# CRANBERRY RESEARCH REPORT – 15

**Project title:** Evaluation of Fungal Populations in British Columbia Cranberries as it Relates to Fruit Rot Incidence

**Commission Research Priority Addressed:** Although Fruit Rot is not listed as one of the priorities, it is ever increasing in all three growing areas of the Pacific Northwest and may be an important component of the recent "soft fruit" issue affecting the BC industry.

**Objectives:** (1) Three 'Stevens' beds will be selected from three different areas for this study; (2) Rotted and healthy fruit from these beds will be sampled at regular intervals from early berry development until harvest, and at similar intervals when berries are in storage, and fruit will be cultured for fungal pathogens and, (3) Fungal pathogens will be identified to genus (and species where necessary) and populations will be identified throughout the sampling period from the different beds. Knowledge of the fungi that infect cranberries and are subsequently responsible for fruit rot in BC will help growers better manage fruit rot at acceptable levels in succeeding growing seasons by using the most appropriate fungicides at the proper times.

**Project Summary and Procedures:** 1) Three cranberry beds (all 'Stevens' with higher percentages of fruit rot/soft fruit in the most recent growing seasons) will be selected from three separate cranberry growing areas for sampling – Langley, Pitt Meadows and Richmond with the help of Brian Mauza, Ocean Spray Cranberries. These beds will all be processed fruit beds, as it is important to have the same cultivar in each bed. It was hoped that a fresh fruit bed could be substituted for one of the processed fruit beds, but that would introduce a different cultivar. Instead, it is better to have the same cultivar for all three beds to avoid introducing another variable.
(2) Rotted and healthy fruits will be sampled from the three beds (by Brian Mauza), beginning in early August as soon as decent-sized fruit are available. Subsequent samples will be taken at three-week intervals up to harvest. 100 berries (each type) will be sampled in transects across the bed so that a good cross section of the bed is sampled. It is expected that there will be five pre-harvest samples to evaluate. Just prior to harvest, a bulk sample will be harvested with a large enough volume of berries so that three post-harvest samples can be evaluated until late November. Berries will be cut in half through the calyx and stem ends, surface-sterilized in 10% Clorox + 0.01% Tween 20 for 20 minutes, blotted dry on sterile paper towels and plated on acidified cornmeal agar (5 berry halves per plate). Plates will be incubated at 25-28$^{o}$ C (room temperature) for three weeks. Berries for storage rot evaluation will be sampled and cultured as those for the field rot evaluation.
(3) At this point, fungi will be identified to genus and species (for *Colletotrichum* and *Phyllosticta*) and percent recovery calculated for each bed per sampling time, and for fresh and processed fruit berries in storage after harvest. Non-sporulating fungal isolates may need to be subcultured onto another medium (V-8 or half-strength potato dextrose

agar) in order to induce sporulation for identification to genus. Fungal recovery from the different beds will be tabulated for each bed. These results will give a good incidence of the fungal population in each one of these beds as part of the longer-term study. Fungicide records will be collected and used to compare populations from different beds. Particular attention will be focused on *Phyllosticta*, to see whether *P. vaccinii* or *P. elongata/Botryosphaeria* is more prevalent. The latter fungus is not a key component of fruit rot. In addition, special attention will also be devoted to the newly characterized fungal pathogens from 2013 in Washington, *Cryptosporiopsis* and *Cadophora*. Artificial inoculations showed that both of these new fungi infect cranberry fruit. Results from these studies will be presented at the Cranberry Congress in February 2015.

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**Project Duration:** Start date: July 2014; Projected completion date: January 2015

**Location:** Samples will be taken from Langley, Pitt Meadows and Richmond; Processing of samples, plating of berries and fungal evaluations will be performed in the plant pathology laboratory of Dr. Debra Inglis at the WSU Research & Extension Center, Mount Vernon, WA

