

RESEARCH REPORT

March 1, 2016

Fruit Rot Pathogens and their Impact on Cranberry Production in British Columbia [2014 & 2015 Studies]

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)



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, years, and even during the growing season. 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 can reach greater than 50% if they are not strategically managed. Because fruit rot involves multiple pathogens, an accurate diagnosis of the causal agents and their biology and disease epidemiology should be taken into consideration when applying appropriate disease management strategies to manage pre- and post-harvest fruit rot. In BC, information on fungal pathogens associated with cranberry fruit rot has not been documented as rigorously as in the major cranberry growing areas in the Northeastern United States. 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 production. 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 also lead to introduction, establishment and spread of new and more aggressive strains of plant pathogens.

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

Year 1, 2, 3 & 4 (November 2013 to March 2017)

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 Identify gaps in the information gathered to plan and execute the proposed research program.

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

2. Conduct field surveys during 2014 and 2015 cropping seasons, focusing on cranberry fields in different geographical locations in the Fraser Valley, and identify the occurrence and distribution of the major fungal plant pathogens associated with fruit rot incidence,
3. Assess the impact of fruit loss in cranberry fields at pre- and post-harvest, and
4. Evaluate the efficacy of fungicides and identify effective management tools for managing major fruit rot pathogens (objective for 2016/2017)

MATERIALS & METHODS

Identification of fungal pathogens associated with cranberry fruit rot diseases

Field survey/sampling: In 2014, fourteen fields located in Chilliwack, Langley, Pitt Meadows, Richmond and Delta were sampled, and in 2015, fourteen different fields located in Surrey, Langley, Pitt Meadows, Richmond and Delta were sampled. In all fields, only 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 m² 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 m² transect placed within each plot (i.e. a total of 60 flowers or berries per field); at each sampling time, a new area within the 2 m² 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.

In 2015, the Cranberry Research Farm in Delta, BC was also sampled at the ripe fruit stage for the same purpose, to assess the prevalence of fruit rot pathogens among different cranberry varieties. Five cranberry varieties, Mullica Queen, Crimson Queen, Demoranville, Haines, and Welker, were sampled using the same protocol as described above.

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 then cutting them 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 through the use of genetic markers via PCR and DNA sequencing. The most prevalent fungal pathogens associated with fruit rot and their prevalence at each farm and overall distribution in the Fraser Valley were determined. 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 m² sampling plot, as described previously, forty fruits were collected from each of two 1 m² transects that were placed diagonally inside the sampling plot. A total of 120 fruit were collected from 3 replicate plots per field. All samples were placed in Ziploc bags, kept in a cooler with ice-packs and brought to the laboratory.

In 2015, the Cranberry Research Farm in Delta, BC was also sampled at the ripe fruit stage for the same purpose, to assess the amount of fruit loss among different cranberry varieties. Five cranberry varieties, Mullica Queen, Crimson Queen, Demoranville, Haines, and Welker, were sampled using the same protocol as described above.

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 the fields 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 newly “symptomatic” fruits following 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 identify them by microscopy. The percentages of “symptomatic” fruits prior to harvest, at 3 weeks of post-harvest incubation, and cumulatively were calculated based on the number of fruits (n = 120) sampled from each of 3 replicate plots.

RESULTS & DISCUSSION (2014 & 2015)

Fruit Loss Assessment

- The percentage fruit loss as estimated at harvest, after 3-weeks of post-harvest incubation and cumulatively in 14 farms in 2014 and 14 farms in 2015 is shown in Figure 1 and Figure 2, respectively. The percentage incidence of fruit loss at harvest varied considerably from farm to farm in both years. The highest incidence of fruit loss at harvest was estimated at 24% in 2014 and 18% in 2015. The differences between farms and years could be due to differences in production and disease-management practices adopted by each grower, prevalence and distribution of pathogens, and environmental factors (weather conditions) pertaining to each field, location and year.
- The highest percentage fruit loss estimated at harvest at the Cranberry Research Farm was in Demoranville (9%), followed by Crimson Queen (8%) and Haines, Mullica Queen, and Welker (1%).

After 3-weeks of incubation, cumulative levels of fruit loss rose to between 33% and 78%. The levels of fruit loss at harvest and after incubation that we see at the research farm were comparable to the commercial bogs throughout the Fraser Valley.

- In all 28 farms sampled in 2014 and 2015, the percentage fruit loss increased considerably when harvested fruit was held for 3 weeks at ambient temperature. The highest increase in fruit rot after the incubation period was from 4% at harvest to 50% after incubation (Figure 3). This indicates that any delay in fruit harvest or holding harvested fruit at the farm or receiving station can result in further fruit spoilage. Adopting an appropriate fungicide application program during crop production, timely harvest, and storing harvested fruit at cool temperatures can reduce post-harvest fruit loss due to microbial activity.
- In 2014, the cranberry farms in Chilliwack and Pitt Meadows had the highest fruit loss incidence at harvest and after 3-week post-harvest incubation (on average) followed by the farms in Delta, Richmond and Langley. In 2015, the cranberry farms in Delta and Pitt Meadows had the highest fruit loss incidence at harvest and after 3-week post-harvest incubation (on average) followed by the farms in Richmond, Langley and Surrey. The average amount of fruit loss in different regions in 2014 and 2015 at-harvest ranged between 1% and 13% (Figure 3). These differences may be due to regional variations in the prevalence and distribution of different fungal pathogens that contribute to the cranberry fruit rot disease complex (please see below) and also differences in management practices (including selection of fungicides, timing and number of application).

Fungal Pathogens Associated with Fruit Rot

- Figures 4 and 5 show the mean percentage of fungal pathogens responsible for fruit rot that were recovered from flowers and immature- and ripe-fruit samples from 14 farms in 2014 and 2015, respectively. The most commonly recovered fruit rot pathogens over the two-year study, as shown in the cumulative summary in Figure 6, were *Phyllosticta* (Early rot/Berry speckle), *Allantophomopsis* (Black rot), *Physalospora* (Blotch rot), *Phomopsis* (Viscid rot), *Coleophoma* (Ripe or White rot), *Colletotrichum* (Bitter rot), *Botrytis* (Yellow rot), and *Fusicoccum* (End Rot). Collectively in 2014 and 2015, *Phyllosticta*, *Allantophomopsis*, *Physalospora*, *Phomopsis*, *Coleophoma*, *Colletotrichum*, *Botrytis*, and *Fusicoccum* were isolated from 59%, 55%, 49%, 9%, 7%, 6%, 6%, and 5%, respectively, of the ripe-fruit samples from the 28 farms at harvest. However, the incidence of fruit rot pathogens varied from farm to farm.
- In 2014 and 2015, the predominant pathogens in the Fraser Valley were found to be *Allantophomopsis*, *Phyllosticta* and *Physalospora*, responsible for well over 50% of the fruit infection at harvest.
- Besides the pathogens that are known for causing fruit rot diseases of cranberry, *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Mucor*, *Penicillium*, *Pestalotiopsis*, *Trichoderma* and yeasts were also recovered from the samples in 2014 and 2015 (Figure 7 & 8). These organisms may contribute to pre- and post-harvest damages, particularly when harvested fruit are not properly stored.
- Based on the location of cranberry fields in the Fraser Valley sampled in 2014 and 2015, the most commonly recovered pathogens from flowers, green-fruit and ripe-fruit in 2014 and 2015 are shown in Figure 9a to 9e and Figure 10a to 10e, respectively. The following table summarizes the two-year average percentage recovery of the major fruit rot pathogens from ripe-fruit prior to harvest from

28 cranberry fields in the Fraser Valley (also see Figure 12).

Fruit rot pathogen	Surrey (1 field)	Chilliwack (2 fields)	Delta (6 fields)	Langley (5 fields)	Pitt Meadows (6 fields)	Richmond (8 fields)
<i>Allantophomopsis</i>	63%	79%	48%	55%	54%	54%
<i>Botrytis</i>	8%	2%	3%	13%	7%	3%
<i>Coleophoma</i>	3%	8%	8%	4%	8%	8%
<i>Colletotrichum</i>	2%	10%	9%	5%	6%	5%
<i>Fusicoccum</i>	3%	1%	3%	11%	9%	5%
<i>Phomopsis</i>	5%	27%	11%	7%	9%	7%
<i>Phyllosticta</i>	70%	39%	68%	57%	71%	49%
<i>Physalospora</i>	15%	28%	49%	38%	63%	52%

- In the Research Farm, the most frequently recovered fungal pathogens from the five varieties (Mullica Queen, Crimson Queen, Demoranville, Haines, and Welker) were *Physalospora* (47%), *Allantophomopsis* (32%), *Phyllosticta* (15%) and *Coleophoma* (13%). However, the prevalence of each of these pathogens varied among the five varieties (Figure 11); further samplings would be required to understand the varietal differences in susceptibility to each fruit rot pathogen. Although the Research Farm has no pesticide application program, it is otherwise being managed like a commercial farm, it shows a similar trend in the fruit rot pathogen types and populations as we see in commercial bogs in the Fraser Valley.
- The recovery of fruit rot pathogens from cranberry fields at flowering 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 an appropriate fungicide spray program starting at flowering.
- Application of fungicides at flowering and during fruit development is very important for controlling fruit rot diseases in cranberry fields and during post-harvest handling and storage. Use of appropriate and effective fungicides, timing of application (with regards to different stages of crop development, weather conditions and type of pathogens and their infection periods) and application intervals are critical for reducing the pathogen/disease pressure in the field. Alternate use of fungicides from different chemical groups will minimize the risk of resistance development to fungicides by pathogens and prolong the efficacy and lifespan of fungicides.

The following fungicides are registered for use on cranberries in BC:

Group M - Bravo (chlorothalonil) & Copper oxychloride

Group 3 - Funginex (triforine), Jade or Topas (propiconazole), & Proline (prothioconazole)

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, Delta, Langley, Pitt Meadows, and Richmond.

