2007 NW Cherry Research Review
November 16-17, 2006
Richland, Washington
Thursday Agenda

Time	Page	PI	Project Title	Funding period
8:00		McFerson	Introduction & technology roadmap update	
			Final Reports	
8:15	1	Grove	Modeling and managing cherry PM	04-06
8:30	11	Calabro	Biology & control of fruit infection phase of PM	03-06
8:45	22	Olmstead	Fruit & foliar resistance mechanisms in cherry	06
9:00	30	Eastwell	Virus control strategies to assist cherry production	04-06
9:15	39	Yee*	Temperate fruit fly workshop report & strategic plan	06
Group			**POSTER SESSION - Continuing Reports - 9:45 - 11:30**	
1	44	Azarenko	Horticulture management systems for fresh & brine cherries	90
1	53	Azarenko	On-farm sweet cherry training system trials	06-08
1	60	Azarenko	Flowering, pollination & fruit set of sweet cherry	05-07
1	64	Elfving	Branch induction in cherry trees in orchards & nurseries	05-07
2	68	Whiting	Breeding and genetics program for PNW sweet cherries	05-07
2	76	Iezzoni	Sweet cherry dwarfing rootstocks	05-07
2	84	Whiting	Causes and prevention of pistil doubling	06-07
3	91	Neilsen	Understanding N requirements for cherry production	05-07
3	98	Yin	Alternative nutrient, water and floor mgmt strategies	05-07
3	105	Riga	Post-plant management of dagger nematodes	06-08
3	107	Smith	Cherry fruit fly control options	06-08
			Final Reports	
1:45	114	Yee	Insecticide effects on CFF biology	04-06
2:00	126	Yee	CFF feeding ecology and food-based lures and baits	04-06
2:15	137	Schrader	Improving cherry fruit quality & post harvest shelf life	05-06
2:30	147	Bai	Edible coating to improve storage and marketing quality	04-06
2:45	157	Elfving	Bioregulators to manage growth, flowering, and cropping	04-06
3:00	163	Whiting	High density orchard systems	04-06
3:15	172	Whiting	Sweet cherry source-sink relations	04-06
3:30	183	Whiting	Clonal rootstock evaluation	04-06
3:45	191	Bliss	Northwest cherry improvement project	05

FINAL PROJECT REPORT
WTFRC Project Number: CH-04-406

Project Title: Modeling and Managing Powdery Mildew of Sweet Cherry

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Budget History:

Item	**Year 1:**	**Year 2:**	**Year 3:**
Salaries	17,520	17,409	19,167
Benefits	5,606	5,426	6,708
Wages	12,000	12,200	10,688
Benefits	1,952	1.952	1,176
Equipment			
Supplies	6,700	6,700	1,700
Travel	4,700	4,700	3,700
Miscellaneous			
Total	48,678 (funded 41,000)	47,487 (funded 41,000)	43,139

Objectives:

I. Develop a risk index model (utilizing rainfall, irrigation, temperature, relative humidity, and pathogen presence/activity) for initiating fungicide spray programs and adjusting subsequent spray intervals.

II. Develop means of detecting, identifying, and quantifying airborne propagules of *P. clandestina* early in epidemic progress.

III. Develop and refine economically viable conventional and organic powdery mildew management programs.

IV. Determine the effects of temperature and wetness on acute petroleum oil phytotoxicity. Determine the chronic effects of oils on tree health (reported in 2005).

Objective de-emphasized in 2005 and 2006 due to insufficient funding:

V. Develop baseline sensitivities for resistance-prone compounds. Preliminary studies focused on the DMI fungicides. Future studies will concentrate on QoI and quinoline fungicides.

Significant Findings:

- Cleistothecia (the primary inoculum supply) viability declined from 58% at bud burst to 0% about 1 week after pit hardening. For the third year, the degradation of the ascospore supply required slightly less than 200 cumulative degree-days > 10 C (50 F).

- Investigations on the temperature and humidity *ranges* over which the cherry mildew fungus colonizes (grows on) cherry foliage were completed in 2006. Disease developed at 10 (50 F) -28 C (82.4 F) but did not develop at 7.5 (45.5 F) and 28.5-35 C (86-95 F). The effect of relative humidity (between 80% and 100%) alone was insignificant but there were significant temperature/humidity interactions (equation 1). Multiple regression analyses indicated that disease development on cherry foliage was best described by the equation:

Disease severity = $38.9 + 1.3\ T + -0.052\ T^2 * RH + 0.008\ T^{3*} RH$ (equation 1)

where T = temperature and RH = relative humidity. The equation accounted for about 82% of the variability in the raw data ($R^2 = 0.82$). The most significant aspects of these findings are the identification of the temperatures above and below which the fungus does not actively colonize cherry foliage. The temperature algorithm for the secondary infection risk index was partially derived from this equation and previously published information on the latent period. The optimum temperature for colonization was 20.5 C (68.9 F).

- The results of our controlled-environment studies on spore production commenced in 2005 and were completed in 2006 (Figure 1). Sporulation occurred at 12.5 C (54.5 F) -27.5 C (81.5 F) at relative humidities of 80-100% Multiple regression analyses of the raw data indicated that sporulation on cherry foliage was described by the equation:

(log) $Y = -0.003 + 0.05T + 0.09\ T*RH + 0.0001\ T^2 + -0.0004T^3RH$ (equation 2)

with an R^2 of 0.74. The optimum temperature for sporulation was 21.5 C (70.7 F).

• Studies to ascertain the effects of high temperature on the viability of powdery mildew spores were initiated in 2006. It was found that 24 hours of exposure at 40 C was required to kill spores. Exposure times of 0, 4, 8, and 24 hours at 40 C (104 F) resulted in germination levels of 28.1%, 20.9%, 17.0%, and 0%, respectively. Temperatures of 30-39 C (86-102 F) were not lethal regardless of incubation time.

• The PCR assay (using primers developed by the R.A. Spotts group) was found to be extremely sensitive. More thorough sensitivity testing was accomplished in 2006. The assay was found to be sufficiently sensitive to amplify DNA from 100-500 conidia placed directly on glass air sampling medium. Regression analysis revealed a significant ($F = 47.27$; $P = 0.0054$) relationship [y=1.6*exp(-exp(-(x-74.1)/75.4))] (Figure 2) between the numbers of conidia placed on glass sampling rods and successful PCR amplifications with a coefficient of determination (R^2) of 0.93. A new non-phenol extraction procedure developed in 2006 further improved sensitivity to 5-10 conidia placed on glass rods. Intensive testing indicated that the primers did not amplify the DNA of other powdery mildew fungi common in the region.

• For three consecutive years (Figure 3), the PCR assay facilitated the detection of low levels of *P. clandestina* inoculum in air samples within hours of collection in field studies *prior to disease onset*. Air sampling results also confirmed the presence of ascospores in the orchard when their presence was predicted by the temperature/rainfall (primary infection) component of the model. Parallel studies using a Burkard volumetric spore trap indicated the initial detections were a result of ascospore releases during all years of the study.

• *Model and detection based disease management.* The basic code for the model is nearly complete and the current version is in beta on 2007 iteration of the AgWeatherNet web site. The client enters the date of bud burst to activate the model. At this point the model begins degree-day calculations to determine the point of exhaustion/degradation of the primary inoculum supply (model component A; Figure 5). The supply is (conservatively) considered exhausted at $\geq$ 250 cumulative degree-days after bud burst. The model looks for 0.1" precipitation at 50 F or greater prior to the exhaustion of the primary inoculum supply (model component B, Figure 5). When primary infection occurs, the secondary infection/risk index component of the model is activated (model component C, Figure 5). When a significant epidemiological event occurs model output is hyperlinked to pertinent management recommendations.

The beta version of the model and/or the results of detection studies were used to initiate and schedule orchard fungicide applications in 2006. Spray programs were applied according to tree phenology or as specified by 1) the primary infection component of the model or 2) the initial detection using the molecular air sampling techniques. In cases 1 and 2, the secondary infection risk index was initiated at primary infection or first detection and subsequent spray intervals adjusted accordingly. The initiation of the model- and sampling-driven regimes began at least two weeks after the initiation of the phenology-based program and resulted in an elimination of 2 fungicide applications without compromising disease control. For example (Figure 4), a program initiated at the first primary infection identified by the model resulted in disease incidence and severity values of 2.2% and 16.0%, while programs initiated at first detection were 0.4% and 6.8%, respectively. Disease incidence and severity in the industry standard and untreated controls was 3.9% and 32.3% and 48.9% and 78.3%, respectively. Disease incidence and severity values in the model- and detection-based programs were not statistically different from the phenology/calendar program. However, six fungicide applications were made using the standard (phenology/calendar) approach, while four applications were made using the model-driven and detection approaches.

- Five new (significantly less expensive) formulations of tebuconazole were tested for efficacy against powdery mildew and compared to Elite, the commercially available form of the chemical. All formulations provided mildew control equal to that attained using Elite. Different fungicide regimes that conform to FRAC recommendations for resistance management were evaluated in a second trial. There were no significant differences between programs (Table 1).

Results and Discussion:

Our vision for improved management of powdery mildew of cherries involves the use of a forecasting model that incorporates components to predict the exhaustion of the overwintered inoculum supply (model component A), primary infection (component B, i.e. ≥ 0.1" of precipitation at ≥ 50 F), disease pressure once primary infection has occurred (secondary infection risk, model component C), and eventually the initial presence of *P. clandestina* in the orchard air (component D).

Ascocarp degradation model (component A) and primary infection. This model component identifies the period of time over which primary infection (from ascospores) can occur provided adequate moisture and conducive temperatures. Model component "B" was validated using the air sampling technique described below. Ascospores of *P. clandestina* were detected only when predicted to be by present by the rules of model component B.

Secondary infection risk index (component C). The results of studies on the effects of temperature on foliar infection and latent period were used to develop the basic rules for secondary infection risk index. The index is initiated (following primary infection) when

1) there are four consecutive days with ≥ 6 consecutive hours at 15-28.5 C (59-83.3 F). When these conditions are met the index is initiated by adding the first 20 index points

2) on each day when there are ≥ 6 consecutive hours between 15-28.5 C (59-83.3 F), add 20 index points

3) index decreases10 points each day with < 6 consecutive hours between 15-28.5 °C (59-83.3 F)

4) Index decreases 10 points on any day with ≥ 6 hours ≥ 28.6 °C (83.3 F)

5) If none of the above are true, then no change

The index, which ranges between 0 and 100, will be used to adjust spray intervals at low (indices of 0-40), moderate (indices of 40-50), and high (indices of 60-100) disease pressures. At this juncture the powdery mildew model is in the experimental or "beta" stage ready for extensive field-testing and the development of cherry-specific spray intervals for various fungicide classes. Further improvements will result from in-depth studies on the effects of relative humidity and temperature on spore production and high temperatures on colony and spore survival. We need to emphasize that the results used to develop the basic model rules were obtained in controlled environments and that adjustments to algorithms may need to be made after extensive field studies and further controlled-environment research.

PCR techniques and air sampling studies. The primers developed by R.A. Spotts were tested for sensitivity for detection of powdery mildew in reaction mixtures and on glass rods used in orchard air

sampling studies. The PCR assay was demonstrated to be extremely sensitive, e.g. DNA extracted from 1 and 5 spores placed directly into reaction mixtures was detected 83% and 100% of the time, respectively. The PCR assay consistent detected DNA extracted from 100-500 conidia placed directly on glass rods used for air sampling. The air sampling technique described herein shows promise as a research and disease management tool. The method utilizing a Rotorod air sampler operated continuously was the only assay that detected *P. clandestina* in the orchard air early enough to be of practical significance during all years of the study. During 2004-2006, *P. clandestina* was not detected in the orchard air during March and early- to mid- April, indicating that "background" DNA from previous epidemics should not result in "false positives". The initial detection of the fungus in the orchard air in 2005 occurred during a rain event in late-April, while in 2006 this occurred during a rain event in late May. The presence of ascospores in the orchard air (which was predicted using component B of the predictive model) during these rain events was confirmed using a Burkard volumetric air sampler. Positives did not occur for the following 5-10 days after the initial detection. The resumption of "positives" preceded the appearance of visible symptoms by 3-5 days. The air sampling/PCR technique confirmed the presence of the fungus in the orchard throughout the fruiting season. Results of this study should represent the initial step in the incorporation of an inoculum availability component into a cherry powdery mildew risk assessment model. The significance of this component has several potential benefits. The plant disease triangle dictates that any plant disease results from the interaction between host, pathogen, and environment. If the pathogen were absent, even the most disease-conducive weather conditions would not result in disease. Results of the fungicide program initiated upon initial detection of the pathogen in the orchard indicate the potential value of this air sampling technique: control measures are instituted only upon *actual* pathogen presence rather than *predicted* presence. The new and more sensitive non-phenol extraction should make the technique significantly more sensitive in the orchard.

Treatment and rate/A[z]	Spray Timing[y]	% Mildew Severity[w,x]	% Mildew Incidence[v,w]
Non-treated		13.3 a	89.3 a
Stylet oil 97% (conc. 1%).....	1,2		
Pristine 38WG 14.5 oz +			
Sylgard 309 0.03% v/v	4		
Quintec 250SC 7 fl oz	6	3.2 b	39.8 bc
Pristine 38WG 14.5 oz +			
Sylgard 309 0.03% v/v	1,4,7,8		
Rally 40W 5 oz.....	2,6	2.7 b	36.3 bc
Rally 40W 5 oz.....	1,4		
Pristine 38WG 14.5 oz +			
Sylgard 309 0.03% v/v	2,6,7,8	1.5 b	24.0 c
Procure 480SC 12 fl oz.....	1,2,4,6	2.4 b	31.8 bc
Procure 480SC 16 fl oz.....	2,6		
Flint 50WG 3 oz	4,8	3.5 b	42.3 bc
Rally 40W 5 oz.....	1,2,		
Quintec 250SC 7 fl oz	4,6	2.1 b	28.8 bc
Rally 40W 4 oz.....	1,4,		
Quintec 250SC 7 fl oz	2,6	2.0 b	32.5 bc
Elite 45WP 6 oz +			
Induce 0.06%.....	3,5,7	4.2 b	46.8 bc
Flint Max 50WG 6 oz.....	3,5,7	3.5 b	54.3 abc
Gem 500SC 3 fl oz	3,5,7	3.9 b	63.3 ab

[z]Formulated rate per acre, percent spray mix or volume per volume.
[y]Dates for spray applications: 1 = 9 May, 2 = 23 May, 3 = 1 Jun, 4 = 6 Jun, 5 = 15 Jun, 6 = 20 Jun, 7 = 27 Jun, 8 = 5 Jul.
[x]Percentage of leaf area affected.
[w]Means within a column followed by the same letter are not significantly different according to Tukey-Kramer HSD P=0.05.
[v]Percentage of leaves with mildew symptoms.

Table 1. Various fungicide regimes used to manage powdery mildew of cherries. Note that regimes that conform to FRAC resistance management guidelines provide mildew control equal to that obtained using one product and that the oil-based management program (Stylet Oil, Pristine, Quintec) provided control statistically equal to other regimes.

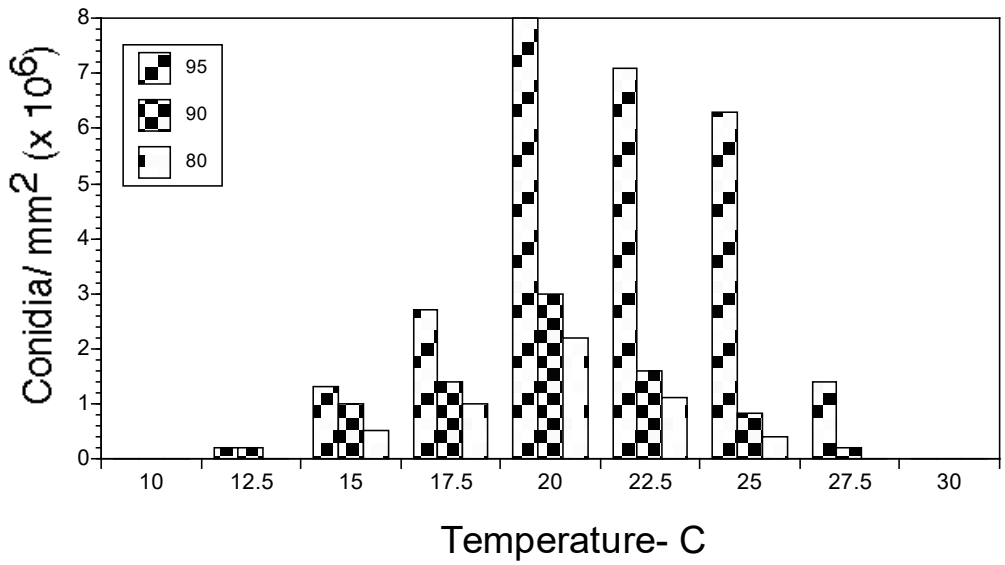

Figure 1. Production of conidia of *Podosphaera clandestina* a 10-30 C under various humidities. Only a trace of sporulation occurred at 70% RH.

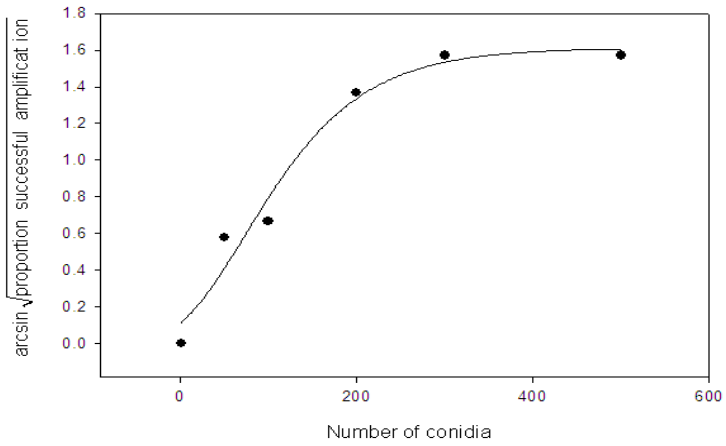

Figure 2**.** Results of PCR assay sensitivity tests: proportion of positive amplifications versus inoculum level. Conidia of *Podosphaera clandestina* were placed directly on glass air sampling rods coated in silicon grease using a human eyelash and extracted using a FastDNA kit and procedure. Specimen DNA was amplified using primers specific to *P. clandestina*.

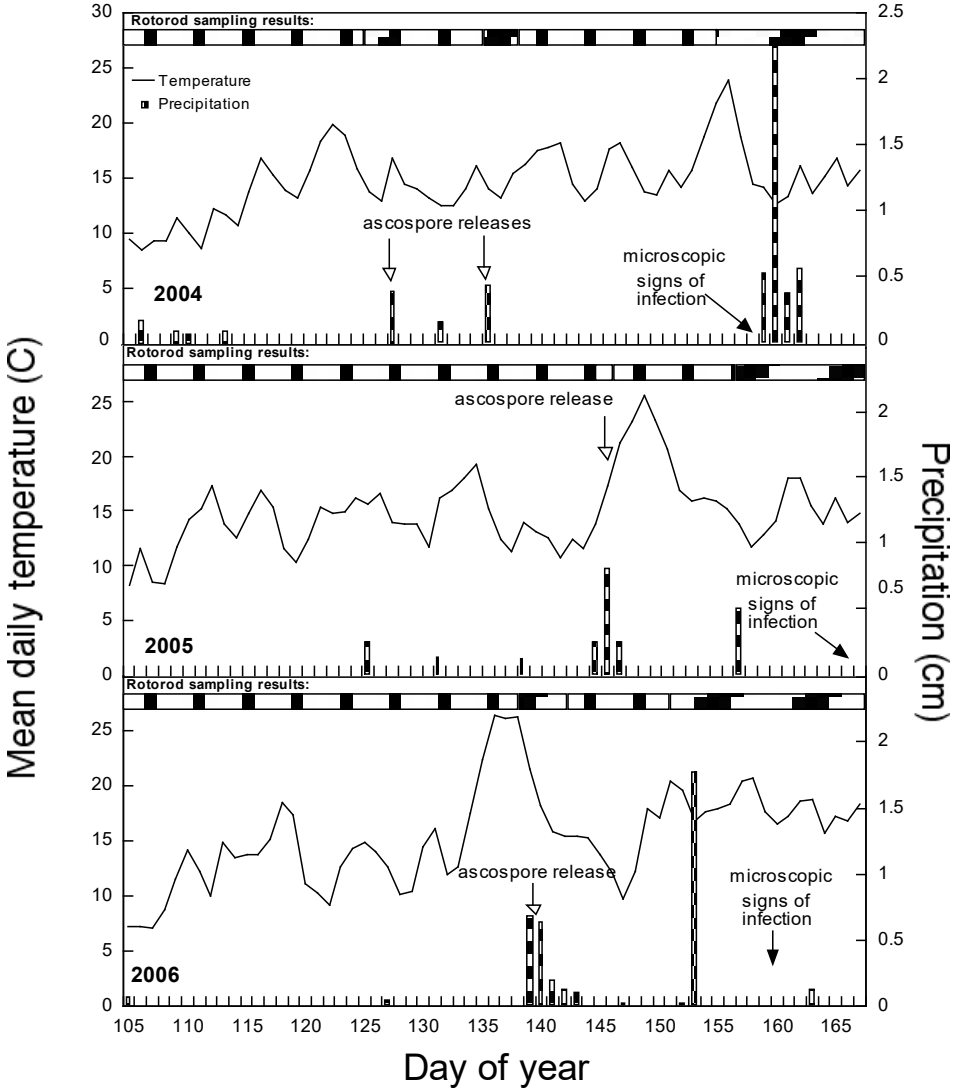

Figure 3. Results of 2004-2006 studies using the PCR-based technique for detection of *Podosphaera clandestina* in orchard air samples. The horizontal bar in the upper portion of each graph indicates the time period over which vineyard air was sampled during the growing season. The segement with vertical lines indicates the period where no amplification of *P. clandestina* DNA occurred. Segments represented by diagonal lines indicate sampling periods where positive PCR amplification indicated the presence of *P. clandestina* in the orchard air. Displayed is daily mean temperature (Celsius (20 C = 68 F; solid line), precipitation (cm; bars), ascospore releases (open arrows) confirmed using a Burkard air sampler and morphological features for propagule identification, and appearance of powdery mildew signs (visible mycelia) over the sampling periods (solid arrows).

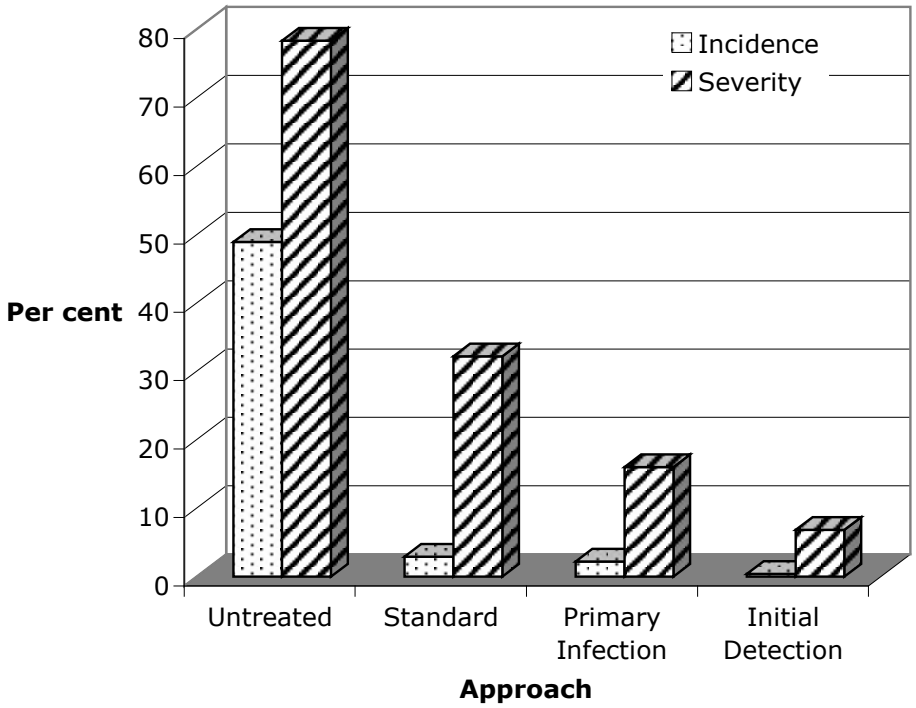

Figure 4. Cherry powdery mildew incidence and severity values attained using different fungicide strategies. Standard program was initiated at shuck fall without regard to weather conditions. "Predicted infection" program was initiated 24 hours after the first post-bud break occurrence of 0.1" of precipitation at 50 F or greater. "Actual detection" treatment regime commenced 24 hours after initial detection of *P. clandestina* in the orchard air using the Rotorod air sampler/PCR identification technique.

2006-05-20	A 224	B 0.17	C 64.5	Primary infection has occurred. Fungicide treatment recommended. Secondary infection/risk Assessment Index initiated.* Powdery Mildew of Cherry Management Options

Cherry Powdery Mildew Secondary Infection/Risk Assessment Index Summary
For station: Moses Lake

Date	Risk Index	Disease Pressure	Status or Event	Consecutive Hours between 59 and 82 F	Hours Temp > 86 F	Spray Interval	Notes
2006-05-20	n/a		Primary Infection	3	0		
2006-05-23	0	Low	Pathogen present	6	0		
2006-05-24	20	Low	Pathogen present	13	0		
2006-05-25	40	Moderate		8	0		
2006-05-26	60	High	Pathogen reproducing every 5 days	D 7	0	Short	Treatment recommended if fungicide protection has expired Powdery Mildew of Cherry Management Options

Figure 5. Output of model components A, B, and C during a critical period during the season. Events at A (degree-day threshold), B (precipitation), C (temperature) coincided to result in primary infection. Note that 0.17" of precipitation was received at 64.5 F prior to the accumulation of 250 cumulative degree days > 50 F; *at this point primary infection occurs which initiates secondary infection risk index.* Note crossing of high-pressure threshold in the Risk Index Column in D. The epidemiological events a A-C and D are hyperlinked to management recommendations.

FINAL PROJECT REPORT

Project Title: Biology and control of powdery mildew on sweet cherry.

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Budget History:

Item	**Year 1:** 2003	**Year 2:** 2004	**Year 3:** 2005	**Year 4: 2006**
Salaries	15000	15675	16560	16560
Benefits	524	548	1418	1418
Wages				
Benefits				
Equipment				
Supplies	500	500	500	500
Travel	300	300	300	300
Miscellaneous				
Total	16324	17023	18778	18778

Project Objectives:

1. Determine when fruit infection occurs in relation to maturity.
2. Examine the effect of temperature and relative humidity on fruit infection.
3. Develop an early detection method for PM on fruit.
4. Establish a baseline for powdery mildew (PM) resistance to demethylation inhibitors (DMI's) fungicides.
5. Evaluate foliar mildew levels under various management regimes.
6. Study the relationship between powdery mildew infection and pitting.

Significant Findings:

- ✓ Fruit remain susceptible to PM throughout the growing season, potentially gaining some resistance after reaching 15 ºBrix.
- ✓ Powdery mildew is able to effectively infect fruit under a range of temperature and relative humidity combinations.
- ✓ qPCR - quantification
- ✓ PM isolates resistant to DMI's have been found.
- ✓ Orchard management practices impact the amount of PM infection. Rootstock selection, training system, and cultivar selection all influence the development of PM.
- ✓ Pitting responses to injury vary by cultivar and are related to the temperature at which the injury occurred. PM worsens pitting.

Results and Discussion:

<u>Objective 1: Determine when fruit infection occurs in relation to maturity. Figures 1, 2, & 3.</u>

The timing of cherry fruit infection is a key piece of information that can be invaluable in control and management strategies of PM. A study designed to answer this question was initiated in 2003 and was repeated in subsequent years of this project. Bags were removed from each fruit cluster for a one-week period throughout the growing season so that fruit were exposed to PM spores and infection. Fruit were assessed for mildew incidence upon harvest, and a sampler monitored the daily number of conidia in the orchard air. Three cultivars, Bing, Lapins, and Sweetheart, were included. In 2003, bags were made of Typar material that was not conducive to fruit development. Sensors confirmed that relative humidity within the Typar bags was extremely elevated compared with ambient conditions (data not presented). In the three remaining years of the project, bags were made of a nylon fabric that allowed gas and moisture exchange, yet excluded PM conidia. Sensors confirmed that the relative humidity and temperature within the nylon bags was similar to ambient condition (data not presented).

Among the cultivars, Sweetheart consistently had the highest incidence of PM infection and Bing the lowest. The 2004 growing season had the highest infection level of the three years, and the 2006 season had the lowest for each cultivar. The air sampler indicated similar amounts of conidia present in the orchard for both 2004 and 2006 (maximum values of 531.31 and 553.06 conidia/m^3, respectively) and much lower conidia in 2005 (maximum value of 293.11 conidia/m^3). Data were collected for each of the three cultivars in season 2004, but only cultivar Sweetheart in 2005, and Bing and Sweetheart in 2006. The cool, wet weather conditions in spring 2005 and 2006 were such that pollination was poor and/or flowers suffered frost damage, resulting in loss of fruit for this study in cultivars Bing and Lapins in 2005 and only Lapins in 2006.

In the case of Lapins and Sweetheart, the incidence of powdery mildew was significantly greater on fruit never covered with a bag (positive control) than fruit always covered with the bag (negative control). With Bing, the two controls were not statistically different. This may be due to lower infection rates observed in Bing. Other studies have indicated that cultivar Bing is less

susceptible to foliar infection by PM when compared with cultivars Sweetheart and Lapins (report below). Fruit infection was not statistically different among the treatments in which bags were removed for a one week period. In each of the three cultivars, fruit remained susceptible to PM throughout the growing season. Fruit infection declined somewhat near the point at which fruit reached 15 °Brix, about 1 ½ weeks before harvest. Perhaps some slight resistance is gained once 15 °Brix is reached, but these studies show evidence to the contrary of this theory. Powdery mildew incidence was statistically the same in the weeks before and after 15 °Brix was reached consistently.

Objective 2: Examine the effect of temperature and relative humidity on fruit infection. Tables 1 & 2.

Optimal conditions for fruit infection, including temperature and relative humidity, have not been well studied. Studies by Grove found that leaf infection is optimal at 20 and 25 °C, and that infection could occur under a range of temperature and relative humidity combinations. Conidia produced from leaf infections most likely initiates infection on cherry fruit. Thus, knowledge of the optimal temperature and relative humidity for fruit infection will aid in the development of a PM prediction model for fruit infection.

A laboratory assay using detached fruit of cultivar Bing was developed to evaluate temperature and relative humidity combinations. Preliminary studies in 2004 led to the successful inoculation of detached cherry fruit with PM. In 2005, this assay was done with twelve combinations of temperature and relative humidity chosen to represent common environmental conditions during four key stages of fruit development, full bloom, initiation of pit hardening, completion of pit hardening, and harvest. In 2006, this assay was repeated but modified to include only one temperature, 18 °C, and six levels of relative humidity. In each year, immature fruit were inoculated by touching a sporulating leaf to the fruit surface. After being held at the appropriate temperature and relative humidity treatment for six days, fifty spores per fruit were assessed for germination. A conidium was deemed germinated if it had at least one hypha twice the length of the conidium. Extensive colonization of the fruit surface and any sporulation were also noted.

In both 2005 and 2006, the differences between treatments on percent germination were not statistically significant, indicating that PM has excellent fitness for infecting sweet cherry fruit under the wide range of temperature and relative humidity conditions tested. Germination was quite low in 2006 in comparison with 2005. An average of 6.8% of the evaluated conidia germinated, while 14.2% germinated in 2005. The highest percentage of spore germination in 2005 occurred at 18.6 °C, which corresponds to average day time high temperature during pit hardening, usually the last week of May at MCAREC. Analysis revealed that the number of fruit with extensive hyphae was not independent of the relative humidity at which fruit were incubated. Thus, humidity had an effect on the growth capabilities of PM. This temperature was then used for the 2006 studies. About 10% of the inoculated fruit developed secondary hyphae and/or an extensive colony of hyphae at the point of inoculation but did not sporulate in 2005. In 2006, this percentage was 5.6%. The majority of fruit in this category were incubated at 18.6 °C regardless of relative humidity. In 2006, fruit held at 92% relative humidity had the highest germination rate. Of all the fruit inoculated in both studies, only one sporulated; it was incubated at 15.5 °C and 68% relative humidity for six days.

These results contrast a similar study by G. Grove, where spore germination increased with increasing relative humidity. This study found no such relationship. Grove's study, however, defined spore germination as when the germ tube length exceeded the width of the spore, only included one temperature (20 °C), and held the detached fruit for a maximum of 24 hours. These results indicate that PM is capable of infecting cherry fruit under a wide range of environmental conditions.

Objective 3: Develop an early detection method for PM on fruit.

A polymerase chain reaction (PCR) technique was successfully developed to identify cherry PM in both fruit and leaf tissue. PCR uses certain primers designed specially for cherry PM that are specific to enough to delineate cherry PM from other common PM fungi. PCR is a molecular tool

that clones cherry PM DNA so that it can be quickly detected on plant tissue, even before it can be seen with the naked eye. Techniques for using both regular and quantitative PCR, which allows cherry PM DNA to be quantified, have been worked out. Future diagnostic, population, and genetic studies will benefit from this.

Objective 4: Establish a baseline for powdery mildew (PM) resistance to demethylation inhibitors (DMI's) fungicides. Tables 3 & 4.

Several orchardists in the PNW have expressed concern about losing effectiveness of certain fungicides used to control PM, particularly in the class of DMI's. Studies on several other crops have positively identified resistance of PM to certain DMI's; so, a preliminary study was undertaken in 2005 to assess DMI resistance in cherry PM.

A leaf disk assay was used to test five commercially available DMI's: Elite (tebuconazole), Orbit (propiconazole), Procure (triflumizole), Rally (myclobutanil), and Rubigan (fenarimol). Of the ten orchards evaluated, five were suspected as having PM resistance to one or more fungicides and were located in Hood River, The Dalles, and Prosser. Procure was the only fungicide that seemed to retain its effectiveness in all of the orchards. In 2006, techniques were refined and the orchard area expanded to best determine whether or not an orchard has DMI resistant PM.

One of the changes in 2006 was to use monoclonal isolates as the inoculum source as opposed to the mix of isolates from one orchard used in 2005. To obtain a monoclonal isolate of PM, a single conidium is transferred to a leaf using a hair. Furthermore, inoculation was done by transferring a single chain of conidia with a hair to each leaf disk in the assay following the application of the fungicide. PM isolates were collected from orchards in Wenatchee, Yakima, Parkdale, Mosier, Hood River, and The Dalles, and at least ten monoclonal isolates per orchard were attempted. Sweet cherry PM was found to be difficult to culture, and many monoclonal isolates did not survive or did not produce sufficient conidia to carry out the leaf disk assay. In total, thirteen monoclonal isolates were successfully evaluated. Procure again held up remarkably well in that none of the isolates showed any loss of sensitivity to it. A great variability existed among isolates, even when collected from the same orchard. For example, one isolate collected from an Orchard 8 was resistant to Orbit, Rally, and Rubigan, but another isolate from Orchard 8 was not resistant to any of the DMI's. Two isolates from two different orchards, Orchard 6 and 8, were sensitive to all of the DMI's tested and showed no signs of resistance. One of these, Orchard 6, is a certified organic operation.

DMI fungicides have a single mode of action and target only one gene to control fungi. Resistance develops much more quickly in single mode of action fungicides, because a simple mutation in the fungus can decrease the effectiveness of the fungicide. The life cycle of PM ensures great genetic variability among this group of fungi, so that resistance to DMI's is a valid concern. These results confirm that resistance to DMI's does exist among populations of cherry PM in the PNW. However, this study was not thorough enough to determine the prevalence of these isolates in orchards. A great effort should be made to educate growers about the importance of engaging strategies to preserve the effectiveness of the DMI's currently available, such as using the maximum labeled rate and rotating DMI's with fungicides from other classes.

Objective 5: Evaluate foliar mildew levels under various management regimes. Figures 4 - 7.

Management practices, such as pruning, cultivar selection, and rootstock selection, influence the development of PM in an orchard. In 2003 and 2004, ten shoots of current year's growth were collected per tree, and the incidence of PM was recorded for the outer most ten leaves. No more than ten trees per management practice were surveyed. Three training systems (steep leader, central leader, and Spanish bush), four rootstocks (Edabriz, Maxima 14, Pontileb, and Mazzard), and five cultivars (Bing, Lapins, Regina, Staccato, and Sweetheart) were compared. All of the trees were part of other, ongoing studies.

In both years, PM incidence was significantly greatest in trees trained with the Spanish bush system. Spanish bush pruning strategies promote heavy branching and dense foliage that would diminish air movement through the canopy, thereby creating a more favorable environment for PM. Both central and steep leader are more conducive to encouraging air flow through the canopy. With rootstocks, Mazzard consistently had the significantly highest PM incidence and Edabriz the least. This is also likely related to air flow in the canopy, as trees with Mazzard rootstock have a much larger, denser canopy. Edabriz is a more dwarfing rootstock, which enables greater air circulation.

A range of PM resistance was evident among the five cultivars. In both 2003 and 2004, cultivar Regina had the lowest incidence of PM. In 2003, Sweetheart had the highest incidence, and Staccato had the highest incidence in 2004. Bing was always the cultivar with the second lowest incidence, and Lapins was the third.

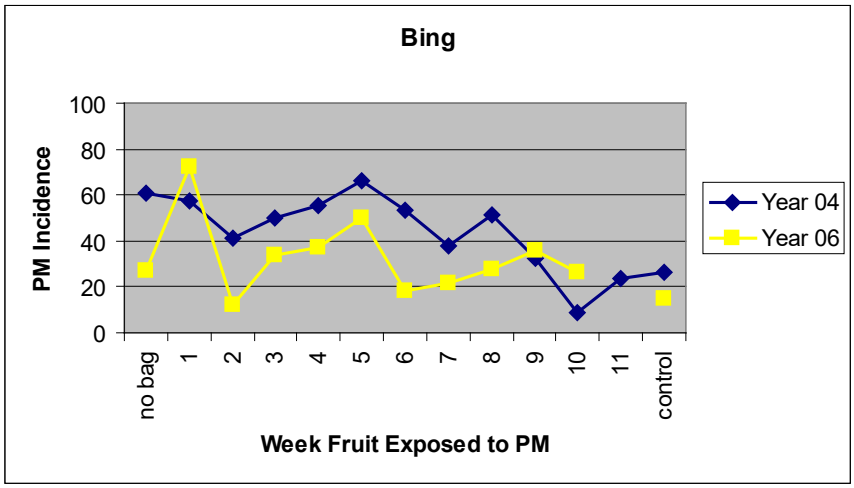
These results illustrate the importance of PM consideration when selecting a training system, rootstock, and cultivar, particularly for new orchards. If the orchard is known or has the potential to have high PM infection levels, then cultivar selection, training system, and/or rootstock should be carefully chosen. Selecting a cultivar such as Regina and/or using a central leader training system can lower or delay a PM epidemic and ultimately dependence on fungicides.

Objective 6: Study the relationship between powdery mildew infection and pitting. Tables 5 - 11.

PM has been proposed to worsen the effects of pitting, resulting from handling/injury to the fruit. Studies in 2004, 2005, and 2006 were designed to clarify the relationship between PM and pitting. Infected fruit were collected and categorized based on their percent surface area infected: no PM (0%), slight PM (1-33%), moderate PM (34-66%), or severe (67-100%). Cultivars Bing, Lapins, and Sweetheart received a standard injury at 1, 4, or 20 °C with a special tool. After two weeks of storage at either 1 or 4 °C, pitting was rated on a scale where 1 = no pitting, 2 = slight pitting, 3 = moderate pitting, and 4 = severe pitting. Due to poor fruit set, only cultivar Sweetheart was included in 2005, and only Lapins with no PM or slight PM infection levels were included in 2006.

In each of the three years, cultivar Sweetheart had the greatest injury due to pitting, suggesting a possible cultivar effect. Storage temperature had no effect on pitting. With both Bing and Sweetheart, pitting was related to the temperature at which the injury occurred; the impact delivered at 1 °C resulted in significantly greater pitting than those at 20 °C. With Lapins, this effect was inexplicably opposite in 2004 but held true in 2006. The effect of temperature is in accordance with previous reports by Lidster and Tung.

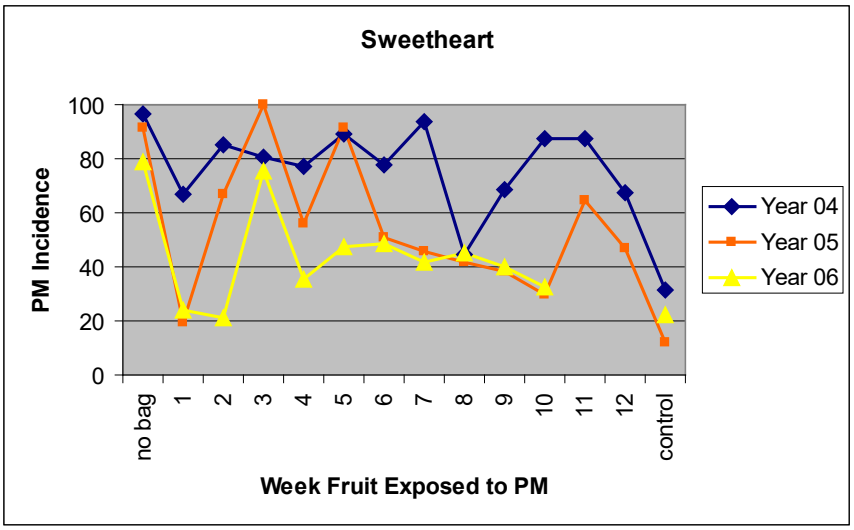
PM worsened pitting in all of the studies except Bing 2006, where PM had no effect on pitting. Whether or not PM is directly responsible for this trend is unknown. In 2004 and 2005, mildewed fruit were less mature in terms of size, color, and °Brix. Previous studies by Facteau and Rowe have shown that immature fruit are more susceptible to pitting than mature fruit. Mildew might be delaying fruit maturity and thus causing an increase in pitting damage, or mildew impacts the integrity of the fruit, somehow rendering it more susceptible to pitting.

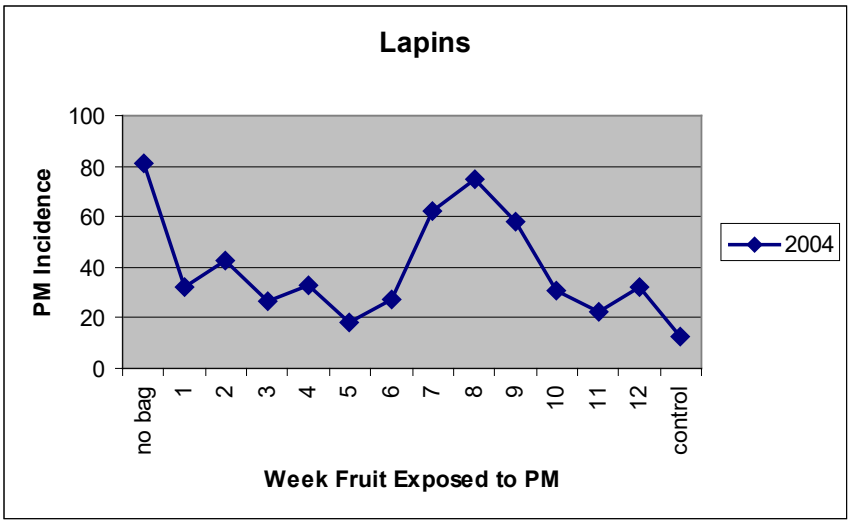


Figures 1, 2 & 3. Average percent fruit infected, or PM incidence, in the bagging study for each cultivar. The positive control where

fruit were never bagged is indicated by "no bag. " Subsequent weeks correspond to the period of time when fruit were exposed (bag removed) and vulnerable to PM infection. The negative control where fruit remained covered by a fabric bag the entire season is indicated by "control." PM incidence ratings were done following harvest.

Tables 1 & 2. Results from the detached fruit inoculation study. The average percent of germinated conidia with hyphae at least twice as long as its conidium, are listed as % Germinated Conidia relative the temperature-relative humidity treatment. The number of fruit with extensive hyphal colonization from the detached fruit inoculation study is presented. A Chi-squared test for homogeneity revealed that extensive, secondary hyphae production is not independent of the temperature – relative humidity treatment in 2005. In 2006, there were no differences among treatments.





2005				
Temperature		Relative Humidity	% Germinated Conidia	# Fruit with Extensive Hyphae
°C	°F			
12.79	55	70	12.25	2
		79	15.17	2
		88	11.42	1
15.46	60	68	9.17	3
		75	6.50	1
		83	14.00	5
18.62	65	68	12.33	6
		74	22.50	4
		80	9.25	2
23.76	75	70	7.25	0
		75	7.50	1
		79	16.58	0

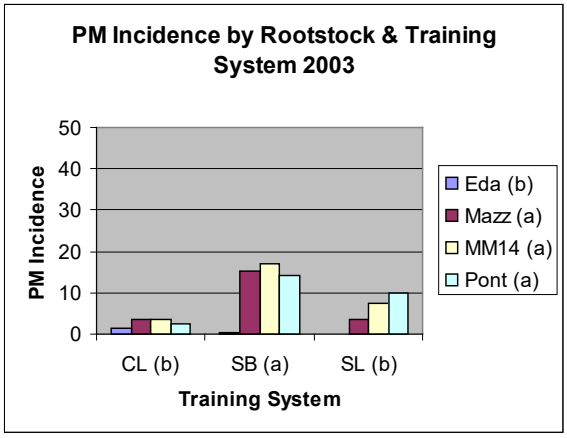
2006; temp = 18.6 °C		
Relative Humidity	% Germinated Conidia	# Fruit with Extensive Hyphae
68	8.08	2
74	4.67	2
80	5.75	0
86	4.42	1
92	11.92	0
98	6.08	3

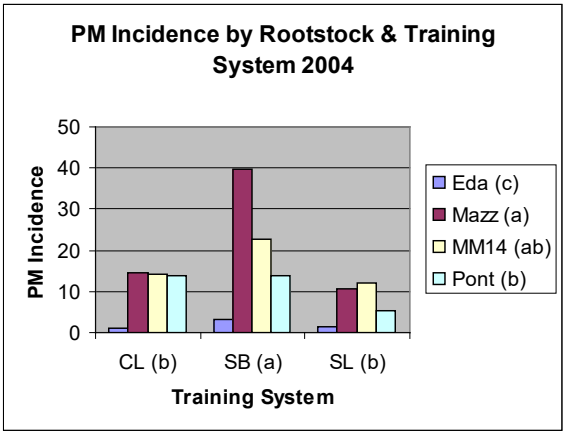
Table 3 & 4. ED50 values (mg/L) from resistance studies 2005 and 2006.

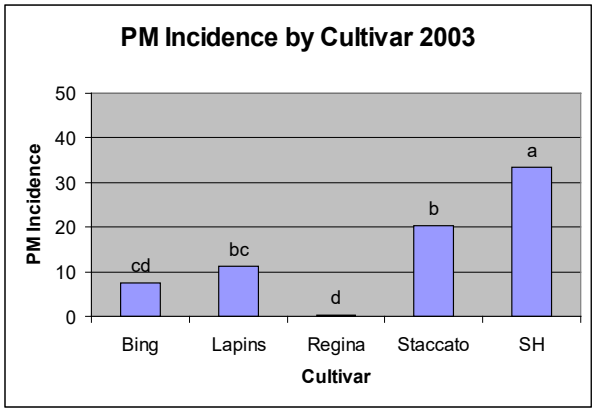
2005 Orchard	Location	Elite	Orbit	Procure	Rally	Rubigan
1	**The Dalles**	54.20	47.86	15.85	42.66	5128.00
2	**The Dalles**	9.77	3.8×10^{37}	11.75	10.47	1.5×10^{39}
3	**The Dalles**	18.62	12.88	19.05	15.85	1.1×10^{46}
4	**Prosser**	18.62	10.47	12.02	7.94	11.22
5	**Hood River**	12.30	12.59	15.14	14.13	9.12
6	**Hood River**	8.71	7.08	10.96	9.77	17.38
7	**Hood River**	66.68	10.96	20.89	10.23	7.59
8	**Hood River**	8.13	53.70	13.49	21.88	8.71
9	**Hood River**	11.22	7.41	10.96	9.77	13.49
10	**Prosser**	8.71	7.94	12.88	7.41	77.62
	Max Labeled Rate	**67.41**	**43.17**	**149.80**	**59.92**	**62.60**

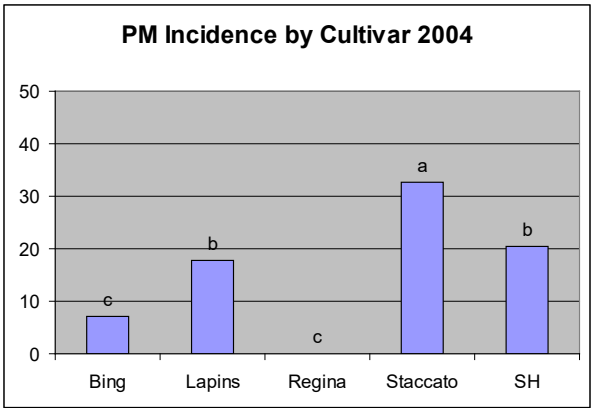
2006 Orchard	Location	Elite	Orbit	Procure	Rally	Rubigan
1	**Wenatchee**	419.76	79.43	25.18	15.21	97.28
2	**The Dalles**	1570.36	5023.43	31.48	33.65	688.65
3	**Prosser**	74.30	23.55	17.02	21.88	5.12×10^{21}
4	**Hood River**	65.31	47.32	24.49	212.32	35.89
5	**Hood River**	737.90	1559.55	14.39	19.72	1741.81
5	**Hood River**	25.24	12.27	13.84	35.34	100.23
6	**Mosier**	32.66	9.33	14.09	19.06	11.75
7	**Wenatchee**	28.84	82.22	11.78	10.09	11.75
7	**Wenatchee**	633.87	8.47	10.86	11.43	51.40
8	**Yakima**	21.98	3.27×10^{8}	11.35	4560.37	3.1×10^{17}
8	**Yakima**	31.48	17.50	10.94	10.52	42.76
9	**Yakima**	18.62	81.66	12.82	17.91	21.48
9	**Yakima**	112.20	864.97	22.54	31.33	583.45
	Max Labeled Rate	**67.41**	**43.17**	**149.80**	**59.92**	**62.60**

Figures 4 - 7. Average percent PM infected leaves by different management systems. Rootstock abbreviations are as follows: Eda = Edabriz, Mazz = Mazzard, MM14 = Maxima 14, and Pont = Pontileb. Training system abbreviations are as follows: CL = central leader, SB = Spanish bush, and SL = steep leader. In all figures, statistical differences are indicated by the lower case letter in parentheses.









Tables 5 - 11. The average rating of pit development following a standard impact on fruit with varying degrees of PM infection. A pit rating of 0 = no damage, 1 = slight damage, 2 = moderate damage, and 3 = severe damage. Prior to the injury, fruit were sorted based on their level of PM infection where No PM = no visible PM, Slight PM = 1 – 33% of the fruit surface colonized, Moderate (Mod.) PM = 34 – 66% of the fruit surface colonized, and Severe PM = 67 – 100% fruit surface colonized by PM. Statistical differences in the severity of pitting among the fruit infection levels and temperature at which the fruit were injured are indicated by different letters.

Bing 2004					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (a)	Mod. PM (a)	Severe PM (b)
1 (c)	1	3.08	2.72	2.92	3.20
1 (bc)	4	2.48	2.68	2.48	3.52
4 (b)	1	2.52	2.48	2.88	2.92
20 (a)	1	2.08	2.16	2.20	2.36

Lapins 2004					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (ab)	Mod. PM (bc)	Severe PM (c)
1 (ab)	1	2.28	2.40	2.68	2.96
1 (a)	4	2.32	2.48	2.56	2.40
4 (ab)	1	2.48	2.48	2.68	2.64
20 (b)	1	2.56	2.68	2.72	2.84

Sweetheart 2004					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (b)	Mod. PM (b)	Severe PM (b)
1 (b)	1	2.56	2.88	2.76	2.96
1 (b)	4	2.56	2.88	2.84	2.88
4 (ab)	1	2.44	2.68	2.68	2.80
20 (a)	1	2.32	2.56	2.80	2.68

Sweetheart 2005					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (a)	Mod. PM (b)	Severe PM (b)
1 (a)	1	2.12	2.12	2.64	2.60
1 (a)	4	2.08	2.20	2.44	2.60
4 (a)	1	1.80	1.84	2.52	2.84
20 (a)	1	1.72	1.84	2.56	2.68

Bing 2006					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (a)	Mod. PM (a)	Severe PM (a)
1 (b)	1	2.24	2.04	1.96	1.74
1 (b)	4	1.96	2.28	2.36	2.28
4 (b)	1	2.12	1.80	2.24	2.00
20 (a)	1	1.67	1.36	1.64	1.40

Lapins 2006			
Injury Temp C	Storage Temp C	Mildew Rating	
		No PM (a)	Slight PM (a)
1 (b)	1	2.21	2.17
1 (b)	4	2.24	1.96
4 (b)	1	2.00	1.96
20 (a)	1	1.40	1.30

Sweetheart 2006					
Injury Temp C	Storage Temp C	Powdery Mildew Rating			
		No PM (a)	Slight PM (b)	Mod. PM (b)	Severe PM (ab)
1 (b)	1	2.60	2.72	2.76	2.64
1 (ab)	4	2.24	2.60	2.44	2.56
4 (ab)	1	2.28	2.76	2.88	2.48
20 (a)	1	2.12	2.40	2.60	2.20

FINAL PROJECT REPORT
WTFRC Project Number: CH-06-600

Project Title: Fruit and foliar powdery mildew resistance mechanisms in cherry

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Budget History:

Item	**Year 1:** 2006
Salaries	0
Benefits	0
Wages	4,500
Benefits	450
Equipment	0
Supplies	500
Travel	1,000
Miscellaneous	0
Total	6,450

Introduction

Powdery mildew (PM) is the most important pre-harvest disease of sweet cherry in the Pacific Northwest (PNW). Great strides have recently been made in conventional control methods, including the development and registration of new fungicides, elucidation of the life cycle and infection periods for cherry PM, and the development of forecasting models. These are all tools that have helped to prevent or ameliorate devastating epidemics. However, even with excellent control methods, PM continues to be one of the highest research priorities listed by PNW growers. As the production season continues to lengthen into late summer with extremely late-ripening cultivars and new production areas, the time period control measures are necessary increases, as well as the financial expenditures necessary for application. In addition to conventional control methods, genetic resistance to PM is likely to be an important trait for sustainable cherry production, providing another tool for growers to use in disease and resistance management programs.

Because of this, development of PM resistant cultivars is a primary goal for the WSU Sweet Cherry Breeding Program. Fortunately, five cultivars have been determined to possess foliar resistance to PM ('PMR-1', 'Chelan', 'Venus', 'Moreau', and 'Hedelfingen') (Olmstead et al., 2001; Olmstead and Lang 2002a). Additionally PM resistance in all five cultivars was shown to be controlled by a single dominant gene (Olmstead and Lang 2002a,b). However, to most effectively use this genetic resistance(s) in a breeding program, three critical questions remain:

<u>Is the gene controlling PM foliar resistance the same in all five selections?</u>

If the selections possess two or more resistance genes it will be possible to pyramid these genes, thus providing a more stable resistance. This is conceptually similar to the fungicide resistance management programs that PNW growers now use. The more and diverse genes available, the less chance there is for the fungal organism to overcome the host plant genetic resistance. Crosses to answer this question were made in 2005 and PM screening was initiated in 2006 as a routine procedure in the Sweet Cherry Breeding Program.

<u>Is the mechanism(s) of PM foliar resistance suggestive of a high or low risk of being overcome by pathogen mutation?</u>

An answer to this question is critical for designing a breeding strategy that results in the most durable foliar resistance possible. For example, many single dominant resistance genes have had a low level of durability under field conditions. In these cases, the plant host –pathogen interaction involves specific *R*-genes (plant host) and *avr* genes (fungal organism) that are both part of the recognition reaction (Staskawicz, 2001). Resistance breakdown happens when a mutation occurs in the *avr* gene of the fungal organism so that the resistance interaction is no longer possible. A hypersensitive response involving local cell death in the host plant is often indicative of this host-pathogen reaction. This localized cell death prevents further colonization by the fungal organism. This initial plant response to attempted infection can be observed microscopically.

To date, evaluations of PM resistance have not been done at this level. Instead, phenotypic evaluations have been done at the visual level (Figure 1). For continued improvement in the Sweet Cherry Breeding Program, a better characterization of the type of interaction between PM and the resistant cultivars currently available is necessary. Most importantly, if the different PM resistant cultivars disrupt pathogen growth and reproduction in different ways, pyramiding these resistant mechanisms would improve the stability of the resistance under field conditions.

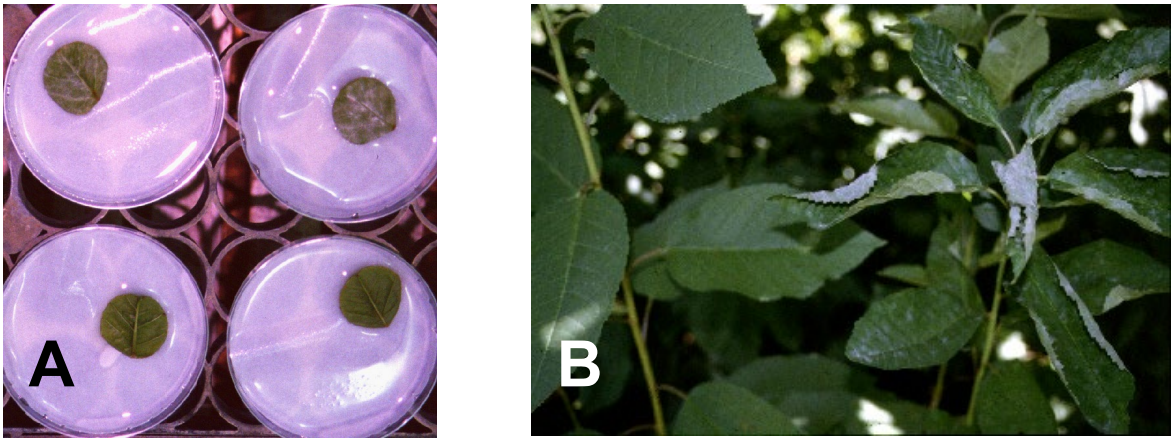

Figure 1. Resistance phenotype exhibited by progeny from the cross PMR-1 x Rainier. **A.** Leaf disks taken from four different progeny and inoculated with PM. Top: leaf disks are susceptible to PM and show significant sporulation. Bottom: leaf disks completely resistant to PM infection. **B.** A resistant plant (left) and susceptible plant (right) during field screening.

<u>Do any of the five sweet cherry cultivars exhibiting foliar resistance to PM possess fruit resistance to PM?</u>

To date, none of the PM resistance screening in the Cherry Breeding Program has been done on fruit. Instead disease screening has been exclusively on the leaves. Although foliar resistance will likely reduce the available inoculum during periods of fruit susceptibility, fruit resistance to PM is an important goal, due to the potential economic costs to the grower community.

The second objective of this proposal addresses this question. Specifically, fruit from the five resistant cultivars exhibiting foliar resistance will be collected weekly to determine whether the resistance response is also present in the fruit.

- In summary, this proposal brings together the genetic and plant pathology expertise to answer two questions critical to the success of the development of PM cultivars that possess durable resistance to foliar and fruit infection. The information gained will be immediately implemented into breeding decisions.

Objectives

The specific objectives of this research were to:

1. Microscopically evaluate the resistant host/PM pathogen interaction at the cellular level to precisely determine the affects of host resistance on PM growth/inhibition using PM resistant selections from the Cherry Breeding Program.
2. Determine whether fruit from the same PM resistant sources as Objective 1 exhibit the same resistance response as foliar plant material.

Significant Findings and Accomplishments

- Fruit from cultivars exhibiting foliar PM resistance were also resistant to the disease.
- No hypersensitive reaction was observed for any of the resistant cultivars.

- Although PM growth progressed as far as hyphae growth, no spore producing structures (conidiophores) were produced on resistant cultivars.
- Conidial germination, appressoria formation, and hyphae production on resistant cultivars were significantly lower than susceptible cultivars.
- Among the resistant cultivars, 'PMR-1' and 'DD' (a selection from the cross between 'PMR-1' and 'Rainier') were the only that differed significantly for conidial germination, appressoria formation, and hyphae production.
- For the resistant cultivars, conidial germination, appressoria formation, and hyphae production generally peaked three days after inoculation.

Methods

Objective 1. Microscopically evaluate the resistant host/PM pathogen interaction at the cellular level to precisely determine the affects of host resistance on PM growth/inhibition using PM resistant selections from the Cherry Breeding Program.

Plant material: Young, newly expanded, foliar samples visibly free of PM infection were collected from 'PMR-1', 'Chelan', 'Venus', and 'Moreau', all resistant parents, and selected progeny from existing populations at the WSU-Roza farm using 'PMR-1' as a resistant parent. Additionally, the susceptible cultivars 'Bing', 'Lambert', 'Rainier', 'Sweetheart', and selected susceptible progeny from the above populations were sampled. Leaves at this developmental stage are most susceptible to PM infection, and were collected from orchard blocks with no PM control methods applied.