**Literature Review:** Fruit rot has traditionally been an issue for cranberry growers in Massachusetts and New Jersey where they must apply 3-6 fungicide applications on a yearly basis in order to control the disease. Because weather patterns have evolved into a warmer climate during the past decade, and many cranberry beds have been renovated and planted with high-producing new hybrid cultivars, and fungicide use patterns have changed with newly-registered materials, the fungal population responsible for field and storage rot is a constantly changing variable. Although *Phyllosticta vaccinii* and *Physalospora vaccinii* remain the most important causal agents of fruit rot in the East, *Coleophoma empetri* and *Colletotrichum acutatum* have emerged as more important pathogens in fruit rot in the past decade there. Growers in Wisconsin now must regularly apply fungicides in order to manage fruit rot, although these levels do not approach those levels in the East. Although sporadic, growers in Oregon, Washington and British Columbia have encountered higher fruit rot levels in the past decade, and fungicide use has increased there as well, especially for those growers growing fresh fruit. Since the retirement of Dr. Pete Bristow, WSU small fruit pathologist, in 2005, fungal populations in fruit in the Pacific Northwest are largely unknown, and that population has likely significantly changed from what it was in the early 2000's. Previous isolations from the fruit were only performed at harvest, thus missing a large part of the true fungal population. Recently, Dr. Siva Sabaratnam, plant pathologist at the BC Ministry of Agriculture in Abbotsford, has isolated fungi from beds affected with fruit rot and dieback, and consequently, there is more of a fungal data base in BC than in OR and WA.

Knowing what fungi are responsible for the fruit rot directly relates to which fungicides should be applied for optimal control of the disease, particularly because new fungicides are now available to growers. New cranberry cultivars not available a decade ago have been planted which will also account for a different fungal population in the region. This project aims to continue an investigation begun in 2013 in Washington, expanding it to British Columbia, to evaluate the pathogenic fungi involved in the infection of cranberries. This will form a multi-year evaluation of this fungal population as the cranberry industry makes changes moving forward. These findings will also help devise the necessary strategies for the best management of fruit rot in processed and fresh fruit beds.

Fruit rot (field rot for all cranberry growers, and storage rot for fresh fruit growers) is a problem growers must address every growing season. It appears that it will be an increasing problem with climate change. If climatic trends continue, it will be certainly be a larger issue in the Pacific Northwest. With increasing levels of fruit rot, it will be necessary to identify which fungi are responsible for infection in order to devise the best fungicide program for management of the disease. It is important that these fungicides are used judiciously to avoid fungicide resistance in the causal fungi, since all of the newer registered fungicides with narrow modes of action will be prone to this occurrence. Although Fruit Rot is not listed among the research priorities, recently BC growers have been delivering "soft fruit" at harvest. Some of this may be due to the fruit being over mature or due to handling in the field, but much of it may be due to fruit rot. In 2013, the percentage of poor fruit at delivery in BC doubled. Fungal populations have routinely been monitored in Massachusetts and New Jersey and more recently in Wisconsin on a less intensive basis, as fruit rot has only emerged as a significant problem in certain areas of that growing area. There is very little current data for BC except for a sample evaluated here and there on an irregular basis. Most of the isolations of fungi are typically done at harvest time, missing the entire profile of fungi in the fruit during its maturation. It is the intent of this project to supplement fungal population data elsewhere with data from BC. What is especially important is to determine how widespread the early rot pathogen, *Phyllosticta vaccinii*, is in BC, if at all. This fungus is very important in Massachusetts and New Jersey and has emerged as a key pathogen in Wisconsin. The fungus does not only cause field rot, but it can also cause significant leaf spot and leaf drop in newly-renovated cranberry beds, resulting in consequential buildup of inoculum for fruit rot in subsequent growing seasons. Results from Year 1 of fruit isolations in WA indicated that *Phyllosticta vaccinii* was not present in the six sampled beds, which is good news. Studying these populations in WA and BC while fruit rot is a relatively small problem may help to avoid the disease becoming a more significant problem requiring great expenditures of money to apply fungicides.

This project will also help address two additional industry priorities. The ability of the west coast industry to supply adequate fresh fruit to the market is severely limited by poor keeping quality. The resulting data will help provide key information to growers to

enable them to potentially reduce storage rot. In addition, a better understanding of the pathogen complex and the infection periods could help eliminate unnecessary fungicide residues that are often problematic for fresh and export fruit, particularly in the Pacific Northwest.

**Results**
**:** Field rot percentages for the three beds can be seen in Table 1, while storage rot percentages can be seen in Table 2. Fungicides utilized in each bed are shown after Table 2. Field rot levels were low in Beds 1 and 2 and significantly high in Bed 3 where only one fungicide was utilized and the timing of this fungicide application may not have been at an optimal time. Storage rot levels were high in all three beds and highest in Bed 3, again, probably due to the poorest fungicide utilization.

