spur; stem intact– spur attached.

Five levels of wheel speed was used for testing: 1) Slow-Slow, 2) Slow-Medium, 3) Medium-Medium, 4) Medium-Fast, 5) Fast-Fast, corresponding to each wheel. Direction was classified into three levels: 1) CW-CW, 2) CW-CCW, 3) CCW-CCW, corresponding to each individual wheel.



Fig. 2: Prototype harvester wheel placement on an apple.

The two wheels were place on either side of an apple as shown in Fig. 2. Adequate pressure was applied so that the wheel does not slip on the apple skin. As the wheels spun, a twisting motion was applied to the apple about the stem abscission point.

#### **Results and Discussion- Harvest**

Fruit removal with stem intact–no spur ranged from 36% for 'Granny Smith' to 86% for 'Pacific Rose'. Apple cultivar had a significant effect on the fruit removal condition (p=0.000) at a 95% confidence level. Apples removed with stem not intact ranged from 8% on 'Pacific Rose' to 64% on 'Golden Delicious'. The highest percentage of apples removed with spurs attached was 10% in 'Jazz'. The direction of rotation had a significant effect on the fruit removal condition (p=0.000) at a 95% confidence level.

	Jazz	Pacific Rose	Golden Delicious	Granny Smith	Jonagold
Stem Intact – No Spur	70%	86%	80%	36%	58%
Stem Not Intact – No Spur	20%	8%	18%	64%	40%
Stem Intact – Spur Attached	10%	6%	2%	0%	2%

#### Table 1 Harvesting results for five cultivars of Washington apples.

The desired classification is stem intact – no spur. When variables were set at slow-slow and CW-CCW, 100% of removed apples, across all cultivars, did not meet this classification. When the wheel speed changed to medium-medium, at CW-CCW, 89% of the removed apples did not meet this classification. Practically speaking, when the rotation of the wheel is opposing (CW-CCW), the apple is pulled away from the branch rather than twisted. For opposing rotational direction and speeds set at either slow-medium or medium-fast, 33% and 56% of apples were not classified as stem intact– no spur, respectively. Although the same principle of pulling rather than twisting holds true, the slight

variation in speed tended to apply a minor twist or rotation. This potentially helped increase the percentage of stem intact– no spur classified apples.

The highest rate of stem intact– no spur for all varieties occurred at CW-CW direction for slowmedium and medium-medium speeds. Fruit removal at these speeds resulted in 89% of the apples being categorized as stem intact– no spur. For 'Golden Delicious', 100% of the harvested apples that were categorized as stem not intact– no spur or stem intact– with spur attached occurred when the speed of the wheels were equal to each other. For 'Granny Smith' and 'Jonagold' cultivars, 66% and 67% of the harvested apples that were categorized as stem not intact– no spur or stem intact– with spur attached occurred when the speed of the wheels were equal to each other.

## **Discussion- Punctures**

Initial tests, on 'Gala' apples, show that twisting in a vertical direction has the potential to remove fruit from limbs. It was also observed that rolling an apple across a branch can cause damage to the apple. More specifically, uncontrolled rolls across a limb can puncture the fruit. Based on this observation, a separation barrier was fabricated to facilitate a controllable shoulder to roll the apple on. A simple wireframe structure separated the apples from the limbs (Fig. 3).

In efforts to reduce the number of stem pulls, razor blades were mounted to the separation barrier to slice or cut the stem entirely. This concept deviates slightly from the initial proposal but collectively focuses on the overall objective of fruit removal. Tests will be continued during the 2013 apple harvest season. Additional adjustments in the structural design of the harvesting system are expected throughout the remainder of the project. We do expect that stem pulls will continue to be a challenge but we will investigate different ways to minimize them as we develop and evaluate improved prototypes.



Fig. 3: Wireframe separation barrier used for puncture reduction testing.

The remainder of year 1 will focus on the improvement and scaling of the harvesting prototype for trellised orchards trained to both random and formal architectures. During the winter season, the focus will be on developing a multiple wheel structure, and the addition of the bioyield pressure applicator (Fig. 4). It is expected that phase II of the prototype harvester will be completed by early summer 2013.



Fig. 4: Phase II of the prototype apple harvester.

## Conclusions

The initial results from year 1 show a successful fruit removal technique applicable to trellised apples. Signs of branch punctures were visible on some of the apples. A method to seclude the apple from any branch or ensure that no branches can be pressed between the wheel and the apple skin was attempted. Although no initial measurements were made to classify the varying degrees of this type of damage, it will be persistently examined in year 2 and 3\*. Apples located on short spurs tended to be removed easier than apples growing on long flexible branches. apples located on long branches moved more freely and this flexibility reduced the twisting, or torque, that was ultimately transferred to the apple and stem resulting in less effective removal rates. Horticulture and genetics or phenotype can play an important role in aiding the fruit removal technique described in the above research.

Table 2 shows the project timeline for the originally proposed duration of the project. Year one focuses mainly on hand harvest evaluation, prototype design and evaluations. All of these tasks are currently being carried out and will be continued through the 2013 harvest season. Prototype end effector design and improvement will continue through the winter. Complete evaluation of the prototype will be during the 2013 and 2014 harvest seasons. Grower feedback, suggestions, and evaluation will continue to occur in informal interviews and a symposium during the winter. It is noted that the activities proposed for Year 2 and Year 3 are contingent upon our success on securing further funding for the project.

	Year 1	Year 2	Year 3
Grower Input			
Grower Feedback			
Grower Evaluation			
Hand Harvest Evaluation			
Prototype End Effector Design			
End Effector Phase 2			
Lab and Field Evaluations			
Preliminary Economic Evaluation			
Machine Integration and Demonstration			

\*Note: Activities in Year 2 and Year 3 are contingent upon our success on securing further funding for this project.

# **CONTINUING PROJECT REPORT** WTFRC Project Number: TR-11-101

Project Title: 3D machine vision for improved apple crop load estimation

PI:	Manoj Karkee	<b>Co-PI (2):</b>	Qin Zhang
Organization:	Center for Precision and	<b>Organization</b>	Center for Precision and
Telephone:	Automated Ag Systems, WSU 509-786-9208	Telephone:	Automated Ag Systems, 509-786-9360
Email:	manoj.karkee@wsu.edu	Email:	qinzhang@wsu.edu
Address:	24106 N. Bunn Rd.	Address:	24106 N. Bunn Rd.
City/State/Zip	o: Prosser, WA 99350	City/State/Zij	p: Prosser, WA 99350
Co-PI (3): Organization:	Karen Lewis WSU Extension		

**Telephone**: 509-760-2263 Email: kmlewis@wsu.edu Address: Courthouse Address 2: P.O. Box 37 City/State/Zip: Ephrata, WA 98823

Cooperators: None

**Total Project Request: Year 1**: \$33,104 **Year 2**: \$34,402

**Percentage time per crop:** Apple: 100% Pear: 0% Cherry: 0% Stone Fruit: 0%

# Other funding sources: None

Budget 1		
<b>Organization Name: WSU</b>	Contract Administra	ator: Carrie Johnston
Telephone: 509.335.4564	Email address: carrie	ej@wsu.edu
Item	2011-2012	2012-2013
Salaries <sup>1</sup>	\$22,901	\$23,817
Benefits <sup>1</sup>	\$1,821	\$1,893
Wages <sup>2</sup>	\$6,264	\$6,515
Benefits <sup>2</sup>	\$601	\$625
Supplies	\$1,000	\$1,000
Travel <sup>4</sup>	\$517	\$552
Plot Fees		
Miscellaneous		
Total	\$33,104	\$34,402

Footnotes:

<sup>1</sup> Salary and benefit for a graduate student
 <sup>2</sup> Wages and benefits for hourly help to fabricate sensor platform and collect field data

<sup>3</sup> Cost to purchase materials and build a sensor platform

<sup>4</sup>Travel cost for field data collection and testing

# **OBJECTIVES**

The following were the specific objectives of this project.

- 1. Develop a sensor system with 3D and color vision cameras for imaging apple trees from both sides of the tree canopy
- 2. Develop an image processing technique to create 3D maps of the fruit and estimate apple crop-load
- 3. Evaluate and improve the accuracy of crop-load estimation

# SIGNIFICANT FINDINGS

- Visibility of apples increased substantially when images were taken from both sides of the tree canopy.
- The mapping algorithm developed in the laboratory setting showed promise for reducing repeat counting by co-registering of 3D images taken from both sides of the tree canopy.
- Over-the-row sensor platform with a tunnel structure minimized variability in lighting condition and background, which helped improve image processing techniques for fruit identification and mapping.

# **METHODS**

To reduce occlusion caused by nearby fruit, leaves and branches, images of apple trees were taken from both sides of the tree canopy. Because some apples were visible from both sides of the canopy, a single apple could be counted twice. A 3D camera was incorporated into the system to minimize recounting of the same apple by measuring the position of the apples on the tree. In the following paragraphs, we describe the sensors, platform, and algorithms we have been developing to capture and analyze images for improved crop-load estimation.

<u>Spatial Calibration of Sensors:</u> The sensor system consists of a color camera and a 3D camera (Fig. 1). A Prosilica camera (GigE 1290c, Allied Vision Technologies, Stadtroda, Germany) was used to capture color images of apple trees with fruit. A PMD camera (CamCube 3.0, PMD Technologies, Siegen, Germany) was used to take 3D images. These 3D images are used in conjunction with the color images to minimize repetitive counting of apples.





# Fig. 1: Sensors used for image acquisition in laboratory setup (a), and in field tests (b). Prosilica GigE 1290c color camera is on the top of the camera mount and PMD CamCube 3D camera is on the bottom.

A checkerboard-based camera calibration technique was used to identify intrinsic and extrinsic parameters of the color camera and 3D camera. A checkerboard was placed in front of the imaging system in such a way that it appeared within the imaging field-of-view of both cameras. The image from the color camera (Fig 2a) and the intensity image (Fig 2c) obtained from the 3D camera were used to calibrate for intrinsic and extrinsic camera parameters. The extrinsic parameter gives the relative position of two cameras. Using these parameters, the 3D coordinates from the 3D camera (Fig 2c) were projected onto the image plane of the color camera to obtain distance-mapped color images.



