FINAL PROJECT PROPOSAL

Project Title: Sources of primary cherry powdery mildew inoculum - revisited

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Cooperators: Ms. Neusa Guerra[3] (WSU-IAREC, Prosser), Washington and Oregon State Growers

[1] Research lead on fruit quality aspects and identification of volatiles of objectives 2
[2] Research lead on modeling component of objective 2
[3] Technical assistant

Total Project Request: **Year 1:** 24,872 **Year 2:** 25,040 **Year 3:** 31,219

Other funding sources
None

Budget 1
Organization Name: WSU-IAREC Contract Administrator: Lisa Bruce
Telephone: 509-786-2226 **Email address:** lisa-bruce@wsu.edu

Item	**2016**	**2017**	**2018**
Salaries			
Benefits			
Wages[4]	4800	4800	~~4800~~
Benefits	480	480	~~480~~
Equipment			
Supplies[5]	6000	6000	~~6000~~
Travel[6]	2500	2500	~~2500~~
Miscellaneous			
Plot Fees			
Total	13780	13780	~~13780~~

2018 funding will not be requested due to ……………………………….

Budget 2

Organization Name: **OSU-MCAREC** **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2016	2017	2018
Salaries[1]	4,584	4,722	~~4864~~
Benefits[2]	1008	1038	~~1069~~
Wages			
Benefits			
Equipment			
Supplies[3]	5,000	5,000	~~5,000~~
Travel[4]	500	500	~~500~~
Miscellaneous			
Plot Fees			
Total	11,092	11,260	~~11,433~~

Footnotes:
[1]300hr for a Biological Science Tech. at $15.28/hr. 3% increase is factored into Year 2 and 3.
[2]OPE: 22% of the wage, with a 3% annual increase.
[3]Supplies: renting and maintaining cold rooms; GC/MS supplies including gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals; shipping fees.
[4]Travel to field

Budget 3

Organization Name: WSU-AgWeatherNet **Contract Administrator:** Lisa Bruce
Telephone: 509-786-2226 **Email address:** lisa-bruce@wsu.edu

Item	2016	2017	2018
Salaries[1]			~~4,620~~
Benefits			~~1,386~~
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total			~~6,006~~

Footnotes:
[1]Dr.Melba Salazar

Significant findings

- The stem cavity (stem-fruit attachment zone) is the primary infection court for the fungus. Fruit infections are initiated in this area. If the cavity is unavailable for the fungus (in our study it was sealed with Vaseline), fruit did not become infected, even if disease pressure was high (100% disease incidence in the tested orchard). Mycelium radiates away from the stem cavity towards the cheeks and down to the stylar end. (Figure 2, Table 1)
    - If the stem cavity is sealed early during the growing season, fruit development is impaired. However, knowing how fruit infection starts has major implications for future disease management strategies.

- Attachment of conidia to the leaf is a quick process (about 2h). With the exception of Sweetheart. Here, conidia attached nearly instantly (Figure 1).
    - The importance of leaf properties should be investigated. For example, it is known that hydrophobicity of the host substrate plays a major role in conidial attachment and germination success; younger leaves are more hydrophobic than older leaves. This may explain why young leaves are much more susceptible to infection than older leaves (ontogenic resistance). Observational evidence from work in the breeding program also showed that some “odd” looking cherry leaves were not (or less) infected by the fungus.

- Overwintering of powdery mildew as mycelium on cherry seeds is unlikely. Overwintering of powdery mildew as chasmothecia followed by ascospore release in the spring is the only known means of initiating an epidemic (Grove 1998).
    - Survival rate of cherry pits after overwintering on the orchard floor is low (less than 2%, on average). With the onset of winter (December), no conidial viability could be detected on cherry pits. No powdery mildew infection was observed on seedlings, even if the seed came from a previously infected cherry.

Objectives

1. Characterize the role of cherry fruit in fungal life and disease cycle

2. Identify cherry volatiles and characterize their impact on spore germination
Due to the tragic passing of Dr. Yan Wang this objective has been cancelled and it has been decided to end the study in 2017 (and not 2018).