Measurements: Fresh tissue was cut into 20 mm diameter leaf disks, surface sterilized with a dilute bleach solution, and artificially inoculated with fresh PM conidia using a spore settling tower. After inoculation, replicated experiments were cultured in a controlled environment using previously identified environmental conditions (Olmstead et al., 2000). Leaf disks were sampled at 1, 2, 3, 5, and 7 d intervals and placed in chemical fixative to kill the leaf and PM organism and preserve the sample. Differential staining using aniline blue and solophenyl flavine 7GFE (Hoch et al., 2005) were used to examine fungal growth on the leaf surface.

Objective 2. Determine whether fruit from the PM resistant sources listed in Objective 1 exhibit the same resistance response as foliar plant material.

Plant material: Fruit from the same cultivars and selections listed under Objective 1 were examined for PM growth in the field and under controlled conditions.

Measurements: Weekly observation of fruit from shuck fall to maturity for visible PM infection was the primary method for determination of potential fruit resistance. Controlled inoculation of fruit from resistant cultivars using a spore suspension was used to determine the extent of PM growth. Inoculated fruit were chemically fixed and stained for microscopic observation as in Objective 1.

Results and Discussion

After the primary PM infection cycle of cherry is initiated following ascospore release from the overwintering cleistothecia (Grove and Boal, 1991), disease progression occurs through production and release of vegetative spores called conidia. These conidia are produced as long chains on hyphal outgrowths termed conidiophores, and conidia production continues through much of the growing season. When a conidial spore is released and lands on the appropriate tissue, it forms a germination tube structure that contacts the epidermal plant cell (Green et al., 2002). After contact

between the fungus and the plant is made, an enlarged structure called the appressoria is formed, and a penetration peg attempts to enter the plant cell to form an absorption structure known as a haustorium (Figure 2). Once the haustorium is established, hyphae grow across the surface of the plant tissue, repeating the penetration process and producing additional conidia. In resistant cultivars, the plant host –pathogen interaction generally involves specific *R*-genes (plant host) and *avr* genes (fungal organism) that are both part of the recognition reaction (Staskawicz, 2001). Thus, conidial germination and appressoria formation occur prior to plant cell penetration, the site of initial host-pathogen recognition. A hypersensitive response involving local cell death in the host plant is often indicative of this host-pathogen reaction. This localized cell death prevents further colonization by the fungal organism. These initial fungal growth attributes were examined on both resistant and susceptible cherry cultivars to more precisely determine the affects of host resistance on PM growth/inhibition.

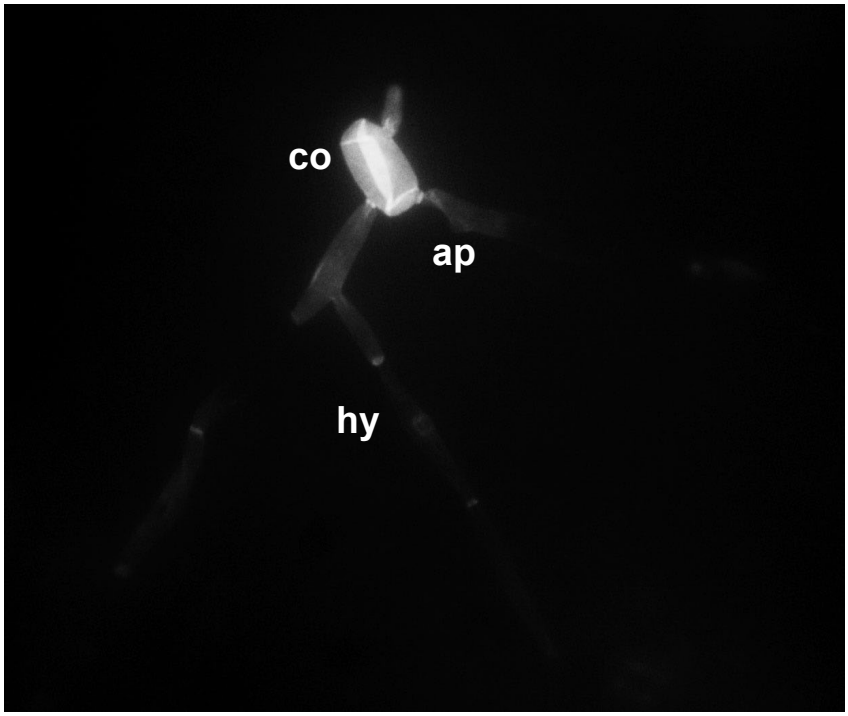

Figure 2. Example of initial powdery mildew growth on a sweet cherry leaf. Fungal structures were stained with solophenyl flavine 7GFE and viewed at 40x magnification. Co = conidia, ap = lobed appressorium, hy = hyphae.

Comparison of resistant and susceptible cultivars indicated that all of the resistance sources initially delayed PM infection and growth (Figure 3). By three days after inoculation, conidial germination, appressoria formation, and hyphae growth were equal between resistant and susceptible cultivars. After three days post-inoculation, hyphae production was significantly reduced among resistant cultivars. No conidiophore production was observed on resistant cultivars, although limited conidiophore production was evident on susceptible cultivars beginning in the fifth day after inoculation (Figure 3).

Among the resistant cultivars examined, only 'PMR-1' and 'DD' differed significantly for the observed fungal growth characteristics. 'DD' had significantly less conidial germination and appressoria formation, while 'PMR-1' had significantly less appressoria and hyphae formation than the other resistant cultivars (Table 1). Both 'PMR-1' and 'DD' carry the *Pmr-1* resistance gene and therefore are expected to exhibit similar resistance phenotypes. The similarity between 'Chelan', 'Moreau', and 'Venus' may indicate a common resistance gene among these cultivars; allelism tests will be made from crosses made between these cultivars in the Sweet Cherry Breeding program and currently under evaluation.

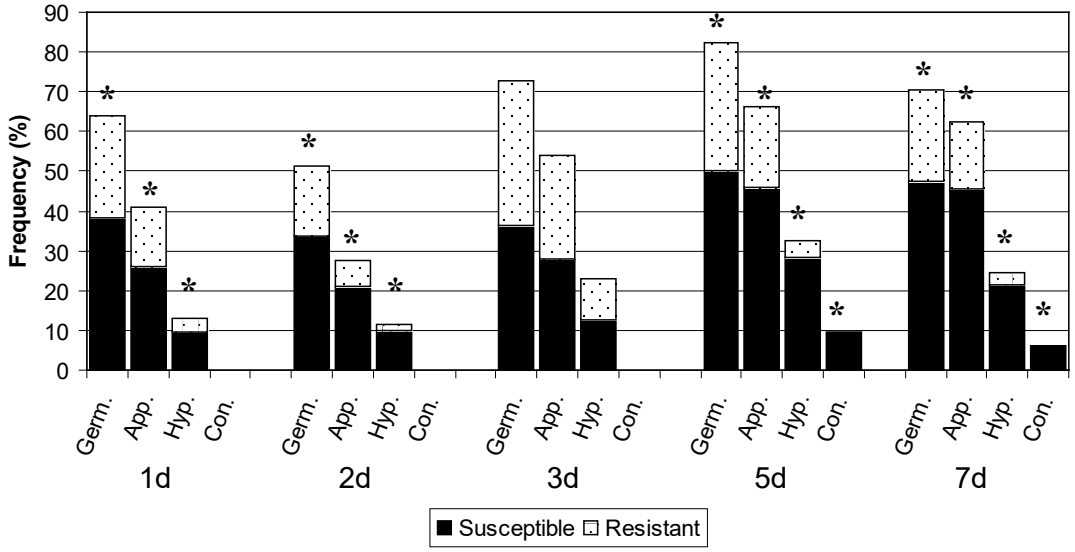

Figure 3. Frequency of observed powdery mildew growth stages on resistant and susceptible sweet cherry cultivars. Germ. = germ tube formation emergence from conidia; App. = appressorial lobe evident; Hyp. = hyphae present; Con. = conidiophore present. Significant differences ($P < 0.05$) were determined by t-tests between resistant and susceptible cultivars for each day post-inoculation and are indicated by an asterisk.

Table 1. Cumulative frequency of observed powdery mildew growth stages on resistant sweet cherry cultivars. Significant differences between cultivars within columns ($P < 0.05$) are indicated by letters.

	Germination (%)	Appressoria formation (%)	Hyphae formation (%)
Chelan	33.3 a	20.0 a	4.4 ab
DD	16.2 b	8.6 b	2.9 ab
Moreau	36.4 a	25.5 a	7.3 ab
PMR-1	30.0 a	14.3 ab	1.4 b
Venus	27.0 a	23.0 a	8.1 a

Conidial germination, appressoria formation, and hyphae production for the resistant cultivars generally peaked by three days after inoculation with the following exceptions: conidial germination for 'Chelan' peaked at seven days, 'PMR-1' and 'Chelan' had the highest rates of observed appressoria immediately after inoculation, and hyphae production in 'PMR-1' was highest five days post-inoculation. 'Moreau' had the highest incidence of hyphae, although conidiophore production was never observed.

No evidence of hypersensitive response was seen in any of the resistant cultivars examined. Although a hypersensitive response with concomitant localized cell death is often evident in resistant reactions, the lack of autofluorescent compounds exhibited in this reaction has been documented in *Arabidopsis* (Vogel and Somerville, 2002).

The second objective of this research was to characterize the reaction of fruit from the same resistant cultivars to PM. Although each exhibits foliar disease resistance, fruit resistance had not been documented. Visual observations of disease progression were conducted in orchards that had no PM control applications made during 2006. PM colonization on the fruit of susceptible cultivars in these orchards was epidemic (Figure 4), while no visible PM colonies were observed on fruit of cultivars exhibiting foliar resistance.

Figure 4. Powdery mildew colonization of 'Sweetheart' fruit grown in Prosser, Wash. with no fungicide applications during 2006.

To further examine PM growth on fruit of 'Chelan', 'DD', 'Moreau', 'PMR-1', and 'Venus', immature fruit from each were inoculated and incubated for two weeks in a controlled environment conducive to disease progression. As with the foliar samples described previously, conidia on the fruit germinated and progressed as far as initial hyphae growth, but did sporulate (Figure 5).

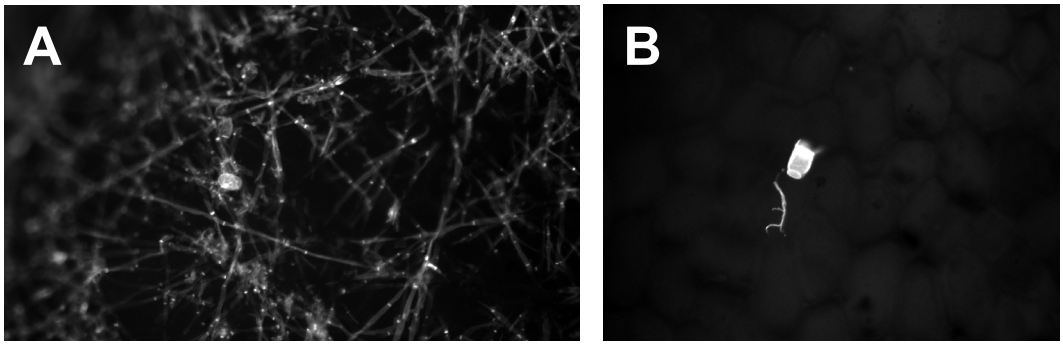

Figure 5. Powdery mildew colonization of susceptible 'Rainier' (A) and resistant 'DD' (B) fruit two weeks after inoculation.

Conclusions

For the cultivars examined, resistance was primarily exhibited as a lack of secondary spore production. However, differences in initial disease infection and growth on 'PMR-1' and 'DD' compared to the other resistant cultivars are promising given that both carry the same resistance gene. If 'Chelan', 'Moreau', and 'Venus' carry at least one different resistance gene, the two genes can be pyramided together in future breeding selections for more durable PM resistance. Fruit from the cultivars examined were also resistant to PM, an important finding given that fruit, not foliar, infection is economically important for PNW growers.

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FINAL PROJECT REPORT
WTFRC Project Number: CH-04-404

Project Title: Virus control strategies to assist cherry production in the PNW

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Fran Pierce, Director, CPAS, WSU-Prosser
Tom Unruh, USDA-ARS, Wapato
Various growers

Budget History:

Item	**Year 1:** $26,616	**Year 2:** $31,655	**Year 3:** $36,973
Salaries	14,084	14,648	17,946
Benefits	4,084	5,292	7,282
Wages	1,960	4,000	0
Benefits	313	640	0
Equipment	0	0	0
Supplies	6,106	6,800	11,200
Travel			
- to plots/growers	24	0	0
Miscellaneous	45	45	45
Total	$26,616	$31,655	$36,973

Objectives of this three year project were:

1. Develop progressive strategies to control virus diseases that contribute to the decline of sweet cherry productivity in the Pacific Northwest.
2. Develop laboratory tests that will make virus testing more accessible to growers.
3. Monitor commercial sweet cherry orchards for emerging virus diseases.
4. Evaluate the use of remote sensing to identify areas of declining cherry production that may be associated with virus infections.

Significant findings:

- *Cherry leafroll virus* is detected in the pedicels of fruit and in fruiting spurs collected from trees that were not previously infected with the virus. This has significant implications for understanding natural spread of this virus and strategies for its control.
- Root grafting is a major route of tree-to-tree spread of several important diseases of cherry.
- *Cherry raspleaf virus* infects several agronomic crops and we demonstrated that the sequence of their coat proteins differ significantly across sources (host plant and geographical region). Furthermore, the sequence of local isolates of *Cherry raspleaf virus* is consistent with its classification as a member of the genus *Cheravirus* rather than as a *Nepovirus* as previously thought.
- The protein shell of *Cherry raspleaf virus* consists of three different peptides. We identified CP2 as the one most likely involved in soliciting and reacting with antibodies. This information is used to develop serological reagents for virus detection.
- The virus that causes little cherry disease in Europe (*Little cherry virus-1*) is widely distributed in the PNW. This is a particularly difficult virus disease to control because there are no readily discernible symptoms other than poor fruit production (low yields and smaller fruit than is currently profitable).
- The incidence of Western X disease is showing an alarming resurgence throughout WA.
- Viruses of the genus *Foveavirus* are associated with diseased and declining trees in many orchards west of the continental divide. An enzyme-linked immunosorbent assay (ELISA) was developed for Montmorency stem pitting foveavirus. This proved effective in discriminating between samples infected with this damaging virus from those infected with related viruses whose long term consequences are less significant.
- Virus infection may be detected using light reflectance. The silicon detectors required for this technology are inexpensive and can be incorporated into one of several different formats.

Results and discussion:

Objective 1: Develop progressive strategies to control virus diseases that contribute to the decline of sweet cherry productivity in the Pacific Northwest.

The identification of *Cherry leafroll virus* in sweet cherry orchards of the USA is still a relatively recent event being identified in the PNW for the first time in 1999. We have elucidated many key factors in disease epidemiology associated with this new virus disease. *Cherry leafroll virus* is unique in that, experimentally, it infects a wide range of host plants from many different plant families, but each host is associated with a distinct virus strain. In nature, infection of a host plant with a strain from a different host genus has not been reported. Thus, virus isolates from each host present unique biology and challenges.

Our program demonstrated that pollen of infected sweet cherry trees contains very high concentrations of infectious *Cherry leafroll virus* particles. Moreover, these infectious particles can be transported by bees. This presented two questions that required response: Does virus-laden pollen play a role in the transmission of *Cherry leafroll virus*? Do other factors play critical roles in pollen-mediated transmission?

During the first two weeks of April, 57 bees were collected from orchard trees and tested to determine their capacity to be carriers of *Cherry leafroll virus*; two bees contained detectable *Cherry leafroll virus* in their pollen sacs and on their bodies as indicated by ELISA. Virus in the pollen sacs was infectious. On April 22, approximately two weeks after peak cherry bloom in the vicinity, 58 bees were collected and tested. Although no virus was detected by ELISA, the more sensitive reverse transcription polymerase chain reaction (RT-PCR) assay revealed three bees still bearing trace amounts of *Cherry leafroll virus*. These experiments indicate that bees are able to transport *Cherry leafroll virus*. Substantial amounts of virus-laden pollen are moved as the bees forage. Based on bee feeding behavior, the greatest potential for tree-to-tree spread of pollen is to nearby trees. However, the potential for long distance dissemination of virus by this method is relatively small but significant.

Using ELISA, we demonstrated that *Cherry leafroll virus* can infect cherry pits of fruit on healthy sweet cherry trees growing adjacent to an infected pollinator variety. The *Cherry leafroll virus*-free status of subject trees was determined by ELISA performed on five leaves collected randomly from each tree each year. Obviously, the most likely source of *Cherry leafroll virus* detected in the pits was virus-infected pollen. To examine the role of flowers in pollen epidemiology in greater detail, pits, mesocarp (fruit flesh), and pedicels from subject trees were extracted separately and tested for *Cherry leafroll virus*. No virus was detectable in the fruit flesh. Since the mesocarp is derived solely from the tree bearing the fruit, this provided confirmation that the parent tree was not infected with *Cherry leafroll virus*. Virus was detected in up to 22.5% of the pits by ELISA, and none was found in the pedicels (Table 1).

Table 1: Fruit was harvested from trees that had previously tested negative for *Cherry leafroll virus*, but were located adjacent to an infected pollinator. Fruit flesh, pits and pedicels were tested separately by ELISA.

Tree identification	**ELISA results** *number positive/number tested (percentage positive)*		
	Pedicel	Pits	Mesocarp
'Bing' R1T5	0/200 (0.0%)	45/200 (22.5%)	0/200 (0.0%)
'Van' R15T10	0/400 (0.0%)	7/400 (1.8%)	0/400 (0.0%)

The inability of ELISA to detect *Cherry leafroll virus* in pedicels suggested that although the virus is entering the pit from infected pollen grains, the virus is not moving from the flower/fruit structures into the recipient tree. However, when RT-PCR is used to examine the same question, results differed significantly. With the increased sensitivity offered by RT-PCR (100- to 1,000-fold increase in sensitivity relative to ELISA), *Cherry leafroll virus* was detected in a significant number of fruit pedicels (Table 2). This result was confirmed in two growing seasons. One concern was that the increased sensitivity of RT-PCR would detect residual virus from pollen contaminating the surface of the pedicel, thus giving positive results in the RT-PCR assay even though the surface contamination would not be biologically significant. This issue was addressed by two strategies. The test of fruit pedicels was repeated throughout the growing season until two weeks past commercial harvest. *Cherry leafroll virus* was consistently detected in some of the pedicels at each sampling time. During this period, the virus in potentially contaminating virus-laden pollen is dissipated below the limits of detection by RT-PCR. This suggests that the virus that was detected by RT-PCR was internal to the pedicels. In a second approach, immunolocalization of virus particles in tissue sections from fruit and flower parts was used to differentiate between virus particles on the pedicel surface from those within cells. Tissues were collected from subject trees as well as from known infected and non-infected trees for comparison. Tissues collected at various times during fruit development were embedded, sectioned, and labeled with gold via virus-specific antibodies. Gold-label was silver enhanced and observed with a confocal microscope. Examination of sections reveal *Cherry leafroll virus* particles

Table 3. *Cherry leafroll virus* is detected in tissues at three weeks post harvest by RT-PCR.

Table 2. *Cherry leafroll virus* is detected by RT-PCR in pedicels of fruit collected from 'Van' cherry trees adjacent to *Cherry leafroll virus*-infected 'Bing' trees.

Tree identification	**RT-PCR results** *number positive/number tested*			
	2005	2006		
	Pedicel	Pedicel	Pit	Fruit flesh
'Van' 1	5/5	†	†	†
'Van' 2	0/5	2/10	10/10	2/10
'Van' 3	0/5	0/10	10/10	0/10
'Van' 4	0/5	1/10	8/10	0/10
Positive control	5/5	5/5	5/5	5/5
Negative control	0/5	0/5	0/5	0/5

† In winter 2006, *Cherry leafroll virus* was detected in tree 'Van' 1 by ELISA, indicating that this tree had become systemically infected. The tree was not used for further pollination studies in 2006.

solutes through vascular tissue is unidirectional, toward the developing fruit, and no virus would move contrary to this source-sink flow. Cell-to-cell movement through parenchyma cells beneath the epidermis is still possible as demonstrated by this study. These experiments confirm that virus particles are entering the flower tissues from infected pollen and are capable of migrating into the structure that connects the flower tissue to the maternal tree.

We next examined the possibility that *Cherry leafroll virus* is capable of entering the vegetative portions of trees from the flowers. To explore the possibility that the virus is able to breach the abscission layer between the pedicel and the tree, samples were collected three weeks after commercial harvest and the flower spurs and associated pedicels were tested by RT-PCR. The results are summarized in Table 3. As in the previous experiments, *Cherry leafroll virus* RNA was detected in the pedicels. Moreover, in a significant number of instances, the viral RNA was detectable in the fruiting spurs of the maternal tree. Of the 58 spurs tested, three contained detectable virus. This strongly suggests that virus is being translocated from the reproductive tissues into the tree. That is, the virus is likely entering the tree through the flower. Further analysis is needed to confirm this phenomenon.

Pollen appears to play a key role in the epidemiology of *Cherry leafroll virus*. Based on our results, we established protocols to detect *Cherry leafroll virus* in commercial pollen sources. This protocol is being utilized by some commercial pollen companies to insure that their pollen is a virus-free product. In some instances, this procedure has alerted growers to the presence of *Cherry leafroll virus* in an orchard that had gone undetected before the pollen test. Consequently, the infected tree was identified and removed. Monitoring of cherry seedling rootstock production has also been initiated. This will help minimize the distribution of *Cherry leafroll virus* into new areas through infected propagation material. Both of these practices adopted by sectors of the industry are the direct result of our studies in the transmission of *Cherry leafroll virus*.

Branch number	**Assay results** *number of positive samples/ number of samples tested samples*					
	'Van' 2		'Van' 3		'Van' 4	
	Pedicel	Spur	Pedicel	Spur	Pedicel	Spur
1	0/4	0/4	0/4	0/4	0/5	0/5
2	0/3	0/3	0/4	0/4	0/3	0/3
3	2/3	1/1	0/4	0/4	0/4	0/4
4	0/5	1/5	0/4	0/4	0/4	0/4
5	1/5	0/5	0/4	0/4	1/4	1/4
Total	3/20	2/18	0/20	0/20	1/20	1/20

Transmission through infected pollen is likely the major route of infrequent long distance movement of *Cherry leafroll virus*, and also a factor in transmission to neighboring trees. In the latter case, there is a second mode of transmission that we demonstrated. Within an orchard, root grafting plays a significant role in tree-to-tree spread. This was illustrated by the number of trees reacting to herbicide treatment after cutting a nearby diseased tree and treating the resultant trunk with herbicide. Using herbicide damage as a guide, in the orchards that were studied, typically one in eight of the neighboring trees were root grafted to the virus-infected tree. These orchards were 10- to 18-years old at the time. Thus, our research on the epidemiology of *Cherry leafroll virus* demonstrated that transmission through root grafts is an important route of tree-to-tree spread in the orchard. To explore this further, a small pilot project was established in two separate commercial orchards. Cherry trees planted on 'Colt' rootstocks in the *Cherry leafroll virus* infested orchards did not become infected with the virus, whereas two-thirds of those on Mazzard did become infected. This occurred in both orchard settings. The difference was not related to bloom because any flowers that developed during the time of the study were removed manually. This suggests that 'Colt' offers some resistance to *Cherry leafroll virus* in the field setting. We are now beginning to explore other rootstocks that may offer protection against root grafting of *Cherry leafroll virus*.

Field studies revealed that different cherry cultivars respond to virus infections with different severity of symptoms. Of those tested, 'Tieton' is the mostly severely affected cultivar and shoot tip death was common in plants inoculated with *Cherry leafroll virus*, whereas the majority of cultivars such as 'Bing' display such symptoms only when the tree is infected with *Cherry leafroll virus* plus one of the ilarviruses. In our tests, 'Chelan' was a symptomless carrier of *Cherry leafroll virus* and hence, it may facilitate unintentional distribution through propagation material. Infected 'Chelan' trees could also be sources of infected pollen in the orchard that will be very difficult to identify because there are no outward symptoms of virus infection.

Objective 2: Develop laboratory tests that will make virus testing accessible to growers.
Substantial progress was made in developing diagnostic procedures for viruses associated with little cherry disease. Using these techniques, we demonstrated that the virus associated with the disease in Europe is also well established in western North America. This is in addition to the little cherry virus that had been previously identified in British Columbia and Washington.

The virus characterized in Europe, *Little cherry virus-1*, exhibits extreme sequence variability. This thwarted early efforts to develop reliable molecular assays. However, we successfully identified and characterized isolates obtained from many locations including Washington, Oregon, California, Pennsylvania, British Columbia and Europe. The result of our analysis is a molecular assay that provides a much greater level of confidence in identifying *Little cherry virus-1*. These advancements are coupled to our previous studies on *Little cherry virus-2* to develop a multiplex RT-PCR assay that will detect both viruses in a single reaction, thus providing an opportunity for enhanced identification and management of little cherry disease.

Serological reagents are desired for the viruses associated with little cherry disease because they would reduce the cost and increase the availability of routine testing to growers, fieldmen and researchers. Substantial progress was made in the development of these diagnostic reagents. We characterized the gene encoding the two coat proteins of local strains of *Little cherry virus-2*. These were expressed in bacteria and monoclonal antibodies produced in response to the expressed proteins. We identified two hybridomas producing antibodies that recognize the major coat protein of the virus particle and have the potential to work well in ELISA for the detection of *Little cherry virus-2.* Further development of the assay is required and the use of these antibodies in routine assays will be validated through ongoing research.

In a major advancement this year, we also identified the major coat protein gene of *Little cherry virus-1* and using a strategy similar to that described above, we sought to develop antibodies suitable for the detection of this virus. After screening approximately 4,000 hybridomas for antibody production, we identified approximately 150 hybridomas producing antibodies that recognize the coat protein of *Little cherry virus-1* expressed in bacteria, and one of these was demonstrated to detect by ELISA virus particles in crude leaf extracts. During the course of the coming season, we will evaluate the ability of this antibody to detect virus at all seasonal stages of tree growth.

Studies by others identified *Green ring mottle virus* and *Cherry necrotic rusty mottle virus* as members of the *Foveavirus* genus. Our research has now shown that the viruses associated with cherry rusty mottle, cherry twisted leaf, and Montmorency stem pitting are also caused by closely related foveaviruses. As we accumulate information, it is apparent that there is extensive sequence variability between the different viruses associated with these diseases in cherry, but there are also areas of sequence conservation. This is allowing us to develop both broad spectrum and virus-specific molecular assays. Furthermore, we have produced antibodies against the Montmorency stem pitting virus that are very effective as a diagnostic aid. The antibodies also react with a number of other foveaviruses of cherry, albeit with lower avidity. This enables a quick response to growers for determining if a foveavirus might be associated with their diseased trees. *Green ring mottle virus*, which is generally regarded as latent in most sweet cherry varieties, does not react with the antiserum that we developed, thus, its presence does not interfere with efforts to detect disease-causing viruses.

We are developing molecular and serological methods to detect *Cherry raspleaf virus.* Although the distribution of this virus through the PNW is quite limited, where it does occur, it is devastating. The virus is transmitted by nematodes and also infects a wide range of broad-leaf weeds. Once it is introduced into orchard land, there are few options available to the grower. The virus also causes flat apple disease so converting to apple production is not an appropriate response. In on-going research, we are exploring the ability of certain rootstocks to offer resistance against *Cherry raspleaf virus*. In order to execute these studies, refined diagnostic tools are required. Towards this objective, we characterized the three peptides that make up the coat of the virus particles. We determined that CP2 is most likely involved in serological reactions, and hence, the best candidate to solicit antibodies for detection. The gene sequence for CP2 was expressed in bacteria and antibodies solicited in response to this peptide. Initially, as a preliminary trial, a small amount of polyclonal antibodies were produced. We are currently developing monoclonal antibodies that should provide a more specific and more reliable reagents to be integrated into an ELISA.

Objective 3: Monitor commercial sweet cherry orchards for emerging virus diseases.

Little cherry disease has re-emerged as a potentially serious virus. The disease that was recognized and so destructive from 1940 to 1960 was associated with *Little cherry virus-2*. *Little cherry virus-1* is now emerging as a serious problem. It can be very damaging because the leaf symptoms are non-existent or mild. On the surface, this statement seems contradictory. *Little cherry virus-1* causes a reduction in fruit size and quality but the reduction is not as great as that observed in response to *Little cherry virus-2*. Therefore, the involvement of a virus is not immediately suspected. The reduced fruit size in orchards with trees infected by *Little cherry virus-1* is usually thought to be the

result of other causes such as water and nutrition management. Valuable resources are inevitably used in efforts to improve fruit size with no success. Early and correct determination of *Little cherry virus-1* status of trees will permit correct response to these conditions; that is, tree roguing in the case of virus diseases or altered horticultural practices if viruses are not detected. There is growing concern world-wide that *Little cherry virus-1* may not yield a sufficiently strong response by the traditional biological indicator to be reliably identified. The development of laboratory tests is therefore very timely and critical. We have identified a growing number of orchards where poor production is associated with the presence of *Little cherry virus-1*. These orchards are located in Yakima, Grant and Chelan counties in Washington. Continued monitoring is required to establish the level to which this virus has penetrated cherry production in the PNW.

Foveaviruses emerged as an important group of viruses in cherry production over the past few years. Many different molecular forms of these viruses were detected. We have developed the means to discriminate one virus from another by their molecular properties and by the degree and nature of symptoms that they cause. Availability of detection strategies is greatly enhancing the ability to identify and react to virus infections. A general molecular assay for the foveaviruses of cherry was developed that greatly enhanced our ability to detect and characterize foveaviruses from a number of orchards and disease situations. A complex pattern that has arisen from the data is still being resolved.

Continued surveillance of cherry production areas over the past five years revealed a dramatic increase in the frequency with which Western X disease is encountered. This disease severely impacted WA cherry production in the 1950's and 1960's; careful management of blocks in which the disease occurs is necessary to minimize further impact. Recent Western X infections occur in all cherry production regions of WA State.

Objective 4: Evaluate the use of remote sensing to identify areas of declining cherry production that may be associated with virus infections.

Trees in a commercial orchard were analyzed for the presence of *Cherry leafroll virus* (CLRV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV). Based on the results, eight trees were selected for analysis representing trees that were not infected, infected with either *Prune dwarf virus* OR *Cherry leafroll virus,* or with both *Prune dwarf virus* and *Cherry leafroll virus*. Three spur leaves from each tree were collected and two spectral measurements per leaf were obtained using an ASD field spectrometer. It generates a reflectance curve from 400-2500nm at 2nm intervals, and the curve represents the reflectance of a single point of a leaf. To emphasize wavelengths where the virus-infected plants exhibit the greatest differences, the average spectrum for each infection type is normalized by the average of the healthy leaves (Figure 1). The further the spectrum deviates from 1.00, the greater the difference in reflectance. Spectra from *Prune dwarf virus*- and *Prune dwarf virus* plus *Cherry leafroll virus*-infected trees look similar, but different from trees infected with *Cherry leafroll virus* alone. Reflectance values of 582nm, 697nm, 1458nm and 1975nm, as well as derived stress indices of normalized difference vegetation index (NDVI), modified chlorophyll absorption in reflectance index (MCARI), photosynthetic response index (PRI), water band index (WBI), and red edge vegetation stress index (RVSI) were evaluated. Analysis of Variance (ANOVA) was used to quantify the differences among plants with and without *Cherry leafroll virus*. Only RVSI significantly differentiated non-infected leaves from infected leaves with an F value of 7.89 (> 0.001) and at a 95% confidence limit. When only the healthy and *Cherry leafroll virus* infected leaves were used in the analysis, RVSI again produced the most significant contrast between the infected and non-infected leaf measurements with an F value of 18.55 (> 0.001). Thus a hand-held device could distinguish leaves from healthy trees from leaves from virus infected trees.

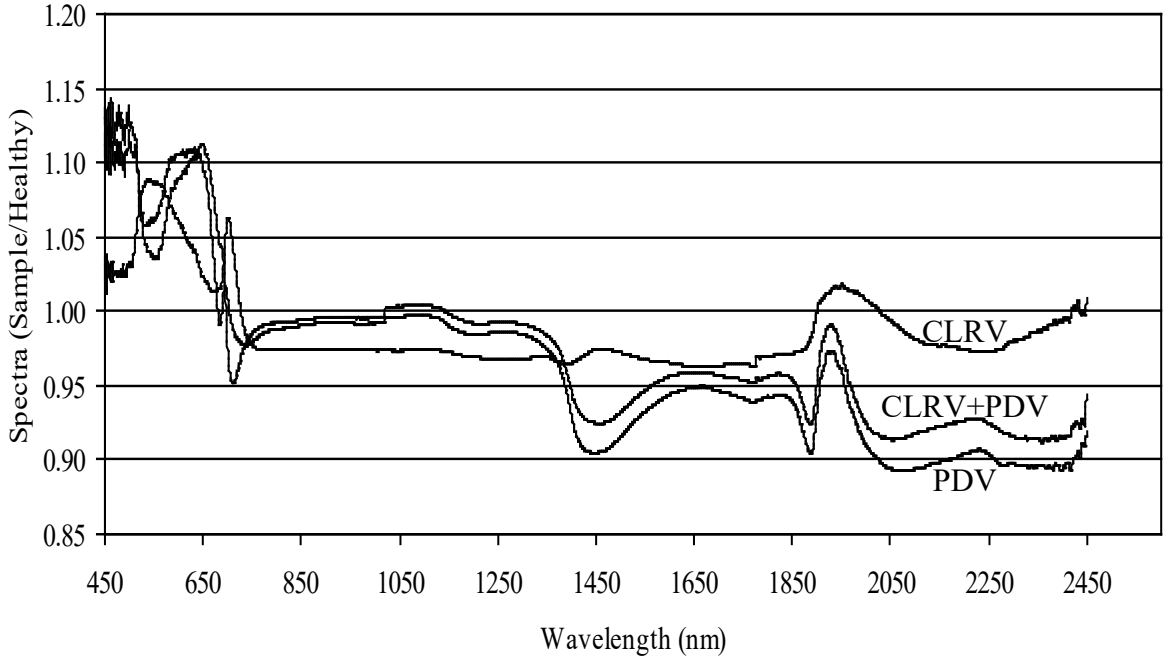

Figure 1. Averaged spectra from *Cherry leafroll virus*, *Prune dwarf virus* and *Cherry leafroll virus* plus *Prune dwarf virus* infected trees in a commercial orchard. The spectra were normalized using the averaged spectrum from healthy trees. Therefore, the graph represents deviation from readings from healthy trees which have the value of 1.00.

The RVSI and other indices that characterize the shape and location of the actual chlorophyll red-edge utilize narrow spectral bands measured with a portable spectrometer that makes point measurements. To gain an overview of an orchard disease status, a multispectral camera was used to view trees in a commercial orchard. The multispectral camera is based on broad spectral bands (green, red, and near IR) that cannot characterize the red-edge, thus image processing analogous to RVSI is not available, however, the image bands can be used to determine NDVI. The NDVI images use the red and near infrared bands in which infected trees appear as dark red and healthier canopy appears bright. Thus, the difference between *Cherry leafroll virus*-infected and non-infected trees becomes more noticeable. These results suggest that a small, portable, lightweight video system sensitive to the red and near infrared bands to produce a real-time NDVI would be of value in locating stressed trees in the orchard. This system could be worn by someone, or attached to a PDA to generate NDVI images. All of this preliminary data was obtained with rather generic interpretations of vegetation images. With the detailed spectral information obtained from individual leaf reflectance measurements, a dedicated imaging system based on this information could be developed. Perhaps in the future a method will become available to make narrow spectral band measurements with an imaging (camera) system.

Project Title: Temperate Fruit Fly Workshop
WTFRC Project #: CH-06-604

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Budget History:

Item	**Year 1:** 2006
Salaries	0
Benefits	0
Wages	0
Benefits	0
Equipment	0
Supplies	0
Travel	5,000
Miscellaneous	0
Total	5,000

The $5,000 (and another $5,000 from Apple Entomology) was used to reimburse 7 scientists for their travel to and stay in Yakima for the workshop.

Objectives 2006

1) Have a focused update on the nature of our problem with fly pests.
2) Provide updates on what research is ongoing and relevant – both in the Pacific NW and nationally.
3) Discuss how we ought to revise our research strategy to develop more collaborative, productive research and implementation.

Significant Outcomes:

At the end of the workshop, the following were identified as items of high research priority for cherry fruit fly:

- **IDENTIFICATION (WHAT IS IT?)**
  A. Identification of western cherry fruit flies (*Rhagoletis indifferens*) on wild hosts versus flies on commercial cherries.
  B. Identification of young larvae of western cherry fruit fly versus those of black cherry fruit fly (*Rhagoletis fausta*).

- **DETECTION (WHERE IS IT?)**
  A. Trapping of adult cherry fruit flies to determine whether an orchard needs to be sprayed; creation of fly-free areas.
  B. Develop better, more sensitive methods to determine the presence of cherry fruit fly larvae in fruit at packinghouses.

- **CHERRY FRUIT FLY CONTROL AND MANAGEMENT**
  A. Improve bait formulations to improve attraction and expedite cherry fruit fly kill.
  B. Modes and mechanisms of kill and control using neonicotinoid and other newer insecticides and relation to application timing.
  C. Develop attractants and use in baits and trapping (monitoring).
  D. Comparison of economics of GF-120 bait sprays versus integrated techniques using chemicals, parasitoids, nematodes, and cultural practices.
  E. Determine effects of crop phenology and loads on fly populations.
  F. Control of different life stages of flies: pupae, adults.
  G. Post-harvest practices, including use of systemic/translaminar insecticides.
  H. Determine source of flies in commercial orchards; where flies are coming from.
  I. Host plant resistance to cherry fruit fly.
  J. Use of systems approaches for management to satisfy domestic and foreign markets.

- Collaboration between Wee Yee and Diane Alston on work addressed by research priorities under "Cherry Fruit Fly Control and Management," topics A and B: "Improve bait formulations to improve attraction and expedite cherry fruit fly kill" and "Modes and mechanisms of kill and control using neonicotinoid and other newer insecticides and relation to application timing".

History and Methods

The Washington Tree Fruit Research Commission initiated a discussion to address research that could expedite the management of cherry fruit flies on January 7, 2005 at the Cherry Institute Meeting in Yakima. Discussion initially centered on the cherry fruit fly, but at a second discussion that took place at the Apple Entomology Research Review in Yakima on January 28, 2006, it was expanded to include apple maggot (which will be dealt with at the Apple Entomology Review). At the Review, discussion was centered on what fly problems occur in the Northwest and how the Commission can help in funding projects that potentially can solve these problems. A third discussion took place at the USDA's Yakima Agricultural Research Laboratory (YARL) on March 1, 2006, and it was here at YARL that the idea arose to hold a fly workshop at the Cherry Research Review in The Dalles in November 2005. Between this time and the Cherry Priority Setting session in Ellensburg on August 11, 2005, various fruit fly researchers were contacted for their possible participation for a November meeting. However, the November meeting conflicted with the Entomological Society of America's Annual Meeting, which is attended by almost all professional entomologists, and it was decided to postpone the fruit fly workshop to a later time. At the Cherry

Research Review in The Dalles on November 4, 2005, a question and answer session about the proposed fruit fly workshop was conducted in which Commission support for the workshop was gauged (research participants were Wee Yee, Pete Landolt, Vince Jones, and Mike Willett, moderated by Jim McFerson).

After more discussion, it was decided a meeting to plan the workshop should be held at the Western Orchard Pest and Disease Management Conference in Portland from January 11-13, 2006 to come up with a tentative agenda. The 8 attendees in Portland were: Diane Alston, Utah State University, Rufus Isaacs, Michigan State University, Vince Jones, Washington State University, Gary Judd, Ag Canada, Pete Landolt, USDA-ARS, Howard Thistlewood, Ag Canada, Tom Unruh, USDA-ARS, and Dave Biddinger, Penn State University.

One of the main conclusions of the group was that several areas of research are relevant and need to be emphasized; a few additional ones (f and g) were added after the meeting in Portland:

a. Identification problems (esp. apple maggot and snowberry maggot) using molecular techniques
b. Behavioral studies, particularly migration of mated females, population biology, and phenology
c. Detection/security
d. What happens in the soil? Including biological control, possible use of nematodes
e. Management, including bait sprays, area-wide approaches, pesticide efficacy
f. Host range – likelihood of a fruit fly species infesting a certain host. Any objective measure of a commercially significant host range that could be explored
g. Survival of flies in different habitats in Washington

It was further agreed that the idea was not to ask Washington people to present their research, but to let experts from other parts of the country do this. Because there were seven identified areas, potentially seven outside researchers would participate, although there may be two participants under some of the areas. The plan was to have a maximum of 10 people invited to the workshop.

On April 10, 2006 in Ellensburg, a meeting was held to draft a workshop agenda and to decide which fruit fly researchers to invite to the workshop. The meeting was attended by Wee Yee, Vince Jones, Tom Unruh, and Jim McFerson, with Mike Willett calling in. From this meeting a draft was generated.

After much more correspondence, the list of invited researchers and the researchers' general areas of expertise was finalized:

1- Dr. Sue Opp, California State University – Dispersal of walnut husk fly
2- Dr. Charles Linn, Cornell University – Attraction of apple maggot races to fruit volatiles
3- Dr. Jeff Feder, University of Notre Dame – Genetic differences among apple maggot fly host races
4- Dr. Stewart Berlocher, University of Illinois – Genetics of and taxonomic relationships among fruit flies
5- Dr. Russ Messing, University of Hawaii – Biological control of fruit flies
6- Dr. Diane Alston, Utah State University – Insecticide control of western cherry fruit fly
7- Dr. Larry Gut, Michigan State University – Management of eastern cherry fruit fly and apple maggot fly using insecticides and baits

After several revisions, the final agenda was as follows:
Date and Location: August 28-29, 2006, USDA-ARS Lab in Wapato, WA
SUN, AUGUST 27
– Researchers fly in; evening get together of researchers, at Tom Unruh's house.

MONDAY, AUGUST 28
1. WELCOME & INTRODUCTION – WEE YEE – 8:00-8:10

2. OVERVIEW 8:10-8:30
MIKE WILLETT -- NORTHWEST HORTICULTURAL COUNCIL
Magnitude of Problems of Apple Maggot and Cherry Fruit Fly,
Quarantine issues; Distribution of apple maggot, etc.
3. OVERVIEW OF WASHINGTON RESEARCH LAST FIVE YEARS
Wee Yee and Tom Unruh USDA-ARS Wapato (8:35-8:55)
SECTION I. GENETICS AND LIFE HISTORY*
4. GENETIC VARIATIONS: (9:00-9:20)
Jeff Feder and Stewart Berlocher
- Host Use
- Identification
- Implications for Management

5. LIFE HISTORY: (9:25-9:45)
Charlie Linn
- Behavior
- Odor and Visual Cues
- Learning
- Detection- Trapping

BREAK (9:50-10:05)
6. FACILITATED DISCUSSION AND SYNTHESIS – SECTION I (10:05-12:00)
- Focus on areas ripe for collaboration, areas where info is missing or inadequate

LUNCH (12:-1:30)
***each person summarizing should give us at the end of their presentation, 3 areas that are researchable and key to understanding the life history and management of the flies**

SECTION II. POPULATION BIOLOGY AND MANAGEMENT*
7. POPULATION BIOLOGY: (1:30-1:50)
Sue Opp
- Dispersal
- Phenology
- Survival, abiotic and dietary factors

8. BIOLOGICAL CONTROL: (1:55-2:15)
Russ Messing
–Parasitoids & Predators
–Potential to reduce problems

9. MANAGEMENT: (2:20-3:05)
Larry Gut **(2:20-2:40)**
Diane Alston **(2:45-3:05)**
- Area Wide Suppression
- Bait Sprays
- Attract-and-Kill
- Pesticide Efficacy
- Thresholds

BREAK (3:10-3:25)
10. FACILITATED DISCUSSION AND SYNTHESIS – SECTION II (3:25-4:30)
4:30-5:15 – BREAKOUT GROUPS FOR FUTURE COLLABORATION
5:15-5:45 - CONTINUE BREAKOUT GROUPS AND/OR LAB TOUR
6:00 – 9:00 SILVERLAKE WINERY TOUR & SOCIAL WITH INDUSTRY REPS
***each person summarizing should give us at the end of their presentation, 3 areas that are researchable and key to understanding the life history and management of the flies**

TUESDAY, AUGUST 29
1. INTRODUCTIONS INDUSTRY AND SCIENTISTS (8:20-8:30)
SCIENTISTS' AND ORGANIZER'S MEETING (Synthesis of Monday's presentations and discussions) 8:30-10:30

(**Scientists and** Willett, Brunner, Landolt, McFerson, Yee, Unruh, Jones)

1. Give synthesis of where we are in PNW as of now
2. Emphasize the areas of needed research, areas ripe for collaboration, areas where info is missing or inadequate
3. Address in the presentation issues that we can't control
    a. Zero tolerance effects on IPM

10:30-10:45 BREAK
2. DISCUSSION WITH INDUSTRY (10:45-11:45)

Willett & McFerson

– Go through each area again (summary points only up on screen)
    - Ask for questions, comments, and suggestions in each area
    - Was anything missing?
    - Throw open for interactions

END BY 12:00; after lunch, researchers can leave.

1:30-3:00; ORGANIZER'S COMMITTEE (Willett, Brunner, Landolt, McFerson, Wee, Unruh, Jones)

– **Meets and modify presentation dependent on interactions in morning**
– **Set research priorities for industry. Or should this be done over a week's time?**

PARTICIPANTS IN ADDITION TO THE 7 SCIENTISTS:

Industry	Researchers and Others
McFerson	Klaus
Willett	Brunner
Craver	Landolt
Doornink	Yee
Hayden	Jones
Tim Smith	Unruh
Milne	Barcenas
Dan Griffith	

On August 28 and 29, 2006, the workshop was held at the YARL. All invited scientists were present: Sue Opp, Charles Linn, Jeff Feder, Russ Messing, Stewart Berlocher, Diane Alston, and Larry Gut. The Washington entomologists and industry people were: Mike Willett, Vince Jones, Jim Doornink, Jim McFerson, Timothy Smith, Michael Klaus, Jay Brunner, Brent Milne, Dain Craver, Tom Unruh, Pete Landolt, and Wee Yee.

The August 29 attendees were: Brent Milne, Jay Brunner, Dan Griffith, Charlie Linn, Russ Messing, Mike Willett, Vince Jones, Diane Alston, Larry Gut, Jim Doornink, Michael Klaus, Sue Opp, Tom Unruh, Pete Landolt, and Wee Yee.

The workshop in general followed closely the agenda outline for the first day.

The invited scientists were asked prior to the workshop to come up with three key research areas that will help understand fly biology and fly management:
The following were ones pertinent to cherry fruit fly:

Stewart Berlocher:
1-Surveys to determine basic ecological and biogeographical data on western *Rhagoletis*
2-Find molecular markers to distinguish eastern cherry fruit fly from western cherry fruit fly

Russ Messing:
1 -Selectivity of parasitoids (long-term, high risk)
2-Mass-rearing technology and field testing of augmentation (medium-term, high risk)
3-Comparative economics of weekly GF-120 sprays area-wide, systems approach to population management strategies with integrated techniques (chemicals, parasitoids, nematodes) (medium-term, low risk)

Larry Gut:
1-Mode of insecticides; relationship to application timing
2-Improve bait formulations; potential use in non-commercial setting
3-Test, develop attractants/use in baits/monitoring

Diane Alston:
1-Effect of crop phenology, crop loads, reduced cop loads
2-How flies forage, how far, carry over effects
3-Target different points of life stages: pupation, adult emergence

On the second day, the workshop ended at 12:00 pm, earlier than scheduled. Pete Landolt suggested that, based on the hours of discussion on Monday, the research areas could be placed under three categories of: identification (what is it?), detection (where is it?), and control and management. From this and the lists of research areas provided by the invited scientists, a list of items of high research priority was generated.

The plan for the next few years arose from this list. Specifically, Wee Yee and Diane Alston will collaborate on work on research priorities under "Cherry Fruit Fly Control and Management," topics A and B: "Improve bait formulations to improve attraction and expedite cherry fruit fly kill" and "Modes and mechanisms of kill and control using neonicotinoid and other newer insecticides and relation to application timing".

CONTINUING PROJECT REPORT **YEAR**: Continuous

Project Title: Horticultural management systems for fresh & brine cherries

PI:	Anita Nina Azarenko	**Co-PI(2):**	Annie Chozinski
Organization:	Oregon State University	**Organization:**	OSU
Telephone/email:	541-737-9877	**Telephone/email:**	541-737-8959
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City:	Corvallis	**City:**	Corvallis
State/Province/Zip	OR 97331	**State/Province/Zip:**	OR 97331

Cooperators: Don Nusom; John and Karen Carter; David, Karen and Stacey Cooper; Mike, Mel and Linda Omeg; John McClaskey and Clark Seavert.

Budget 1:
Organization Name: Agricultural Research Foundation **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	**Last Year :** 2006-07	**Year 2:** 2007-08	**Year 3:**
Salaries (0.75FTE)		(0.75FTE) 25,350	
Benefits		(67%) 17,000	
Wages (1 students)			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous (plot charges)		7,800	
Total	45,000	50,150	

Footnotes:

Objectives:

1. Identify cherry cultivars and rootstocks suitable for the processing cherry industry (e.g. brine, freezer) and those that may have potential for fresh market production in the Willamette Valley and cooler cherry growing districts.
2. Evaluate the effects of training system, rootstock and variety on tree performance, fruit quality and yield.
3. Refine and test growing degree hour model for fruit growth in main cultivars of dark sweet cherry.
4. Determine plant growth regulator effects on stem pull force and fruit quality of cultivars. (Discontinue)
5. Evaluate alternative cherry cropping systems (ie. protected culture and alternative orchard floor management) for orchard performance and profitability.
6. Determine if rootstock influences susceptibility of a scion cultivar to *Pseudomonas syringae* pv. *syringae.*

Significant findings and results

1. *Rootstock and varieties*
   a. *2002 PiKu 1 and 3 trial*- Tree mortality is highest on PiKu 1 rootstock. PiKu 3 has begun to produce more fruit of larger size than PiKu 1 (Table 1). 'Regina' and 'Sweetheart' trees produced exceptionally firm fruit. Stem pull force was low for 'Black Gold', 'Skeena', and 'Sweetheart'.
   b. *2002 'Sweetheart'/MxM trial*- MxM2 trees had the largest TCSA followed by 2, 39, 46 and 14 which had the smallest TCSA (Table 2). Yields were not significantly different but yield efficiency was greatest for MxM39 and 46 trees. Fruit size was well suited for the brine market. MxM60 trees had a higher percentage of fruit remaining on the tree after mechanical harvest.
   c. *2005 Regina rootstock trial* – The trunk cross-sectional area of trees grafted onto Gisela 5, Gisela 6, Gisela 12 and Mazzard were not different from each other after the second leaf.
   d. *2006 Variety and rootstock trial* – Plantings of 'Bing', 'Tieton', 'Sunset Bing', 'Sylvia', 'Benton', 13N07-39, 'Early Robin', 'Rainier', 'Regina', 'Sweetheart', and 'Skeena' grafted onto Gisela 6, MxM14, Gi196-4 and Mazzard trees were established at the Lewis-Brown Farm, Corvallis, OR and at two on-farm sites (see report for on-farm trials). Trees are being trained to a central leader system and became well established.
   e. *2006 NY blush and dark cherry cultivar trial*- Planting of 12 NY selections with 'Skeena', 'Regina' and 'Rainier' were established at the Lewis-Brown Farm, Corvallis, OR and at two on-farm sites (see report for on-farm trials). Trees are being trained to a central leader system and are well established.

2. *Training systems*
   a. *2002 Top-worked trees* – MxM 60 and MxM 14 trees have the largest TCSA followed by Mazzard and Gi 196-4 (Table 3). Yields were greatest on Gi196-4 as was the yield efficiency.
   b. *2003 Training systems and rootstock trial trees* – Multiple leader trees had higher yields across all three cultivars (Table 4). Central leader 'Sweetheart' and 'Stardust' trees produced larger fruit. Fruit firmness and stem pull force were not affected by training system. Gisela 6 trees have higher yields for all cultivars. Gi 196-4 trees had intermediate yields for 'Stardust' and 'Sweetheart'. Fruit size was smaller on MxM 14 trees. Fruit on M x M 14 trees tended to be less firm and have a lower stem pull force.

3. *Growing degree hour model*- Growing degree hours appear to be useful in predicting the end of the lag phase or beginning of final swell. The end of final swell does not appear to relate well to the fruit maturity at which orchardists harvest their fruit.

4. *Plant growth regulator effects*- MaxCel, KT30, Promalin and GA had no effect on the fruit quality attributes of fruit size, firmness, color, or stem pull force in comparison to control on either Nusom's or Omeg's 'Skeena' nor Omeg's Lapins.

5. *Alternative cropping systems*
   a. Protective culture and spectral light management- The 3-bay tunnel system was installed. Each bay is 120m (400') long, 8.2m (~28') wide, and 5.2m (17') tall (at the highest point). A planting outside of the structure serves as a commercial comparison. The total planting is approximately 0.6 ha. The study is arranged in a randomized complete block design with three replications and 30 trees per plot. The tree density is approximately 1655 trees/ha (670 trees/acre). Trees are trained to a central leader system.
   b. Alternative orchard floor and fertility management- moisture probes and lysimeters were installed. Trees were trained to a central leader. Compost was applied in autumn. Tree growth was less when grown with a straw or bark mulch.

6. *Pseudomonas trial*- Trees will be delivered in the late winter and the trial will begin then.

Materials and Methods:

- Train trees, maintain orchard and obtain data on yield, fruit size, tree vigor, bacterial canker tolerance and other relevant data from the existing cherry trials (~3.3 ha) which include:
  - 2002 PiKu 1 and 3 trial (0.20 ha)
  - 2002 'Sweetheart'/MxM rootstock trial (0.12 ha)
  - 2002 Topworked mechanical harvest trial (0.90 ha)
  - 2003 Training systems and rootstock trial (0.50 ha)
  - 2006 Variety and rootstock trial (0.20 ha)
  - 2006 NY blush and dark cherry cultivar trial (0.20 ha)
  - 2005 Alternative fertility management trial (0.60 ha)
  - 2006 Protective cultivation with light spectral management (0.60 ha)

- Continue testing and refine *growing degree hour* model of cherry fruit growth. Test models over several sites where weather stations are located. Collaborating orchardists will provide peak bloom, straw and harvest dates.

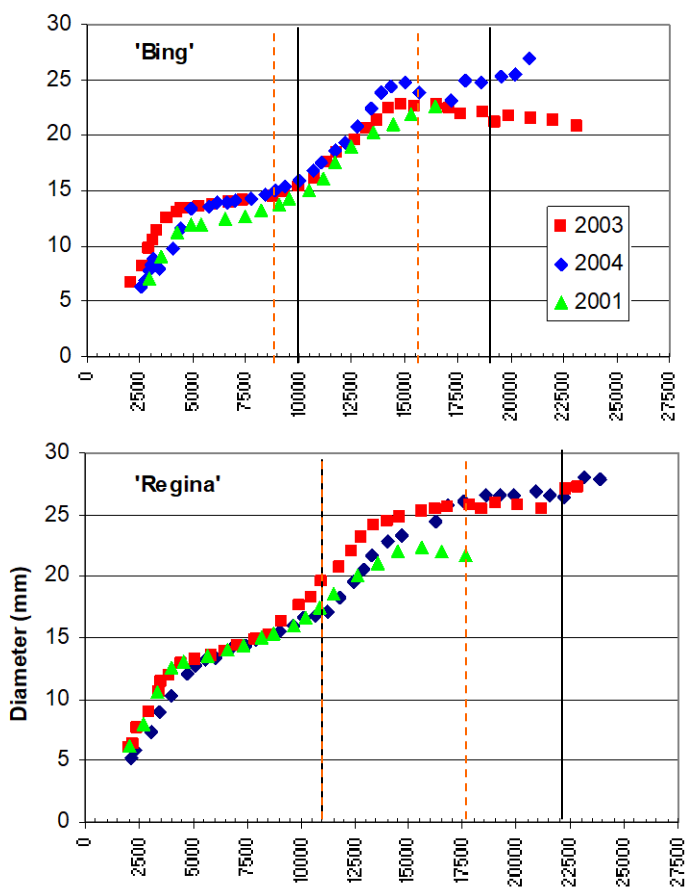
- *Alternative cropping systems-*
  - <u>Alternative orchard floor and fertility management</u>- The USDA competitive grant is covering the cost of all activities and services and supplies with the exception of the plot charges for 0.6ha and the in-kind match of Annie Chozinski's salary. Biological and economic effects of two different methods of orchard floor and fertility management during orchard establishment and early production are being compared. The research orchards of 'Regina' on Gisela 6 were established in 2005. Geotextile cloth and straw/bark mulch followed by compost are used in the tree row (Fig. 2). Plots are planted in two locations: Lewis-Brown (LB) (Corvallis) and MCAREC (Hood River). Trees are being pruned and trained to a central leader system. Soil water content and quality are being measured at the LB Farm; and leaf analyses performed; soil chemistry, physics, and biology (nematode, enzymes and molecular) characterized; and tree performance evaluated at both locations. In addition, 13 commercial orchard sites are being sampled to determine if soil community structure is an indicator of the effects of different management practices on soil health

and orchard performance via the collection of soil chemical, physical, and biological (nematode, enzymes and molecular) data.

- Protective cultivation with light spectral management- Three different colored nets of red, blue and pearl will be installed 1 March 2007 to alter light quality. The control is a standard film and an exterior planting serves as a commercial comparison. Trees will continue to be trained as central leaders (Fig. 3). Temperature data will be collected inside and outside of the tunnels. Light quality will also be measured.

➢ *Bacterial canker tolerance* will be tested on 20 trees of 'Sweetheart', 'Regina', 'Sylvia' and 'Bing' varieties low-budded onto Mazzard, Gisela 6 and MXM14 rootstocks. Trunks will be injured and sprayed with a mix of isolates of *Pseudomonas*, held overnight in a 0°C cooler, taken outside and observed for symptom development and assessment.

Results: See detailed findings in the following figures and tables.



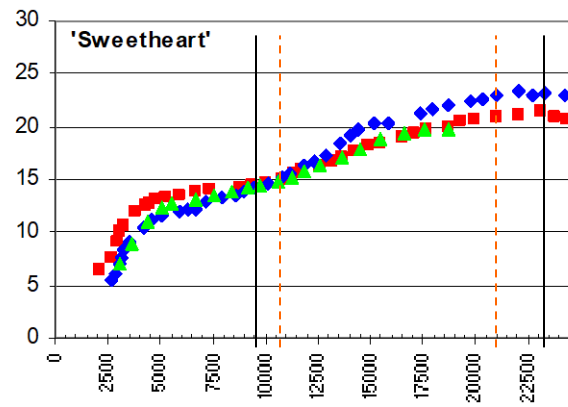


Fig. 1. Fruit growth (mm) as related to growing degree hours (GDH) in 2001, 2003 and 2004. GA applied (solid line on left), fruit harvested (solid line on right), estimated beginning of final swell (dotted line on left), and the end of final swell (dotted line on right) in 2006.

Fig. 2. Corvallis planting for the alternative orchard floor and fertility management after the second growing season.

Figure 3. Protected culture of ‘Early Robin’, ‘Rainier’ and 13N07-39 sweet cherry in a high tunnel that is120m (400’) long, 8.2m (~28’) wide, and 5.2m (17’) tall (at the highest point. Materials donated at cost from Wilson Irrigation.

Table 1. The influence in 2006 of PiKu 1 and 3 rootstocks on trees of 10 cultivars planted in 2002.

Variety	PiKu rootstock	(n)	First bloom (April)	Peak bloom (April)	Harvest date	Yield (kg)	Firmness (g/mm)	Fruit size (mm)	Color (1-7)	Cracks (%)	Pullforce (g)	SSC (°brix)	Fruit wt (g)
Sonata	1	2	4	11	21 June	0.4	245	23.5	5.7	4.0	869	29.6	6.7
	3	4	4	12		8.4	213	24.5	5.5	4.0	907	27.7	7.0
White	1	4	10	18	23 June	4.9	211	24.4	.	35.0	816	18.3	7.5
Gold	3	4	13	19		7.6	222	25.2	.	16.0	796	18.0	7.7
Royal	1	4	9	21	27 June	8.8	204	23.2	.	0.0	665	23.0	5.8
Ann	3	4	8	15		6.3	206	22.2	.	0.0	687	22.3	6.4
Bing	1	4	7	17	27 June	4.6	188	24.5	5.0	9.0	769	22.6	7.7
	3	4	7	13		11.3	190	24.6	4.9	4.0	731	22.1	7.8
Black	1	2	17	24	30 June	1.8	263	20.9	.	0.0	552	21.4	5.2
Gold[z]	3	4	19	24		6.2	249	24.4	.	1.6	594	23.0	7.8
Attika	1	4	13	24	3 July	11.2	265	24.3	5.0	0.0	608	21.1	8.0
	3	4	12	23		9.5	255	26.1	5.2	2.0	630	20.8	9.2
Lapins	1	4	4	14	6 July	6.9	248	27.1	4.9	2.0	604	20.1	9.4
	3	4	6	12		11.3	267	26.6	4.8	1.0	568	19.7	9.0
Skeena	1	3	13	21	10 July	14.7	288	27.8	5.5	0.0	416	20.8	9.6
	3	4	14	22		6.0	283	28.2	5.4	1.0	449	21.4	10.0
Sweet-heart	1	4	6	16	12 July	7.6	334	26.4	4.2	2.0	543	22.3	8.9
	3	4	7	14		6.9	333	26.9	4.1	1.0	591	20.7	9.0
Regina	1	4	13	23	18 July	2.3	321	22.8	6.5	1.0	807	24.6	6.5
	3	4	17	24		9.1	245	27.3	6.8	4.0	732	22.4	9.8

[z]Highlighted cells are significantly different.