Fig. 2: Checker board-based camera calibration; a) original color image of checker board, b) distance image of the board, and c) intensity image of the board

**Distance Calibration:** The 3D camera we have used requires distance calibration to improve the accuracy of 3D mapping. For distance calibration, measurements of about two hundred reference points from a known camera distance were taken. A checkerboard surface (Fig. 3) was placed and imaged at six different positions from the camera in a range of 0.5 - 2.0 m. Images were taken with different combinations of integration times (200, 250 and 300 µs) and frequencies (19, 20 and 21 MHz) of the 3D camera. Random points within the checkerboard surface were selected and their actual distances from the camera were measured manually. Estimated distances from the camera to the corresponding points were provided by the 3D camera.



Fig. 3: Imaging of checkerboard surface for distance calibration

Manually measured distances were compared with distances provided by the camera at different combination of frequencies and integration times. The combination that caused the least root-mean-squared error between actual and estimated distances was selected for future imaging. Then a mathematical model was developed to calculate corrected distance using 180 new measurements. The model is represented by,

$$\begin{split} D &= 0.044 - 0.028 * x + 0.075 * y + 1.009 * d + 0.004 * d^2 - 0.005 * d^3. \\ & \text{Where } D = \text{corrected distance,} \\ & d = \text{measured distance, and} \\ & x, y = \text{ image coordinates.} \end{split}$$

The calibration model was tested with 53 points that were not used for model development. The predicted distance was compared with distance measured manually to evaluate the accuracy of the model. The root-mean-squared distance error for the test points was found to be 2 cm.

<u>3D Mapping Algorithm Development:</u> Images captured from both sides of a tree canopy in 2011 and 2012 have shown substantial increase in apple visibility. However, it is also evident that some apples are visible from both sides (Fig. 4), thus requiring 3D mapping of apples to avoid duplicate counting.



Fig 4: a) and b) are the images captured from two opposite sides of an example tree canopy. Apples visible from both sides are circled.

An algorithm was developed using a laboratory set-up to register images captured from both sides. Color and 3D images of a model of an apple tree (a real, dead tree with fake leaves and fruit on it; Fig. 5a) were captured. The 3D coordinates of objects in the field-of-view were transformed from the 3D camera coordinate system to project on the imaging plane of the color camera so as to obtain a distance-mapped color image. Each pixel in this distance-mapped color image included color information with the corresponding 3D location information.



Fig. 5: a) and b) Color images from front and back side of the tree; c) 3D-mapped apples of corresponding color images in (5a) as hollow circles and (5b) as solid circles.

The center of apples visible from each side of the canopy was located as shown in Fig. 5a and 5b. 3D locations of the four corners of the reference frame (GI pipe square in Fig. 5a, and 5b) were used to obtain the rigid transformation between these two camera positions. Using the rigid transformation, all the corresponding apple locations from one side of the canopy were transformed to the coordinates on the other side. Fig. 5(c) shows 3D locations of apples viewed corresponding to Fig.5a (hollow

circles) and Fig. 5b (solid circles) respectively. The apples visible from both sides of the canopy can be seen overlapping each other. Apples within a distance of less than the diameter of corresponding apples were considered to be the same apple mapped from the opposite sides.

**Platform Modification and Data Collection:** Field data was collected in the 2011 harvest season with the first prototype of the over-the-row sensor platform. An improved sensor platform was designed and fabricated in 2012 (Fig. 6) based on knowledge learned in 2011. The new platform was lighter and more robust than the earlier platform. A fixed platform width was used in the new design to reduce unnecessary degrees of freedom. The platform included a sliding mechanism for convenient mounting and positioning of cameras. The images acquired during daylight in 2011 harvest season were affected by variation in lighting conditions such as presence of direct sunlight and shadows. To eliminate variations in lighting conditions, a tunnel structure was added to block direct sunlight in the tree canopy during imaging. An artificial lighting system was integrated to create a controlled lighting environment while taking images. The artificial lighting system also added capability for nighttime operation (Fig. 6b and 6c).





Fig. 6: a) A tall spindle commercial orchard of Allan Bros., Inc. in Prosser, b) and c) new overthe-row platform taking images of Jazz apples during daylight and night-time.

Data collection in commercial orchards with the improved platform began the week of September 24<sup>th</sup> and continued until the last week of October. We collected daylight images of Jazz apple trees in tall spindle architecture (row spacing 9'0" and inter-plant spacing 3'10") in Prosser, WA (Yakima Valley Orchards) (Fig. 6(a)). The night-time images in Jazz and Fuji apple trees were collected in Prosser and Grandview, WA (commercial orchards of Allan Bros., Inc.). Human manual counts included number of apples visible from each side of the tree canopy, number of apples visible from both sides of the canopy (repeat counts) and total number of apples.

<u>Apple Identification Algorithm Development:</u> An algorithm was developed to identify and count apples from images captured in commercial orchards (Fig. 7). To minimize the counting error due to clustering of apples, the ratio of major axis and minor axis of identified apples was determined. If the ratio was greater than three, it was assumed that the identified object is a cluster of two apples. Currently, it is assumed that a cluster does not include more than two apples. This limitation will be

addressed in the remaining period of the project. The apple count estimated from both sides of the tree canopy was added to obtain the total number of apples. Finally, the count of apples visible from both sides was obtained (so far manually) from the images and was subtracted from the total count. Application of 3D mapping technique for automatic removal of duplicate counting and for apple sizing will also be addressed in the remaining period of this project.



Fig. 7: Elliptical shapes indicate apples identified from side A (a) and side B (b) of a sample tree canopy of Fuji apples In Grandview, WA

# **RESULTS AND DISCUSSIONS**

The improved sensor platform increased the efficiency of data collection in the field. It was easier to move in the orchards since it was lighter and more robust. The new sliding mechanism improved camera mobility. Images could be taken from different heights to ensure proper overlapping between images. The tunnel helped to reduce variability in lighting conditions. Images taken in a controlled lighting environment will make image processing much easier. Images taken at nighttime with LED lights showed promise for night time operation of the system.

Manual counting of apples revealed that the visibility of apples increased substantially when dualsided canopy imaging was used (Table 1). Average visibility of apples was 60-70% when imaged from one side, which increased to more than 95% (Table1) when imaged from two opposite sides. The apple identification algorithm was applied to day and night-time images. The algorithm was able to identify and count the number of apples in the image. Preliminary results showed root mean square error (RMSE) of 15.4% on identifying the apples from image analysis.

The algorithm developed in 2012 to co-register 3D and color images as well as 3D images from both sides of a canopy was able to register images captured in the laboratory setup. Results from the laboratory tests showed that duplicate counts of apples can be avoided by using distance between apples presented in a co-registered 3D map (Fig.8). The algorithm has shown promise for application to field data.

	Арј	Apple Count from Image (#)				V	/isibility	(%)
S.N	Side A	Side B	Duplicate	Total	Field Count (#)	Side A	Side B	Total
1	64	57	18	103	106	61	54	98
2	55	53	20	88	75	74	71	118
3	52	68	39	81	90	58	76	90
4	40	53	26	67	94	43	56	71
5	50	69	25	94	92	54	75	102
6	45	43	28	60	84	54	51	71
7	43	63	22	84	115	37	55	73
8	35	24	15	44	40	88	60	110
10	47	32	19	60	50	95	65	121

Table 3: Visibility of Apples for 10 sample trees



Fig 8: 3D mapped apples visible from front (hollow circles), back (solid circles) and both (hashed circles) sides (axes in millimeters).

In 2013, we will focus on improving and applying image processing and 3D mapping technique to the dataset collected in commercial orchards. A new set of images will be collected in 2013 using the improved sensor platform developed in 2012. We will also develop techniques to obtain position and orientation of the cameras on one side relative to the cameras on the other side of a canopy so that the accuracy of 3D mapping can be improved. Geometric information of the over-the-row sensor platform and orientation sensors (if necessary) will be used to obtain relative position and orientation information.

#### CONTINUING PROJECT REPORT WTFRC Project Number: TR 11-100

**YEAR**: 2 of 2

**Project Title**: Intelligent bin-dog system for tree fruit production (Phase II)

PI:	Qin Zhang	<b>Co-PI(2)</b> :	Karen Lewis
Organization:	Washington State Univ.	Organization:	Washington State Univ.
Telephone:	509.786.9360	Telephone:	509.754.2011 X 407
Email:	<u>qinzhang@wsu.edu</u>	Email:	kmlewis@wsu.edu
Address:	24106 N. Bunn Rd.	Address:	PO Box 37 Courthouse
City/State/Zip:	Prosser, WA 99350	City/State/Zip:	Ephrata, WA 98823

Co-PI(3):Long HeOrganization:Washington State UniversityTelephone:509.786.9257Email:long.he@wsu.eduAddress:24106 N. Bunn Rd.City/State/Zip:Prosser, WA 99350

Cooperators: WA Producers, Yakima Valley Orchards

**Total Project Request:** Year 1: 99,397 Year 2: 69,454

#### **Other funding sources:** None

Budget 1				
<b>Organization Name: W</b>	VA State University	<b>Contract Administrator: Carrie Johnston</b>		
Telephone: 509.335.76	67	Email address: carriej@wsu.	edu	
Item	2011-12	2012-13		
Salaries <sup>1</sup>	65,352	47,966		
Benefits	16,045	9,488		
Wages				
Benefits				
Equipment <sup>2</sup>	7,000			
Supplies &	5,000	6,000		
Fabrication Costs <sup>3</sup>				
Travel (Zhang) <sup>4</sup>	2,000	2,000		
Travel (Lewis) <sup>4</sup>	3,000	3,000		
Miscellaneous <sup>5</sup>	1,000	1,000		
Total	99,397	69,454		

**Footnotes:** <sup>1</sup> one Post-doctoral research associate (12 months) and one Ph.D. graduate student (12 months) for yr-1; one Post-doctoral research associate (12 months) for yr-2; <sup>2</sup> Budget for purchasing an existing bin-carrier platform; <sup>3</sup> Budget for fabricating bin-dog prototypes (yr-1 for the research prototype and yr-2 for the demonstration prototype (including NAPA parts); <sup>4</sup> Budget for travel will cover the expenses for research personnel traveling to experiment sites for conducting project activities; <sup>5</sup> A small miscellaneous budget is for all other project related expenses.