3. Monitor flag shoot like development and post-harvest fruit management in commercial orchards in WA and OR

Objective 1

Conidia adhesion assays. Conidial adhesion (attachment) is the first interaction between fruit and pathogen. Upon contact with a host surface, fungal conidia secrete a liquid exudate that tightly binds the conidia to the host. This process is time and substrate dependent and needed to protect the fungal conidia from being removed by wind and rain. The time required for conidial adhesion to leaf surfaces was assessed on Bing, Black Republican, Early Robin and Sweetheart. Briefly, circular leaf disks were cut from a clean leaf and gently dipped onto an actively sporulating powdery mildew culture. After various time periods (0 to 8 min, 15, 45, 60, 75, 90, 105, 120, 135, 150, 180, 201, and 240 min) two inoculated leaf disks were removed and gently immersed into 1ml of water. The number of conidia transferred from the leaf disk in the water was quantified. The leaf disk was allowed to air-dry and adhesive tape was used for sampling of conidia from leaf disk surfaces for subsequent microscopic analyses. The proportion of conidia found in the water was compared to the proportion of conidia found on the leaf disk for each time point. In theory, once the spore has successfully attached to the leaf surface, it should not be easily removed by water. Hence, the proportion of conidia found in the water should decrease and the proportion of conidia found on the leaf disk should increase over time. This general trend can be observed for all but Sweetheart (Figure 1). In all trials, the proportion of conidia attached to Sweetheart leaves was greater than the proportion of conidia flushed into the water starting the moment conidia were brought into contact with the leaf surface. In the other cultivars, conidia were first detected on leaf surfaces after 1 min (Black Republican and Early Robin) and 5 min (Bing). The proportion of conidia retained on the leaf increased over the 2h time period. It has been shown that with increasing leaf age the hydrophobicity of the leaf decreases significantly, which could be a factor for the increased susceptibility of young leaves to powdery mildew infection (Bringe, Schumacher et al. 2006). All leaf disks used in this study were the same age to account for hydrophobicity. Still, attachment of powdery mildew conidia to Sweetheart leaf disks was very rapid and it would be interesting to investigate the leaf properties of this cultivar and compare it to other cultivars. Observational evidence from work conducted in the breeding program points toward an effect of leaf properties and ability of the fungus to cause disease.

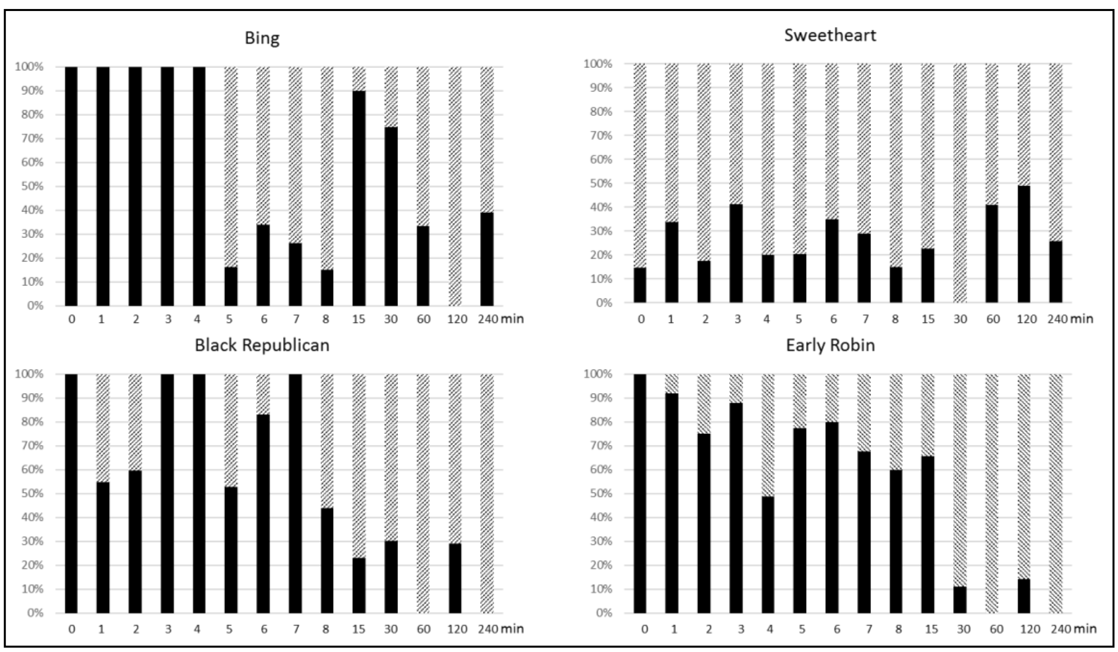
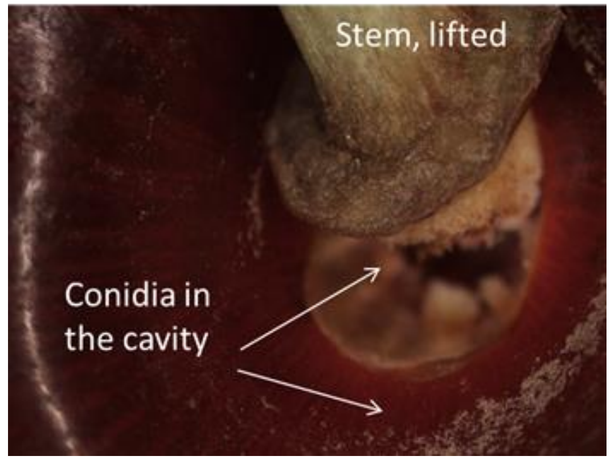