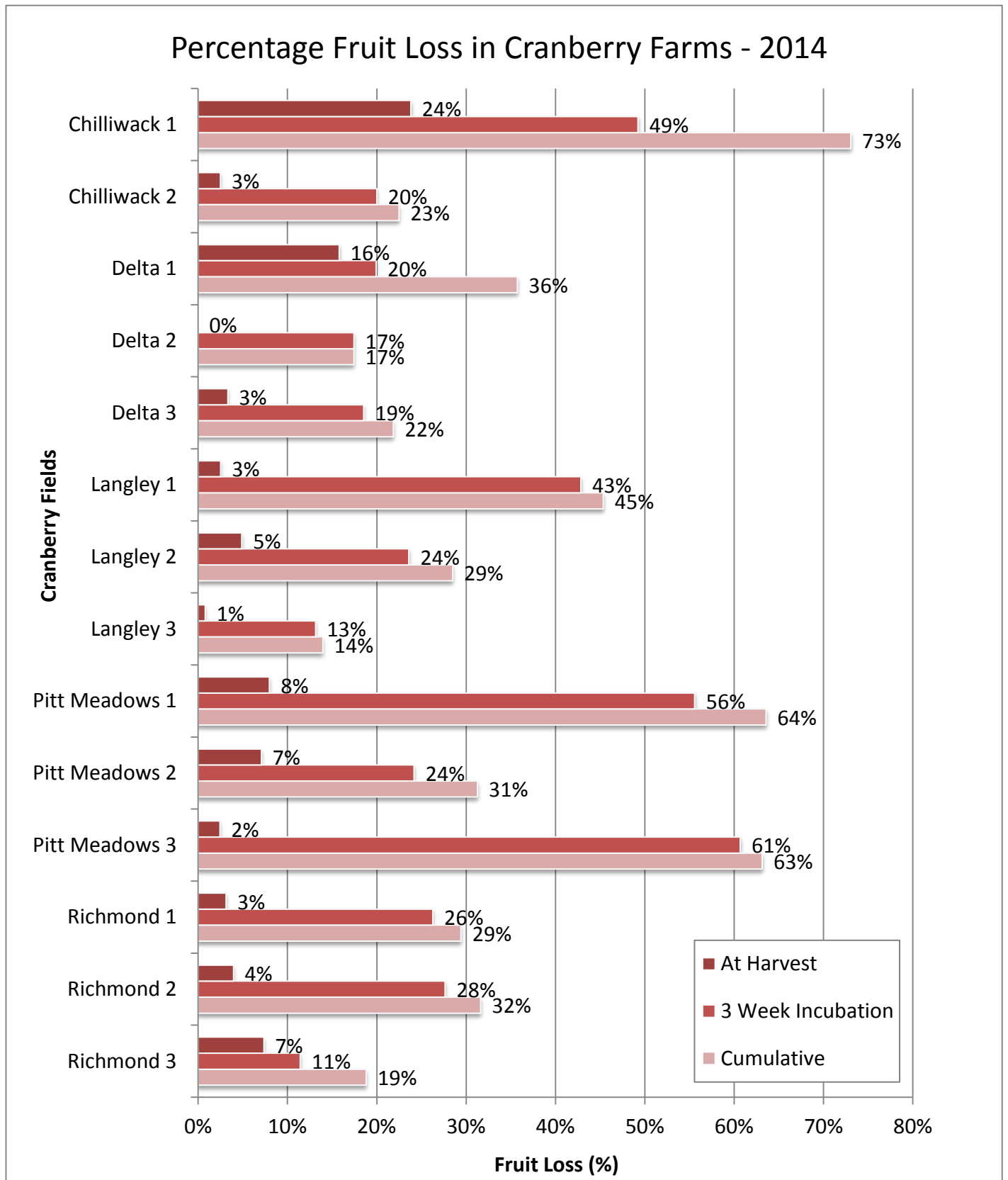


Figure 2. Percentage fruit loss at harvest, 3-week post-harvest incubation and cumulative fruit loss during 2015 growing season in 14 farms, located in Surrey, Delta, Langley, Pitt Meadows, and Richmond.

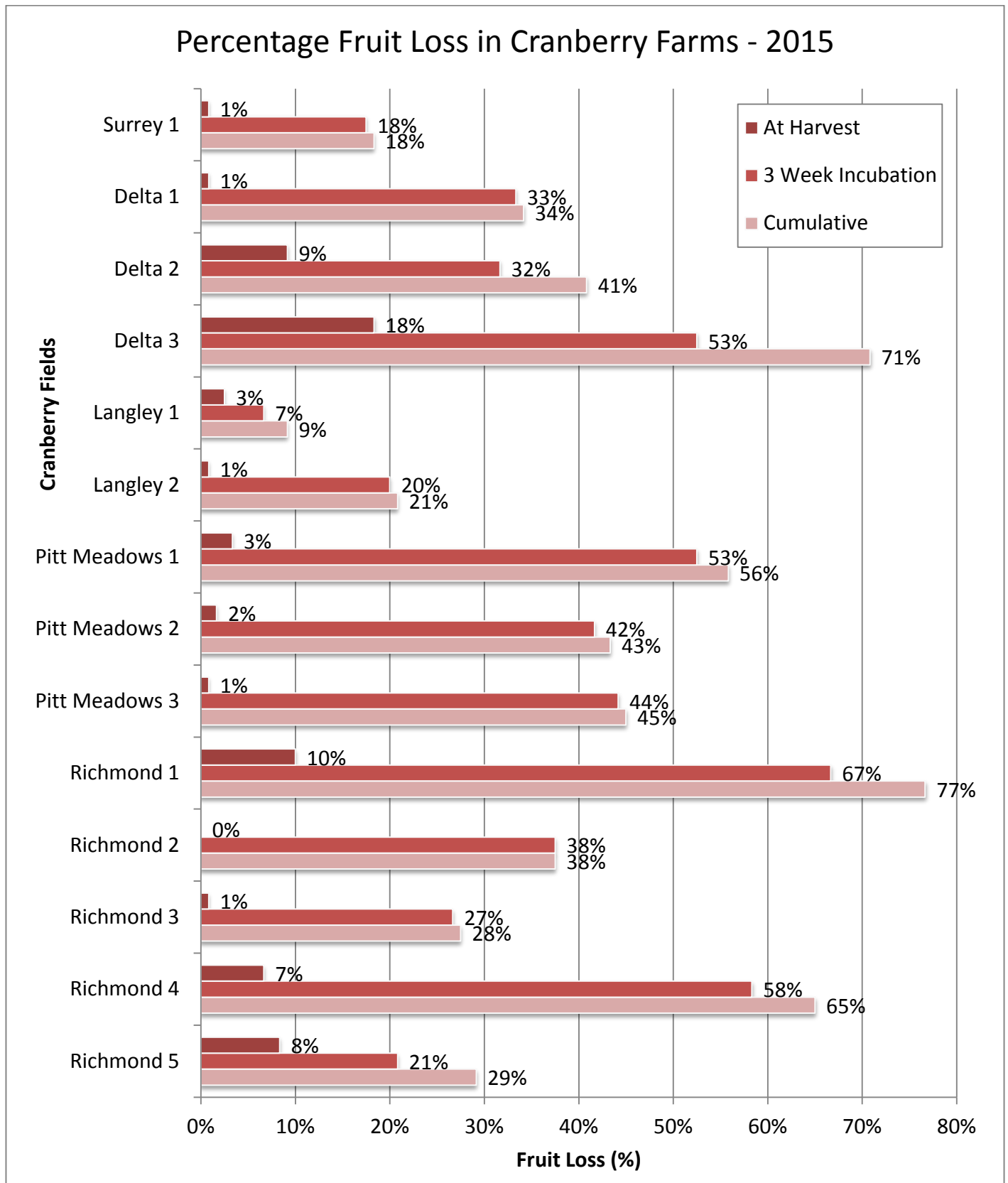


Figure 3. Average percentage fruit loss at harvest, 3-week post-harvest incubation and cumulative fruit loss during the 2014 & 2015 growing season at each location - Chilliwack, Delta, Langley, Pitt Meadows, Richmond, and Surrey.

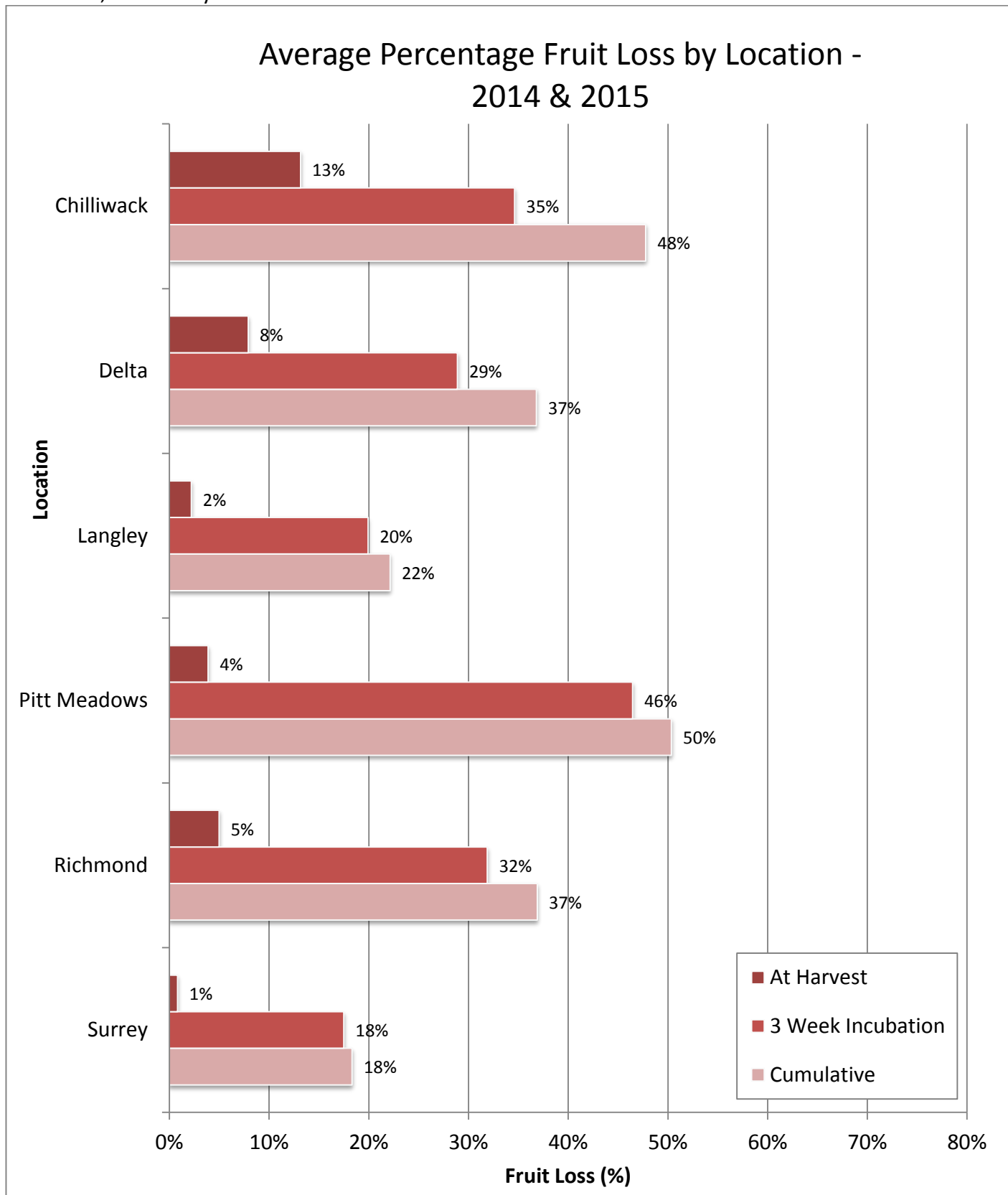


Figure 4. Mean percentage of fungal pathogens **known** to cause fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2014 growing season in 14 cranberry fields.

