March 6, 2015 – **Updated (March 1, 2016)**

# Fruit Rot Pathogens and their Impact on Cranberry Production in British Columbia [2014 Study]

Prepared by:
**Siva Sabaratnam, Brandon Wood** and **Keiko Nabetani**
Abbotsford Agriculture Centre, Ministry of Agriculture, Abbotsford, B.C.

For correspondence: Siva. Sabaratnam
(Tel: 604 556 3029, E-mail: Siva.Sabaratnam@gov.bc.ca)

Growing Forward 2 | British Columbia Ministry of Agriculture | Canada

## BACKGROUND INFORMATION

Cranberry fruit rot is caused by several fungal plant pathogens and their distribution, incidence and disease severity can vary among cultivars, geographical locations, from farm to farm or year to year, and even during the growing season (Olatinwo, 2003). Fungal pathogens can cause substantial damages to cranberry as both pre- and post-harvest fruit rot, resulting in reduced yield and poor quality fruit. Potentially, yield losses due to fruit rot diseases can reach 50% to 100% if they are not strategically managed. Because fruit rot diseases involve multiple pathogens, an accurate diagnosis of the causal agents and their biology and disease epidemiology should be taken into consideration when adopting appropriate disease management strategies to manage pre- and post-harvest fruit rot. In BC, information on fungal pathogens associated with cranberry fruit rot was documented almost 20 years ago, and therefore there is an absolute need for understanding the current status of plant pathogens that are responsible for fruit rot incidence and their impact on BC cranberry farms. Furthermore, gradual changes in the climate over the years and movement of plant materials (e.g. cranberry transplants, vines and fruit and other *Vaccinium* spp.) nationally and internationally can lead to introduction, establishment and spread of new and more aggressive strains of plant pathogens.

Therefore, the overall objective of this 4-year (2013/2014 to 2016/2017) research project is to identify and characterize the major plant pathogens involved in fruit rot of BC cranberries, assess their impact on fruit yield, understand their disease cycle (epidemiology), and develop/adopt and recommend appropriate disease management strategies to help BC cranberry growers.

### Year 1 (November 2013 to March 2014)

Objectives:

1. Conduct literature review and summarize available information on cranberry fruit rot diseases and associated pathogens; their biology and epidemiology and disease management strategies, and

2. Identify gaps in the information gathered under Objective-1 to help planning and executing the proposed research program.

*This work was completed and the REPORT was submitted to the BC Cranberry Research Committee*.

## Year 2 (April 2014 to March 2015)

Objectives:

1. Conduct field surveys during cropping season, focusing on cranberry fields in different geographical locations in the Fraser Valley, to identify the occurrence of major fungal plant pathogens associated with fruit rot diseases, and
2. Assess the incidence/impact of fruit rot diseases in cranberry fields at pre- and post-harvest

## MATERIALS & METHODS

### Identification of fungal pathogens associated with cranberry fruit rot diseases

***Field survey/sampling:*** Fourteen fields, located in Chilliwack, Langley, Pitt Meadows, Richmond and Delta were included in this study. In all fields, cultivar Stevens was included to ensure consistency; furthermore it is the most commonly grown cultivar in the Fraser Valley. Samples were collected at three developmental stages of cranberry, flowers at 50% flowering, and fruits at immature- and ripe-fruit stages. In each field, three 2 $m^2$ area of sampling plots (replicates) were selected diagonally from one corner to the centre of the field. For large fields, sampling distance was covered only to a part of the field, consistent with other fields. At each sampling time, at least twenty flowers or berries were collected randomly along the 2 diagonal lines of a 1 $m^2$ transect placed within each plot; at each sampling time, a new area within the 2 $m^2$ plot was sampled. Samples were collected in Ziploc bags, placed in a cooler box with ice-packs and transported to the laboratory. The samples were placed at 4°C until they were processed.

***Laboratory analysis:*** Flowers and fruits were surface sterilized with 0.5% NaOCl solution for 1 min and washed 3 times with sterile distilled water. Excess moisture on flower or fruit was removed by placing them on sterile paper towel and cut longitudinally into two-halves along the stem- and calyx-end. Dissected halves of two flowers or a single fruit were placed on a 90 mm Petri plate containing acidified ¼-strength potato dextrose agar medium (acidified ¼-PDA) and incubated in the dark at 22°C. Plates were periodically examined for fungal growth, originating from flower or fruit tissue. Based on the visual appearance of fungal colonies, representative fungal isolates were transferred to fresh acidified ¼-PDA and maintained in the dark at 20°C. For each set of samples (i.e. 60 flowers or berries per field), information on fungal colony types and their frequency (incidence) of occurrence on culture medium were recorded. All fungal isolates (pathogens and others) were identified to their genus or further to their species by the morphology of spores produced in culture and using genetic markers via PCR and DNA sequencing. The most prevalent fungal pathogens associated with fruit rot and their incidence at each farm and overall distribution in the Fraser Valley were determined. The representative fungal pathogens are maintained in the laboratory for future studies/reference.

### Assessment of fruit loss

***Field sampling:*** To assess the amount of fruit loss due to fungal pathogens or otherwise, cranberry fruit samples were collected at the same time as when ripe-fruits were collected just before harvest for the identification of pathogens associated with fruit rot incidence (as stated above). From each 2 $m^2$ sampling plot, as described previously, forty fruits were collected from each of two 1 $m^2$ transects (i.e. 20 per transect) that were placed diagonally inside the sampling plot. A total of 120 fruit were collected from 3

replicate plots per field. Samples were placed in Ziploc bags, kept in a cooler with ice-packs and brought to the laboratory.

***Laboratory analysis:*** *It is important to note that the assessment of fruit loss incidence, i.e. "symptomatic" fruit, was solely based on visual examination of fruit and accounted for symptoms caused by both microorganisms (fungal pathogens) and abiotic/physical damages (e.g. sun scorch, mechanical damage, etc.). It is not feasible to separate symptomatic fruit due to pathogens from abiotic/physical damages based on visual observation. Therefore, as described previously, isolation and identification of pathogens from symptomatic fruit is very important.*

Fruits collected from each of the three replicate plots from each field were counted and examined visually for any signs of fruit damage and the number of "healthy" and "symptomatic" fruits was counted and separated into two groups. The "symptomatic" fruits were separated based on colour, shape or appearance of necrotic/soft lesions that differed from "healthy" fruits. Some of the commonly noticed symptoms were softening of tissue with circular or irregular shaped lesions, dark bulls-eye like lesion, and softening and swelling of berry. Symptoms were recorded and photographed. Fruits that were separated as "healthy" were incubated in a moist chamber for 3 weeks at ambient temperature (~24-25°C) to assess for any development of symptoms and the number of "symptomatic" fruits upon incubation was counted. All "symptomatic" fruits were then incubated in a moist chamber for at least 7-14 days at ambient temperature (~24-25°C) to enhance sporulation of fungal pathogens, if associated with the "symptoms", and identified them by microscopy. The percentages of "symptomatic" fruits prior to harvest, at 3-weeks of post-harvest incubation and cumulative fruit loss were calculated based on the number of fruits (n = 120) sampled from each of 3 replicate plots.