## **OBJECTIVES**

This report is the fourth progress report of the second phase of intelligent bin-dog research. The primary goal of this phase is to develop a prototype of a self-propelled "bin-dog" implementable in typical Washington/Oregon tree fruit orchards. To achieve this goal, the bin-dog prototype should have the following critical functionalities to be considered a success of this research: (1) capable of traveling in typical WA/OR tree fruit orchards using electrical maneuvering systems; and (2) capable of placing an empty bin at target locations in the row to support efficient picking and transporting a full bin to the designated bin landing area. The following specific project activities have been conducted to date for accomplishing the proposed project goal:

- 1. Defined a set of design specifications for this bin-dog prototype (completed in February 2012 and reported in 2012 Spring Progress Report);
- 2. Designed and built prototype-one of the bin-dog (including a remote control system) using off-the-shelf components based on the defined specifications for accomplishing the designated critical functionalities (completed in September 2012 and reported in 2012 Fall Progress Report); and
- 3. Conducted field tests to assess the capability of prototype-one in accomplishing the designated critical functionalities, and to investigate its usability and efficiency in both research and commercial orchards (completed in September 2012, and partially reported in 2012 Fall Progress Report).
- 4. Based on obtained test results, the limitations/problems of prototype-one have been analyzed, and a new prototype (prototype-two) is under design to accommodate the modifications for solving identified problems (the main reporting item of this Progress Report).

In summary, we have completed the design and fabrication of one bin-dog prototype (prototypeone), and tested it in both off-field environment and orchard environment over the past three reporting periods (September 2011 to January 2013) to prove the concept and validate the major functionalities. Obtained resulted verified that prototype-one of the bin-dog was capable of accomplishing the designated functionalities in both research and commercial orchards in Yakima valley region. However, we did find some limitations and/or weaknesses from those field tests. We discovered the swivel wheels turned so easily that the bin-dog occasionally got stuck, had difficulty traveling in a straight line, and its capacity to drive on slopes was limited. To solve the problems identified in field tests, we have designed a new prototype (prototype-two), which will be fabricated and available to conduct field testing in the 2013 harvest season in varied orchard conditions, including sloping orchards.

## SIGNIFICANT FINDINGS

## A fully functional self-propelled bin-dog prototype (prototype-one)

To prove the proposed concept of placing an empty bin at target location and transporting a full bin to bin landing area, the developed prototype-one, namely the remotely controlled self-propelled bin-dog prototype, has been tested for its capability of performing all defined operation steps in orchard environment. As depicted in Figure 1, the complete prototype-one consisted of five modules for performing those defined operations:

1) The main frame on which all other modules were installed;

2) A power unit consisting of a set of batteries and three DC motors with speed and direction control capabilities;

3) A front-wheel-driven electrical drive-train system with two DC motors installed directly on the two driving wheels;

4) A passive turning system accomplished using the speed difference of motors at both sides to push/pull two idle wheels making a desirable turn; and

5) An electro-mechanical bin handling system for picking up the bin as well as either lifting an empty bin for passing on a full bin or lifting a full bin for stacking it on another full bin at the collection area.



Figure 1. Illustration of the developed concept-approval bin-dog prototype

# In orchard tests of prototype-one in 2012 apple harvest season

In 2012 apple harvest season, functionality tests for the bin-dog prototype-one were conducted both in off-field environment and orchard environment (Figure 2).



Figure 2. Bin-dog prototype tested at WSU station in Prosser (a) and apple orchard (b)

The results of both laboratory and field tests verified that the developed Bin-dog prototypeone could accomplish all the functionalities defined by the concept.

Partial results have been reported on 2012 Fall Progress Report.

In that report, two empty bins were used in field tests. More field tests were conducted from the end of September through October 2012 after that report. In these tests, bins with full of apples were used to operate the functionalities of bin-dog. Table 1 shows the time for each step of the bin-dog operation process in the apple harvest orchard with the bins full of apples approximately 50' from the bin landing area of six randomly picked data sets out from numerous test runs. The result was very similar to that of the empty bin test (reported on 2012 Fall Progress Report). From Table 1, the time for each step varied a lot due to the limitations and/or weakness which will be illustrated in the following section.

Test		Ti	me for each s	step of Bin-	dog operatio	n process (s)		Total
set	Load an empty bin	Drive to full bin	Lift up empty bin	Go over full bin	Unload empty bin	Back to load full bin	Back to landing area	time (s)
1	12	40	4	20	12	20	39	147
2	13	35	4	17	7	21	38	135
3	13	39	4	23	13	27	33	152
4	13	45	4	32	6	22	44	166
5	15	38	4	26	6	24	39	152
6	25	38	4	24	7	13	43	154

Table 1. Time record for each step of the Bin-dog operation process (from six randomly picked tests)

## Issues which need to be addressed on prototype-one

According to the field tests using prototype-one in 2012, we found some problems and/or limitations with this prototype which could affect the efficiency and the reliability of the bin-dog when it is used for continuous operation and in complex orchards conditions.

- 1) In the developed prototype-one, two swivel wheels were used to aid steering. The swivel wheels turned too easily causing the bin-dog to get stuck and preventing it from moving in a straight path.
- 2) Prototype-one lacked a leveling adjustment and sufficient power to enable use in sloping orchards.
- 3) The reliability of the lifting system was hindered by the use of a winch with two wires connected to the lifting frame which occassionaly got stuck. Additionally, the winch was mounted on the top of the frame which increased the overall height of the bin-dog.
- 4) One or more lifting fingers might not engage during loading bins when the bins were on an uneven surface.
- 5) Batteries were insufficient, needing to be charged every few hours even with intermittent operation. If more functions are expected to be added on the bin-dog, the current power is a limitation.

To improve reliability and efficiency of the bin-dog, a new prototype (prototype-two) has been designed incorporating the needed modifications.

#### Modifications/improvements to be done on the new prototype (prototype-two)

Based on the problems/limitations found in field and laboratory testing, the following modifications or improvements will be addressed in prototype-two:

- 1) In order to be capable of working in sloping orchards, a novel bin-dog frame leveling system and a four-wheel-drive system have been designed for prototype-two.
- 2) To provide more precise steering control and make the steering wheels less load-sensitive, the two swivel wheels are replaced by two positive steering wheels in the new design.
- 3) To improve the reliability of bin lifting process, a scissor lifting system actuated by two cylinders will be used to replace the winch lifting system in prototype-two. Additionally this will decrease the height of the overall frame and added the capability of being a general platform for future use.

- 4) To improve the effectiveness of bin loading, two forks are used to load full bin/empty bin instead of four small fingers.
- 5) To provide sufficient power, a gas engine-driven hydrostatic powertrain will used to replace the battery-powered electrical-driven powertrain.

## PLANNING OF THE PROTOTYPE-TWO

#### Basic structure of prototype-two

Based on the basic concept of the bin-dog and the required functions, the newly designed prototype-two consists of a drive system, a steering system, a bin lifting system, and a frame leveling system (Figure 3). Gas engine-driven hydraulic power system will be used in this prototype.



Figure 3. Overview of the new designed prototype (prototype-two)

Based on the row spacing in typical high density orchards of 8-11 feet width and the typical bin size of  $48 \times 48$  inches used in those orchards, and referencing the developed bin-dog prototypeone, we have defined the initial design specifications for the bin-dog system as follows:

- Overall dimension (L x W x H):  $7.0' \times 6.0' \times 5.0'$  (7.0' high when goes over a full bin)
- Wheelbase (space between front and rear wheels): 6.0'
- Wheel space (space between two front/rear wheels): 5.0'
- Maximum working speed: 3.0 mph
- Four-wheel-drive/two-wheel-steer
- Capability of working in up to 30% grade of sloping orchards

#### Capability of working in slope road (bin-dog frame leveling system)

As approximately10% of WA/OR fruit tree acreage are on slopes of 20% or greater, the capability of working in sloping orchards for bin-dog is expected. A four cylinder actuated frame leveling system has been added in prototype-two design, which will allow the bin-dog functioning normally at sloping orchards up to 30% grade. Figure 4(a) shows the condition of bin-dog working uphill/downhill, and Figure 4(b) shows the condition on the side sloping orchard. For the individual adjustability of these four cylinder systems, the prototype-two is also capable of working in the orchards of combined slopes.





a) Drive on the sloping orchard

b) Drive on the side slope orchard

Figure 4. Illustration of bin-dog prototype-two working in sloping orchards

#### Four-wheel-drive

In order to provide the capacity to drive in sloping orchards, four-wheel-drive has been designed for the new prototype. The two-wheel-drive is switchable for higher speed requirements. In order to achieve required traction, low speed high torque hydraulic motors are selected for the drive system (Model: TL0170LS080AAA @ Parker).

## Steering system

Two separate hydraulic cylinders are used to actuate two steering wheels respectively. A  $30^{\circ}$  maximum steering angle is designed for this prototype. Based on the calculation, the specifications of cylinder for steering system are: 1.5" of bore size and 6" of stroke.

## Bin lifting system

A scissors mechanism is used for bin lifting system in the new prototype, and two cylinders are used to actuate the scissors at both sides to lift up/down bins. Based on the calculation, the specifications of cylinder for scissors bin lifting system are: 2.0" of bore size and 10" of stroke.

## Hydraulic maneuvering system

A gas engine-driven hydraulic power system is adopted in the new prototype. The specifications of selected power system are: a 9 Hp of gas engine and an 8 GPM of drive pump.



Figure 5. Hydraulic maneuvering system flow chart for bin-dog prototype-two

#### Remote control system design

The remote control is still used in this prototype-two, while some improvements will be added. More specifically, an auto guidance control system is is planned to be added to prototype-two if we have sufficient time.

- 1) A control pad will be used to replace the joysticks and buttons for simplifying bin-dog maneuvering in orchards.
- 2) An ultrasonic range finder will be added to gain a scrounging awareness in the vicinity of operating bin-dog to reduce the risk of collision caused by the incorrect operations.
- 3) A PID control algorithm will be added to the drive system and steering system to enhance the robustness of the system and the accuracy of the operations.