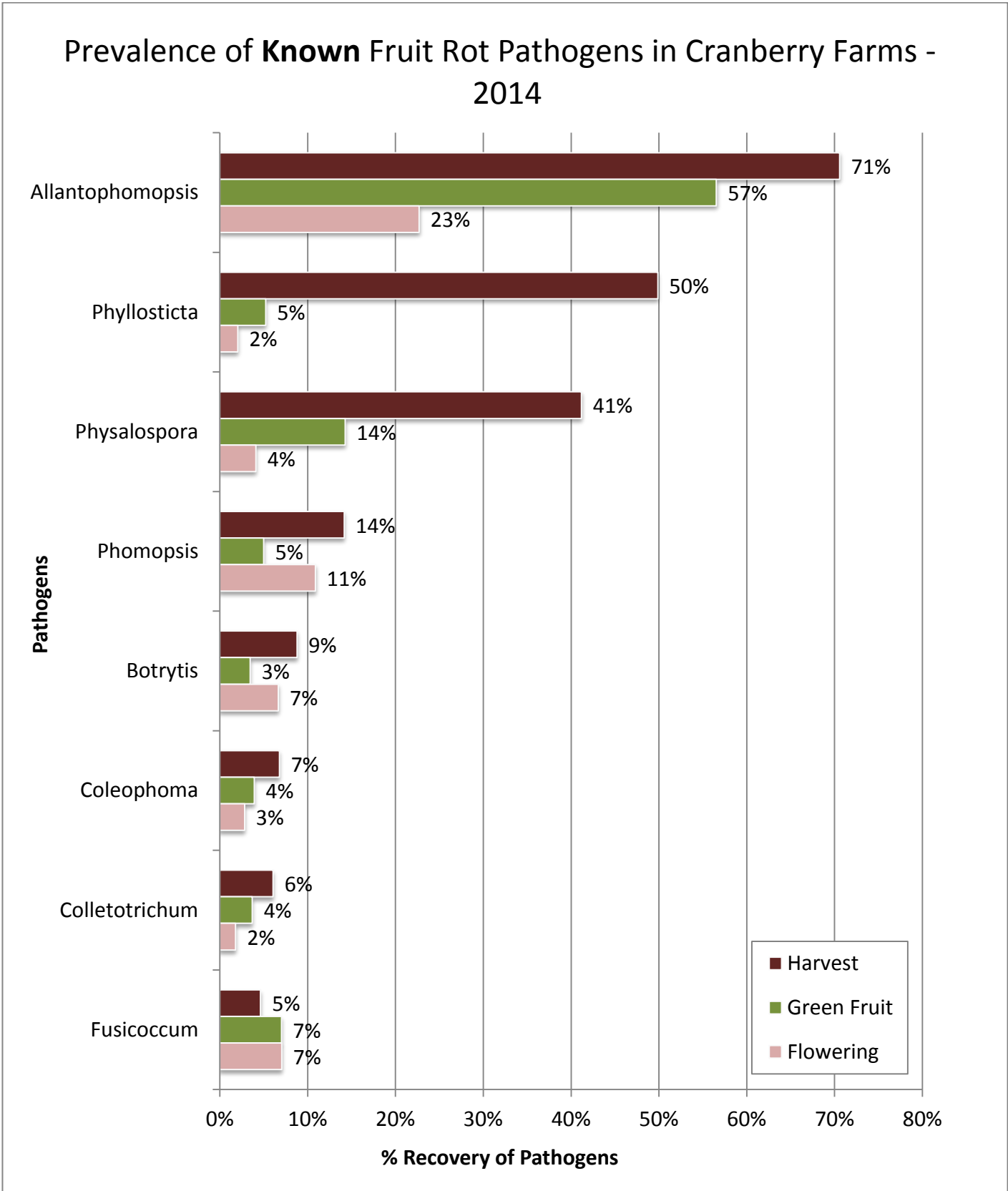


Figure 5. Mean percentage of fungal pathogens, **known** to cause fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2015 growing season in 14 cranberry fields.

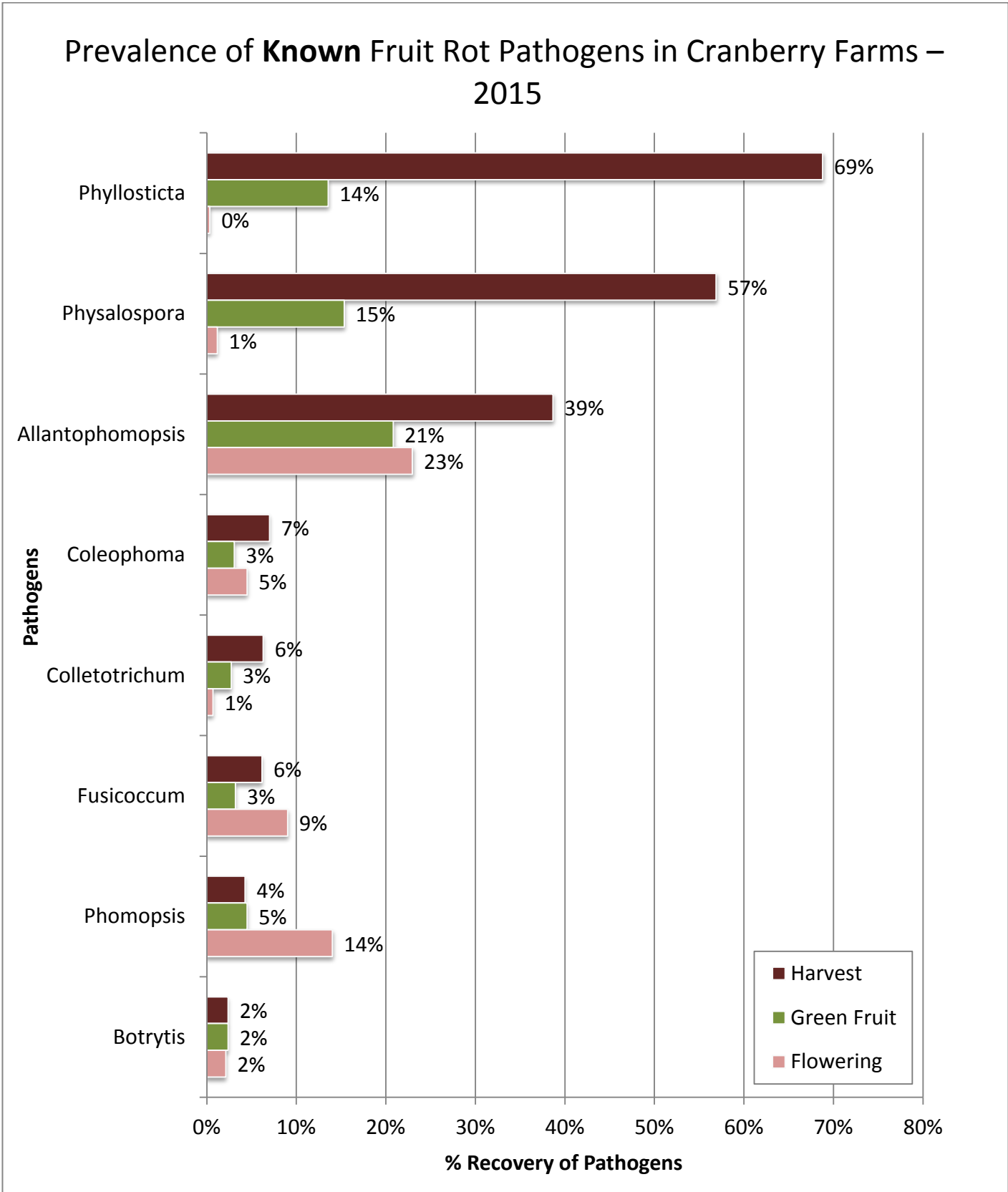


Figure 6. Mean percentage of fungal pathogens, **known** to cause fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during the 2014 and 2015 growing season in 28 cranberry fields.

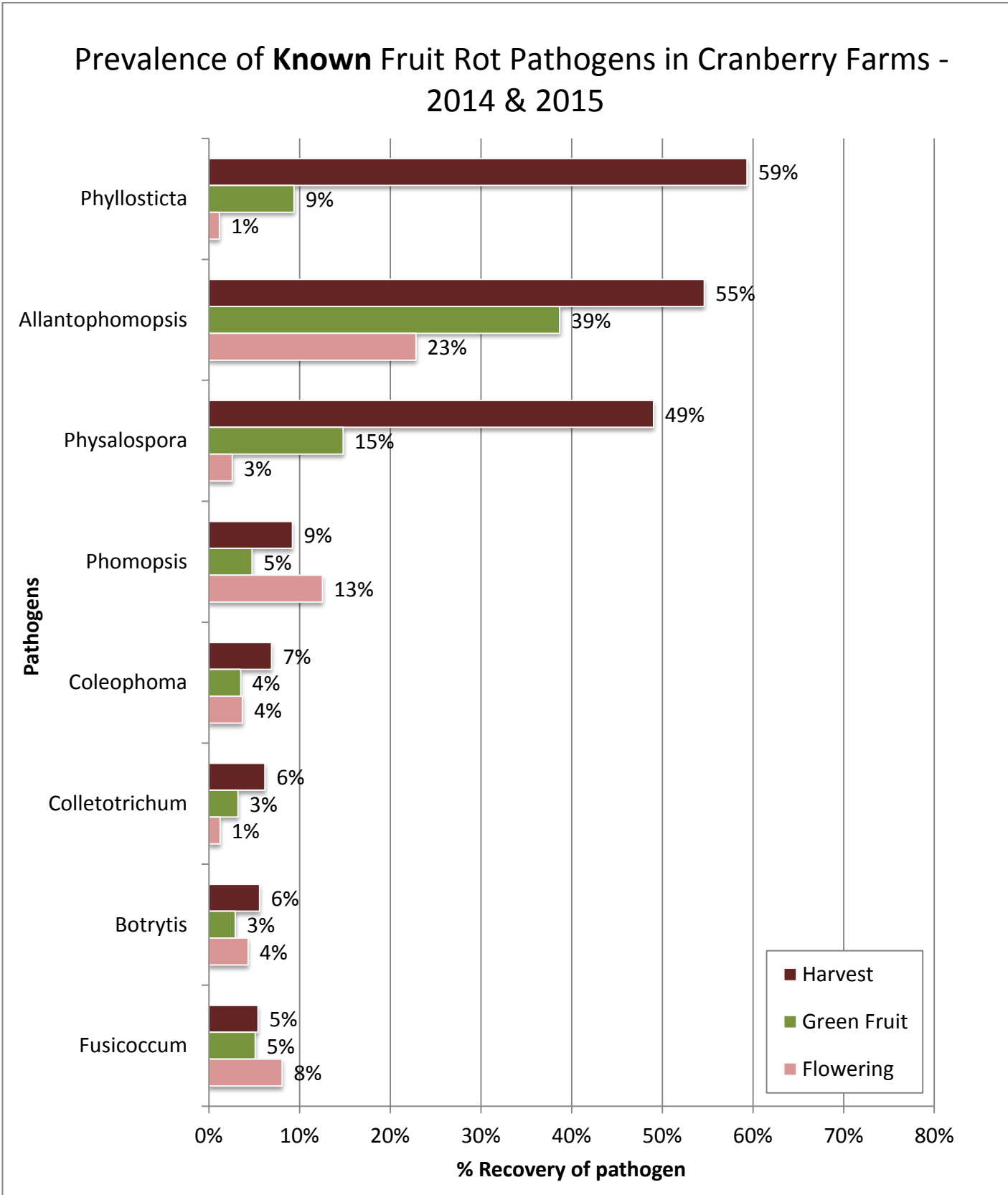


Figure 7. Mean percentage of fungal pathogens that may contribute to fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2014 growing season in 14 cranberry fields.