Figure 1. Proportion of conidia flushed off into the water (black bar) versus retained on the leaf (stripped bar) after various time periods (0 min to 2h).

Stem-fruit attachment area. Previous observations pointed toward the stem-fruit attachment area (from here on referred to as stem cavity) as the preferred powdery mildew infection court. The experiment was designed to establish the importance of this specific region during initiation of fruit infection. Sets of cherry clusters were uncovered and the fruit bowl was sealed off with Vaseline. Fruits were left uncovered and exposed to natural, airborne conidia. Negative control clusters remained covered all season. Cherries were evaluated for powdery mildew incidence and severity at harvest using a 0-3 severity scale (Calabro 2007). The results of this study were spectacular. Vaseline treated fruit (as well as the always covered negative control) remained disease free during both study years. This clearly shows that the primary infection court for the fungus is the stem cavity and that infection do not usually start on the cheeks or the stylar end. A possible explanation could be an easier access point for the fungus to penetrate the fruit epidermis. The cavity also provides a natural hideout for conidia, which can usually be seen (with the microscope) if the stem is lifted from the cherry. However, fruit development was significantly impaired in fruit receiving Vaseline treatment on May-21 and somewhat impaired in fruit treated on June 4. Only fruit treated on June 19 developed without impairment. Disease management strategies should take this into account.



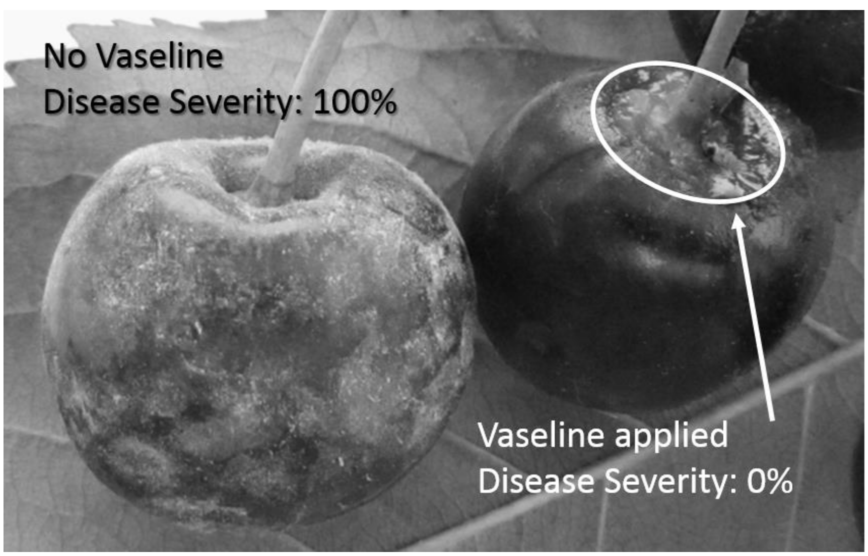


Figure 2. Two Sweetheart cherries from the same cluster. The cherry on the left was left untreated; the cherry on the right had Vaseline applied to the stem cavity on June 19. Onset of fruit infection was June 26 – 28 (2017).