## **RESULTS AND DISCUSSION**

We have finalized the scheme of the new prototype, selected the key component for the new prototype, with some of them being ordered or under fabrication. We plan to complete the fabrication of the new prototype before 2013 apple harvest season, and test/validate the functionalities and efficiency of the prototype-two in a variety of orchard conditions. Table 2 summarizes the project management plan and the expected accomplishments for the remaining time of this project.

No.	<b>Planned Milestone</b>	<b>Time Period</b>	Planned Deliverables	Expected Accomplishments
1	Bin-dog prototype-two	01-03/2013	Calculation manual and	Structure and functionality
	design		design drawings	design of prototype-two
2	Bin-dog prototype-two	03-06/2013	A fully functioned bin-dog	A bin-dog prototype ready to
	fabrication/assembly		prototype-two	test in the lab/orchards
3	Remote control system	01-04/2013	Control programs for all	Hardware system and software
	development for		functionalities of prototype-	system for bin-dog remote
	prototype-two		two	control
4	Bin-dog prototype-two	07-10/2013	Data/photos/videos collected	Pre-tests/tuning before harvest
	pre-tests and orchard		from different orchards	season, and in-field tests in
	tests		operation to support the	sweet cherry/apple harvest
			functionality and effective of	operations
			bin-dog prototype-two	
5	Demonstrations and	11-12/2013	Final report	
	documentations			

 Table 2. Project Management Plan, Expected Outcomes and Accomplishments

# FINAL REPORT WTFRC Project Number:

## YEAR: 2012

#### Project Title: Protein-based foam for applying lacewings eggs to fruit trees by ATV

PI:	Thomas Unruh	<b>Co-PI(2)</b> :	Christopher Dunlap
Organization:	USDA-ARS	<b>Organization</b> :	USDA-ARS
Telephone:	(509) 454-6563	Telephone:	(309) 681-6339
Email:	thomas.unruh@ars.usda.gov	Email:	christopher.dunlap@ars.usda.gov
Address:	5230 Konnowac Pass Rd.	Address:	Room 3323
Address 2:		Address 2:	1815 N University St
City/State/Zip:	Wapato WA 98951	City/State/Zip:	Peoria IL 61604

Cooperators: David Horton, USDA-ARS Wapato, WA Gene Miliczky, USDA-ARS Wapato, WA Sinthya Penn, Beneficial Insectary, Redding CA

# Other funding sources

WTFRC/ Apple Crop Protection Amt. requested/awarded Total Project Request: Requested: \$239,663 / awarded: Year 1 (2010): \$79,117; Year 2(2011): \$79,866; Year 3 (2012): \$80,680. **Notes:** The lacewing portion of this grant overlaps with the foam project

# Pending:

Western SARE:	Total request: \$178,954
WTFRC Crop Protection:	Total Request \$ 237, 702

# **Budget History**

# **Organization Name: USDA-ARS**

Item	2012-Unruh	2012-Dunlap	TOTAL
Salaries			
Benefits			
Wages GS-3 (90/90 days)	\$7431	\$7431	
Benefits	\$569	\$569	
Equipment	\$ 400		
Supplies	\$600	\$1200	
Travel		\$800	
Miscellaneous			
Total	\$9000	\$10000	\$19,000

Footnotes:

# **OBJECTIVES**

- 1. Test formulations of various foaming agents using a foam generator and adapt foam generation to a modified 12-volt pump sprayer suitable for use on an ATV We have tested keratin and whey protein hydrolysates, saponin-containing Yucca extract and <u>Qullaja sopninaria</u> extract as foaming agents were tested using off-the-shelf foam sprayers and a sprayer under development. The latter device drops dry lacewing (LW) eggs into the foam stream after it leaves the pressurized portion of the sprayer and appears close to a final product. The remaining problem is in the geometry of the egg delivery system which allows the eggs to be blown out ahead of the foam and falling before the target is reached.
- 2. Test adhesion of foam to waxy, water repellent, surfaces and leaves of seedling apples and on bark

Initial efforts have been restricted to tests on artificial surfaces including Tyvec sheets plastic cafeteria trays (Wapato) and Plexiglass (Peoria. We have found that the foam produces by keratin, <u>Yucca</u> and <u>Quillaja</u> stick well to tree trunks

*3.* Test survivability of lacewing eggs in laboratory conditions when eggs are immersed in and sprayed with these foams

With each new formulation of foam producing liquid, measurement of survival after 30 minute submersion in the product is compared to submersion in water. With new spray technique where eggs are dropped into a trough and swept up in a stream of foam, survival has been tested with egg sprayed onto Tyvec surface or sprinkled into foam

- 4. Test adherence of LW eggs in foam on apple, pear and cherry trees in the greenhouse and the field and estimate hatch rates of eggs in those settings. In field experiments using tarps below trees, collect and estimate bounce-off and drop of sprays are desirable but have not yet been addressed.
- 5. Estimate colonization rates (proportion of eggs recollected as larvae) on test trees. Studies remain to be conducted in pears and in apples infested with aphids and pear psylla at the Moxee Farm. Preliminary studies could not be made in 2012 because of lack of aphids in our experimental farm orchards and our incapacity to apply eggs in foam during June-July

## SIGNIFICANT FINDINGS

- ✓ Keratin and whey protein hydrolysates, *Quillaja* and *Yucca* saponins can produce rich foam suitable for initial contact adherence to water repellent surfaces and tree trunks
- ✓ Passage of eggs through rotary diaphragm pumps damages >25% eggs requiring eggs be introduced into the stream of the foaming agent distal to the pump
- ✓ Eggs e introduced in a suspension medium separate from the foaming medium using Venturi aspirator has proven problematic accurate
- $\checkmark$  Eggs can be dropped into the spray stream of foam after foam leaves spray nozzle.
- ✓ Mixing of eggs with dry bulking material is necessary to meter eggs for above gravity feed.
- ✓ Long term adherence depends on volume deposited, concentration of foaming agent and presence of other additives
- ✓ Psyllium husk (Metamucil), a potential bulking agent, expands on wetting, absorbs water as foam collapses and causes eggs to stick securely to Tyvec substrate

- ✓ A two trigger spray gun has been developed which has a sliding plate that collects a fixed amount of eggs with bulking material, drops eggs into a trough, and toggles the spray.
- ✓ The addition of eggs to the foam stream after it leaves the spray nozzle eliminates mechanical damage from pressure and shearing in the pump, and from long term submersion of eggs in the foaming agent or other liquids. Optimization of this spray device is required.

## **RESULTS & DISCUSSION**

**Objective 1** - Initial testing evaluated the suitability of a variety of natural products and proteins to serve as a foaming agent in this application. A variety of food grade or OMRI approved proteins or natural surfactants were evaluated to serve as foaming agents. Preliminary screening evaluated keratin

hyrdolysate, egg albumin, gelatin, whey protein isolate and concentrate (Glanbia inc.),  $\beta$ lactoglobulin and  $\alpha$ - lactalablumin (Davisco foods inc.) and *Yucca schidigera* extract and recently *Quillaja saponaria* extract.. The suitability was evaluated by measuring their physical properties including dynamic surface tension, expansion ratio, half-life, and density using standard procedures. This analysis was conducted us a pestifoamer PF-2 (Richway Industries) to generate foam in continuous mode.



In general, full size proteins evaluated lacked sufficient dynamic surface tension to produce suitable foam under these conditions. The protein hydrolysates (keratin hydrolysate and whey protein isolate) provided better dynamic surface tension due to their smaller molecular size and faster diffusion rates. Still only keratin hydrolysate produced acceptable foam characteristics under these conditions. While keratin hydrolysate had the potential to serve a suitable foam agent, it had other potential limitations. Keratin hydrolysate, while derived from agricultural products (bovine hooves and horns), is not currently OMRI approved. In addition, while it is produced on a commercial scale for fire-fighting foams, it is not readily available without antimicrobial biocides included as preservatives. These limitations and the need to use OMRI products in some field testing sites caused to take a closer look at existing OMRI certified surfactants that could be adapted with other adjuvants to serve as suitable foaming agents. This search identified Yucca schidigera and Quillaja saponaria extracts, both of which are OMRI approved agricultural surfactants(with a reputation of undesirable tank foaming in standard spray applications). Initial screening identified it as having acceptable dynamic surface tension to meet our requirements. Preliminary screening of hatch rate of eggs after being submerged in the vucca extract showed no appreciable differences from water controls up to 5% yucca. These studies suffered from low survival of eggs in the water control. The following foaming systems were evaluated Moultrie MFH-SPR15P ATV sprayer (Moultrie inc), Pump up bullet foamer, model#925008 (LaffertyEquipment) Pestifoamer (Richway Industries LTD), a variety of TeeJet venturi spray tips on a generic variable pressure sprayer. In each case these sprayers were used with 3.5% keratin hydrolysate. Mixtures have not been tested.

The secondary goal under this objective was to determine the best method to introduce lacewing eggs to foam. The solution to this objective was confounded by the competing engineering requirements needed for foam generation and introducing the lacewing eggs. After much trial and error, we concluded that the ideal system would produce a transient pulse of foam with some ability to cast it and introduce the eggs in a batch mode. The eggs would be metered in a dry state on a tree by tree basis. **Figure 2** shows a prototype design of an applicator sprayer that fulfills these design requirements.

Figure 2. Two prototype designs of a two stage foam generating LW egg sprayer are shown. Sprayer in upper panel shows: A) sliding plate in the refill position - when the beveled hole would be below and this refilled by the hopper (=the plastic bottle which would contain eggs mixed with dry bulking agent); simultaneously the trigger fully squeezed (foam spray is on) or trigger is half depressed; B) sliding plate is above spray trough and has released the eggs – at this time the spray is off and the trigger is at rest; C) spray trough, inside are found air induction nozzles that produce the foam and a trough that the spray slides across picking up the LW eggs on the way out. Sprayer in lower panel does not show the egg delivery system but instead shows and describes a new design where foam quality is improved by introducing compressed air and adding a sparging system(mixing chamber)



**Objective 2** was to test of adhesion of foam to waxy, water repellent surfaces in the laboratory in Peoria using a foam generator/sprayer. Fulfilling this objective was limited by the ability to settle on a preferred method of foam generation and egg introduction, which greatly impact the physical properties (such as velocity and droplet size) of the emitted foam solution. However, efforts were made to

identify suitable materials that mimic the properties of apple tree surfaces. A literature survey and analysis of local tree stock determine apple leaves are generally considered easy to wet with water contact angles in the 60-800 range. The bark of local apple trees, at the estimated site of application, was variable with an average water contact angle of  $74 \pm 9^{\circ}$ . It was decided to use Plexiglas with a water contact angle of  $76^{\circ}$  as the leaf mimic, due to its low cost and wide availability. The branches of the canopy will imitated with small diameter polyvinyl chloride pipe, which has a water contact angle of  $85^{\circ}$ . Once a suitable foam generation system has been identified these mimics will be used to evaluate the influence of additional adjuvants on adhesions. These adjuvants will include viscosity modifiers, polymers to promote egg suspension and adhesion. Recent tests using psyllium as a dry

bulking agent for metering out eggs shows exceptional promise in assisting sticking of the foam because the water leaving the foam as it collapses is taken up by the psyllium. In preliminary tests it appears the larges problem from psyllium is using too much, then eggs become trapped in a mat of cross-linked psyllium fibers. Finer grinding of the psyllium husks may also alleviate this problem.