**Table 1. Percent field rotted fruit**

| Bed # | August 15 | September 5 | September 26 | Total |
|---|---|---|---|---|
| 1 | 0 | 3.25 | 2.36 | 2.13 |
| 2 | 0 | 0.83 | 3.91 | 2.67 |
| 3 | 1.98 | 6.46 | 19.51 | 14.73 |

**Table 2. Percent storage rotted fruit**

| Bed # | October 17 | November 12 | November 25 | Total |
|---|---|---|---|---|
| 1 | 15.48 | 36.40 | 17.73 | 53.30 |
| 2 | 11.10 | 40.18 | 20.07 | 53.60 |
| 3 | 46.27 | 75.68 | 20.72 | 83.87 |

Fungicides used in each bed:

1 = Bravo 500 (3 L/A) on 5/24; Bravo 500 (3L/A) on 6/19

2 = Oxychloride 50 (1.62 kg/A) on 4/25; Echo 720 (2L/A) on 5/22; Jade (200 ml/A) on 5/22; Jade (200 ml/A) on 6/3; Echo 720 (2L/A) on 7/22

3 = Bravo 500 (3 L/A) on 5/20

Fungal isolations from healthy (symptomless) berries in the six samples for the three beds can be seen in Tables 3-8. Fungal isolations from rotted berries in the six samples for the three beds can be seen in Tables 9-14. Rotted berries were only found in the first sample for Bed 3.

**Table 3. Sample #1 – Healthy berries – August 15**

Percent incidence

| Fungus | #1 | #2 | #3 |
|---|---|---|---|
| Allantophomopsis | 26 | 16 | 67 |
| Coleophoma | 3 | 4 | 1 |
| Colletotrichum | 1 | 0 | 1 |
| Fusicoccum | 2 | 37 | 2 |
| Phomopsis | 3 | 6 | 2 |
| Phyllosticta elongata | 0 | 20 | 0 |
| Physalospora | 11 | 7 | 7 |
| Yellow spreading | 1 | 1 | 0 |
| Sterile | 44 | 9 | 16 |

**Table 4. Sample #2 – Healthy berries – September 9**

Percent incidence

| | #1 | #2 | #3 |
|---|---|---|---|
| Allantophomopsis | 39 | 10 | 69 |
| Coleophoma | 13 | 4 | 23 |
| Colletotrichum | 8 | 0 | 3 |
| Fusicoccum | 12 | 9 | 2 |
| Phomopsis | 3 | 15 | 0 |
| Phyllosticta elongata | 0 | 0 | 0 |
| Physalospora | 34 | 38 | 16 |
| Yellow spreading | 4 | 3 | 0 |
| Sterile | 9 | 14 | 2 |

**Table 5. Sample #3 – Healthy berries – September 30**

Percent incidence

| Fungus | #1 | #2 | #3 |
|---|---|---|---|
| Allantophomopsis | 45 | 10 | 41 |
| Coleophoma | 6 | 12 | 30 |
| Colletotrichum | 0 | 0 | 0 |
| Fusicoccum | 4 | 29 | 0 |
| Phomopsis | 4 | 15 | 4 |
| Phyllosticta elongata | 0 | 0 | 0 |
| Physalospora | 17 | 16 | 16 |

| | | | |
|---|---|---|---|
| Yellow spreading | 1 | 1 | 2 |
| Sterile | 23 | 12 | 18 |

**Table 6. Sample #4 – Healthy berries – October 20**

Percent incidence

| Fungus | #1 | #2 | #3 |
|---|---|---|---|
| Allantophomopsis | 44 | 16 | 65 |
| Coleophoma | 8 | 14 | 44 |
| Colletotrichum | 1 | 0 | 0 |
| Fusicoccum | 6 | 28 | 1 |
| Phomopsis | 0 | 9 | 1 |
| Phyllosticta elongata | 0 | 0 | 0 |
| Physalospora | 24 | 15 | 8 |
| Yellow spreading | 3 | 5 | 1 |
| Sterile | 19 | 16 | 4 |

**Table 7. Sample #5 – Healthy berries – November 12**

Percent incidence

| Fungus | #1 | #2 | #3 |
|---|---|---|---|
| Allantophomopsis | 41 | 19 | 76 |
| Coleophoma | 5 | 14 | 10 |
| Colletotrichum | 0 | 1 | 0 |
| Fusicoccum | 2 | 21 | 0 |
| Phomopsis | 4 | 4 | 0 |
| Phyllosticta elongata | 0 | 0 | 0 |
| Physalospora | 16 | 16 | 7 |
| Yellow spreading | 8 | 7 | 0 |
| Sterile | 24 | 10 | 12 |