Table 1 **Powdery mildew (PM) development on cherries treated with Vaseline**

		Severity[2] scale				July 25, 2017	
		0	1	2	3		
Bing	**Date^**	**0**	**1-33%[2]**	**34-66%**	**>67%**	**PM % Incidence[3]**	**Avg PM Severity[4]**
Experiment 1[1]	21-May	8	0	0	0	0	0
	4-Jun	12	0	0	0	0	0
	19-Jun	16	0	0	0	0	0
	UTC[5]	6	9	10	25	88	2.1
Experiment 2	21-May	12	0	0	0	0	0
	4-Jun	16	0	0	0	0	0
	19-Jun	12	0	0	0	0	0
	UTC	0	2	22	26	100	2.5
Experiment 3	21-May	8	0	0	0	0	0
	4-Jun	20	0	0	0	0	0
	19-Jun	11	0	0	0	0	0
	UTC	0	0	8	42	100	2.8
Sweetheart	**Date^**	**0**	**1-33%[2]**	**34-66%**	**>67%**	**PM % Incidence[3]**	**Avg PM Severity[4]**
Experiment 1	21-May	15	0	0	0	0	0
	4-Jun	12	0	0	0	0	0
	19-Jun	9	0	0	0	0	0
	UTC	6	2	12	26	87	2.3
Experiment 2	21-May	9	0	0	0	0	0
	4-Jun	16	0	0	0	0	0
	19-Jun	12	0	0	0	0	0
	UTC	0	0	10	40	100	2.8
Experiment 3	21-May	8	0	0	0	0	0
	4-Jun	16	0	0	0	0	0
	19-Jun	12	0	0	0	0	0
	UTC	0	4	8	38	100	2.7

^ Day Vaseline was applied to the stem-fruit area
[1] Single tree experiments. One tree per experiment.
[2] % fruit surface area affected by powdery mildew
[3] Proportion of fruit diseased.
[4] Severity was averaged based on the following formula: $\bar{x} = \frac{\sum_{i=1}^{5} C_i \cdot N_i}{\sum_{i=1}^{5} N_i}$ where: $\bar{x}$ = weighted average of infection severity, C_i = infection severity [1, 2, 3], N_i = number of samples sorted into severity of infection
[5] UTC = Untreated control. Never bagged. Natural disease occurrence.

Shoot assays. After harvest, infected cherries (cvs 'Bing' and 'Sweetheart') were sorted into four categories (0 = no infection,1 = 1-33% fruit surface area colonized; 2 = 34-66% fruit surface area colonized; 3 = >66% fruit surface area colonized). Cherries free of powdery mildew were used as a negative control. To simulate overwintering, 100 cherries per category will be buried just below the soil surface in the experimental cherry orchard at WSU-Prosser. Cherries harvested from commercial orchards (The Dalles, OR) were also left in the respective orchard for the winter. Germinated seedlings were recovered the following spring, individually potted and grown in isolation in a greenhouse. Percentage of seedlings developing flag shoots were recorded. Not a single seedling grown from overwintering pits developed powdery mildew. It has to be mentioned that the survival rate (measured as germination rates) of cherry pits in the orchard was below 10% at WSU and below 5% in The Dalles. This study did not give any indication that powdery mildew survives as mycelium on cherry pits.

Fungal activity was measured on overwintering cherry seeds. Briefly, 3x 20 seeds from decaying, previously infected fruit were obtained from the orchard floor (WSU) in the middle of October, November and December. Seeds were washed in sterile water and subjected to the PMA viability assay. The assay was able to detect minute amounts of the fungus in October and November with viability of conidia below 2%. No fungal viability was detected in December.

Objective 3

The possibility of powdery mildew surviving on cherry pits in the orchard was monitored in commercial orchards in Oregon and Washington. Surveys started with the onset of vegetative tree growth and ended when irrigation was turned on. First irrigation is linked to ascospore dissemination, which is the common route of powdery mildew overwintering. Briefly, nine transects (10 trees x 10 trees each) were evaluated for on-ground shoots and overwintering pits were collected from the orchard floor surrounding one random tree in the transect. The number of seeds was recorded and seeds were evaluated for fitness. Empty seeds were discarded and healthy seeds were germinated. Seedlings were grown in isolation in a greenhouse. Occurrence of powdery mildew on seedlings was noted. **No early season flag shoots were detected** in any participating orchard or in seedlings grown in the greenhouse. All orchard floors had a vigorous occurrence of root shoots (98-99%) compared to true seedlings (1 to 2%). The low occurrence of seedlings is also explained by the low viability (2%) of seeds found on the orchard floors after the winter. Powdery mildew started growing on root shoots. However, the source of the powdery mildew on root shoots could not be determined and most likely was related to ascospore release from overwintering chasmothecia (given shoot proximity to bark fissures).