## RESULTS & DISCUSSION

### Fruit Loss Assessment

- The percentage fruit loss, as estimated at harvest, 3-week of post-harvest incubation and cumulative fruit loss, in 14 farms is shown in **Figure 1**. The percentage incidence of fruit loss at harvest varied considerably from farm to farm. The highest incidence of fruit loss at harvest was estimated as **24%** and the lowest was **0%**. This could be due to differences in production and disease-management practices adopted by each grower, nature of distribution and spread of pathogens within each farm or field location, and environmental factors (weather conditions) specific to field location and conducive to fruit rot development.

- In all 14 farms, the percentage fruit loss increased considerably when harvested fruit was held for 3 weeks at ambient temperature. In one of the farms, the percentage fruit loss increased from **24%** at harvest to **73%** at 3-week post-harvest incubation and, in another farm, the percentage fruit loss of **0%** at harvest was increased to **17%** at 3-week post-harvest incubation (**Figure 1**). This indicates that any delay in harvest or holding harvested fruit at farm or receiving station can result in further fruit loss due to post-harvest rot. Adopting appropriate fungicide application program during crop production, timely harvest and keeping harvested fruit at cool temperatures can reduce post-harvest fruit loss.

- The cranberry farms in Chilliwack and Pitt Meadows had the highest fruit loss incidence at harvest and 3-week post-harvest incubation (on average) followed by the farms in Delta, Richmond and Langley, respectively (**Figure 2**). The farms in Langley had the lowest fruit loss incidence at harvest

(**Figure 2**). This may be due to the regional variations in the distribution and occurrence of different fungal pathogens that contribute to cranberry fruit rot diseases (please see below).

**Fungal Pathogens Associated with Fruit Rot**

- **UPDATED. Figure 3** shows the mean percentage frequency of fungal pathogens responsible for fruit rot diseases that were recovered from flowers and immature- and ripe-fruit samples from 14 farms. The most commonly recovered fruit rot pathogens in all 14 farms were *Allantophomopsis cytisporea* (Black rot), *Phyllosticta* (early rot/berry speckle), *Physalospora vaccinii* (Blotch rot), *Phomopsis* spp. (Viscid rot), *Botrytis* (Yellow rot), *Coleophoma empetri* (Rip or White rot), and *Colletotrichum* spp. (Bitter rot). Collectively, *Allantophomopsis*, *Phyllosticta, Physalospora*, *Phomopsis*, *Botrytis*, *Coleophoma* and *Colletotrichum* were isolated from **71%**, **50%**, **41%**, **14%**, **9%**, **7%** and **6%**, respectively, of the ripe-fruit samples collected from all 14 farms at harvest. However, the incidence of these fruit rot pathogens varied from farm to farm.

- Besides the pathogens that are known for causing fruit rot diseases of cranberry, *Epicoccum*, *Cladosporium*, *Trichoderma*, *Mucor*, *Aspergillus*, *Penicillium*, *Alternaria, Fusarium, Pestalotia/Pestalotiopsis* and yeasts were also recovered from the samples (**Figure 4**). These organisms may contribute to post-harvest damages if harvested fruit are not properly stored.

- **UPDATED.** Based on the location of the fields in the Fraser Valley, the most commonly recovered pathogens from the ripe-fruit samples at harvest are shown in **Figure 5a to 5e**; **Chilliwack farms**: *Allantophomopsis* (**79%**), *Phyllosticta* (**39%**), *Physalospora* (**28%**), *Phomopsis* (**27%**), *Colletotrichum* (**10%**) and *Coleophoma* (**8%**), **Langley farms**: *Allantophomopsis* (**83%**), *Phyllosticta* (**44%**), *Physalospora* (**37%**), *Botrytis* (**18%**) and *Phomopsis* (**12%**), **Pitt Meadows farms***: Phyllosticta* (**64%**), *Allantophomopsis* (**58%**), *Physalospora* (**58%**), *Phomopsis* (**13%**), *Botrytis* (**12%**), *Colletotrichum* (**9%**) and *Coleophoma* (**9%**), **Delta farms**: *Allantophomopsis* (**61%**), *Phyllosticta* (**57%**), *Physalospora* (**42%**), *Phomopsis* (**14%**), *Coleophoma* (**8%**), and *Colletotrichum* (**6%**) and **Richmond farms**: *Allantophomopsis* (**74%**), *Phyllosticta* (**41%**), *Physalospora* (**38%**), *Phomopsis* (**9%**), *Coleophoma* (**8%**), *Fusicoccum* (**7%**), and *Botrytis* (**5%**).

- Recovery of the fruit rot pathogens from flowers (**Figure 3**) indicates that infection by the major fruit rot pathogens can take place as early as at flowering. Therefore, it is important to protect the crop from such infection by implementing appropriate fungicide spray program, starting at flowering.

- Application of fungicides before fruit set at flowering and during fruit development is very important for controlling fruit rot diseases in field and, perhaps, during post-harvest/storage. Use of fungicides alternatively from different chemical groups will reduce the development of resistance to fungicides by pathogens and, therefore, prolong the efficacy of fungicide use. Registered fungicides on cranberry:
  **Group M** - Bravo (chlorothalonil) & Copper oxychloride
  **Group 3** - Funginex (triforine), Jade or Topas (propicanazole), & Proline (prothiocornazole)
  **Group 4** – Fontelis (penthiopyrad)
  **Group 7** - Isofetamid
  **Group 11** - Quadris (azoxystrobin)

**Figure 1**. Percentage fruit loss at harvest, 3-week post-harvest incubation and cumulative fruit loss during 2014 growing season in 14 farms, located in Chilliwack, Langley, Pitt Meadows, Richmond and Delta.

