FINAL PROJECT REPORT

Project Title: Identification of powdery mildews attacking apples and cherries in Washington State

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Cooperators: Gary Grove, Washington State Univ.
Growers across the state

Other funding sources: None

Total Project Funding: $37,860

Budget History:

Item	2010	2011 (extension)
Salaries[1]	0	
Benefits[1]	0	
Wages	$18,122	
Benefits	$1,740	
Equipment	0	
Supplies[2]	$5,700	
Travel[3]	$4,298	
Plot Fees	0	
Miscellaneous[4]	$8,000	
Total	$37,860	No request

Footnotes:

[1]hourly worker (Tess Barlow)

[2]laboratory supplies (reagents, DNA extraction kits and associated materials, plastic ware, supplies for microscopy, collecting bags, misc)

[3]travel (7 trips from Seattle to collect research specimens in orchards, 3 nights, 4 days, per diem, est. at $614/trip)

[4]Gene sequencing (estimated 800 runs @ $10 each)

OBJECTIVES

1) To develop accurate, up-to-date information on the species of powdery mildews attacking apples in Washington.
2) To develop preliminary information on the species of powdery mildews attacking cherries in Washington.

Activities include:

- Collecting specimens from about 10 sites within apple orchards and 10 sites within cherry orchards in each of five fruit-production regions, including: Brewster to Okanogan area (north); Wenatchee area (north central); the greater Royal Slope area (the Columbia Basin); Yakima area (south central); Tri-Cities; and Walla Walla area (south).
- Characterizing about 300 specimens using bright-field and differential interference microscopy
- Generating ITS and 28S rDNA sequences from about 50 apple powdery mildew specimens (10 from each production region)
- Generating ITS and 28S rDNA sequences from about 50 cherry powdery mildew specimens
- Analyzing data by various methods including comparing morphological data with published descriptions of powdery mildew species and comparing DNA sequence data against reference sequences in GenBank
- At the conclusion of the study specimens will be deposited in the Mycological Herbarium at Washington State University and sequence data will be deposited in GenBank

SIGNIFICANT FINDINGS

This project is nearly complete, final results will be available this year once data analysis is finished..

Summary of significant findings:

- To date, only *Podosphaera leucotricha* has been detected on apple and only *Podosphaera clandestina* has been found on cherry.
- No variation in ITS and 28S rDNA sequences have been detected in *P. leucotricha* (from apple)
- No other species of powdery mildews known to attack apples have been detected
- No variation in ITS and 28S rDNA sequences have been detected in *P. clandestina* (from cherry)
- A wild species of *Prunus* was found to host *P. clandestina* but this strain differed in 4 of 620 bp when compared to ITS in specimens collected from commercial cherries

RESULTS & DISCUSSION

We compiled data on 692 apple powdery mildew specimens collected from 61 sites. Specimens sequenced so far were selected to maximize geographic diversity. To date, only *Podosphaera leucotricha* has been detected. The following information summarizes the apple varieties collected, the number of collecting sites and the number of specimens from which variety that were collected.

Variety	# Collecting Sites	# Specimens
Braeburn	1	9
Fuji	10	119
Gala	17	193
Ginger Gold	2	23
Golden Delicious	3	34
Granny Smith	11	110
Honey Crisp	9	92
Jonathan	2	23
Pink Lady	2	26
Rubens	1	12
Variety to be confirmed	3	60

161 cherry powdery mildew samples were collected from 13 sites. Specimens sequenced were selected to maximize geographic diversity. Only *Podosphaera clandestina* was detected from samples taken in commercial orchards. Collections from a single wild *Prunus* tree yielded what might possibly be a different species; further work is under way on those specimens. The following information summarizes the cherry varieties collected, the number of collecting sites), and the number of specimens from which variety that were collected.

Variety	# Collecting Sites (# sequencing completed)	# Specimens
Bing	7	89
Rainier	1	6
Sweetheart	2	20
Van	1	2
Wild *Prunus*	1	3
Variety to be confirmed	4	41

We detected only *Podosphaera leucotricha* on apple and only *Podosphaera clandestina* on commercial cherry cultivars. Information from this study may facilitate exporting apples and cherries to other countries.

The discovery of a distinct strain of *P. clandestina* on wild cherry raises some questions of significance to the industry. Can this strain attack commercial cherry varieties or breeding lines? How diverse are *P. clandestina* strains on wild hosts? Do they increase the likelihood that resistance to powdery mildew could be overcome in future varieties? Can wild strains play a role in the emergence of fungicide-resistant strains in orchards? Additional work to collect on wild hosts and test the host ranges of P. *clandestina* strains on them would help answer these questions.

Results have suggested that ITS and 28S regions from strains of powdery mildews attacking apples and cherries are invariable. Results suggest that these regions are potentially very useful for PCR-based approaches to detecting these species.

Collecting cherry powdery mildew was hampered because incidence was lower than in previous years, and the development of populations was much slower than for apple powdery mildew. Consequently the plan to collect both apple powdery mildew and cherry powdery mildew on the same collecting trips proved to be less than successful. For future projects it would be prudent to plan separate collecting trips if both diseases are being studied.

EXECUTIVE SUMMARY

During the study a total of 692 specimens of apple powdery mildew were collected from 10 cultivars in 61 sites. A total of 522 ITS and 28S rDNA sequences were obtained as part of the characterization of the specimens. *Podosphaera leucotricha* was the only species found on apple. 161 specimens from four cultivars in 13 sites were collected from cherry. *Podosphaera clandestina* was the only species found on commercial cherry, although a different strain (and possibly species) was found on a wild cherry in a single location.

Results were somewhat surprising as older literature suggested several species might be found on these hosts. The results do suggest that at present *P. leucotricha* is the primary (and perhaps only) species to be found on apple, and *P. clandestina* is the primary (and perhaps only) species on cherry. The results did not provide any indication that other species occurred on these hosts.

Significance of the findings includes the following:

- This is the only extensive study of apple and cherry powdery mildew in Washington that included analysis of DNA sequences. The sequence data was useful in characterizing the mildews and reinforced the conclusion that apples and cherries each were attacked by a single species.

- There appeared to be no variation in ITS and 28S rDNA species in the specimens studied. This finding suggests that rDNA sequence data could be very useful in developing PCR-based detection systems for these powdery mildews.

- Findings did not support the possibility that unusual powdery mildews occur on these crops, possibly simplifying exporting them to international markets.

- Results also suggest that apple and cherry breeding programs can focus their efforts on developing cultivars with resistance to *P. leucotricha* and *P. clandestina*, respectively. While it is possible that population-level differences in virulence among powdery mildew species may exist, there is no evidence that multiple species attack these crops in Washington.

Implications for future research include the following:

- Because the apple and cherry powdery mildews behaved differently in the field (the cherry mildew populations appeared to increase more slowly), it did not prove efficient to collect species during the same collecting trips. Future research projects on these powdery mildews should take this difference into account (i.e., it is not practical to collect both species at the same time).

- It proved more difficult to amplify powdery mildew DNA as the season progressed. Many of the sequences amplified late in the season represented yeasts rather than powdery mildews. Because some yeasts are known to parasitize or suppress powdery mildews, this incidental observation suggests that interactions between powdery mildews and yeasts might deserve future study, both to provide a clearer picture of powdery mildew behavior and the possibility that naturally-occurring powdery mildew parasites might have beneficial activity in the field (such as reducing primary inoculum during the following season).

- Because of rather low incidence of cherry powdery mildew during the study period, relatively few specimens were collected. It may be useful do additional work to characterize more specimens.