Table 2. Effect of rootstock on performance in 2006 of 'Sweetheart' trees topworked onto M x M rootstocks.

M x M rootstock	Yield (kg)	Remaining (%)	SSC	Weight (g)	Pullforce	Size (mm)	TCSA	Change	YE (kg/cm^2)
14	32.6	1.5 c	17.3	5.2	624	20.9	98 c	28 ab	0.27 c
46	48.1	1.7 c	16.3	5.3	615	20.7	119 bc	24 b	0.36 ab
2	43.5	3.2 bc	17.1	5.1	703	20.7	152 ab	64 ab	0.32 cd
39	45.8	7.3 ab	16.3	4.7	669	19.9	127 bc	45 ab	0.40 a
60	50.3	9.0 a	15.9	5.0	653	20.7	171 a	80 a	0.29 c
MSD	ns	5.3	ns	ns	ns	ns	34	52	0.06

[z]Rootstocks were planted in 2000 at a 18' x 18' spacing in a completely randomized design with 6 replications. Trees were topworked in 2001.
[y]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.

Table 3. Effects of rootstocks on the tree and fruit characteristics of topworked 'Sweetheart', 'Stardust', and 'Royal Ann' in 2006.

Rootstock	First Bloom	Peak Bloom	SSC (°Brix)	Weight (g)	Pullforce (g)	Fruit size (mm)	Yield (kg/tree)	TCSA (cm^2)	ΔTCSA (cm^2)	YE (kg/cm^2)
'Sweetheart'[z]	April									
MXM60	4 b	12 a	17.4 ab	6.9	865 b	24	5.1 b	103 a	66 a	0.05 b
MXM14	4 ab	11 c	14.9 b	6.9	913 b	24.2	3.1 c	95 a	54 a	0.03 b
Mazzard	5 a	11 bc	18.7 a	6.7	853 b	23.7	2.3 c	54 b	25 b	0.04 b
Gi 196-4	4 ab	12 ab	17.7 ab	7.1	1013 a	24.1	8.0 a	50 b	17 b	0.15 a
MSD[y]	<1 day	<1 day	3.3	ns	85	ns	1.6	14	13	0.02
'Stardust'										
MXM60	13 b	22	13.3	7.3 ab	1084 b	24.8 a	5.6 b	93 a	57 a	0.06 b
MXM14	15 a	22	14.6	7.7 a	1086 b	24.6 a	3.6 b	72 b	41 b	0.05 b
Mazzard	15 a	21	14.2	7.2 ab	1067 b	24.2 a	3.1 b	56 c	23 c	0.06 b
Gi 196-4	13 b	22	13.2	6.6 b	1248 a	24.5 b	15.1 a	58 bc	24 c	0.25 a
MSD	1 day	ns	ns	1.1	64	0.6	2.9	15	14	0.03
'Royal Ann'										
MXM60	6 b	15	15.8 b	6.2 ab	925 b	23.0 b	1.4 b	91 a	56 a	0.03
MXM14	8 a	15	16.0 b	6.1 b	986 b	23.0 b	1.4 b	87 a	59 a	0.03
Mazzard	7 ab	16	17.0 a	6.3 ab	817 c	23.2 ab	1.1 b	49 b	21 b	0.05
Gi 196-4	6 b	15	14.8 c	6.5 a	1243 a	23.5 a	4.8 a	48 b	19 b	0.05
MSD	1 day	ns	0.6	0.3	66	0.5	1	11	14	ns

[z]Rootstocks were planted in 2002 at an 18' x 18' spacing in a completely randomized design with 18 plots. Trees were topworked in 2003. Trees were mechanically harvested.
[y]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.

Table 4. Effect of training system and rootstock on the performance of ‘Sweetheart’, ‘Stardust’ and ‘Royal Ann’ trees planted in 2003.

	Yield (kg)			Size (mm)			Firmness (g/mm)		Pullforce (g)		
	Sweet♥	Stardust	Royal Ann	Sweet♥	Stardust	Royal Ann	Sweet♥	Stardust	Sweet♥	Stardust	Royal Ann
Training system											
ML[z]	10.4 a	12.3 a	1.6 a	27.0 b	28.5 ab	23.3	358	232	552	588	845
CL	2.9 b	3.0 b	0.4 b	27.9 a	28.8 a	23.7	386	259	648	602	865
Tatura	0.46 b	0.34 c	0.0 b	27.3 b	27.5 b	24.6	368	269	599	609	910
Sign.[y]	.0002	.0004	.0026	.0039	.0454	.2568	.2963	.0675	.0679	.8099	.8990
Rootstock											
Gisela 6	7.4 a	7.3 a	0.7 a	27.5 a	28.5 a	24.2 a	379 ab	257 a	626 b	618	925 a
Gi196-4	2.9 b	5.7 b	0.4 b	27.8 a	28.6 a	23.9 a	394 a	262 a	668 a	593	883 b
M x M 14	3.9 b	3.0 c	0.2 c	26.7 b	27.8 b	23.5 ab	350 b	231 b	537 c	576	883 b
Mazzard	4.0 b	.	0.3 bc	27.6a	.	22.9 b	362 ab	.	569 c	.	829 c
Sign.	<.0001	.0007	<.0001	<.0001	.0298	.0002	<.0001	.0001	<.0001	.2753	.0006
Training system x rootstock											
Sign.	<.0001	.0206	<.0001	<.0001	.6721	.4664	.0796	.0727	.0007	.6733	.0301

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

Project Title: On-Farm Research for Sweet Cherry Farming Systems

PI:	Anita Nina Azarenko	**Co-PI(2):**	Annie Chozinski
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State/Province/Zip	OR 97331	**State/Province/Zip:**	OR 97331

Cooperators: Kristi Barckley, Mike, Mel and Linda Omeg; John and Karen Carter; Stacey, David and Karen Cooper; and Marcus Morgan

Budget 1:
Organization Name: Agricultural Research Foundation **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	Year 1: 2006-07	Year 2: 2007-08	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies and services		12,000	
Travel			
Miscellaneous			
Total	12,000	12,000	

Footnotes:

Objectives

1. Evaluate the effects of three different tree pruning and training systems; central leader, bush, and steep leader, on early tree growth and development of 'Early Robin/Gisela 6' and 'Rainier/Gisela 6' cherry trees (Carter and Morgan).

2. Establish a variety and rootstock trial of 'Bing', 'Tieton', 'Sunset Bing', 'Sylvia', 'Benton', 13N07-39, 'Early Robin', 'Rainier', 'Sweetheart', and 'Skeena' grafted onto Gisela 6, Gi196-1, and Mazzard at two on-farm sites (Cooper and Omeg) (total of 90 trees per location)

3. Establish a variety trial containing NY113 (bi-color (bc)), NY132, NY213, NY288, NY1913 (bc), NY7679 (bc), NY7690 (bc), NY8033 (bc), NY8039 (bc), and NY9116 on Gisela 6 as compared to 'Bing', 'Rainier', 'Regina', and 'Skeena' (total of 42 trees per location) at two sites.

4. Assist in the tree pruning, training, sample and data collection at the MCAREC station in the ecological soil management planting. Help collect pollen and perform flowering research.

Significant findings:

All trials are well established after the first growing season.

Methods:

1. Orchard establishment and tree training trials

YEAR 1 (2006)- 'Early Robin' and 'Rainier' trees grafted on Gisela 6 were planted by Coopers and Morgans, respectively. Five replicates of five tree plots arranged in a completely randomized design were pruned and trained as with central leader, bush or steep leader trees at Coopers. Six replicates of at least four tree plots were pruned in a similar fashion at Morgan's. Central leader and multiple leader systems were pruned in mid-summer, while steep leader pruning will be performed in the dormant season. Measure growth (trunk circumference) and photograph changes during the growing season.

YEAR 2 (2007) will focus on continued tree and canopy development with the appropriately time heading cuts and tree training. This will again be done in consultation with the grower cooperators.

YEAR 3 (2008) will begin focusing on the vegetative-reproductive plant status.

2. Establish two on-farm variety x rootstock trials in two locations

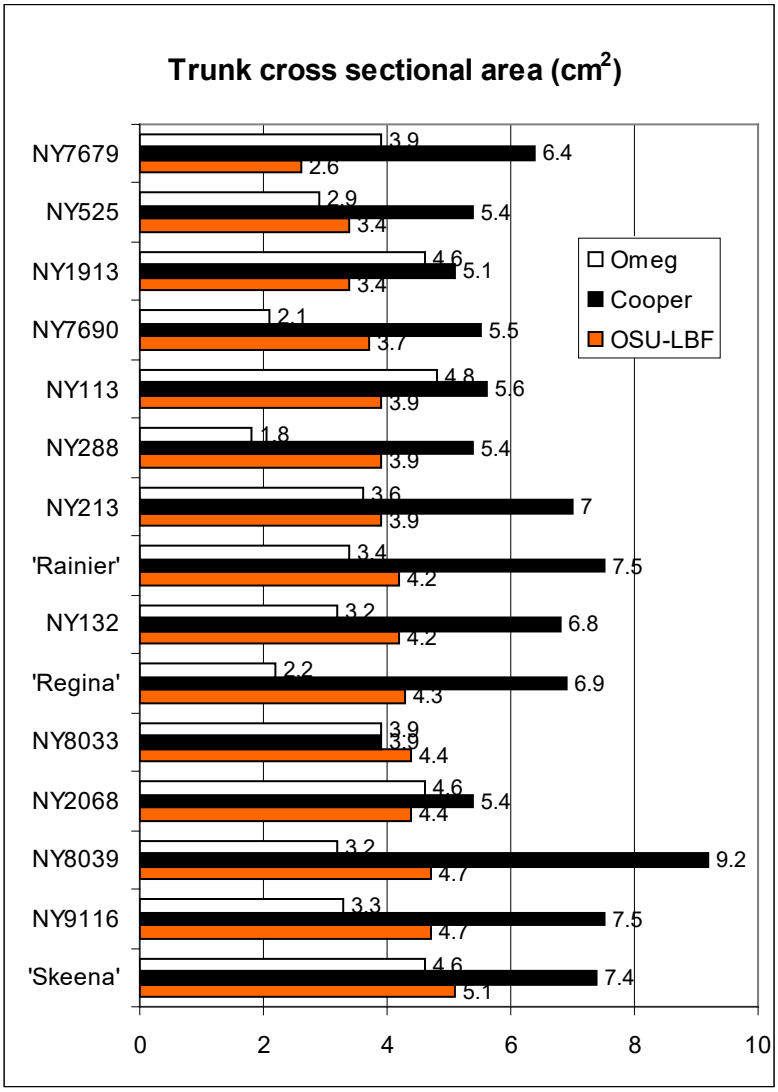
a. Plant new *2006 sweet cherry trial* (Omegs and Coopers) that contain 'Rainier', 'Regina', 'Skeena', NY113, NY132, NY213, NY288, NY1913, NY7679, NY7690, NY8033, NY8039, and NY9116 on Gisela 6, implement grower's desired training system and evaluate performance after one year.

b. Distribute experimental trees to orchardists (Omegs and Coopers) for a *2006 cultivar x rootstock trial* that includes 'Bing', 'Tieton', 'Sunset Bing', 'Sylvia', 'Benton', 13N07-39, 'Early Robin', 'Rainier', 'Sweetheart', and 'Skeena' grafted onto Gisela 6, Gi196-4, and Mazzard. Design training system approaches collaboratively with the orchardists for each combination and evaluate performance after one year.

Results:

Table 1. Trunk cross-sectional area of trees in the training systems trials.

	'Early Robin' on Gisela 6	'Rainier' on Gisela 6
	TCSA (cm^2)	
Steep leader	10.9 a	6.0
Central leader	10.0 a	6.0
Multiple leader (Bush)	6.4 b	6.6
MSD	0.9	ns



Cooper's planting grew more vigorously than the other two locations but at all sites trees grew well.

Fig. 1. Trunk cross-sectional area (cm^2) of the NY trial planting in three locations; Lewis-Brown Farm, Omegs and Coopers.

Table 1. Trunk cross sectional area (cm^2) of the 2006 variety x rootstock trial planted at three locations in Oregon.

	OSU				Cooper				Omeg			
Selection	Gi196-4	Gisela 6	Mazzard	MSD	196-4	Gisela 6	Mazzard	MSD	Gi196-4	Gisela 6	Mazzard	MSD
'Sweetheart'	8.9 a	5.7 b	5.1 b	1.6	8.1	7.7	7.4	ns	7.6 a	5.0 b	5.2 b	1.8
'Benton'	8.2 a	5.7 b	4.7 b	2.2	12.3	8.2	8.1	ns	10.8 a	5.2 b	2.5 b	4.7
'Skeena'	7.9 a	6.9 a	4.9 b	1.4	7.2	7.5	7.9	ns	6.3 a	4.6 b	7.0 a	1
'Sunset Bing'	7.5 a	5.3 b	7.7 a	2.2	8.5	6.6	8.1	ns	8.2 a	4.9 b	7.7 a	1.3
'Tieton'	6.9 a	3.5 b	6.2 a	1.7	9.5	7.4	7.2	ns	6.7 a	5.8 a	6.7 a	2.3
'Early Robin'	6.7 a	4.3 b	3.4 b	1	7.9 a	8.4 a	6.5 b	1	6.9	4.5	5.4	ns
'Rainier'	6.5 a	4.2 b	5.6 ab	1.4	8.7 a	7.9 ab	6.3 b	1.8	5.7	4.4	5.2	ns
13N 7-39	6.4 a	.	3.7 b		5.1	7.6	7.4	ns	6.4	4.8	5.3	ns
'Regina'	6.2 a	4.8 b	3.3 c	1.2	8.6	7.4	7.3	ns	7.0 a	3.0 c	4.5 b	1.5
'Sylvia'	5.6 ab	4.9 b	6.4 a	1.4	6.1 b	7.6 ab	9.1 a	2.1	5.3 b	4.3 b	7.1 a	1.7
'Bing'	.	6.8 a	6.3 b		.	7	5.6	ns	.	4.8	4.5	na

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Flowering and pollination of 'Regina' and 'Bing' sweet cherry trees

PI:	Anita Nina Azarenko	**Co-PI(2):**	Annie Chozinski
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Address 2:	Department of Horticulture	**Address 2:**	Dep't of Horticulture
City:	Corvallis	**City:**	Corvallis
State/Province/Zip	OR 97331	**State/Province/Zip:**	OR 97331

Cooperators: Mike, Mel and Linda Omeg; John and Karen Carter; and Don Nusom

Budget 1:
Organization Name: Agricultural Research Foundation **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	Year 1:	Year 2: 2006-07	Year 3: 2007-08
Salaries		8,300	8,450
Benefits		4,900	5,660
Wages		2,500	3,200
Benefits			10
Equipment		500	
Supplies			
Travel		1,500	680
Miscellaneous			
Total		17,700	18,000

Objectives for 2006-2007:

1. Determine ovule longevity of 'Regina' and 'Bing' flowers.

2. Assess pollen viability of 'Attika', 'Sam', 'Sandra Rose', 'Stark's Gold', 'Sylvia', 'Skeena', 'Regina' and 'Schneider's Späte Knorpel'.

3. Compare pollen tube growth rates when 2-4 standard pollinizers are used in 'Regina' and 'Bing' plantings.

Significant findings and results:

- *In situ ovule longevity studies* – 'Regina' flower senesce at a faster rate than 'Bing' flowers (Fig. 1). Rate of ovule senescence for a genotype appears to be highly dependent on temperature (growing degree hours) as observed by the similar slopes of the lines.

- *Pollen tube growth rates* –Pollen tubes reached the base of the style faster at the Lewis Brown Farm in 'Regina' flowers than at Carter's in The Dalles (Fig. 2). No clear patterns emerged on the best pollen source nor combinations thereof although in Corvallis, 'Sylvia' and 'Schneider' pollen appears to have reached the base of the style more quickly than other pollen sources. In 'Bing', when 'Rainier' pollen was used alone, it never traveled through more than 60% of the style for the duration studied (1200hrs).

- *Pollen viability* – Viability was extremely variable for the genotypes studied (Table 3). Pollen from the Willamette Valley generally had higher germination than when compared to The Dalles.

Materials and Methods:

- *In situ ovule longevity*- Ovule longevity of 'Regina' and 'Bing' flowers were and will continue to be determined in two locations, The Dalles and Lewis Brown Farm, over a two week period. A minimum of three trees were and will be covered with netting to prevent pollination. Flowers were and will be removed at 250 GDH intervals in 2007 for durations of no less that 2500 GDH. Flowers are placed in a fixative. Ovules are excised after rinsing out the fixative, stained with aniline blue, and observed under a fluorescence microscope. Fluorescence of callose at the chalazal end indicates ovule senescence. In 2006, duration of sampling of 'Bing' flowers was inadequate as 100% ovule senescence was not observed by 1200 GDH. Over 400 ovules were evaluated in 2006 requiring over 310 hrs. These and future data will determine the significance of bloom overlap of pollinizer cultivars.

- *Pollen tube growth rates*- 'Regina' and 'Bing' flowers were and will be hand-pollinated with pollen from 2-4 standard pollinizers, alone and in combination. Ten flowers were and will be collected at 12 hr intervals, placed in a fixative, stained with aniline blue, and observed under a fluorescence microscope. The percent of the style traveled by the pollen tube was and will be measured for each sampling date. Callose plugs and tubes are observed. In 2006, over 5000 pistils were collected and 1500 were evaluated (550 hrs). Seed were collected from mature fruit, and will still be analyzed for s-alleles using molecular markers and PCR technologies. The s-alleles in the seed will indicate the pollen parent. These data will help to identify the most suitable pollinizer(s) and estimate time required for growth of pollen tubes to the base of the style.

- *Pollen viability*- Flowers are collected and brought back in garbage bags to prevent dessication. Bases of twigs are cut and put into water. As flowers opened each day, for 3-7 days, anthers were cut off, induced to dehisce pollen, and pollen collected for observing pollen germination and viability. Pollen was collected and put into vials plugged with cotton into the freezer with dessicant

in a plastic container. Pollen collection required 80 hrs. A simple liquid sucrose medium was and will continue to be used to induce pollen germination and pollen viability was tested prior to placing in the freezer. Pollen viability studies required 30 hrs of labor. Through evaluation of pollen germination over multiple years, we hope to determine compatible genotypes that have better and more predictable pollen viability.

Results: See details of the findings in the following figures and tables.

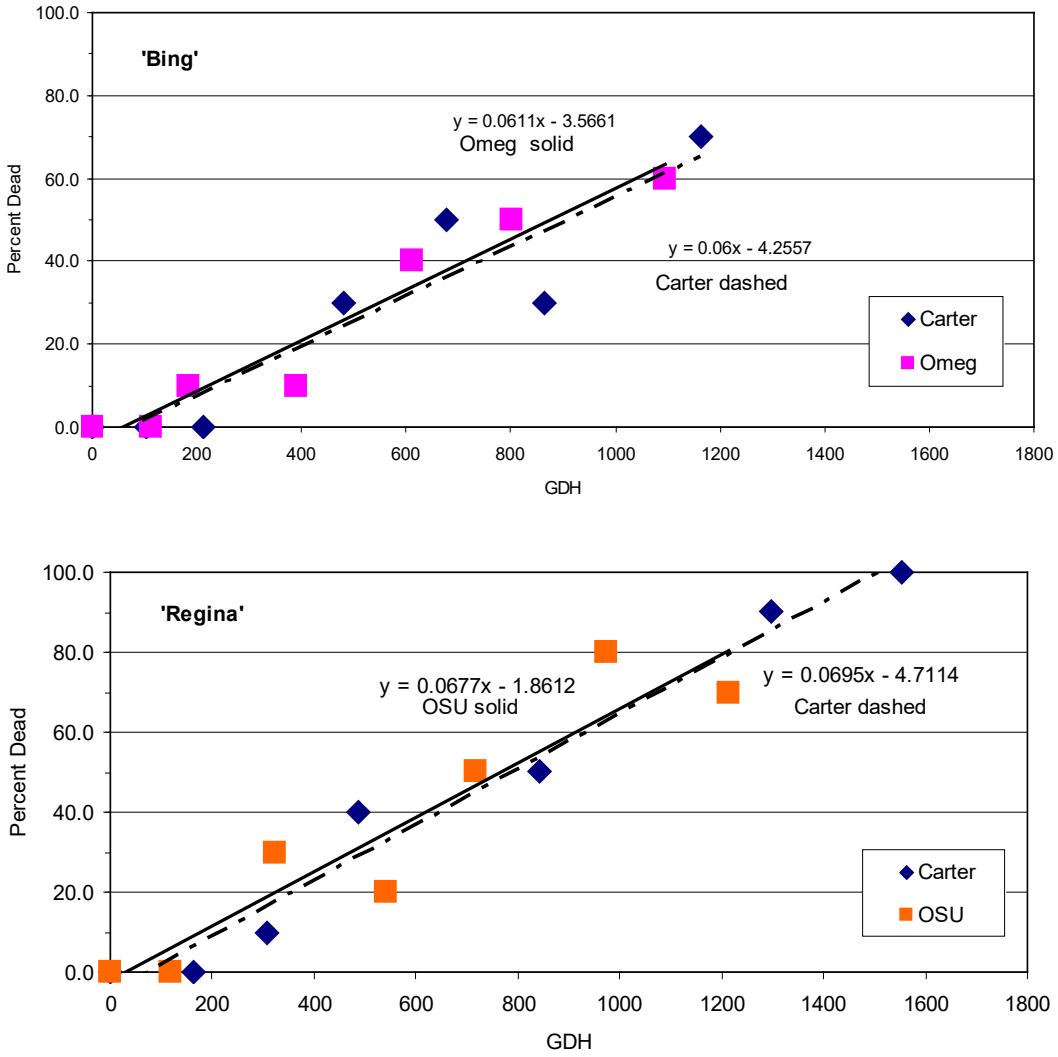

Fig. 1. Ovule senescence as a function of growing degree hours for 'Bing' and 'Regina' flowers from two locations each.

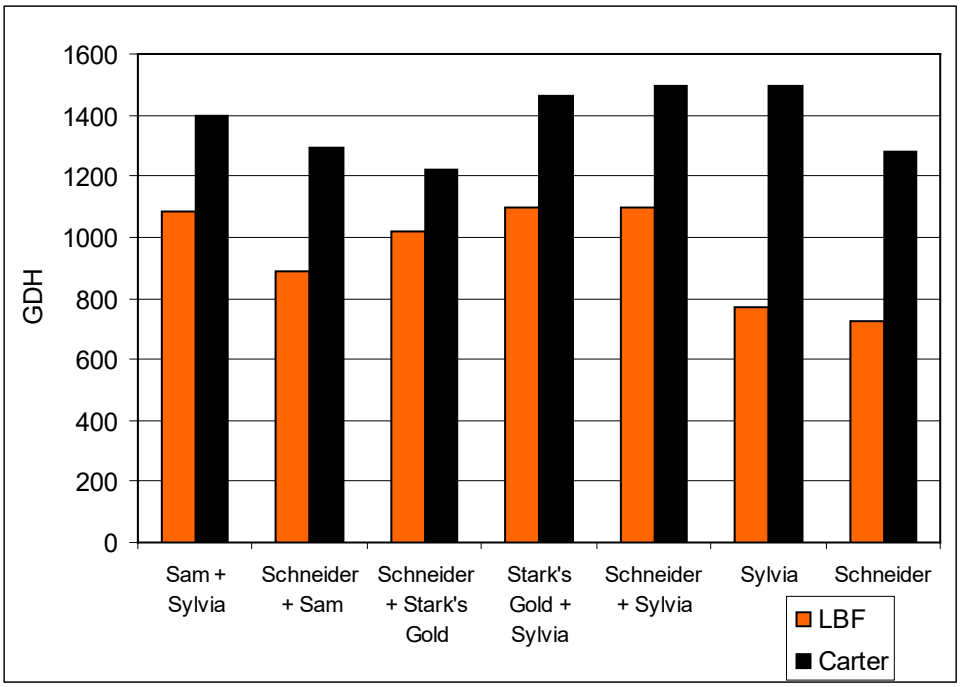

Fig. 2. Number of growing degree hours (GDH) required for pollen tubes in 'Regina' flowers to reach the base of the style when pollinated with different combinations of pollen sources.

Table 1. Number of growing degree hours (GDH) and maximum observed distance that pollen tubes traveled within the style in 'Bing' flowers pollinated by 'Rainier' and 'Van'.

Pollen source on 'Bing'(s_3s_4)	GDH	Distance of style with pollen tubes (%)
Omeg		
Van (s_1s_3)	863	90
Rainier (s_1s_4)		Never exceeded 30% of style
Van + Rainier	756	80
Carter		
Van	808	90
Rainier		Never exceeded 60% of style
Van + Rainier	863	100

Table 2. Pollen viability of compatible pollinizers for 'Bing' and 'Regina' in 2006.

Pollen genotype	s-alleles	n	2006 Viability (%) Range	Mean
Bing	s_3s_4			
Rainier	s_1s_4	6	1.3 - 23.0	9.7
Van	s_1s_3	4	2.0 - 28.3	9.9
Regina	s_1s_3			
Sam	s_2s_4	1	18.3	18.3
Schneider's Späte Knorpel	s_3s_{12}	3	8.0 - 32.3	22.4
Stark's Gold	s_3s_6	1	24.0	24.0
Skeena	$s_1s_{4'}$	3	5.7 - 43.3	22.8
Sandra Rose	$s_3s_{4'}$	3	5.3 - 63.7	33.4
Sylvia	s_1s_4	3	1.0 - 42.7	22.1

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-05-508 (WSU Project 13C-3655-7299)

Project Title: Induction of Branches in Sweet Cherry Trees in the Orchard and Nursery

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Cooperators: Matt Whiting, WSU Prosser, Dwayne Visser, WSU Wenatchee

Budget 1:
Organization: Washington State University **Contract Administrator:** ML. Bricker / Sally Ray
Telephone: 509-335-7667 / 509-663-8181 x221 **Email:** mdesros@wsu.edu / saray@wsu.edu

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries (Technical)[1]	4,200	4,368	**4,543**
Benefits[2]	1,302	1,485	**1,545**
Wages (Time-slip)	1,500	2,000	**2,400**
Benefits[2]	240	220	**276**
Equipment	0	0	**0**
Supplies[3]	500	800	**1,000**
Travel[4]	2,000	2,500	**3,000**
Miscellaneous	500	500	**500**
Total	10,242	11,873	**13,264**

Footnotes:
1 Technical and time-slip help to set up trials, apply treatments and collect data as needed.
2 Salaries benefit rate for year 1 was 31%. Years 2 and 3 benefit rate is calculated at 34%. This increase is due to an increase in the contribution made by WSU on behalf of the employee. Time-slip benefit rate for year 1 was 16%; benefit rate for year 2 was 11%; and year 3 is calculated at 11.5%. This was due to a change in policy at WSU.
3 This category includes a variety of miscellaneous supplies, non-capital equipment, consumables, etc. that are needed to carry out the research project. Cell phone charges are allowable under this grant.
4 Travel to distant research sites is expensive. These funds will be used to defray costs of vehicular operation, maintenance, and travel costs for Dr. Elfving, Mr. Visser and their employees to research plots in grower-cooperator orchards through the south-central and north-central Washington fruit production areas.

Objectives:

1. Relate green-tip branching treatment effectiveness to timing of the bioregulator and bark-manipulation treatments in relation to bud development to determine how critical timing is as a factor controlling the branching response.
2. Assess the relation of timing of branching treatments to development of branching, location of branch development on the tree, and number, angle and quality of the lateral branches formed.
3. Assess the effect of cultivar and branch orientation on branching response to bioregulator and bark-manipulation treatments. Examine the potential for combining chemical/physical branch induction with later treatments of ethephon for stimulation of flowering and cropping.
4. Develop new combinations of bioregulator and physical treatments for enhanced cost effectiveness in branch-induction techniques.
5. Determine through longer-term monitoring the benefit in terms of both onset of productivity and sustained productivity of increased lateral branch development during the early years of canopy formation.
6. Explore the opportunity for developing new tree training systems based on the application of effective branch-induction methodology.

Significant findings:

- Where half-score cuts plus cytokinin treatment were made on the outside half of vertical, 1-year-old leader shoots, the number of shoots formed was increased by 2- to 5-fold, with a large number of those shoots appearing on the lower two-thirds of the treated leader shoots.
- Half-score cuts on the outside portion of the leaders definitely promoted preferential lateral branching on the outside half of the treated leader shoots, mainly on the lower portions of those leaders.
- Shoot development was induced to some extent above a score plus cytokinin application as well as below the point of treatment.
- There was very little movement of the stimulative effect of a score plus cytokinin treatment laterally around the treated stem.
- Branch-induction results clearly showed that making cuts every 12 inches along the leader was just as effective for stimulating shoot development as making 2 or 3 times as many cuts plus cytokinin treatment (every 4 or 8 inches), thus reducing the labor input required.
- Successful lateral-branch induction did not require any attention be paid to the location of a half-scoring cut relative to nearby buds.
- Where various mixtures of cytokinin plus adjuvants were applied to unscored bark, no beneficial effect of the treatment on shoot development was observed.
- Scoring plus treatment with 5,000 ppm GA_{4+7} in 'Skeena' cherry was almost as effective for shoot induction as treatment with 6-benzyladenine alone, a surprising result that should be confirmed with further testing.
- Application of thidiazuron (TDZ) at 1,000 ppm or of chlorophenylurea (CPPU, Prestige) at 500 ppm without added GA produced a weaker shoot induction response than normally observed when Promalin (5,000 ppm) is painted on scoring cuts. When GA_{4+7} was added to either cytokinin and painted on scoring cuts, shoot induction was the same as for the standard Promalin treatment.
- The only shoot-induction treatment with positive results on newly planted 'Rainier'/G.5 trees was disbudding. The low vigor of their growth in year 1 did not allow for significant expression of bioregulator-mediated shoot induction.
- When cytokinin was mixed with 10 ppm of fixed Cu and applied to half-scoring cuts, the branch-induction effect was undiminished; thus Cu has no negative effect on the efficacy of cytokinin.
- Again in 2006, we observed no bacterial canker infections on any of the hundreds of scoring cuts we made in the spring.

Methods:
Five trials were initiated in the 2006 growing season to test effects of cytokinin-containing bioregulator products on stimulation of lateral branch development in sweet cherry trees at green-tip. Objectives this year included clarifying the role of gibberellic acid in the branching response, testing several new cytokinins for efficacy in lateral-shoot induction, examining the number of scoring cuts plus cytokinin in relation to the amount of branching observed, and attempting to develop a method for successful treatment of cherry shoots with cytokinin without the need for cutting the bark. One trial was designed to assess the potential for effective branch induction treatment in the year of planting.

Results and discussion:
Making bark cuts at approximately 12-inch intervals is our "standard" approach at the present time. Since it is more difficult to induce branching from the lower portions of vertical leader shoots, we tested the notion that "more cuts are better" but discovered no evidence for improvement in branching with more cuts. Since cutting the labor cost of this program is of paramount importance, this finding is very significant. It may be that we can reduce the number of cuts we apply in our scoring program even more; only further testing will determine if that conclusion is valid.

By limiting the bark cuts to a half-circle around the outside portion of the vertical shoots, we were able to demonstrate that the cytokinin effect on bud stimulation moves somewhat upward as well as moving downward, likely in the phloem. There is relatively little lateral (around the stem) transport so using half-scoring cuts promoted preferential branching to the outside of the leader shoots. This response was most clearly observed in the lower portions of the treated leaders. A substantial amount of shoot growth still develops from near the terminal of the 1-year-old shoot. This is to be expected since this growth is what establishes the overall apical-dominance effect that we observe. When we induce more lateral shoots, the vigor of those naturally occurring shoots near the tip is reduced.

Results in 2006 validated the concept that it is not necessary to place scoring cuts on the leader in any specific relation to the location of buds. Because of the ability of the cytokinin effect to translocate vertically away from the cut, labor efficiency in applying the cuts is significantly improved.

Because of the directionality of the movement of the cytokinin effect, and because the stimulative effect of any cut is limited in distance, the possibility of specific programs of targeted cuts, planned locations for new shoot development, and efficient use of labor for shoot induction is now possible.

Although we did not observe an improvement in branch development when cytokinin treatments were applied to the bark in the absence of cuts, we plan to continue this aspect of the research program. If we can figure out a way to make cytokinin applications effective without the need for cuts to be made, we would radically improve labor efficiency and eliminate the risk of possible bacterial canker infection. In this year's trials, we found that mixing fixed Cu with cytokinin did not affect branch induction, but elimination of the need for making cuts would diminish the risk of infection, though low, to zero.

A surprise this year was the observation that GA_{4+7} alone was capable of inducing shoot development in sweet cherry. Because GA does not have cytokinin-like effects, its role in the shoot-induction process in sweet cherry is unclear. Further trials should be undertaken to verify that this result can be repeated.

Other cytokinins besides 6-benzyladenine are capable of inducing new shoot growth in sweet cherry. CPPU is now available commercially from Valent Americas under the trade name Prestige, but this product has no registration for any use on sweet cherry. Thidiazuron (TDZ) is still an experimental material and is unavailable at this time for use on sweet cherry.

Acknowledgments:
The assistance and support of the following persons and organizations is gratefully acknowledged: Tom Auvil, John Griggs, Dr. Chris Ishida, Brandon Lewis, Pete Savage, John Steffen, Griggs Orchards, Lewis Orchards, Savage Orchards, Valent BioSciences, the Washington Tree Fruit Research Commission, and the WSU Agricultural Research Center.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-05-504B

Project Title: Breeding and Genetics Program for PNW Sweet Cherries
PI: Matthew Whiting
Organization: WSU-IAREC
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City: Prosser
State/Province/Zip WA/99350

Cooperators: James Olmstead, Amy Iezzoni

Total Amount Requested : $94,485

Budget 1:
Organization Name: WSU **Contract Administrator:** ML. Bricker /S. Brock
Telephone: 509-335-7667/509-786-1224 **Email address:** mdesros@wsu.edu/sabrock@wsu.edu

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3: 2007**
Salaries		30,000	**30,804**
Benefits		11,400	**10,781**
Wages	3,500	8,500	**8,000**
Benefits			**920**
Equipment	500	500	**1,500**
Supplies	500	1,000	**1,500**
Travel		1,000	**1,500**
Plant Material	3,200	2,700	**2,900**
Virus Indexing	1,000	1,000	**1,000**
Miscellaneous		800	**500**
Total	8,700	56,900	**59,405**

Footnotes:

Budget 2:
Organization Name: Michigan State University **Contract Administrator:** Lorri Busick
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Item	**Year 1 -** 2005	**Year 2 -** 2006	**Year 3 -2007**
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
DNA genotyping svc	10,000	10,000	**15,000**
Miscellaneous			
Total	10,000	10,000	**15,000**

Budget 3:
Organization Name: Amy Iezzoni **Contract Administrator:** consulting fees
Telephone: 517-355-5191 ext. 391 **Email address:** iezzoni@msu.edu

Item	**Year 1 -**2005	**Year 2 -** 2006	**Year 3 -2007**
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Breeding Consultant	10,000	10,000	**10,000**
Expenses	3,000	3,000	**3,000**
Miscellaneous			
Total	13,000	13,000	**13,000**

Budget 4:
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: 509-655-8271 **Email address:** Kathy@treefruitresearch.com

Item	**Year 1 -**2005	**Year 2 -**2006	**Year 3 -2007**
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot Fees	0	1,200	**7,080**
Miscellaneous			
Total	0	1,200	**7,080**

Objectives

New sweet cherry cultivars with production and fruit traits superior to current cultivars are needed to provide differentiated products and production advantages that will allow the Washington and Oregon sweet cherry industries to remain competitive. Currently, there is no breeding program, either domestic or foreign, able and/or designed to provide a series of new cultivars that target the demands of the Washington and Oregon industries. New cultivars are needed to extend the ripening season, minimize production costs and post-harvest cullage, and that have supreme fruit size, firmness, texture and flavor. Genetically improved cultivars could also reduce the risks and costs from biotic (e.g., powdery mildew) and abiotic (e.g., temperature stress) factors. The breeding goal of this project is to meet this need by developing a full-season series of sweet cherry cultivars that exceed current cultivars for a range of characteristics desired for current and future domestic and foreign market opportunities. To ensure the success of the breeding program, strategies have been put into place to (1) use an extensive array of unique germplasm, (2) achieve large seedling populations for evaluation, (3) reduce the juvenility period, and (4) utilize genetic and genomic approaches that increase the efficiency and capability of the breeding program.

The specific objectives of this research project are to:

1. Develop a comprehensive framework for a sweet cherry breeding and genetics program.

2. Produce through hybridization and selfing, genetically-variable sweet cherry selection populations that segregate for important target traits.

3. Select outstanding families and elite individual seedlings in multiple target environments for important production and fruit traits.

4. Establish elite selections in replicated multi-site locations for pre-commercial evaluation.

5. Install a data base for storage and analysis of critical information that will effectively support the breeding goals.

6. Acquire and develop molecular information and tools that facilitate rapid and efficient marker-assisted selection for production and fruit traits critical for commercial success.

Significant Findings and Accomplishments

<u>End of YEAR 1 (2005)</u>

- A comprehensive oversight and communications framework was established and implemented. Oversight of the breeding program is provided by an Advisory Committee (AC) consisting of cherry growers from WA and OR.

- Germination of seeds from YR 2004 crosses in the seed beds at Willow Drive Nursery was extremely poor with only 250 seedlings resulting from the 4,466 seeds (6% germination). Thus, new seed stratification and germination strategies were employed.

- Novel germplasm was successfully imported by Amy Iezzoni as pollen.

- Despite the consistently cold weather conditions, 84 crosses including reciprocal crosses were successfully accomplished at Prosser in spring 2005 resulting in 7,166 hybrid seed.

- Matt Whiting planted a new orchard to evaluate the potential for rootstock and nursery production system to hasten productivity. 'Bing' and 'Tieton' on Mazzard seedling, Gisela®6, and Maxma14 trees were planted at the Roza farm as sleeping eyes (i.e., fall-budded, spring dug and planted), and standard nursery trees (i.e., fall-budded, nursery-grown, spring dug and planted +1yr).

- It was decided that the Oregon selection site will be located at the Hood River Station.

- Amy Iezzoni received a $400,000 three year USDA-CSREES-NRI grant entitled "Genomic resources to improve fruit size and quality in sweet cherry". This grant which started June 1, 2005, provides funds to obtain the baseline genetics/genomics research required for the breeding program. The grant team members are Drs. Wayne Loescher (fruit biochemistry/physiology), Dechun Wang (quantitative genetics) and Esther van der Knaap (molecular genetics).

<u>End of YEAR 2 (2006)</u>

- Jim Olmstead began as manager of the breeding program January 1.

- The existing database system used in the apple breeding program was effectively utilized for the cherry breeding program with minor modifications. The platform used for this database can be user-modified, a function not readily available from many commercial breeding database systems.

- Germination of year 2005 seedlings was significantly better than those in 2004 (30% vs. 6%), with 1,462 seedlings currently available.

- The spring 2006 crosses were extremely successful, with 17,848 seed generated from 111 crosses including reciprocal crosses.

- Shoot-tip micrografting did not increase efficiency over traditional chip budding due to low success rate and slow growth after grafting.

- No MxM 14 or Gisela rootstocks were available in 2006 for budding of 2004 seedlings. Due to continued shortages of these rootstocks key to successful implementation of the rapid cycling protocols devised for the cherry breeding program, the decision was made to advance several promising rootstock selections from Amy Iezzoni's rootstock selection program.

- The project to identify a molecular marker linked to the gene controlling powdery mildew resistance was completed. Because of a high level of genetic similarity, no candidate markers linked to the *Pmr-1* gene have been identified. Therefore, in collaboration with Drs. Angela Baldo and Gayle Volk, we initiated an alternate project to identify Resistance Gene Analogs (RGA) using a set of conserved DNA primers that will specifically target resistance genes in the cherry genome. If any RGA are found from the powdery mildew resistant parent that are not in susceptible parents, Amy Iezzoni will screen them on the available segregating progeny to determine if they are linked to *Pmr-1*.

- Greenhouse incidence of powdery mildew was used as a screening method to select seedlings resistant to the disease. Amy Iezzoni's lab will use molecular markers to screen those resistant seedlings for self-fertility. Combining the two early screening methods is expected to reduce by three-quarters the number of seedlings sent to field testing.

- An early-season testing site was selected in the Pasco, WA area to complement the MCAREC testing site in OR, and a yet to be determined high elevation, late-season site in the Chelan-Okanagon area.

Methods to be employed by objective

1. Develop a comprehensive framework for a sweet cherry breeding and genetics program**.**

   The framework for a sweet cherry breeding program strategy was developed through consultation by Amy Iezzoni and implemented after the project proposal was accepted in 2004. An industry Advisory Committee was developed consisting of members from the WA and OR industries.

2. Produce through hybridization and selfing, genetically-variable sweet cherry selection populations that segregate for important target traits.

   Determine the final crosses taking into account seed quality, bloom time, self-fertility or incompatibility group, and virus status. Diverse germplasm will be obtained from Europe as pollen. The goal is to produce enough seed to generate 5,000 seedlings per year for each of the three years. Seedling germination and establishment still did not reach target levels in 2006. However, experimentation conducted in 2006 indicated that we could improve germination and establishment by cracking seed coats prior to the onset of germination (early January). This enables seedling establishment before the hottest period of summer growth.

3. Select outstanding families and elite individual seedlings in multiple target environments for important production and fruit traits.

   Effective methods to accelerate seedling flowering and fruiting are essential to increase efficiency in the breeding program. One potential method to do this is to propagate seedling trees on a precocious rootstock. In year 2005, we proposed propagation of the first year seedlings on MxM 14 rootstock. No MxM14 or Gisela rootstock liners were available commercially in 2006 or for the immediate future. Due to inconsistent and short supply of available precocious rootstock, several promising selections from Amy Iezzoni's rootstock selection program were started in tissue culture at Duarte Nursery in California. Rootstock liners should be available during the 2007 growing season for propagation. Because of the greenhouse facilities available, propagation is not limited to fall chip-budding, and the container-based production system does not rely bare-root digging and dormant shipping. This represents a potential time savings of up to one year over traditional nursery propagation, with field planting of full size trees at a similar time as sleeping-eye dormant trees would be planted.

   Additional horticultural-based methods to accelerate seedling flowering implemented in 2006 were green shoot-tip grafting of seedlings with Gisela 6 rootstock and cold room cycling to approximate two growing seasons in the first calendar year of seedling life. Green shoot-tip grafting resulted in a 30% success rate. Furthermore, slow initial growth after grafting resulted in no increased efficiency over traditional fall budding. The potential for cold room cycling to reduce the time to flowering will be evaluated in future years as those seedlings begin to fruit. We speculate that those seedlings which are genetically predisposed to a short juvenility period

would respond well to the cycling strategy and would flower profusely in Year 3. However, those seedlings that are genetically predisposed to a longer juvenility period may need to be grafted to a precocious rootstock to ensure early flowering. In order to determine what procedure to use for each seedling, we will explore the possibility of identifying molecular marker(s) linked to juvenility time. The onset of flowering and fruiting will be recorded in the Emperor Francis x New York 54 population and subjected to QTL analysis using the developing sweet cherry linkage map (part of Obj. 6). This initial QTL discovery objective will be funded by the USDA-CSREES Cherry Genomics Project. Results from 2006 are promising as a QTL [LOD ~ 5.0] for early flower abundance was identified on linkage group 3.

In consultation with the Advisory Committee, descriptions of target cultivars that are desired outcomes of the breeding program were finalized. A multi-site selection and testing strategy will be implemented to emphasize adaptation to specific site and production requirements.

4. Establish elite selections in replicated multi-site locations for pre-commercial evaluation.

   Planting will begin in Spring of 2007.

5. Install a data base for storage and analysis of critical information that will effectively support; 1) parental choices, 2) pedigree information, 3) selection criteria and progeny performances, 4) trait and tree performance at multiple sites, and 5) filing and prosecution for optimal Intellectual Property protection.

   The existing database from the apple breeding program was installed for use in the sweet cherry breeding program.

6. Acquire and develop molecular information and tools that facilitate rapid and efficient marker-assisted selection for production and fruit traits critical for commercial success.

   No candidate molecular markers were identified for powdery mildew resistance. Therefore, progeny populations resulting from YR 2005 crosses were screened in the greenhouse for resistance. Amy Iezzoni's lab will use existing molecular markers to screen for self-fertility among these seedlings.

   For successful implementation of marker-assisted breeding, high-throughput DNA extraction and marker screening is critical. Contact was made with STA Laboratories to test the feasibility of DNA marker genotyping for the sweet cherry breeding program. Additionally, WSU has recently hired two positions in Horticultural Genomics; these labs would be expected to cooperate with the sweet cherry breeding program.

Summary List of YR 2007 Activities

- Implement the multi-site testing strategy with the spring planting at four test sites.
- Crack, germinate, plant, and maintain the seedlings resulting from the 17,848 seeds generated in year 2006.
- Develop a crossing plan, import needed pollen, and carry out the spring 2007 crosses.
- Harvest the fruit, clean, and stratify the seed from the spring 2007 crosses.
- Explore collaborations for DNA marker development and screening.
- Implement containerized budded tree production at Duarte Nursery.

Results and Discussion

A comprehensive oversight and communications framework was established and implemented in year 2005. Oversight of the breeding program is provided by an Advisory Committee (AC) consisting of cherry growers from WA and OR. Quarterly executive summaries of progress will be and were provided to the AC on a quarterly basis. These quarterly summaries can be freely distributed. Matt Whiting established a secure web site to enhance communication with the AC. This web site also contains seedling and pedigree information that must remain confidential to protect IP. Communication with the entire grower community was accomplished through one article in the Good Fruit Grower in 2006, an overview presentation at the 2006 Cherry Institute, and participation in the 2006 Cherry Field Day at WSU-Prosser.

Since the initial crossing year in 2004, 213 different crosses (including reciprocal crosses) have been attempted. Included in this list of crosses are those utilizing imported pollen. To date, the project has seedlings resulting from 17 newly utilized foreign sweet cherry varieties. These foreign selections were chosen specifically to add the following superior traits to the breeding program: heat tolerance, very late ripening time, very early ripening time, rain cracking tolerance, fruit firmness, large fruit size, suitability for mechanical harvest, a novel source of self-fertility, and different sources of powdery mildew resistance.

Crosses made in 2006 were those that combined elite parents anticipated to yield potential cultivars in the first generation, used novel germplasm sources to incorporate useful traits (see above), and pyramided potential sources of powdery mildew resistance. Additionally, some crosses that were lost in 2004 or could not be completed due to frost in 2005 were repeated. Although seed germination of the 2005 crosses was significantly better than those from 2004, the overall germination percentage did not reach our target goal of 50%. Given the fact that poor germination continues to be a problem and may be limited due to the parental genotypes used in the breeding program, a greater number of crosses were made in 2006. Thus, even if we are unable to increase the germination percentage from that in 2005 (30%), we will meet the target goal of 5,000 seedlings from the 2006 crosses. Using seed handling techniques (seed cracking, altered storage and germination temperatures) devised based on our experiences with the 2005 seed, a higher germination percentage is not unlikely. At our target of 50% germination, the total number of seedlings from 2006 crosses would make up for the deficit in 2005.

In 2005, leaves were collected and DNA was extracted from the parents and 375 seedlings from crosses between PMR-1 with Van, Bing, and Rainier that had previously been screened for powdery mildew resistance. Also included were four other cultivars that are powdery mildew resistant; Venus, Moreau, Chelan and Hedelfingen. Resistant and susceptible bulk populations were designed and screened using AFLPs generated from *Eco*RI with *Mse*I and *Pst*I with *Mse*I selective primer pair combinations. Because of a high level of genetic similarity, no candidate markers linked to *Pmr-1* have been identified to date. The limited number of polymorphic fragments identified among the four sweet cherry cultivars (PMR-1, Bing, Van and Rainier) highlights the genetic uniformity present in sweet cherry cultivars. For this reason, an alternate approach using conserved DNA primers to target resistance genes was initiated in collaboration with Drs. Angela Baldo and Gayle Volk at USDA-ARS. Using this RGA approach may increase our chances of identifying *Pmr*-1 or flanking regions. With the absence of candidate markers, powdery mildew resistance screening for 2006 was performed under greenhouse conditions. Priority crosses for powdery mildew resistance screening in 2006 included those combining self-fertility and disease resistance. Because the powdery mildew resistance source is a single dominant gene, all crosses with susceptible, self-fertile parents segregated 1:1 for resistance and susceptibility. Thus, of the 235 seedlings screened for powdery mildew resistance to date, 116 are resistant. Since the self-fertility allele used in these

crosses also segregates 1:1, a further reduction of ~58 seedlings is anticipated when the genotyping is completed. For practical purposes, this represents a reduction in the required field space to less than 1/10th of an acre. The combined screening for these two traits took less than one year from germination. Furthermore, identification of self-fertility at this stage of seedling development is only possible using molecular markers.

The breeding project includes a budget line of $15,000 for genotyping services, not the genetic research that would be required to elucidate the genetic control of complex fruit quality traits. The USDA-CSREES project was designed specifically to fill this void. The goal of the USDA grant is to develop the genomic resources required to implement marker-assisted selection in cherry (*Prunus* sp.) breeding programs. We plan to accomplish this goal using a QTL strategy focused on fruit size and quality traits, followed by QTL validation and allele mining using a newly-developed pedigree genotyping approach. The research consists of the following steps: (1) Construct a sweet cherry genetic linkage map for comparative mapping with the *Prunus* reference map and other *Prunus* linkage maps. (2) Identify QTL for fruit size and quality traits. (3) Fine map the major QTL identified and design markers for marker assisted selection. (4) Validate the QTL across genetic backgrounds and identify QTL alleles. This supplemental funding will allow us to greatly exceed our prior expectations for objectives 5 and 6, database capability and acquisition of molecular information, respectively. In addition, one of our team members, Dr. Wayne Loescher, is studying the biochemical basis of the differences in fruit quality using fruit from the varieties used as parents in the breeding program. This information will greatly enhance our selection and QTL discovery capabilities. The capacity for genetic research within the WSU tree fruit research community will also increase after the hiring of two Horticultural Genomics positions this summer.

Two powdery mildew resistant genotypes from the WSU sweet cherry breeding program are being propagated for advanced testing based on performance of second test trees at Prosser. Both were identified from crosses between 'Rainier' and 'PMR-1' and have been given testing names of DD (Fig. 1) and GG (Fig. 2). DD averaged 8.5 row size on Gisela 6 rootstocks and matures near 'Lapins' timing. GG averaged 9.5 row size on Gisela 6 rootstocks and matures mid-season. Further quality and storage/shipping characteristics will be evaluated as larger plantings become available.

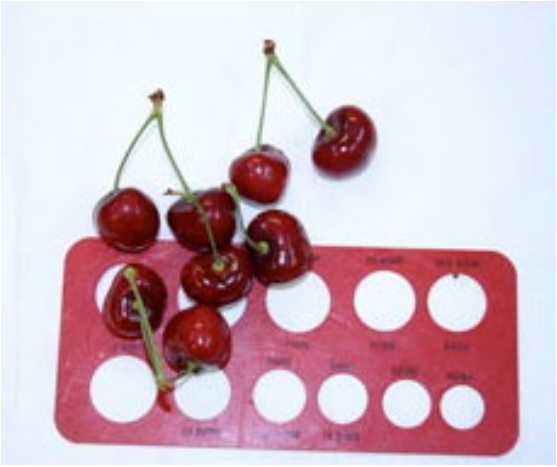

Figure 1. DD cherry selection from the WSU cherry breeding program

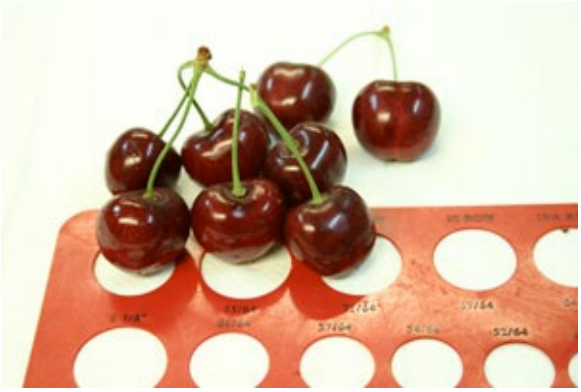

Figure 2. GG cherry selection from the WSU cherry breeding program

CONTINUING PROJECT REPORT **YEAR**: 3
WTFRC Project Number: CH-05-503

Project Title: Sweet cherry dwarfing rootstock

PI:	Amy Iezzoni	**Co-PI(2):**	Matt Whiting
Organization:	Michigan State Univ.	**Organization:**	Washington State Univ.
Telephone:	517-355-5191 ext 391	**Telephone:**	509-786-9260
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Address:	Dept. of Horticulture	**Address:**	WSU-IAREC
Address 2:	MSU	**Address 2:**	24106 N Bunn Rd.
City:	East Lansing	**City:**	Prosser
State/Province/Zip	MI 48824	**State/Province/Zip:**	WA 99350

Total funding requested: $17,884
Budget 1: (Due to a change in procedures since the proposal was submitted, certain budget lines highlighted in bold have increased. The reasons for these increases are briefly noted in the footnotes and discussed more fully in the project detail.)

Organization Name: MSU **Contract Administrator:** Ms. Lorri Busick
Telephone: 517-355-3591 **Email address:** busick@msu.edu

Item	**Year 1**: 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries	$ 4,725	$ 4,867	$ 6,343 [1]
Benefits	2,183	2,351	3,191 [2]
Wages/Benefits	3,500	5,000	1,500 [3]
Equipment	0	0	0
Supplies	400	1,500	600 [4]
Travel	1,500	2,500	2,500 [5]
Tree & freight cost	100	500	750 [6]
Plot cost at MSU	1,000	1,000	1,000[7]
Greenhouse cost	0	3,000	0
Miscellaneous	0	0	0
Total	$ 13,408	$ 20,718	$ 15,884

Footnotes: [1] This represents partial funding for technical support to oversee the technical aspects of this project and data collection and summarization. This amount has increased from the $5,013 originally proposed as two tasks were included: (1) development of DNA markers to fingerprint the MSU rootstock candidates, and (2) use these DNA markers to assure trueness to type of the shoot cultures at Duarte's Nursery.
[2] Benefits for YRs 2005, 2006, and 2007 are calculated at 46.2 %, 48.3%, and 50.3%, respectively. The salary increase for the fingerprinting resulted an associated increase in benefits.
[3] Student labor will assist with data collection. The increased labor cost in YR 2 reflects the increased labor needed for virus indexing and vegetative propagation at MSU.
[4] Supplies include mouse guards, tags and other field supplies. In YR 2, the additional cost is due to the purchase of ELISA kits and propagation supplies. In YR 3, the supply budget was increased by $200 to cover the cost of DNA genotyping supplies.
[5] Travel to WSU in April for planting at Willow Drive Nursery and summer for plot evaluation. An additional $1,000 was added for travel of A. Iezzoni to Duarte Nursery.
[6] The cost of transporting the 2080 cuttings from MSU to Seattle is $1250. $500 was budgeted in 2006. The remaining $750 was added to the 2006 budget. The previous budget line was zero.
[7] Plot fees are required at all MSU Horticultural Research Stations. These costs are based upon a fee structure that reflects the cost of standard plot maintenance.

Budget 2:
Organization Name: WSU
Telephone: 509-355-7667

Contract Administrator: Mary Lou Bricker
Email address: mdesros@wsu.edu

Item	Year 3 (2007)
Salaries	
Benefits	
Wages/Benefits	$ 2,000
Equipment	
Supplies	
Travel	
Tree & freight cost	
Plot cost at MSU	
Greenhouse cost	
DNA fingerprinting	
Miscellaneous	
Total	$ 2,000

Objective

Identify MSU rootstock selections that may have commercial potential as dwarfing precocious rootstocks for sweet cherry.

Specific objectives for 3 year project:

1. Complete the planting of the rootstock candidates at the WSU-Prosser and MSU-Clarksville test sites.

2. Identify the most promising rootstock candidates by evaluating tree health, precocity, trunk cross-sectional area, flower density, crop load, fruiting habit and fruit size.

3. Vegetatively propagate the most promising rootstock selections to provide grafted trees for advanced trials at multiple test locations.

Specific objectives for 2006:

1. Identify the most promising rootstock selections by evaluations for tree health, precocity, flowering and fruiting characteristics.

2. Vegetatively propagate the most promising rootstock selections to provide grafted trees for advanced trails at multiple locations.

Significant findings/accomplishments in 2006

- Twenty-eight rootstock selections were chosen for advanced testing.

- Approximately 4,300 cuttings from these 28 selections were rooted in Michigan. A total of 2,080 cuttings were obtained. These cuttings will be shipped air freight to Willow Drive Nursery in March 2007.

- Final arrangements were made for two of the rootstock test sites. The Prosser test site will be at the Rosa Farm and will have 'Benton' as the scion. A second test site will be in Pasco with grower cooperator Ken Waliser with 'Chelan' as the scion.

- Quarantine restrictions were met to permit shipment of rootstocks to Willow Drive Nursery and budwood to Duarte's Nursery. The rootstocks were given county code names to reduce the likely hood of identify mix ups at the commercial nurseries and throughout the testing process.

- At the time this proposal was submitted negotiations were underway between MSU and John Duarte that would result in Duarte's Nursery propagating the MSU rootstock selections and providing trees for the test sites.

Specific Objectives for 2007

1. Continue the evaluation of the 28 MSU rootstock candidates in plots at Clarksville, Mich. and Prosser, Wash. to identify any rootstock candidates that should be eliminated from future testing.

2. Identify the grower cooperators and locations of the additional test site in Washington and the test site in Oregon. Determine the scion to be tested at each of these locations.

3. Work with Willow Driver Nursery and Duarte's Nursery to assure the availability of grafted trees for 2009 spring planting in the test sites.

4. Develop diagnostic DNA fingerprints for each of the 28 MSU rootstock selections and use this method to verify the identity of the MSU rootstocks in shoot culture at Duarte's Nursery.

Methods by Objective

1. *Data collection from existing plots*: The trees on the 28 MSU rootstocks selections in the MSU and WSU test plots will be evaluated for tree health, structure, survival and trunk cross-sectional area. Whole tree reproductive traits to be evaluated include visual estimates of bloom density and crop load based on a scale of 0 to 5. Yield potential, yield components and fruiting habit of the promising rootstock selections will be determined by evaluating the two- and three-year-old-wood on two branches per tree. The following traits will be measured: number of spurs for each branch section, fruit size from each branch section, and branch cross sectional area to calculate branch yield efficiency.

2. *Identify grower cooperators and test sites*: Meet with the Oregon and Washington grower organizations to identify grower cooperators and test sites plus the desired scion cultivar.

3. *Propagation of grafted trees on the MSU rootstock selections*:

    - Willow Drive Nursery: ~ 2,080 rooted cuttings representing 28 MSU rootstock selections will be shipped air-freight to Seattle in March and trucked to Willow Drive Nursery. These liners will be field planted in the spring. Iezzoni will be present at the planting to oversee the field labeling. In August these liners will be budded to provide trees for the test sites.
    - Duarte Nursery: Budwood of the 28 MSU selections were sent to Duarte's Nursery in October 2006. These selections will be established in shoot cultures to provide liners for grafted trees.

4. *Development of diagnostic DNA markers and use of these markers to verify the identity of the rootstocks at Duarte's Nursery*: DNA will be extracted from all 28 MSU rootstock selections and genotyped using SSR primers that have been identified in the Iezzoni lab as exhibiting maximum polymorphisms. Markers will be screened until a set of markers is identified that provides a unique fingerprint for all 28 MSU rootstock selections. DNA will be extracted from liners produced through shoot tip cultures at Duarte's Nursery for all 28 MSU rootstock selections. Iezzoni will visit Durate's at a time appropriate to collect the samples. The marker profile of these liners will be compared to the marker profile of each selection. A comparison of these marker profiles will provide assurance that the rootstock liners are labeled correctly and no error has occurred during plant material transfer and establishment.

Results and Discussion

Identification of superior rootstocks: Twenty-eight MSU rootstocks were selected from the 77 total rootstocks under evaluation. At Prosser, some of the MSU rootstock selections selected had statistically equivalent numbers of spurs on two and three year old wood compared to GI 6 (18 to 20 for the MSU selections and 21.5 for GI 6). However, due to potential over-cropping challenges with self-fertile cultivars we did keep in the program some rootstock candidates that had reduced spur numbers (13 to 15) but exhibited other desirable attributes such as reduced tree structure compared to GI 6 and/or wider branch angles compared to GI 6.

At MSU's Clarksville Station, some of the rootstocks planted in 2002 were evaluated with both Bing and Hedelfingen scions. With Bing, most of the MSU rootstocks resulted in improved fruit size and two of the MSU rootstocks had improved yield efficiencies (Figure 1). Interestingly, with Hedelfingen scion, GI 6 performed better even when the rootstock was held constant. This scion by rootstock interaction showcases the importance of scion selection for our next set of rootstock trials. Of particular interest were the MSU rootstocks planted at Clarksville in 2004 and fruited for the first time in 2006. Our favorite was 'Curry' as its truck cross sectional area was equivalent to GI 6, indicating that it was growing with sufficient vigor (Figure 2). In addition, trees with 'Curry' as their rootstock had on average 50 fruit per tree and fruit averaged 2.4 grams larger than the fruit on the GI 6 trees of the same age. Due to the superior traits of this rootstock, tree vigor and exceptional early fruiting capacity, we would like to use it as a rootstock in the breeding program for those selections where we urgently need to move to the next generation. Fortunately, achieving this should be technically easy as this rootstock exhibited 80% rooting.