Management strategies ranged from frequent or moderate chemical root shoot removal (‘burn-back’) to general mowing. Overall, the orchards with frequent root shoot removal had the least amount of powdery mildew disease. Mowing seemed less effective since root shoots located close to the base of the trees were not removed. In all cases, orchard floor management stopped shortly after harvest. Root shoots growing during this period frequently (in 8 out of 9 orchards) were infected with powdery mildew. This infection spread to the trees likely resulting in the production of chasmothecia (‘Next Year’s mildew’).

Taken all of these observations into account, survival of powdery mildew on seeds and the successful growth of both the seed and the fungus in the spring may be an event with a very low incidence and likely no commercial importance.

Executive Summary:

What we know so far about the infection process of cherry fruit:
In general, fungi explore their habitat with rapidly-growing, sparsely-branched hyphae, then, when some of those hyphae find a nutrient resource, the extension rate declines, rate of branching increases, and the mycelium captures and exploits the resource, from which it subsequently send out a new generation of exploratory hyphae and/or populations of conidia are produced. It is important to recognize that fungal growth is dynamic. One part of a mycelium may be growing as a rapidly extending, sparsely branched exploratory sector, another part may be a highly branched and interconnected network exploiting a nutrient resource, while a third region maybe actively involved in formation of conidia (mode of dispersal). Conidia (spores) are produced at greater quantities when nutrient become limited.

On cherries, conidia accumulate on cherry fruit surfaces throughout the cherry growing season. Once powdery mildew shows up on leaves*, powdery mildew can be detected on the fruit. The early fruit infection is invisible but characterized by a steady increase in fungal mass throughout the cherry growing season. At this point, the fungus is still resting and not actively growing. The increase of fungal mass on cherries is likely due to increasing foliar infections and airborne conidia concentrations that steadily land on fruit. The onset of visible fruit infection is sudden and not related to fruit maturity. Once the fruit infection has begun, it can be found on all cultivars. In order for a cherry to become infected, the conidia have to be located in the stem cavity area. Those conidia will germinate and produce hyphae to explore and feed at an exponential rate and in a pattern that produces the characteristic morphology of the mycelium. Mycelium radiates away from the stem cavity towards the cheeks and down to the stylar end, and not in reverse order. This is also contrary to leaf infection. Here, several powdery mildew colonies may appear on different locations and/or upper or lower leaf surface, grow which eventually leads to a fusion of these colonies. Also, while leaf infections start with a few infected leaves which constantly re-infect new leaves, cherry fruit seem to become (visibly) infected simultaneously. The exact trigger for fruit infection to start is not known, but could be related to a change of fruit volatiles or be a density-dependent process. Even though abundant fungal mycelium is produced on cherry fruit, it is likely not associated with overwintering (asexual form of overwintering). The main route of overwintering remain chasmothecia (sexually produced overwintering structures) which harbor ascospores that become released in the spring (Grove and Boal 1991). The start of first irrigation, wetness and temperature are main factors in such releases, which have been described by Grove (1998). Managing the production of chasmothecia post-harvest is still essential to contain next season's epidemic. However, since such productions occur post-harvest, powdery mildew management has usually ceased in the orchard.

** For reference: In 2015 and 2016, foliar powdery mildew started 30 days after full bloom (dafb) and 14dafb in 2017*

Literature cited

Bringe, K., C. F. Schumacher, M. Schmitz-Eiberger, U. Steiner and E.-C. Oerke (2006). "Ontogenetic variation in chemical and physical characteristics of adaxial apple leaf surfaces." Phytochemistry **67**(2): 161-170.

Calabro, J. M. (2007). Biology of sweet cherry powdery mildew, Oregon State University.

Grove, G. G. (1998). "Meteorological factors affecting airborne conidia concentrations and the latent period of Podosphaera clandestina on sweet cherry." Plant disease **82**(7): 741-746.

Grove, G. G. and R. J. Boal (1991). "Overwinter survival of Podosphaera clandestina in eastern Washington." Phytopathology **81**(4): 385-391.