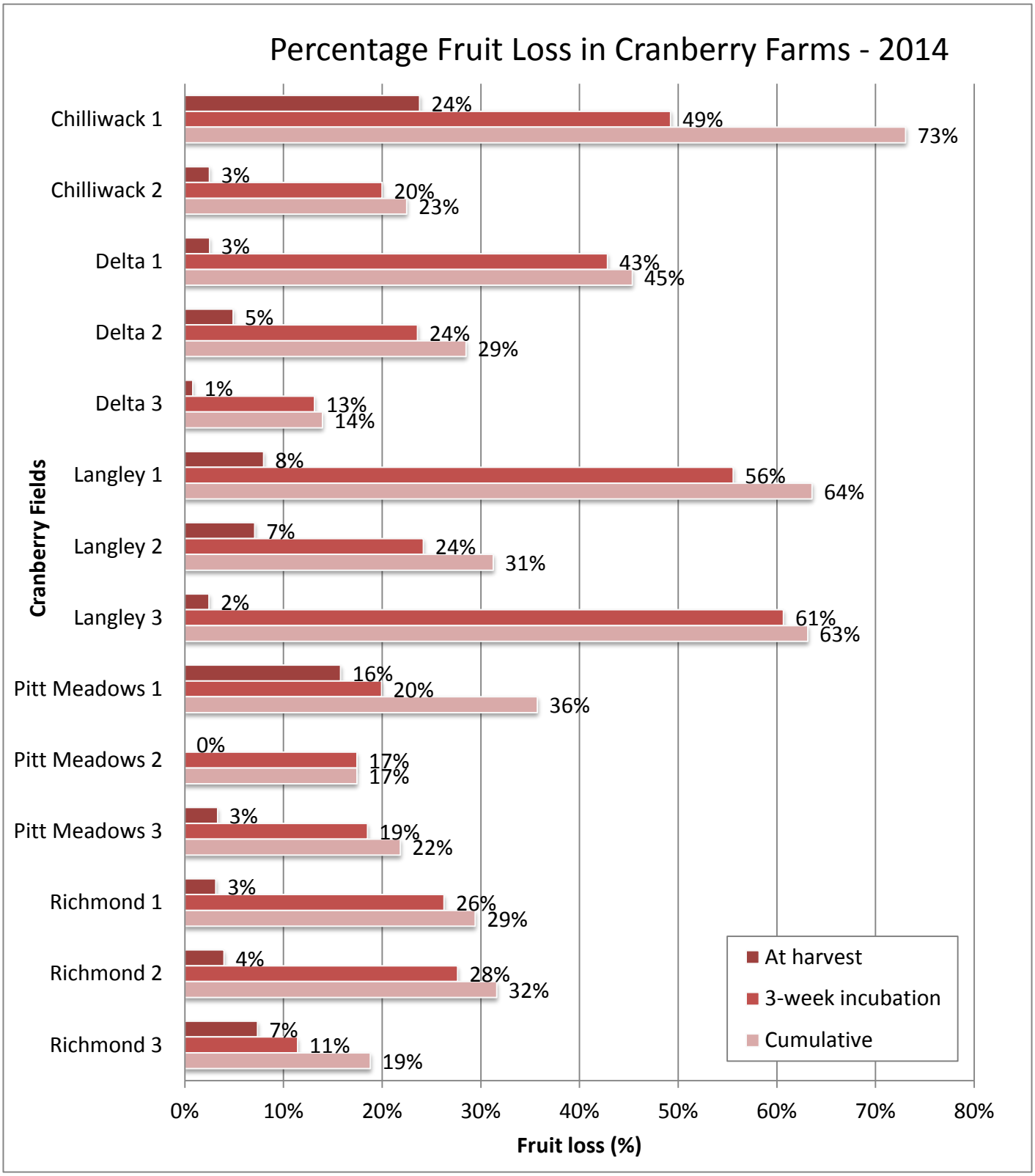


**Figure 2**. Mean percentage fruit loss at harvest, 3-week post-harvest incubation and cumulative fruit loss during 2014 growing season at each location - Chilliwack, Langley, Pitt Meadows, Richmond and Delta.