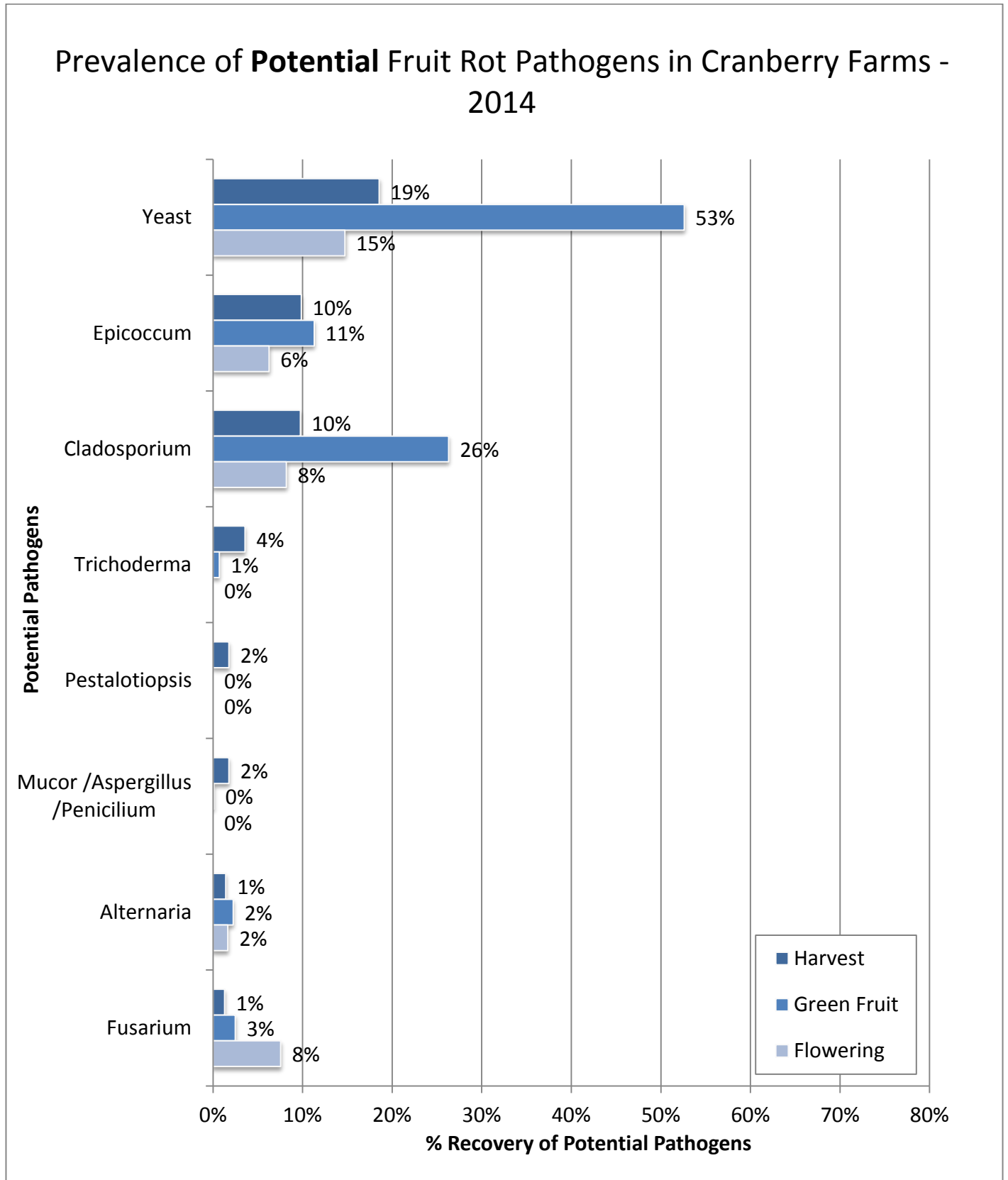


Figure 8. Mean percentage of fungal pathogens that may contribute to fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2015 growing season in 14 cranberry fields.

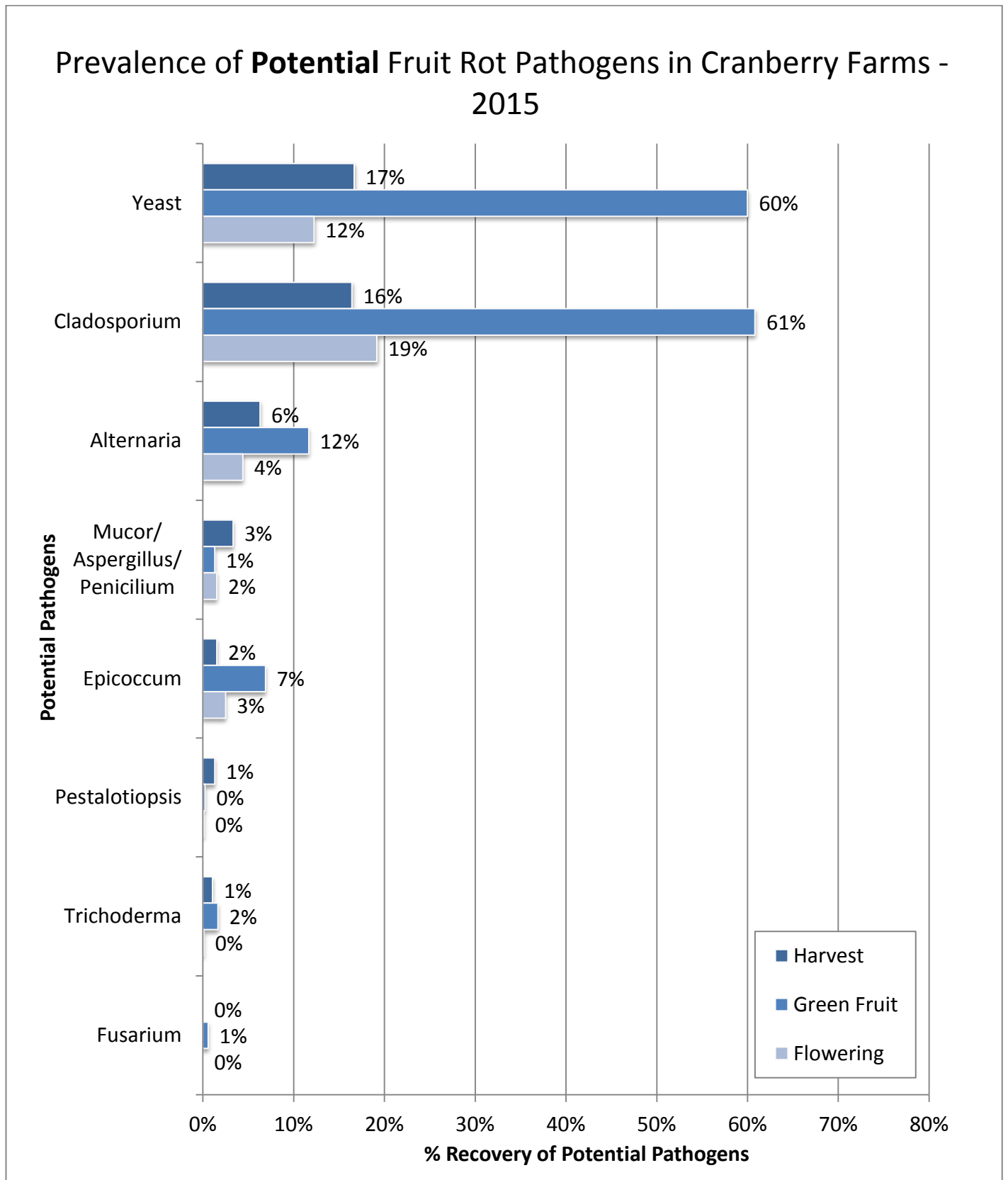
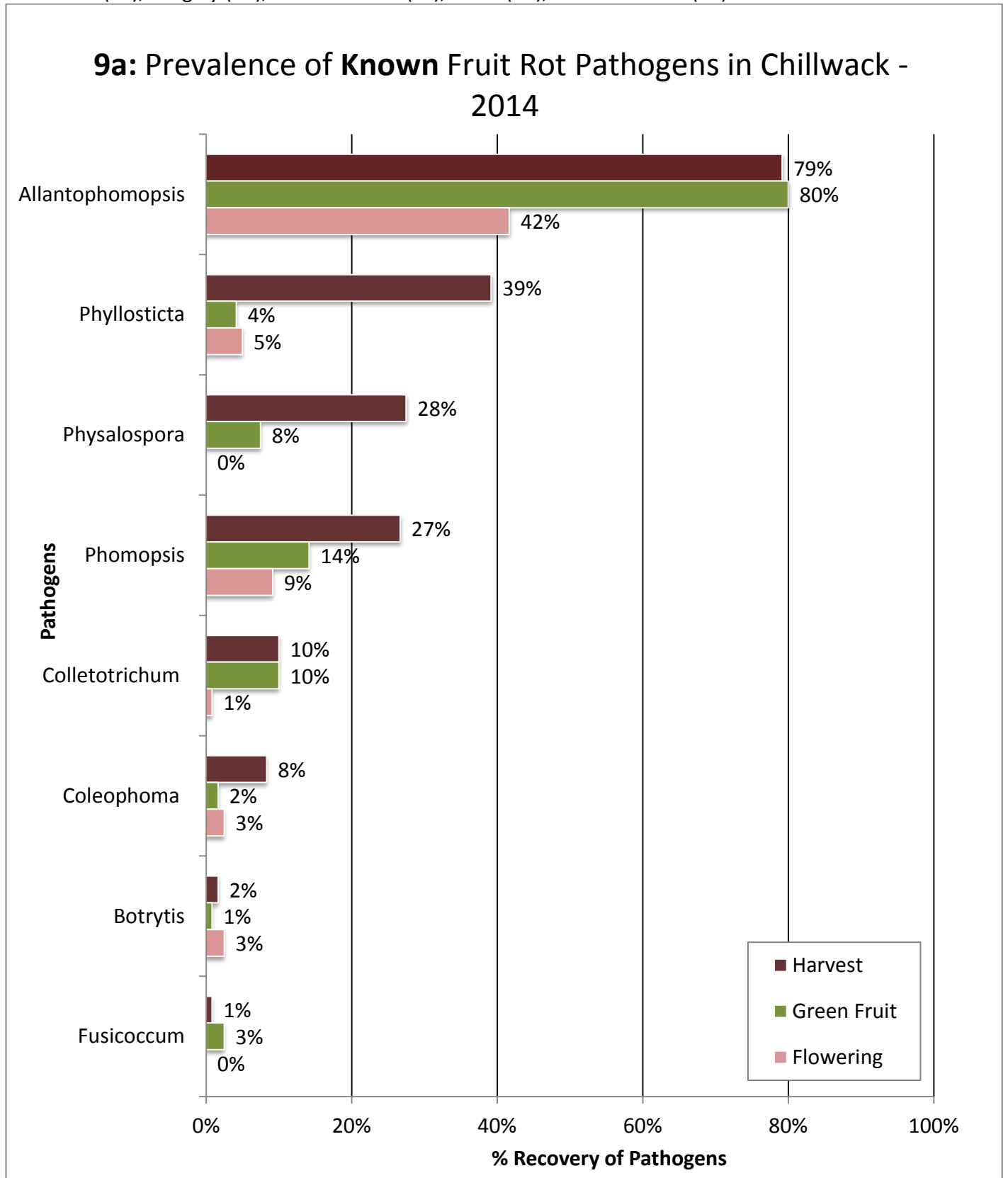
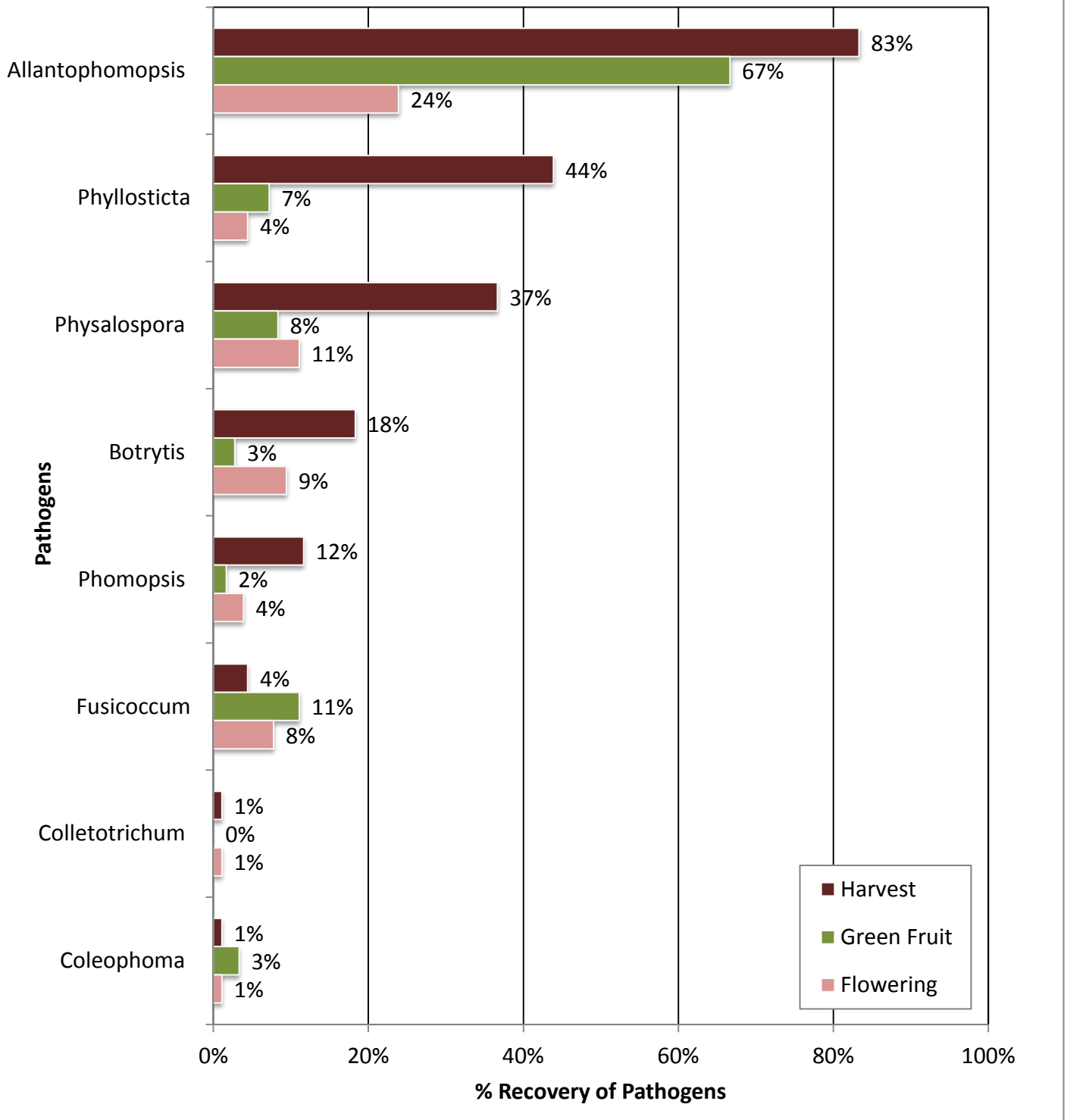