**Objective 3.** Tests of LW egg survival following submersion in protein hydrolysates show promise, but survival less than 50% has been seen in the firefighting foam and in both the two saponin extracts from Yucca and Quillaja solutions. We have found that lower survival is cause by excessive storage of eggs prior to use for testing. Additional testing will be done once a foam generation and spraying system is finalized. The most recent test (Dec 17, 2012) is shown in Figure 3.



control. Eggs were dried following immersion by placement on a towel and allowed to dry and then dropped on a dry adhesive (back side of an adhesive label) which allows hatch but prevents lacewing larvae from moving to other eggs and feeding on unhatched eggs.

**Objective 4 and 5.** A preliminary study was conducted at the Moxee farm which consisted of sprinkling dry eggs onto fresh foam solution, wet white glue and water alone painted onto leaves. Best retention of eggs occurred on leaves with glue, followed by foam and no retention at 3 days was observed with water. Improvements in adhesiveness of foam is provided using bulking agents (Metamucil) as observed by greenhouse studies subsequent to field tests. Objectives 4 and 5 remain incomplete until we have an optimal foam and egg sprayer.

# **EXECUTIVE SUMMARY**

Project Title: Protein-based foam for applying lacewings eggs to fruit trees by ATV

Participants: Tom Unruh and Christopher Dunlap; USDA-ARS

Budget: \$19,000 for 1 year.

Second year is not being requested and a no-cost extension of funding is requested for continued on project with funds remaining from year 1.

# **OVERVIEW**

This project was designed to discover a organically useful foaming agent that could be used to apply lacewing eggs onto trees using an ATV that is only mildly modified from standard spray programs they are currently used for in apple and cherry orchards. There have been two sides to our efforts: 1) chemical, specifically to find an OMRI-approved foaming agent that preserves the health of the lacewing eggs and provides adhesion to foliage or tree bark; 2) mechanical, develop a sprayer that both produces the foam and delivers the lacewing eggs to the trees while an applicator is on the ATV. We have made progress on both fronts, but have not completed the project. We do not ask for funds for a second year because we received funds rather late in the granting cycle and had delays in hiring assistants. Given that, we intend to reach the goals stated with the funds provided in year 1.

# Accomplishments:

Keratin hydrolysates, *Yucca* and *Quillaja* saponin extracts all produce suitable foam which adheres well to foliage and tree bark in test application. Only the saponin extracts are OMRI approved.

Survival of lacewing eggs in foaming agents exceeds 80% in many trials in the laboratory.

A modified hand gun that sprays foam through a cylinder where dry lacewing eggs are placed can accurately deliver the eggs in foam to a target 6-8 feet distant. Additiono of compressed air to this system has provided a very rich foam.

Eggs are dropped in the cylinder after a single spray cycle (=trigger pull and release) and addition of bulking agents (ground rice hulls or sphagnum) together with a sticking agent (dry chopped psyllim hulls – the ingredient of Metamucil) result in significant adhesion of the foam.

## Work Needed:

The mechanical sprayer needs to be optimized to have reliable retrieval and carrying of the eggs from the spray gun and in metering the egg numbers accurately.

Demonstration of the utility and efficacy of application of LW eggs in foam must be demonstrated in the field.

# NEW PROJECT PROPOSAL

#### **PROPOSED DURATION:** 1 year

Project Title: New woven pesticide applicator protective garments with repellent

PI:	Carol (Rams	ay) Black						
<b>Organization</b> :	Washington	Washington State University						
Telephone:	509-335-922	22						
Email:	ramsay@ws	u.edu						
Address:	WSU Enton	nology						
Address 2:	PO Box 646	382						
City/State/Zip:	Pullman, W	A 99164-6382						
Cooperators:	Dr. Anugrah Washington	Shaw University of State University; W	f Maryland Easte SDA Farmwork	ern Shores; Dr. k er Education Pro	Karen Leonas, ogram			
Total Project I	Request: Y	ear 1: \$15,000						
Percentage tim	e per crop:	Apple: 65%	Pear: 10%	Cherry: 20%	Stone Fruit: 5%			

#### Other funding sources: None

#### WTFRC Collaborative expenses: None

Budget 1			
Organization Name: WSU CAHN	NRS Contra	ct Administrator: C	Carrie Johnston
Telephone: 508-335-4564	Email a	ddress: carriej@w	su.edu
Item	2013	2014	2015
Salaries	0		
Benefits	0		
Wages	1500		
Benefits	30		
Equipment	0		
Supplies	3470		
Travel	10000		
Miscellaneous	0		
Plot Fees	0		
Total	\$15,000		

Footnotes:

Wages and benefits (1500 + 30) for work study students in developing the presentation, travel logistics, and collating data. 125 hours @12/hour.

Supplies include sample PPE garments from US and Brazil vendors (\$1,000), miscellaneous meeting goods and services (\$2,470)

Travel \$10,000: 2 trips from Maryland to Washington, 3 preparatory meetings with WSDA and WSU Educators, 12 grower meeting expenses.

Carol Black has been on the road training for January and February. Will start work on the project in March with the bulk of the work effort in May and June.

# Timeline

- 1. Get survey questions approved from WSU Institutional Review Board in March
- 2. Schedule meetings with county faculty in April
- 3. Schedule meetings with growers, field managers, and applicators for May and June
- 4. Meet with Anugrah Shaw in Washington State May
- 5. Meet with WSDA Farmworker Education Program staff
- 6. Summarize survey findings June and July
- 7. Conduct follow-up survey in late July if necessary
- 8. Prepare presentations for final report and winter training season.
- 9. Prepare proposal to further efforts on garment implementation if study warrant this effort

# **CONTINUING PROJECT REPORT** WTFRC Project Number: AP-12-104

# **YEAR**: 1 of 3

PI:	Gerrit Hoogenboom	Co-PI (2):	Melba Salazar
Organization:	Washington State University	Organization:	Washington State University
Telephone:	509-786-9371	Telephone:	509-786-9281
Email:	gerrit.hoogenboom@wsu.edu	Email:	m.salazar-gutierrez@wsu.edu
Address:	AgWeatherNet	Address:	AgWeatherNet
Address 2:	24106 North Bunn Road	Address 2:	24106 North Bunn Road
City/State/Zip:	Prosser, WA 99350	City/State/Zip:	Prosser, WA 99350
Co-PI(3): Organization: Telephone: Email: Address: Address 2: City/State/Zip:	Tory Schmidt WTFRC 509-665-8271 tory@treefruitresearch.com 1719 Springwater Avenue Wenatchee, WA 98801	Co-PI (4): Organization: Telephone: Email: Address: Address 2: City/State/Zip:	Nairanjana Dasgupta Washington State University 509-335-8645 dasgupta@wsu.edu Department of Statistics Neill 103 Pullman, WA 99164

**Project Title:** Development of apple bloom phenology and fruit growth models

Cooperators: Karen Lewis (WSU-Extension), Felipe Castillo (WTFRC)

<b>Total Project Request:</b>	Year 1: \$70,000	Year 2: \$82,500	Year 3: \$85,000
-------------------------------	------------------	------------------	------------------

## Other funding sources

Indirect support through the existing infrastructure of AgWeatherNet and its network of 138 weather stations.

Item	2012	2013	2014
Salaries	3,000	3,500	4,000
Benefits	1,200	1,400	1,600
Wages <sup>1</sup>	7,500	7,500	*0 or 7,500
Benefits			
<b>RCA Room Rental</b>			
Shipping			
Supplies			
Travel <sup>2</sup>	2,400	2,700	3,000
Plot Fees			
Miscellaneous			
Total	\$14,100	\$15,100	*\$8,600 or \$16,100

Footnotes: \*Additional field data collected only if needed in 2014

<sup>1</sup> Labor calculated as 2 persons at \$16.00/hr working 12 hrs per week for 13 weeks during the growth season.

<sup>2</sup> In-state travel to research plots

#### Budget

Organization Name: ARC-WSU

Contract Administrator: Carrie Johnston

<b>Telephone:</b> 509-555-4504	Eman address: carriej@wsu.edu					
Item	2012	2013	2014			
Salaries	53,936	65,536	67,496			
Benefits	12,564	13,464	14,004			
Wages						
Benefits						
Equipment						
Supplies	1,000	1,000	1,000			
Travel	2,500	2,500	2,500			
Miscellaneous						
Plot Fees	0	0	0			
Total	\$70,000	\$82,500	\$85,000			

**Footnotes:** The budget that is requested through this proposal includes partial support for a Research Associate (Melba Salazar) who will be responsible for the overall evaluation and implementation of the various growing degree models that are applicable for conditions in the Pacific Northwest and partial support for an Application Programmer (Sean Hill) for integration of the model on the web portal of AgWeatherNet (www.weather.wsu.edu). We also have budgeted for a Graduate Student (to be hired) who will be responsible for the development of a physiological fruit growth model. The proposal includes a request for a computer for the graduate student during the first year of the project. Additional budget items include operating expenses for computer software and related costs and travel to participate in field data collection. Finally, this proposal includes support for Professor Dasgupta in the Department of Statistics to complete her statistical model development and evaluation (objective 2).