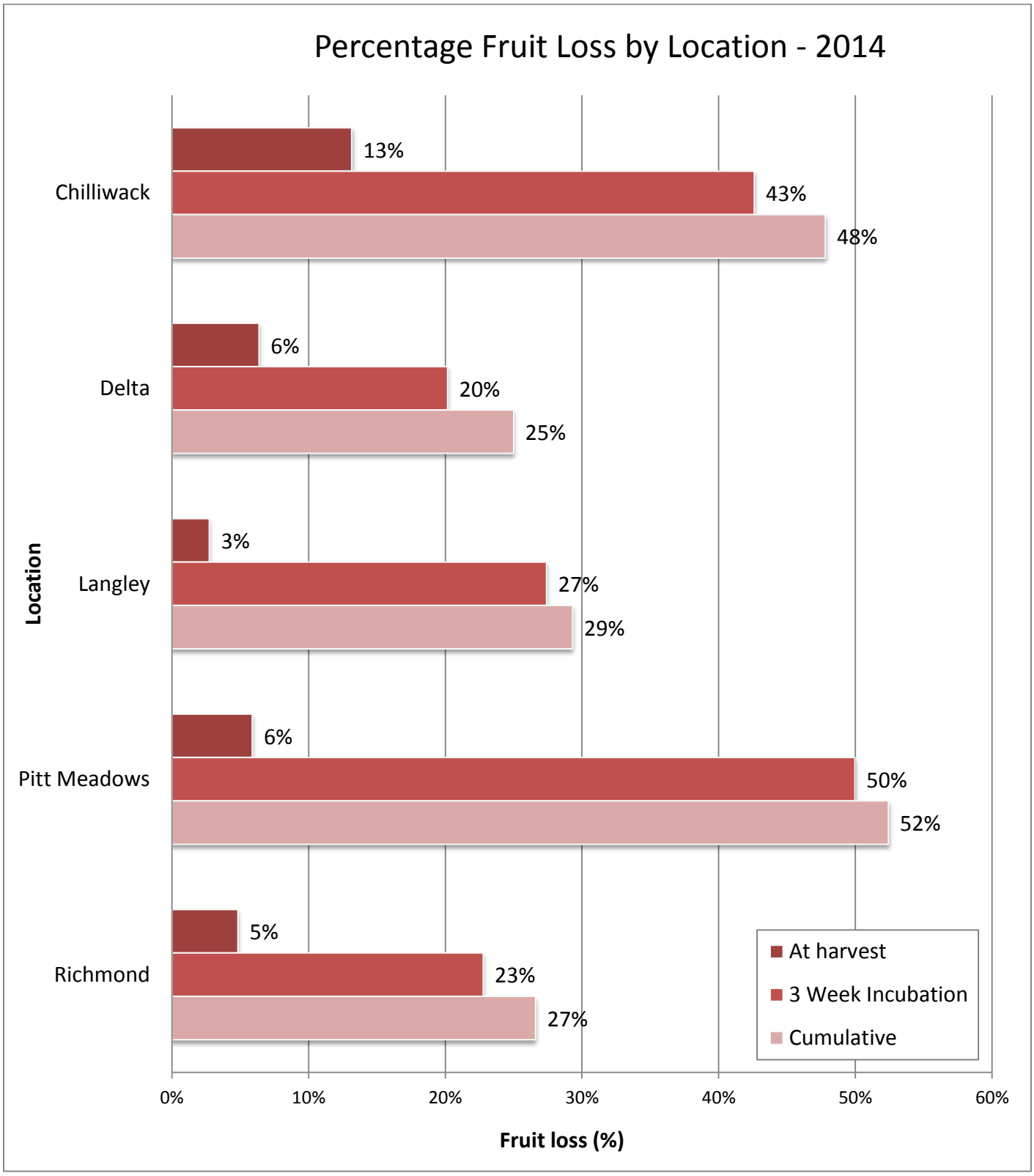


**Figure 3**. **UPDATED.** Mean percentage of fungal pathogens, **known** to cause fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2014 growing season in 14 cranberry fields.

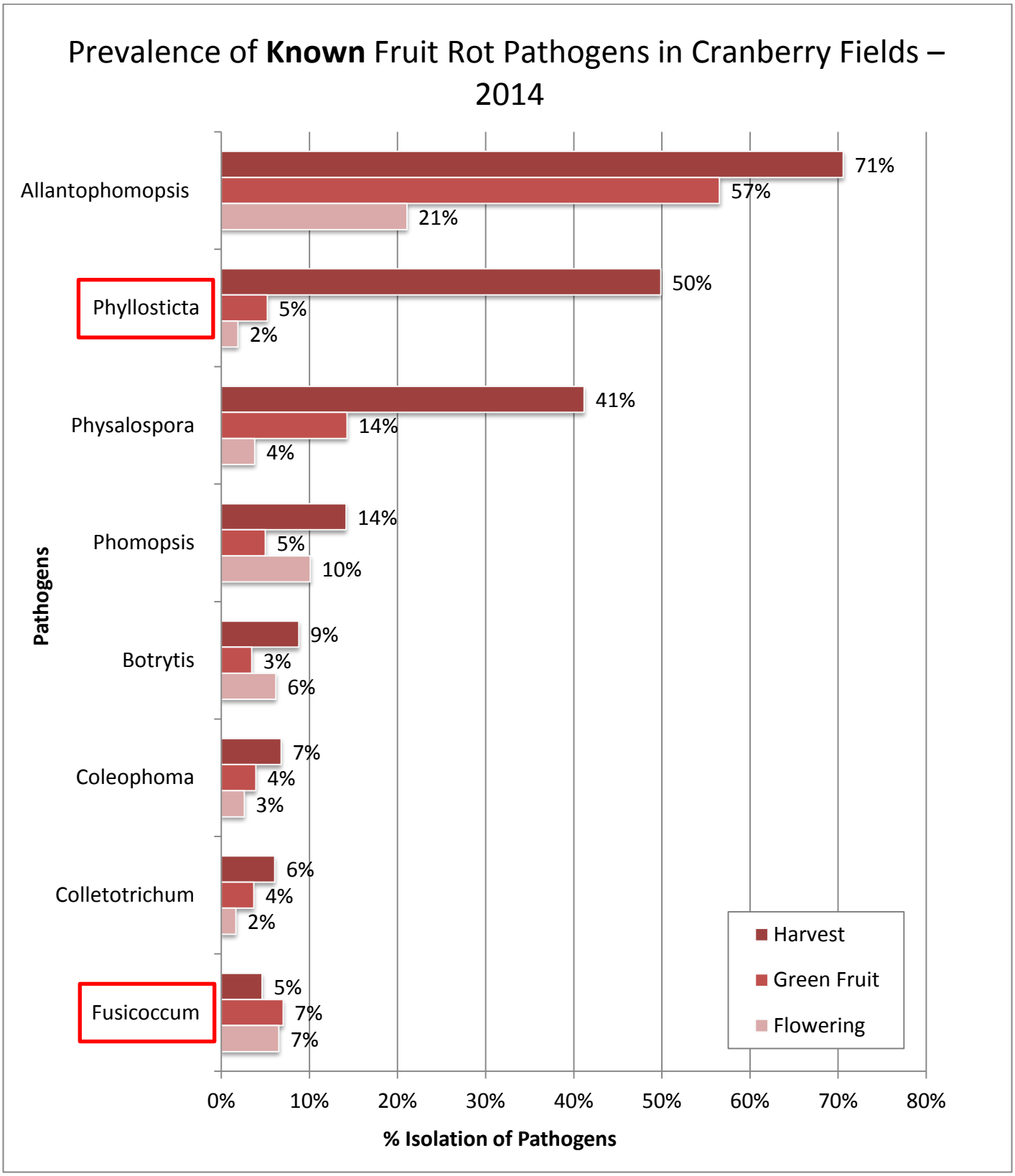


**Figure 4**. Mean percentage of fungal pathogens that **may** contribute to fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2014 growing season in 14 cranberry fields.