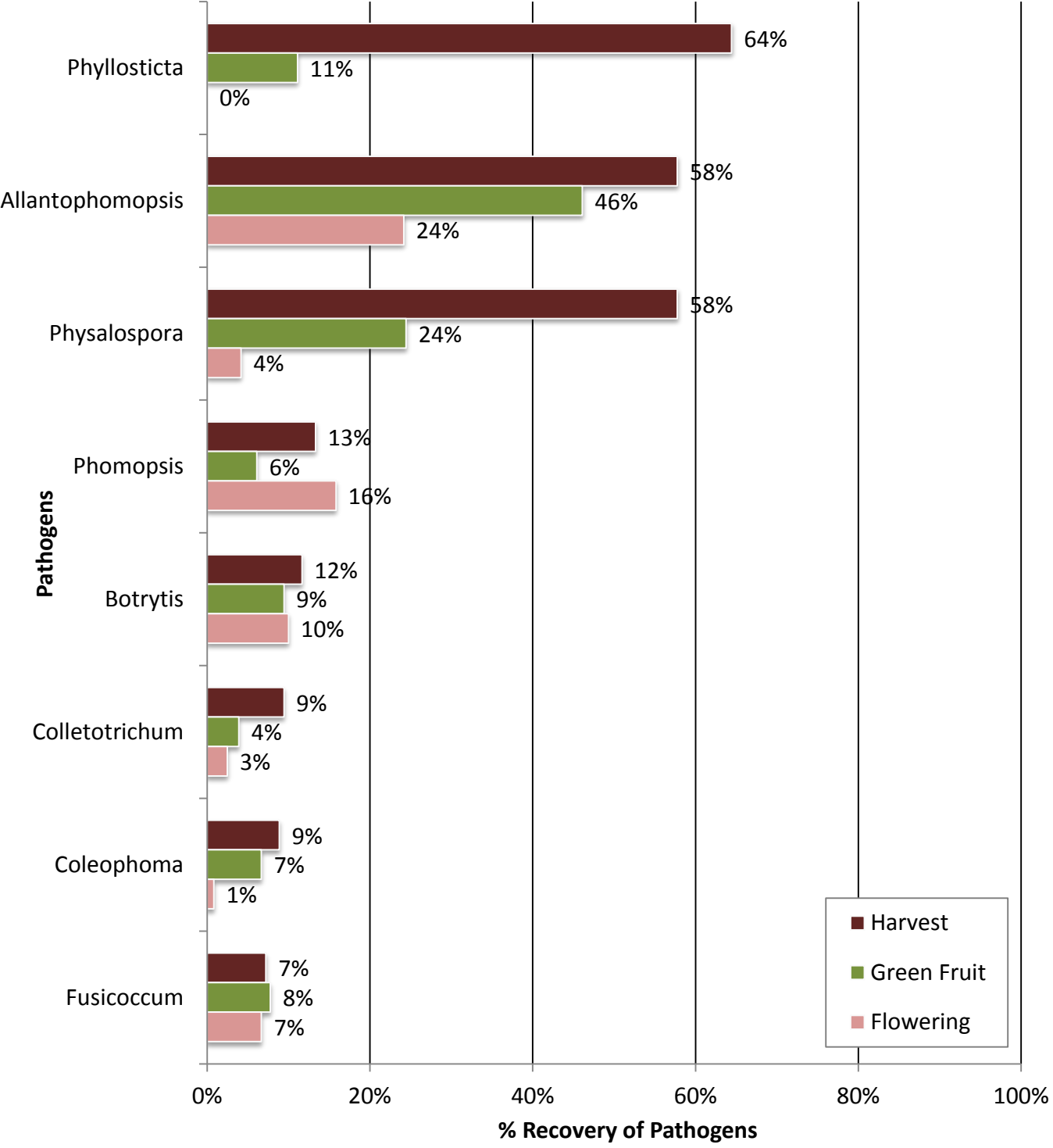
Figure 9a-e. Mean percentage of fungal pathogens known to cause fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2014 growing season at each location – Chilliwack (9a), Langley (9b), Pitt Meadows (9c), Delta (9d), and Richmond (9e).



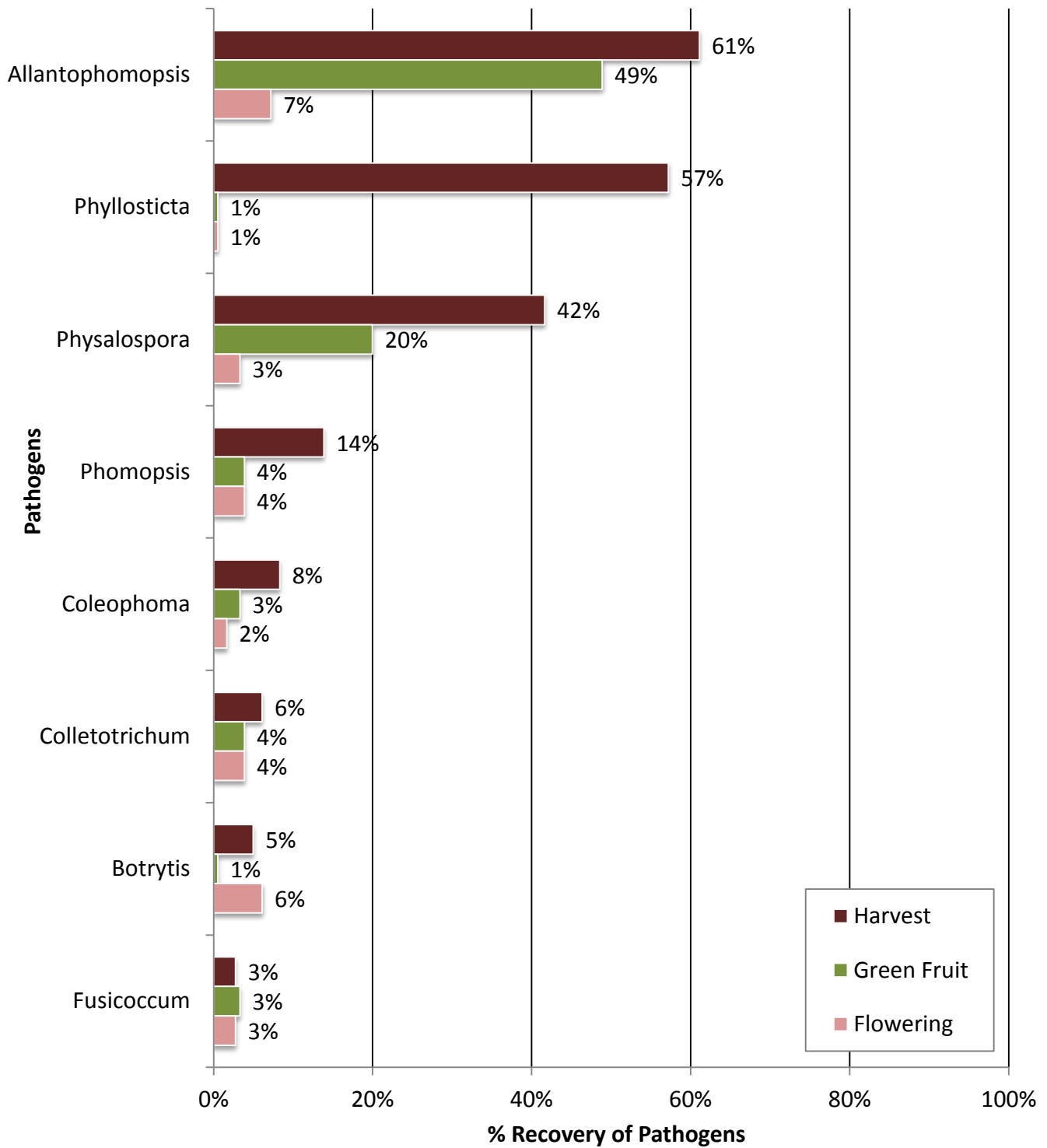
9b: Prevalence of **Known** Fruit Rot Pathogens in Langley - 2014