**Table 8. Sample #6 – Healthy berries – December 1**

| Fungus | #1 | #2 | #3 |
|---|---|---|---|
| Allantophomopsis | 38 | 11 | 63 |
| Coleophoma | 3 | 10 | 19 |
| Colletotrichum | 0 | 0 | 1 |
| Fusicoccum | 6 | 23 | 0 |
| Phomopsis | 7 | 13 | 2 |
| Phyllosticta elongata | 0 | 0 | 0 |
| Physalospora | 16 | 17 | 4 |

Percent incidence

1 = 100 berries; 2 = 100 berries; 3 = 94 berries

**Table 9. Sample #1 – Rotted berries – August 15**

| | Percent incidence | | |
|---|---|---|---|
| Fungus | #1 | #2 | #3 |
| Allantophomopsis | 0 | 0 | 92 |
| Coleophoma | 0 | 0 | 0 |
| Colletotrichum | 0 | 0 | 8 |
| Fusicoccum | 0 | 0 | 0 |
| Phomopsis | 0 | 0 | 15 |
| Physalospora | 0 | 0 | 0 |
| Yellow spreading | 0 | 0 | 0 |
| Sterile | 0 | 0 | 0 |

1 = 0 berries; 2 = 0 berries; 3 = 13 berries

**Table 10. Sample #2 – Rotted berries – September 9**

| | Percent incidence | | |
|---|---|---|---|
| Fungus | #1 | #2 | #3 |
| Allantophomopsis | 45 | 50 | 32 |
| Coleophoma | 36 | 0 | 52 |
| Colletotrichum | 18 | 25 | 0 |
| Fusicoccum | 14 | 0 | 0 |
| Phomopsis | 0 | 0 | 4 |
| Physalospora | 14 | 25 | 4 |
| Yellow spreading | 0 | 0 | 0 |
| Sterile | 0 | 0 | 16 |

1 = 22 berries; 2 = 4 berries; 3 = 25 berries

**Table 11. Sample #3 – Rotted berries – September 30**

| | Percent incidence | | |
|---|---|---|---|
| Fungus | #1 | #2 | #3 |
| Allantophomopsis | 44 | 20 | 32 |
| Coleophoma | 26 | 22 | 28 |
| Colletotrichum | 2 | 2 | 2 |
| Fusicoccum | 0 | 20 | 0 |
| Phomopsis | 2 | 0 | 0 |
| Physalospora | 8 | 6 | 12 |
| Yellow spreading | 0 | 4 | 2 |
| Sterile | 24 | 36 | 30 |

1 = 50 berries; 2 = 50 berries; 3 = 50 berries

**Table 12. Sample #4 – Rotted berries – October 17**

| | Percent incidence | | |
|---|---|---|---|
| Fungus | #1 | #2 | #3 |
| Allantophomopsis | 70 | 46 | 38 |
| Coleophoma | 32 | 54 | 80 |
| Colletotrichum | 0 | 0 | 0 |
| Fusicoccum | 0 | 6 | 0 |
| Phomopsis | 0 | 0 | 0 |
| Physalospora | 2 | 4 | 0 |
| Yellow spreading | 0 | 0 | 0 |
| Sterile | 2 | 8 | 0 |

1 = 50 berries; 2 = 50 berries; 3 = 50 berries

**Table 13. Sample #5 – Rotted berries – November 12**

| | Percent incidence | | |
|---|---|---|---|
| Fungus | #1 | #2 | #3 |
| Allantophomopsis | 66 | 36 | 36 |
| Coleophoma | 36 | 62 | 88 |
| Colletotrichum | 0 | 0 | 0 |

| | | | |
|---|---|---|---|
| Fusicoccum | 6 | 4 | 0 |
| Phomopsis | 0 | 0 | 0 |
| Physalospora | 0 | 0 | 0 |
| Yellow spreading | 0 | 0 | 0 |
| Sterile | 0 | 8 | 0 |