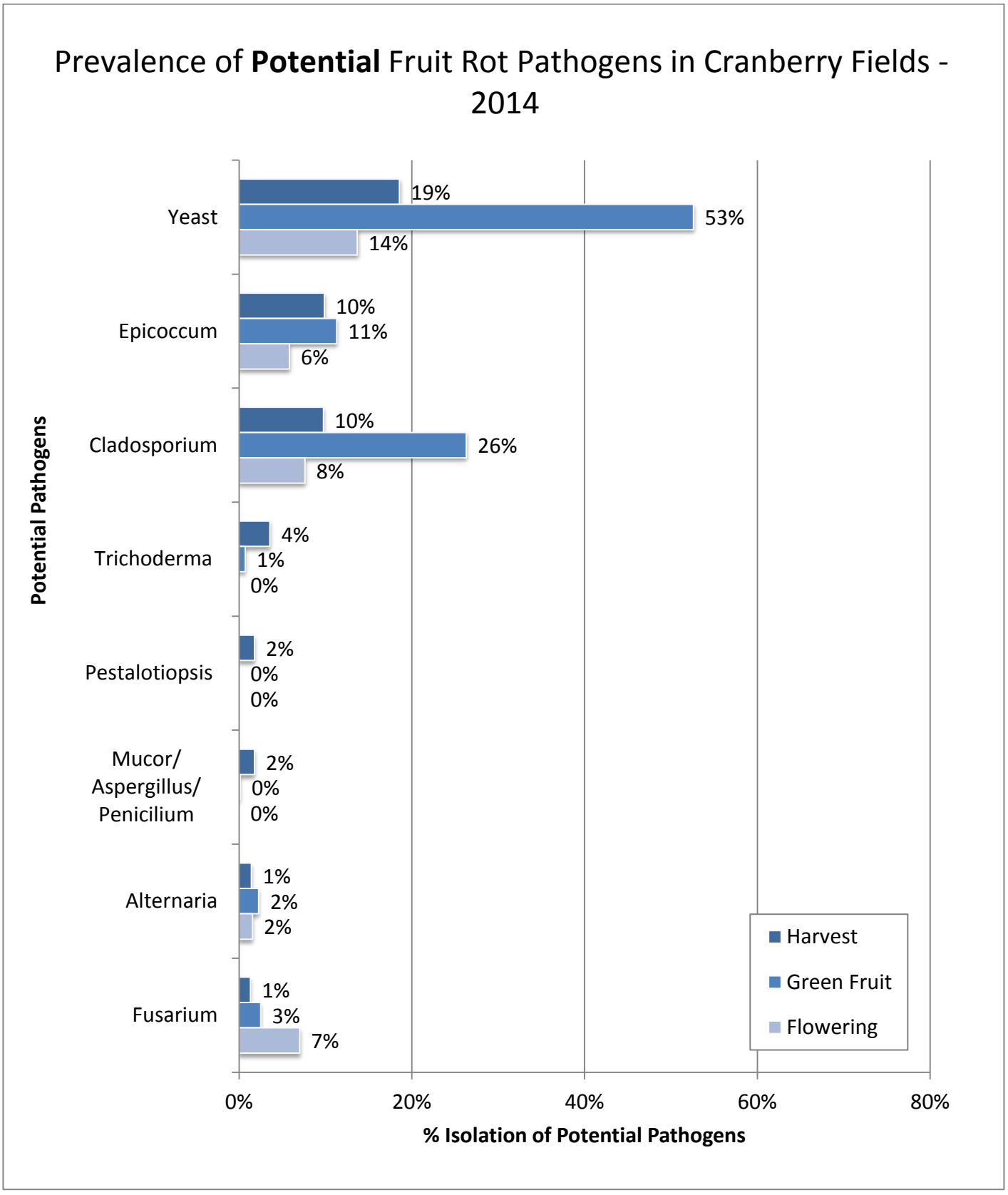
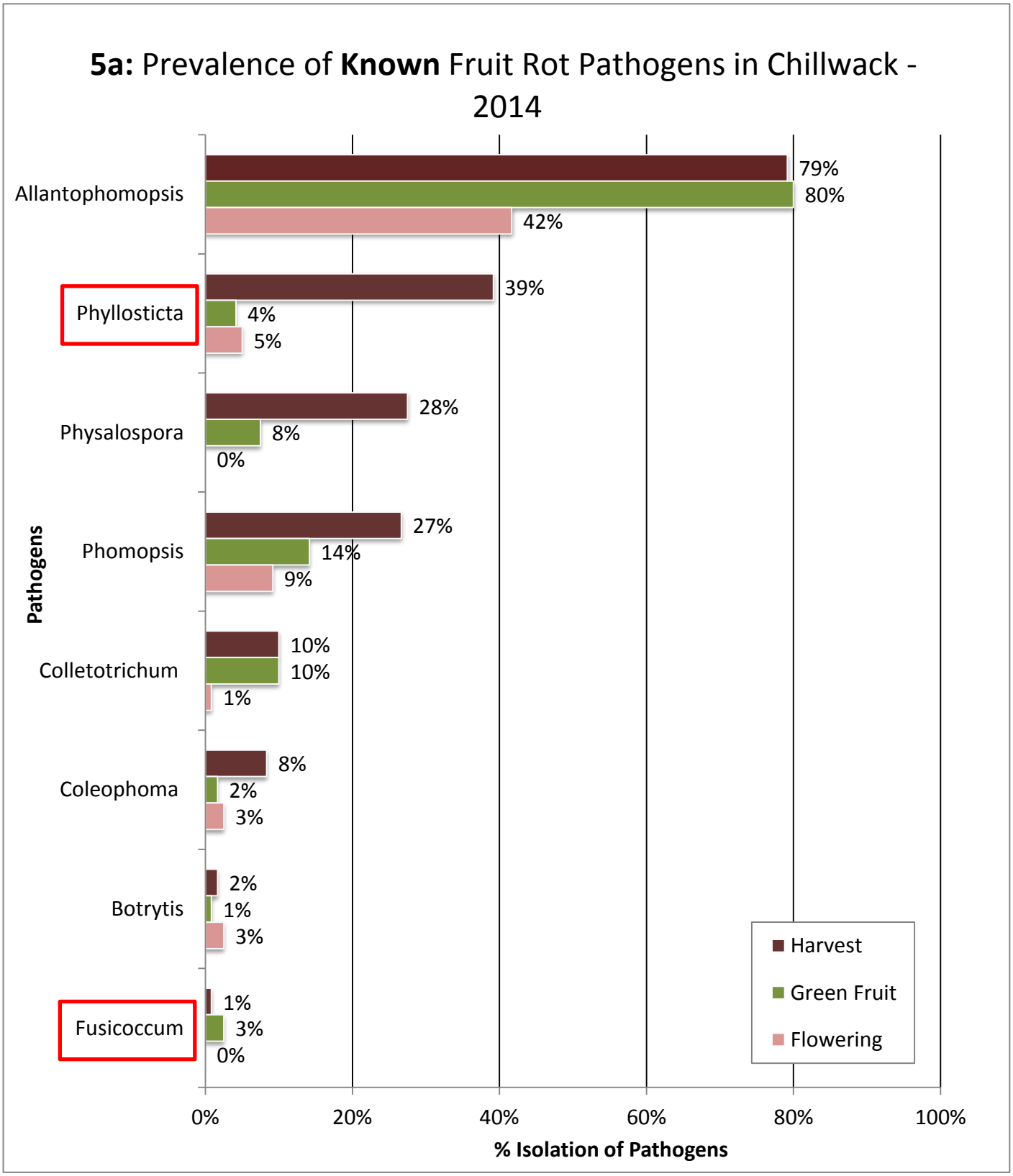
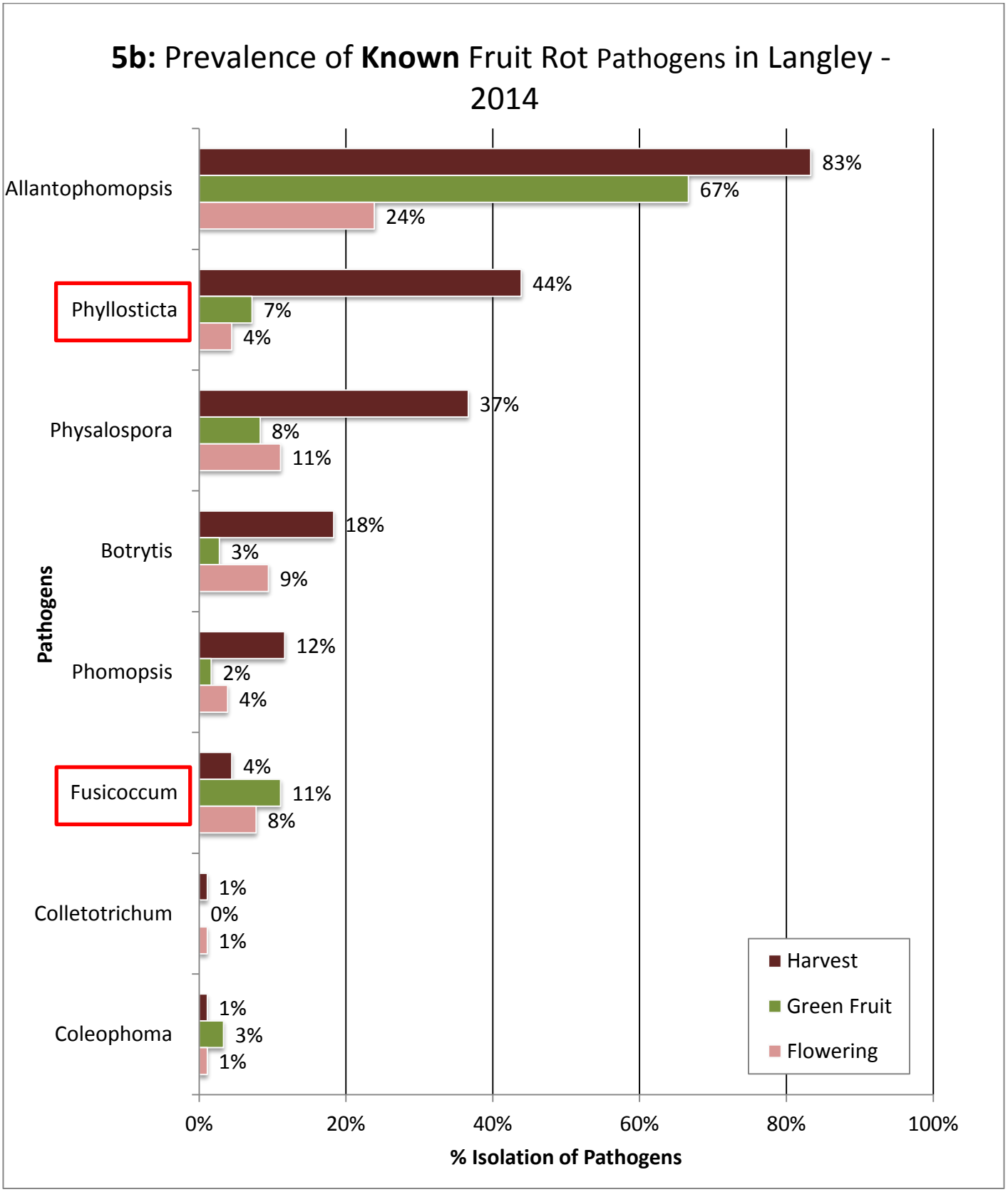