9c: Prevalence of **Known** Fruit Rot Pathogens in Pitt Meadows - 2014



9d: Prevalence of **Known** Fruit Rot Pathogens in Delta - 2014



9e: Prevalence of **Known** Fruit Rot Pathogens in Richmond - 2014

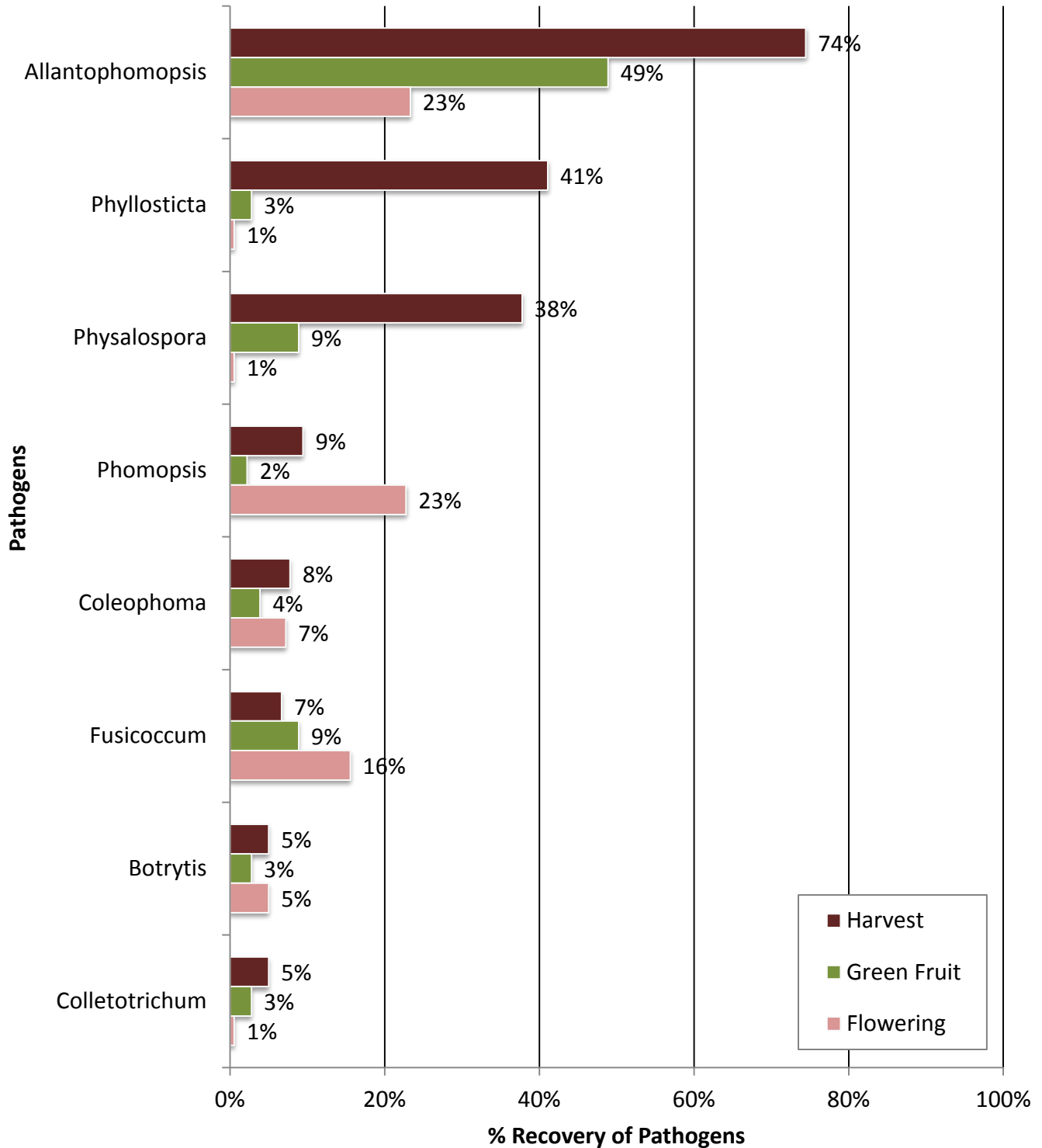
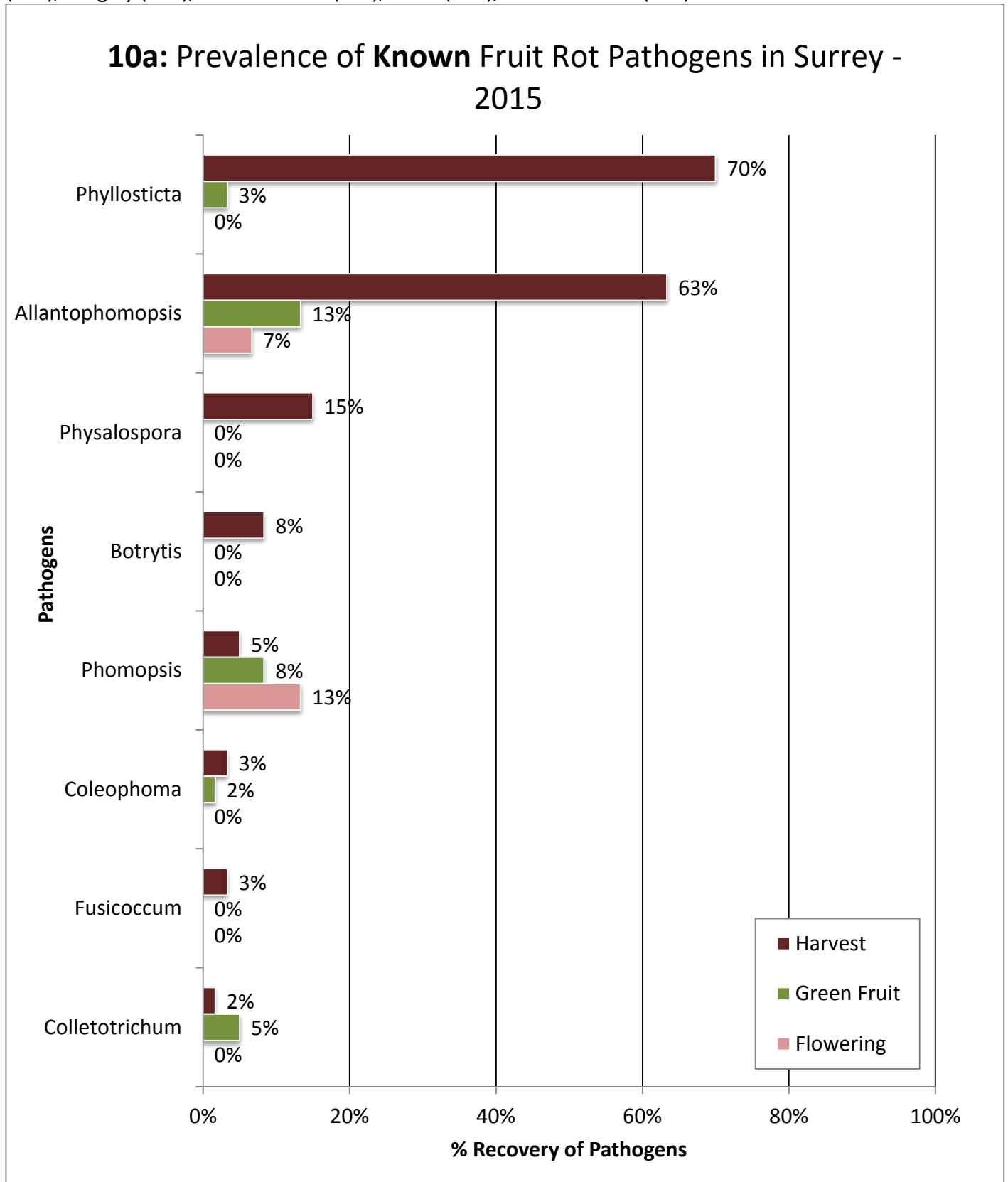
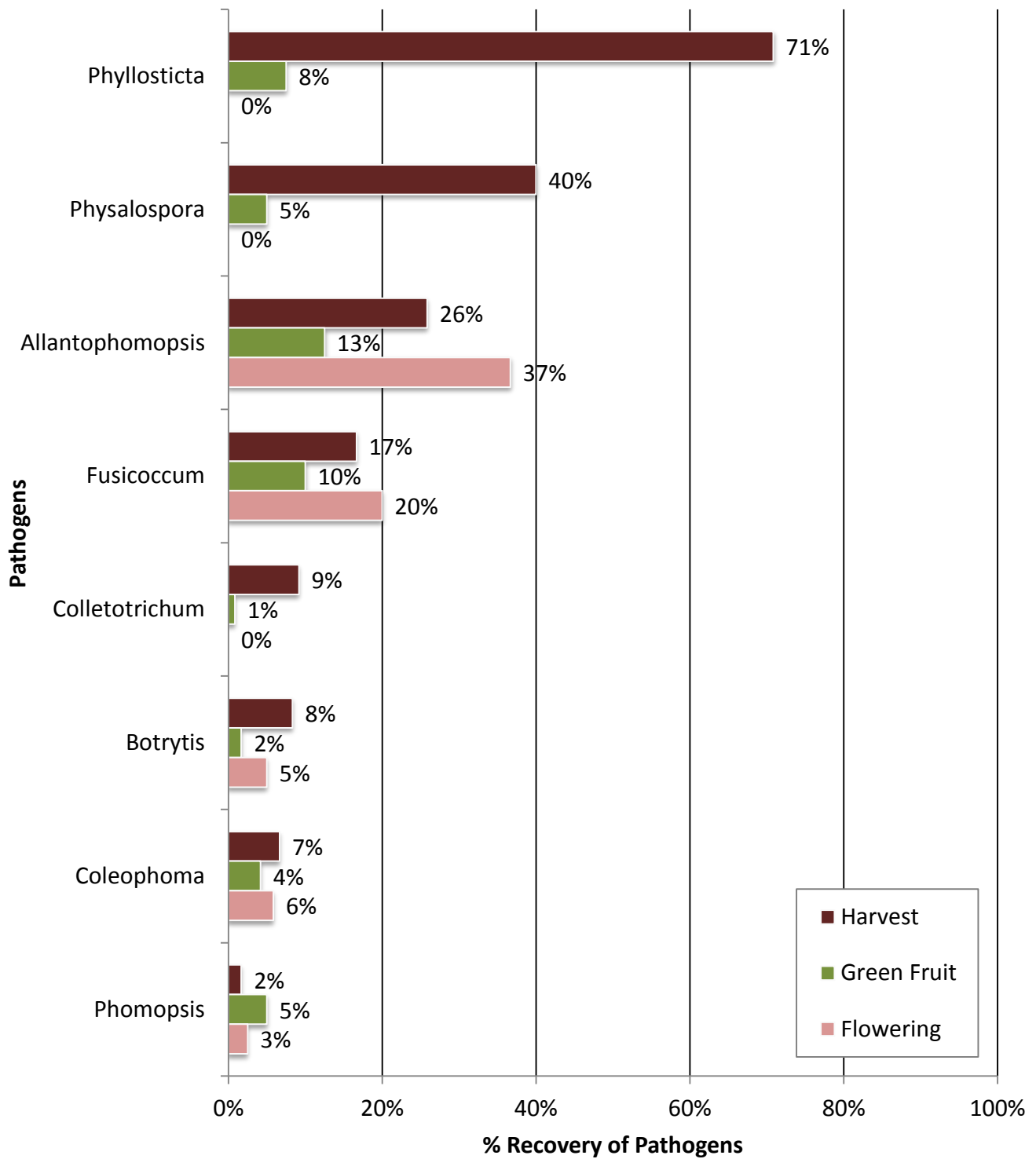