# **OBJECTIVES**

- 1. Continue data collection on bloom phenology and fruit growth for selected sites and cultivars to enhance model accuracy and vigor. (Schmidt in collaboration with Castillo)
- 2. Continue refinement of statistical models for bloom phenology and fruit growth. (Dasgupta)
- 3. Develop physiological-based models for bloom phenology and fruit growth of apples. (Hoogenboom, Salazar)
- 4. Implement and evaluate models as decision support aids on the AgWeatherNet portal using industry beta-testers. (Hoogenboom, Salazar and Dasgupta in collaboration with Lewis)
- 5. Improve model/portal user interface based on feedback from beta-testers and other stakeholders. (Hoogenboom, Salazar in collaboration with Lewis)

# **Timetable for Project**

Activities		2013		2013-2014			2014-2015						
1. Ex	xperimental data collection		Х	Х			Х	Х			Х	Х	
2. Sta	atistical model development and evaluation	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х		
3. Ph	nysiological model development and	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х
eva	valuation												
4. We	eb-based user interface development			Х	Х	Х	Х	Х	Х				
5. We	eb-based user interface evaluation by WSU						Х	Х	Х	Х	х	Х	х
Ex	xtension and stakeholders; final												
im	plementation												

# SIGNIFICANT FINDINGS

- Differences among locations and among cultivars for the different phenological stages where found in terms of Growing Degree Days.
- Fruit growth was modeled using Growing Degree Days at each location. Differences among cultivars were fund for each location.
- Red Delicious and Gala had significantly larger diameters than Cripps Pink, the final size of the fruit varied from year to year and location to location.

# **METHODS**

1. Data collection

WTFRC staff will continue collecting bloom phenology and fruit growth data from established sites to augment data sets from the previous project.

## 2. Continue refinement of statistical models for bloom phenology and fruit growth

For the growth models, data have been compiled for Gala for 2010 and 2011, while for Red Delicious and Cripps Pink data have been compiled for 2010. For the bloom models, data have been compiled for 2010 and an ordinal logit model has been used to fit the data. All data for phenology, growth and temperature will be compiled for 2011. For the growth model the data for 2010 and 2011 have to be combined and new parameters have to be estimated. For the bloom model similar procedures will be followed.

Following a successful development of both the statistical bloom phenology model and the statistical fruit growth model, they will be evaluated with the new data that will be collected during the 2012 and 2013 growing seasons.

## 3. Develop physiological models for bloom phenology and fruit growth

Physiological time will be used as input of the model for the different phenological stages for apple, referred to as Growing Degree Days (GDD) or Thermal time. A comparison among three of the most traditionally methods for GDD accumulation is planned. The requirements for the different phenological stages of the most important apple cultivars using hourly temperature records from the AgWeatherNet will be summarized. An evaluation is planned to determine the most accurate model using historical observed dates under different environmental conditions. The performance of the model will be compared using the weather data collected with the Hobo data loggers that have been part of the data collected by WTFRC.

# 4. Implement and evaluate models as decision support aids on the AgWeatherNet portal

To assist the growers for making decisions, an information delivery system and media tool will be posted in the web page using the models developed. This tool will provide, in an easy and userfriendly way, thermal time, cumulative chilling, and cumulative degree hours in real-time (current) for different environmental conditions where local weather data are available through tables and graphs as well as information about the current phenological and development stages and the climatic requirements to complete the next stage.

Alerts to the growers will be generated when the crop can be at risk due to the actual temperatures in excess of the threshold temperatures. The system will be available through a link created on the AgWeatherNet web portal and other web portals where information for apples is provided, including the Decision Aid System (DAS).

## 5. Improve model/portal user interface and release for general use

The overall goal is to develop a web portal that will provide a guideline and advisory for the growers who are monitoring their individual apple orchards in terms of weather conditions and weather predictions. Closely work with WSU Extension and industry representatives as beta testers during the second and third year of this project is planned to try to include the comments to improve the tool and decision aid to the benefit of the local apple growers.

# **RESULTS & DISCUSSION**

## 1. Data collection

Observations of bloom phenology were recorded in 2012 by WTFRC internal staff every Monday, Wednesday, and Friday in 29 blocks clustered around 10 location nodes. Current varieties include Red Delicious, Cripps Pink and Gala. WTFRC staff also collected fruit size data starting at petal fall until final harvest with a brief break during thinning. Similar procedures are planned for 2013, with possibly inclusion of one or two warmer sites.

## 2. Continue refinement of statistical models for bloom phenology and fruit growth

For the statistical growth models, we obtained the following potential explanatory variables were: GDD, Julian date, DAFB, Cum Av Temp, Cum Av Soil Temp, Cum Av Dewpoint, Cum Av RH, Cum Min, Cum Max., Daily Min and Max and Solar Radiation. We studied the correlations of the above list with Diameter, at first for the variables based on time: Julian Date, DAFB and GDD. For the cultivar Gala we found that the diameter was correlated to all three but mostly to DAFB (r=0.928), while Julian Date was 0.923 and GDD was .847. As these are highly correlated it makes sense to have only one of these in our model. We then studied the correlations among diameter and the weather variables. These are as follows:

CumAvgRHCumAvgTempCumMIN CumMAXCumAvgDewPtCumAvgSoilTemp-0.592110.73947-0.440270.796680.511140.70750

It is evident that all these are fairly good predictors of diameter with Cumulative Average Temperature (CumAvgTemp) having the highest correlation .73947. It makes sense that both Cumulative Minimum temperature and Cum Average Relative Humidity (CumAvgRH) are negatively associated with diameter.

We used stepwise method in SAS to look at model selections for each cultivar. In each case, we report the best model. If GDD was not in our best model we looked at the best model with GDD in it and commented upon it. We did account for multi-collinearity among the weather variables and for each cultivar we report the two models and the corresponding R-squares. We report the data for 2010 as an illustration.

#### Gala 2010:

Model: Diameter = 0.89834 + 0.01918 DAFB - 0.00979 CumAvgRH -0.00905 CumMin + 0.00764 CumAvgDewPt

This model has an R-square of 89.29%. Using the same logic as above the best model for Red Delicious is given as:

Model: Diameter = 2.70984 + .00055 GDD - .0119 CumAvgRH - .0209 CumMin + .0043 CumAvgDewPt.

This model included GDD and the R-square was 86%. Using the same logic as above the best model for Cripps Pink is given as:

Model: Diameter = 1.618 + .0121 DAFB - .0336 CumAvgRH - .00813 CumMin + .08842 CumAvgDewPt - .0444 CumAvg SoilTemp + .00564 CurrentDewPoint

With an R-square of 89.9% while the model based on GDD had an R-square of 82%.

It is clear that all three cultivars have the following variables in common: GDD/DAFB, cumAvgRH, CumMin, and CumAvgDewPt. Cripps Pink also has CumAvgSoilTemp and the current Dew Point in the model. The R-squares of these models range from 86% to 90% which is very promising, given the noisy nature of the data and the fact that location was not used in the models.

We have similar results for the 2011 data. For the growth data we are incorporating the weather variables and running ordinal logit models. Our results using cross validation are promising with r-squares over 90% for all three cultivars.

#### 3. Develop physiological models for bloom phenology and fruit growth

The dynamics of the different phenological stages were analyzed in terms of Growing Degree Days (GDD) using a base temperature of 43 °F for each location and each cultivar for the 2011 and 2012 growing season. The analysis shows differences among locations and among cultivars for the different phenological stages. An example for East Wenatchee, Royal and Chelan for Gala, Red Delicious and Cripps pink are presented (Figure 1).

Fruit growth was observed in 10 locations during 2011 and 2012. The duration in Growing Degree Days was determined for Gala, Red Delicious and Cripps Pink for each season from petal fall to harvest, using a base temperature of 43°F, an example of the total accumulation for Gala, Red Delicious and Cripps pink are presented for East Wenatchee Royal and Chelan (Table 1). Differences among cultivars were found for each location (Figure 2). In general for all locations Red Delicious

and Gala had significantly larger diameters than Cripps Pink, the final size of the fruit varied from year to year and location to location. Cultivar differences in fruit diameter reflect differences in mean fruit diameter as well as fruit growth period.

4 Implement and evaluate models as decision support aids on the AgWeatherNet portal No activity to report

## 5 *Improve model/portal user interface and release for general use* No activity to report

**Table 1**. Growing degree days (GDD) using a base temperature of 43 °F starting on Petal Fall to Harvest for East Wenatchee Royal and Chelan.

Location	Cultivar	2011	2012	Average
East				
Wenatchee	Gala	2637	3026	2831
	Red			
	Delicious	3316	3656	3486
	Cripps Pink	3570	3667	3619
Roval City	Gala	2600	2748	2674
- 9 9	Red			
	Delicious	3296	3467	3381
	<b>Cripps</b> Pink	3413	3485	3449
Chelan	Gala	2895	3008	2951
Chiefun	Red	2000	2000	2/01
	Delicious	3337	3595	3466
	Cripps Pink	3438	3558	3498



**Figure 1.** Progression of the Phenological stages for apples at the different locations evaluated, for Gala, Red Delicious, and Cripps Pink cultivars during 2011 and 2012 growing season respectively.



**Figure 2.** Relationship of the seasonal changes in fruit diameter and Growing Degree Days of three apple cultivars: Gala, Red Delicious, and Cripps Pink in 10 locations, during the growing seasons of 2011 and 2012.

#### **CONTINUING PROJECT REPORT** WTFRC Project Number: TR-12-102

## **YEAR**: 1 of 3

**Project Title:** Effect of early spring temperature on apple and sweet cherry blooms

PI:	Gerrit Hoogenboom	<b>Co-PI (2):</b>	Melba Salazar
<b>Organization</b> :	Washington State University	<b>Organization</b> :	Washington State University
Telephone:	509-786-9371	Telephone:	509-786-9281
Email:	gerrit.hoogenboom@wsu.edu	Email:	m.salazar-gutierrez@wsu.edu
Address:	AgWeatherNet	Address:	AgWeatherNet
Address 2:	24106 North Bunn Road	Address 2:	24106 North Bunn Road
City/State/Zip:	Prosser. WA 99350	City/State/Zip:	Prosser, WA 99350
Co-PI (3): Organization: Telephone: Email: Address: Address 2: City/State/Zip:	Matthew Whiting Washington State University 509-786-9260 <u>mdwhiting@wsu.edu</u> IAREC 24106 North Bunn Road Prosser, WA 99350	Co-PI (4): Organization: Telephone: Email: Address: Address 2: City/State/Zip:	

Cooperators: John Ferguson and Markus Keller, IAREC-WSU

<b>Total Project Request:</b>	Year 1: \$95,000	Year 2: \$80,0	00 Year	<b>3:</b> \$80,000
Percentage time per cro	<b>p:</b> Apple: 50%	Pear: 0%	Cherry: 50%	Stone Fruit: 0%

#### **Other funding sources**

Indirect support through the existing infrastructure of AgWeatherNet and its 13 weather stations.