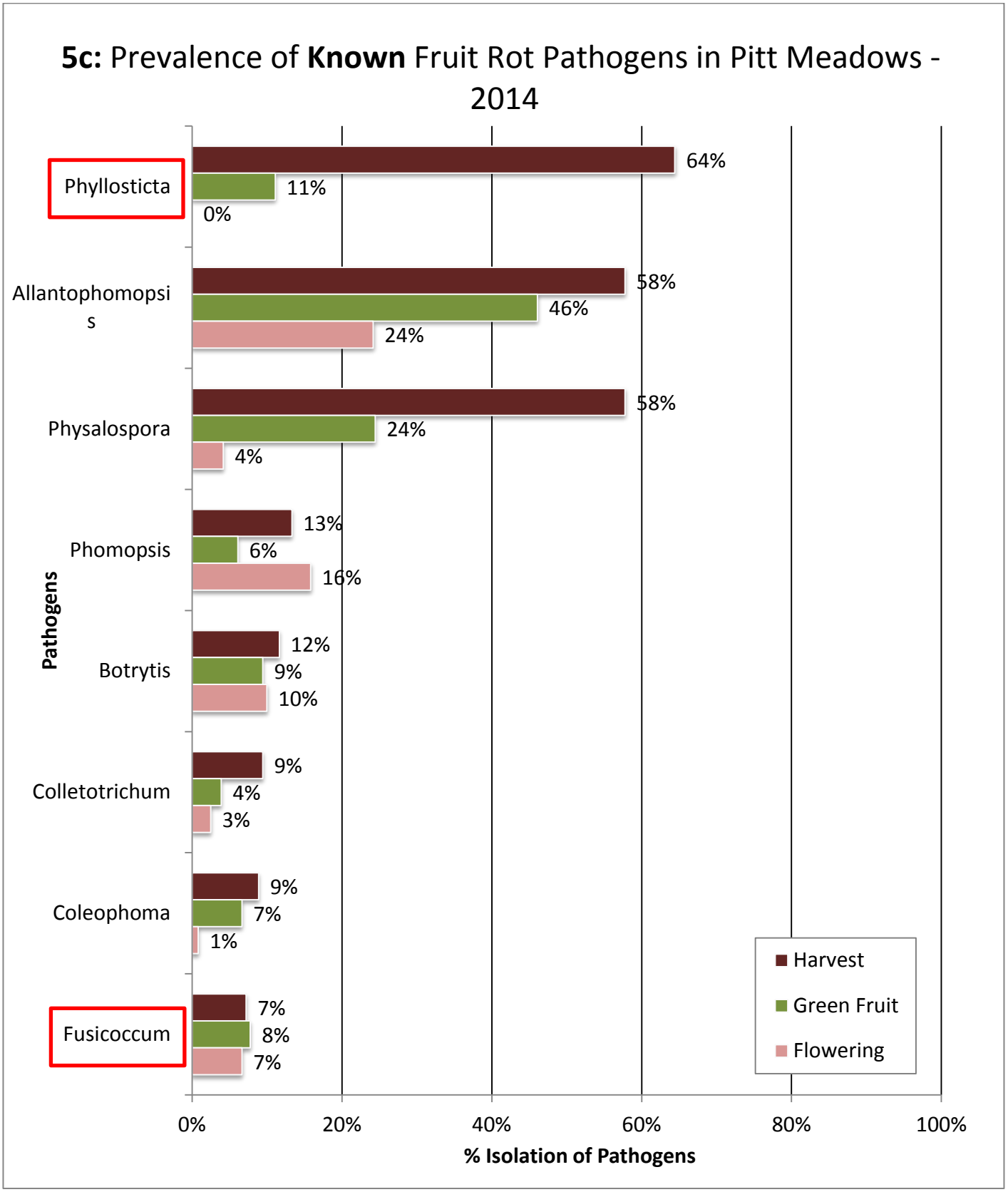
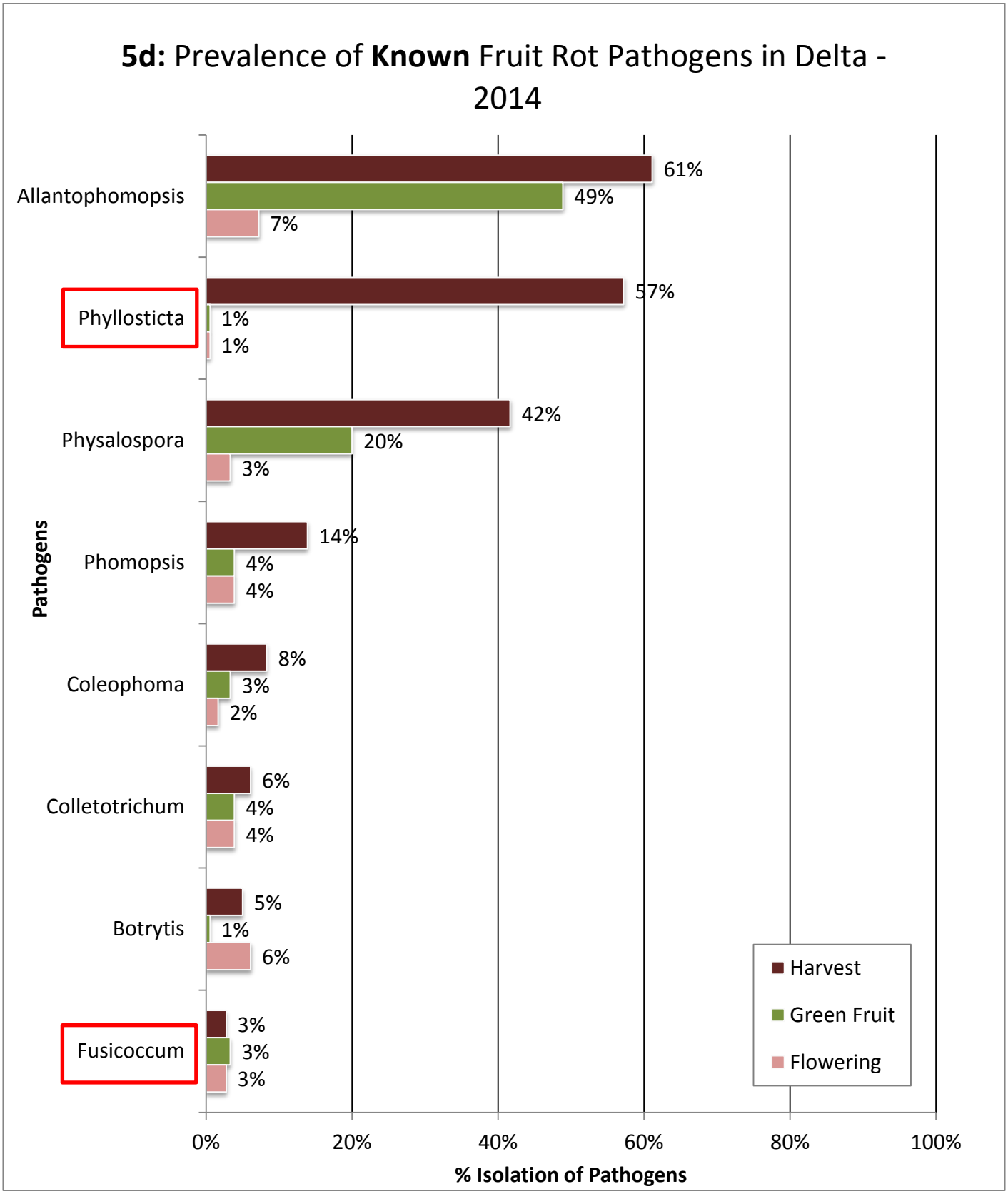