Of the 28 MSU selections advanced for further testing, six of the selections, including 'Curry', are from one population that is a complex cherry species hybrid mix that I collected on a dry hillside location in Europe. Interestingly the latitude of this location is almost equivalent to that of Chelan, Washington. The MSU rootstock project was started years ago based on the premise that the MSU germplasm collection offered a unique opportunity to sample a broad base of cherry germplasm that just might yield superior rootstocks for the Washington and Oregon sweet cherry industries. The fact that one fifth of the advanced selections trace back to this unique germplasm indicates that we are moving forward with plant material that is not in commerce and to my knowledge is not currently in test anywhere else in the world.

Rootstock propagation: A total of 4,300 cuttings were vegetatively propagated for the 28 MSU rootstock selections in June 2006 (Figure 3). From this propagation we obtained 2,080 cuttings. Percentage rooting for the selections ranged from 87% to 3% and the number of final cuttings per selection ranged from 156 to 3. All 28 selections were recoded with MI, WA, and OR county names as we felt that letter names would reduce the likely hood that there would be any number mix up through out the propagation and testing process. These cuttings will be shipped air-freight to Seattle and trucked to Willow Drive Nursery for spring planting. All APHIS requirements have been met thanks to the timely assistance of Tom Wessels. Scion buds will be inserted in August 2007 and the resulting trees will be dug in time for Spring 2009 planting.

For some of the rootstocks, we fell short of our goal of 100 rooted cuttings. This number was needed to provide enough trees for 5 test sites using the following experimental plot design: 20 trees per rootstock selection organized into 4 replicates of 5 trees each. This experimental design has worked well for the Geneva apple rootstock trails currently planted in Washington. To increase liner, and ultimately tree number, we decided to explore an alternative propagation strategy, shoot propagation in culture, in collaboration with Duarte's Nursery (see discussion below).

Plans for the test sites: The next rootstock experiments will be planted at five different locations: three in Washington, one in Oregon and one in Michigan. To date, the details of just two of the test sites have been completely worked out. The Prosser site will be at the Roza Farm and 'Benton' will be the scion cultivar. There will be a Pasco site with Kent Waliser as the grower cooperator and 'Chelan' will be the scion cultivar. In August 2006 we visited the Brewster and Lake Chelan areas but failed to find a grower cooperator and an available site. We had considered that 'Sweetheart' would be a possible scion for this high-elevation Washington site. We will work with the Oregon Sweet Cherry Commission to determine the details of the Oregon site. The rootstock controls will be GI 6 and Mazzard.

Plans for future liner and tree production: We need to make sure that two potential problems do not impede the progress of the MSU rootstock testing effort: (1) shortage of rootstock liners, and (2) non-uniform nursery stock. To avoid these two problems, Duarte Nursery was approached to see if they would be willing to collaborate to produced additional liners and eventually trees for the rootstock testing phase. Duarte's Nursery has a tissue culture lab that puts each rootstock into shoot culture and therefore liner number can be ramped up as needed. In addition, they make their trees by budding onto liners that are in pots in the greenhouse, therefore providing very uniform plant material. As initial discussions were promising, and budwood from the MSU rootstock selections met California APHIS requirements, budwood was sent to Duarte's Nursery's tissue culture lab in October 2006. At the time this report/proposal was submitted, discussions regarding this collaboration were on-going.

Timetable for the next two years.

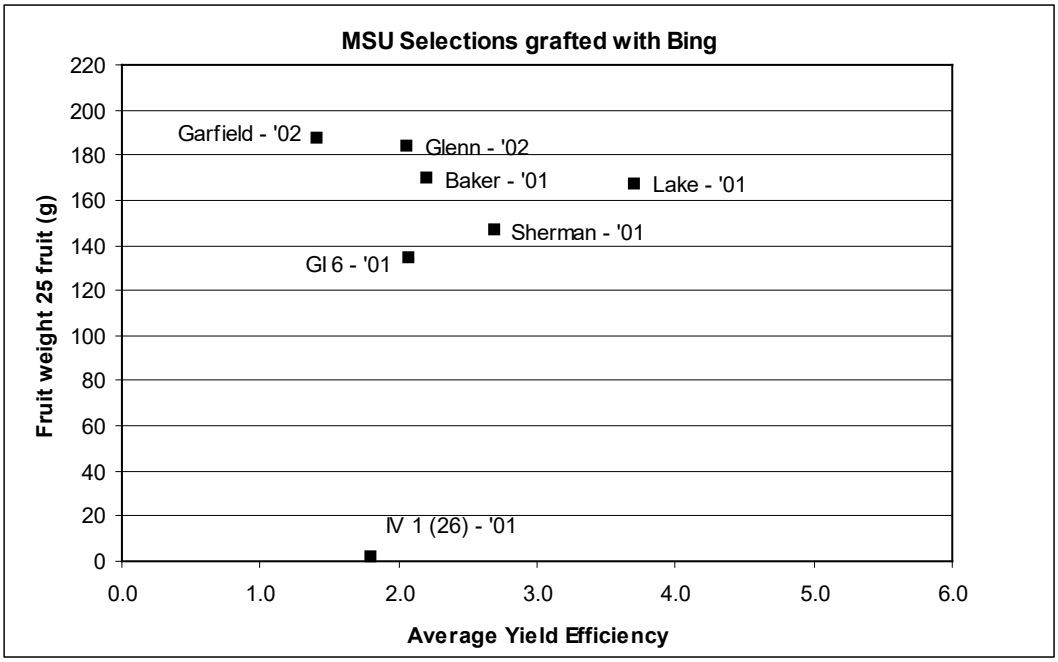
2007

- Ship the MSU rooted cuttings air freight to Wash. APHIS regulations have been met.
- Plant the cuttings (liners) at Willow Drive Nursery (WDN).
- Continue the evaluation of the MSU rootstock selections at MSU and WSU.
- Grow the rootstock liners that were produced in 2006 in Michigan at WDN.
- Establish shoot propagation for the MSU rootstock selections at Duarte Nursery.
- Based on YR 2007 data, delete any undesirable rootstock candidates.
- Determine the additional grower cooperators, test locations, and scion cultivars to be tested.
- At WDN, chip bud the most promising selections for advanced testing.

2008

- Continue the evaluation of the MSU rootstock selections at MSU and WSU.
- Grow the grafted trees at WDV and dig the trees.
- Grow the grafted trees at Duarte Nursery.
- Prepare the test sites for planting in 2009.

Figure 1. Average yield efficiency (g/mm^2) and fruit weight (g) for 25 fruit from branches from MSU selections graphed with Bing (top) and Hedelfingen (bottom). Planting year for the trees is indicated as 2001 or 2002, ’01 or ’02, respectively. Trees are planted at MSU’s Clarksville Station.



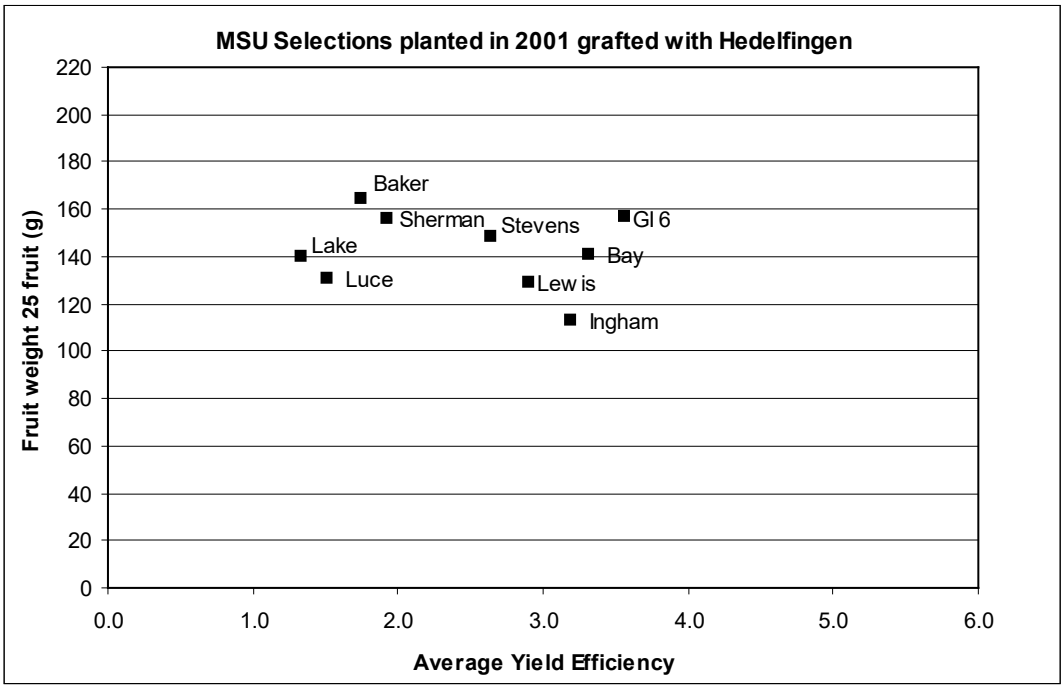


Figure 2. Whole tree yield (g) versus trunk cross sectional area (mm^2) for MSU selections grafted with Hedelfingen planted at MSU’s Clarksville station in 2004. Data was taken in July 2006.

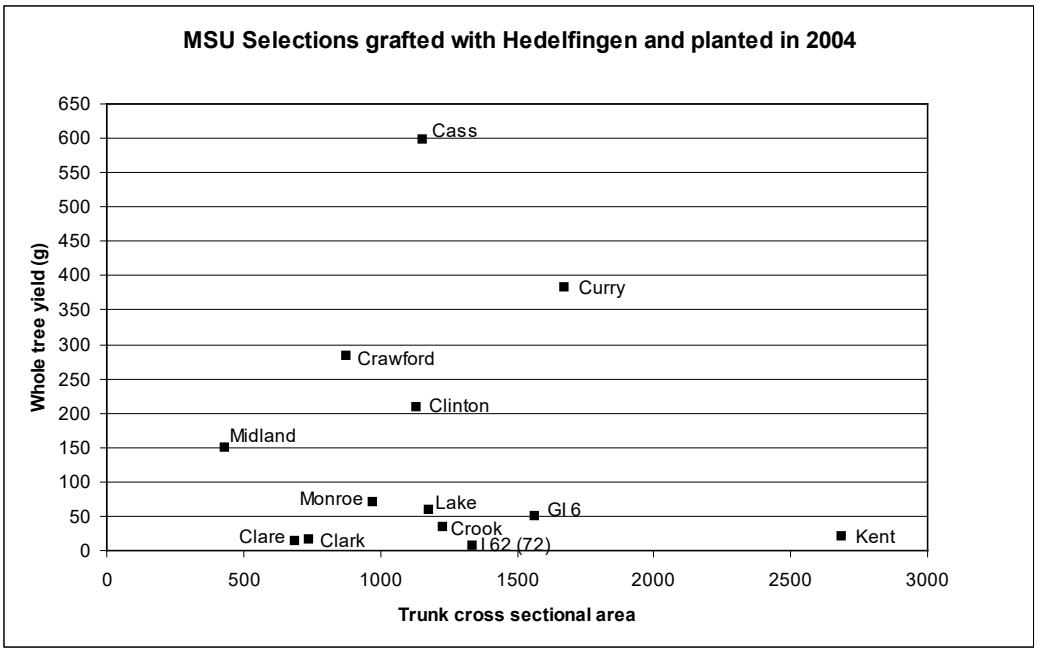


Figure 3. MSU cuttings propagated under mist in June 2006.

CONTINUING PROJECT REPORT **YEAR**: 2 of 2
WTFRC Project Number: CH-06-601

Project Title: Causes and Prevention of Pistil Doubling
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Budget 1:
Organization Name: WSU-Prosser **Contract Administrator:** ML. Bricker
Stephanie Brock
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Item	Year 1: 2006	Year 2: 2007	Year 3:
Salaries	18,747	**19,508**	
Benefits	1,827	**1,844**	
Wages	5,300	**6,000**	
Benefits	530	**690**	
Equipment	1,500	**1,500**	
Supplies	1,500	**1,500**	
Travel	1,500	**2,000**	
Miscellaneous			
Total	30,904	**33,042**	

OBJECTIVES:

For early-, mid-, and late-season sweet cherry varieties:

1. Elucidate the seasonal trends in flower bud initiation & organ differentiation

2. Determine seasonal susceptibility to pistil doubling

3. Determine seasonal relationship between tissue and air temperature and pistil doubling

4. Compare efficacy of practical means for reducing pistil doubling

5. Investigate potential to incorporate Schrader's fruit surface temperature logger/controller for doubling control.

SIGNIFICANT FINDINGS:

- the system for manipulating tissue temperature *in situ* was able to heat and cool developing fruit buds by ± 5°C from untreated
- both tissue temperature and timing are important factors in pistil doubling
- in 2005, 'Bing' flowers were more susceptible to doubling in late July vs. early July and early August
- buds kept below 37.3 C (99.1 F) throughout the preliminary trial in 2005 did not exhibit doubling
- over-tree evaporative cooling shows great potential for moderating tissue temperature (ca. 8.5 F reduction) and reducing pistil doubling
- under-tree microsprinklers are ineffective at reducing canopy tissue temperature
- shade and Surround® are moderately effective at reducing canopy temperature (ca. 3.5 to 4 F reduction)

MATERIALS & METHODS:

Objective 1 – Axillary meristems will be collected at a two-week (or similar growing degree day) interval from mid May (ca. bloom + 45 days) until late September. Samples (10 buds per date) will be collected from among the axils of the oldest leaves on two-year-old fruiting spurs and placed directly in standard FAA fixative (10% formalin, 50% ethanol, 5% glacial acetic acid). Meristems/buds will be collected from varieties whose fruit mature over approximately a 5-week interval: Chelan, Tieton, Bing, Skeena, and Sweetheart. Throughout the season, and at each collection date, records of tree phenology will be made (e.g., full bloom, fruit size, harvest date, shoot development) to serve as bio-reference points to relate the microscopic development of flower buds. In addition, timing of bud initiation and differentiation will be related to accumulated growing degree days (GDD) and other local environmental conditions so that predictive models may be developed. Temperature data will be collected in the orchard by AgWeatherNet stations located within 100' of experimental trees.

Floral bud initiation/differentiation will be analyzed by scanning electron microscopy. Briefly, buds will be dissected using a dissecting microscope, rinsed in 50% ethanol, dehydrated with increasing concentrations of ethanol, ending with two rinses at 100% ethanol. Samples will then be dried and mounted on stainless steel stubs with carbon tape. Gold coating will be applied by

sputter coater. Samples will then be examined by scanning electron microscopy at 15kV. Digital images will be collected and analyzed for meristem diameter.

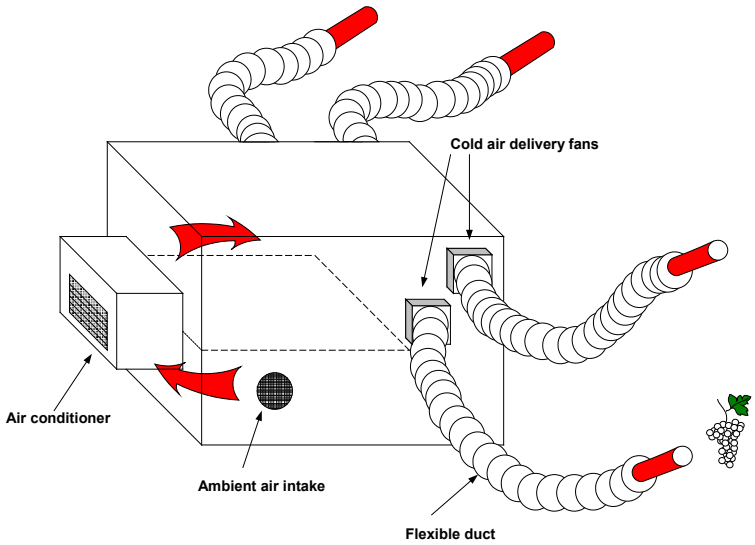

Figure 1. Schematic diagram of the apparatus used to cool tissue by forced convection.

Objectives 2 & 3 – Entire 'Bing' spurs will be heated and cooled *in situ* throughout floral bud induction and differentiation to determine temperature response and periods of susceptibility. Two-year-old fruiting spurs will be exposed one of four treatments:

- untreated control
- ambient air by blower
- heated air blown
- chilled air blown

We will use a system capable of low-velocity heating and cooling tissue as described in Tarara et al. (2000). Briefly, for chilling spurs, two standard room-size air conditioners are mounted in a ca. 1.8 m^3 insulated enclosure, creating a cold air reservoir (Fig. 1). Flexible, insulated ducts deliver air from the reservoir to PVC delivery tubes. To heat spurs, heaters (100-W) are constructed from 1.4Ω resistance wire mounted inside identical PVC delivery tubes (Fig. 2). Blower tubes are identical to the heater delivery tubes but lack the heating element. Hot, cold, or ambient air is delivered by in-line fans installed at the lower end of the delivery tubes. Air velocity at the outlet is minimal at ca. 1.9 $m \cdot s^{-1}$ (≈ 4 mph). We field tested this technique in 2005 and found it reliably heated and cooled spur tissue ca. 9°F compared to ambient to create a range of 18°F (Fig. 3). To evaluate the relative susceptibility of fruit buds to pistil doubling throughout the season, we intend to deploy this technology in the field for seven 14-d intervals, from June to September. During each run, bud tissue temperature and air temperature will be monitored by fine wire thermocouples coupled to a Campbell CR10x datalogger. Pistil doubling will be assessed several ways. First, dormant buds will be harvested and analyzed by microscopy (as outlined above), and second, in the subsequent spring, visual records of doubles will be made during bloom and subsequently at harvest. Doubling will be assessed as % of available flowers on a bud and spur basis and related to tissue temperature.

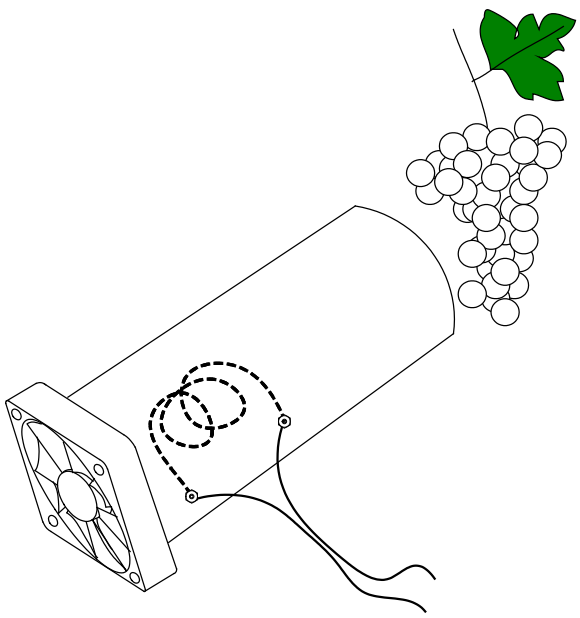

Figure 2. Schematic diagram of the forced-air delivery tube used for heaters, chillers, and the ambient air blowers. The resistance element installed in heater tubes is shown.

Objective 4 – The efficacy of potential practical strategies for reducing doubling will be compared. Treatments will include:

A. under-tree microsprinklers
B. over-tree microsprinklers
C. sprayable reflective materials (e.g., Surround®)
D. shade cloth
E. untreated control

In 2006, without knowing critical temperatures for doubling or periods of greatest susceptibility, treatments A and B will be applied whenever air temperatures exceed 95F between immediately after harvest and the end of August. Treatments C and D will be applied following harvest and remain until the end of August. The effect of each treatment on tissue and air temperatures will be monitored within the canopy at 1, 2, and 3 m above the orchard floor. These temperature data, combined with temperature threshold data from objectives 2 & 3 will allow comparisons to be made among potential strategies for reducing doubling. In addition, at each canopy height, pistil doubling will be assessed in the subsequent season by visual observation during bloom. A minimum of 100 flowers per tree per height will be assessed for doubling. In addition, a similar doubling assessment will be made at harvest. In 2006 each treatment will be applied throughout predicted flower bud differentiation (i.e., ~ early July – early September, though this will vary by variety). In 2007, applications will be targeted to protect buds during periods of high susceptibility (as determined in 2006). To increase the likelihood of significant doubling in untreated controls, treatments will be evaluated at Sagemoor Farms on drip-irrigated 'Bing' trees where doubling pressure is high.

RESULTS AND DISCUSSION:

In 2006, we accomplished each of our goals outlined above.

Floral bud initiation and organ differentiation In 2006 we collected at approximately 4-week intervals from May through November entire spurs from 'Chelan', 'Tieton', 'Bing', 'Skeena', and 'Sweetheart' trees. Spurs were stored immediately in FAA fixative for analyses by scanning electron microscopy. At this stage, samples are being dissected and prepared for microscopy. We anticipate having all samples analyzed and images prepared by the end of December. Because of

the difficulty in completing sample preparation and image collection in time for this report, we will post images on the program's website as soon as possible. In addition, we will submit an article this winter summarizing our analyses of floral bud initiation and organ differentiation to the Good Fruit Grower.

Critical timing and temperatures Results from the 2006 heating and cooling in situ are not yet available – treatment effects on pistil doubling will be assessed in April, 2007. We learned from our preliminary trial in 2005 that microscopic assessment of doubling in dormant buds is prohibitively time-consuming. Therefore, we will assess doubling at bloom, as proposed originally. Currently we are analyzing 2005 data by tissue time-temperature threshold; this approach accounts for the relationship between temperature and the length of exposure to those temperatures with respect to doubling.

We assessed doubling, in response to the artificial heating and cooling of buds in 2005, during bloom in 2006. The heating/cooling apparatus worked well and was able to increase and reduce bud tissue temperature by 5°C from ambient throughout the day (data not shown). It is clear that susceptibility to pistil doubling is affected by temperature and the period (i.e., stage of bud differentiation) during which high temperatures were encountered (Table 1). From our preliminary analyses, it appears that in 2005, 'Bing' flower buds were most susceptible to doubling between 18 and 25 of July (i.e., about one month after harvest). Flowers within buds artificially heated during this period exhibited 19% doubling in 2006. Timing appears to have an effect because exposure to similar temperature regimes in the subsequent period (i.e., Aug. 2 – 14) did not cause as much doubling as during late July. However, temperatures were higher during the latter half of July and early August than they were during the earliest interval (July 5 – 14). It is not known how much doubling would have occurred in the early July timing in response to similar high temperatures. What is clear, is the role of high temperature – we did not record a single double pistil from cooled spurs, irrespective of timing. In addition, flowers that were cooler than 37.3°C (99.1°F) throughout our trial period did not have doubled pistils – we only observed doubling, albeit variable (0 – 60%), when tissue temperatures exceeded 37.3°C (data not shown). The variability in doubling above 37°C also reinforces the need to analyze time-temperature threshold rather than a particular temperature alone.

Table 1. Preliminary data on doubling in 2006 and tissue temperature from heating/cooling trials conducted in 2005 on 3rd-leaf 'Bing'/'Gisela5' trees.

	July 5-14, 2005			July 18-28, 2005			August 2-14, 2005		
Treatment	% Doubles	Avg Temp (˚F)	Max Temp (˚F)	% Doubles	Avg Temp (˚F)	Max Temp (˚F)	% Doubles	Avg Temp (˚F)	Max Temp (˚F)
Ambient	0	75.0	84.9	6.4	81.2	103.5	4.8	80.8	99.7
Cool	0	67.6	72.7	0.0	73.2	91.9	0.0	73.2	93.4
Heat	0	82.8	90.3	19.0	90.0	105.4	13.3	89.1	104.4
Blow	0	74.1	82.6	5.1	80.8	100.8	1.5	80.2	98.8

Practical strategies for reducing doubling In 2006 we initiated several trials evaluating programs for reducing pistil doubling. Orchard and treatment details are outlined below:

Trial location	Variety/Rootstock	Treatments	% Doubling in 2006
WSU-Roza Farm, Prosser	'Tieton'/'Gisela5'	Control, Surround®	28%
	'Bing'/'Gisela6'	Control, Surround®, Over-tree cooling, Under-tree irrigation, Shade	
Wild Willow Ranch, Benton City	'Tieton'/'Gisela5'	Control, Surround®, Over-tree cooling	22 %
Auvil Fruit Company, Vantage	'Tieton'/'Gisela5'	Control, Surround®, Over-tree cooling, Shade	26%
Sagemoor Farms, Pasco	'Chelan'/Mazzard	Control, Surround®, Raynox®	
	'Bing'/Mazzard	Control, Surround®, Over-tree cooling	

Results from these trials will be summarized in early 2007, following doubling evaluation during bloom, as outlined above. In each trial using sprayable reflectives, Surround® and Raynox® were applied in early July and re-applied as necessary to maintain good coverage (no more than 3 applications). Over-tree cooling was applied at the cooperator's discretion – usually at ca. 95ºF+ air temperature.

At the Roza experimental farm, we initiated a trial to model canopy tissue temperature response to various potential strategies for reducing pistil doubling. Six 11-yr-old 'Bing'/'Gisela6' trees were selected for similar canopy architecture. Within each tree, 3 horizontal planes were identified at 1 m, 2 m, and 3 m above ground level. Within each plane, 3 or 4 clusters of 3 thermocouples each (connected in parallel) were positioned just beneath the bark to record tissue temperatures (i.e., 8 or 9 thermocouples per tree).

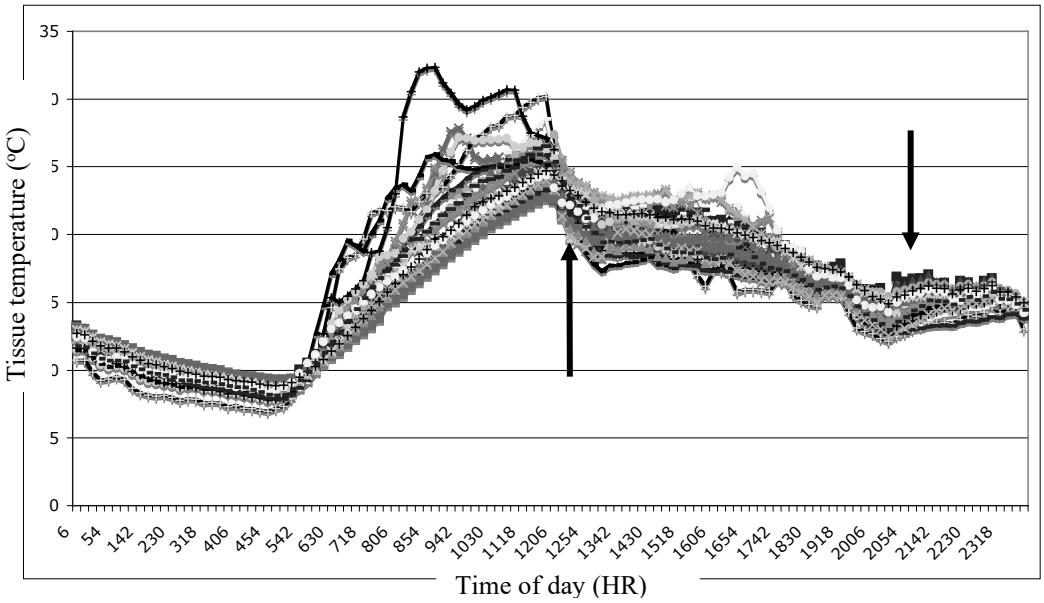

Figure 3. Diurnal trend in tissue temperature within an 11-yr-old 'Bing'/'Gisela 6' tree. Over-tree evaporative cooling was initiated approximately at noon and terminated at 2100 HR (indicated with arrows).

Evaporative cooling by over-tree sprinklers (26 gph nominal output microsprinklers) was the most effective treatment for reducing tissue temperature (Table 2, Figures 3 & 4). An immediate reduction in tissue temperature was evident, throughout the canopy (Fig. 3). On average, over-tree irrigation reduced daily tissue temperature by 4.8ºC vs. untreated canopies (Table 2). Interestingly, when examining only the upper canopy regions (i.e., those in the highest light and temperature environment), we recorded a similar reduction in temperature. Remarkably, tissue temperature was reduced by nearly the same degree as the cooling treatment reported above. The potential for evaporative cooling to reduce tissue temperature is further highlighted in Figure 4. These data show that during two days of evaporative cooling (1200 to 2100 HR), cooled trees had lower tissue temperatures (ca. 1.2ºC lower) during the afternoon/evening than in the morning. In contrast, without over-tree cooling during the subsequent two days, normal canopy tissue temperatures were drastically warmer (ca. 5.1ºC) during the afternoon/evening. Over-tree evaporative cooling shows great potential for lowering canopy tissue temperature and reducing pistil doubling. In 2007 we intend to study various over-tree evaporative cooling strategies to reduce water application and maximize cooling effect.

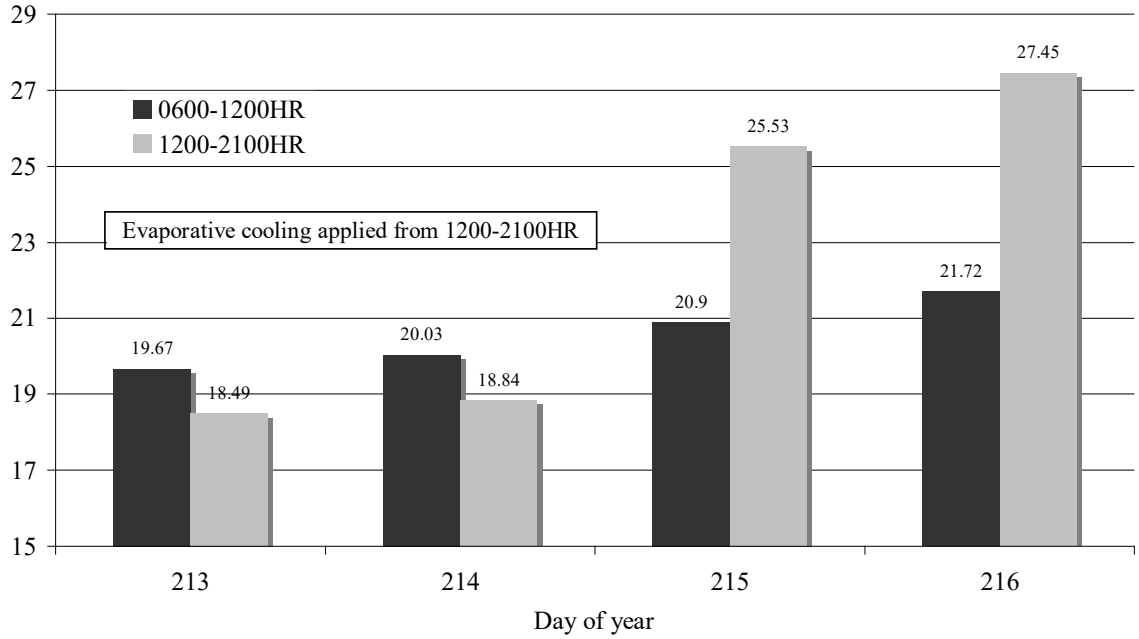

Figure 4. Comparison of 'Bing'/'Gisela6' canopy tissue temperatures on two days with afternoon evaporative cooling (Days 213 & 214) and two days without any cooling treatment (Days 215 & 216). Environmental conditions were similar on all four days.

Table 2. Effect of various cooling treatments on whole-canopy temperature (ºC ± untreated, n = 25) and temperature of selected, sun-exposed canopy regions (ºC ± untreated, n = 6). Data are means from 0600 – 2100 HR collected by thermocouples throughout 11-year-old 'Bing'/'Gisela 5' trees planted in N–S rows spaced 8.5´ × 15´.

Treatment	Whole-canopy mean temperature (ºC ± ambient)	Sun-exposed canopy temperature (ºC ± ambient)
Whole-tree shade	– 0.50	– 2.25
Under-tree microsprinklers	+ 0.81	+ 0.80
Over-tree evaporative cooling	– 4.81	– 4.92
Surround®	– 1.21	–1.75

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-05-506

Project Title: Understanding N requirements for sweet cherry production

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Country/Zip: Scotland UK AB15 8QH

Budget 1:
Organization Name: Agriculture Canada **Contract Administrator:** Denise Neilsen
Telephone: 250-494-7711 **Email address:** neilsend@agr.gc.ca

Item	**Year 1: 2005**	**Year 2:2006**	**Year 3:2007**
Salaries	$4,500	$4,500	$4,500
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$500	$500	$500
Travel			
Miscellaneous			
Total	$5000	$5000	$5000

Footnotes: This funding has been matched, 2005-2007 by an equal cash grant from AAFC 's Matching Investment Initiative Program

OBJECTIVES

The initial proposal (2005) included objectives for the determination of sap composition and N requirements for sweet cherry, based on xylem N flux and the use of ^{15}N labeled fertilizer. In 2005 funding was re-targeted to the assessment of sap flow probes in greenhouse and field experiments. The effects of intervening in Fall N withdrawal and spring remobilization to determine their contribution to cherry nutrition and production will be determined over three seasons.

Technique development for sap flow (2005-2006 objectives)

1. Test and compare several heat balance/heat dissipation/heat pulse probes to monitor sap flow, including a low-cost, self-built system.
2. Calibrate in field and green house to determine the feasibility of making whole tree transpiration measurements.
3. Determine the effects of environmental factors (temperature, solar radiation, soil moisture) on sap flow in the greenhouse and field.

Basic understanding of N requirements for cherry

4. Determine the contribution of spring remobilized N to total N requirements

2007 objectives

1. In the greenhouse, test calibration of thermal dissipation and heat pulse probes with heat balance probes, prior to field season.
2. In the greenhouse, determine methodology for identification and automation of water stress trigger points
3. In the field, test the effect of mulches and irrigation frequency on plant water use and stress using sap flow gauges in two cherry varieties with different fruiting characteristics (young Cristalina/Gi.6 and Skeena/Gi.6).
4. In the PARC lysimeter facility, test the use of sap flow gages in water balance studies using young Skeena/Gi.6.
5. Continue to monitor long term effects of fall N cycle in Lapins/Gi.5.

Significant findings

- Sap flow gauges respond well to water stress events in cherry in both lab and field conditions, with the best relationships found for thermal dissipation probes (TDP) made at PARC.
- None of the probes consistently predicted the absolute amount of water used by the trees. The best probe type for predicting total water use was the Tranzflo heat pulse probe.
- These results suggest the need for development of a calibration factor for use in field studies to determine total water use.
- The rapid adjustment of TDP probes to tree water stress could allow the development of a trigger for automated watering.
- For sweet cherry on dwarfing rootstocks Premature leaf fall (simulated by removal) affects over-winter N nutrition and reduces fruit number and yield two seasons later.

METHODS

Sap flow

Sap Flow Probes. There are three major probe types all of which are based on the measurement of heat transfer in relation to sap movement. Heat dissipation probes (e.g. Parc TDP probes constructed on site and Dynamax TDP10 commercially available probes) compare temperatures of a constantly heated probe with an unheated reference probe, both of which are inserted into the xylem. The amount of heat dissipated away from the heated probe by convection as a result of sap flow is proportional to the rate of sap flow. Heat pulse probes (e.g. Tranzflo) compare the temperatures of two probes containing thermocouples at specified depths placed into the xylem. These probes are located a given distance from a heater installed between the probes. A pulse of heat is transferred through the plant material and carried by the sap. The rate of sap flow is calculated from the time required for the temperature at the midpoint between the two probes to equilibrate following the heat pulse. Calculations of flow volume for Granier and Tranzflo probes require an estimate of the area of functional xylem.

Greenhouse experiments Granier style Parc TDP probes constructed according to the procedures of James et al., (2003), Dynamax TDP-10 probes and Tranzflo heat pulse probes were installed in two year-old Van/Gisela.6 and two year old Ambrosia/M.9 trees with a Fuji inter stem in the greenhouse. The trunk areas surrounding each probe were insulated appropriately to reduce thermal noise. Each pot was sealed with plastic to prevent evaporative loss from the soil surface and placed on a weighing platform calibrated to within a resolution of 10g that used a sheer beam force transducer (Omega Engineering) supported on a steel tripod to record weight change. Two watering regimes were imposed: 1) full water, re-watered daily (to field capacity) and 2) multi-day dry-down followed by re-watering to field capacity. There were two replications. Changes in weight and in temperature due to sap flow were sampled every 10 seconds and averaged each 10 minutes. Calculations of sap velocity and flux were made using Granier's equation (Granier, 1985) and heat pulse equations which were then compared to the measured weight loss over a give time period.

Field Trial Parc TDP probes were installed in the trunks of 8 year-old Lapins/Gisela.5 at 30 cm above the graft on the opposite side to the rootstock. Two probes were installed in each tree and the pair was insulated appropriately. Trees received irrigation at 100% ET until one week before harvest when deficits of either 50% or 25% of atmometer estimated ET were imposed. There were three replications. Sap flux and velocity were calculated using Granier's equation (Granier, 1985). Plant response to stress was measured using the Licor LI-1600 porometer and the soil moisture drydown was monitored using trace TDR..

Cherry N nutrition

Field experiment. An experiment was established in 2004 with 4 treatments, each with 5 single-tree replicates, on young (4th field growing season) Lapins sweet cherry on Gisela 5 rootstock. Treatments included all combinations of 2 N rates (zero and 220 pounds of N per acre) applied as ammonium nitrate (34-0-0) to trees with early (Sept 17th) or no leaf stripping prior to dormancy. The leaf stripping treatment was designed to test the effects of restricting the availability of stored N the next year. Systematic mid shoot leaf sampling was undertaken throughout 2004-05 and crop yield was also measured in both years. In 2006, crop yield, numbers and fruit size were measured to determine on-going effects of the 2004 leaf removal.

RESULTS AND DISCUSSION
Greenhouse experiments

Comparison and calibration of probes for total transpiration

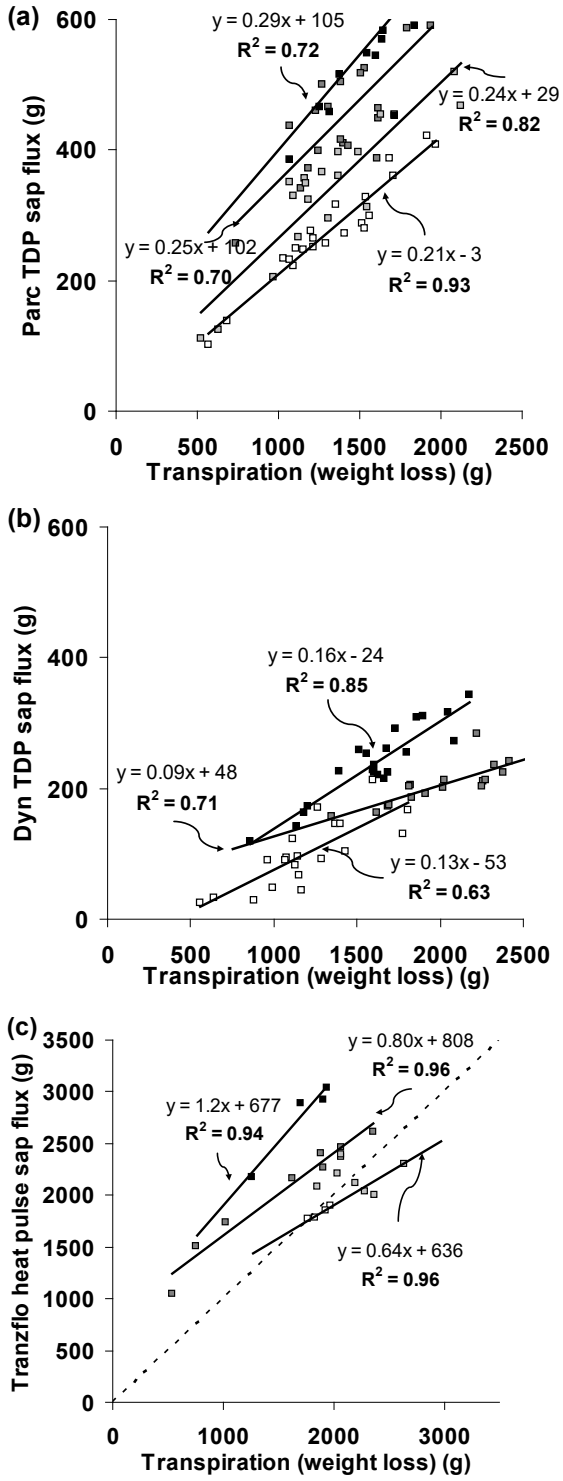


Figure 1. Relationship between transpiration measured by weight loss and sap flux for (a) Parc TDP (b) Dynamax TDP and (c) Tranzflo heat pulse probes in sweet cherry.

There were strong relationships (R^2 values close to 1.0) between transpiration measured by weight loss of pots and sap flux for all probes (Fig. 1). However, sap flux measured by TDP-type probes (Fig. 1a and b) did not accurately predict transpiration measured by weight loss of the pots. For Parc-TDP probes the percentage of transpiration estimated from sap flux ranged between 14% and 33% and for Dynamax-TDP probes ranged from 10% to 16%. Sap flux measured by the Tranzflo probes was much closer in magnitude to transpiration measured by weight loss (Fig. 1c), although the percentage of transpiration measured was still variable (100% to 160%). Similar results were found for apple (data not shown). None of the probes were consistently reliable for field estimates of total tree transpiration. Thus the amount of water transpired was best estimated by Tranzflo probes, but the most consistent relationships between transpiration and measured sap flow occurred with PARC-TDP probes. This suggests the possibility of developing a calibration factor for use in the field to estimate total tree water use.

Probe responsiveness to water supply

Typical sap flow profiles for PARC-TDP and Tranzflo heat pulse probes are shown in Fig. 2. For both types of probes, deficit trees were not watered after day 233. PARC-TDP probes were highly responsive to imposed stress, as both peak height and peak shape changed on day 235 compared to previous day values and also to well watered trees (Fig. 2a). This may allow a threshold for water-up to be determined which has possibilities as a trigger point for automated irrigation scheduling (Fig. 2b). Tranzflo probes showed a steady decline in peak height, but not in peak shape (Fig. 2c).

This may hinder the development of a trigger point for re-watering (Fig. 2d). All trees responded rapidly to re-watering, but effects were more pronounced in PARC-TDP probes (Fig. 2b), compared to Tranzflo probes (Fig. 2d).

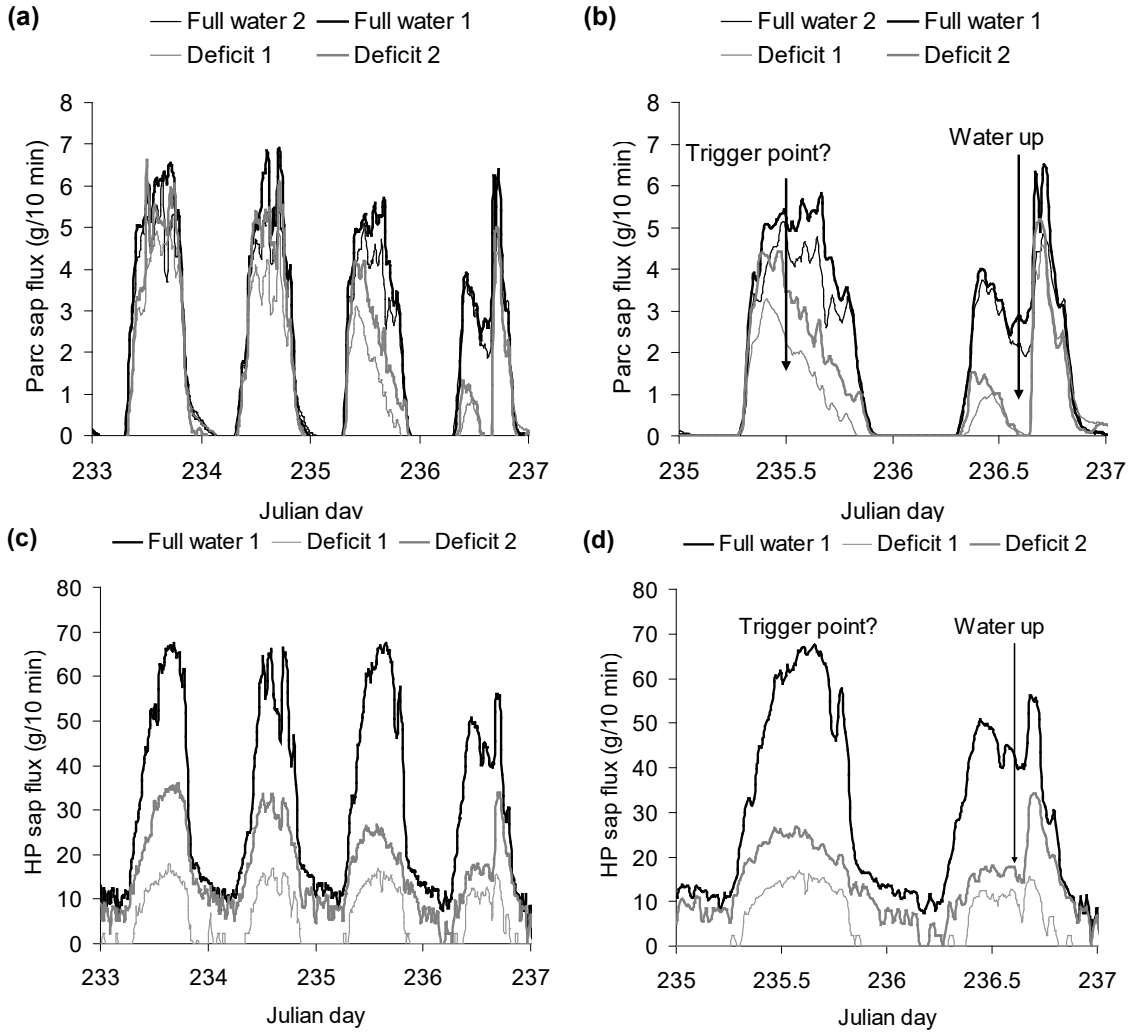
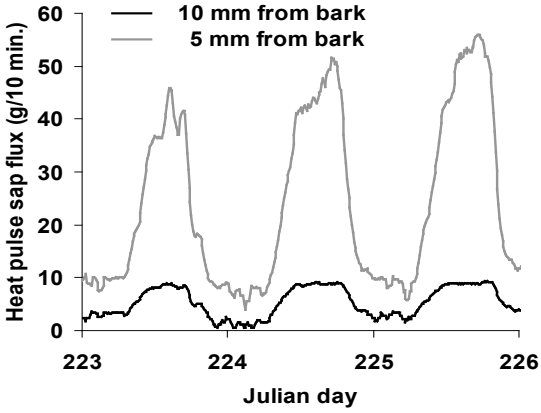


Figure 2. Responses of sap flow probes to imposed drought (a) PARC-TDP full cycle (b) PARC-TDP with potential trigger point and water-up response (c) Tranzflo heat pulse (HP) full cycle (d) Tranzflo heat pulse (HP) water-up response for sweet cherry in the greenhouse



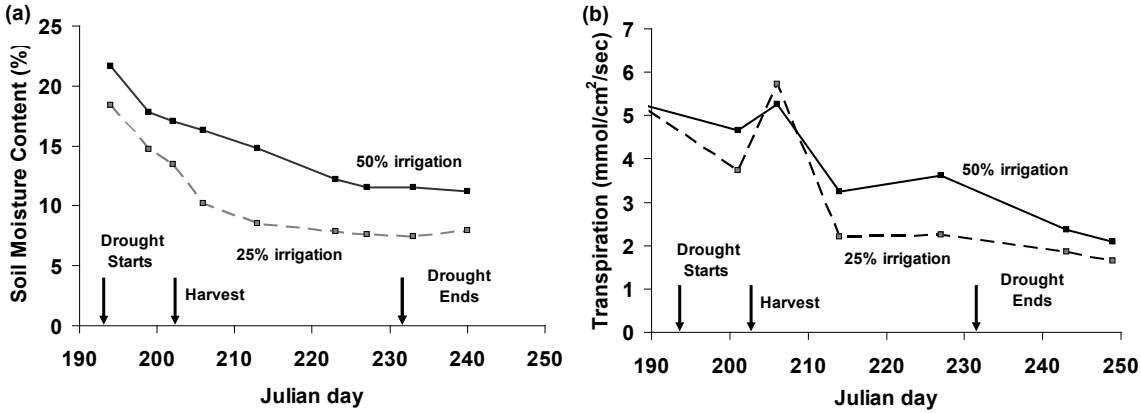
Spatial distribution of xylem flow

Figure 3. Spatial distribution of sap flow measured in Tranzflo probes.

In 2005, we discussed the possible effects of differences in xylem conductivity and probe location on estimates of transpiration. The Tranzflo probes have two 5mm segments and indicated that the majority of flow occurred in the outer 5 mm of the xylem (Fig. 3). This indicates the need for shallow placement of probes. Under stress, changes in flow only occurred in the outer ring (data not shown).

Field experiments

Cherry sap flow



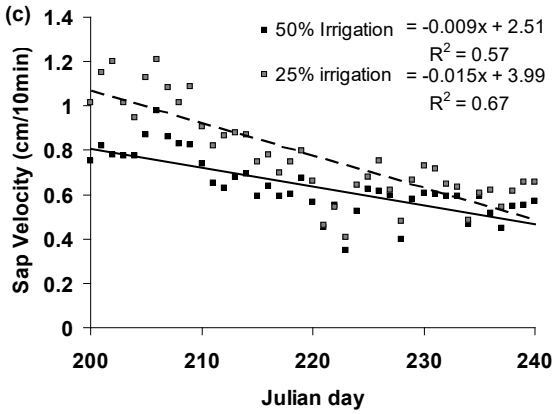


Figure 4. Effects of drought on (a) soil moisture, (b) individual leaf transpiration and (c) sap velocity in field grown Lapins/Gi.6

Soil moisture (Fig. 4a) and leaf transpiration (Fig. 4b) declined after moisture deficits of 50% and 25% ET were imposed. Sap velocity of trees with 25% irrigation declined faster than in trees receiving 50% irrigation (Fig. 4c). This indicated that sap velocity (transpiration) decreased as irrigation supply decreased.

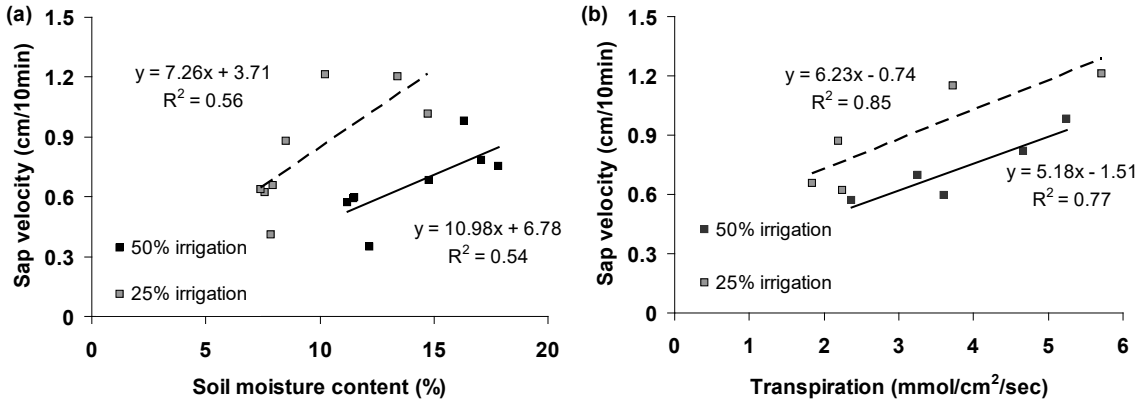


Figure 5. Relationship between sap velocity and (a) soil moisture content and (b) transpiration in field grown Lapins/Gi.6. Average of three trees per graph point.

Sap velocity increased as soil moisture (Fig. 5a) and leaf transpiration increased (Fig. 5b) indicating that the sap flow gauges gave a useful measurement of whole plant water use and water stress as they are sensitive to changes in water supply.

Practical use of sapflow in the cherry orchard

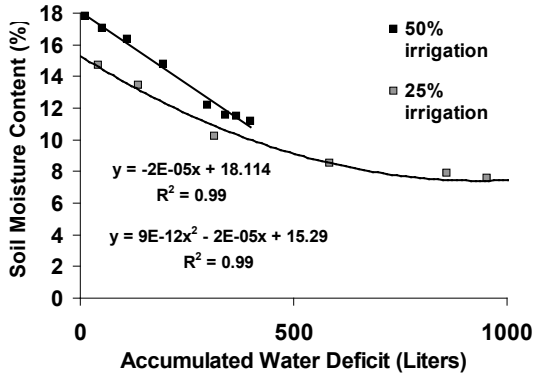
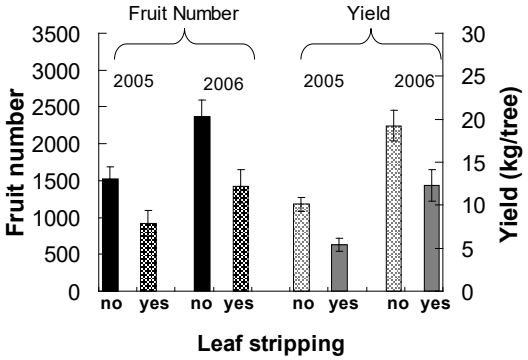


Figure 6. Comparison of soil moisture content and estimates of accumulated soil water deficit calculated from sap flux in field grown Lapins/Gi.6. Average of three trees per graph point.

The possibility of using a universal calibration factor based on the greenhouse study was tested using sap flux data and soil moisture measurements. Accumulated water deficit was calculated as a difference between water added from irrigation and the water used by the tree using the sap flux values adjusted with calibration factors ranging from 3 to 7, calculated from the greenhouse trials. The daily surplus or deficit values were then accumulated for each tree and averaged and compared to soil moisture content(Fig. 6). The best relationship was found using the higher multiplier of 7. The accumulated water deficit for the 25% irrigation trees levelled off at a soil moisture content of ~9% indicating that the trees could not extract water stored in the soil at that point and were totally dependent on daily added irrigation water. However, greenhouse studies also showed that calibration is both probe and trunk location specific. A second possibility is to derive in-field calibration methods for individual probe/tree combinations. Many field studies of forest stands (Granier, 1987; James et al., 2002) have compared TDP probe fluxes to estimates of ET (e.g. Penman Monteith calculations from weather station data). However, this would be dependent on having a good understanding of crop coefficients (canopy development factors) which would change over the growing season and would also depend on whether soil moisture supply was limiting transpiration. Dragoni et al. (2005) calibrated Tranzflo probes using whole canopy gas exchange chambers and found strong but inconsistent relationships between the two methods of measuring transpiration. We propose to calibrate TDP or Tranzfllo probes with heat balance sap flow gauges, which provide good estimates of total transpiration, but cannot be used for long periods of time due to thermal damage to bark (see 2005 report). This could be achieved by moving heat balance gauges from tree to tree.

Cherry nutrition

Figure 7. Comparison of fruit number and yield for Lapins/Gi.5 trees with (yes) or without (no) leaf stripping on Sept. 17th 2004.



The effects of early leaf removal in 2004 were long tern and continued to depress fruit number and yield into 2006. Premature leaf drop or poor N nutrition before senescence has long-lasting implications for cherry production.

Literature

Dragoni, D., Lakso, A. N. and Piccioni, R. M. 2005. Agric. For. Meteorol. 130:85-94

Granier, A. 1987. Tree Physiology. 3:309-320.

James, A.S., M.J., Clearwater, F.C. Meinzer and G. Goldstein. 2002. Tree Physiology. 22:277-283.

CONTINUING PROJECT REPORT **YEAR**: 3 0f 3

Project Title: Alternative Nutrient, Water, and Floor Management Strategies
PI: Xinhua Yin
Organization: Oregon State University (OSU)
Mid-Columbia Ag. Ctr. (MCAREC)
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City/State/Zip: Hood River, OR 97031

Cooperators: Jinhe Bai, Post-Harvest Physiologist, OSU-MCAREC
Clark Seavert, Agricultural Economist, OSU-MCAREC

Budget 1:
Organization Name: Oregon State Unversity **Contract Administrator:** Peggy S. Lowry
Telephone: 541-737-4933 **Email address:** sponsored.programs@oregonstate.edu

Item	**Year 1: 2005**	**Year 2:** 2006	**Year 3:** 2007
Salaries	8,500	8,670	**8,670**[1]
Benefits	4,165	4,248	**4,248**[2]
Wages	2,800	2,800	**2,606**[3]
Benefits	224	224	**651**[4]
Equipment			
Supplies	2,661	2,661	**2,688**[5]
Travel	450	450	**448**[6]
Miscellaneous			
Total	18,800	19,053	**19,311**

1. One-fourth FTE for a faculty research assistant who will oversee the technical aspects of this project (plot establishment and maintenance, sample collections, field measurements, data entry, and harvest).
2. Benefits for a research assistant are calculated at 50% according to 2007 OSU regulations.
3. Hourly help for field measurements and harvest.
4. Benefits for hourly help are calculated at 25% according to 2007 OSU regulations.
5. Supplies for tissue and soil analysis. There are 128 soil and leaf samples each year. The cost for each sample is $21.
6. Travel to plots: 15 round trips to The Dalles (65 miles each), the total mileage will be 65*17= 975 miles. 10 round trips to Hood River orchard (13 miles each), the total mileage will be 13*10=130 miles. Therefore, the total mileage to both locations will be 1,200 miles. Each mile costs 40.5 cents.

Objectives

1) Compare drip irrigation with micro sprinkler on water use efficiency, fruit set, quality, storability, and yield of sweet cherry, and grower profitability.
2) Examine the effects of fertigation on nitrogen (N) use efficiency, fruit yield, quality, and storability of sweet cherry and grower profitability as compared with broadcast application of dry N fertilizer on soil surface.
3) Evaluate the impacts of an integrated drip irrigation and fertigation production system on fruit set, quality, storability, and yield of sweet cherry, grower profitability, and water and N use efficiencies as compared with our current micro sprinkler irrigation plus surface broadcast application of dry N fertilizer system.
4) Compare ground cover (straw mulch or fabric) vs. no ground cover, mulch cover vs. fabric cover, and white fabric cover vs. black fabric cover on fruit quality, storability, and yield of sweet cherry, and on soil fertility, soil quality, soil microbial biology, and plant nutrition as well.

Significant Findings

- Drip irrigation saves 79% of irrigation water compared with micro sprinkler irrigation.
- Fruit yield under drip irrigation is similar to that under micro sprinkler. However, there is a trend of yield increase with straw mulch and fabric covers.
- Fruit quality including fruit sugar content, firmness, and size does not differ regardless of irrigation and ground cover system.
- Drip irrigation significantly increases the percentage of marketable fruits by reducing cherry surface pitting and bruising compared with micro sprinkler. Black fabric may also increase the percentage of marketable fruits.
- Fabric cover over the row area of young sweet cherry significantly improves tree N uptake and leaf N content.
- Application of organic fertilizers directly on the top of fabric cover is equally effective as the application of these fertilizers to the beneath of fabric cover.
- Split nitrogen fertigation systems produce competitive cherries with more flexibility for N fertilizer applications.

Methods

Drip Irrigation and Straw Mulch Trial

A field experiment initiated on Mel Omeg's orchard at The Dalles, Oregon in 2005 was continued in 2006. Two irrigation systems (drip irrigation, micro sprinkler irrigation) and four ground management systems [mulch with straw, white fabric cover, black fabric cover, and control (no mulch or fabric cover, but herbicides was used to control weeds)] were evaluated in a split-plot design with four replications. Soil moisture measurements were taken weekly at the soil depths of 12 inches from May to September. Irrigation scheduling for each treatment was based on soil moisture monitoring, and each plot was irrigated separately. Soil available nutrients at the depth of 12 inches, total nutrient concentrations in leaf, and tree vigor were measured after harvest. Fruit yield, firmness, size, color, and sugar were determined for each plot. Visual evaluation of fruit surface pitting was conducted after the fruits had been stored in a cold storage room at 33ºC for three weeks. Four categories of excellent, slightly pitted, pitted, and bruised fruits were used in this evaluation.

IFP Cherry Trial

The IFP experiment was initiated in 2001 on a 3-acre sweet cherry orchard that was planted in April 2001 on a sandy loam soil at the Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, Oregon. Two ground management systems [synthetic fabric

cover (an 8-ft wide synthetic fabric cover made of black, woven polypropylenec), control (no cover, but with herbicide applications in the tree row area)] were evaluated. Soil fertility, plant nutrition, and ground management effect on cherry surface pitting were measured in this trial in 2006. Other measurements including soil moisture, soil temperature, tree growth, and fruit yield were continuously evaluated by Dr. Roberto Núñez-Elisea at OSU-MCAREC.

Organic Fertilizer Placement Trial
A field experiment was initiated in 2005 on a 1-acre black fabric-covered adult sweet cherry orchard that was transited into organic production in 2003 at MCAREC. This trial was continued in 2006. Two types of organic fertilizers (fish mill, blood mill) and two placement methods of these fertilizers (broadcast application on the top of fabric cover, broadcast application to the beneath of fabric cover) were evaluated in a split-plot design with four replicates. Soil available nutrients to 12 inches deep, total nutrient concentrations, and tree vigor were measured after harvest. Fruit yield was determined for each plot.

Fertigation and Drip Irrigation Trial
A field experiment was initiated on Regina cherry trees that were planted in 2001 on John Benton's orchard at Hood River, Oregon in 2006. Five N fertigation and irrigation systems [1. control (micro sprinkler + broadcast application of dry N fertilizer to the soil surface), 2. micro sprinkler irrigation + fertigation of N fertilizer injected at the same time and interval as irrigation, 3. drip irrigation every day + fertigation of N fertilizer every day, 4. drip irrigation every two days + fertigation of N fertilizer every two days, and 5. drip irrigation every four days + fertigation of N fertilizer every four days] were evaluated in a randomized complete block design with four replications. Soil moisture measurements were taken weekly at the soil depths of 12 inches from May to September. Irrigation scheduling for each treatment was based on soil moisture monitoring, and each treatment was irrigated separately. Soil available nutrients at the depth of 12 inches, total nutrient concentrations in leaf, and tree vigor were measured after harvest. Fruit yield, firmness, size, color, and sugar were determined for each plot. Visual evaluation of fruit surface pitting was conducted after the fruits had been stored in a cold storage room at 33ºC for three weeks. Four categories of excellent, slightly pitted, pitted, and bruised fruits were used in this evaluation.