Organization Name: ARC-WSU	Contract Administrator: Carrie Johnston			
Telephone: 509-335-4564	Email address: carriej@wsu.edu			
Item	2012	2013	2014	
Salaries	14,040	38,646	37,661	
Benefits	5,616	7,803	7,102	
Wages	42,400	20,860	21,694	
Benefits	4,240	2,086	2,169	
RCA Room Rental	0	0	0	
Equipment	10,000	0	0	
Supplies	10,204	2,605	2,874	
Travel	8,500	8,000	8,500	
Plot fees	0	0	0	
Miscellaneous	0	0	0	
Total	95,000	80,000	80,000	

**Footnotes:** Salary for a Post-doctoral Research Associate (Dr. Melba Salazar) for four months during the first and second year of the project and for three months during the final year of the project. Dr. Salazar will be supported by a graduate student, budgeted for two years of the project. One year of 0.5 FTE technical support (Mr. John Ferguson) to design and build the automated sampler system. The automated sampler will be integrated with a freezer, which is budgeted at \$10,000. Additional budget items include part-time hourly labor to help with sample collection and sample analysis for all three years, goods and services for the parts associated with the automated sampler and travel for collection of the samples in the region.

# **Goal and Objectives**

The overall goal of this proposal is to investigate the effects of early spring temperature on apples and sweet cherries at different developmental stages and to determine the hardiness. We propose to use a traditional methodology through exposure to freezing temperatures, and to automate part of this procedure. The outcome will be updated hardiness charts for apples and sweet cherries.

The following are our specific objectives:

- 1. To determine the effect of early spring temperature on bloom development for different apple and sweet cherry cultivars.
- 2. To develop a cold resistance curve from dormancy to bloom for apples and sweet cherry.
- 3. To update the charts for the different stages of blossom buds of apples and sweet cherry cultivars for local weather conditions in the Pacific Northwest.

## **Significant Findings**

- Differences in hardiness and lethal temperature were found during different phenological stages for the same cultivar as well as among the sweet cherry cultivars that were initially evaluated.
- The automated sampler, referred to as the "vending machine," has been completed and was installed as part of a dedicated temperature-controlled freezer.
- We are testing the automated sampler "vending machine" to determine the hardiness of the crops when DTA is not effective. Preliminary results indicate differences between apples and sweet cherries and among cultivars.
- The results from preliminary dissection indicate that there is a variation in cold hardiness for different bud sizes of apples for the same sampling date.
- Growth chamber data revealed differences in hardiness of flower bud progression for the three temperature environments that were evaluated.

## Methods

Bud samples were collected throughout late winter and early spring in 2012 season to determine the effect of temperature on bloom development for apple and sweet cherry cultivars. We started our measurements in February 2012 and ended them around early bloom. For apples we evaluated the varieties Gala, Red Delicious and Fuji. For cherries we evaluated the varieties Bing, Chelan and Sweetheart. The sweet cherry and apple cultivars at different bud development stages were sampled from the field and tested in the laboratory. We restarted our sampling on October 1, 2012 for the current growing season for both cherries and apples and for the same varieties.

Cold hardiness was assessed using differential thermal analysis (DTA) for the first phenological stages and when the DTA was not effective, beyond open cluster, a new automated sampling device was developed and used. For the new device we load the tissue samples into color coded cans and expose the material to different durations and controlled cold temperatures combinations in a freezer. After the cold temperature treatment has been completed each tissue sample is dissected to determine frost damage.

Simultaneously to the process described above we collected dormant apple and cherry shoots that were 6 to 10 inches long with terminal flower buds. The shoots were kept in containers filled with water. The base of the shoots was recut every week and water was replaced every other day and forced in 3 different growth chambers with days/nights at a controlled temperature each one (12/4°C; 18/6°C; 24/12°C) similar to the procedures of Proebsting and Mills (1978), to simulate tree different spring environmental conditions. The samples were processed at three-day intervals and classified accordingly with its hardiness.

Digital pictures were taken for the different growth stages to illustrate, identify, and define the key growth stages for apple and sweet cherry to update the charts, these pictures will be combined with the data obtained from the cold hardiness exposure described previously. All information will be integrated to develop both traditional hard copy charts as well as digital systems that can be accessed via the web, including AgWeatherNet and apple and cherry decision aids, as well as via smart and hand-held devices.

#### **Results and Discussion**

This report refers to the results for cherry only. The same procedures are being applied to apples and data collection for both crops will continue in 2013. Critical injury temperatures for buds of Bing, Chelan, and Sweetheart have been evaluated continuously every week since October 1, 2012. However, only five dates are presented in this report corresponding to each month of the evaluation.

The relationship of the cumulative percentage of dead buds and the temperature was modeled using a logistic function (Fig 1). The following equation represents the fitted model:

$$CDF = c + \frac{(d-c)}{1+e^{-K(t-G)}}$$
 (1)

where CDF is the cumulative dead bud flower, in a logistic growth curve (Eq. 1), c and d represent the lower and the upper asymptote respectively which means the percentage of mortality presented already in the field (c) and the maximum percentage of mortality (d), K is the so called 'slope parameter', t is the gradient of temperature in the freezer and G is the temperature where the inflexion point of the curve occurs.

Significant logistic curves (p < 0.01) were adjusted for each of the cherry cultivars and for each of the different dates of sampling (Fig 1). The estimated parameter values of the model and the corresponding dates are presented in Table 1. As the confidence intervals for the *G* parameter are different, the overlapping curves are different. This means that the cultivars are different with respect to their resistance to lethal temperature (Table 1.)

The Probit procedure was used to calculate the percent of mortality (LT) for 10, 50 and 90. The resulting  $LT_{10}$ ,  $LT_{50}$ , and  $LT_{90}$  values for each cultivar and each date of sampling were then used to model the behavior over time. A quadratic function was initially developed. However, it will be necessary to complete the measurements until bloom to develop the full model. The comparison among cultivars shows that there are variations in the temperatures at which injury occurs for each of the cultivars. The pattern of the injury is different at 10, 50 and 90 for each cultivar (Figs 2 and 3).

The cold hardiness is greatly affected by bud development, since the temperature at which the buds become injured changes over time. These results support the earlier report that changes in hardiness were observed for different dates of sampling among cultivars and size of the buds. Buds from the first two sampling dates were less sensitive to cold temperature as compared to the latest sampling dates (Fig 3). This shows that plants at the latest dates had less hardiness and that the deacclimation process has begun.

Deacclimation for the different cherry flower buds resulted in a moderate increase in the LT temperatures as the season advanced. The results presented here are consistent with the previous reports by Proebsting (1987). There was a quadratic relationship between LT and the day when the sampling was conducted (Fig 3). Each point represents the value of the temperature where the buds was frozen and dead on that date. For each LT, a quadratic regression was adjusted to estimate the

mortality trough time or bud development, the parameters of the adjusted model are presented in Table 2. In general the goodness of fit was good enough since the lowest  $R^2$  was equal 80%.

A new experiment has just set been started for apples and cherries for three different environments using growth chambers. The measurements will be done initially every week and then when a phenological change is observed, the measurements will be done every other day. The goal of this experiment is to determine the sensitivity of the buds assuming three different environmental conditions during spring.

#### Limitations

The results presented her are limited to one location and conditions of the orchards where the samples were collected. Additional will be required to expand research sites. The current chamber also does not have relative humidity control due to the high cost. Additional research might be required for different relative humidity conditions representative of those found in an orchard.

Table 1. Estimated parameters values of the logit model fitted for each of the different samp	ling dates
for the three sweet cherry cultivars that are being evaluated.	-

Cultivor	Data	מ	C	V	G	95% Conf	ïdence
Cultival	Date	D	ι	Λ	0	Limits (G)	
Bing	23-Oct	0.82	0.0	-2.4	18.9	18.8	19.1
	13-Nov	1.00	0.0	-0.6	5.7	5.6	5.7
	18-Dec	1.00	0.1	-1.1	-1.8	-1.9	-1.7
	8-Jan	1.00	0.1	-1.3	-3.4	-3.5	-3.3
	6-Feb	1.00	0.1	-0.9	-1.4	-1.5	-1.3
Chelan	23-Oct	1.00	0.0	-0.9	16.3	16.2	16.5
	13-Nov	0.99	0.0	-0.7	8.7	8.7	8.8
	18-Dec	0.99	0.0	-0.8	-1.7	-1.8	-1.6
	8-Jan	1.00	0.0	-0.9	-3.1	-3.2	-3.0
	6-Feb	1.00	0.1	-1.0	-2.1	-2.2	-2.0
Sweetheart	23-Oct	1.00	0.0	-0.8	14.8	14.6	15.0
	13-Nov	0.99	0.0	-1.0	8.4	8.4	8.5
	18-Dec	1.00	0.1	-0.7	0.5	0.3	0.7
	8-Jan	1.00	0.0	-1.1	-2.4	-2.5	-2.3
	6-Feb	1.00	0.1	-0.6	0.7	0.6	0.9

Cultivar	LT	Equation	$\mathbf{R}^2$
Bing	10	$y = 0.0028x^2 - 0.590x + 31.13$	0.87
	50	$y = 0.0027x^2 - 0.559x + 24.68$	0.91
	90	$y = 0.0030x^2 - 0.577x + 19.91$	0.84
Chelan	10	$y = 0.0024x^2 - 0.538x + 31.52$	0.90
	50	$y = 0.0026x^2 - 0.551x + 26.43$	0.94
	90	$y = 0.0026x^2 - 0.571x + 22.10$	0.95
Sweetheart	10	$y = 0.0023x^2 - 0.489x + 28.69$	0.90
	50	$y = 0.0021x^2 - 0.457x + 22.23$	0.91
	90	$y = 0.0021x^2 - 0.457x + 15.52$	0.83

Table 2. Quadratic equations for  $LT_{10}$ ,  $LT_{50}$ , and  $LT_{90}$  as a function of time for the three sweet cherry cultivars that are being evaluated.





temperature for cherry cultivars at different evaluated dates.