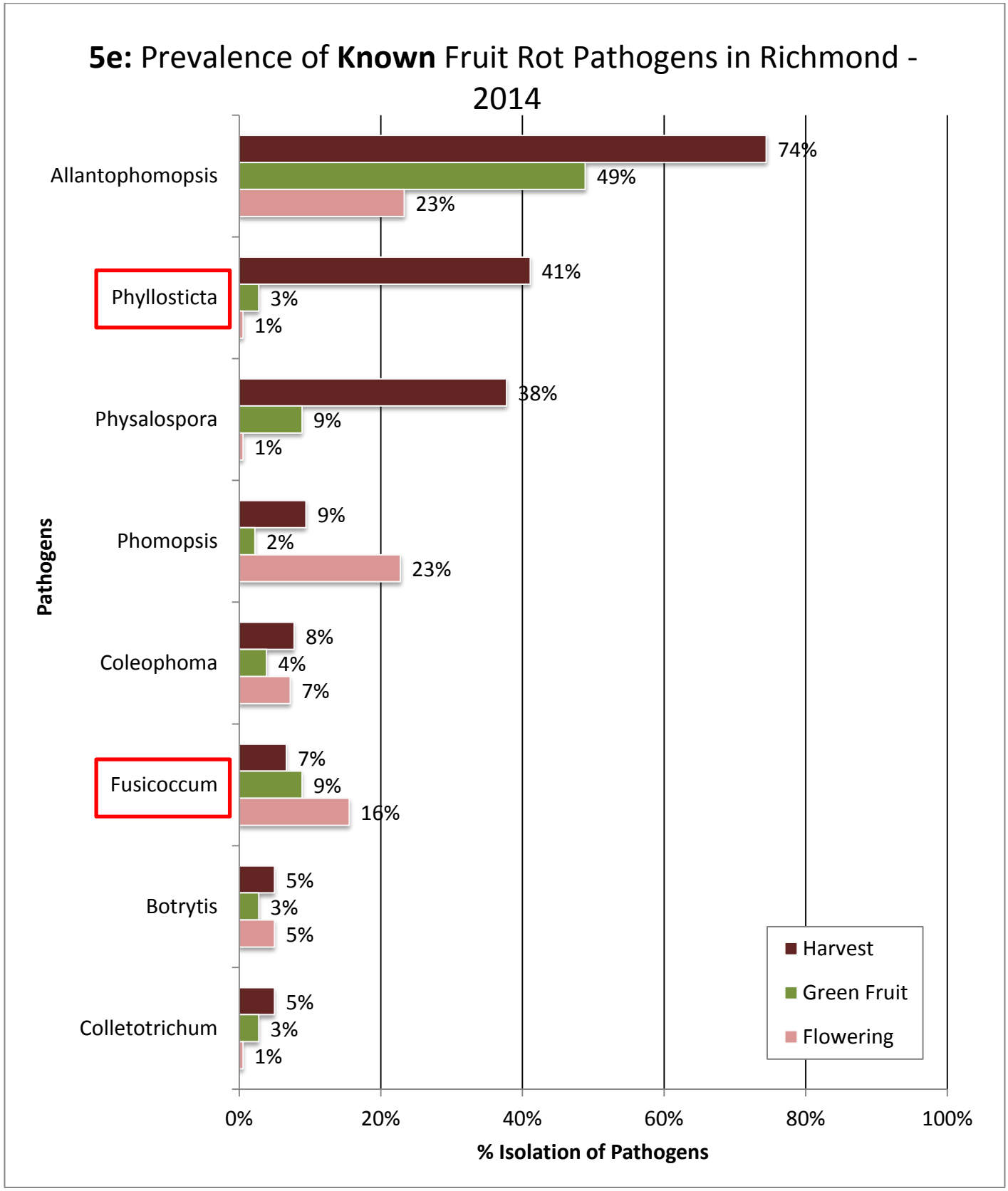
**Figure 5a-e. UPDATED.** Mean percentage of fungal pathogens, known to cause fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2014 growing season at each location – Chilliwack (5a), Langley (5b), Pitt Meadows (5c), Richmond (5d) and Delta (5e).