Results
Drip irrigation had significantly higher nitrogen (N) and manganese (Mn), but lower potassium (K) concentrations in leaf than micro sprinkler in August, about one month after harvest (Table 1). The concentrations of other nutrients were statistically similar between these two irrigation systems. The above results suggest that the uptake of all these nutrients except K by roots is not reduced due to the switch from micro sprinkler to drip irrigation in the second year. The four ground cover systems had similar leaf nutrient concentrations except Cu (Table 1). The biggest benefit with drip irrigation was water saving. During the entire season from May to September, drip irrigation saved as much as 79% of irrigation water relative to micro sprinkler (Table 2). Compared with no cover, straw mulch reduced seasonal water consumption by less then 1%, and black fabric and white fabric had a 3 to 5% increase in water use. Fruit yield with drip irrigation was similar to that under micro sprinkler (Table 2) averaged over the four ground cover systems. There was a trend of yield increase with straw mulch and fabric covers, relative to no cover, although these yield increments were statistically insignificant. Fruit quality including fruit firmness, size, and color did not differ regardless of irrigation or ground cover system (Table 2); but sugar content was greater with drip irrigation than micro sprinkler. Drip irrigation increased marketable fruits (excellent + slightly pitted) by over four percent (absolute value) via reducing

cherry surface pitting compared with micro sprinkler (Table 3). It seemed there is a benefit with black fabric in reducing fruit pitting and bruising relative to no cover.

IFP Cherry Trial

Soil NO_3^- was lower although statistically insignificant with the covered than non-covered grids after harvest in 2006 (Fig. 1). Similar to previous years, differences in soil available P were not significant between the covered and non-covered trees in 2006 (Fig. 2). Covered grids had similar soil available K as no cover in 2006 (Fig. 3). Significant effects of synthetic fabric cover on soil Ca, Mg, S, B, Mn, Cu, pH, or organic matter were not observed in 2006 (data not presented). Consistent with 2002, 2003, 2004, and 2005, leaf N content was 14% greater with the covered than non-covered grids in 2006 (Fig. 4). Because tree size in the covered grids was greater than that in the non-covered grids (data not presented), this suggests that the total N uptake by roots is greatly enhanced due to fabric cover. Unlike 2002, 2003, 2004, and 2005, leaf P content was similar between the covered and non-covered grids in 2006 (Fig. 5). Leaf K was 6% greater with the covered grids than non-covered grids in 2006 (Fig. 6). Leaf S and Cu contents were increased by 8% and 16%, respectively, in 2006 because of fabric cover (Table 4). The effects of fabric cover on Ca, Mg, B, Zn, and Mn contents were not significant in 2006 (Table 4). Fruit quality, such as sugar content, firmness, and fruit size, did not differ between the covered and non-covered trees (Table 5). However, fruit pitting evaluation showed that fabric cover increased the percentage of marketable fruits (excellent + slightly pitted fruits) by reducing fruit pitting (Table 5).

Organic Fertilizer Placement Trial

Similar to 2005, concentrations of soil available nutrients, such as NO_3^-, P, K, etc. after applying fish mill or blood mill on the top of fabric cover were similar to those following the application of the same fertilizer to the soil surface by removing the fabric cover (data not presented). Neither did leaf nutrient concentrations differ between the two placement methods (Table 6). The above results suggest placement method did not affect the availability of applied organic N. Fruit yield in 2006, the second year of experimentation, was not different between the two placement methods (data not presented). It seems there is no need to apply fish mill or blood mill to the beneath of fabric cover. Application of these organic fertilizers directly on the top of fabric cover is equally effective and could save labor.

Fertigation and Drip Irrigation Trial

Micro sprinkler irrigation plus N fertigation system resulted in significantly higher concentrations of N, Mn, and Cu in leaf than micro sprinkler plus dry N fertilizer system after harvest (Table 7); which suggests that the uptake of these nutrients by roots may be improved due to the switch from dry N fertilizer application to the soil surface to split N fertigation under micro sprinkler in the first year. However, almost no increase in leaf nutrient concentration was observed with the three drip irrigation plus N fertigation systems compared with the current water and N management system – micro sprinkler irrigation and the application of dry N fertilizer to soil surface. The biggest benefit with drip irrigation and N fertigation systems was water saving. During the entire season from May to September, the three drip irrigation plus fertigation systems saved over 60% of irrigation water relative to the two micro sprinkler systems (data not presented). Fruit yield and quality including fruit sugar content, firmness, and size were statistically similar among the five irrigation and N management systems (Table 8). Furthermore, no difference in fruit surface pitting was observed among the five irrigation and N management systems (data not presented). Overall, drip irrigation plus N fertigation systems produce competitive cherries with much less irrigation water and more flexibility for N fertilizer applications.

Table 1. Effects of irrigation system and ground cover on leaf nutrient concentrations.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
Micro sprinkler	2.36	0.32	2.64	1.70	0.34	0.18	75.7	14.7	52.3	5.0
Drip irrigation	2.54	0.31	2.31	1.81	0.38	0.18	72.6	14.7	62.7	5.5
Significance	*	ns	**	ns	ns	ns	ns	ns	*	ns
No cover	2.43	0.33	2.45	1.73	0.38	0.18	74.6	14.7	53.9	5.1
Straw mulch	2.40	0.33	2.54	1.87	0.36	0.18	73.3	15.2	58.5	5.2
Black fabric	2.46	0.29	2.48	1.76	0.35	0.18	75.5	15.7	60.4	4.7
White fabric	2.52	0.33	2.44	1.66	0.36	0.18	73.2	13.1	57.2	5.9
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	*

* indicates the treatment effect is statistically significant at 5% probability level. Non significant effect is denoted by ns.

Table 2. Effects of irrigation system and ground cover on irrigation water consumption and fruit yield and quality.

Treatment	Water consumption (gallon/tree)	Yield (lbs/tree)	Sugar (^{o}brix)	Firmness (g/mm^2)	Size (mm)
Micro sprinkler	4323.8	178.8	17.3	259.7	25.8
Drip irrigation	928.0	174.0	18.5	268.9	25.5
Significance	*	ns	*	ns	ns
No cover	2575.3	170.7	17.9	264.7	25.4
Straw mulch	2564.0	181.3	18.0	266.6	25.7
Black fabric	2654.0	175.5	17.5	270.5	25.7
White fabric	2710.3	178.0	17.9	255.8	26.0
Significance	ns	ns	ns	ns	ns

* indicates the treatment effect is statistically significant at 5% probability level.
Non significant effect is denoted by ns.

Table 3. Effects of irrigation system and ground cover on fruit surface pitting.

Treatment	Excellent (%)	Slightly Pitted (%)	Excellent + Slightly Pitted (%)	Pitted (%)	Bruised (%)
Micro sprinkler	42.0	24.4	66.4	20.3	13.3
Drip irrigation	44.2	26.6	70.8	19.2	10.0
Significance	ns	ns	*	ns	ns
No cover	41.8	24.7	66.4	23.0	10.6
Straw mulch	41.8	26.7	68.6	18.7	12.7
Black fabric	48.2	23.7	71.8	16.8	11.4
White fabric	41.3	26.9	68.2	19.7	12.1
Significance	ns	ns	ns	ns	ns

* indicates the treatment effect is statistically significant at 5% probability level. Non significant effect is denoted by ns.

Table 4. Effects of row cover on leaf Ca, Mg, S, B, Zn, Mn, and Cu concentrations.

Year	Treatment	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
2001	Not covered	0.95a†	0.24a	0.05a	35.6a	22.8a	48.9a	14.3a
	Covered	0.96a	0.26a	0.05a	35.9a	25.6a	64.0a	13.6a
2002	Not covered	1.55a	0.41a	0.19a	52.4a	24.3a	92.4a	9.1b
	Covered	1.35b	0.31b	0.18a	50.5a	20.0a	88.9a	10.4a
2003	Not covered	1.34a	0.32a	0.09a	52.4a	12.8a	57.4a	8.4a
	Covered	1.19b	0.28b	0.10a	48.6a	13.0a	55.1a	8.6a
2004	Not covered	1.38a	0.32a	0.13a	78.8a	10.4b	50.3a	4.0b
	Covered	1.35a	0.30b	0.13a	77.8a	12.7a	50.9a	4.4a
2005	Not covered	1.53a	0.39a	0.14a	70.9a	12.9a	43.1a	5.9b
	Covered	1.39b	0.37a	0.15a	68.1a	14.8a	45.1a	6.4a
2006	Not covered	1.61a	0.44a	0.13b	63.9a	16.3a	45.0a	4.5b
	Covered	1.60a	0.42a	0.14a	65.4a	13.3a	49.6a	5.2a

† Values in column within each year followed by the same letter are not significantly different at 5% probability level.

Table 5. Effects of row cover on fruit quality and surface pitting in 2006.

Treatment	Sugar (°brix)	Firmness (g/mm^2)	Size (mm)	Excellent (%)	Slightly Pitted (%)	Excellent + Slightly Pitted (%)	Pitted (%)	Bruised (%)
Not covered	19.2a†	245.5a	26.2a	32.4b	31.3a	63.8b	27.2a	9.0a
Covered	19.6a	245.6a	25.9a	43.7a	29.7a	71.6a	18.5b	9.9a

† Values in column followed by the same letter are not significantly different at 5% probability level.

Table 6. Effects of organic fertilizer types and placement methods on leaf nutrient concentrations.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
Fish mill	2.10	0.40	2.32	2.18	0.48	0.15	66.2	16.0	51.9	6.1
Blood mill	2.06	0.39	2.37	1.88	0.46	0.16	75.5	15.2	56.9	6.1
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
On top of fabric	2.10	0.42	2.32	2.13	0.47	0.16	71.1	15.7	55.3	6.3
Beneath fabric	2.07	0.38	2.37	1.93	0.47	0.15	70.6	15.5	53.5	6.0
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

* indicates the treatment effect is statistically significant at 5% probability level. Non significant effect is denoted by ns.

Table 7. Effects of fertigation and irrigation on leaf nutrient concentrations.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
1	2.13	0.26	1.84	1.61	0.33	0.14	63.5	19.6	42.6	8.6
2	2.42	0.23	1.74	2.36	0.34	0.15	62.8	25.8	58.4	13.7
3	1.90	0.24	1.64	1.74	0.34	0.15	56.4	22.0	67.0	6.9
4	2.03	0.22	1.84	2.03	0.29	0.15	58.2	20.1	48.6	7.3
5	2.08	0.25	1.76	1.69	0.31	0.14	59.2	21.5	57.4	8.8
Significance	**	ns	*	ns	ns	ns	*	ns	*	**
LSD	0.17		0.14				5.4		15.4	1.9

* and ** indicate the treatment effect is statistically significant at 5% and 1% probability level, respectively. Non significant effect is denoted by ns.
If difference between two treatments in a column is greater than the LSD value, the two treatment values are statistically different.

Table 8. Effects of fertigation and irrigation on fruit yield and quality.

Treatment	Yield (lbs/tree)	Sugar (°brix)	Firmness (g/mm^2)	Size (mm)
1	23.7	23.9	259.3	26.4
2	24.6	23.8	247.1	25.9
3	24.8	23.2	247.2	26.2
4	23.8	23.6	252.7	26.9
5	23.8	23.1	228.0	26.6
Significance	ns	ns	ns	ns

* indicates the treatment effect is statistically significant at 5% probability level. Non significant effect is denoted by ns.

Fig. 1. Soil NO_3^-

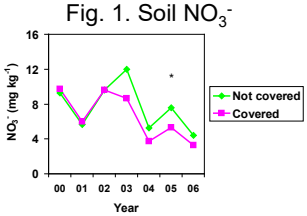


Fig. 2. Soil P

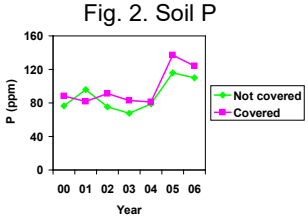


Fig. 3. Soil K

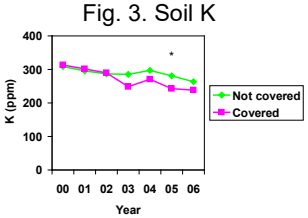


Fig. 4. Leaf N

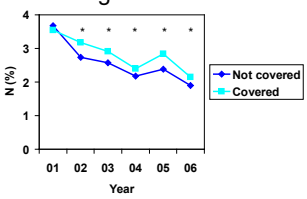


Fig. 5. Leaf P

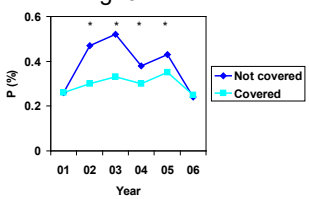
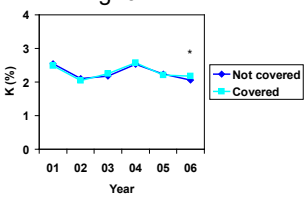


Fig. 6. Leaf K



CONTINUING PROJECT REPORT **YEAR**: 2 of 3

WTFRC Project Number: CH-06-602

Project Title: Post-Plant Management of Dagger Nematodes
PI: Ekaterina Riga
Organization: WSU
Address: 24106 N. Bunn Road
City/State/Zip: Prosser, WA 99350
Telephone: 509-781-9256
Email: riga@wsu.edu

Cooperators:
1) Mr. Don Jagla; Cherry Grower, Wenatchee, WA
2) Mr. Jerry Gutzwiler
3) Dr. C. Ishida, Field R&D Scientist, Valent Biosciences Co.
4) Dr. Ken Eastwell, Virologist, Washington State University, IAREC, Prosser, WA.

Budget 1:
Organization Name: WSU **Contract Administrator:** ML. Bricker/S. Brock
Telephone: 509-335-7667 / 509-786-9242 **Email address**: mdesros@wsu.edu / sabrock@wsu.edu

Item	Year 1: 2006	**Year 2: *2007**	Year 3: 2008
Salaries			
Benefits			
Wages	4,185		
Benefits	460		
Equipment			
Supplies	450	**1,000**	1,000
Travel	400	**1,000**	1,000
Miscellaneous			
Total	5,495	**2,000**	2,000

Footnotes:
For 2007 and 2008, less funding is requested as Mr. Jagla applies Ditera on his own. In addition, Mr. Jagla collects the soil and root samples. I am responsible of picking up the samples and processing them for nematodes.

Objectives: The short term objective is to use DiTera as means to control both lesion and dagger nematodes in post-plant cherry orchards. The long term objective is to find alternatives for Nemacur by testing new compounds with nematical properties.

In 2007, Ditera will be tested in two cherry orchards (the 2nd orchard was added in our study in fall 2006) with history of dagger and lesion nematodes. In addition, new compounds, green manures and meals are screened in my greenhouses against lesion and dagger prior to field testing.

Significant findings for 2006:
After 1 year of applying Ditera in one of the cherry orchards, a significant reduction in dagger nematode population was achieved.
No significant reduction of lesion nematodes was observed during the first year of treatment.

Methods: The methods are the same as in 2006. Soil samples and root samples are collected prior and post Ditera application in the beginning of the season, mid-season and post harvest. Nematodes are extracted from soil and roots, and data from treated trees is compared to untreated controls.

New compounds are tested in the greenhouse on pots infected with lesion and dagger nematodes. The effectiveness of the new compounds is evaluated against untreated controls. The following compounds are evaluated: *Brassica carinata, Muscodor albus* and STO.

Results and discussion: After 1 year of applying Ditera in a cherry orchard with high densities of dagger and lesion nematodes, a significant reduction in dagger nematode population was achieved. In one of the samples, dagger nematodes were reduced from 700 individuals per 250 cc soil to 24 individuals per 250 cc. Similar reduction was recorded from all soil samples (Fig. 1). However, no significant reduction of lesion nematodes was observed during the first year of treatment (Fig. 1). Ditera did not have negative effects on the beneficial free living nematodes.

The reduction of dagger nematodes will be of importance to the cherry industry as controlling these nematodes will lead to reduction of virus transmission, yield increase and tree survival.

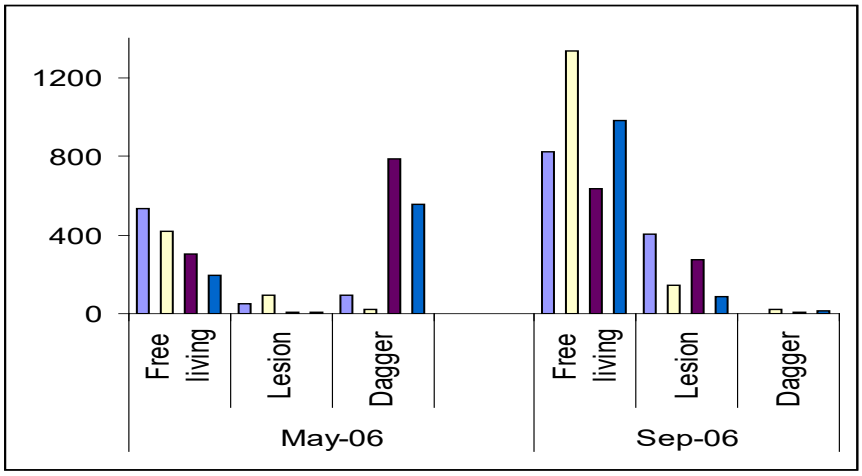


Figure 1. The effect of Ditera on dagger and lesion nematodes – 2006 field trial

CONTINUING PROJECT REPORT **YEAR**: **2** of 3
WTFRC Project Number: CH-06-603

Project Title: Cherry Fruit Fly Control Options
PI: Timothy J. Smith
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Budget 1:
Organization Name: WSU Extension **Contract Administrator:** ML. Bricker / J. Jansen
Telephone: 509-335-7667 /509-335-2867 **Email address:** mdesros@wsu.edu / jjansen@wsu.edu

Item	**Year 1:** 2006	**Year 2: 2007**	**Year 3:** 2008
Salaries	10,773	**11,916**	12,393
Benefits	4,094	**4,051**	4,214
Wages			
Benefits			
Equipment			
Supplies	300	**300**	300
Travel	1,940	**1,869**	1,869
Miscellaneous			
Total	17,107	**18,136**	18,776

Introduction and Justification
At the time this project was initiated, cherry fruit fly was identified as the top priority in the TFRC Cherry Research Committee yearly priority setting sessions. The objective of this project is the discovery and demonstration of safe and effective new CFF control materials and methods, as the carbamate and organophosphate class insecticides available at the inception of this work were (and continue to be) under regulatory pressure.

Significant Results Summary:
Objective 1: Identify new conventional and organic cherry fruit fly control products and methods.

! Twelve products have been tested in these trials, most for the first time on cherry fruit fly.

! Two other promising products are proposed for test in 2007.

Objective 2*:* Assess new insecticides and control methods for cherry fruit fly.

! Most of the ten candidate products tested in 2006 were quite effective, especially when applied at "moderate" or "full" proposed label rates and at 7 or 10 day spray intervals. Rate and interval data will be used for future label directions.

! This project first recognized and demonstrated the efficacy of GF-120 Bait as a Cherry Fruit Fly control. Early adoption of this control method is **saving the PNW Cherry growers about $1.5 million each year** by reducing labor, application and material costs. This bait may now be the most commonly used insecticide on Washington cherries.

! Three products were identified as alternatives to dimethoate as post-harvest "clean-up" sprays. The EPA-proposed lower rate of dimethoate was found to be ineffective.

! PNW Organic growers are now fully able to control this pest with the bait and/or Entrust. One organic product was proven ineffective, another product was found to be suppressive, but not entirely effective.

Results and Discussion:
Products included in this project during the 2006 trials included Assail, Provado, Rynaxypyr (an "anthranilic diamide," a new class of insecticide), Entrust, GF-120NF Bait, XDE-175 (a new synthetic spinosin), Pyganic, Rimon (an IGR, applied as a spray and as a bait), and another numbered product. Most of the products had never been tested in the field for effect on cherry fruit fly when first included in this project. At least two promising new-chemistry products will be included in 2007.

Efficacy Trials: Most tested products controlled CFF very well at moderate or full rates applied at 10 day intervals. As in past trials, effective products became less effective when applied at 14 day intervals, even with full standard rates. This interval and rate information will be used during the development of use directions for these products, and during educational programs. See table 1 for 2006 season result details.

GF-120 bait treatment was applied to four new sites in 2006, and 10 sites previously treated from two to four seasons. All sites were well infested prior to initiation of GF-120 application, and no other control method or material has been applied during the 49 "treatment years." (Treatment year = one site treated for one season.) During the past five years, two larvae were found in 35,400 cherries crushed from these 49 treatment-year sites. No larvae were found after treatment

of the four new infested 2006 sites. ***Use in the first three years of registration has saved Washington cherry growers over $2,750,000*** in reduced labor, machinery and material costs, and economic benefits will continue at about $1.5 million per season at current use levels. Adoption of this new technology has essentially eliminated a serious and increasing problem with cherry fruit fly in organic orchards. Due to use of this product, applicator exposure to products with potential to inhibit cholinesterase was reduced by about 8,000 hours during May, June and July of 2006. Due to the data gathered in this trial, GF-120 was registered in Canada for 2006, and extensively used in their organic orchards. They report excellent control in previously infested orchards.

Three materials were demonstrated as effective for control of cherry fruit fly larvae inside the fruit, as possible alternatives for post-harvest dimethoate. The dimethoate data has been submitted to the EPA by Northwest Hort Council. See the post-harvest section and table 4 for details.

Provado, Assail and Calypso controlled black cherry aphid (Myzus cerasi) when used at rates and application timings intended for cherry fruit fly control.

An insect growth regulator (Rimon), previously untested on CFF, was very suppressive of larval infestation. Test efforts were greatly expanded this season after interesting results in 2005. The product suppressed larva numbers in fruit from highly infested trees, especially when used as an active ingredient in bait, applied in the same way as GF–120. The single tree treated in 2005 had 110 flies caught in that season and 14 captured in 2006. Infestation levels on that tree have dropped from nearly 100 percent in 2004, to 1 percent in 2005, and 0.2 percent in 2006.

Table 1. Details of 2006 Trials:

Treatment	Trees / Sites	Days Interval Spray	Flies / Trap 2006	Fruit Sample Number	Larvae Found in Fruit
"Standard" Control. Provado 1.6F, 6 oz/A 1st. Treatment, Carbaryl 4 pints/A 2nd, Provado 6 oz/A 3rd treatment, Success 4 oz/A 4th treatment + GF-120 BAIT weekly during and after harvest.	2/2	10	289 13	1000 1000	0 0
Untreated Check Trees	3/3	na	846 605 275	1000 1000 1000	263 428 131
Rynaxypyr 2 oz/a + silicone wetter @ 2 fl.oz./100 gal.	4/4	10	57 289 13 515	1000 1000 1000 1000	0 0 0 0
Rynaxypyr 3 oz/a + silicone wetter @ 2 fl.oz./100 gal.	3/3	10	48 15 515	1000 1000 1000	0 0 0

Table 1, Continued. **Treatment**	**Trees / Sites**	**Days Interval Spray**	**Flies / Trap 2006**	**Fruit Sample Number**	**Larvae Found in Fruit**
Rynaxypyr 4 oz/a + silicone wetter @ 2 fl.oz./100 gal.	4/4	10	57 289 13 515	1000 1000 1000 1000	0 3* 0 0
Rynaxypyr 2 oz/a, NO wetter	4/4	10	21 535 60 13	1000 1000 1000 1000	0 1* 0 0
Rimon 32 fl.oz/a (An Insect Growth Regulator)	3/3	10	20 2 14	1000 1000 1000	0 0 2
Rimon /Bait 2 fl. oz. Rimon per 20 fl.oz NuLur Bait / Acre. 0.2 fl.oz. Bait mix per tree	3/3	7	62 55 55	1000 1000 1000	0 0 0
Assail 30SG, 5 oz / A 10 day spray + interval	3/3	10	21 289 535	1000 1000 1000	0 0 0
Assail 30SG, 5 oz / A 14 day spray interval	4/4	**14**	19 19 19 19	1000 1000 1000 1000	3 11 0 2
Provado 1.6F 6 fl oz /a	3/3	10	21 289 13	1000 1000 1000	0 1* 0
Provado Pro 192 NT 4 fl oz/a	4/4	10	21 15 535 13	1000 1000 1000 1000	0 0 0 0
Provado Pro 192 NT 6 fl oz/A	4/4	10	21 289 13 515	1000 1000 1000 1000	0 0 0 0
XDE-175 (GF-1640) **4.5 oz. /a**	4/4	10	21 6 214 535	1000 1000 1000 1000	0 0 0 0

Table 1, Continued. Treatment	Trees / Sites	Days Interval Spray	Flies / Trap 2006	Fruit Sample Number	Larvae Found in Fruit
XDE-175 (GF-1640) **3.0 oz. /a**	3/3	10	21 214 535	1000 1000 1000	0 0 0
Entrust **1.9 oz./a**	4/4	10	21 535 6 214	1000 1000 1000 1000	0 0 0 0
Numbered Product Z **Moderate rate**	4/4	10	289 60 214 515	1000 1000 1000 1000	0 0 0 0
Numbered Product Z **Higher rate**	4/4	10	48 535 214 515	1000 1000 1000 1000	0 0 0 0
Pyganic 5 (5% pyrethrum) 12 fl.oz./a **with buffer**	4/4	7	53 53 53 11	1000 1000 1000 1000	0 1 2 2
Pyganic 5 (5% pyrethrum) 12 fl.oz./a **NO buffer**	2/2	7	18 75	1000 1000	3 2
GF-120NF Bait **20 fl.oz./a, 1:3 dilution** **0.20 oz product / tree**	18/14	7	see details in text	14,000	0

*The test tree with this light infestation was adjacent to a tree where control failed. Female CFF were free to fly from the infested tree to the nearby test tree with fully mature eggs. This might explain the control breakdown, as the other three replicates treated with this product and rate were free of larvae, despite high pressure.

Post-harvest Treatments:
Provado, Assail and Calypso applied to severely infested fruit on a tree prevented all or most subsequent larval emergence. As in the 2005 post-harvest trial, Calypso was effective to a practical degree, but did not completely control larva inside the fruit. The lowest effective rate for Provado has not yet been determined. The currently recommended rate of Dimethoate (1.33 lb. ai / a, or 4 pints of the 2.67 lb/gal. formulation) was also effective. The lesser rate of Dimethoate, (1.0 lb./ai/A, or three pints of the 2.67), recently proposed by the EPA as the high

legal rate during the re-registration process, was not as effective. This research was submitted to the EPA by the Northwest Hort Council and WSU in an effort to persuade them to reconsider the rate reduction.

Methods: Portions of an unharvested CFF infested cherry tree were treated with the various test products on a date that would have been "post-harvest," under normal conditions. The test products were applied in a volume of water that results in "full drip," which we judged to be equivalent to about 300 gallons per acre. At the treatment date, some of the larvae in the fruit were late in their third (and final) instar, and were soon to emerge, as they had cut the characteristic breathing and emergence holes in some of the fruit. Most of the larvae are in the third and second instar at this stage of population development. One day after treatment, 250 fruit were harvested from each treatment and suspended over sand. The larvae were allowed to emerge at room temperature over the next three weeks. Larvae emerged from the untreated fruit most rapidly during the first five days after treatment, when 72 percent of the total emerged. After that time, emergence rapidly tapered off, and was complete by the 11th day. Judging by the number of larvae that emerged, about 30 percent of the fruit on the test tree was infested.

All products tested appear to be very acceptable replacements for dimethoate, the only product currently recommended for controlling larvae in fruit remaining on harvested trees. This "post-infestation effect" may give products with this chemistry an advantage as a pre-harvest product, as application may control newly hatching eggs or larvae that may have slipped through earlier control programs. At this time, dimethoate is not a popular pre- or post-harvest choice, as it sometimes causes leaf yellowing, necrosis and drop. Many growers avoid using it.

Table 2. Post harvest "Clean-up" Spray Options:

Product	Rate	Fruit Sample	Larvae Emerged
Dimethoate 267	64 oz./300 gal./A 1.33 lb. ai/Acre	250	0
Dimethoate 267	48 oz./300 gal./A 1.0 lb. ai/Acre	250	9
Provado 1.6F	8 oz./300 gal./A	250	0
Provado 1.6F	6 oz./300 gal./A	250	0
Calypso SC 480	8 oz./300 gal./A	250	3
Assail 30 SG	8 oz./300 gal./A	250	1
Untreated	0	250	76

Other effects: Despite as many as five weekly applications at higher than necessary rates, no treatment in this project has resulted in leaf marking, yellowing or shedding, fruit marking, or excessive mite flare-ups leading to significant leaf damage. Some moderate leaf symptoms induced by mite feeding were observable by late summer on some of the trees treated with up to five weekly applications of Provado, Assail, and Calypso. Many of the candidate products have not yet been tested on all common sweet cherry varieties, so, while there are no indications of these potential problems to date, potential for leaf drop sensitivity in some varieties, or marking of light colored cherries is unknown.

FINAL PROJECT REPORT
WTFRC Project #: CH-04-402

Project Title: Evaluation of Insecticide Effects on Biology of Cherry Fruit Fly
PI: Wee Yee
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Cooperators: Various homeowners in Tri-Cities and Yakima, WA

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	22,050	22,050	22,050
Benefits	2,450	2,450	2,450
Wages	0	0	0
Benefits	0	0	0
Equipment	1,000	1,000	1,000
Supplies	1,000	1,000	1,000
Travel	0	0	0
Miscellaneous	500	500	500
Total	27,000	27,000	27,000

Objectives 2004-2006:
1) Determine effects of insecticides on adult fly mortality, oviposition, and preference for cherries.
2) Determine relative importance of contact or ingestion mechanisms of kill of adult flies.
3) Determine residual activity of insecticide formulations in the field.
4) Determine effects of insecticide on larval infestations in the field.
5) Determine feeding times on insecticide droplets.
6) Determine effects on egg production of flies treated with sublethal amounts of insecticides.
7) Determine translaminar effects on cherries with various stages of fly eggs and larvae during early, mid, and late season.

Significant findings in 2004-2006:

● Entrust and GF-120 (spinosad insecticides) were the most effective insecticides against adult cherry fruit flies and caused 100% mortality within 1-4 days, followed by Provado (imidacloprid) and Calypso (thiacloprid). Results show there are fairly toxic non-organophosphate materials for use against the fly.

● GF-120, Entrust, Provado, Actara, Avaunt, and Guthion reduced oviposition into cherries by flies; Guthion was most effective, followed by Entrust, Provado, and Actara, which were similar. Mortality caused by all insecticides except Avaunt was >90% after 2 days, but Guthion caused 100% mortality after this time. This suggests the toxicity of materials need to be increased to improve their ability to reduce oviposition or that other, more toxic materials need to be identified.

● Flies laid equally in insecticide-treated versus untreated cherries, suggesting the insecticides are not a deterrent and that the flies cannot tell the two types of cherries apart.

● Spinosad in GF-120 and Entrust had equally high contact and oral activity; Provado and Calypso had higher oral than contact activity. This suggests flies may not need to feed on spinosad or Provado to be killed.

● GF-120 had residual activity of 14 days; Entrust, Provado, and Calypso had no activity after 14 days of aging. This suggests that under ideal conditions of no rain and moderate weather, the bait in GF-120 is able to protect the spinosad from degradation from environmental factors or prevent or reduce it from being absorbed rapidly into leaves.

● A field trial indicated Entrust, GF-120, Provado, and Calypso can reduce larval infestations of cherries significantly, at least when fly densities are low; however, except for GF-120 (in this one trial), no materials eliminated infestations.

● Flies fed more on all insecticides when they were mixed with sugar than without. This suggests sugar mixed in insecticide solutions can speed up kill.

● Calypso had the highest ovicial (egg-killing) and young larval-killing activity, suggesting it can be used in a post-harvest sprays.

● Provado when sprayed on cherries reduced larval emergence the most, suggesting it also could be used as an effective post-harvest spray.

● Further study showed that all insecticides tested except Avaunt reduced larval emergence from cherries, with Guthion, GF-120, and Provado most effective; however, Guthion was the only one that prevented emergence. Despite reducing emergence, the other materials should be improved for their

ability to be absorbed into fruit tissue (at post-harvest) or that their toxicities need to be increased against the eggs or larvae, to be as effective as Guthion.

1) Determine Effects of Insecticides on Adult Fly Mortality, Oviposition, and Preference for Cherries

Experiments using the label rates of the following were conducted: (1) Entrust (spinosad, no bait), (2) GF-120 (spinosad mixed with bait of sugar, protein, attractants), (3) Provado (imidacloprid), and (4) Calypso (thiacloprid). An untreated control (water) was included. Insecticides were tested without sugar (except GF-120). Ten male and 10 female flies 2-7 days old were placed inside a pint-size paper container with food and water with no insecticides (control) or with 100 μl (test 1) or 500 μl (test 2) of each of the 4 treatments. Treatments were presented as 5 (test 1) or 25 (test 2) 20 μl drops spread uniformly on a shallow plastic dish on the bottom of a container over 4 d. Fly mortality was determined at 1, 2, 3, and 4 days after exposure. Flies were classified as dead if they could not walk. There were 3 or 4 replicates of the control and treatments in test 1 and 5 of each in test 2.

To determine effects of insecticides on fly oviposition, flies were exposed to cherries sprayed with insecticides. Flies were aged from emergence inside half gallon white paper containers containing food and water. Flies were tested at 14-16 days old. Treatments were (1) Entrust, (2) GF-120, (3) Provado, (4) Calypso, (4) Avaunt (indoxacab, an oxadiazine), (5) Actara (thiamethoxam, a neonicotinoid), and Guthion (azinphos-methyl). A control was included. Insecticides were applied on cherries the day before testing. Thirty cherries were spread as one layer on a piece of aluminum foil on a plastic tray in a fume hood. Using a spray bottle, 4.8 ml of solution was evenly applied onto the cherries. Cherries were left on trays for 24 h at 20-21 ºC. On the test day, the 30 cherries were gently poured onto the bottom of a half gallon paper container. The container had 2 water wicks and a strip of food. Six males and 6 females were then introduced into each container. Males were placed in with females to allow for mating. Female fly mortality was checked at 1, 2, and 3 days after exposure to treatments. At 3 days, all 30 cherries were removed and stored in alcohol to check for eggs later. There were 5 replicates of the control and all treatments.

The same treatments were compared in an experiment to determine if there is an oviposition preference by flies for insecticide-treated versus untreated cherries. Procedures were essentially the same as in the previous experiment, except that 15 cherries were treated and 15 were left untreated in each container. A strip of paper acted as a barrier and separated the 2 types of cherries on the bottom of the cage. There were 5 replicates of the control and treatments.

2) Determine Importance of Ingestion or Topical Application Mechanisms of Kill of Adult Flies

Entrust, Provado, and Calypso were mixed with 20% sucrose (wt:wt) to stimulate feeding and tested against 3-11 day old flies. The control was 20% sucrose only. Newly-emerged flies were held inside pint-size containers with food and water. Food was removed 16-20 hours before tests. Individual flies were immobilized at 1.7-3.3 ºC for 5-6 min. For testing effects of topical application, a 2 μl drop of solution was placed on top of the thorax of a single fly under a microscope. For ingestion effects, a 2 μl drop of solution was placed in a glass vial 15 min after a fly was introduced into the vial. The fly was closely observed and given a maximum of 15 min to drink the solution. All flies drank the insecticide solutions within this time, some consuming the entire 2 μl drop. After topical application and ingestion treatments, each fly was placed inside a pint-size paper container with food and water. Mortality was checked daily up to 30 days. There were 15-25 flies of each sex for the control and treatments.

3) Determine Residual Activity of Insecticide Formulations in the Field

Entrust, GF-120, Provado, and Calypso aged for 0, 3, 7, and 14 days on sweet cherry leaves at the USDA experimental orchard in Moxee, WA were exposed to field-collected flies in 2005. A hand-held sprayer was used to deliver about 10 ml of spinosad bait and 20 ml of the other materials to tops and bottoms of approximately 30 leaves on the south sides of trees on 6, 13, 17, and 20 June. Bait sprays are not intended to provide 100% coverage and thus a lower spray volume of GF-120 was used. Unsprayed leaves served as controls. There was 1.3 mm precipitation on 7 June and a trace

amount on 18 June. Most days were sunny. For the "0" day treatment, leaves were sprayed and then air dried for one hour before being exposed to flies. Flies were collected from Kennewick and Zillah, WA over a 2.5-week period before the experiment, and maintained at 20-21 ºC with food and water inside pint-size paper containers. Each replicate container held 8 male and 4 female flies. Flies that died before the start of the experiment were replaced as needed. For each replicate, one randomly chosen control or treated leaf was placed inside a container. The leaf was laid on its edge to maximize exposure of flies to insecticide residues on both sides of the leaf. Flies were provided with food and water. Mortality was checked at 1, 3, and 7 days after fly exposure to the leaves. There were 5 replicates of the control and each treatment. The experiment was conducted at 24-29 ºC.

4) Determine Effects of Insecticides on Larval Infestations in the Field

In Washington, one spray trial was conducted in 2004 at the USDA experimental cherry orchard in Moxee using single trees, which simulated unmanaged homeowners' trees. Yellow sticky traps baited with ammonium carbonate were placed in selected trees in May to detect first fly emergence. A control and Entrust, GF-120, Provado, and Calypso treatments were compared. The test was set up as a randomized complete block design. Each tree was separated from others by one untreated tree. There were 7 replicate blocks. Applications were made within 5 days after the first fly capture. Because of the low fly density, traps were removed afterwards to reduce the possibility they would capture too many flies. GF-120 was applied using a hand-held sprayer at 532 ml of spray/tree (per label for single trees). The other treatments were applied using a Nifty Pul-Tank and a handgun at 100 psi in a volume of 7.56 liters per tree. Applications were made every 8 or 10 days. Two hundred cherries were removed from each tree on 1 July. Mature cherries were laid on emergence trays over one month to collect pupae.

5) Determine Feeding Times on Insecticide Drops

Observations were made of flies exposed to 2.5 μl drops of insecticide solutions with and without 20% sucrose inside clear glass vials. One fly was tested at a time and exposed to one drop. Insecticides tested were GF-120, Entrust, Provado, and Avaunt, and Actara. Water with or without sucrose was the control. Numbers of feeds and durations of feeds over a 5-minute period were recorded.

6) Effects of Sublethal Insecticide Amounts

For Entrust and Provado treatments, egg production of flies exposed to 2.5 μl drops of insecticide placed on the dorsa or that ingested the drops were determined by allowing flies to lay eggs into untreated cherries at days 10-14 inside pint-size containers.

7) Determine Translaminar Effects on Cherries with Eggs and Larvae

In 2005, sweet cherries with stems attached were picked from 5 infested trees on 7 and 8 June in Kennewick, WA and treated with water, Entrust, GF-120, Provado, and Calypso on 8 June. The cherries were ripe. There were 110 cherries for the control and each treatment from each tree. The 110 cherries were placed on hardware cloth and then sprayed with 10 ml of each material using a squirt bottle. They were then suspended above a tub containing dry soil and held outdoors in the shade for 30 days (8 June to 8 July). At 8 days, 10 cherries were randomly selected from each sample and opened to determine numbers of dead and live larvae. Each larva was measured to determine if growth was affected. The other 100 cherries were held on the hardware cloth for an additional 22 days. At 15 and 30 days after treatment, numbers of pupae in the soil at the bottom of each tub were counted. Pupae were stored in sealed cups at 21 ºC in moist soil. All pupae were dissected at 30-37 days post treatment to determine mortality. There were 5 replicates of the control and treatments.

In 2006, cherries from 7 infested cherry trees in Kennewick were collected and treated with Entrust, GF-120, Provado, Calypso, Avaunt, Actara, and Guthion. A water control was included. Samples from the 7 trees were considered replicates. Cherries were collected on 3 dates that represented early, mid, and late season: 31 May, 7 June, and 14 June. Stems were retained on the cherries. Cherries were brought back to the laboratory, and spread among hardware cloths, with 30 cherries each. The cherries were sprayed with 2.8-3.2 ml insecticide solutions using a squirt bottle on the day of collections and again one week later. Cherries were kept outdoors in the shade for 30 days

for larvae to emerge. All pupae in tubs were counted. Pupae were then chilled at 3 ºC and removed after 4 months to determine survival of flies.

2004-2006 Results and Discussion:

1) Determine effects of insecticides on adult fly mortality, oviposition, and preference for cherries.

In test 1 using 100 μl solutions, mortality among all treatments was higher than in the control and rankings of effectiveness were similar among days (Table 1). Entrust caused the highest mortality, although statistically it was not different from GF-120. Provado did not differ from GF-120 and Calypso, but it was less effective than Entrust at 1, 3, and 4 days and Calypso was less effective than Entrust at 1, 2, 3, and 4 days (Table 1). Exposure to 5 times greater volume in test 2 resulted in higher mortality in the Provado than Calypso treatment at 3 and 4 d. Mortality caused by Entrust or GF-120 and Provado was not different except at 2 days after exposure (Table 1). These results indicate there are fairly toxic non-organophosphate materials against adult cherry fruit flies.

In the oviposition deterrence test (Table 2), all materials suppressed infestations and numbers of eggs laid by 14-16 day old flies, but none of the materials prevented oviposition. The ranking of effectiveness in percentages infestation that were reduced and numbers of eggs laid in cherries was: Guthion > Provado >GF-120 >Actara > Entrust > Avaunt > control, although statistically Guthion = Provado = GF-120, with Entrust and Actara and Avaunt similar and all more effective than the control. The data on mortality (Table 2) indicate that even though mortality was highest at day 1 for Actara and Guthion, flies were not killed quickly enough in these 2 treatments to prevent oviposition. At days 1 and 2, mortality among all treatments increased and was more similar, with Avaunt causing the lowest mortality. Results suggest that despite relatively high toxicity to adults, more toxic materials are still needed, to be comparable to Guthion.

In the oviposition choice test (Table 3), contrary to expectations, there were no differences between infestations in cherries that were treated or untreated with insecticides, except for GF-120 (Table 3). Even though half of the cherries in a container were untreated, mortality of females was high, especially at days 2 and 3. The results suggest the insecticides are not a deterrent and that the flies cannot tell the two types of cherries apart. There is thus no reason to believe flies will leave insecticide-treated trees because of a repellent effect.

2) Determine Importance of Ingestion or Topical Application Mechanisms of Kill of Adult Flies

Contrary to expectations, spinosad in GF-120 and Entrust had equally high contact and oral activity; Provado and Calypso had higher oral than contact activity (Table 4). Although it is possible the materials may have spread down the thorax of flies and caused the flies to feed on the materials, the fact is that flies did not need to directly ingest the materials to suffer high mortality. Thus sprays of GF-120 and the other insecticides may affect control through both contact and ingestion of materials. However, which mechanism accounts for the control in the field is not known, although the less toxic materials may be more effective if ingested.

3) Determine Residual Activity of Insecticide Formulations in the Field

There were clear effects of aging insecticides and baits and of different insecticides on fly mortality (Table 5). Entrust lost effectiveness when aged over the 14 days, whereas GF-120 did not lose any effectiveness over 14 days. Provado lost effectiveness after only 3 days of aging. Calypso did not lose any effectiveness over 14 days of aging, but it also was ineffective, causing lower mortality than any of the other materials (Table 5). At 1 DAE, mortality within 0-day old residues ranked as follows: GF-120=Entrust> Provado> Calypso. With 3-day old residues, the ranking was GF-120>Entrust= Provado=Calypso. With 7- and 14-day old residues, GF-120 caused greater mortality than all other materials, with Calypso least effective. These same rankings were generally seen at 3 and 7 DAE, even though there was increased overall mortality, including in the controls. Significantly, GF-120 was the only material that caused 100% mortality by 7 days after exposure when aged 0, 3, and 14 days (Table 5). The results clearly suggest that GF-120 has the longest residual activity when there is little precipitation. It is possible that the bait component of GF-120

(sugars, ammonium acetate, oil, etc.) protected the spinosad from degradation or that it concentrated the spinosad on the leaves. It is also possible that the bait reduced the absorption of spinosad into the leaves. Entrust possibly was more easily washed off or subjected to degradation by the sun's rays. Under idea conditions, it appears that GF-120 can be applied every 14 days and remain effective. The others need to be applied every 7 days. Increasing the longevity of insecticides may make them more useful for fly management.

4) Determine Effects of Insecticides on Larval Infestations in the Field

Numbers of flies in the test trees at Moxee were low and significant reductions in larval infestations were obtained using Entrust, GF-120, Provado, and Calypso (Table 6). No larvae emerged from the GF-120 treatment.

5) Determine Feeding Times on Insecticide Drops

Whether flies fed on insecticide drops depended on the feeding history of the fly and also on whether sugar was mixed with the insecticide solution (Table 7). When flies were not starved and no sugar was in the insecticide solutions, flies rarely fed on any treatment. When flies were starved and no sugar was in the insecticide solutions, flies still fed. There was no evidence of any deterrence, but flies fed longest on GF-120 because sugar and protein were present in this bait. When flies were starved and there was sugar in the insecticide solutions, flies fed the most and longest. Feeding again was longest on GF-120, but flies fed for relatively long periods on the other materials as well. The exception was Actara, which may have had some deterrent effect (Table 7). The results suggest that insecticides mixed with sugar alone may increase the speed of fly kill, especially if the flies are in a food-deprived state. Sugar mixed in insecticide solutions perhaps can result in better control than using insecticides only if the less toxic materials are used.

6) Effects of Sublethal Insecticide Amounts

Results using sublethal amounts of insecticides were inconclusive, although there was some evidence that sublethal doses of Entrust reduced oviposition by flies (control, mean of 31.8 eggs; lethal Entrust, 0.2 eggs; sublethal Entrust, 5.0 eggs). More tests are needed to determine if insecticides can reduce oviposition of flies that are exposed to but not killed by insecticides.

7) Determine Translaminar Effects on Cherries with Eggs and Larvae

When eggs were exposed to insecticides for 15 seconds, hatch in the Calypso treatment was significantly lower hatch in the control and Entrust and Provado treatments (Table 8). Greater than 90% of eggs in all treatments hatched between 3-7 days old, and thus it appeared the insecticides did not delay hatching. Up to 12, 48, and 24% of unhatched eggs exposed to Entrust, Provado, and Calypso, respectively, contained fully developed but dead larvae. In contrast to a 15-second exposure, eggs exposed continuously to all insecticides never hatched. Up to 60, 68, and 62% of eggs exposed to Entrust, Provado, and Calypso, respectively, contained fully developed but dead larvae. This shows that despite its relatively low toxicity to adults, Calypso is highly toxic to eggs. Calypso could be used during early season when cherries have only eggs as the life stage. An obstacle to overcome is how to make Calypso penetrate the fruit quickly.

When small larvae were exposed to insecticides, Calypso caused the highest mortality (Table 9). Large larvae were affected differently, because there was either no effect or a negative effect on larval survival when they were exposed to insecticides (Table 9). When infested cherries were sprayed, there was no evidence the large larvae were killed by any of the insecticides, based on dissections of fruit. However, larval emergence rates were reduced, indicating movement of all materials into the cherries. The materials either prevented egg hatch or killed a small percentage of the small larvae. The results strongly suggest that sprays applied on unpicked fruit after harvest can reduce larval populations.

In 2005, larval emergence from treated fruit was lower than in the control at days 1-15 (Table 10). At days 16-30, emergence was lowest from the Provado and Calypso treatments, although Calypso was not different from GF-120. Entrust was not different from the control. Over the 30 days, all treatments had significantly lower emergence than the control, with Provado having the lowest

numerically, even though it was not different from GF-120 (Table 10). There was no effect of any material on mortality of pupae at 30-37 days post-treatment (Table 10). Results here also suggest Provado could be used as an effective post-harvest spray.

In 2006, all materials reduced larval emergence from cherries, except for Avaunt (Table 11). Of those that did reduce emergence, Guthion was the most effective, and Entrust was the least effective numerically (Table 11). The numbers of larvae that emerged increased as the season progressed, but the relative effects among materials were fairly constant. This suggests that, despite reducing emergence, the other materials should be improved for their ability to be absorbed into fruit tissue or that their toxicities against eggs or larvae need to be synergized and increased.

Significance to the Industry and Potential Economic Benefits

The significance of the results from this project to the cherry industry is that it identifies potential alternatives to the use of one type of chemistry for fly control and identifies the mechanisms of kill of some of the insecticides and materials. Specifically, GF-120, Entrust, and Provado appear to be the most effective products tested against all life stages of the fly, with Calypso having effects mostly against eggs. The use of the most effective insecticide reduces the risk of infestations in orchards and of bins being rejected at the packinghouse. The continued use of one material, including spinosad, may potentially result in resistance, if not in fruit flies, then perhaps in other, non-target pests on cherries such as leafrollers, thrips, or even beetles. The use of insecticides with different chemistries may reduce the chances that control will be needed for these pests. Insecticidal control of other insect pests on cherries would clearly incur more spray costs.

Table 1. Effects of insecticides and bait on mean cumulative percent mortality of adult cherry fruit flies at 1-4 days after exposure in the laboratory

Test 1: 100 μl solution				
Treatment	1 day	2 days	3 days	4 days
Control	2.5	3.8	8.8	16.3
Entrust	77.0	94.8	100.0	100.0
GF-120	57.3	79.3	89.7	96.3
Provado	40.5	58.5	63.3	68.5
Calypso	45.0	50.0	60.0	63.3
1-way ANOVA				
df = 4, 13 *F*	16.17	9.66	8.89	7.76
P	< 0.0001	0.0007	0.0011	0.0020
Test 2: 500 μl solution				
Treatment	1 day	2 days	3 days	4 days
Control	0.0	0.0	2.0	5.0
Entrust	75.0	88.0	99.0	100.0
GF-120	70.2	86.6	93.8	97.0
Provado	50.0	65.0	87.0	89.0
Calypso	38.0	43.0	60.0	65.0
1-way ANOVA				
df = 4, 13 *F*	16.30	21.25	23.56	26.20
P	<0.0001	<0.0001	<0.0001	<0.0001

Test 1: 3 or 4 replicates; Test 2: 5 replicates; 20 flies/replicate
Means followed by the same letter within columns are not significantly different (LSD test, $P > 0.05$).

Table 2. Effects of insecticides on mean oviposition by cherry fruit fly and mortality of flies in the laboratory

	% Cherries		Cumulative % Female Mortality[b]		
Insecticide	Infested[a]	No. Eggs[a]	Day 1	Day 2	Day 3
Control	88.7a	141.2a	0.0e	3.3d	3.3c
GF-120	9.3cd	4.6cd	76.7b	96.7a	100.0a
Entrust	22.7bc	16.6bc	66.7bc	90.0ab	96.7a
Provado	7.3cd	3.0cd	42.5cd	81.7b	94.2a
Actara	14.7bc	7.0cd	80.0ab	86.7ab	93.3a
Avaunt	34.0b	27.8b	23.3d	56.7c	73.3b
Guthion	1.3d	0.4d	100.0a	100.0a	100.0a
1-way ANOVA					
F (df = 6, 28)	19.08	26.69	17.18	23.53	49.45
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

5 replicates of 6 females and 6 males; [a]Per 30 cherries; [b]Days after exposure to insecticides.
Means within columns followed by the same letter are not significantly different (LSD test, $P > 0.05$).

Table 3. Effects of insecticides on mean oviposition on treated and untreated cherries by cherry fruit flies and mortality of flies in the laboratory

	% Cherries Infested[a]		No. Eggs[a]	
Insecticide	Treated	Untreated	Treated	Untreated
Control	96.0	98.7	105.6	153.8
GF-120	12.0	24.0	2.4	10.0
Entrust	18.7	17.3	3.0	3.2
Provado	30.7	20.0	5.0	4.6
Actara	25.4	33.4	5.0	9.6
Avaunt	60.0	62.7	25.0	41.2
Guthion	4.0	6.7	0.6	1.4

5 replicates of 6 females and 6 males; [a]Per 15 cherries.

Table 4. Effects of contact with and ingestion of insecticides on mean days survived post treatment[a] by single adult cherry fruit flies

Contact with Water or Insecticide[b]					Ingestion of Water or Insecticide[c]			
Treatment	N	Males	N	Females	N	Males	N	Females
Water	17	19.1a(b)	25	1 5.5a(b)	17	16.1a(b)	18	17.3a(b)
Entrust	18	4.9a(a)	20	2.8a(a)	15	1.0a(a)	17	1.2a(a)
Provado	15	7.5a(ac)	18	10.2a(b)	17	4.3a(a)	18	7.0a(a)
Calypso	16	13.3b(bc)	18	12.3b(b)	17	6.1ab(a)	19	4.3a(a)

Water and all treatments contained 20% sucrose.
Means followed by the same letter within rows outside parentheses are not significantly different (LSD test, $P > 0.05$);
Means followed by the same letter within columns inside parentheses are not significantly different (LSD test, $P > 0.05$).

Table 5. Effects of field-aged insecticide and bait residues on leaves on mean cumulative percent mortality of adult cherry fruit fly in the laboratory at 1-7 days after exposure (DAE)

Age of Residues on Cherry Leaves at Initial Exposure to Flies				
1 DAE	0 d (fresh)	3 d	7 d	14 d
Control	3.3 a(a)	1.7a(a)	3.5a(a)	1.7a(a)
Entrust	70.0c(c)	26.0b(b)	14.6ab(a)	0.0a(a)
GF-120	78.3a(c)	72.4a(c)	70.1a(b)	79.4a(c)
Provado	34.2b(b)	17.0b(b)	23.0b(a)	5.0a(ab)
Calypso	8.3a(a)	3.5a(ab)	8.8a(a)	13.3a(b)
3 DAE	0 d (fresh)	3 d	7 d	14 d
Control	10.1a(a)	3.3 a(a)	8.6a(ab)	3.3a(a)
Entrust	95.0b(c)	76.7b(c)	65.0b(c)	8.3a(ab)
GF-120	98.3a(c)	100.0a(c)	91.1a(d)	96.9a(c)
Provado	63.0c(b)	42.3bc(b)	32.3ab(b)	11.7a(ab)
Calypso	16.7a(a)	19.3a(ab)	8.8a(a)	26.7a(b)
7 DAE	0 d (fresh)	3 d	7 d	14 d
Control	23.0a(a)	17.6a(a)	20.4a(a)	15.0a(a)
Entrust	98.3b(bc)	88.3b(c)	98.3b(c)	17.2a(b)
GF-120	100.0a(c)	100.0a(c)	98.2a(c)	100.0a(a)
Provado	91.7c(b)	57.4b(b)	41.0ab(b)	23.3a(a)
Calypso	31.7a(a)	35.1a(ab)	23.8a(ab)	35.0a(a)

5 replicates of 12 flies each (8 males, 4 females).
Means followed by the same letter within rows outside parentheses are not significantly different (LSD test, $P > 0.05$); Means followed by the same letter within columns inside parentheses are not significantly different (LSD test, $P > 0.05$).

Table 6. Effects of insecticide sprays on cherry fruit fly larval infestations of cherry in 2004 at Moxee, WA

Treatment	No. Larvae/100 Cherries	RBD ANOVA
Control	2.45a	$F = 6.35$
Entrust	0.2b	df = 4, 24
GF-120	0b	$P = 0.0012$
Provado	0.1b	
Calypso	0.2b	

7 single tree replicates of each treatment.

Table 7. Effects of different feeding histories on cherry fruit fly feeding responses to insecticide drops under laboratory conditions

Flies not starved before testing; kept on 5% sucrose up to testing; no sucrose in insecticide solutions						
	Males			Females		
Treatment	*N*	No. Feeds	Tot. Dur (min)	*N*	No. Feeds	Tot. Dur (min)
Water	11	0	0	11	0	0
GF-120	11	0.5	7.2	11	0.3	8.1
Entrust	11	0	0	11	0	0
Provado	11	0.1	0.2	11	0.1	0.5
Actara	10	0	0	10	0	0
Avaunt	11	0	0	10	0	0
Guthion	10	0	0	10	0.1	0.4
Flies starved 16-20 hours before testing; kept on 20% yeast and 80% sucrose before starving; no sucrose in insecticide solutions						
	Males			Females		
Treatment	*N*	No. Feeds	Tot. Dur (min)	*N*	No. Feeds	Tot. Dur (min)
Water	18	0.2	1.1	21	0.5	10.3
GF-120	21	0.5	27.7	28	0.8	26.7
Entrust	14	0	0	15	0.2	1.0
Provado	17	0.4	7.8	20	0.2	2.1
Actara	11	0.3	3.5	23	0.2	1.8
Avaunt	12	0.2	3.2	15	0.2	3.1
Guthion	14	0.1	0.5	21	0.3	2.6
Flies starved 16-20 hours before testing; kept on 5% sucrose before starving; 20% sucrose in insecticide solutions						
	Males			Females		
Treatment	*N*	No. Feeds	Tot. Dur (min)	*N*	No. Feeds	Tot. Dur (min).
Water	20	0.4	9.7	20	0.1	1.9
GF-120	21	1.6	74.4	20	1.6	75.1
Entrust	20	1.0	53.1	20	2.0	70.2
Provado	20	0.7	26.8	22	0.6	27.8
Actara	20	0.4	16.4	20	0.8	33.8
Avaunt	20	1.3	56.8	20	1.4	71.2
Guthion	21	0.8	45.6	20	1.4	79.2

Table 8. Effects of insecticides and insecticide exposure time on percent egg hatch of cherry fruit fly

Treatment	15-second Exposure	Continuous Exposure
Control	41.0a	42.4a
Entrust	37.0a	0.0b
Provado	25.0a	0.0b
Calypso	3.3b	0.0b
1-way ANOVA		
F	15.3 (df = 3, 20)	55.7 (df = 3, 16)
P	< 0.0001	< 0.0001

15-second exposure: 6 replicates of 50 eggs; continuous exposure: 5 replicates of 50 eggs each.
Means followed by the same letter inside parentheses within days are not significantly different (LSD test, $P > 0.05$).

Table 9. Effects of treating small and large larvae with insecticides on percent mortality of larvae and pupae of cherry fruit flies after 2 days

Small Larvae (2-3 mm long)			
Treatment	% Larvae Dead		Larval Lengths (mm)
Control	6.9a		3.5a
Entrust	69.7b		3.3a
Provado	77.3b		3.0a
Calypso	91.0c		3.1a
Large Larvae (5.5-7 mm long) Test 1			
Treatment	% Larvae Dead	% Larvae Alive	% Pupae
Control	35.0a	30.0a	35.0ab
Entrust	72.5a	10.0a	20.1a
Provado	37.5a	20.0a	42.5b
Calypso	32.5a	2.5a	65.0c
Large Larvae (5.5-7 mm long) Test 2			
Treatment	% Larvae Dead	% Larvae Alive	% Pupae
Control	38.0a	22.0a	40.0b
Entrust	90.0b	4.0 b	6.6a
Provado	72.4b	4.0 b	23.6b
Calypso	77.3b	0.0 b	22.7b

5 or 4 replicates of 10 larvae each.
Means followed by the same letter inside parentheses within dates are not significantly different (LSD test, $P > 0.05$).

Table 10. Effect of spraying cherries with insecticides and bait on larval mortality and numbers of larvae of cherry fruit flies emerging from cherries collected in Kennewick, WA, 2005

	Dead Larvae/10 Fruit[a]		Live Larvae/10 Fruit[a]	
Treatment	No.	Length (mm)	No.	Length (mm)
Control	0.2a	6.8	12.6b	4.9a
Entrust	1.0a	4.5	12.0b	4.2a
GF-120	0.8a	4.6	11.4b	4.2a
Provado	1.2a	3.5	6.4a	3.8a
Calypso	0.8a	2.1	6.4a	4.9a
	No. Pupae/100 Fruit			
Treatment	Days 1-15	Days 16-30	30-Day Total	% Pupae Dead
Control	130.8b	44.4d	175.2d	36.9a
Entrust	70.4a	33.3cd	103.4b	39.6a
GF-120	66.0a	18.6bc	84.6ab	40.4a
Provado	58.2a	6.0a	64.2a	42.3a
Calypso	89.0a	12.0ab	101.0b	39.4a

5 replicates of the control and each treatment; [a]At 8 days post-treatment; one application only.
Means followed by the same letter inside parentheses within dates are not significantly different (LSD test, $P > 0.05$); [b]Too few to analyze statistically.

Table 11. Numbers of mean numbers of cherry fruit fly larvae from detached cherries after 3 weeks of treatment with different insecticides, fruit collected in Kennewick, WA, 2006

	Cherries Collected On (Color):					
	31 May (orange)		7 June (red)		14 June (dark red)	
Insecticide	No. Larvae	% fewer	No. Larvae	% fewer	No. Larvae	% fewer
Control	1.7a	-----	10.9a	-----	32.0a	-----
GF-120	0.3bc	82	1.3cd	88	11.2bc	65
Entrust	1.1abc	35	6.9b	37	20.9ab	35
Provado	0.1bc	94	3.1bc	72	11.7b	63
Actara	0.3bc	82	6.3b	42	17.1ab	47
Avaunt	1.3ab	24	11.1a	----	30.6a	4
Guthion	0.0c	100	0.0d	100	2.3c	93
1-way ANOVA						
F	2.30		14.51		5.93	
P	0.0519		<0.0001		0.0002	

7 replicate (trees) of control and treatments. No. larvae per 27 fruit per replicate. Means within columns followed by the same letter are not significantly different (LSD test, $P > 0.05$).

