INVESTIGATION OF THE CASUAL AGENTS ASSOCIATED WITH CRANBERRY DIEBACK DISORDER IN BRITISH COLUMBIA

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EXECUTIVE SUMMARY

PHASE I (Year 2007): Cranberry fields in the lower mainland of British Columbia (B.C.) have been affected by a severe vine decline and death, referred to as cranberry dieback disorder (CDD), resulting in substantial yield losses. A comprehensive field and grower survey in spring/summer 2007 excluded insect pests, particularly cranberry girdler, weevil or Dearness scale damage, herbicide injury or poor field conditions as causes of CDD. To identify the possible casual agent(s), systematic field observation, sampling and laboratory analyses of roots, runners, uprights and soils from 32 affected cranberry beds belonging to 24 farms were carried out in spring and summer 2007. *Phytophthora cinnamomi* and an unidentified *Phytophthora* sp. were recovered from the soils of two separate fields, confirming for the first time their presence in cranberry soils in B.C. Although several fruit rot and foliar pathogens, including *Allantophomopsis* sp., *Coleophoma* sp., *Colletotrichum acutatum*, *Pestalotia* sp., *Phomopsis vaccinii* and *Phyllosticta vaccinii*, were occasionally encountered during microscopic examination and culturing of tissue samples, they were ruled out as the casual agents of CDD. Among the several other potential fungal pathogens isolated from the tissue samples, *Coniothyrium sporulosum*, *Cylindrocarpon destructans* and an unidentified *Phomopsis* sp., were consistently recovered at high frequencies from nearly 80% of the symptomatic fields, suggesting that these pathogens may be responsible for CDD, either acting solely or synergistically. Although the pathogenicity of *C. sporulosum*, *C. destructans* and *Phomopsis* sp. on cranberry is yet to be determined, they have previously been suspected or reported to cause diseases on other plants. This investigation (Phase I) strongly suggested that CDD may be caused by a complex of pathogens, perhaps, including plant-parasitic nematodes, whose pathogenicity, epidemiology and association with CDD need to be investigated (Fitzpatrick et al, 2007, Sabaratnam et al, 2008).

PHASE II (Year 2008 / 2009): In 2008, a greenhouse study was initiated to assess the pathogenicity (Koch's Postulates) and virulence of the potential pathogens and to confirm the casual agent(s) of CDD. Cranberry plants (var. Stevens), propagated from disease-free stem (upright/runner) cuttings and grown on peat-sand medium in 1 gallon pots, have been challenged with the pathogens, *Coniothyrium sporulosum*, *Cryptosporiopsis actinidiae*, *Cylindrocarpon destructans*, *Gymnopus* sp., *Phomopsis* sp., *Rhizoctonia* sp. and *Phytophthora cinnamomi*, individually or in combinations. A total of thirteen treatments have been included in this study. The inoculated plants have been maintained at the Pacific Agri-Food Research Centre, AAFC, in Agassiz until the plants express the symptoms of CDD. Since the inoculation of cranberry plants with the potential pathogens was completed by the end of August 2008, it may take 3-6 months or longer for the plants to express the expected symptoms of CDD. At the time of symptoms expression (spring/summer 2009), plants will be assessed for symptoms, disease incidence and disease severity and the inoculated pathogens will be reisolated from the symptomatic plant tissues to confirm the casual agent(s) of CDD.

In addition to the pathogenicity study, *in vitro* growth-rate of the potential pathogens, grown on potato-dextrose or V8-juice medium, at various temperatures (4^{o}C to 32^{o}C) and their ability to produce infectious propagules such as spores, sporangia etc. are being investigated under laboratory conditions. The assessment of growth-rates of the potential pathogens and their ability to produce infectious propagules at various temperature regimes will aid in developing effective control/management strategies for CDD.

