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

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

PI: James Olmstead
Organization: WSU-IAREC
Telephone/email: 786-9249/jwolmstead@wsu.edu
Address: 24106 N. Bunn Rd.
City: Prosser
State/Province/Zip WA 99350

Cooperators: Gary Grove, phone: (509) 786-2226, email: grove@wsu.edu;
Matthew Whiting, phone: (509) 786-9260, email: mdwhiting@wsu.edu

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:

Is the gene controlling PM foliar resistance the same in all five selections?

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.

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

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.

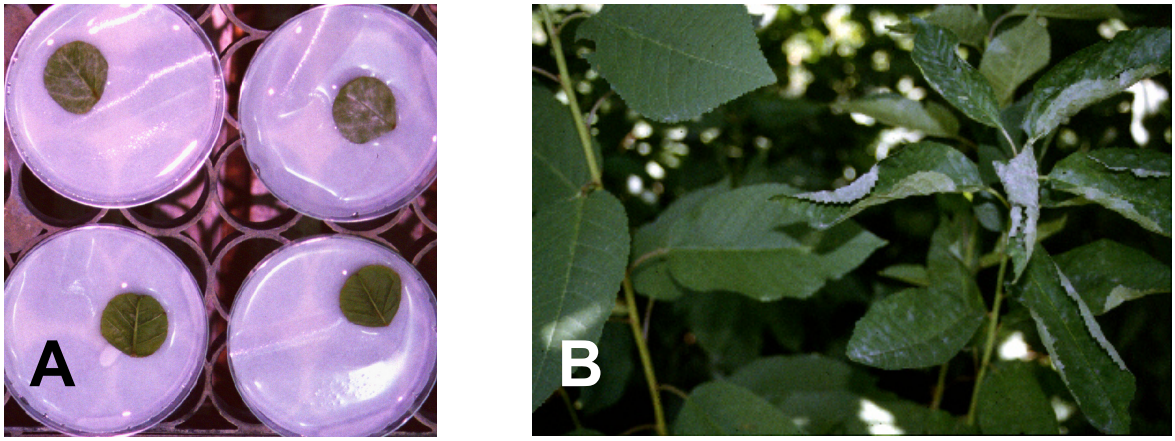

Figure 1. Resistance phenotype exhibited by progeny from the cross PMR-1 x Rainier. **A.** Leaf disks taken from four different progeny and inoculated with PM. Top: leaf disks are susceptible to PM and show significant sporulation. Bottom: leaf disks completely resistant to PM infection. **B.** A resistant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

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

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.

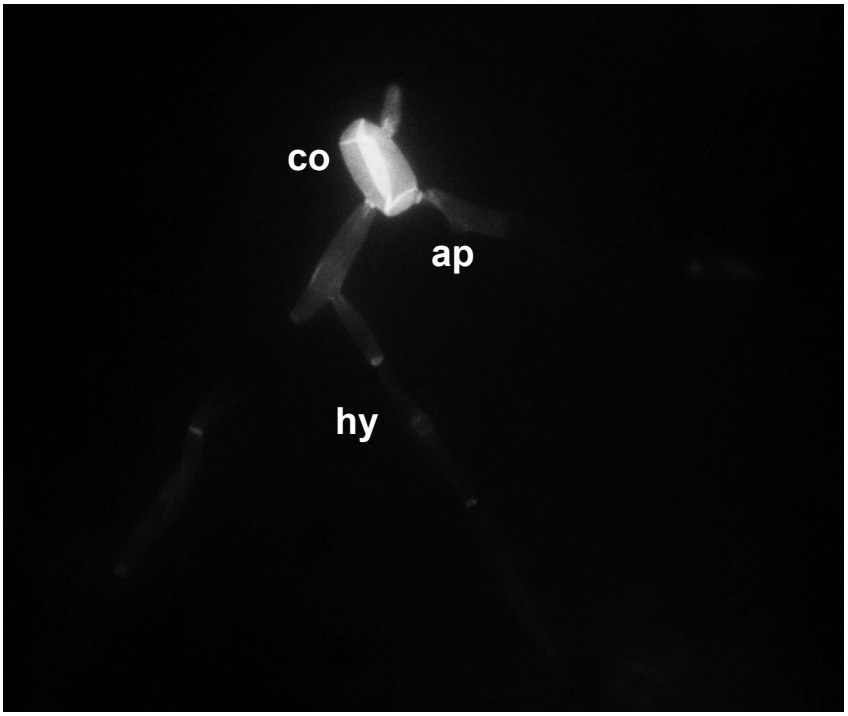

Figure 2. Example of initial powdery mildew growth on a sweet cherry leaf. Fungal structures were stained with solophenyl flavine 7GFE and viewed at 40x magnification. Co = conidia, ap = lobed appressorium, hy = hyphae.