FINAL PROJECT REPORT

WTFRC Project #: CH-04-401

Project Title: Fly Feeding Ecology and Food-Based Lures and Baits

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Organization: USDA-ARS

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Address: 5230 Konnowac Pass Rd

City/State/Zip: Wapato, WA 98951

Cooperators: Various homeowners in Tri-Cities and Yakima, WA

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	18,000	18,000	18,000
Benefits	2,000	2,000	2,000
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	2,000	87	87
Travel	0	0	0
Miscellaneous	0	0	0
Total	22,000	20,087	20,087

Objectives 2004-2006:
(1) Identify foods of western cherry fruit flies in nature.
(2) Determine when the flies feed, both daily and seasonally, and how much sugar and protein flies feed on in nature; amounts of foods in the environment.
(3) Determine the most attractive protein and sugar baits in the field and laboratory; baits that stimulate highest feeding and cause highest mortality.
4) Determine effects of enhancing baits with attractive compounds.
5) Determination of most effective bait sprays.

Significant Findings in 2004-2006:
● Feeding occurred mostly on leaf surfaces, with grazing behaviors most common. Bacteria and sugars on leaf surfaces are likely foods. Extrafloral leaf nectaries, cherry juice, and bird feces are food sources. The extensive grazing on leaf surfaces may result in flies finding baits frequently.
● Analyses of nutrients on leaf surfaces suggest flies need to forage over large areas to obtain their food requirements. This also suggests the extensive grazing behaviors result in chance encounters with baits and that this may be the main mechanism of fly control using baits.
● GF-20, Mazoferm, and Nulure protein and sugar baits tested were less attractive than ammonium hydroxide lures, suggesting attractiveness of baits need to be improved.
● Flies were not attracted to GF-120 or other baits from mid to long distances, suggesting mechanism of control is not through bringing flies in from around the tree.
● Flies were not attracted to GF-120, Mazoferm, or Nulure at close distances, suggesting the mechanism of control is not a close-range attraction.
● In the laboratory, mortality caused by GF-120, Mazoferm, Nulure, yeast (with Entrust [spinosad]), and Entrust alone was similar at all times post exposure to treatments, suggesting flies were encountering the drops through normal movement.
● Adding ammonia compounds to GF-120 increased its attraction in the field, suggesting attractiveness of baits can be enhanced.
● Adding ammonium acetate and ammonium carbonate did not increase feeding times by flies; feeding was longest on GF-120 alone or GF-120 with uric acid, suggesting some deterrence of feeding when ammonia compounds were added to baits.
● Mortality caused by GF-120 with or without enhancement with ammonia compounds was similar, suggesting that even short feeding times or contact with the baits (with spinosad) is sufficient to cause high mortality.
● GF-120, Mazoferm, and Nulure (with spinosad) sprayed on single cherry trees reduced the infestation levels of larval flies, but did not eliminate them; there was no evidence any one bait was superior to another. This suggests any bait with spinosad might have the same effect as GF-120.
● Spinosad alone sprayed on trees performed inconsistently; effective in one trial, not in the other. This suggests baits of any sort are more effective than Entrust alone, but this is unclear.

Methods 2004-2006:

(1) Identify Foods of Flies in Nature
In 2005, observations of feeding on different natural foods were made at one site in Zillah on 2 trees from 19 May to 12 June between 0830 and 1345 hours. In Roslyn, observations were made on 3 trees from 5 July to 4 August between 0900 and 1300 hours. A fly on a leaf or fruit was randomly selected and its feeding activities followed for a maximum of 10 min. The first fly that came into view and that could be watched from a distance of 15-25 cm was chosen. A timer was used to record the numbers and durations of fly feeding events on the leaves or fruit. The flies' mouthparts were observed closely. If a fly contacted the substrate with its proboscis, feeding was presumed to occur. Grazing consisted of rapid up and down movements of the mouthparts onto the substrate surface. Behaviors were recorded as: (1) grazing on undefined matter on leaves or fruit; (2) feeding on

discrete substances: (a) nectar from extrafloral nectaries (EFNs), located on the distal part of the leaf petiole, 0-8 mm from the leaf; (b) cherry juice stains or splatters from damaged fruit; (c) bird feces, and (d) honeydew on or near aphid colonies. Attempts were made to follow at least 5 females and 5 males on leaves and fruit each sample day. A minimum observation of 30 sec was required for data to be included in analyses. At Zillah, flies were observed on 19, 24, 26, 27, and 31 May and 3, 6, 7, 10, 13, and 14 June. At Roslyn, flies were observed on 5, 7, 11, 14, 15, 18, 20, 25, 27, 29 July and 1, 3, and 4 August.

In 2006, methods for recording feeding on different substrates were similar to those in 2005, but there were also several differences: (1) to determine if flies grazed more frequently on top versus bottom of leaves, data of grazing on the 2 locations were kept separate; (2) to increase the numbers of flies observed, each fly was followed for a maximum of 5 instead of 10 min; (3) all flies were captured using a small glass vial after observations to reduce chances of repeated observations on the same fly; (4) observations were made earlier, usually between 0800-1100 hours, because 2005 observations suggested more flies foraged during this time than later. Flies were observed on 1, 7, 9, 12, 14, and 19 June. On 9 June, observations were made for one hour from 0600-0700 hours.

(2) Determine When Flies Feed, Daily and Seasonally

In 2005, 200 leaves and 200 cherries were collected from 3 trees on 23 May and 1, 8, 15, and 22 June. Leaves and fruit were dipped in water to remove sugars and other materials. Washings were placed inside bottles. At the same time, as many flies as possible were collected from the 3 trees using glass vials. Flies were immediately frozen in the field inside metal cans inserted in dry ice in Styrofoam boxes. All samples were then frozen at -80 ºC for later processing. Sugars and other substances from leaf and fruit washings and flies were analyzed using HPLC.

(3) Determine Most Attractive Baits; Baits that Stimulate Feeding and Cause Mortality

To test attraction of flies to ammonia and GF-120, Mazoferm, and Nulure, bait drops were applied on 5 leaves on the south sides of 3 to 6 cherry trees (0900-1500 hours) in Zillah and Roslyn in 2004. The ammonia lure tested was a Nalgene bottle with a 0.05 cm hole. The bottle contained 10 ml of ammonium hydroxide saturated in cotton. Total bait volumes applied were 500 or 1,000 μl per 5 leaves. Flies seen feeding on the bait or within a 30 cm distance of the lure or baits were counted. For each test, fly numbers on leaves and fruit were recorded every 2 min for 30 min. After observations were made, leaves were removed and discarded. Branches were shaken to dislodge flies from the leaves or fruit. Positions of treatments were randomized after the first observation.

To determine long- to medium-range attraction to and feeding on baits, 2 tests were conducted in 2005 in Zillah using 40% concentrations of GF-120, Nulue, and Mazoferm. Test 1 was conducted using a total of 500 μl of water or baits: a volume of 100 μl water or bait (no spinosad) was applied on each of 5 randomly selected leaves. The control and each bait were applied on the south side of the same tree. Treatments were about 1-1.5 m apart, 1-2.5 m above the ground. Test 2 used 10 ml of the same solutions applied on a 45-60 cm stretch of a randomly selected branch with 20-40 leaves and fruit, using a 32-oz volume spray bottle. In both, numbers of female and male flies within 15 cm of droplets or that fed on the bait were recorded every 1.5-2 min for 30 min. Flies that clearly were the same at successive intervals were counted as one. All sprayed leaves were immediately removed after observations. Observations were made between 0900 to 1400 hours. An observer made observations of flies higher in trees while standing on a ladder. On each date, observations from one tree constituted a replicate, each with a control and one of each treatment. Test 1 was conducted on 24, 27, and 31 May. There were 5 replicate trees on 24 and 27 May and 3 trees on 31 May. Test 2 was conducted on 7, 10 and 14 June, with 3 replicate trees each day.

To determine close-range attraction, in 2005, one test was conducted in Zillah. The method used was to simulate situations where drops were detected by flies as they looked for food. About 25 μl of 40% GF-120, 40% Mazoferm, 40% Nulure or water were slowly placed 1-2 cm from a randomly selected fly on the top or underside of a leaf using a micropipette. This method was used in place of spraying because spraying would have resulted in direct contact of bait with the flies. Drops were placed in front of still or walking flies, but because flies sometimes moved, drops often ended up

behind or on the side of flies. Most flies did not fly off or move when drops were placed near them, and thus did not appear to the observer to be disturbed by the presence of the observer and pipette tip. A fly was watched for a maximum of 5 minutes, and numbers and durations of all feeding events were recorded. Flies that flew off before 5 minutes were also used in analyses as long as they stayed a minimum of 15 seconds. After observations, flies were collected whenever possible using a glass vial to reduce chances that the same fly would be observed again. Treated leaves were removed. Observations were made 1-3 m above ground with the observer standing on a ladder if necessary. One to 3 trees were used on 25, 26, 27, 31 May and 2, 3, 6, 7, 9, 10, and 13 June. On each day, treatments were alternated so that each of the treatments and the control were tested before another set of the same materials were tested. There were 1 to 3 flies of each sex tested per treatment or control/day. Solutions were made fresh daily.

In 2006, the protocol to determine close-range attraction to baits was similar to that used in 2005, except for 3 differences. First, spinosad (Entrust) was added to all treatments. Second, in addition to a control and GF-120, Mazoferm, and Nulure treatments, spinosad alone was tested. Third, droplets were applied only to the top surface of leaves. Observations were made on 19, 22, 23, 24, 25, 26, 30, and 31 May and 5, 6, 8, and 9 June between 0800 and 1100 hours. There were large differences in weather during the roughly first half and second half of the season (the season based on 4 weeks when fly numbers are highest), unlike in 2005, so data from the 2 periods were compared.

4) Determine Effects of Enhancing Baits with Attractive Compounds

To test attraction of flies to ammonia and ammonia-enhanced GF-120, tests similar to those described for regular baits (above, objective 3) were done in Zillah and Roslyn in 2004. An ammonia lure was compared with Mazoferm + 10% ammonium carbonate (AC) (wt:wt), NuLure + 10% AC, and GF-120 + 10% AC.

In the laboratory, a test was conducted to determine the feeding responses of flies to enhanced bait. Water, GF-120, GF-120 + 10% uric acid (component of bird feces), GF-120 + 10% AA, GF-120 + 10% AC, and Entrust (spinosad) only were applied on an artificial leaf inside a half gallon cage. Five males and 5 females were released inside the cage. Observations were made over one hour of numbers of flies that fed, the feeding durations, and the time spent on the leaf not feeding. Another test was conducted to determine fly mortality caused by these treatments. A volume of 50 μl bait (as 3 drops) was placed on a dish on the bottom of a cage. A total of 30 flies was released inside cage. Mortality was determined at 2, 4, 6, 8, 24, and 48 hours after exposure.

5) Determination of Most Effective Bait Sprays

In 2005, 40% GF-120, 40% Mazoferm, and 40% Nulure were sprayed on residential cherry trees from May to June. Trees were 4-5 m tall and isolated or occurred in groups of 2 to 5. To determine the presence of and the approximate first emergence of flies, a sticky yellow panel was placed on each tree on 9 May and checked every day or 2 days for flies. Traps were baited with a lure containing 10 g of ammonium carbonate with two 1-mm holes. Three days after the first fly capture (on 13 May), the first application was made. Sprays were delivered using 1.18 liter RL Flo-Master© pressurized sprayers. For the GF-120 treatment, 90 ml of GF-120 was mixed in a total volume of 225 ml and applied on one tree (recommended rate for "spot spray of individual plants"). Mazoferm and Nulure were applied at the same rate. There were 8 control and 4 or 5 treatment trees. Sprays were applied as ~8 streaks using an upward motion around the periphery of each tree. Droplets varied in diameters, ~4-6 mm. Some of the "droplets' were streaks of spray. Sprays were applied every 7 days, except once when it rained, in which case they were applied 3 days after a previous spray. Applications were made on 16, 19, 26 May, and 2, 9, and 16 June. Numbers of flies on traps were counted on all spray dates. Fruit from all trees were picked by 24 June. Fruit loads were low in most trees due to frost during fruit set in April and May, so a wide range in numbers of cherries were picked, from 14 to 506 per tree. For determining larval infestations, fruit were laid on hardware cloth on tubs and held outdoors. Numbers of pupae in the tubs after $\geq$ 30 days were recorded. Trees with traps that yielded no flies were dropped from the study.

In 2006, a spray protocol similar to that in 2005 was followed, except for the following, in order to match the feeding response test in 2006. First, in addition to a control and 40% GF-120, 40% Mazoferm, and 40% Nulure treatments, a spinosad (Entrust) only treatment was used, the same as in the feeding response test. Two tests were run. In test 1, a site in Zillah was used and 75 ml of spray was applied per tree, because trees were pruned and thus had much less foliage than trees used in test 2 (below). Each tree was 3-5 m tall. There were 3 replicate blocks of trees at this site, each with the control and 4 treatments. Blocks were different locations within the yard. In test 2, data from sites used in Zillah, Toppenish, and Yakima were pooled. The spray volume was 150 ml per tree. Each tree was 4-5.5 m tall. There were 7 control and 3 or 6 treatment trees. Only trees that had at least one fly captured on traps were used.

In both 2006 tests, the first sprays were made within 7 days of first fly capture. In test 1, traps were hung on trees on 8 May; there were 5 spray applications. In test 2, traps were hung 11 to 12 May; there were 5 to 7 applications. The range of applications was needed because there were different varieties of cherries with early or late developing fruit within the test. Also, birds threatened to remove all the cherries on some. Due to these factors, there was also variability in fruit picking dates, with 1 to 4 per tree. Unlike in 2005, trees bore heavy cherry fruit loads.

Results and Discussion

(1) Identify Foods of Flies in Nature

For simplicity, data over the season and not on a daily basis are shown. Grazing on leaves occurred much more frequently than feeding on cherry juice on leaves, bird feces on leaves, and extrafloral nectaries (EFNs) (Table 1). This was true on every date. Grazing occurred on every date, whereas feeding on EFNs, cherry juice, and bird feces was seen only on one, three, and one of the 11 dates, respectively. No aphid colonies were seen. On fruit, female and male flies rarely fed (Table 1). Males frequently stayed on fruit for entire 10-min observations. There were no differences between sexes (Table 1). In 2006, as in 2005, grazing on leaves occurred more frequently than feeding on cherry juice on leaves, bird feces on leaves, and EFNs (Table 1). This was true on every date. Grazing occurred on every date, but flies were seen feeding on EFNs, cherry juice, and bird feces only on 2, 4, and 3 of 6 dates, respectively. Nectar was seen in EFNs on every date. No aphid colonies were seen. The extensive grazing on leaf surfaces may result in flies finding baits.

Similarly, in Roslyn, of 49 females and 34 males on leaves, 10.2% and 11.8%, respectively, grazed leaves whereas none fed on EFNs and bird feces. Two females fed on cherry juice on leaves. Grazing and feeding on cherry juice were seen on 5 and 2 of the 13 dates, respectively.

Overall results suggest the extensive grazing behaviors observed can result in chance encounters with baits and that this may be the mechanism of control using baits.

(2) Determine When Flies Feed, Daily and Seasonally

Sugar analyses of flies throughout the season indicated consistently high levels, suggesting flies are able to find and feed on sugars regardless of the absence or presence of ripening cherries. Early analyses of sugars in the environment suggest diffuse food sources on cherry trees (flies and leaf samples have not all been processed and many are still frozen). The diffuse food sources may force flies to graze over large areas of the tree.

(3) Determine Most Attractive Baits; Baits that Stimulate Feeding and Cause Mortality

In the field, flies were not attracted to GF-120, Mazoferm, or Nulure baits from far distances, although they were to ammonium hydroxide lures (Table 2). A repeat of a similar test in 2005 revealed similar results: flies were not drawn to the GF-120 or Nulure and Mazoferm (Table 3). In 2005, when GF-120, Nulure, or Mazoferm were placed close to flies on leaves, flies were also not attracted to them (Table 4). In 2006, when spinosad (Entrust) was added, this same pattern was observed (Table 5). There was, however, a seasonal effect on fly responses. Feeding responses were greater during the second half of the season (Table 5). Results suggest that control should be similar using the different baits and that the baits should result in faster kill later in the season because either

more flies respond to them or they respond more quickly to them. Results suggest there is no benefit of using GF-120 over Nulure or Mazoferm with spinosad.

In the laboratory, exposure of flies to GF-120, Nulure, Mazoferm, yeast, and Entrust all resulted in similarly high mortality of flies that were exposed to sugar only (Table 6). However, low mortality was seen across all treatments when flies were exposed to a sugar and yeast strip during the test (data not shown). The similar mortality among treatments is consistent with observations that none of the baits was superior to the others tested.

4) Determine Effects of Enhancing Baits with Attractive Compounds

When GF-120, Mazoferm, and Nulure were enhanced with ammonium carbonate, attraction to the baits was higher than to the control (Table 7). However, despite the greater attraction to enhanced GF-120, feeding on the GF-120 enhanced with AA or AC was not increased in the laboratory. In fact, the numbers of feeds and durations of feeds were highest on GF-120 alone and GF-120 + uric acid (Table 8). When flies were exposed to GF-120 alone or to GF-120 with ammonia compounds, mortality over time was similar (Table 9). The results suggest there are differences between attraction and feeding on ammonia-enhanced baits. An ideal enhanced bait should attract flies to the bait and once there, stimulate the flies to feed. However, it could be that even short feeding times (and therefore small amounts ingested) are sufficient to kill the flies.

5) Determination of Most Effective Bait Sprays

In 2005, when cherry trees were sprayed with 225 ml bait/tree, there were no differences in numbers of adult flies trapped among control and treatments, although numerically there were fewer flies in treatment than control trees (Table 4). Numbers of larvae per fruit were not significantly different in the control and the GF-120 treatment, but numbers in Nulure and Mazoferm treatments were significantly lower than in the control (Table 4). During the first 14 days of the test, there were 4 days of rain and 1.02 cm of precipitation. During the entire 40-day test (first spray to last fruit picking), there were 7 days of rain, for 1.25 cm total precipitation. Because there was relatively little rain, it is unlikely it affected results.

In 2006 in test 1, when trees were sprayed with 75 ml bait/tree, there were no significant differences in adult flies trapped and in larval infestations among control and treatment, although numerically there were fewer larvae per fruit in all treatments than in the control (Table 4). Larval infestations per fruit were much lower than in 2005. This was also true in test 2, when trees were sprayed with 150 ml bait/tree (Table 4). Fly populations in test 2 were lower than in test 1, and larval infestations were low even in control fruit. During the first 14 days of the test 1, there were 10 days of rain and 2.63 cm of precipitation. During the entire 36-day test (first spray to last fruit picking), there were 13 day of rain, for 3.17 cm total precipitation. For test 2, precipitation the first 14 days was the same as in test 1, but over the 50-day test, there were 15 days of rain, for a total of 3.32 cm precipitation. The rain may have affected results if they diluted the bait sprays.

The overall conclusion of the bait spray tests is that, under the fly densities and precipitation conditions encountered, the baits were unable to prevent larval infestation. At the very least, this could mean that the baits with spinosad did not kill the flies quickly enough to prevent egg laying. It is possible that after the flies laid the eggs, they fed on the spinosad in the baits and died. Whether the failure to prevent egg laying was the result of flies that matured (over 7 days) while on test trees and did not find the bait or the result of mature flies migrating in from surrounding trees was not determined. However, in isolated trees, the chances of this occurring seemed low.

Significance to the Industry and Potential Economic Benefits

The results of this project are significant to the cherry industry because they identify a mechanism of cherry fruit fly control using baits, explaining why GF-120, Mazoferm, and Nulure are similar in their effectiveness. Results using GF-120, Mazoferm, and NuLure show none is attractive and suggest flies find baits through normal foraging behavior rather than through a strong directed orientation towards odors. Identification of preferred foods for the flies is one step towards determining attractants or stimulants that can be incorporated into baits to make them more attractive and possibly

more effective. Results suggest that use of Mazoferm and Nulure could reduce costs to growers, who need to spray baits often during the cherry season, especially when there is much rainfall. Additional work on the use of effective and long-lasting baits may help reduce spray frequencies and may further reduce chances larvae are ever found in fruit.

Table 1. Percentages of cherry fruit flies engaged in feeding on various substrates on sweet cherry leaves and fruit in over the season in 2005 and 2006, Zillah, WA

2005: On Leaves						
	Sex	N	Grazing On Leaf	EFN	Cherry Juice	Bird Feces
Season Totals	F	77	37.7	1.3	3.9	1.3
	M	70	22.9	1.4	4.3	0
F vs. M		X^2	3.76	-----	-----	-----
		P	0.0526	-----	-----	-----

2005: On Fruit					
	Sex	N	Grazing	Cherry Juice	Feces
Season Totals	F	29	13.8	3.4	0
	M	99	0	1.0	0

2006: On Leaves							
	Sex	N	Grazing On Leaf	EFN	Cherry Juice	Bird Feces	EFN, Juice, Feces: X^2, *P*
Season Totals	F	131	45.0	1.5	6.9	6.1	4.57, 0.1016
	M	130	45.4	1.5	4.6	2.3	2.39, 0.3029
F vs. M		X^2	0.00	-----	0.60	2.27	
		P	1.0000	-----	0.4386	0.1317	

2005 - each fly observed for maximum of 10 min; 2006 – observed for 5 min; observations made between 0830 and 1430 hours (PST). EFN, extrafloral nectary; Data not analyzed when cells <5. 2005: season totals from 11 d. 2006: Season totals from 6 d.

Table 2. Effects of ammonium hydroxide lure and protein baits on mean numbers of cherry fruit flies attracted, May-June 2004, Zillah, WA

	Days[a]	Control	NH_3	Mazoferm	Nulure	GF-120
500 ul/leaf[b]	5	0.62	3.71	0.88	0.47	0.69
1,000 ul/leaf[c]	4	0.05	1.84	0.62	0.20	0.41

[a]3-6 replicate trees per day; [b]Zillah; [c]Roslyn

Table 3. Effects of baits applied on leaves of cherry trees on numbers of cherry fruit flies feeding on or near baits, May-June 2005, Zillah, WA

Test 1: 500 μl of bait spread on 5 leaves						
	No. Feeding			No. 15 cm From Bait, No Feeds		
Treatment	5/24	5/27	5/31	5/24	5/27	5/31
Water	0	0	0	0	0	0
40% GF-120	2	0	0	4	5	0
40% Nulure	0	0	0	2	0	4
40% Mazoferm	0	0	0	0	7	0
Test 2: 10 ml of bait sprayed on 20-40 leaves						
	No. Feeding			No. 15 cm From Bait, No Feeds		
Treatment	6/7	6/10	6/14	6/7	6/10	6/14
Water	0	0	0	4	5	1
40% GF-120	1	2	1	4	7	1
40% Nulure	1	0	1	5	0	1
40% Mazoferm	2	0	1	3	2	2

5 replicate trees on 24 and 27 May; 3 replicate trees on other dates.
On each date, recordings were made every 2 min for 30 min on each tree with the control and 3 treatments; totals of 39 females and 29 males recorded.

Table 4. Effects of placing bait droplets near cherry fruit flies on leaves on numbers of feeds and feed durations (min) on cherry trees, May-June 2005, Zillah, WA

Bait	Sex	*N*	% Response	No. Feeds	Feed Duration
Water Only	F	25	48.0	0.72	0.07
	M	20	35.0	0.80	0.10
Blank 40% GF-120	F	27	48.1	1.15	0.62
	M	20	50.0	1.05	0.50
Blank 40% Nulure	F	20	35.0	1.20	0.19
	M	21	42.9	0.33	0.09
Blank 40% Mazoferm	F	23	47.8	1.04	0.23
	M	20	25.0	0.30	0.03
2-Way ANOVA	Bait	df = 3, 168	$F = 0.93$; $P = 0.4252$	$F = 7.30$; $P = 0.0001$[a]	
	Sex	df = 1, 168	$F = 1.57$; $P = 0.2124$	$F = 1.46$; $P = 0.2288$	
	Bait × Sex	df = 3, 168	$F = 0.59$; $P = 0.6235$	$F = 0.37$; $P = 0.7770$	

[a]Feeding duration on GF-120 > on water, Nulure, and Mazoferm.

Table 5. Effects of placing bait droplets near cherry fruit flies on leaves on numbers of feeds and feed durations (min) on cherry trees, May-June 2006, Zillah, WA

		N		% Response		No. Feeds	
Bait	Sex	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
Water Only	F	18	9	44.4	55.5	0.56	1.11
	M	17	24	47.1	62.5	0.71	1.04
40% GF-120	F	27	16	40.7	75.0	0.74	2.19
	M	12	19	16.7	57.9	0.33	1.26
40% Nulure	F	18	20	27.8	50.0	0.78	1.25
	M	23	17	21.8	58.8	0.43	1.24
40% Mazoferm	F	21	20	23.8	65.0	0.38	1.10
	M	16	15	18.8	66.7	0.19	1.00
Spinosad Only	F	14	16	7.1	68.8	0.07	1.38
	M	26	15	34.6	46.7	0.54	0.87
3-Way ANOVA	Bait	df = 4, 343				$F = 1.30$; $P = 0.2708$	
	Sex	df = 1, 343				$F = 1.72$; $P = 0.1905$	
	Period	df = 1, 343				$F = 39.59$; $P < 0.0001$	
	Bait × Sex	df = 4, 343				$F = 0.93$; $P = 0.4447$	
	Bait × Period	df = 4, 343				$F = 0.81$; $P = 0.5170$	
	Sex × Period	df = 1, 343				$F = 0.75$; $P = 0.3878$	
	Bait × Sex × Period	df = 4, 343				$F = 1.01$; $P = 0.4033$	

Each bait and the spinosad solution contained 0.0096% spinosad; Period 1, 19 to 30 May; Period 2, 31 May to 9 June.

Table 6. Cumulative percent mortality of cherry fruit flies exposed to various baits with spinosad at different times after exposure in the laboratory

Treatment	2 hours	4 hours	6 hours	8 hours	24 hours	48 hours
Water	0	0	0.7	0.8	1.6	2.7
GF-120	3.3	11.3	19.1	34.7	66.0	90.0
Nulure	2.7	8.7	29.3	46.7	88.0	97.3
Mazoferm	1.3	6.0	15.3	26.7	67.3	90.7
Yeast	0.7	10.7	22.7	42.7	78.0	94.7
Entrust	4.0	16.7	24.0	32.7	72.0	94.0
Blank GF-120 + Entrust	4.7	14.0	24.7	42.0	70.0	83.3

5 replicates, 30 flies each.

Table 7. Effects of adding ammonium carbonate (AC) on mean numbers of cherry fruit flies attracted to baits, Zillah, and Roslyn, 2004, WA

	Days[a]	Control	NH_3	Mazoferm +AC	Nulure +AC	GF-120 + AC
500 μl/leaf[b]	3	0.10	-----	0.98	1.33	0.32
1000 μl/leaf[c]	4	0.03	0.41	0.34	0.30	0.29

[a]Each day with 3 or 6 replicates; [b]Zillah; [c]Roslyn.

Table 8. Feeding responses of cherry fruit flies to GF-120 enhanced with various compounds in the laboratory

Treatment	No. Feeds	Feed Durations (min)	Dur. Non-Feeds (min)[a]
Control	0.17	0.01	2.55
GF-120	3.83	2.46	53.82
GF-120 + Uric Acid	4.17	2.06	28.30
GF-120 + AA	1.00	1.04	21.87
GF-120 + AC	0.33	0.07	20.93
Entrust Only	0.17	0.02	14.37

[a]On artificial leaf; 6 replicates each of 5 males and 5 females; AA, ammonium acetate; AC, ammonium carbonate.

Table 9. Cumulative mortality of cherry fruit flies exposed to GF-120 enhanced with various compounds at different times post exposure in the laboratory

Treatment	2 hours	4 hours	6 hours	8 hours	24 hours	48 hours
Control	0	0	0	0	3.3	20.0
GF-120	3.3	13.3	18.4	28.4	51.7	81.6
GF-120 + Uric Acid	1.7	10.0	20.8	34.2	51.6	76.6
GF-120 + AA	3.3	3.3	3.3	3.3	30.0	73.3
GF-120 + AC	2.5	5.0	7.5	20.0	51.7	76.7
Entrust Only	5.0	8.3	10.0	13.3	33.3	48.3

2-4 replicates per treatment; AA, ammonium acetate; AC, ammonium carbonate.

Table 10. Effects of bait sprays on mean numbers of cherry fruit fly adults and larvae from cherries using single tree replicates in Yakima County, WA, 2005 and 2006

2005: Yakima (16 May -24 June), 225 ml spray/tree					
Treatment	*N*	Flies/Trap[a]	Larvae/Fruit	% Fewer	No. Fruit Picked/Tree
Control	8	257.2	0.907a	-----	214.6
40% GF-120	4	16.0	0.501ab	45	73.8
40% Nulure	5	49.0	0.412b	55	233.4
40% Mazoferm	5	53.0	0.125b	86	273.2
1-way ANOVA df = 3, 18		$F = 2.31$ $P = 0.1111$	$F = 6.90$ $P = 0.0027$	-----	-----
2006: Test 1: Zillah (22 May – 26 June), 75 ml spray/tree					
Treatment	*N*	Flies/Trap[b]	Larvae/Fruit	% Fewer	No. Fruit Picked/Tree
Control	3	40.0	0.086	-----	194.3
40% GF-120	3	71.7	0.050	42	401.0
40% Nulure	3	115.7	0.004	95	496.7
40% Mazoferm	3	57.7	0.034	60	236.3
Spinosad Only	3	48.3	0.007	92	284.7
RBD ANOVA Df = 4, 8		$F = 1.22$ $P = 0.3760$	$F = 0.86$ $P = 0.5267$	-----	-----
2006 Test 2: Zillah, Toppenish, Yakima (22 May-10 July), 150 ml spray/tree					
Treatment	*N*	Flies/Trap[c]	Larvae/Fruit	% Fewer	No. Fruit Picked/Tree
Control	7	14.9	0.031	-----	368.0
40% GF-120	6	7.0	0.001	97	590.7
40% Nulure	6	6.0	0.004	87	576.0
40% Mazoferm	3	16.0	0.004	87	419.0
Spinosad Only	6	20.0	0.027	13	454.0
1-way ANOVA df = 4, 23		$F = 0.70$ $P = 0.6007$	$F = 2.04$ $P = 0.1215$	-----	-----

Each bait and spinosad solution contained 0.0096% spinosad.
Dates inside parentheses are first spray to last fruit picking; [a]over 42 d; [b]over 46 d; [c]over 57 d.
Means within columns followed by the same letter are not significantly different (LSD test, $P > 0.05$).

FINAL PROJECT REPORT
WTFRC Project Number:

Project Title: Improving Cherry Fruit Quality and Postharvest Shelf Life
PI: Larry Schrader
Organization: WSU Tree Fruit Research and Extension Center
Address: 1100 N. Western Avenue
City/State/Zip: Wenatchee, WA 98801

Cooperators: Jizhong Xu and Cindy Kahn
WSU Tree Fruit Research and Extension Center, Wenatchee, WA 98801

Budget History:

Item	Year 1: 2005	Year 2: 2006
Salaries	10,608	11,032
Benefits	4,031	4,192
Wages	2,000	2,000
Benefits	320	220
Equipment		
Supplies	3,000	3,000
Travel	500	500
Miscellaneous		
Total	20,459	20,944

Objectives:

1. Investigate the effects of formulations on stem browning and water loss of cherries after harvest.
2. Compare water loss in stem-free cherries to water loss in cherries with stems.
3. Apply gibberellic acid at different stages of maturity and study its effect on fruit quality.
4. Study efficacy of GA when tank mixed and applied with RainGard.
5. Conduct microscopic studies to determine anatomical differences among cultivars that differ in their susceptibility to rain cracking.
6. Conduct further studies to improve efficacy of RainGard, cherry cracking suppressant.

Significant findings:

1. Water loss in stem-free cherries during cold storage was reduced by the following dip treatments: 20 ppm GA_3, 5% RainGard, and 20 ppm GA_3 + 5% RainGard for 10 seconds after harvest. The most effective treatments were 5% RainGard and 20 ppm GA_3 + 5% RainGard.
2. Water absorption by cherries dipped postharvest in RainGard alone or RainGard + GA was also reduced significantly as compared to controls or GA alone.
3. Quality factors such as firmness, soluble solids content, and water loss in stem-free cherries was not significantly different from cherries with stems during cold storage.
4. Cracking in Bing cherries was decreased by 47%, on average, with four weekly applications of RainGard in three orchards in The Dalles, Oregon, during 2005.
5. Cracking in Rainier was more severe than in Bing, and RainGard was less effective in suppressing cracking of the Rainier cherries. The suture of Rainier appears to be very susceptible to cracking.
6. For Sweetheart, four RainGard applications at weekly intervals during 2005 decreased cracking by 38%.
7. With Tieton, four RainGard applications during 2005 decreased cracking by 35%.
8. Based on many studies, a program that includes four weekly applications of RainGard prior to harvest is recommended for best protection of sweet cherries from rain cracking.

Materials and Methods:

Objectives 1 and 2: Stem-free Bing cherries were harvested on June 26, 2006, from trees treated with 550 ppm Ethephon at one and two weeks before harvest. The method for harvesting stem-free cherries consisted of shaking tree limbs and catching cherries on a raised plastic tarp. For each replication, 25 cherries of uniform quality were placed in plastic clamshells. Cherry firmness was measured weekly with a FirmTech 2 fruit firmness tester (Bio Works, Inc. U.S.A.). Cherries were removed from cold storage and warmed to room temperature two hours prior to testing. Water loss was determined by weighing the cherries weekly. Water loss was calculated with the following formula: (W1-W2)/W1. W1 = initial weight and W2 = final weight. Soluble solids content was measured weekly with a digital refractometer, and results were expressed in degrees Brix.

Objectives 3 and 4: Initial experiments to test efficacy were done during 2005 on single trees in a two-way factorial randomized complete block with four replications. GA was applied alone at different concentrations and also tank mixed with RainGard before application. Applications were sprayed on cherries at different intervals prior to maturity.

In 2006, stem-free cherries at room temperature were dipped in different solutions for 10 seconds after harvest. Treatments included water, 20 ppm GA_3, 5% RainGard, and 20 ppm GA_3 + 5% RainGard. A control with no treatment was also included. Treatments of 20 ppm GA_3 + 5% RainGard

were done for 30 and 60 seconds. Treatments contained four replicates of 25 fruit each. The fruit were placed in cold storage and evaluated for quality and water loss.

Water absorption: Cherries were weighed prior to treatments, dipped in the various treatments for different amounts of time and then blotted dry with paper towels. Cherries were weighed again when they were dry. Water absorption was expressed by the difference in cherry fruit weights (weight of 25 cherries after treatment minus weight of cherries before treatment).

Fruit firmness: Cherry firmness was measured weekly with a FirmTech 2 fruit firmness tester (Bio Works, Inc. U.S.A.). Cherries were removed from cold storage and warmed to room temperature two hours prior to testing.

Water loss in storage: The cherries were weighed weekly. The water loss rate was calculated with the following formula: W1-W2)/W1. W1 =initial weight and W2 = final weight.

Soluble solids content (SSC): SSC was measured weekly with a digital refractometer, and results were expressed in degrees Brix.

Objective 5: Digital images were taken with a Nikon SMZ-U dissecting microscope to observe differences in the structure of the stylar scar end of each of several cultivars.

Objective 6: In 2005, 13 grower/cooperators were selected for efficacy testing of RainGard, a new experimental product to protect cherries from cracking. Locations of these test sites varied widely from Kennewick and Pasco, Washington, on the east to Tonasket, Washington, on the north and to The Dalles, Oregon, on the west. Sufficient rain to cause measurable cracking occurred at only six sites. The predominant cultivar studied was Bing although at least one of the trials included Staccato, Sweetheart, Rainier or Tieton. Quality data (fruit weight, color, firmness, soluble solids and titratable acidity) were collected on fruit from 10 of the 13 trials.

All treatments were applied by grower/cooperators. The four treatments for every trial were as follows:

A. 10% (v/v) RainGard, two applications—at straw color of fruit (or slightly earlier if rain were imminent) and two weeks after the first application;

B. 5% (v/v) RainGard, two applications with same timing of applications as with treatment A;

C. 5% (v/v) RainGard, four weekly applications—first application same as above, and then weekly thereafter;

D. Untreated control (i.e., no application of RainGard).

Results and discussion:

***Objectives 1 and 2*:** In cold storage, water loss in 2005 in stem-free Sweetheart cherries as well as stemmed cherries was reduced significantly when cherries were dipped into RainGard for 10 seconds immediately after harvest (data not shown).

Water loss studies in 2006: The weekly changes in cherry water loss during cold storage are shown (Fig. 1). The water loss of the stem-free fruit was slightly higher than that of fruit with stems during cold storage, but differences were not statistically significant. At 8 weeks after harvest (WAH), the total water loss was 5.52% for the stem-free cherries and 5.05% for the stemmed fruits.

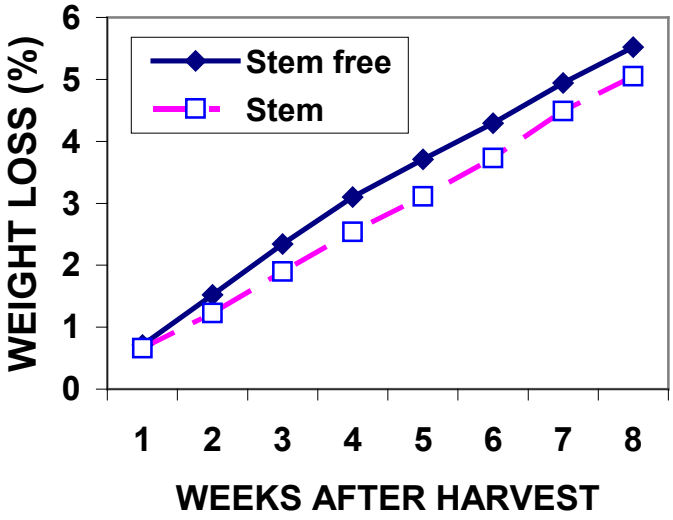

Figure 1. Cumulative water loss from stem-free and normal cherries during cold storage.

Fruit firmness: Changes in firmness during cold storage were similar between stem-free cherries and cherries with stems. The firmness of cherries with stems increased at 5 WAH, while the stem-free cherries began to decrease in firmness (Fig. 2). Firmness of fruit with stems was higher than that of stem-free fruit during cold storage, but differences between the two types of fruit were not statistically significant. Firmness of the stem-free fruit was 218.5 g/mm at harvest and 223.7 g/mm for the fruit with stems. The firmness of the cherry fruit usually increased from 1 WAH to 5 WAH (for stem-free fruit) and from 2 WAH to 6 WAH (for fruit with stems) during cold storage. The firmness of the stemmed fruits was 253.4 g/mm at 6 WAH, which was an increase of 13.3% from the firmness at harvest. Firmness was the highest (237.6 g/mm) at 5 WAH for the stem-free fruit, an increase of 8.7% from harvest. These observed increases in firmness during cold storage were not expected but may be attributable to loss of water during storage.

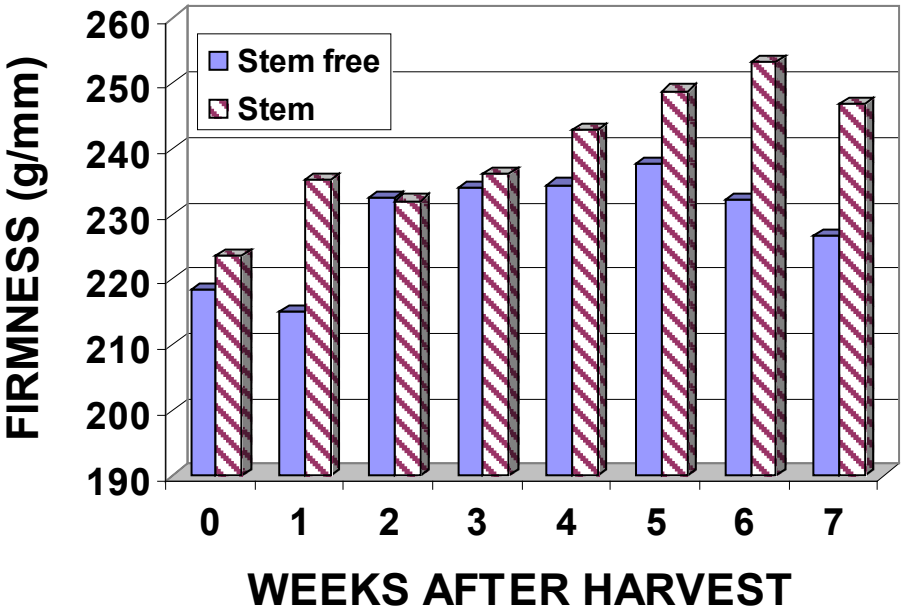

Figure 2. Cherry firmness during cold storage.

Soluble solid content (SSC): SSC was 16.7% for fruit with stems and 16.9% for the stem-free fruit at harvest, respectively. The SSC in the fruit with stems was lower than that of the stem-free fruit, but differences were not significant statistically (Fig. 3).

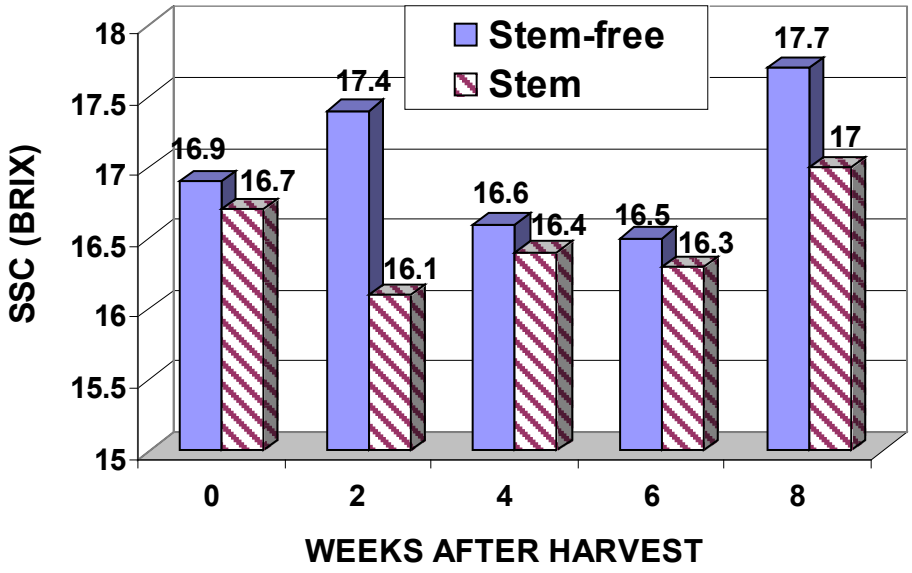

Figure 3. Soluble solids content (SSC) of stem-free cherries and cherries with stems.

***Objectives 3 and 4*:** In 2005, GA, RainGard alone, and RainGard + GA were applied by spraying on Rainier and Bing at various intervals prior to harvest to determine if GA and RainGard can be applied together. No significant differences were observed among the treatments for SSC, fruit weight, firmness, titratable acidity or color.

Postharvest water absorption: In 2006, cherry water absorption was influenced by the type of treatment applied to the cherries postharvest. The GA_3, RainGard + GA_3, and RainGard only treatments significantly reduced the amount of water absorbed compared to the untreated control ($P<0.01$) (Fig. 4). Water absorption for cherries treated with water, GA_3, RainGard, and GA_3 + RainGard were 4.54, 3.26, 1.64, and 1.07 g/unit, respectively (one unit=25 cherries).

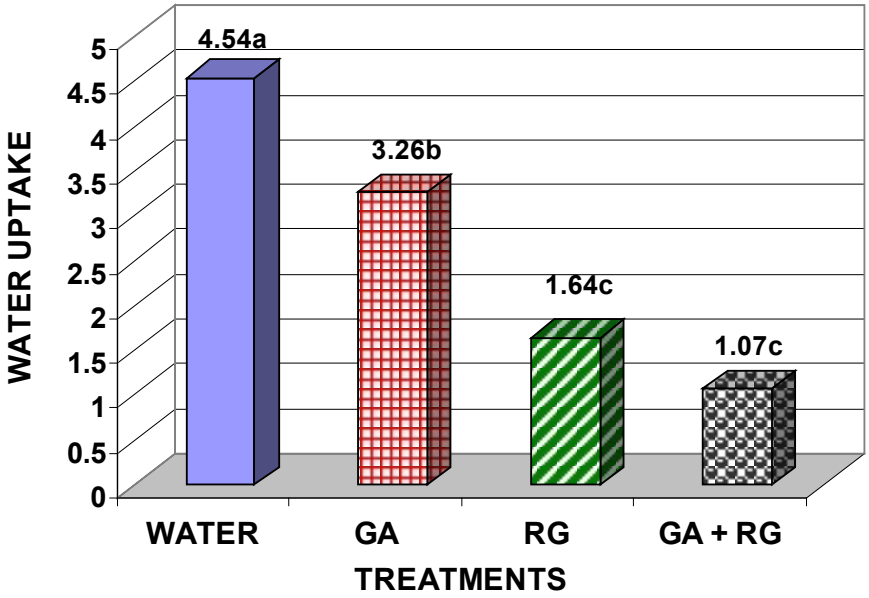

Figure 4. Water absorption after 30 minutes by cherries treated for only 10 seconds with different formulations. Water (undipped control), GA=GA_3, RG=RainGard, GA + RG=GA_3+RainGard. Bars with different letters after the values above the bars are significantly different ($P<0.01$).

Postharvest water loss: Cherry water loss during cold storage was examined weekly. The GA_3, RainGard, water treatment, and GA_3+RainGard treatments significantly reduced water loss at one week after treatment (WAT) as compared to the untreated control cherries (for water treatment $P<0.05$ and all other treatments $P<0.01$). Water loss in cherries treated with water, GA_3, RainGard, and GA_3+RainGard at 1 WAT was 0 .55%, 0.20%, 0.14%, and 0.21%, respectively, while the untreated control cherries had a water loss of 0.82% (data not shown). Water loss gradually increased during cold storage (Fig. 5). Cumulative water losses from cherries treated with RainGard and GA_3+RainGard were the lowest of all treatments during six weeks of cold storage and were significantly lower than that of the untreated control ($P<0.01$ between 2 and 4 WAT, or $P<0.05$ at 6 WAT). Cumulative water loss from cherries treated with GA_3 was significantly lower than that of the control ($P<0.01$ at 4 WAT or $P<0.05$ at 2 and 6 WAT). Cumulative water loss of cherries treated with water, GA_3, RainGard, and GA_3+RainGard were 3.52%, 3.13%, 3.12%, and 3.09%, respectively, at 6 WAT and 3.93% for the untreated control cherries. Firmness and SSC were not significantly affected by these treatments. We conclude that postharvest applications of RainGard alone or RainGard + GA are effective in reducing water absorption postharvest and also water loss of cherries during cold storage.

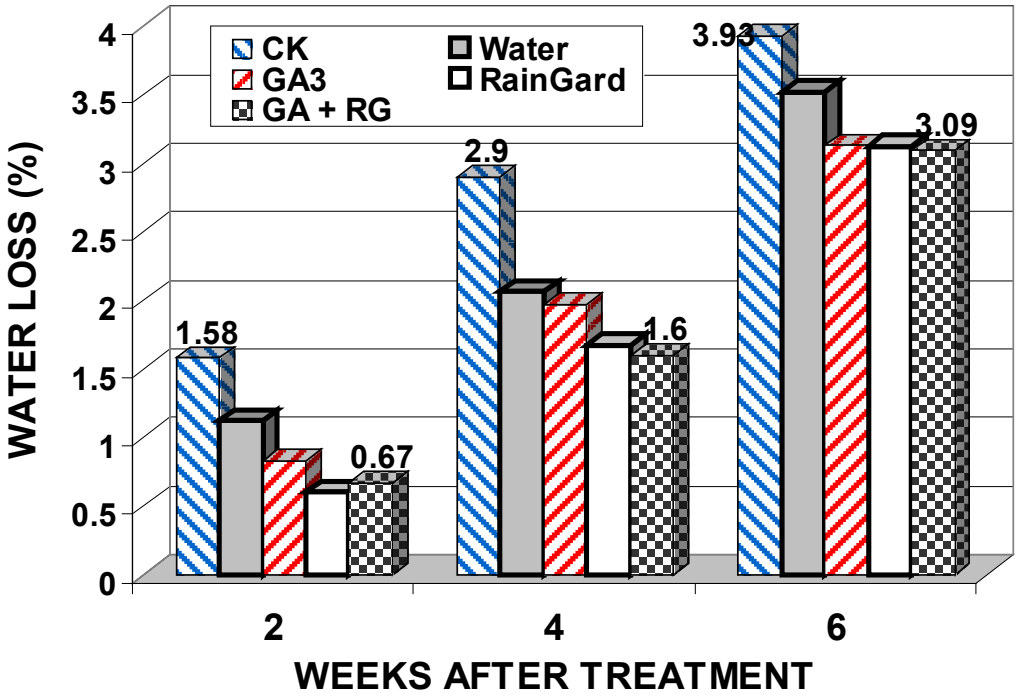

Figure 5. Cumulative water loss in cherries treated with different formulations (GA + RG=GA_3+RainGard; CK= untreated control; Water=cherries treated with water only).

***Objective 5*:** We previously observed that the junction between the stylar scar tissue and the cuticle appears to be open in Bing cherries, partially open in Van and closed in Lapins. "Conductive" tissue appears to be more pronounced in Bing, somewhat less in Van and even less apparent in Lapins. Tieton's anatomy seems similar to Bing and may account for its susceptibility to cracking. Rainier cherries were also examined in this manner but showed a tight junction between the stylar scar and the cuticle. However, we have observed that the suture of Rainier cherries is especially susceptible to cracking, but this needs more examination.

Objective 6: To test efficacy, RainGard was applied to Bing cherries in three orchards near The Dalles, Oregon, during 2005. These trials were funded by other extramural funding. The mean of all three trials is shown (Fig. 6). Total cracking in all three RainGard treatments was significantly lower than in the untreated control (D). Treatment C (four weekly applications) had significantly less cracking than the other three treatments.

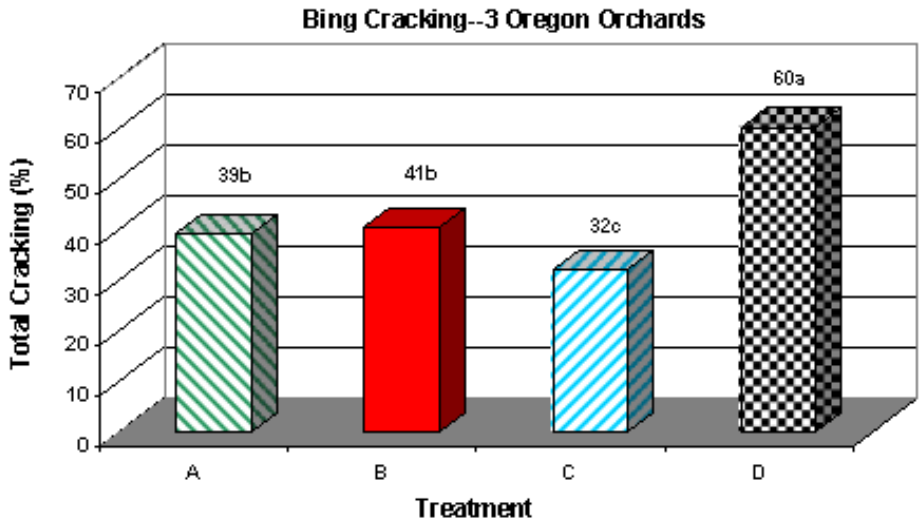

Figure 6. Total cracking of Bing cherries averaged from three orchards near The Dalles, Oregon. Three RainGard treatments are compared to the untreated control (Treatment D). See methods for description of RainGard treatments. If the number above a bar within the graph is followed by a letter different from that above another bar, that bar is significantly different (P<0.05) than the other.

Rainier and Bing were compared in one Oregon orchard, and total cracking was significantly higher in Rainier than in Bing with all four treatments (Fig. 7). With Bing and Rainier, all RainGard treatments significantly decreased cracking as compared to the untreated control (D). Cracking in Bing was lowest again in Treatment C (four weekly applications).

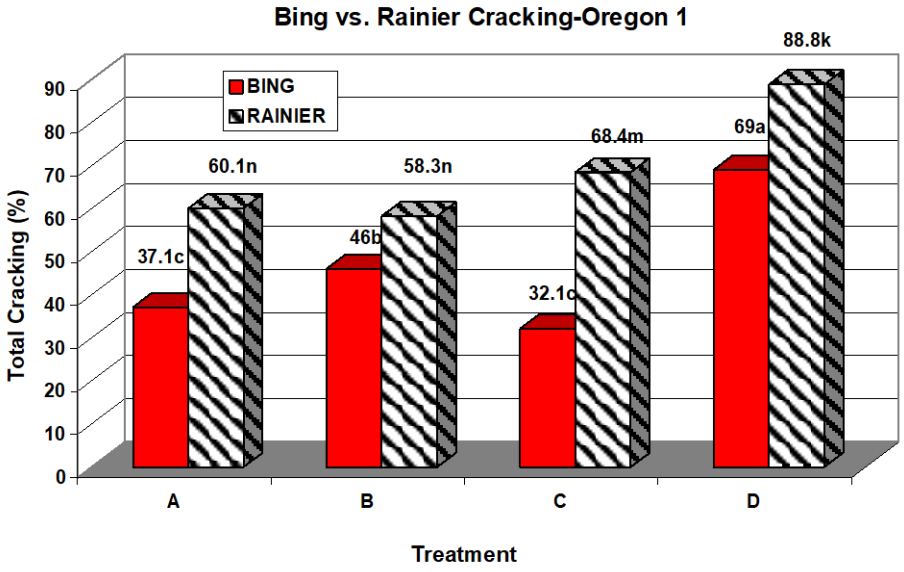

Figure 7. Comparison of cracking in Bing and Rainier sweet cherries in an Oregon orchard with three RainGard treatments versus an untreated control (D).

With Sweetheart cherries, cracking was significantly lower in Treatment C as compared to other treatments (Fig. 8). With Tieton cherries, cracking was also significantly lower in Treatment C as compared to all other treatments (Fig. 9).

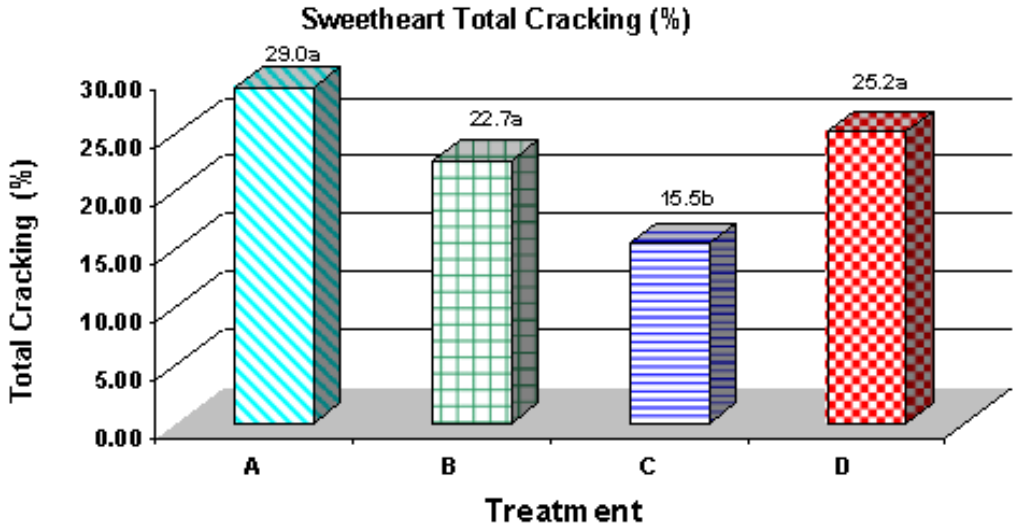

Figure 8. Total cracking of Sweetheart cherries treated with three RainGard treatments versus an untreated control (D).

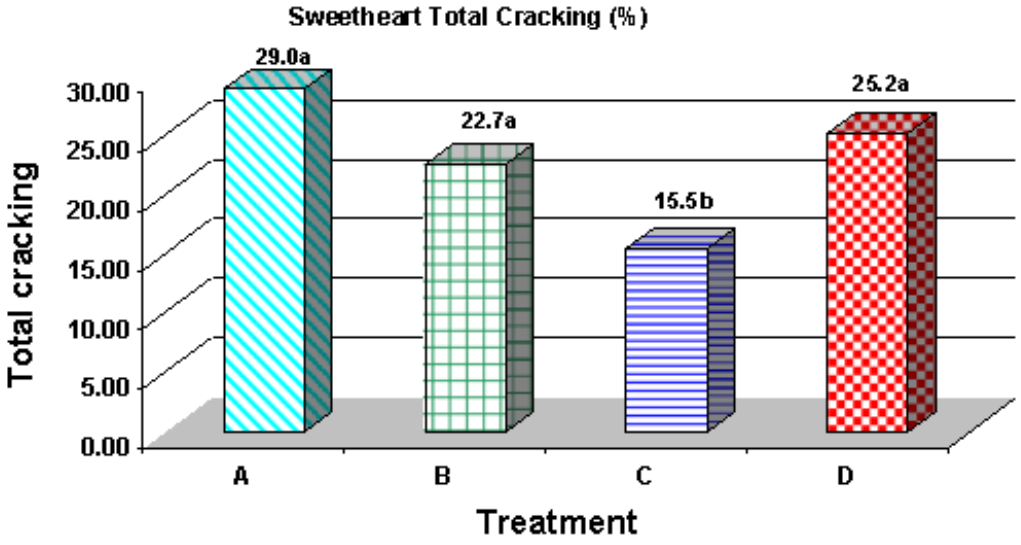

Figure 9. Total cracking of Tieton cherries treated with three RainGard treatments versus an untreated control (D).

The results in Figs. 5 to 9 indicate that more frequent applications (Treatment C—four applications at weekly intervals) provided better protection from rain. The surface area of the cherry expands rapidly during the last few weeks of development and within a few days causes the protective film on the

cherry to become less effective in protecting from the rain (Fig. 10). Note the rapid increases in fruit surface area during the four weeks before harvest. Weekly RainGard applications maintain the protective film for better protection from rain.

The quality analyses completed on 10 trials showed no appreciable differences among the treatments in any cultivar. This included fruit size, color, titratable acidity, soluble solids and firmness.

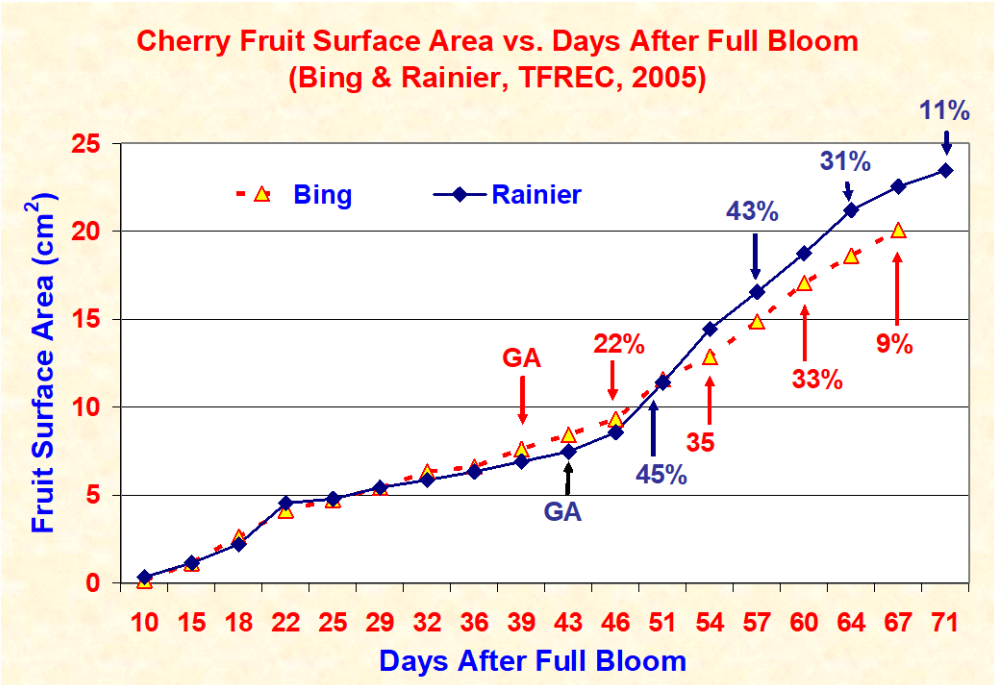

Figure 10. Growth curves for Bing and Rainier cherries. Fruit diameter was determined twice weekly, and then fruit surface areas were calculated.

SIGNIFICANCE OF RESEARCH TO SWEET CHERRY INDUSTRY:
The research conducted under this project has shown that stem-free cherries maintain their firmness and other quality factors during cold storage as well as cherries with stems. Water loss after harvest can be decreased by dipping the cherries in RainGard or RainGard + GA for several seconds. As our research revealed more about the causes of rain cracking of sweet cherries, a protectant called RainGard™ was developed and made available to growers on a limited basis during 2006. RainGard is the most effective protectant available to cherry growers at this time.

NOTE: WSU is including the following 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.
OTHER FUNDING: FruitGard LLC and Pace International LLC provided over $21,000 for Objective 6 (efficacy testing of RainGard) during 2005 and over $9,000 during 2006.