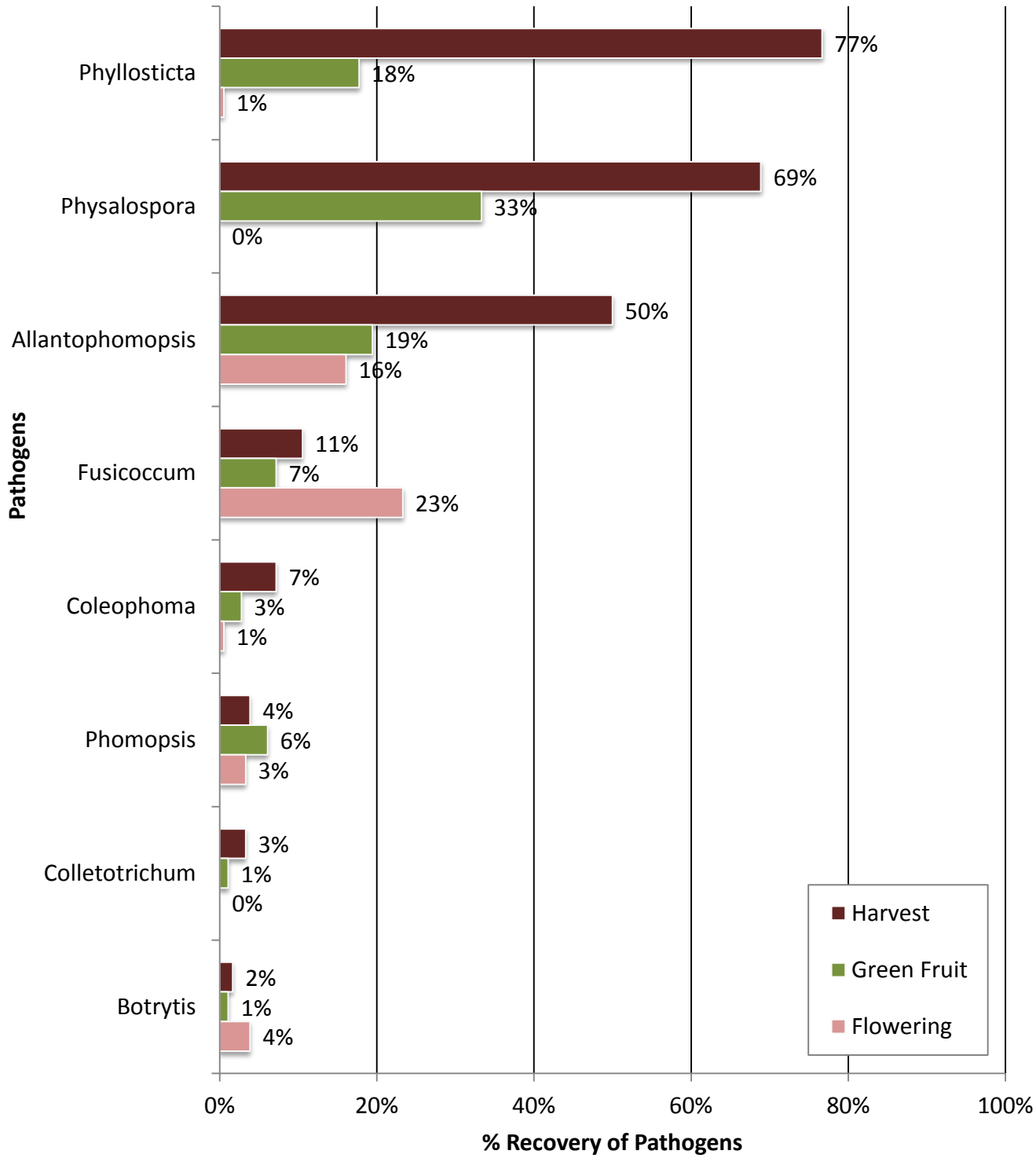
Figure 10a-e. Mean percentage of fungal pathogens, known to cause fruit rot diseases of cranberry, recovered from flowers and immature- and ripe-fruits during 2015 growing season at each location – Surrey (10a), Langley (10b), Pitt Meadows (10c), Delta (10d), and Richmond (10e).



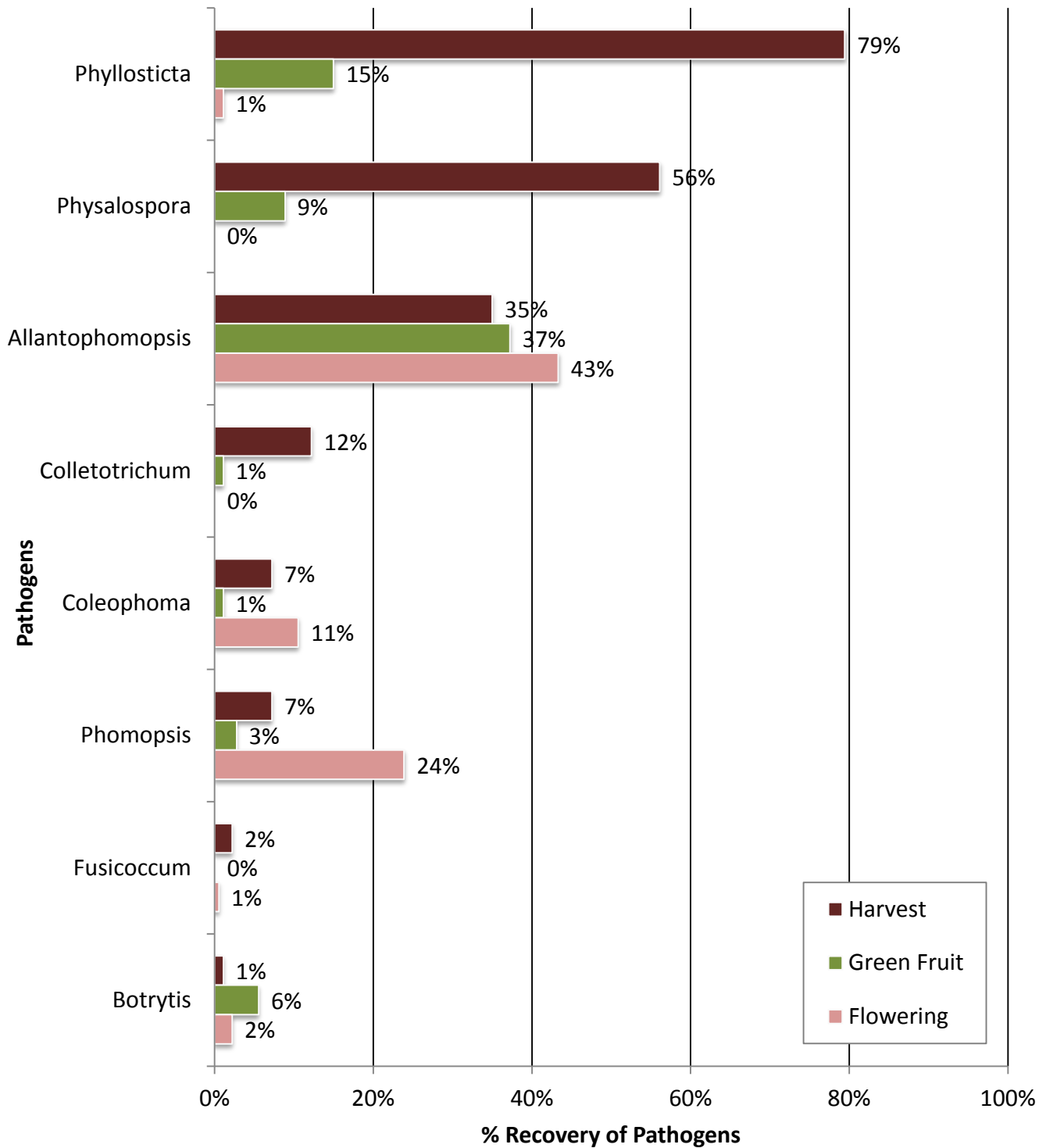
10b: Prevalence of **Known** Fruit Rot Pathogens in Langley - 2015