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.

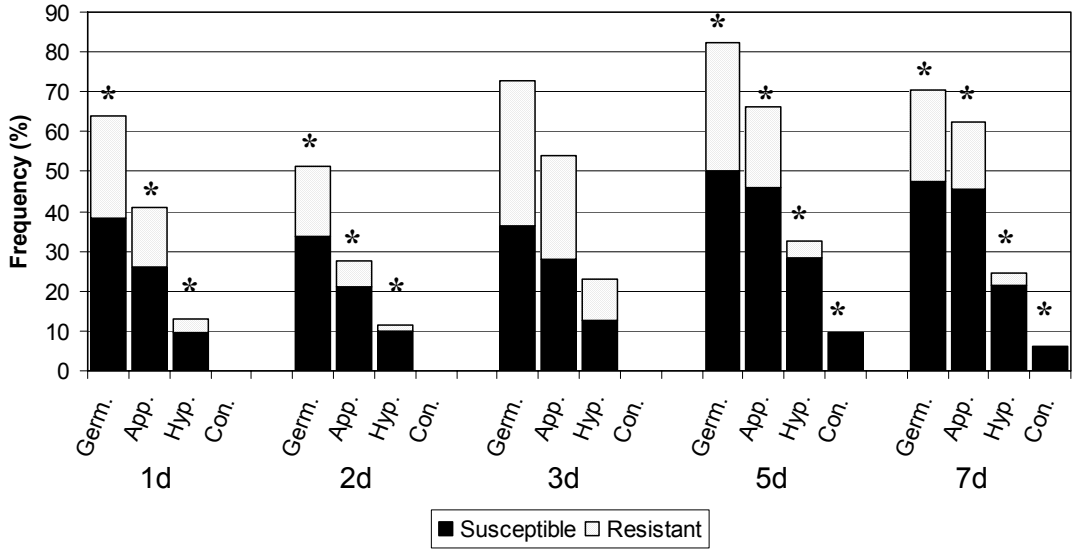

Figure 3. Frequency of observed powdery mildew growth stages on resistant and susceptible sweet cherry cultivars. Germ. = germ tube formation emergence from conidia; App. = appressorial lobe evident; Hyp. = hyphae present; Con. = conidiophore present. Significant differences ($P < 0.05$) were determined 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).

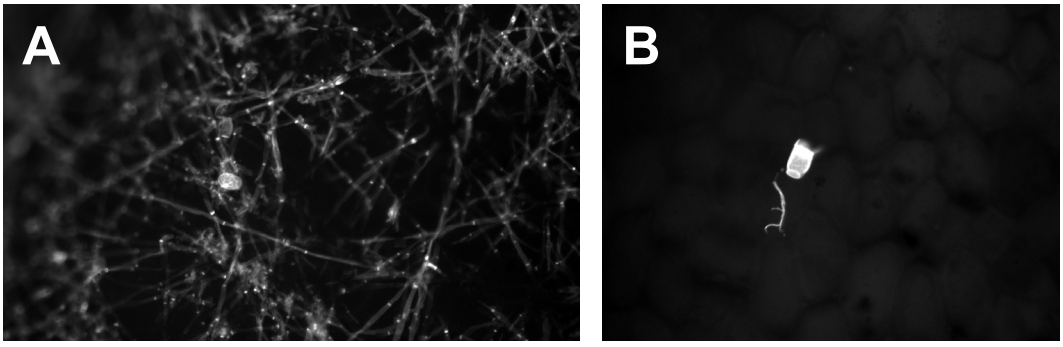

Figure 5. Powdery mildew colonization of susceptible 'Rainier' (A) and resistant 'DD' (B) fruit two weeks after inoculation.

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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