FINAL PROJECT REPORT
WTFRC Project Number: CH-05-501

Project Title: Coatings and other treatments to improve cherry quality

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Budget History:

Item	**Year 1:**	**Year 2:**	**Year 3:**
Salaries		10,200	2,516
Benefits		4,998	1,484
Wages			
Benefits			
Equipment			
Supplies	3,500	3,500	4,000
Travel		500	1,000
Miscellaneous			
Total	3,500	19,198	9,000

Significant findings:

- A new clamshell prototype with smaller openings than the commercial clamshell was developed. The new clamshell decreased moisture loss and doubled the shelf-life of 'Regina', 'Lapins', and 'Bing' fruit.
- An ethanol release pad placed in the clamshell maintained better stem and fruit quality of 'Lapins'.
- The efficiency of coatings to reduce postharvest moisture loss of cherries is coating formulation and fruit variety dependent.
- Sucrose fatty acid ester was the only coating that significantly improved shininess of cherry fruit.
- Chitosan coatings maintained fruit firmness and stem retention better than control and other coatings.
- Ca propionate dips helped maintain fruit firmness but $CaCl_2$ did not.
- Peroxyacetic acid, a sanitizer, maintained better fruit quality than control and other dipping treatments.
- For stem coatings, paraffin + polyethylene decreased water loss and browning, decreased stem detaching, and water loss. However, other film forming formulations did not affect stem quality. GA_3 dips slowed down stem browning of 'Bing' cherries.

Results and Discussion

Clamshell (Fig. 1-4): Currently commercial clamshells are very inefficient because 2-5% of the total surface area is exposed to air (Fig. 1). This opening allows fruit weight to decrease more than 5% when stored at 33°F for 14 – 21 days. The critical point at which fruits and vegetables deteriorate due to water loss is at about 5%. Thus, as a result of this exposure to air, cherry stems dry or turn brown, and fruit shrink and deteriorate.

We developed a better product – our new clamshell significantly decreased fruit weight loss and nearly doubled cherry shelf life. This new clamshell includes smaller openings with the opening ratio to total surface area is from 0.05-0.50% (Fig. 1). Because of the small opening, the relative humidity (RH) inside the clamshell with fruit was 5-6% higher than the commercial clamshell (Fig. 2). The water loss rate of fruit in the new clamshell was only half in comparison with which in the commercial clamshell (Fig. 3). Fruit in the new clamshell had high flesh firmness, less stem discoloration (data not shown), and less incidence of pitting at 33°, 50° and 68°F (1°, 10°, and 20°C), respectively (Fig. 4).

The result with our clamshell is that fruit will store longer, have better quality, and ultimately have happier consumers.

Commercial clamshell Experimental clamshell

Fig. 1. Commercial (left, with large openings) and experimental (right, with small opening) clamshells (2006).

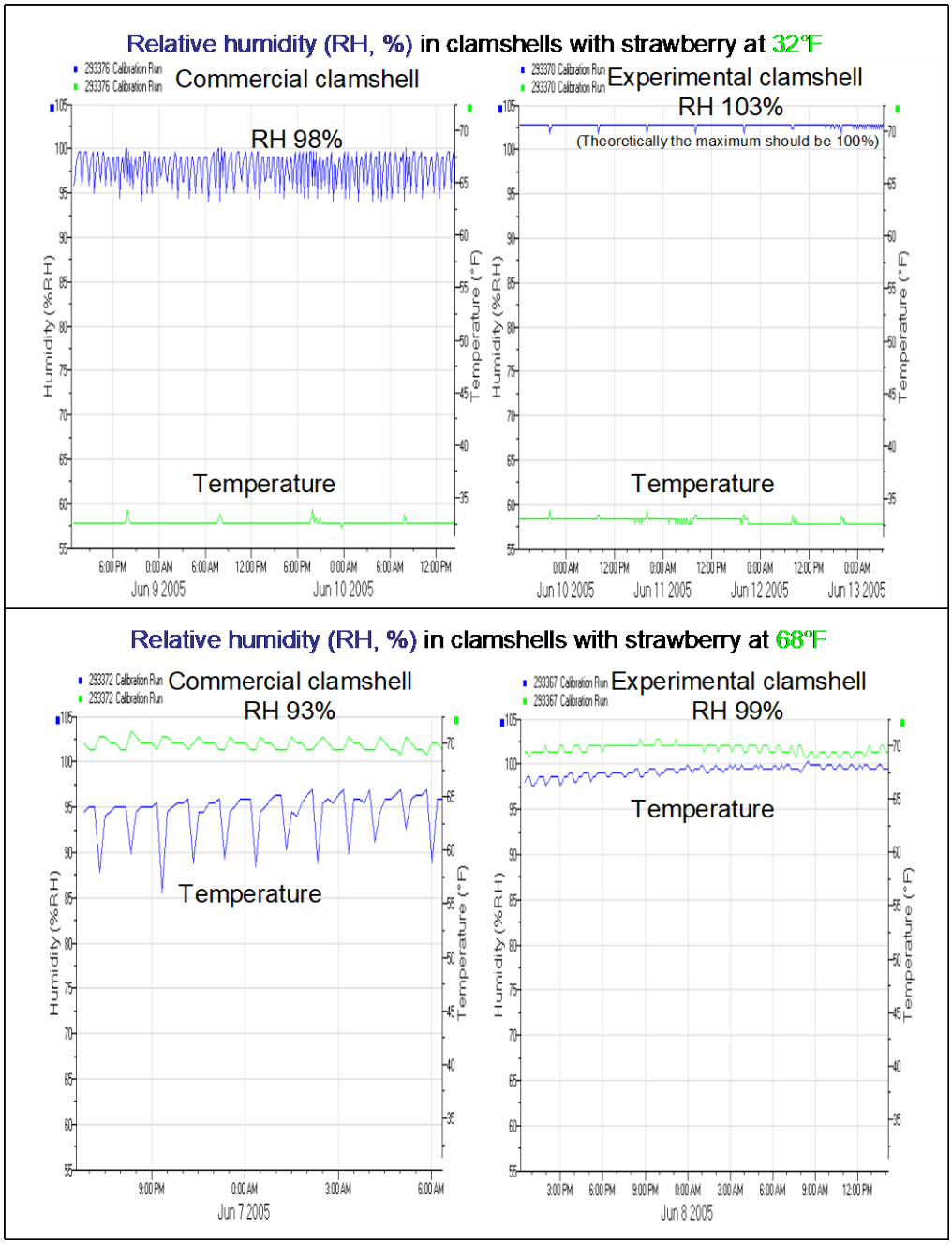

Fig. 2. Effect of clamshell opening on relative humidity (%) in clamshell with one pound of cherries at 32°F (upper) or 68°F (bottom). Opening ratio: Commercial clamshell-3.38%; Experimental clamshell-0.18%. (Average RH and the stability, 2005).

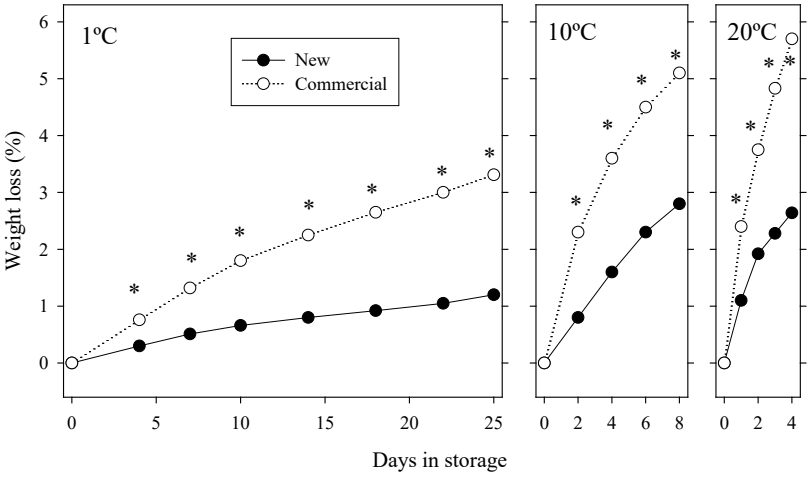

Fig. 3. Effect of clamshell on weight loss of sweet cherries stored at 33°, 50°, and 68°F, respectively (2006).

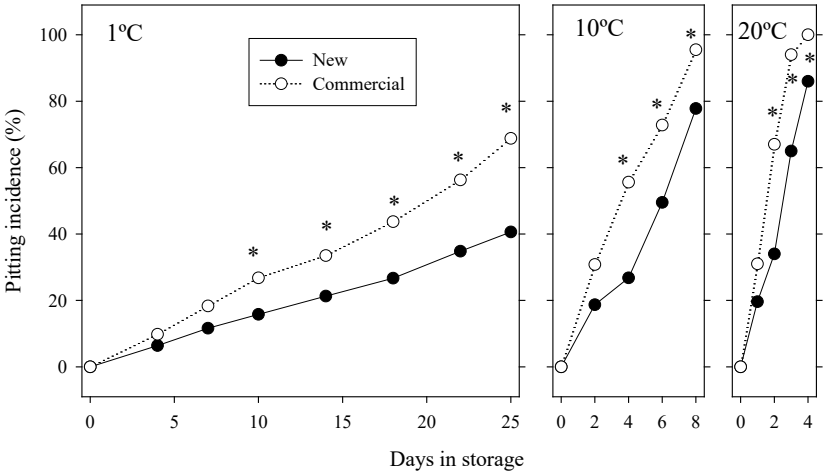

Fig. 4. Effect of clamshell on pitting of 'Lapins' cherries stored at 33°, 50°, and 68°F, respectively (2006).

Ethanol release powder (Fig. 5-6 and **Table 3):** The presence of an ethanol-release pad (Antimold Mild®, Freund Industrial, Japan) in the clamshell allows ethanol vapor to diffuse gradually (Fig. 5). It is made from ethanol absorbed onto silica gel that is packed in a special film, laminated with ethylene-vinylacetate and a proprietary Japanese paper, which regulates ethanol diffusion. The ethanol pad was glued on the top lid of clamshell.

Softening of fruit and browning of stems were retarded by ethanol pads (Fig. 6). Ethanol treatment affects ripening and senescence in some fruit and vegetables (Bai et al., 2004; Plotto et al., 2006; Suzuki et al., 2004). Ethanol vapor treatment of tomato fruit suppressed the climacteric respiratory rise, lycopene synthesis, and chlorophyll breakdown (Saltveit and Mencarelli, 1988). Ethanol injected into the seed cavity of muskmelon and honeydew inhibited softening (Ritenour et al., 1997). Furthermore, ethanol solution prolonged the vase life of cut carnations by suppressing respiration and transpiration (Pun et al., 2001).

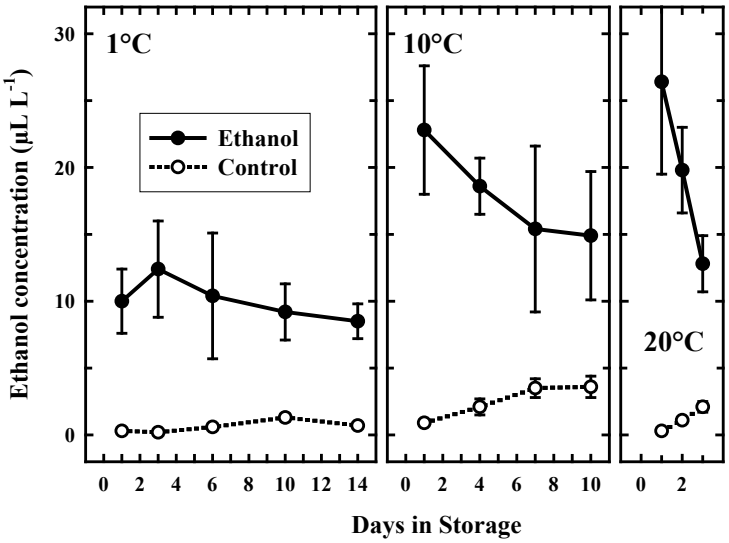

Fig. 5. Ethanol concentration in the headspace of clamshell with or without ethanol pad. 'Lapins' cherries were packed in the clamshell and stored at 33, 50 and 68°F, respectively (2005).

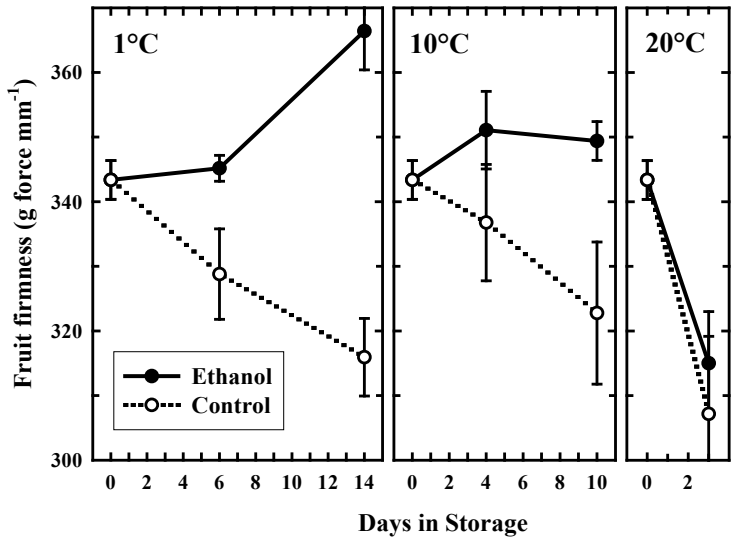

Fig. 6. Effect of ethanol pad on fruit firmness of 'Lapins' cherries. Fruit were packed in the clamshell and stored at 33, 50 and 68°F, respectively (2005).

Fruit coating and other dipping treatments (Table 1-3): The following experimental or commercial coatings and other chemical agents were used, alone or in combination. 1) Natural or artificial film-formers, such as carnauba, resin, chitosan or sucrose fatty acid esters which provide a barrier for protecting against moisture loss while moderately modifying fruit internal atmosphere; 2) Antioxidant agents, such as acetyl cysteine, ascorbic acid or 4-hexyl resorcinol to protect fruit and stems from discoloration; 3) Calcium salts which maintain membrane system of plant cells and increase fruit firmness; 4) Gibberellic acid, a plant regulator which may delay the senescence process of fruit; and 5) A sanitizer, such as peroxyacetic acid. Dipped fruit were stored at 33°, 50°, or 68°C to simulate the commercial storage and marketing in cold room or container car, cold shelf, or ambient shelf. We conducted the coating experiments for two years (2004 and 2005). Treatments that showed good results in 2004 were evaluated again in 2005.

Sucrose fatty acid esters, resins and vegetable oil emulsions are major commercial coatings for cherries. Most of these coatings, more or less, prevented moisture loss of fruit (Table 1). Fruit coated with sucrose fatty acid ester had the highest gloss (data not shown). However, these coatings did not significantly improved appearance of cherries (Table 2). Chitosan is a relatively new coating material. Chitosan forms a film when applied on fruit surface, which resulted in reducing moisture loss, modifying internal atmosphere of fruit, and reducing decay (Bai and Baldwin, 2002). Chitosan also decreased loss of fruit firmness and prolonged stem retention (Table 2). El Gaouth (1991, 1992, and 1997) reported that chitosan coating reduce decay for tomato, pepper and strawberries, therefore it could be a promising coating for cherries, too.

Table 1. Water loss (%) of cherry fruits coated with different formulations and then stored for 28 days at 33 °F (2004)

Treatment	Bing	Lambert	Lapins
Carnauba	-	2.77 b [z]	3.32 ab
Chitosan I	2.14 a	-	-
Chitosan II	2.54 a	3.25 a	2.14 c
Sucrose fatty acid ester	2.32 a	2.20 c	-
Resin I	-	-	2.58 b
Resin II	-	-	2.58 b
Resin III	-	-	1.91 c
Control	2.32 a	3.28 a	3.75 a

[z] Mean value (n = 3) not followed by the same letter are significantly different (P<0.05) by Duncan's multiple range test.

A firming agent, Ca propionate also extended stem retention (Table 2). Ca^{++} has been applied in horticultural crops preharvest and postharvest to improve the postharvest stability of produce (Patterson et al., 1983).

Table 2-1. Bing: effect of coating, gibberellic acid, and calcium salt dipping on fruit and stem quality of sweet cherries after storage at 33 °F for 14 days (2004)

Treatment	Fruit		Stem	
	Firmness (g force mm^{-1})	Appearance index [z]	Detachment force (g force)	Appearance index
Film forming agents				
Carnauba	293 b [y]	0.39 b	511 bc	0.80 a
Chitosan I	332 a	0.46 ab	546 b	0.73 ab
Chitosan II	331 a	0.47 ab	643 a	0.71 ab
Sucrose fatty acid ester	290 b	0.41 b	463 c	0.87 a
GA3				
10 ppm	282 b	0.40 b	401 d	0.73 ab
50 ppm	269 c	0.47 ab	466 c	0.70 ab
100 ppm	264 c	0.49 a	467 c	0.71 ab
Firming agents				
CaCI2	292 b	0.39 b	452 cd	0.61 b
Ca Propionate	317 ab	0.50 a	642 a	0.68 ab
Control	289 b	0.42 b	423 d	0.60 b

[z] Index for stem appearence: 0 = clear; 1 = more than 75% of whole stem length browned; index for fruit appearance: 0 = clear; 1 = inedible.

[y] Mean value (n = 3) not followed by the same letter are significantly different ($P<0.05$) by Duncan's multiple range test.

Table 2-2. Lambert: effect of coating, gibberellic acid, and calcium salt dipping on fruit and stem quality of sweet cherries after storage at 33 °F for 14 days (2004)

Treatment	Fruit		Stem	
	Firmness (g force mm^{-1})	Appearance index [z]	Detatchment force (g force)	Appearance index
Film forming agents				
Carnauba	283 ab	0.71	608 b	0.17 b
Chitosan II	287 ab	0.75	747 a	0.16 b
Sucrose fatty acid ester	288 ab	0.76	492 cd	0.18 b
GA3				
10 ppm	310 a	0.75	588 bc	0.15 b
50 ppm	292 ab	0.69	489 cd	0.15 b
100 ppm	270 b	0.65	528 c	0.17 b
Firming agents				
CaCI2	295 ab	0.71	509 cd	0.22 b
Ca Propionate	296 a	0.72	612 b	0.35 a
Control	261 c	0.71	449 d	0.17 b

[z] Index for stem appearence: 0 = clear; 1 = more than 75% of whole stem length browned; index for fruit appearance: 0 = clear; 1 = inedible.

[y] Mean value (n = 3) not followed by the same letter are significantly different (P<0.05) by Duncan's multiple range test.

Table 3. Effect of coating and other dipping treatments and ethanol-release pad on fruit and stem quality of 'Lapins' cherries. Fruit were stored at 32°F for 14 days (2005)

Treatment	Weight loss (%) [z]		Stem quality index [y]		Fruit quality index	
	Regular	Ethanol	Regular	Ethanol	Regular	Ethanol
Chitosan	12.5 a [x]	13.1 a	1.4 f	1.7 f	2.7 d	3.6 cd
Sucrose fatty acid esters	8.8 b	10.6 ab	6.3 c	7.7 ab	5.1 a-d	5.0 a-d
Ca^{++} + antioxidants	6.6 b	5.6 b	3.6 e	5.7 cd	6.0 a-c	6.8 a-c
Peroxyacetic acid	7.5 b	10.3 ab	4.9 d	7.9 ab	8.1 a	7.3 ab
Control	13.1 a	13.6 a	7.3 b	8.6 a	6.4 a-c	4.0 b-d

[z] + 1 day at 68°F
[y] Stem and fruit index: 1 = best; 10 = worst.
[x] Mean value (n = 3) not followed by the same letter are significantly different (P<0.05) under same attribute.

Stem coating and other dipping treatments (Table 4): In 2004, cherry stems were dipped in different coatings, Ca salts and GA3, respectively, using a screen system which holds the fruit when stems are in the solution. GA3 and chitosan coating decreased stem browning of 'Bing' and 'Lapins' cherries, respectively (Table 4). In 2005, 'Lapins' cherry stems were dipped in chitosan, GA3, paraffin + polyethylene, carnauba or shellac coating/solution. Paraffin + polyethylene coating decreased water loss and browning, and prevented stem detaching (data not shown). We observed surface wax and stomata structure of cherry stem under scanning electricity microscopy (SEM). The results shows that there were clear stomata on the stem and the coating did not cover the stomata well. Surface natural wax was destroyed rapidly at ambient temperature. Shellac and paraffin coating on the surface cracked easily, but chitosan coating showed a good cover on the stem surface (data not shown).

Table 4. Effect of stem coating with different formulations on stem quality of cherries. Fruit were stored for 14 days at 33 °F after coating (2004)

Treatment	Browning index [z]		
	Bing	Lambert	Lapins
Film forming agents			
Carnauba			0.51 b [y]
Chitosan I	0.45 b		
Chitosan II		0.49 ab	0.12 d
Sucrose fatty acid ester	0.4 b	0.53 a	
Resin I			0.67 a
Resin II			0.63 ab
Resin III			0.65 ab
GA3			
10 ppm	0.38 bc	0.46 ab	0.70 a
50 ppm	0.30 c	0.53 a	0.67 a
100 ppm	0.35 bc	0.46 ab	0.4 bc
Firming agents			
CaCI2	0.45 b	0.52 a	0.74 a
Ca Propionate	0.58 a	0.52 a	
Control	0.43 b	0.37 b	0.32 c

[z] Index: 0 = clear; 1 = more than 75% of whole stem length browned.

[y] Mean value (n = 3) not followed by the same letter are significantly different (P<0.05) by Duncan's multiple range test.

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FINAL PROJECT REPORT
WTFRC Project Number: CH-04-405 (WSU Project 13C-3655-7298)

Project Title: Bioregulator Uses for Managing Growth, Flowering and Cropping
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Budget History:

Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries	7,000	7,280	7571
Benefits	1,890	1,966	2,574
Wages	1,000	1,100	1,100
Benefits	160	176	121
Equipment	0	0	0
Supplies	1,160	1,160	1,160
Travel	2,000	2,500	3,000
Miscellaneous	0	0	0
Total	13,210	14,182	15,526

Objectives:

1. Continue to develop improved recommendations for the use of ethephon (Ethrel®, Bayer CropScience) for stimulation of flowering and early fruiting in important sweet cherry cultivars on seedling rootstocks in standard and high-density plantings.
2. Examine the possibility that ethephon treatment for flowering can improve flowering and yield for more than one year after treatment.
3. Explore the potential for use of gibberellic acid (GA_3) as a strategy to reduce flowering the year following treatment on mature sweet cherry trees grown on size-controlling rootstocks where excessive bloom makes crop load control critical for production of fruit of required size and quality.
4. Determine if it is economically feasible to use a single treatment program of GA on cherries to simultaneously obtain both better fruit firmness and quality in the treatment year and also control flowering for the subsequent year as a tool to adjust crop load to benefit fruit quality the next year.
5. Explore in greater detail promising results of preliminary research with SmartFresh® (1-methylcyclopropene, MCP) applied to sweet cherry trees in conjunction with ethephon for loosening fruit for mechanical harvest while reducing negative ethephon effects on fruit quality.
6. If any additional new fruit-abscission products become available, initiate tests for efficacy in loosening sweet cherries while examining effects on fruit quality.

Significant findings over 3 years:

a. Control of flowering and fruit quality with gibberellic acid

1. Both GA_3 and GA_{4+7} at concentrations up to 200 ppm were capable of reducing return bloom when applied during cherry fruit development; GA_3 was more effective than GA_{4+7}.
2. Where bloom was reduced, yield tended to be reduced also. Fruit size was increased or not affected.
3. GA applications at the end of Stage I or Stage II delayed fruit maturity and coloring. Higher than normal GA rates, which were more effective than standard rates (20-30 ppm) for reducing bloom, also produced stronger effects on fruit development and appeared to increase the variability of fruit maturation.
4. Further trials with GA rates from 25 to 100 ppm should be carried out to evaluate whether a compromise concentration range can be found that will both improve fruit flesh firmness and maturation behavior while contributing to a reduction in return bloom in dwarfed cherry trees. GA will not replace other forms of crop-load management in dwarf cherry trees but may give an additional tool to growers seeking a multi-tactic approach to control of crop load.

b. Ethephon for stimulation of flowering

1. The results of the research with ethephon for stimulation of flowering on Mazzard-rooted cherry trees have been extremely variable. Over a 6-year period of research, the flowering response to ethephon has varied from the occasional strong promotion of flowering to the more commonly observed minor effect or total lack of effect. Although significant control over vegetative growth can be obtained with ethephon applications, evidently the juvenility factor created by the use of the seedling rootstock is extremely difficult to overcome with a few ethephon sprays. At this point we consider this part of the project as completed. The final recommendation to growers, should they be interested in trying to reduce the juvenile phase in seedling-rooted trees, is either to wait until the trees flower naturally or to try ethephon, knowing that the ethephon treatment(s) may be ineffective. For more reliable induction of precocity, use size-controlling rootstocks, such as the Gisela series.

c. Fruit loosening and fruit quality effects from ethephon and MCP

1. For the implementation of mechanical harvesting in sweet cherry or to aid hand-harvest of this crop, loosening the fruit from the pedicel must be accomplished by applying ethephon a few weeks before harvest. Unfortunately, ethephon application also accelerates loss of fruit firmness, a key factor in the durability and quality of the fruit after harvest.
2. The degree of negative effect of preharvest ethephon on fruit quality is directly proportional to the amount of product applied per acre.
3. MCP is an inhibitor of ethylene action. In 2003, spraying cherry trees 2 weeks before harvest with the standard SmartFresh formulation resulted in fruit that was firmer than untreated fruit at harvest; MCP also inhibited the flesh softening otherwise normally associated with ethephon treatment. This exciting development created the impetus for further research.
3. Trials in 2004, 2005 and 2006 explored a variety of aspects of spraying MCP on sweet cherry trees, including timing relative to ethephon application, concentration of MCP, air-blast vs. dilute hand-gun treatments, and various formulations of MCP and adjuvants to reduce off-gassing of the MCP molecule once the spray solution was made.
4. In all three years of this project, ethephon application worked as expected to loosen cherries, but application of sprayable formulations of MCP failed to beneficially affect fruit flesh firmness or other fruit characteristics either when applied alone or in combination with ethephon. Spray application technology may be largely responsible for these observations.
5. The gummosis that often accompanies application of ethephon to sweet cherries has not presented any problems in the six years these trials have been underway. The gummosis produced by ethephon is clear to light yellow in color, very different from the dark to black gumming characteristic of pathogen infection in the tree. Yellow gumming appears to have no long-term negative side effects on sweet cherry tree behavior, and normal rates of ethephon for fruit loosening (up to 3 pints/acre) do not normally cause heavy gumming.

Results and discussion:

The increasing importance of size-controlling, precocious rootstocks for commercial sweet cherry culture has highlighted an emerging problem of crop-load management that has not been a concern in the past. Controlling the crop load on dwarf cherry trees is an essential component in meeting increasingly demanding fruit-quality/fruit-size standards. At the present time, pruning is the principal tactic for crop-load control available to growers of dwarf cherry trees. Work is underway on chemical thinning, but this objective is more difficult to achieve with cherries than with pome fruit. One possible strategy that might contribute to the arsenal of crop-load adjustment tools might be the use of gibberellic acid (GA) to control flower formation in sweet cherry trees, thus reducing crop load by limiting the number of flowers available to set fruit the year after treatment. The work done in this project has shown that both commercially available GA products (GA_3 and GA_{4+7}) can reduce flowering in cherry, but GA_3 is more effective. At the same time, GA applications for bloom control must be made during fruit development and thus also affect the maturation and quality of the current season's crop. The current challenge, which we have begun to address, is whether a concentration range of GA can be found that provides satisfactory fruit-quality improvement in the treatment year and a significant reduction in bloom for the following year. If this objective can be achieved, another important tool will be available to help cherry growers cope with demands for improved fruit quality. Such a tool could be worth millions of dollars to the industry.

The use of ethephon to stimulate precocious flowering in Mazzard-rooted trees has met with only limited success. Although we have explored the effects of tree age, cultivar, ethephon concentration, mixtures of Apogee and ethephon, and single vs. multiple sprays, none of these factors, separately or together, has proven to be the key to a reliable flowering response. We suspect that genetic variation in the powerful juvenility behavior of seedlings may exercise a controlling influence that a few

bioregulator sprays simply cannot overcome. Hopefully these results will encourage more growers to change cultural practices to adopt precocious rootstocks. This change alone could mean millions of dollars in benefits for those growers who carefully learn how to properly manage size-controlled sweet cherry trees.

Six years of studies with preharvest ethephon applications for fruit loosening for mechanical harvest have demonstrated the following four main points:

1. The fruit loosening response is a reliable result of ethephon treatment preharvest; the rate of loosening is temperature-dependent but, given enough time, fruit will loosen to the degree needed for effective mechanical harvest.
2. Ethephon treatment also reliably reduces fruit flesh firmness compared to untreated fruit. The effect is concentration-dependent; such fruit must be handled accordingly from harvest to consumer.
3. At this point, ethephon is the only known product that produces a satisfactory fruit-loosening response in sweet cherry. Other possible products are now available for testing.
4. One year of positive results with sprayable MCP suggests that we are still struggling with problems related to the effective spray application of a gas (MCP) to a tree. More research is needed to determine how we can treat the ethylene receptors in the sweet cherry tree with MCP so that their biological activity can be controlled and detrimental effects of ethephon can be reversed. If we can develop a methodology that reliably produces the results we observed in one season, it would remove perhaps the most important limiting factor to the widespread implementation of mechanical harvesting for fresh-market sweet cherries. With the labor shortages that appear to be coming, many millions of dollars of crop value could be conserved with effective mechanical harvest rather than be at risk of loss for lack of sufficient hand labor.

Six years of observations have shown that the gummosis that normally accompanies the use of ethephon for fruit loosening does not appear to be a serious problem. Ethephon-induced gum is light yellow to light brown in color, quite distinct from the black-colored gum associated with a pathogen-infected wound. Ethephon-induced gumming does not appear to result in any negative effects on tree health, tree growth or productivity.

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FINAL PROJECT REPORT
WTFRC Project Number: CH-04-410

Project Title: High Density Orchard Management

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	6199	6301	6553
Benefits	1736	1953	2031
Wages	6000	7000	8000
Benefits	960	1120	1280
Equipment			
Supplies	2000	2000	2000
Travel	1000	1000	1500
Miscellaneous			
Total	17895	19374	21364

OBJECTIVES:

1. Develop and evaluate novel production systems including specific training/pruning strategies, cultivars and rootstocks that improve labor efficiency and yield excellent quality fruit

2. Develop and refine training strategies that facilitate mechanical harvest and/or platform assist of sweet cherries for the fresh market.

3. Continue to evaluate the effect of Ethephon on fruit quality, maturity, and retention force of different cultivars

4. Model tree vegetative and fruit growth in relation to genetic and environmental factors

5. Identify grower cooperators to participate in Competitive Orchard Systems 2015 and initiate high density research plantings with growers.

SINGIFICANT FINDINGS:

- Ethephon applications did not elicit a reduction in fruit-pedicle retention force or fruit firmness in all varieties
- Ethephon induced fruit softening is not problematic for mechanical harvest systems nor characteristic of all varieties
- Ethephon did not advance maturity in all varieties
- Ethephon applications to Bing should be made 2 to 4 weeks before harvest
- Skeena may be harvested mechanically without application of Ethephon
- Bing and Tieton appear better suited for stemfree harvest than Benton and Chelan
- early growth of high density orchards is affected by scion variety, training system, and rootstock
- the relative importance related to tree vigor/growth in our experimental orchard is: scion variety>training system>rootstock
- Bing and Tieton were the most vigorous, Sweetheart and Chelan were the least vigorous
- high yields of excellent quality fruit can be grown within angled fruiting wall orchard system
- in our high efficiency orchard system, 4th leaf Skeena yielded ca. 8.4 tons/acre of fruit that was 10.5-row or larger
- highest fruit growth rates occurred during stage I of development
- alternating trees from E to W was more productive system than splitting tree into the traditional Y-trellis system

METHODS:

High density orchard management. A new high density orchard was planted in 2003 at about 5' within row spacing and 14' between row spacing for a density of approximately 580 trees per acre. It is comprised of cultivars that ripen at approximately weekly intervals (Chelan, Tieton, Bing, Skeena, and Sweetheart) on Gisela 5 and Gisela 12 rootstocks. This block is being trained to a y-trellis system in two different ways: (1) trees headed after planting at approximately 20" and alternately tied to opposite sides of the trellis (*i.e*., three leaders per side in a fan shape) and, (2) trees headed at

approximately 30" and split on the trellis (*i.e.*, two leaders, one per side in a central leader shape). The interactions among training method, cultivar, and rootstock will be evaluated. In the first few years, tree growth and precocity data will be collected, including, trunk cross-sectional area, shoot length, number of laterals, flowering, fruit yield and quality.

Vegetative and fruit growth (when present) in this new orchard will be monitored weekly and related to locally recorded environmental data. Solar radiation, relative humidity, wind velocity, soil and air temperature, and soil water content will be continuously and intensively monitored in this orchard by three AgWeatherNet weather stations located approximately 100' apart. The ultimate goal of this experiment is to model reproductive and vegetative development of distinct germplasm to environmental phenomena (*e.g.*, fruit development, harvest date, and full bloom by degree days/heat units).

A new high density (*ca*. 530 trees/acre) of Tieton on Gisela 5 was planted in 2003. In this block, trees will be trained to either a central leader or multiple leader bush system. Growth, precocity and fruit quality will be monitored and compared between systems. This research program has shown that excellent quality fruit can be grown on a variety of training systems. Therefore, the costs associated with production on these various systems may be an important factor in determining their commercial potential. Each different system will be evaluated for labor efficiency by timing harvest and pruning events on a minimum of 50 trees per system.

Mechanical harvest efficiency. Mature Bing trees trained to various systems (*e.g.*, y-trellis, bush, central leader) will be harvested mechanically. Entire rows will be harvested and efficiency will be documented as harvesting time per tree and the number of impacts per tree. In addition, the efficiency of fruit harvest will be evaluated by collecting and weighing: (1) all fruit remaining on the tree (*i.e.*, those fruit not removed by the harvester), (2) all fruit on the ground (*i.e.*, those fruit removed but not collected), and (3) all fruit in bins (*i.e.*, ostensible yield). Quality of fruit subsamples harvested from each system will be evaluated, in comparison to stemless fruit harvested by hand and control fruit (with stems, harvested by hand), by an independent lab (Allan Bros.) for bruising, pitting, mechanical damage, and stem-end tears at the time of harvest and after two weeks in cold air storage.

Ethrel effects. Whole trees will be treated with Ethrel approximately 14 days before harvest. Cultivars to be treated include Chelan, Tieton, Bing, Benton, Lapins, and Selah. The following data will be collected on each of 40 fruit randomly harvested just prior to application and at 2 – 3 day intervals following application until commercial harvest: fruit retention force, fruit weight, soluble solids, firmness, and color. Fruit from treated trees will be compared to fruit from untreated control trees.

RESULTS AND DISCUSSION:

Varietal response to Ethephon In the first two years of this research trial, we evaluated the response of many varieties to Ethephon applied at a single rate (3 pt/ac) and a single timing (ca. 14 d before harvest). Not all varieties responded similarly to the application of Ethephon. In 2005, Ethephon reduced pedicel-fruit retention force (PFRF) of Bing, Chelan, and Tieton but not of Benton. Tieton and Bing exhibited significantly greater reductions in PFRF than did Chelan. In 2004, each variety tested showed significantly reduced PFRF in response to Ethephon. In 2005, for those affected varieties, the average reduction, measured about two weeks after application, was 35%. Bing and Tieton responded similarly, exhibiting a ca. 41% reduction and Chelan was less-affected – PFRF was only 24% lower in Ethephon treated trees. Regardless, for no variety did Ethephon reduce PFRF below the target of 400 for ideal removal by the mechanical harvester. For those treated with

Ethephon, the lowest values were for Bing at 540 g and the highest were from Benton at 850 g. Our data show that stem retention (% fruit which were removed at the pedicel-spur abscission zone) was high (30% - 90%). Even for Bing, at 540g PFRF, ca. 30% of fruit retained their pedicel. Without Ethephon treatment however, pedicel retention was 90%.

In 2006, we conducted a more detailed analysis of PFRF vs. rate and timing of Ethephon for Bing and Chelan. In general, Chelan PFRF was unresponsive to Ethephon (Figs. 1 and 2). Irrespective of timing and rate of Ethephon, Chelan PFRF did not vary significantly from the untreated control levels. At harvest, in our timing trial on Chelan, mean treated PFRF was 0.72 kg vs. 0.84 kg for untreated. In addition, PFRF at harvest from the rate trial was 0.73 kg across rates (which were similar) vs. 0.79 kg for untreated. The inconsistent response between years with Chelan exemplifies the vagaries of bioregulator research in general and underscores the need to develop/utilize genotypes which naturally develop low PFRF at harvest. In contrast to Chelan, Bing PFRF was reduced significantly in response to Ethephon applications (Figs. 1 and 2). In a rate trial, Bing PFRF at harvest was related negatively to Ethephon rate ($r^2 = 0.74$, data not shown). Mean PFRF across rates was 0.33 kg vs. 0.68 kg for the untreated fruit. This ca. 50% reduction is similar to the reduction in PFRF recorded in previous years. In an Ethephon timing trial on Bing, again, PFRF was reduced significantly – mean values for treated, across timings was 0.39 kg vs. 0.68 kg for the untreated. Single applications of Ethephon applied at 4, 3, and 2 weeks before harvest were equally effective at reducing PFRF (Fig. 2). However, applying Ethephon 1 week before harvest did not reduce PFRF below the 400 kg limit for optimum removal for mechanical harvest.

Bing fruit quality was not affected significantly by rate of Ethephon. Compared to quality at the time of Ethephon application in our rate trial, fruit soluble solids increased similarly across all rates (ca. + 31%). Untreated fruit soluble solids increased similarly over the two week period (+ 28%). Average fruit weight increased by ca. 22% irrespective of rate, whereas untreated fruit increased in weight by 18% over the same period. Firmness of treated fruit declined by ca. 11% to 263 g/mm, again, irrespective of rate of Ethephon. Untreated fruit firmness declined over the same period by 16%, to 281 g/mm, a similar reduction to the treated fruit. Chelan fruit quality responded similarly – we recorded no quality parameter that was affected significantly by Ethephon at rates up to 5 pt/ac. For example, firmness of treated fruit, irrespective of rate declined by ca. 26% to 272 g/mm over the two week period between Ethephon application and harvest. Untreated fruit exhibited a similar decline of 27% to 270 g/mm over the same period. These results contradict slightly results from previous years in which we documented a slight but significant reduction in Bing and Chelan fruit firmness in response to Ethephon (ca. 17% reduction, from 416 g/mm to 347 g/mm in 2005). However, fruit from those previous trials were much firmer overall, this may affect the response to Ethephon. It should be noted however, that, in the current trials and previous research, for no variety/rate/timing did fruit firmness decline to levels which would preclude their being marketed fresh. Therefore, Ethephon induced fruit softening is not problematic for mechanical harvest systems nor characteristic of all varieties. Tieton, for example, though not evaluated in 2006 has consistently shown reductions in PFRF in response to Ethephon treatment without any associated loss of firmness (see continuing reports from previous years). While the different responses to Ethephon are not fully understood, we can select varieties better-suited for mechanical harvest based on their response to Ethephon. Overall, these results suggest that Bing and Tieton are better suited for mechanical harvest than the other varieties we have tested (e.g., Chelan, Benton). However, ideally new varieties with inherent low PFRF at harvest must be identified and utilized in future mechanical harvest systems. At WSU-Roza experimental orchards, we are evaluating several candidate varieties which without Ethephon application, possess low PFRF (e.g., Skeena, Ambrunes).

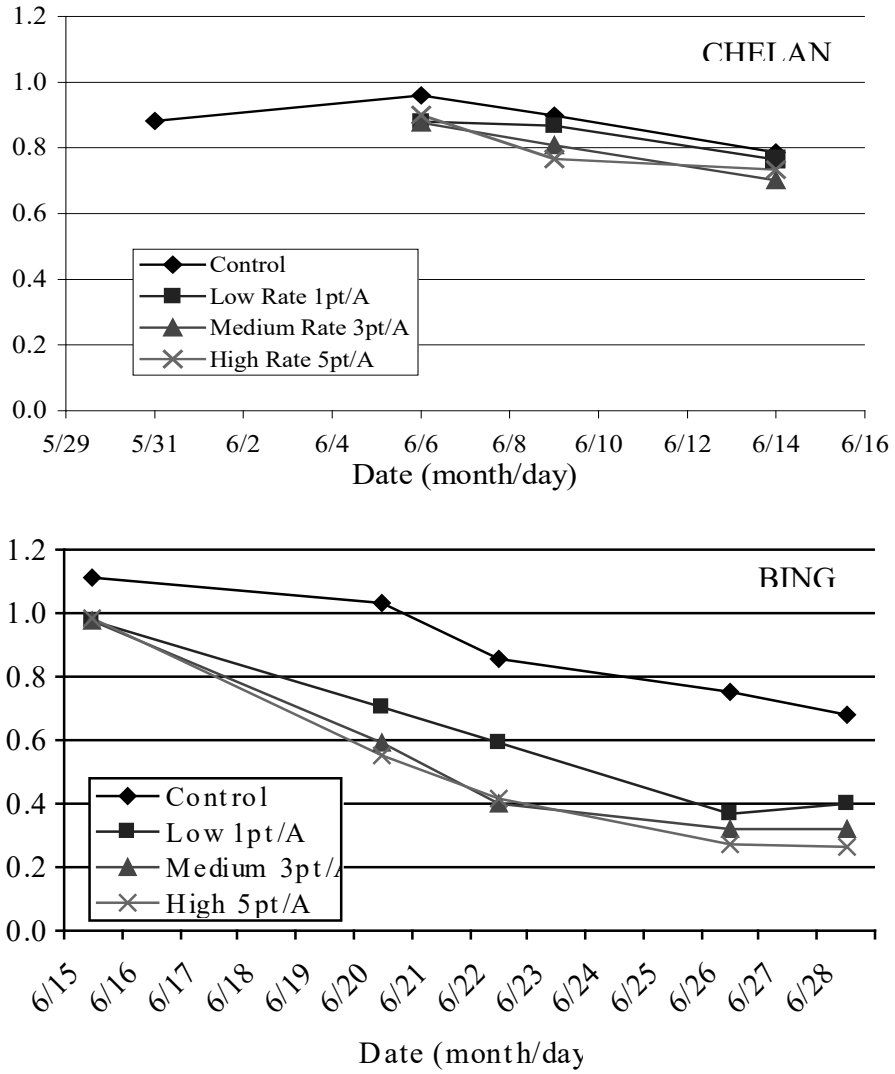

Figure 1. Effect of rate of Ethephon application (applied ca. 14 days before harvest) on pedicle-fruit retention force (kg) for 'Chelan' and 'Bing' sweet cherry. 'Chelan' harvest was on 14 June. 'Bing' harvest was on 29 June. The range between 0.2 kg – 0.4 kg is ideal for mechanical harvest.

Mechanical harvest trials In 2004 we negotiated and signed an agreement with USDA-ARS to transport and house their experimental mechanical harvester in Prosser for a 3-year duration. We will continue to consult with Dr. Peterson and industry cooperators as we refine orchard systems for maximum harvest efficiency. . In 2005 we received funding (ca. $40k) from the IMPACT center at WSU to study the efficiency of the mechanical harvest system, its impact on fruit quality, and consumers' perceptions of stemfree cherries. These projects will complement each other well and lead to a more efficient and rapid analysis of the mechanical harvest system. In 2006 we harvested Skeena mechanically and observed complete removal of fruit without any application of Ethephon. 'Skeena' PFRF declined linearly with fruit ripening and maturation and reached levels sufficiently low (ca. 0.42 kg on 11 July) to facilitate mechanical harvest (Fig. 3). Mechanically harvested Skeena fruit possessed a complete and dry pedicel-fruit abscission zone and we did not observe any 'leaking' of juice from the fruit. This variety appears to be well-suited for mechanical harvest.

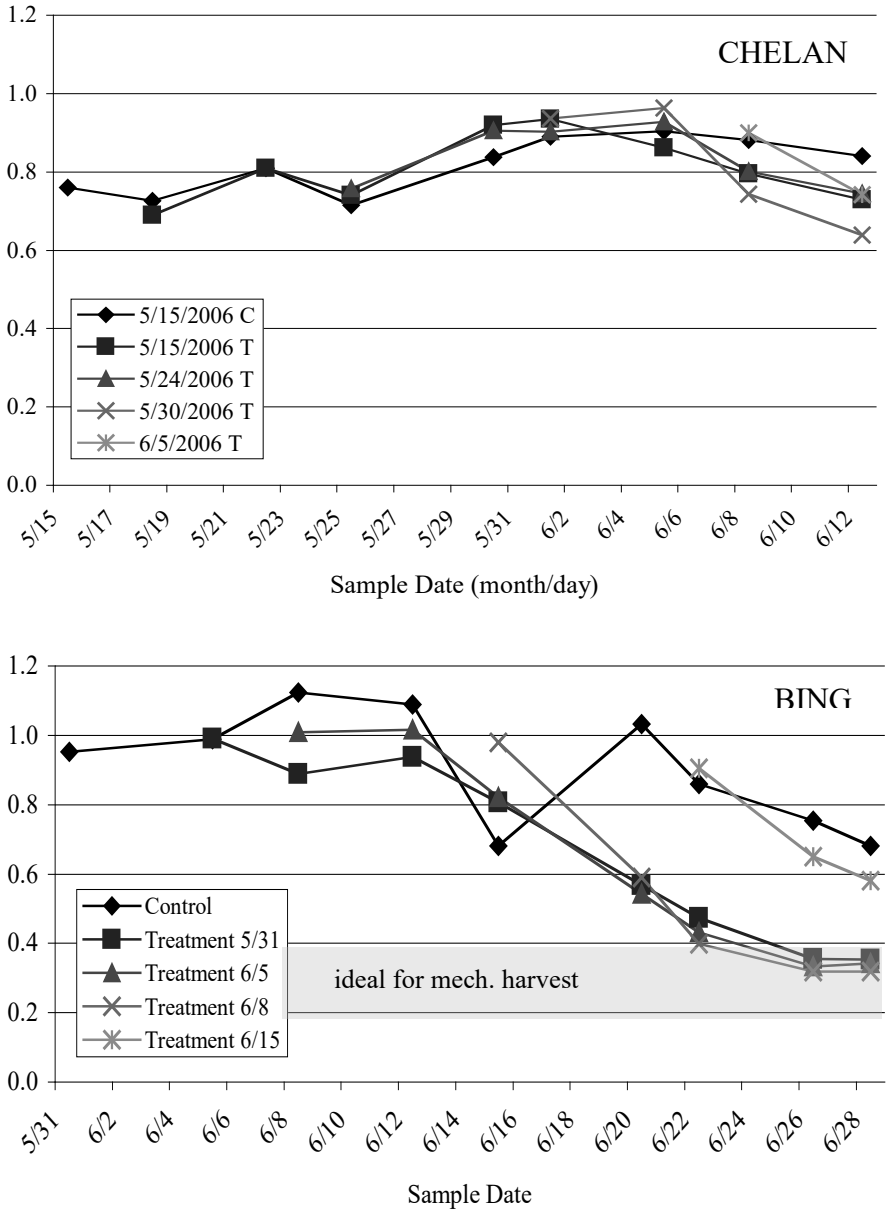

Figure 2. Effect of timing of Ethephon application (at 3 pt/ac) on pedicle-fruit retention force (kg) for 'Chelan' and 'Bing' sweet cherry. 'Chelan' harvest was on 14 June. 'Bing' harvest was on 29 June. The range between 0.2 kg – 0.4 kg is ideal for mechanical harvest.

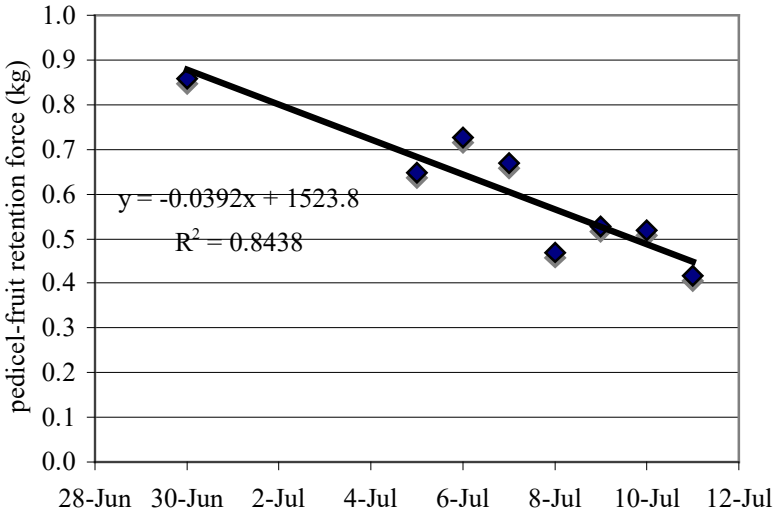

Figure 3. Trend in natural (i.e., without Ethephon) decline in pedicel-fruit retention force of 'Skeena' sweet cherry. Fruit were mechanically harvested on 11 July.

High density orchard management In 2006 we continued to refine training concepts to fit the mechanical harvest system and future integration of other mechanization (e.g., platforms). The original concept remains unchanged – develop homogeneous orchard systems comprised of fruiting 'walls' rapidly and efficiently while optimizing fruit yield and quality. This approach aims to create a system in which training is systematic throughout. The repeating vertical fruiting uprights become 'management units' – structures to be evaluated and treated similarly for crop load management, etc. In the current systems trial we again measured growth and fruiting characteristics of 'Chelan', 'Tieton', 'Bing', 'Skeena', and 'Sweetheart' on 'Gisela 5' and 'Gisela 12' rootstocks. From our ongoing studies of training systems for mechanical or possibly, pedestrian or platform-assisted harvest, we have developed the following principles:

- two single-layer fruiting walls per row in a Y-configuration (one/side)
- ca. 60 – 80° between planes (each plane ≈ 50°- 60° from horizontal)
- each plane consists of vertical fruiting uprights (4 – 7/tree and side though this varies with tree spacing)
- fruiting uprights spaced ca. 18" apart
- horizontal growth is eliminated
- fruiting limbs are renewed below first wire (ca. 28 in) with dormant heading cuts
- upright growth to a height of at least 50 cm (≈ 20 in) above soil

In this orchard's 4th year, yield increased significantly versus 2005. This was predicted due to the dramatic increase in two-year-old fruiting wood present in the 4th leaf trees vs. 3rd leaf trees. Indeed, mean yield per tree across architectures, rootstocks, and varieties was 12.3 kg in 2006 vs. 2.7 kg in the previous season. At 587 trees/ac, this translates into ca. 7.2 tons/ac in 2006. This is excellent productivity for a young orchard and highlights the precocity of the Gisela rootstocks as well as the potential productivity of the Y-trellis system. Overall, rootstock had only a slight impact on productivity. Gisela 12 was about 14% more productive than Gisela 5-rooted trees. This is likely due to greater vigor of Gisela 12 and therefore more fruiting spurs on those trees. In addition, training trees to alternating east and west sides of the trellis vs. the traditional Y-trellis resulted in approximately 10% higher productivity in 2006. Neither rootstock or training system however

affected tree yield as much as variety did. In 2006, 'Bing' was the most productive variety, yielding slightly less than 22 kg per tree (data not shown). The least productive variety was 'Tieton', yielding 6.3 kg per tree. 'Chelan', 'Skeena', and 'Sweetheart' were intermediately productive. The 21.9 kg/tree of 'Bing' translates into ca. 12.8 tons/ac. This again, highlights the potential productivity and precocity of this orchard system. The lowest yielding combination of 'Tieton'/'Gisela 12' yielded 3.2. kg/tree, or ca. 2 tons/ac.

It is not surprising that the lowest yielding system also yielded fruit of the highest quality (data not shown). This research program has documented clearly the negative relationship between fruit yield (canopy fruit-to-leaf area ratio) and quality. 'Tieton' fruit were the largest at 12.1 g/fruit and 100% were 10.5-row and larger. The poorest quality fruit were harvested from 'Chelan'/'Gisela 5' trees – 5.5 g/fruit, 20% smaller than 12-row, though the low fruit soluble solids (ca. 13 ^{o}brix) suggests that these fruit were prematurely harvested and had not fully sized. However, 'Chelan' does not possess the genetic potential for size that 'Tieton' does. 'Chelan' on 'Gisela 12' were significantly higher quality fruit (6.3 g, 24% 10.5-row+), suggesting that poor quality on 'Gisela 5' was related to insufficient carbohydrate supply to developing fruit. Indeed, no attempt at crop load management was made on 'Chelan'. In 2007 and beyond, crop load management via chemical blossom thinning and post-bloom thinning will be utilized to balance crop load with low vegetative vigor of this combination. The scion/rootstock combination yielding the most 10.5-row and larger fruit was 'Skeena'/'Gisela 12' – these trees bore ca. 15 kg/tree, of which 92% was 10.5-row and larger (i.e., 14.3 kg/tree). At 587 trees/ac, this translates into ca. 8.4 tons of 10.5-row and larger fruit per acre, in the 4th leaf. The next most productive combinations of premium quality fruit (i.e., ≥10.5-row) were 'Bing'/'Gisela 12' (6.5 tons/ac), 'Tieton'/'Gisela 5' (ca. 5.5 tons/ac).

Clearly, this orchard system design is precocious, productive, and can yield large quantities of large fruit. At this stage, the greatest challenges for most scion/rootstocks is crop load management to prevent over-production, and vigor control to prevent excessive intra-canopy shading. We will begin renewal of fruiting wood in this orchard this winter for most combinations. By design, this is a simple operation comprised of aggressive dormant heading cuts to remove the most vigorous fruiting upright limbs below the first wire (i.e., just above the point of origin of the limb). It will be critical to adopt an aggressive renewal strategy to maintain excellent light distribution, orchard productivity, and high fruit quality.

Since planting and training this test orchard, we have developed an alternative, novel approach for creating either upright or angled fruiting walls. This new approach may facilitate orchard establishment and creates an architecture comprised of two horizontal, permanent scaffold limbs from which fruiting uprights originate (Fig. 4). Similar to the previous configuration, renewal is accomplished via dormant heading cuts removing all but a short stub from each upright. We anticipate that the naturally vigorous upright growth in response to such pruning will expedite the renewal process. We have established in 2006 a new orchard to this design. It is comprised of Bing, Rainier, and Selah on Gisela 6 rootstock and spaced at 4′ × 15′ and trained to angled fruiting walls at 70° from horizontal.

Vigor varied among varieties most notably in 2006, but also by rootstock and training system. Overall, vigor was lower than in 2005. This is likely due to the significant increase in tree productivity (ca. 6-fold increase in yield vs. 2005) and competition between fruit growth and vegetative growth. Tieton was again the most vigorous variety with ca. 44 cm mean length of new shoots. Bing was similar to Tieton (43 cm). Chelan, Skeena, and Sweetheart were all similar and significantly less vigorous. Mean shoot length for these varieties was ca. 30 cm. This abating in vegetative extension growth will be important for many combinations to minimize intra-canopy shading at the high tree density of the orchard. However, it will be important to keep moderately high

vigor in this orchard to keep canopy leaf area high. In 2006, Gisela 12 was only slightly more vigorous (+ ca. 8%) than Gisela 5, across all varieties. For many scion rootstock combinations, particularly for Tieton and Bing on Gisela 12, trees have completely filled their space and renewal pruning will begin this winter. The least vigorous combinations are Sweetheart and Chelan on Gisela 5. Only with hard dormant heading and prudent water, nutrient, and crop load management will these trees fill their space. Clearly, early growth (first – third leaf) is critically important as trees fill their allotted space and develop future bearing surface. These combinations may never exhibit sufficient vigor to become commercially viable. We will follow the relationships between vigor and precocity and productivity closely in the next few years as some combinations have filled their space and others have not.

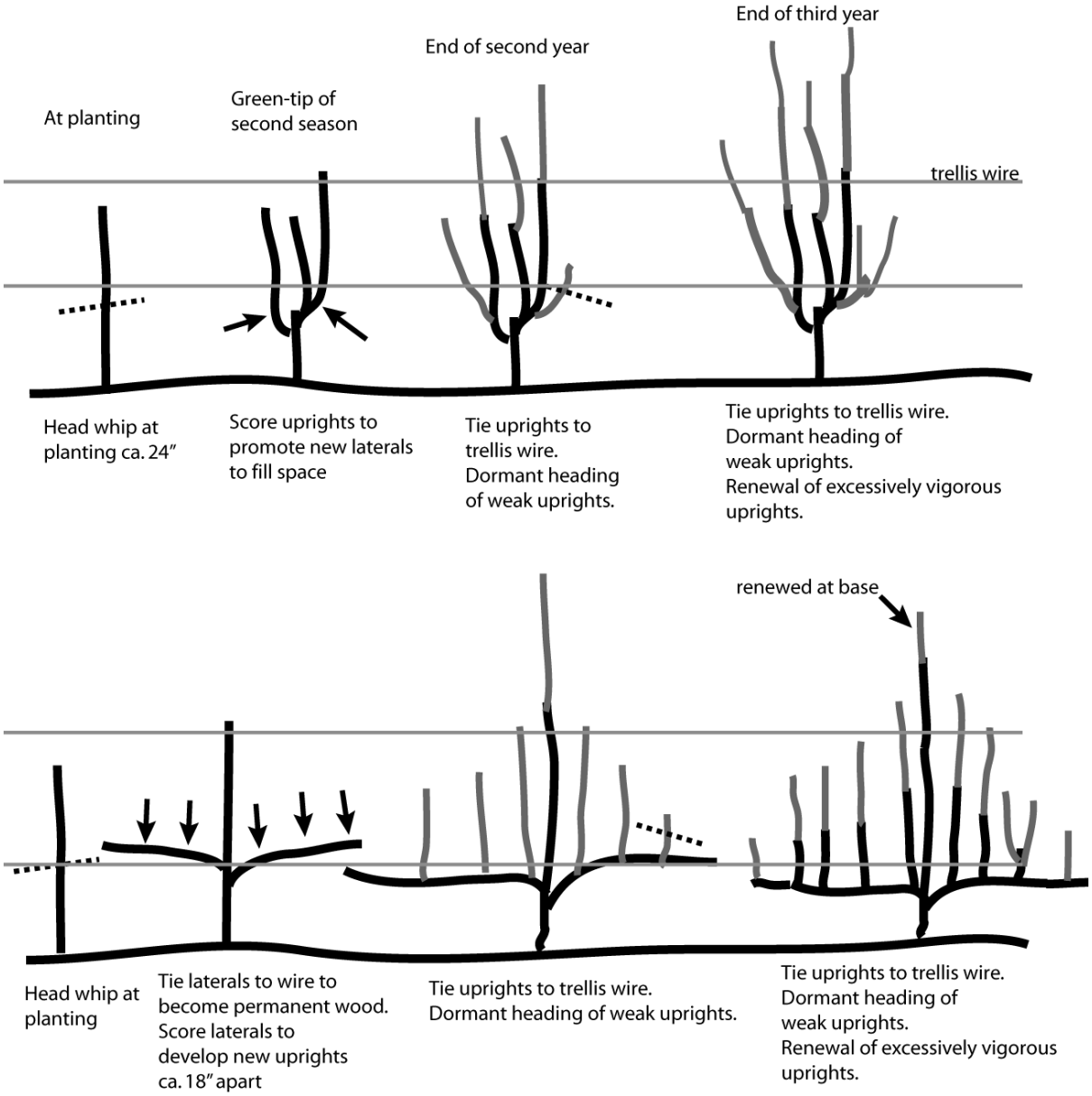

Figure 4. Brief comparison between proposed systems for establishing angled/upright fruiting walls.

FINAL PROJECT REPORT
WTFRC Project Number: CH-04-411

Project Title: Sweet cherry source-sink relations
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Del Feigal, Auvil Fruit Company, Vantage
Jim Doornink, Zillah
John Heffron, Outlook
Mark Hanrahan, Zillah
Angi Harmer
Ines Hanrahan, Yakima

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	6199	6301	6553
Benefits	1736	1953	2031
Wages	13000	13000	9000
Benefits	2080	2080	1440
Equipment			
Supplies	3000	3000	3000
Travel	3000	3000	3500
Miscellaneous			
Total	29015	29334	25524

OBJECTIVES:

1. To develop and evaluate practical strategies for manipulating sweet cherry crop load and maximizing fruit quality.

2. To investigate whole-tree source-sink relations.

3. Investigate the effects of postharvest defoliation on whole-tree physiology and fruit yield and quality.

SIGNIFICANT FINDINGS:

- high quality fruit can be grown on dwarfing, precocious rootstocks with prudent crop load management
- chemical blossom thinners fish oil + lime sulphur (FOLS) and ammonium thiosulphate (ATS) show greatest potential as bloom thinning agents
- chemical blossom thinners vary in their mode of action and efficacy
- ATS and FOLS are most effective applied to flowers whereas tergitol was more effective applied to leaves
- VOE is not an effective bloom thinning agent
- fruit set assessments should not be conducted before late May
- there is a need to develop an effective post-bloom thinning program for sweet cherry
- the optimum timing of post-bloom thinning appears to be between 2 and 4 weeks after full bloom
- FOLS shows efficacy as a post-bloom thinning agent at 14 days past full bloom
- tergitol is not recommended for post-bloom thinning
- Applied to 'Bing', GA_3 is more inhibiting to flower bud induction than GA_{4+7}
- 'Bing' yield in the season subsequent to GA_3 application was related negatively and closely to [GA_3]
- GA_3 and GA_7 between 25 and 100 ppm had no impact on 'Rainier' fruit weight or soluble solids in the season of application
- On 'Rainier', GA_3 increased firmness, proportional to rate, whereas GA_7 did not affect firmness
- Both GA_3 and GA_7 reduced 'Rainier' red coloration and delayed fruit maturation compared to untreated
- gibberellic acid may be an effective crop load management tool for productive orchard systems
- Compared to unpruned trees, summer pruning reduced, by half, whole-canopy NCER
- Summer pruning improved intra-canopy light distribution but had no effect on fruit yield or quality

METHODS:

Objective 1

Chemical blossom thinning. The efficacy of several blossom thinning agents will be evaluated in multiple locations throughout the PNW. Treatments will be applied in the Yakima valley and Wenatchee region as well as in Hood River/The Dalles. Ammonium thiosulphate (ATS), fish oil + lime sulphur (FO+LS), vegetable oil emulsion (VOE), and tergitol will be applied to entire trees at different rates and timings. Treatments will be applied to heavily cropping Bing and Lapins trees on Gisela 5 at the Roza experimental farm as well as other heavily-cropped trees in grower cooperator

orchards. Treatments will be compared for their effect upon floral bud induction (both number of reproductive buds per spur/shoot and floral meristems per bud), fruit set, spur and branch F:LA, and fruit yield and quality. In addition, the tree's physiological response to thinners will be documented by measuring spur leaf gas exchange prior to, and following application, and leaf and shoot expansion rates.
Thinner phytotoxicity will also be evaluated during the winter on trees grown in a greenhouse. Entire potted trees will be sprayed with a wide range of concentrations (0, 1, 2, 4, 8%) of each thinner. Individual leaves will be monitored for rate of expansion, gas exchange, and chlorophyll content (prior to and following treatment).