1 = 50 berries; 2 = 50 berries; 3 = 50 berries

**Table 14. Sample #6 – Rotted berries – December 1**

| Fungus | #1 | #2 | #3 |
|---|---|---|---|
| Allantophomopsis | 74 | 40 | 74 |
| Coleophoma | 16 | 20 | 22 |
| Colletotrichum | 0 | 0 | 9 |
| Fusicoccum | 4 | 32 | 0 |
| Phomopsis | 2 | 2 | 0 |
| Physalospora | 0 | 0 | 0 |
| Yellow spreading | 0 | 2 | 0 |
| Sterile | 8 | 4 | 4 |

Percent incidence

1 = 50 berries; 2 = 50 berries; 3 = 23 berries

Based on the isolations from the healthy and rotted berries during the three pre-harvest samples and three post-harvest samples, it appears that *Allantophomopsis* (*A. cytisporea* and *A. lycopodina*, both which cause black rot and were found at nearly equivalent levels) and *Coleophoma empetri* (cause of ripe rot) were the most prevalent fungi in BC berries, followed by *Physalospora vaccinii* (cause of blotch rot). Other less important pathogenic fungi included *Fusicoccum putrefaciens* (cause of end rot, primarily found in Bed 2), *Colletotrichum acutatum* (cause of bitter rot), *Phomopsis vaccinii* (cause of viscid rot, very low frequency) and the 'yellow spreading fungi' (*Cadophora* and *Cryptosporiopsis*). These latter two fungal genera were isolated for the first time in WA berries in 2013, subsequently also in 2014. The incidence of these new pathogenic fungi is much higher in WA berries than BC berries.

The ability to culture most of these fungi from healthy berries is because they have a latent or endophytic phase in their progression in the berry after infection. Whether the berry rots depends on the individual fungal development in the berry due to physiological maturation of the fruit or stress placed on the fruit. Most fruits will remain symptomless at harvest but may decay in storage.

Other notable findings included:

(1) Berries infected by *Allantophomopsis* in BC rarely showed the typical jet black color (giving the disease its name, black rot) seen in infected berries in MA and NJ. This is also the case in WA berries. Typically, infected berries infected with this pathogen are off-color or of the 'popper' type, exploding upon contact with a knife or scalpel. Healthy berries showed a significantly higher percentage infection than berries encountered in MA. While both species of this fungus were found in nearly equal amounts in BC, *A. lycopodina* is the predominant species in WA.

(2) Berries infected by *Coleophoma* could not be distinguished from *Allantophomopsis* (or *Fusicoccum*, for that matter), and healthy berries also had a higher percentage infection than seen in MA berries. It is possible that both of these genera have a higher ability to exist in the latent state in BC and WA berries (unique strains?).

(3) The early rot pathogen, *Phyllosticta vaccinii*, a major fruit rot pathogen on the east coast, was totally absent in both BC and WA (both 2013 and 2014).

(4) *Phomopsis* is present at a higher incidence in BC berries than WA berries, but it is a minor contributor to fruit rot in both areas.

(5) *Colletotrichum* is a more significant fruit rot pathogen in WA than BC, particularly in field-rotted fruit.

(6) The 'yellow spreading' fungi are also significantly more important pathogens in WA than in BC.

(7) Any of the conclusions reached in this study and the WA study are based on a very small sample size – three beds in BC and six beds in WA. The results are also based on a single cultivar, 'Stevens', which in the past has shown to be particularly susceptible to infection by *Allantophomopsis* (based on the number of black rotted berries observed in numerous storage rot studies performed in MA, compared to other cultivars). Consequently, it is possible that the results of the high incidence of this pathogen in both BC and WA are slightly skewed.

**Probable Subsequent Research Work Required:** Ideally, this should be the first year in a multi-year study to determine which fungi are most prevalent in BC berries, and most likely the contributors to increased fruit rot.

**Budget**

How many years of funding will be required to complete this project? ___at least 2__ year(s)

(in US currency, preferred for payment)

How many years of funding will be required to complete this project? _3-4__

year(s)
**For multi-year Projects please complete this page by Year with a Summary**

| | |
|---|---|
| **This page is for Year # (or Summary)** | **_Year 1____** |
| Wages salaries benefits and contractors fees | |
| Principal Investigator | _____ |
| Others | _____ |
| Travel (between Edmonds and Mount Vernon) | _$2,000_____ |
| Equipment | _____ |
| Consumables (Petri plates, media, etc.) | _$2,000_____ |
| Services (eg contracted lab work) | _____ |
| Other (describe) Shipping costs_____ | _$2,000_____ |
| **Total** | **_$6,000_____** |