5b: Prevalence of Known Fruit Rot Pathogens in Langley - 2014
Allantophomopsis
Phyllosticta
Physalospora
Botrytis
Phomopsis
Fusicoccum
Colletotrichum
Coleophoma
Pathogens
83%
67%
24%
44%
7%
4%
37%
8%
11%
18%
3%
9%
12%
2%
4%
4%
11%
8%
1%
0%
1%
1%
3%
1%
Harvest
Green Fruit
Flowering
0%
20%
40%
60%
80%
100%
% Isolation of Pathogens


5c: Prevalence of Known Fruit Rot Pathogens in Pitt Meadows - 2014
Phyllosticta
64%
11%
0%
Allantophomopsis
58%
46%
24%
Physalospora
58%
24%
4%
Phomopsis
13%
6%
16%
Botrytis
12%
9%
10%
Colletotrichum
9%
4%
3%
Coleophoma
9%
7%
1%
Fusicoccum
7%
8%
7%
Pathogens
Harvest
Green Fruit
Flowering
0%
20%
40%
60%
80%
100%
% Isolation of Pathogens


5d: Prevalence of Known Fruit Rot Pathogens in Delta - 2014
Allantophomopsis
61%
49%
7%
Phyllosticta
57%
1%
1%
Physalospora
42%
20%
3%
Phomopsis
14%
4%
4%
Coleophoma
8%
3%
2%
Colletotrichum
6%
4%
4%
Botrytis
5%
1%
6%
Fusicoccum
3%
3%
3%
Pathogens
Harvest
Green Fruit
Flowering
0%
20%
40%
60%
80%
100%
% Isolation of Pathogens


5e: Prevalence of Known Fruit Rot Pathogens in Richmond - 2014
Allantophomopsis
74%
49%
23%
Phyllosticta
41%
3%
1%
Physalospora
38%
9%
1%
Phomopsis
9%
2%
23%
Coleophoma
8%
4%
7%
Fusicoccum
7%
9%
16%
Botrytis
5%
3%
5%
Colletotrichum
5%
3%
1%
Pathogens
Harvest
Green Fruit
Flowering
0%
20%
40%
60%
80%
100%
% Isolation of Pathogens

**CRANBERRY FRUIT ROT - REVIEW**

| Fruit Rot Disease | Causal Organism | Field/Storage Rot | Time of infection | Notes |
|---|---|---|---|---|
| Black rot | *Allantophomopsis cytisporea* | mostly storage rot | Not certain, most probably during flooding/harvest | disease incidence directly related to elapsed time berries left in flood water. |
| Bitter rot | *Colletotrichum* spp. & *Glomerella* spp. | field rot | from flowering to early-mid fruit development | spore dispersal by rain splash; fungus is latent after infection; symptoms only appear on ripe fruit |
| Blotch rot | *Physalospora vaccinii* | field and storage rot | from July till October (on the East Coast) | fungus is latent after infection; fungus is active at temperatures greater than 16°C. |
| Ripe/White rot | *Coleophoma empetri* | field and storage rot | from flowering till mid fruit development | symptoms only appear around harvest time/in storage. |
| Viscid rot | *Phomopsis vaccinii* | storage rot | from flowering till harvest | spore dispersal by rain splash |
| Yellow rot | *Botrytis* sp. (of *B. cinerea* type) | field and storage rot | Mostly during following | infection by air-born spores; reduce mechanical injuries to fruit during & after harvest. |