GA to inhibit floral bud induction. Trees will be treated with GA at various concentrations (0, 30, 50, and 100 mg a.i./liter) and two stages of flower bud initiation (roughly equivalent to beginning of stage II and III of existing crop). Treatments will be compared for their effect upon fruit quality during the season of application, floral bud induction (both number of reproductive buds per spur/shoot and floral meristems per bud), return bloom density, spur and branch F:LA, and fruit yield and quality. Initial treatments were applied during summer 2003 and consisted of: 1) Control (no treatment), 2) GA_3 30 mg a.i./liter (standard program), 3) GA_3 50 mg a.i./liter, and 4) GA_3 100 mg a.i./liter. Treatments 3 and 4 were applied as single applications at either the beginning of stage II or stage II, or a double application receiving treatment on both dates.

Objective 2
Potential periods of limiting carbohydrate supply will be investigated by establishing a range of F:LA by thinning fruit buds within Bing trees on Gisela 5, Gisela 6, and Mazzard rootstocks. For each scion/rootstock combination, fruit and shoot growth rates will be monitored weekly and canopy and spur F:LA will be determined at harvest.

Newly released cultivars (*e.g.*, Chelan, Tieton, Benton, Selah) and advanced selections (*e.g.*, PC 8011-3, PC 7903-2, PC 7147-9) from the WSU sweet cherry breeding program planted in 1998 will be subjected to one of two crop load treatments: (1) unthinned control, and (2) 50% removal of blossoms by hand. Tree growth, fruit yield and quality (weight, row-size distribution, soluble solids, and firmness) will be evaluated for each scion grown on Gisela 6, Gisela 5, Gisela 195/20, and Edabriz, where possible.
Objective 3
Summer pruning. The impact of summer pruning on canopy gas exchange, light distribution, growth, and fruit yield and quality in the subsequent season will be studied. Comparisons will be made between trees subjected to summer pruning (not dormant pruned) and dormant pruned control trees. Prior to pruning, canopy LA and light distribution will be measured for each tree. The LA removed from pruning will be collected and measured. In addition, for both treatments, pruned wood will be dried to a constant weight and weighed. Light distribution throughout pruned canopies will be assessed by ceptometer following pruning. In addition, rates of single leaf and whole-canopy gas exchange will be assessed prior to, and following summer pruning. In the dormant season, wood samples will be collected and analyzed for tissue carbon and nitrogen. In the subsequent spring, rates of vegetative growth (*e.g.*, leaves and shoots) growth will be monitored weekly. Tree yield and fruit quality will be determined.

RESULTS AND DISCUSSION:
Blossom thinning
Prosser Roza Trials (11-yr-old 'Bing'/'Gisela6'; 4-yr-old 'Skeena'/'Gisela5')
In 2006, at the WSU-Roza research orchard, we conducted two separate thinning experiments. FOLS, ATS, and tergitol were tested as bloom thinning agents when applied at ca. 20% and 80% full bloom to 'Skeena'/'Gisela5' trees and 'Bing'/'Gisela6' trees. On 'Bing', FOLS and ATS were

effective thinners, reducing fruit set to ca. 59% vs. 76% from untreated trees (Table 1). Tergitol however did not reduce fruit set statistically. Fruit set was high overall at about 65% of available flowers. Fruit quality (i.e., weight, firmness, soluble solids, row-size) was not improved by tergitol or ATS, despite numerical reductions in fruit set. Indeed, quality of unthinned control was good (7.7 g, 268 g/mm, 65% 10.5-row and larger) even at a high yield of 29.5 kg/tree (66 lbs), or approximately 10 tons/acre. Only FOLS improved fruit quality. Average fruit weight was ca. 1.1 g higher and FOLS-treated trees yielded about 24% more 10.5-row and larger, on a percentage basis, than control trees (data not shown). However, yield was reduced by FOLS by 10.8 kg/tree and therefore unthinned control trees yielded the most high-quality fruit. Therefore, even a 20% reduction in fruit set can be excessive and have a negative impact on crop value.

Table 1. Effect of chemical blossom thinners applied to 11-year-old 'Bing'/'Gisela 6' trees at ca. 20% and 80% of full bloom on fruit set, yield, and quality. Means within column followed by same letter are not statistically different ($P < 0.05$).

Treatment	Fruit set (%)	Fruit weight (g)	Yield (kg)	Yield ≥10.5-row (kg)
Control	76.3 a	7.7 ab	29.5 a	20.1 a
FOLS	58.9 b	8.8 a	18.7 b	15.6 ab
Tergitol	64.1 ab	7.5 b	22.0 ab	14.0 ab
ATS	59.1 b	7.4 b	21.6 b	11.0 b

Applying the same program to 'Skeena' did not produce similar results. No product effectively reduced fruit set (Table 2). Similar to the 'Bing' trial, fruit set overall was high (ca. 75% of available flowers). Interestingly, we recorded average fruit weight from FOLS and tergitol-treated trees that was lower than the control. In addition, FOLS and tergitol treatments yielded about 30% less fruit that was 10.5-row and larger than the control trees. It appears that FOLS and tergitol delayed fruit maturity – our red color rating and soluble solids were lower in FOLS- and tergitol-treated trees compared to the control (data not shown). It is possible that fruit had not reached a maturity (i.e., size) similar to that of the control at the time of harvest. This is the first evidence of FOLS and tergitol having any negative impact on fruit maturity or quality, and our first trial on 'Skeena'. It is not known whether varieties respond differently to caustic chemical blossom thinners. We intend to repeat this trial in 2007 to further investigate this possibility. In contrast, and consistent with our previous research on 'Bing', ATS was effective at improving 'Skeena' fruit quality without reducing yield significantly. Fruit from ATS-treated trees were 15% heavier (ca. + 1 g/fruit) than control fruit. In addition, ATS-treated trees yielded 93% 10.5-row and larger vs. only 67% from control trees. At the density of the research orchard (580 trees/ac), this improvement in fruit quality translates into an additional 2 tons of 10.5-row and larger fruit per acre from ATS-treated trees vs. untreated.

Table 2. Effect of chemical blossom thinners applied to 4-year-old 'Skeena'/'Gisela 5' trees at ca. 20% and 80% of full bloom on fruit set, yield, and quality. Means within column followed by same letter are not statistically different ($P < 0.05$).

Treatment	Fruit set (%)	Fruit weight (g)	Yield (kg)	Yield ≥10.5-row (kg)
Control	81.1 a	7.5 ab	15.7 a	10.0 ab
FOLS	72.8 a	6.8 b	15.0 a	7.6 b
Tergitol	73.0 a	6.8 b	13.3 a	7.0 b
ATS	72.7 a	8.6 a	14.7 a	13.8 a

Regional thinning trials

In a 'Lapins'/'Gisela 5' thinning trial in Moxee, we found no thinning efficacy from any thinning treatment (FOLS, ATS, or tergitol). Fruit set in this orchard was particularly high at slightly less than 80% across treatments (data not shown). Fruit yield was high, mean across treatments was ca. 90

kg/tree. Quality also was good and unaffected by thinner. Approximately two-thirds of fruit were 10.5-row or larger, and less than 4% was smaller than 12-row. The poor thinning efficacy of the caustic blossom thinners ATS, FOLS, and tergitol on self-fertile varieties such as 'Lapins' and 'Skeena' suggests that these varieties may require more aggressive (i.e., more frequent applications or higher rates) thinning strategies.

In 2006 we also conducted a thinning trial in 'Rainier'. Fruit set was reduced significantly and similarly by each blossom thinner (Table 3). This result supports a previous 'Rainier' thinning trial in which our most promising results were achieved with FOLS, ATS, and tergitol – each reduced fruit set similarly (ca. 33%) and significantly vs. untreated control. At Doornink's orchard, overall, fruit set was about 39% lower in thinned trees. Yield was reduced to a similar extent, 45% lower in thinned trees. Fruit quality was not improved significantly, despite reductions in fruit set. Reductions in fruit set and yield per tree without any improvement in fruit quality is indicative of non-source limiting conditions in unthinned trees. Indeed, it appears that fruit in unthinned trees were not limited in their development by the supply of photoassimilates (96% 10.5-row or larger, 10.6 g). These results again underscore the need for a reliable post-bloom thinning program. Fruit set in untreated trees was low (< 28%) and thinning was not necessary (though this was not apparent during bloom).

Table 3. Effect of chemical blossom thinners applied to 'Rainier' trees at ca. 20% and 80% of full bloom on fruit set, yield, and quality. Means within column followed by same letter are not statistically different ($P < 0.05$).

Treatment	Fruit set (%)	Fruit weight (g)	Yield (kg)	Yield ≥10.5-row (kg)
Control	23.9 a	10.6 a	33.1 a	31.8 a
FOLS	14.0 b	10.3 a	20.0 b	19.3 b
Tergitol	13.3 b	10.1 a	13.4 b	13.2 b
ATS	16.1 b	11.0 a	21.8 b	21.6 b

In another 'Bing' trial we recorded significant reductions in fruit set with FOLS, ATS, and tergitol (data not shown). Similar to the 2006 'Rainier' trial, thinners were similarly effective at reducing set (24% lower than unthinned). Fruit set was high overall however, still greater than 60% in thinned trees. Therefore, due to heavily over-cropped trees, fruit quality was poor across treatments. Thinning did improve mean fruit weight, however, only ATS improved fruit quality significantly (ca. + 1g/fruit, + 1 % soluble solids).

The inconsistent response among thinners and years, and between varieties, underscores our poor understanding of the mode of action of blossom thinners and the factors limiting to fruit set and pollination. Future research should investigate more precisely thinner mode of action on self-sterile and self-fertile varieties. Too often we have elicited thinning at bloom to discover at harvest that over-thinning had occurred. This occurs when improvements in fruit quality of remaining fruit are not sufficient to overcome the reduction in yield. Our data support the need for the development of a reliable post-bloom thinning program for sweet cherry. Having the opportunity to assess fruit set, and therefore the need for thinning would be beneficial for optimizing crop load.

In the 2006 'Bing'/'Gisela6' trial at the Roza experimental farm, we attempted to better understand the thinning response by recording, on 4 spurs per tree, the percent of potential fruit (i.e., number of flowers per spur) actively growing (i.e., exhibiting increase in equatorial diameter measured weekly), the percent attached to the spur but not growing (i.e., no change in fruit diameter), and the percent dropped fruit. These spur characteristics were assessed approximately every 7 days from early May until early June (Table 4). The percent of fruit that were actively growing increased throughout the measurement period. This was due to both an increase in fruit drop and fruit that stopped growing.

In early June, nearly all fruit were either actively growing or had dropped. Most fruit dropped in early May, though treatment affected the timing of the most significant fruit drop. FOLS-treated spurs for example, still exhibited significant drop between 22 May and 31 May – they had significantly more fruit attached and not growing on all but the last two sample dates, compared to control and tergitol treatments. Between 11 May and 7 June, very few fruit dropped from tergitol-treated trees (an additional 7.3%) and untreated control trees (an additional 7.5%). This suggests that fruit from these trees dropped prior to 11 May. In contrast, there was significant fruit drop from FOLS- and ATS-treated trees over the same period, ca 29% and 20.1%, respectively. Expeditious fruit drop would be advantageous, giving the grower an early indication of thinning efficacy and time to plan subsequent thinning, if necessary. In this regard, FOLS is the least favorable thinner because on 11 May, ca. one month after full bloom, 35% of the fruit had not yet dropped. For continued research of blossom thinning, our data also suggest that fruit set determinations should not be collected until the end of May.

Table 4. Effect of chemical blossom thinners applied to 11-year-old 'Bing'/'Gisela 6' trees at ca. 20% (17 April) and 80% (21 April) of full bloom on fruit set. Means within column and category followed by same letter are not statistically different ($P < 0.05$).

Fruit per category (%)						
Actively growing		11 May	18 May	22 May	31 May	7 June
	Control	67.6 a	71.8 a	72.5 a	75.7 a	76.3 a
	FOLS	53.1 b	59.8 ab	61.2 ab	59.4 b	58.9 b
	Tergitol	47.4 b	62.1 ab	56.5 b	63.6 ab	64.1 ab
	ATS	46.2 b	55.7 b	53.4 b	57.2 b	59.1 b
Not growing (attached)						
	Control	16.9 c	13.5 c	6.1 b	2.2 a	0.7 a
	FOLS	35.1 a	30.5 a	12.4 a	2.3 a	0.5 a
	Tergitol	24.3 bc	20.9 bc	5.2 b	1.8 a	0.3 a
	ATS	33.2 ab	23.6 ab	8.5 ab	2.3 a	0.2 a
Dropped						
	Control	15.5	14.7	21.4	22.1	23.0
	FOLS	11.8	9.8	26.4	38.3	40.6
	Tergitol	28.3	17.0	38.2	34.7	35.6
	ATS	20.6	20.8	38.1	40.5	40.7

In 2006 we also initiated research designed to elucidate the relationship between time of thinning and the benefit of the thinning. We conducted a preliminary trial in which 5 'Sweetheart'/'Gisela5' trees were subjected to a 50% removal of fruit every week, beginning 1 week after full bloom. We observed the greatest improvements in fruit quality from thinning 2 to 4 weeks after full bloom (Table 5). Interestingly, the earliest thinning, at 1 week after full bloom was not as effective as the later thinning timings at improving fruit quality. Thinning after about 7 weeks had no benefit on fruit quality. At 7 weeks and later, fruit weight, soluble solids, % 10.5-row and larger were not different from the control. Week 2 thinning caused the greatest improvements in fruit quality – weight was 14% higher, soluble solids were 9% higher, and there were ca. 18% more fruit in the 10.5-row and larger categories. However, unfortunately, yield of unthinned control trees was significantly lower than that of the thinned trees (6.0 kg vs. 13.3 kg). We hypothesize that thinning treatments would have been more beneficial if compared to unthinned trees yielding a similar mass of fruit per tree. We intend to repeat this experiment with greater replication in 2007. These data should be useful as we develop post-bloom thinning programs. From these trials we can comment about appropriate timings of post-bloom thinning. Our preliminary data suggest that fruit quality can be improved significantly with crop removal as late as 7 weeks after full bloom. This supports the conclusion that

final fruit size within a genotype is largely determined by the size of cells in the fruit, rather than the number of cells in each fruit.

Table 5. Effect of timing of thinning (weeks after full bloom) 4-year-old 'Sweetheart'/'Gisela5' trees to 50% crop load on fruit quality and yield. Means within column and category followed by same letter are not statistically different ($P < 0.05$).

Thinning timing	Fruit weight		Soluble solids (%)		% 11- or 12-row		%10.5-row & larger		Yield (kg)	
control	7.7	abcd	20.5	ab	33.4	ab	65.0	ab	6.0	d
week1	7.2	bcd	18.5	b	47.7	ab	49.2	ab	14.8	ab
week2	8.8	a	22.2	ab	16.9	b	82.8	a	12.2	bcd
week3	8.2	abc	21.2	ab	22.6	b	77.8	a	14.5	abc
week4	8.3	ab	22.8	a	23.0	b	76.8	a	12.9	abc
week7	7.9	abcd	22.2	ab	27.5	ab	73.5	ab	11.2	bcd
week8	7.1	cd	19.8	ab	56.8	a	40.2	b	9.9	bcd
week10	7.0	d	19.3	b	52.7	a	42.5	b	17.4	a

In 2006, we also initiated a trial in which we bagged flowering limbs of 'Bing' and 'Rainier' trees on 'Gisela 6' rootstocks with bee exclusion bags at various stages throughout the bloom period. The

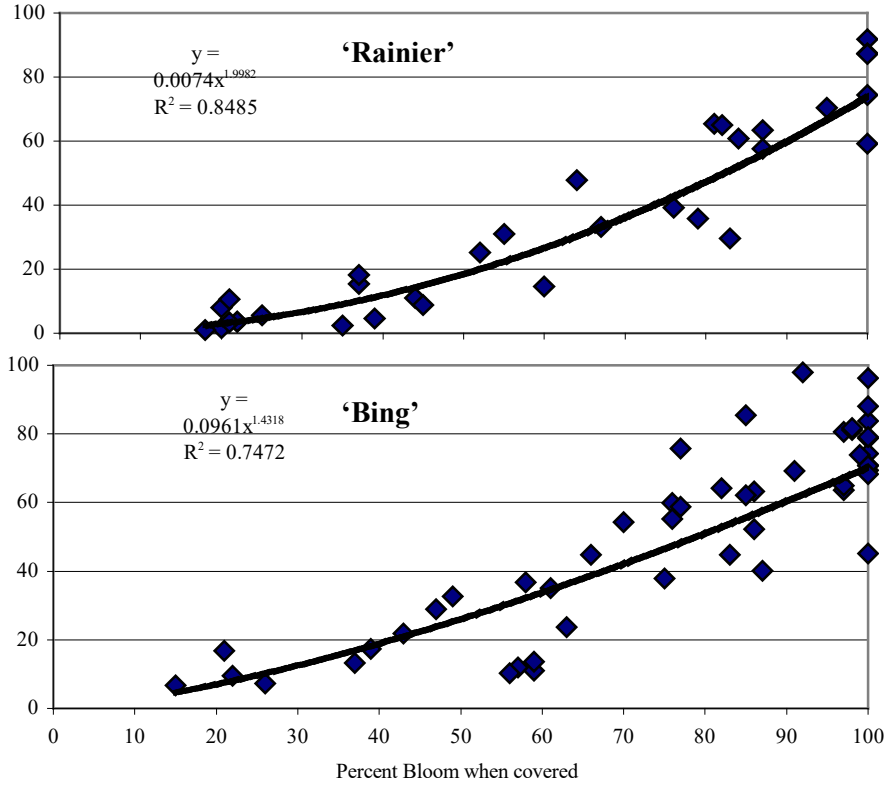

Figure 1. Relationships between percent fruit set and percent open flowers for 'Bing' and 'Rainier' sweet cherry trees.

bags were removed well after bloom. Immediately before covering a limb, each was assessed for percent flowers that were open (i.e., could be accessed by bees). Before harvest, fruit set was assessed as the number of fruit per total flowers on each limb at the time of bagging. Not surprisingly, percent fruit set increased as bloom progressed (i.e., more flowers were open). For both 'Bing' and 'Rainier', the relationship between percent fruit set and percent open bloom at the time of bagging was positive and curvilinear (Fig. 1). Therefore, fruit set is proportional to the amount of open flowers, or percentage of full bloom. These data may be important in designing thinning strategies. For example, between 20% full bloom and 80% full bloom, there exists the potential to set 60% of available flowers (if conditions are good). Unfortunately, we know little of the effect of blossom thinners on the various components of fruit set. We intend to repeat this experiment in 2007 with the addition of a caustic blossom thinner treatment. In addition, these data also suggest the possibility for manipulating pollinator activity to affect fruit set. These data suggest that, in 2006, if one targeted 40% fruit set for their orchard, the removal of bees at 40% full bloom may have achieved this. Alternatively, aggressive thinning strategies imposed immediately after 40% full bloom may accomplish the goal (though again, it is not known how thinners affect previously fertilized fruit vs. interfering with future pollination).

Post-bloom thinning A post-bloom application of 2% FOLS was made in 2005 to investigate the potential for thinning via photosynthetic inhibition. Applications were made at 14 days after full bloom (DAFB) to roughly coincide with the switching from growth supplied by stored resources to being supplied by current season assimilates. In addition, this is a period of high fruit growth rates in

early stage I, and therefore, high sink demand (see High density orchard management report). We hypothesize that by reducing assimilate supply at this stage, we may be able to induce resource limitations and fruit drop. Indeed, fruit set (# fruit/100 flowers) in 2005 was reduced significantly by FOLS applied 14 DAFB (data not shown). This response is likely a result of photosynthetic inhibition from FOLS because pollination/fruit set had already taken place. However, the post-bloom FOLS application was less effective at reducing fruit set than the applications made during bloom. This is likely because post-bloom applications were less phytotoxic compared to applications during bloom and there was no interference with pollination and fruit set – a clear thinning mechanism of bloom applications of FOLS. Despite reductions in fruit set, post-bloom FOLS did not affect fruit yield or quality.

In 2006, we conducted post-bloom thinning timing trials with FOLS and rate trials with both FOLS and tergitol. These were products our previous research showed had phytotoxic effects and reduced photosynthesis. In the timing trial, 2% fish oil mixed with 3% lime sulphur was applied at 14 or 21 days after full bloom or on both dates to 'Bing'/'Gisela 6' trees. No treatment reduced significantly fruit set. This contrasts our previous results with 2% fish oil + 2% lime sulphur applied 14 days after full bloom in 2005. In 2006, fruit set overall was high at 68%. Fruit quality was not affected by any treatment. In the rate trial, 2% fish oil was mixed with either 2%, 3%, or 4% lime sulphur, and tergitol was applied at 1%, 1.5%, and 2% at two weeks after full bloom to 'Bing'/'Gisela 6' trees. Again, no thinning treatment significantly affected fruit set, compared to the control (data not shown). It is not clear why FOLS was ineffective in 2006 and effective in the previous year. In 2005, trials were on 'Bing'/ 'Gisela5' and treatments in 2006 were applied to 'Gisela 6'-rooted trees. The apparent thinning mechanism is via reductions in carbohydrate supply to developing fruit. Larger, 'Gisela 6'-rooted trees may have had greater carbohydrate reserves to supplement the transient reduction from thinner applications. We intend to continue to investigate potential post-bloom thinning programs in 2007, focusing on mode of action.

Table 6. Effect of thinning treatments applied to leaves (not flowers) and flowers (not leaves) on fruit set of 'Bing'. Letters indicate statistical differences by Duncan analysis of variance test within column ($p < 0.05$). Asterisks indicates significant differences within row.

Treatment	Leaves covered/flowers treated	Leaves treated/flowers covered
	Fruit set (% available flowers)	
Control	24.8 ab	34.1 ab
ATS	18.9 b*	42.9 a*
VOE	35.3 a	40.7 a
Tergitol	21.9 ab	19.1 b
FOLS	10.3 b*	21.3 b*

In 2005 and 2006 we attempted to better understand the mechanism by which thinners effect a response. Our previous research and printed reports in other species point to two possibilities – a reduction in tree/spur carbon balance via reductions in net photosynthesis and/or increase in dark respiration, and the interference with pollination and fruit set via causticity to floral structures. In 2005, just prior to the 80% full bloom thinner applications, we covered with plastic bags either the entire spur leaf area (flowers exposed) or all flowers (leaves exposed to thinner). We evaluated fruit set near harvest as a percent of available flowers on a spur basis. When only flowers were treated with thinners, fruit set varied by three-fold though no treatment was significantly different from the control. Both ATS and FOLS however were significantly lower fruit set than VOE (Table 6). FOLS reduced fruit set the most, to about 41% of the control. When only leaves were treated with thinning treatments (i.e., flowers were untreated) fruit set of tergitol- and FOLS-treated spurs showed the greatest reductions in fruit set (ca. 40%) and VOE- and ATS-treated spurs showed numerically greater fruit set than the control (Table 6). These contradict previous reports on the inhibition of pollination by VOE by sealing closed the unopened perianth. In addition, it appears that ATS, despite

significantly reducing NCER (though it was the least phytotoxic thinning treatment) acts by interfering with pollination. Only for ATS and FOLS was fruit set significantly lower when flowers were treated vs. when leaves were treated (indicated by asterisks in Table 6). This suggests that these thinners are most effective when applied to blossoms rather than leaf tissue. In contrast, tergitol was more effective when applied to leaves, rather than flowers only (44% vs. 12% reduction, respectively). In 2006 we covered leaves and flowers separately again but for both the 20% and the 80% full bloom applications. In contrast to the 2005 trial, fruit set was not significantly reduced by any thinner when applied only to the leaves (data not shown). In 2006, fruit set overall was much higher than in 2005. However, when applied to the flowers, each thinner reduced fruit set. FOLS, tergitol, and ATS reduced fruit set vs. the control by ca. 18%, 30%, and 15%, respectively.

Gibberellic acid to inhibit floral bud induction We have shown previously that applications of high rates of GA_3 to 7-year-old 'Bing'/'Gisela 1' trees can inhibit the formation of flower buds, reduce yield, and improve fruit quality significantly in the season after application. In 2004 we conducted an isomer trial on 'Bing'/'Gisela 1' trees to compare the efficacy of GA_3 vs. GA_{4+7} at reducing return bloom and balancing crop load in the season subsequent to application (i.e., 2005). Every application of GA_3 and GA_{4+7} in 2004 significantly reduced return bloom and yield in 2005 compared to the control. At 100 mg/L, GA_3 and GA_{4+7} reduced yield by ca. 71% and 34%. At 200 mg/L GA_3 treatment nearly eliminated all flowers with a 95% reduction in yield; GA_{4+7} was not as inhibiting, reducing yield by 37%. No treatment had a positive effect on crop value though GA_3 at 100 mg/L did improve soluble solid and firmness. Unfortunately, this orchard was not particularly productive – our untreated control trees yielded less than 9 kg (<20 lb). Therefore, fruit growth in untreated trees was not limited by the partitioning of assimilates. Our fruit weight data supports this contention – there was no difference in fruit weight between control trees and those which yielded less than 1 kg.

In 2006 we initiated another trial evaluating the effects of rate of different GA isomers (GA_3 and GA_7) on 'Rainier' fruit quality in the season of application, and, in the subsequent season, return bloom, yield, and fruit quality (to be conducted in 2007). In-season effects of GA isomer and concentration were significant (data not shown). GA_3 caused an increase in fruit firmness that was rate dependent. GA_7 in contrast, had no effect upon fruit firmness. Across 4 picks, irrespective of rate, neither isomer had any consistent impact on fruit soluble solids or weight, though in the first two picks, GA-treated fruit had higher soluble solids. Red coloration of fruit skin was inhibited by both GA isomers and GA_3 was more inhibiting to color development than GA_7. Overall, yield of fruit with greater than 50% surface colored red was reduced by 50%, 64%, and 73% by GA_3 at 25, 50, and 100 ppm, respectively and reduced by 35%, 32%, and 43% by GA_7 at 25, 50, and 100 ppm, respectively. Both GA isomers delayed fruit harvest compared to untreated trees. Approximately 90% of untreated fruit were harvested in the first two picks vs. only ca. 50%, 37%, and 28% for GA_3 at 25, 50, and 10ppm, and 72%, 63%, and 49% for GA_7 at 25, 50, and 100 ppm, respectively (Fig. 2). GA_3 delayed fruit maturation more than GA_7 did. We also recorded significant increase in shoot growth in GA-treated canopies. The greatest increase in vegetative extension growth was from 25 ppm of GA_3 (ca. 18% increase). We will assess treatment effects on floral bud induction by examining buds/spur and flowers/bud during the winter. In addition, return bloom will be assessed in 2007 along with fruit yield and quality.

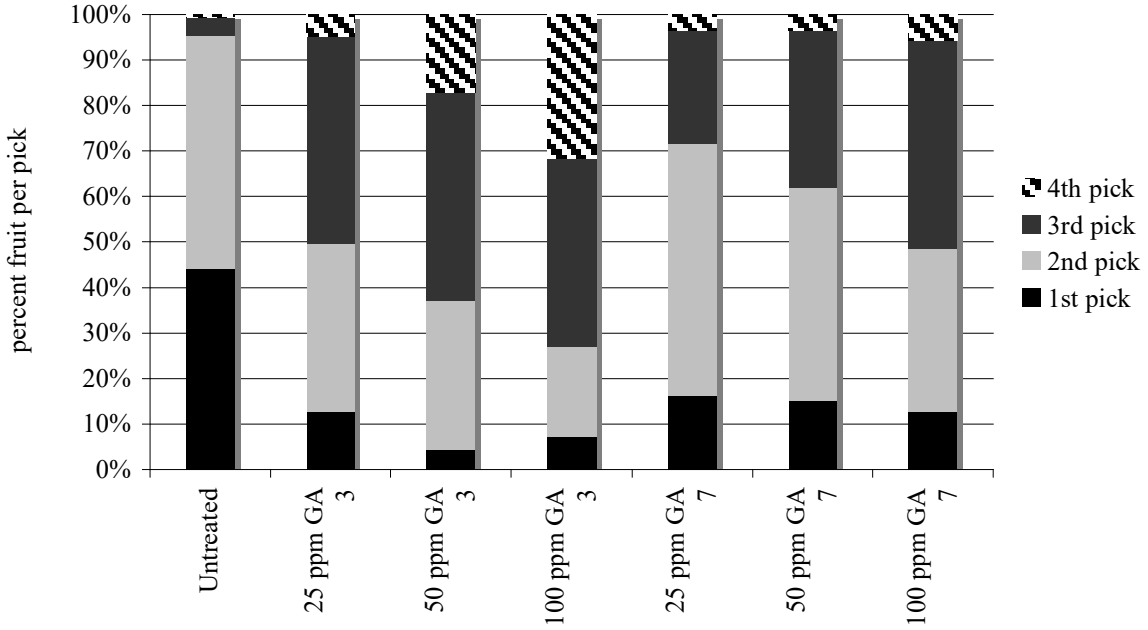

Figure 2. Effect of gibberellic acid isomer and concentration on the percentage of 'Rainier' fruit (of total yield per tree) harvested on 4 separate harvest dates. 1st pick – 23 June, 2nd pick – 1st July, 3rd pick – 10 July, 4th pick – 19 July.

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FINAL PROJECT REPORT
WTFRC Project Number: CH-04412

Project Title: Clonal rootstock evaluations

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	6199	6301	6553
Benefits	1736	1953	2031
Wages	6000	8000	8000
Benefits	960	1280	1280
Equipment	1500		
Supplies	2000	2000	2000
Travel	1500	1500	2000
Miscellaneous			
Total	19895	21034	21864

OBJECTIVES:

1. Continue evaluation of the NC-140 regional project trial ('Bing' on 17 new rootstocks) established in 1998 for horticultural and physiological evaluations and fruit quality. Projected trial duration is 10 years.

2. Continue evaluating vigor and cropping performance of other orchard trials with key PNW cultivars on various rootstocks

3. Analyze the physiology of interactive rootstock/scion horticultural traits (e.g., canopy leaf area, yield efficiency, precocity, graft compatibility).

4. Establish planting of 2005 NC-140 sweet cherry rootstock trial.

SIGNIFICANT FINDINGS:

- high quality fruit can be grown on precocious, dwarfing rootstocks
- Gisela 5 and Gisela 6 are recommended for Bing
- rootstock affected scion vigor, yield, and fruit quality
- rootstock altered 'Bing' fruit maturity (ca. 5 days) and bloom timing (4 days) significantly
- fruit yield was unrelated to tree vigor
- fruit maturity was unrelated to tree vigor
- tree vigor was related negatively to bloom date (i.e., smaller trees bloom earlier than large trees)
- the Gisela series is very precocious/productive
- Gisela 6 induced the greatest cumulative yield (1998 - 2006) of fruit 10.5-row and larger (160 kg/tree), Mazzard induced the third least among rootstocks (49.6 kg/tree)
- Mazzard had the lowest yield in 2006 (19.3 kg, 42.5 lb/tree) but the highest quality fruit
- the worst quality fruit was harvested from Gisela 209/1 and Edabriz
- no rootstock out-performs Gisela 5 or Gisela 6 in the vigorous – semi-dwarfing categories
- PiKu 1 is less vigorous and more precocious than PiKu 3
- novel crop load management strategies will need to be developed to grow high quality sweet cherries on precocious and dwarfing rootstocks

METHODS:

The 1998 NC-140 plot was planted at WSU-Prosser's Roza Experimental Unit, with 'Bing' as the scion cultivar and 'Van' as the pollenizer, on the German rootstock series Gisela 4 (GI 473/10), Gisela 5 (GI 148/2), Gisela 6 (GI 148/1), Gisela 7 (GI 148/8), GI 195/20, GI 209/1, and GI 318/17; the German rootstock series Weiroot 10, W13, W53, W72, W154, and W158; Edabriz (France); P-50 (Japan); and Mazzard and Mahaleb seedlings as controls. There are 8 replications/rootstock, with guard tree around the plot perimeter, and tree spacing of 19.5 x 19.5 ft (6.0 x 6.0 m) to reduce the potential influence of neighboring trees. Irrigation by microsprinklers and frost protection by wind machine were installed. A duplicate plot was planted for potentially destructive analyses, such as physiological stress treatments. The effects of rootstock on tree yield, vigor, fruit quality, first and full bloom dates, fruit maturity, and senescence and cold acclimation will be documented annually.

A research orchard was planted in 1998 with WSU-Prosser varieties (including Chelan, Cashmere,

Benton, Selah, Rainier and Tieton) and elite selections (including 8011-3, 7147-9, and 7903-2) on several Gisela rootstocks (including Gisela 5, 6, 195/20, and 209/1), Mazzard, Mahaleb, and Colt. In this block, tree vigor, fruit yield and quality, and graft compatibility will be monitored. Several of these newly released cultivars (*e.g.*, Chelan, Tieton, Benton, Selah) and advanced selections (*e.g.*, PC 8011-3, PC 7903-2, PC 7147-9) will be subjected to one of two crop load treatments: (1) unthinned control, and (2) 50% removal of blossoms by hand. Tree growth, fruit yield and quality (weight, row-size distribution, soluble solids, and firmness) will be evaluated for each scion grown on Gisela 6, Gisela 5, Gisela 195/20, and Edabriz, where possible.

Another orchard, planted in 2001, will be utilized to evaluate the effects of two new rootstocks (PiKu 1 and PiKu 3) on growth, precocity, fruit quality, and graft compatibility of Celeste, Benton, Selah, Tieton, Regina, Bing, Skeena, Sweetheart, Attika, Rainier, Lapins, Chelan, Summit, Black Gold, White Gold, Glacier, and Sonata.

In a separate trial in cooperation with Amy Iezzoni of MSU, we have planted 21 MSU rootstock selections, totaling 117 trees, in a test plot at the Roza farm. The control rootstock is GI 6 and the scion is Bing with Tieton/GI6 as the pollinator. An additional 243 trees (84 selections) will be planted in 2004. The effects of rootstock genotype on scion growth habit, precocity, and fruit quality will be documented annually.

RESULTS AND DISCUSSION:

1998 NC-140 trial

Productivity 2006 was the sixth fruiting year (9th leaf) for most of the rootstocks in this trial and we recorded tremendous variability in fruit yield per tree (19 – 82 kg/tree) (Fig. 1, Table 1). Clearly, rootstock has a significant effect on 'Bing' precocity and productivity (Fig. 1). In 2006, 16 of the 17 rootstocks in this trial exhibited greater productivity compared to the industry standard, Mazzard. In this 9th season, many trees have reached full production; most notably so are those in the Gisela series (e.g., Gi 7, Gi 5, Gi 6, Gi 195/20). In 2005, mean yield was 52.0 kg/tree, about 2.5 times greater yields than the previous year. The most productive rootstocks were from the Gisela series (Fig. 1, Table 1). The least productive rootstocks were unchanged from 2005: W53, P-50, and Mazzard. Trees on each of these rootstocks yielded about 20 kg (45 lbs) per tree. For W53, by far the most size-controlling of the rootstocks, low yields were due to limited canopy size and therefore inadequate bearing surface. We conclude that, even at a high tree density, this rootstock is too dwarfing to produce commercially acceptable yields. However, for P-50 and Mazzard, low yields are inherent and due to poor floral bud induction. The lack of yield from Mazzard-rooted trees remains a significant concern and its greatest drawback.

Cumulative yield data (2001 – 2006) reveal the overall lack of productivity on Mazzard (Fig. 2). Mazzard-rooted trees yielded only 5.7 tons of 10.5-row and larger fruit per acre between 2001 and 2006 (i.e., less than 2 tons per year). Mazzard was the third-least productive rootstock of 10.5-row+ fruit among all 17. The rootstock yielding the greatest quantity of 10.5-row+ fruit was Gisela 6. Between 2001 and 2006, Gisela 6 and Gisela 5 yielded about 222% and 144% more 10.5-row+ fruit than Mazzard. The trend in tree productivity on Mazzard, Mahaleb, Gisela 6, and Gisela 5 is presented in Figure 3. The precocity and productivity of the Gisela series is again apparent - early yields were 3 to 6-fold greater than those on Mazzard. Bing productivity on Mazzard has increased every year. This suggests that after 9 years, these trees have not yet reached full production. In contrast, our data suggest that Gisela-rooted Bing trees reached full production in their 4th and 5th leaf.

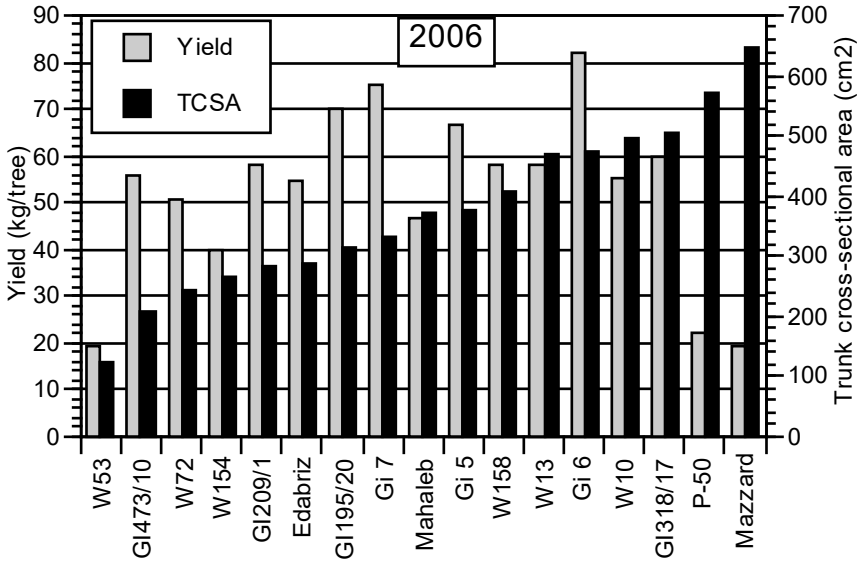

Figure 1. Effect of rootstock on vigor (trunk cross-sectional area, TCSA, black bars) and fruit yield (kg/tree, grey bars) from 9-year-old 'Bing' trees grafted on 17 different rootstocks.

Vigor & quality Vigor varied among rootstocks by more than 5-fold (Fig. 1 & 3). Mazzard is the most vigorous rootstock, W 53 is the least vigorous. Gi 5 and Gi 6 are ca. 58% and 73% the vigor of Mazzard, respectively. P-50, Gi 318/17, Gi 6, W10, and W13 are all vigorous (ca. 75%+ of Mazzard). W 158, Mahaleb, Gi 5, Gi 7, and Gi 195/20 were semi-dwarfing (ca. 50 – 65% of Mazzard). Edabriz, Gi 209/1, W 154, W 72, Gi 473/10, and W 53 are dwarfing rootstocks, reducing TCSA to less than 45% of Mazzard. The tree density of the research orchard is 115 per acre. This is a low density for even the most vigorous rootstocks. It is not known how higher, more appropriate densities would affect tree growth and productivity. However, as we reported previously, yield and precocity are unrelated to vigor (Fig.1). Low-yielding, vigorous rootstocks (i.e., Mazzard and P-50) will not provide growers the early returns on investment or size control necessary to improve labor efficiency, and are not recommended for 'Bing'. However, these rootstocks may be appropriate for very productive and precocious varieties (e.g., 'Chelan', 'Sweetheart'), especially when grown in poor soils.

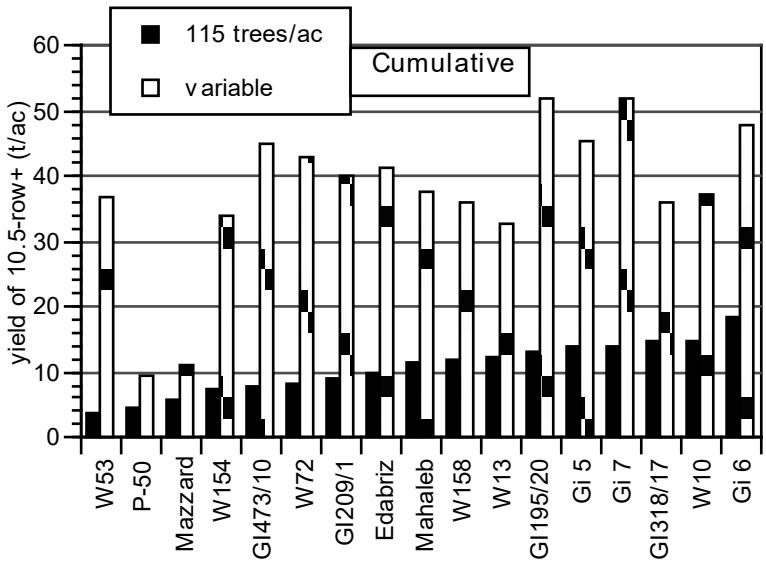

Figure 2. Cumulative yield (2001 to 2006) of 10.5-row and larger 'Bing' fruit (tons/acre) at the test block density of 115 tree/acre and a hypothetical variable density inversely proportional to tree vigor (e.g., Mazzard @ 220 trees/ac; Gisela 5 @ 380 trees/ac; W53 @ 1150 trees/ac).

There are several rootstocks which exhibit moderate – high yield and vigor control (i.e., high yield efficiency) which may be desirable and appropriate for high density, more efficient plantings. In this regard, our analyses suggest almost every rootstock is an improvement upon Mazzard. In a slightly-dwarfing category (70 to 90% of Mazzard), Gisela 6 is the most promising rootstock. In 2006, Gisela 6-rooted trees yielded 82 kg (180 lbs/tree) of fruit that was 9.5 g, 18.0 brix, and ca. 85% 10.5-row and larger (Table 1). This translates into approximately 9.5 tons/ac at the low density of the research orchard. In a semi-dwarfing category (50 to 65% of Mazzard), the most promising rootstock is Gisela 5. In 2006, 'Bing' on Gisela 5 yielded ca. 67 kg (150 lbs/tree) of fruit that was 8.9 g, 18.4 brix, and ca. 75% 10.5-row and larger. At 115 trees/ac, this translates into ca. 7.7 tons/ac.

In 2006, fruit quality overall was good on most rootstocks (Table 1). Fruit weight ranged from a low of 6.8 g on Gisela 473/10 to 11.1 g on Mazzard. Mean fruit weight was 8.9 g. Fruit soluble solids was 18.5 brix, on average, and rootstock had only a subtle effect. Fruit firmness was low in 2006. This is likely due to unseasonably warm temperatures in the days before harvest. Firmness averaged 227 g/mm, down about 23% from the previous season. Percent of fruit that were 10.5-row and larger ranged from a low of 33% (Gi473/10) to 100% (Mazzard). Most rootstocks yielded very few (< 5%) fruit that were smaller than 12-row. Only W53 and Gi473/10 yielded more, 14 and 10%, respectively.

We recorded no clear relationship between fruit yield or yield efficiency and fruit soluble solids or firmness (data not shown). However, there was a clear negative relationship between yield efficiency and fruit weight ($r^2 = 0.69$). In addition, % fruit 10.5-row and larger was related closely and negatively to tree yield efficiency (Fig. 4). This is due to insufficient supply of photosynthate to fruit at high yield efficiency (i.e., high fruit-to-leaf area ratio) and has been reported previously by this lab. Because trees yielded very little fruit that was smaller than 12-row, there was a positive relationship between tree yield efficiency and % 11- and 12-row fruit (Fig. 4). Balanced cropping targets for 2006 can be developed by analysis of these relationships, For example, these data suggest that, to produce a target of no less than 80% 10.5-row and larger fruit, yield should be limited to ca. 0.16 kg per cm^2 TCSA. For Gisela 5, for example, this target translates into approximately 60 kg/tree (0.16 kg cm^{-2}

376 cm^2). Actual yield in 2006 was close to this target at 67 kg. For comparison, on Mazzard, balanced cropping of 0.16 kg/cm^2 would translate into a yield of ca 103 kg/tree. Actual yield from Mazzard-rooted trees was considerably less, at 19.3 kg/tree, suggesting that these trees were very much under-cropped in relation to the capacity of the canopy to support fruit growth.

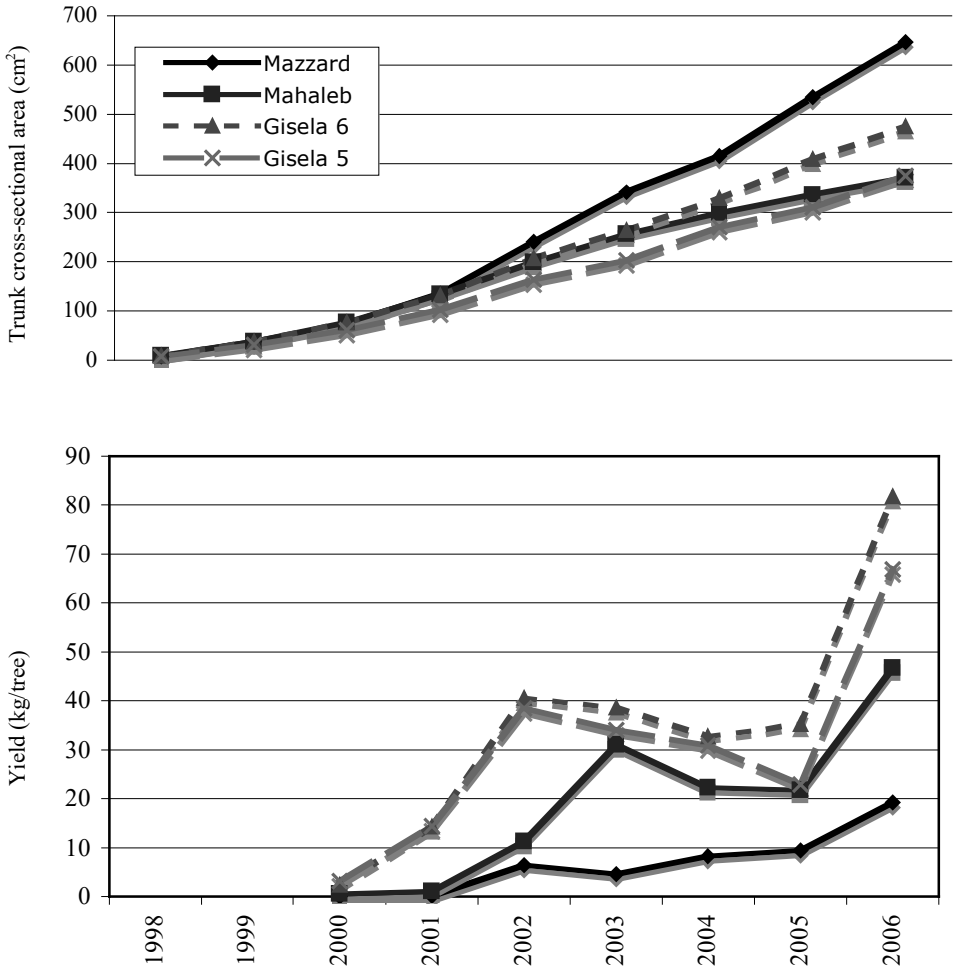

Figure 3. Trend in tree vigor (trunk cross-sectional area) and productivity (kg/tree) of 'Bing' sweet cherry on 4 rootstocks from the 1998 NC-140 trial. Trees were planted in 1998.

In 2005 and 2006, we documented a significant variation in harvest date based on fruit skin color (data not shown). In 2005, 'Bing' on Gi 5, Gi 6, and W158 reached optimum harvest maturity on 22 June while fruit on Mazzard did not reach similar maturity until 1 July. Average harvest date for all rootstocks was 24 June. In 2006, we documented a 4 day variation in harvest maturity. Again, Mazzard-rooted trees were the latest maturing and those on Gi 3, and Gi 473/10 were the earliest to mature. It is not known whether similar discrepancies in harvest maturity would exist for other earlier or later-maturing varieties. However, this result highlights the need to consider rootstock when planning new orchards for a particular harvest season.

Rootstock also affected the date of first and full bloom (data not shown). There was about a four-day (ca. 50 GDD @ base-40) difference between first bloom in Edabriz, Gi 5, W158, and Mahaleb

(earliest-blooming, at ca. 680 – 690 GDD) and Mazzard (latest blooming, at 740 GDD). Mazzard-rooted trees were also the last to achieve full bloom, again about 4 days (ca. 70 GDD) later than Gi 5 and Edabriz. At our orchard site, we have not noticed any relationship between first and full bloom dates and frost damage to flowers but in other sites, this may be a concern. We did document in 2005 a significant positive linear relationship ($r^2 = 0.71$) between tree vigor and bloom date (i.e., the more vigorous the tree, the later the bloom).

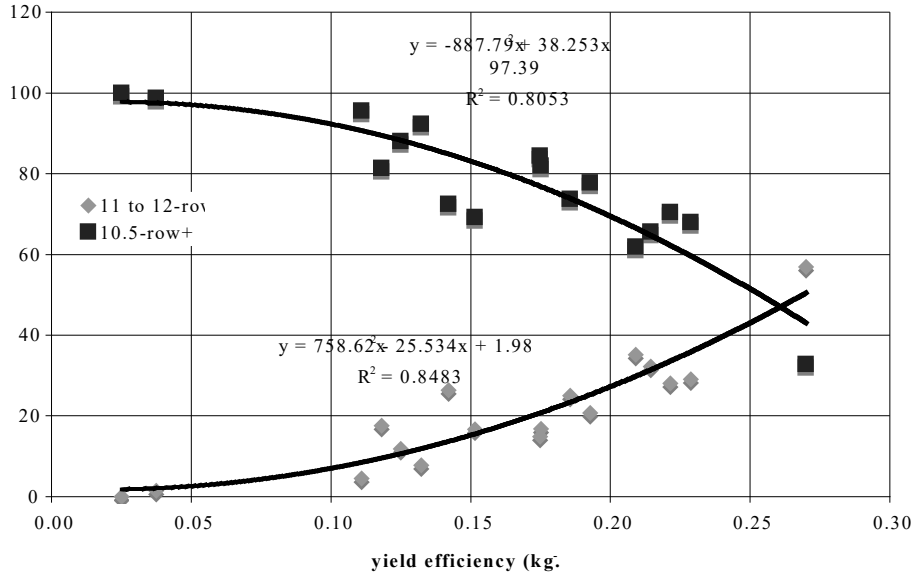

Figure 4. Relationships between tree yield efficiency (kg fruit per cm2 trunk cross-sectional area) and the percent of fruit 10.5-row or larger (black squares) and the percent of fruit 11 to 12-row (grey diamonds) from 9-year-old 'Bing' trees grown on 17 different rootstocks. Each point is a mean of 8 single tree replications per rootstock.

PiKu trial In 2001 we planted an orchard of 16 scion varieties on both PiKu 1 and PiKu 3 rootstocks. 2006 was this orchard's third year of fruiting for most varieties. On PiKu 3, Bing and Selah were the least productive, though, due to poor fruit set overall, yields were low throughout the orchard (Table 1). BlackGold/PiKu1 was the most productive combination, yielding only 2.8 kg (6 lbs) per tree. Several varieties yielded no fruit on PiKu 3 (data not shown). Again in 2005, PiKu 1 was significantly more precocious, out-yielding trees on PiKu 3 by over 7-fold, though this difference was only ca. 2 lbs/tree. In addition, PiKu 1 remains about 40% less vigorous than PiKu 3. Across all varieties, there were significant, albeit subtle, differences in fruit quality between PiKu 1 and Piku 3 in 2005. Fruit firmness and soluble solids were about 6% higher on PiKu 1. Tree mortality was similar for both rootstocks – we have documented ca. 10% tree loss. Particularly poor combinations appear to be Attika/PiKu 3 (75% tree death), Glacier on both PiKus (50% tree death), and Lapins/PiKu 1 (50% tree death).

Fruit quality among scion varieties varied considerably in this third year of production (data not shown). Briefly, Summit, Tieton, Attika, Black Gold, Rainier, Regina, Selah, Sweetheart, and Skeena were among the largest fruit (ca. 10.5 g+ and > 90% 10.5-row and larger) and Sonata, Chelan, and Glacier were the smallest (< 9 g/fruit, less than ca 70% 10.5-row and larger). Sweetheart and Black Gold were the most precocious cultivars, yielding greater than 5 kg (11 lbs) per tree. Overall, productivity is low on these rootstocks, compared to the Gisela series.

Bing, Black Gold, Lapins, Tieton, and Regina were the most vigorous varieties (TCSA > 120 cm^2). Attika, Benton, Celeste, Sonata, and Glacier were the least vigorous (TCSA < 80 cm^2).

Table 1. Effect of rootstock (Piku 1 and 3) on yield and fruit quality of 4-year-old sweet cherry trees. Data are means of 16 scion varieties. Means followed by the same letters within a column are not significantly different (P>0.05).

Rootstock	Date	Weight (g)	°Brix	Firmness (g/mm)	% 11 & 12-row	% ≥10.5-row	Yield(g)	TCSA(cm^2)
2004								
PiKu 1	6/19	8.0a	22.9a	274b	31a	63a	974a	22.7b
PiKu 3	6/18	8.1a	21.1b	296a	25b	66a	406b	36.9a
LSD		0.4	0.9	19	6	8	145	3.6
2005								
PiKu 1	6/26	10.1a	23.8a	347a	8a	91a	1133a	42.2b
PiKu 3	6/28	10.1a	22.4b	327b	11a	88a	152b	72.0a
LSD		0.4	1.1	16	4	5	245	5.6
2006								
PiKu 1	7/1	10.2 b	19.2 a	264 b	13	86	5485 a	74.3 b
PiKu 3	7/2	10.7 a	18.1 b	299 a	9	90	936 b	131.9 a
LSD		0.4	0.6	13.1	4	5	742	7.9

Table 2. Effect of rootstock on 'Bing' fruit yield, quality, tree size, and yield efficiency. Data within a column followed by different letters are significantly different by LSD (P < 0.05).

Rootstock	Vigor (cm^2 TCSA)	Yield (lb)	Yield of 10.5-row+ (lb)	Fruit weight (g)
W53	123.5 g	42.6 g	28.4 g	8.3 cd
Gi473/10	208.4 j	123.1 de	42.1 efg	6.8 e
W72	241.9 ij	111.7 ef	65.1 defg	8.4 cd
W154	264.5 hij	87.9 f	70.7 def	9.1 bcd
Gisela 3	283.4 ghi	128.3 cde	70.8 def	8.1 de
Edabriz	289.7 ghi	120.0 de	96.2 bcd	8.9 bcd
Gi 195/20	315.9 fgh	154.4 abc	110.3 b	8.2 d
Gisela 7	331.8 fg	165.4 ab	114.1 b	8.3 cd
Mahaleb	370.9 g	103.0 ef	75.5 cde	9.2 abcd
Gisela 5	375.8 ef	147.3 bcd	107.4 bc	8.9 bcd
W 158	407.6 de	128.4 cde	97.4 bcd	8.5 cd
W 13	470.0 cd	128.2 cde	118.4 ab	9.7 abc
Gisela 6	475.6 cd	180.3 a	150 a	9.5 abcd
W 10	495.3 c	121.7 de	115.7 b	10.2 ab
Gi 318/17	506.7 bc	132.0 cde	113.8 b	8.4 d
P-50	569.4 b	48.9 g	40.9 fg	9.8 abc
Mazzard	646.2 a	42.5 g	42..3 efg	11.1 a
LSD	72.9	31.1	35.3	1.5

FINAL PROJECT REPORT
WTFRC Project Number: CH-05-511

Project Title: **Northwest Cherry Improvement Project**

PI: **Fredrick A. Bliss**
Telephone/email: **(530) 756-5154**
Address: **214 Inca Pl.**
City/State/Zip: **Davis, CA 95616**

Cooperators: **Matt Whiting, Jim Olmstead, Amy Iezzoni, Jim McFerson**

Budget History:

Item	**2006**
Salaries	
Benefits	
Wages	
Benefits	
Equipment	
Supplies	
Travel	**$1,500**
Miscellaneous	**$7,520**
Total	**$9,020.**

Significant activities and findings:

Presented ideas, evaluations of breeding strategies and plans, development of realistic objectives, and analyses of progress against approved project goals.

- Provided materials for incorporation into new project; "Breeding and Genetics Program for the Pacific Northwest Fresh Market Sweet Cherry Industry".
- Developed document to define suggested primary and secondary tree and fruit characteristics.
- Developed document to define Cultivar Targets for the WA and OR Sweet Cherry Breeding Program

Conducted literature reviews and preparation of reports.

- Reviewed literature associated with cherry breeding and improvement. Provided information to Jim McFerson and references to Amy, Jim O. and Matt for use in the breeding program.

Traveled to research sites in Washington and Oregon to evaluate project and assess progress.

- November 2 - 4, 2005. Traveled to The Dalles, OR to participate in the OSCC/WTFRC cherry research review. Met with Amy Iezzoni, Matt Whiting, Jim Olmstead, Jim McFerson and industry members from Washington and Oregon to discuss activities and progress in the sweet cherry breeding program. Reviewed research proposals and reports to become more familiar with activities related to cherry improvement.

- The Advisory Committee and invited guests met for a working dinner during the 2005 Cherry Research Review in The Dalles, OR. Present were: Denny Hayden, Fred Bliss, Jim McFerson, Bryce Molesworth, Kyle Mathison, Tom Mathison, Norm Gutzwiler, John Carter, Tom Auvil, Brent Milne, Amy Iezzoni, Tim Smith, Tom Butler, Randy McAlister, Jim Doornink, Matt Whiting and Jim Olmstead. The topics discussed included current and future needs such as greenhouse space in Prosser, testing site selection and costs, contracting DNA marker genotyping to outside labs, and commercialization and intellectual property issues for both the scion breeding program and Amy's rootstock evaluation project.

Continued work on developing a panel of sweet and sour cherry cultivars for DNA screening by Cameron Peace at the Kearney Agric. Center, Parlier, CA for polymorphic expression of candidate genes he isolates in his NRI-funded grant.

- October 27 – 28, 2005. Traveled to Kearney Agric. Center, Parlier, CA to meet with Cameron Peace, Carlos Crisosto, and Zaiger Genetics to review molecular marker research in their labs and to assess potential for use in marker assisted selection (MAS) in stone fruits.

Submitted invoices for expenditures on a quarterly basis.

- Quarter one (July 1, 2005 – Sept. 30, 2005 : $ 560.00
- Quarter two ((Oct. 1, 2005 – Dec. 31, 2005): $3,046.50
- Quarter three (Jan. 1, 2006 –Mar. 31, 2006): $ 320.00
- Quarter four (Apr. 1, 2006 – June 30, 2006): $ 360.00
- Total $4,286.50

Results and discussion:

Jim Olmstead started in the Post-doc position and provides on-site breeder guidance and direction for the project. This is especially important for continuity and progress in the breeding program.

Significant progress was made by the breeding team to produce the number of seedlings specified in the program plan. Germination of the 7,166 seed from 2005 began as expected in January of 2006.

Due to lack of available greenhouse space in Prosser, space was contracted out in two commercial greenhouses. In the Spring, 2006, 17,848 seed from 111 crosses were realized.

The Mid-Columbia Ag Research and Extension Center (MAREC) in Hood River was selected as the Oregon testing site for Cherry Breeding Program selections. Amy Iezzoni and Jim Olmstead visited the Mid-Columbia Agricultural Research and Extension Center on Nov 2 and met with Clark Seavert, Anita Azarenko, and Bryce Molesworth about plot establishment. Plans were finalized for scion seedling plantings beginning in Spring 2007.

An initial contact was made with STA Laboratories (www.stalabs.com) to test the feasibility of DNA marker genotyping for the Breeding Program. This will provide opportunities for developing capacity for marker assisted selection in the future. Initial work can be done using markers for the self-fertility allele which are public and the test is relatively easy to interpret. This will be an important step to determine the extent with which the genotyping for selection can be contracted out.

Powdery mildew resistance screening was done for seedling populations segregating for resistance genes. Greenhouse incidence of disease was used as an initial evaluation method. Concurrently, more detailed evaluations of fruit and foliar disease reactions for parental resistance sources and previously identified seedlings are being conducted.

It was planned to develop and validate a DNA screening test for determining seedlings resistant and susceptible to powdery mildew, but no candidate markers were identified despite extensive surveys. This lack of polymorphism adds to the growing evidence that there is a lack of genetic diversity in sweet cherry germplasm. This is a major issue that should be carefully evaluated and properly addressed.

There is excellent progress being made on developing and using genomic tools for research and breeding of rosaceous crops, including sweet cherry. The recent hiring of additional scientists using molecular genetics provides excellent complementary support for the Northwest breeding programs.