10c: Prevalence of **Known** Fruit Rot Pathogens in Pitt Meadows - 2015



10d: Prevalence of **Known** Fruit Rot Pathogens in Delta - 2015



10e: Prevalence of Known Fruit Rot Pathogens in Richmond - 2015

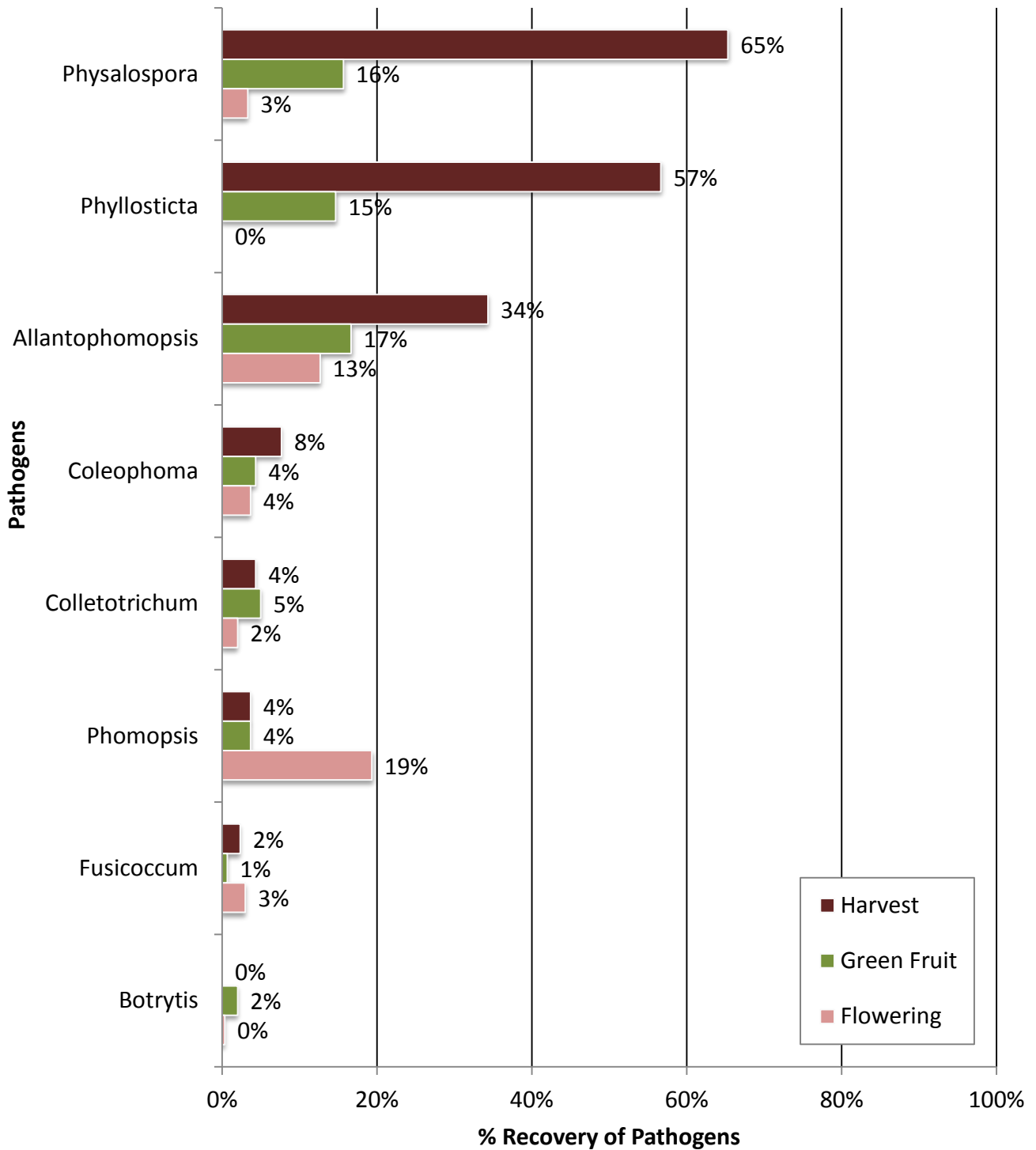


Figure 11. Percentage of fungal pathogens that are **known** to contribute to fruit rot diseases of cranberry, recovered from ripe-fruits during 2015 growing season at the Cranberry Research Farm.

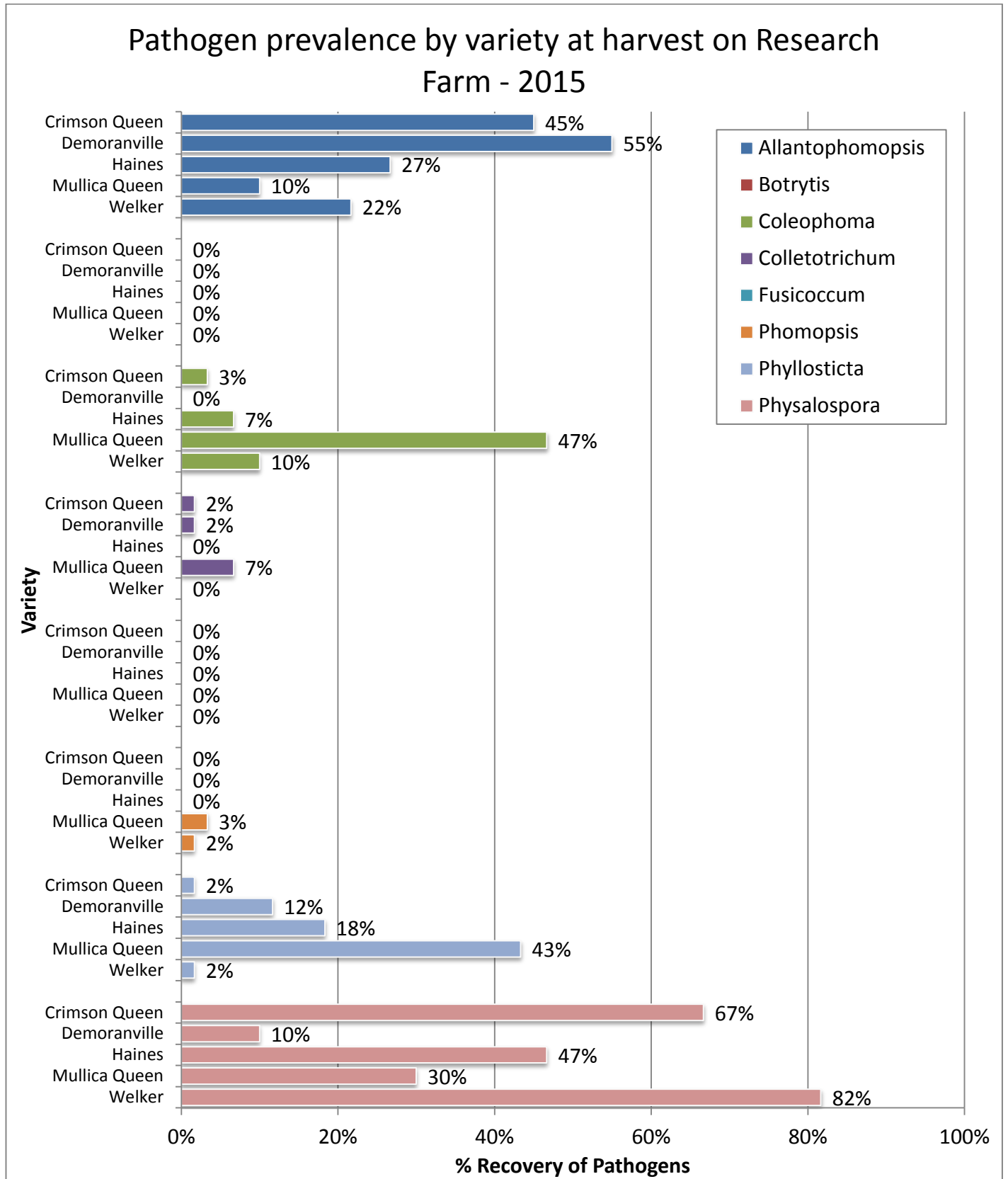
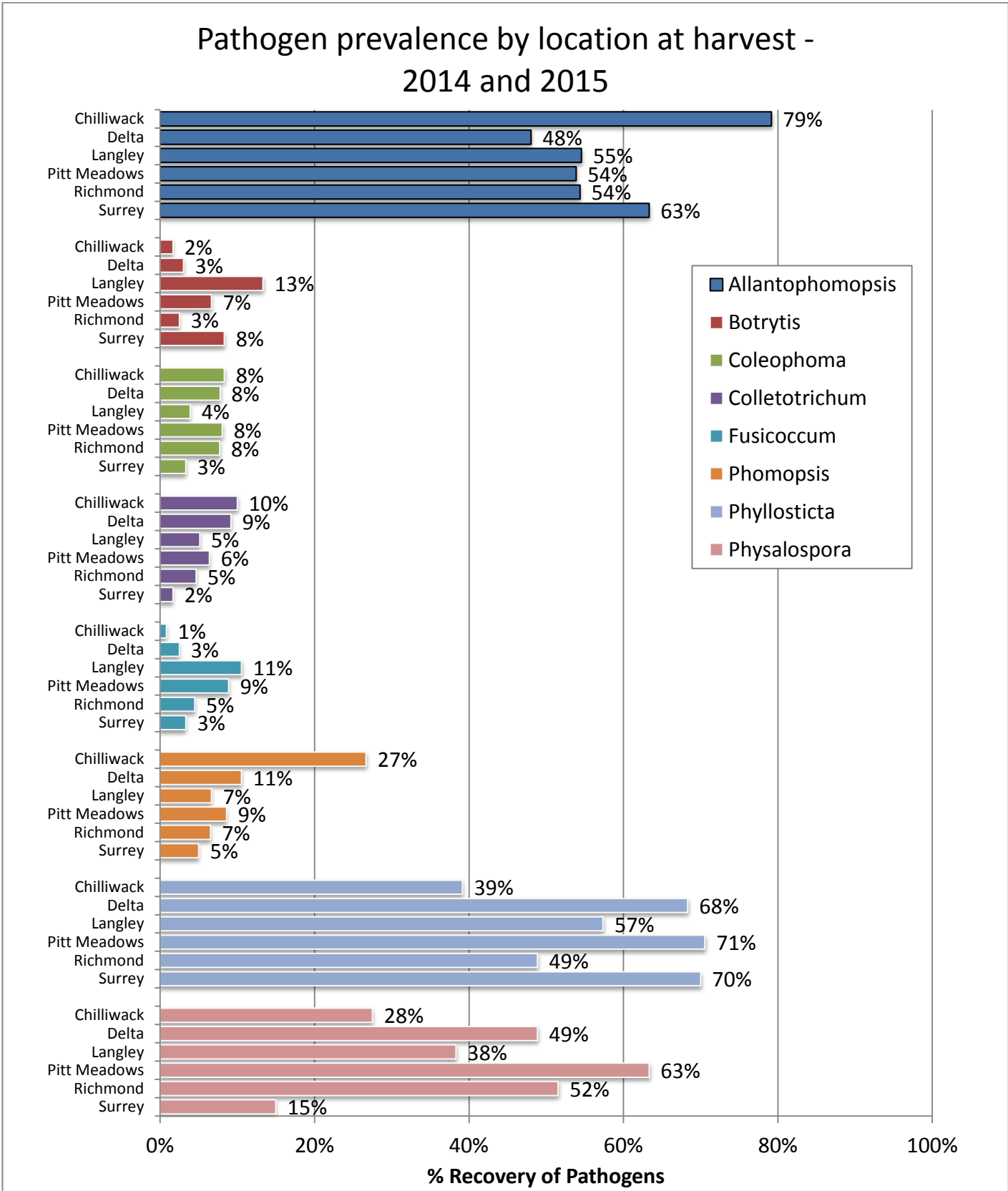


Figure 12. Percentage of fungal pathogens that are **known** to contribute to fruit rot diseases of cranberry, recovered from ripe-fruits during the 2014 and 2015 growing seasons in the Fraser Valley.



CRANBERRY FRUIT ROT - REVIEW

Fruit Rot Disease	Causal Organism	Field/Storage Rot	Time of infection	Notes
Black rot	<i>Allantophomopsis cytispora</i>	mostly storage rot	Not certain, most probably during flooding/harvest	disease incidence directly related to elapsed time berries left in flood water.
Bitter rot	<i>Colletotrichum</i> spp. & <i>Glomerella</i> 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	<i>Physalospora vaccinii</i>	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	<i>Coleophoma empetri</i>	field and storage rot	from flowering till mid fruit development	symptoms only appear around harvest time/in storage.
Viscid rot	<i>Phomopsis vaccinii</i>	storage rot	from flowering till harvest	spore dispersal by rain splash
Yellow rot	<i>Botrytis</i> sp. (of <i>B. cinerea</i> type)	field and storage rot	Mostly during following	infection by air-borne spores; reduce mechanical injuries to fruit during & after harvest.