Figure 2. Seasonal pattern comparison of the LT temperatures (10, 50, and 90) for the three cherry cultivars evaluated on different dates.



Figure 3. Seasonal air temperature (Tmax and Tmin) and LT temperatures (10, 50, and 90) of each of the cherry cultivars buds evaluated on different dates.

## **YEAR**: 2013 (1 of 3)

## **CONTINUING PROJECT REPORT WTFRC Project Number:** TR – 13 - 100

Project Title:	Technology roadmap implementation		
PI:	James Nicholas Ashmore		
<b>Organization</b> :	James Nicholas Ashmore & Associates		
Telephone:	(202) 783 6511		
Email:	nickashmore@cox.net:		
Address:	400 North Capitol Street, N. W.		
	Suite 363		
	Washington, D. C. 20001		

**Cooperators**: None

<b>Fotal Project Request:</b>	Year 1: \$36,000	Year 2: \$36,000	Year 3: \$36,000
-------------------------------	------------------	------------------	------------------

Percentage time per crop: Across Crops

(Efforts focused on policy, programs and procedures, and precedents for all crops)

## Other funding sources: None

#### WTFRC Collaborative expenses: None

 Budget

 Organization Name: James Nicholas Ashmore & Associates

 Contract Administrator: James N. Ashmore

 Telephone:
 (202) 783 6511

 Email address:
 nickashmore@cox.net

Item	2013	2014	2015
Salaries	\$36,000	\$36,000	\$36,000
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	\$36,000	\$36,000	\$36,000

# **Objectives:**

The basic objective of this project is to gather data/information from a wide range of sources, organize that information and work with the Commission Manager and other specialty crops groups to identify and structure flexible options that working with the Congress will lead to a successful effort to reach certain specified goals:

- secure and enhance the continued implementation of the National Technology Roadmap for the Tree Fruit Industry and the gains that have been made to date because of the funding made available for the Specialty Crops Research programs established the 2008 general farm statute;
- support the extension of these Specialty Crops Research programs in any reauthorization of general farm legislation considered by the Congress;
- secure continuation and funding of research programs identified and supported by the Washington tree fruit industry; and,
- seek to obtain funding/support for new initiatives identified and supported by the Washington state industry.

# Findings (To Date):

- In the last Congress, both the Senate-passed farm bill reauthorization and the bill reported to the House from the Committee on Agriculture contained provisions extending and amending the Specialty Crops provisions of the 2008 Farm Act (including language in support of the Clean Plant Network);
- Unfortunately, because of controversies in other areas of the legislation and because of deficit concerns and disagreements about how much "savings" had to be achieved from agriculture spending, the last Congress was unable to complete action on an overall extension of the farm programs;
- As part of a deal that was reached to avoid the "fiscal cliff," the Congress did enact a one year extension of the 2008 Act;
- It is important to note, however, that in an overall effort to address deficit concerns, the one year extension of the 2008 Act moved funding for specialty crops research programs from **mandatory** to **discretionary spending**;
- It is also important to note that the complexities of the "fiscal cliff" agreement have contributed to a delay in the submission of the President's budget for fiscal 2014;
- The actual submission of the President's budget is not expected to occur until March;
- It is also important to note that the "fiscal cliff" agreement delayed the effective date of the agreed-upon sequester until March 1, 2013;
- There have been press reports regarding the impacts of that sequester if it is allowed to go into force, especially on the Department of Defense;
- There have been efforts to develop proposals that could be used as the basis for negotiation to put together legislation to address the sequester issues, including a recent proposal by the Senate Majority that contains agriculture spending cuts but would not include the overall farm bill package;
- That Senate Majority package does not propose agriculture spending reductions that have in the past been acceptable to the House Majority(the House Majority budget from last year seeks larger reductions in agriculture spending), and as a result the Senate Majority proposal t is not likely to pass in its current form;

• It is also important to note that this Congress will be asked to pass continuing appropriations for the current fiscal year, thereby giving rise to yet another debate over the level of Federal spending for agriculture.

# Actions (To Date):

- Attended breakfast meeting (with Chris Schlect, Northwest Horticultural Council; Todd Fryhover, Washington Apple Commission; and Jon S. Alegria, CPC International Apple Commission), and had an opportunity to discuss a wide range of Northwest industry issues;
- Worked with Commission Manager and with Dr. Mike Willett and others in the development of a Pear Research Roadmap; that document has been finalized and submitted to ARS;
- Attended swearing-in parties for Derek Kilmer and Denny Heck, new Members of the Washington State House Delegation;
- Discussed with Delegation offices and House Minority Committee staff; concerns over the implications of the "fiscal cliff deal" and how it has moved specialty crops research funding to "discretionary spending"; those discussions focused on steps that might be taken to urge the Administration to include funding requests for these programs in its budget submission;
- Agreed to attend "Meet and Greet" function for Representative Suzan DelBene, who is from the State and who is a new Member of the House Committee on Agriculture and the House Judiciary Committee;
- Monitored other issues of possible concern to the state growers, including but not limited to on-going discussions about pollinators and colony collapse disorder.

# Actions (Anticipated):

- Review the President's budget once it has been submitted;
- Monitor movement in the House and Senate Budget Committees as they move forward to develop a congressional budget resolution (Senator Murray is the new Chair of the Senate Budget Committee);
- Continue to maintain close contact with the staffs of the authorizing committees of jurisdiction and Delegation offices to determine how they expect to proceed with the farm bill (either as a free-standing measure or as part of an overall budget exercise such as reconciliation);
- Work with the Commission Manager to schedule appropriate meetings for him with Delegation offices and/or Administration personnel whenever he returns to the capital city;
- Be prepared to work with the Commission Manager and Dr. Mike Willett to manage communications with Delegation offices and with the Administration to help move forward with the Pear Research Roadmap; and,
- Follow other developments in Congress and report them as necessary to the Commission Manager to insure that he and the Commission have the best available information necessary to be responsive to the Congress and to the Administration.

# Methods:

There is a remarkable degree of bipartisan support for the Specialty Crops programs and a clear recognition that these programs have been very successful. It is my sense that this is due to a number of things, not least of which is that we have demonstrated the following characteristics:

- Patience based on an understanding that changing culture takes time, that we are moving in the right direction;
- Cooperation based on an understanding that we are stronger as a group, that working together has given specialty crops a "seat at the table" in determining national agricultural policy;
- Recognition based on an understanding that our problems are not unique, that in fact there are common problems that face us and our specialty crops partners;
- Openness based on an understanding that this is necessary for sharing of information and that without full sharing, it is arguably difficult if not impossible to reach a regulatory decision based on sound science and verifiable facts;
- Transparency based on an understanding that we are only as good as our reputation and that we must be a trustworthy and dependable party in the process of moving forward to address our common interests;
- Flexibility based on an understanding that are usually a number of different ways to achieve an identified objective;
- Willingness to continue to work within the process and prove that we are in fact on the right track with respect to changing the research culture and embracing a competitive approach to research awards; and,
- Appreciation based on an understanding that it is extraordinarily important to recognize and thank our partners and our Delegation for their help and their continued support in moving forward.

# **Results and Discussion**

In a general sense, the specialty crops provisions of the 2008 Farm Act are seen on a bipartisan basis in both the House and Senate and in the Administration as being very successful. There is clearly strong support for extension and strengthening of those provisions.

It is reasonable to assume that some form of the specialty crops provisions will be included if the Congress can reach agreement on how to handle the underlying legislation. As I have indicated in previous reports, the conflicts center on how much and where to cut Federal spending on agriculture.

And, those issues are caught up in the even broader and more difficult debate over how to get control over the Federal budget, whether through spending cuts, tax increases, or a combination of the two. This debate has often been quite rancorous from both sides and there have been numerous attempts to set deadlines on Congress and try to force action.

Developments scheduled for March 2013 are likely to set the tone and trajectory for debate on fiscal issues in this Congress. The "sequester" originally scheduled to take effect on January 3, 2013, as result of the "fiscal cliff" was deferred until March 1, 2013. If the Congress fails to act prior to that date, automatic across the board cuts in both defense (\$46 billion) and nondefense (\$39 billion) will take effect and will impact on spending in the current fiscal year (2013), which ends September 30, 2013.

The Continuing Appropriations Resolution, which the last Congress enacted, is scheduled to expire on March 27, 2013. Prior to that date, the Congress will have to take up and pass appropriations legislation funding the Government through the end of the current fiscal year (September 30, 2013. Clearly, if the sequester is allowed to take effect on March 1, 2013, it will have significant and serious consequences for the level of spending allowed in the next Continuing Resolution. The Administration's budget proposal is expected to come up to the Congress sometime in March, where it will be evaluated by the House and Senate Budget Committees and each of those committees will proceed to develop a proposed congressional budget resolution that will be reported to the House and to the Senate respectively. This process is significant because it will establish the baseline for scoring for farm bill reauthorization purposes, and it is very likely that this new baseline will be less than the baseline made available in the last Congress. What this means, simply, is that it is highly likely that there will be less money made available to the committees of jurisdiction to cover farm bill spending.

This reduction will increase pressure on the committees, especially if there is a push for greater cuts in agriculture spending.

It is very difficult if not impossible to predict what will happen and when it will happen. Based on my experience, the process will be contentious and provocative. Because of this, I would suggest that we rely on patience and understanding and that we make every effort possible to continue to work together within the tree fruit industry and within the specialty crops coalition. We need, simply, to help work through the process with an understanding and appreciation of the difficulties that our Delegation and the Congress face.

In the end, we will get a farm bill through the Congress and I fully expect that specialty crops provisions will be retained. We need, I believe, to help move the process forward with as little rancor as possible.

I also believe that we are well-positioned to make progress in our specific areas such as protecting programs of interest to the industry as well as working with USDA to move forward on the pear research roadmap.

There is a lot to be done, and we will have to stay involved and continue to be responsible and responsive to both the Congress and the Administration.