INTRODUCTION

Since 2005, growers have been reporting severe vine decline and death of cranberry beds in the lower mainland of B.C. that could not be attributed to insect feeding, including cranberry girdler, weevil or Dearness scale, herbicide injury, poor drainage or adverse environmental conditions. As depicted in Figure 1, symptoms start to appear in spring when uprights lose their lower leaves and the remaining leaves turn a copper to burgundy-brown colour. Subsequently, the upright stems become grey and appear dead. Later in summer, the affected areas have a patchy appearance of small to large grey-black areas with dying vines. Symptomatic uprights at the outer edges of decline patches can be traced back to runners that often have blackened areas of peripheral tissue, mostly around branching and rooting points, and, sometimes, browning of inner pith tissue that are distinguishable from healthy runners. Affected runners may be well rooted or poorly rooted. When walking through fields, cranberry beds with dying vines feel brittle. The dieback areas seem to spread through the fields over time but individual vines may recover by producing new roots, when a layer of sand is

applied to the affected areas. This unique symptom of vine decline and death has been given the common name of cranberry dieback disorder (CDD) since the cause of the problem could be biotic and/or abiotic in nature and is yet to be determined.

To identify the possible cause or causes of CDD, a systematic field survey followed by analysis of the plant (root, runner and upright) and soil samples from declining fields in Burnaby, Delta, Pitt Meadows and Richmond was conducted in year 2007 (Fitzpatrick et al., 2007, Sabaratnam et al., 2008). Since no study has been done on the pathology of cranberry in B.C. for the past several years, this comprehensive field survey and analysis of plant and soil samples provided an opportunity for understanding the prevalence and distribution of some potential plant pathogens in B.C. cranberry beds (Table 1). As expected, several pathogens that have already been known to cause diseases on cranberry (Caruso and Ramsdell, 1995, Oudemans, et al., 1998), were isolated from the cranberry beds. They were *Allantophomopsis* sp. (causal agent of black rot), *Botrytis* sp. (casual agent of yellow rot), *Colletotrichum acutatum* (causal agent of anthracnose), *Exobasidium* spp. (causal agents of red leaf spot and rose bloom), *Pestalotia* sp. (causal agent of leaf spot and fruit rot), *Phomopsis vaccinii* (causal agent of upright dieback and viscid fruit rot) and *Phyllosticta vaccinii* (causal agent of early rot). Although the field survey and sampling were not targeted for these pathogens, this study confirms the presence of some of the common cranberry pathogens in B.C., and the likelihood of them causing diseases on cranberry under favourable conditions.

In addition to the known cranberry pathogens, several other fungi, including *Alternaria* sp., *Epicoccum* sp., *Coniothyrium sporulosum*, *Cryptosporiopsis actinidiae*, *Cylindrocarpon destructans, Gymnopus* sp*., Fusarium* spp., *Phomopsis* sp., *Rhizoctonia* sp*.* and *Sirococcus conigenus* were also isolated from the samples. Although these organisms have not been previously reported as pathogens on cranberry, pathogens such as *Alternaria*, *Fusarium* and *C. destructans* can be expected to cause diseases on cranberry as they have been known to cause diseases or reported as secondary pathogens on a wide range of host plants, including *Vaccinium* species. The *Rhizoctonia* isolates detected in 6 cranberry beds were predominantly binucleated. Generally, binucleated *Rhizoctonia* spp. are considered as non-pathogens, however some binucleated *Rhizoctonia* spp. can cause root rot on some host plants such as strawberry, raspberry, soy bean and pak choy. Among the pathogens recovered, *C. actinidiae*, *S. conigenus* and an unidentified *Phomopsis* sp. were isolated from the discoloured brown pith tissues; where *Phomopsis* sp. was most frequently isolated. Several species of *Phomopsis* have been reported on a variety of host plants, including *Vaccinium* species, some of them are considered as endophytes in plants (Farr et al., 2002a and 2002b, Schilder et al., 2005). Therefore, the *Phomopsis* sp. isolated from the brown-pith tissue needs to be tested for pathogenicity on

cranberry. Similarly, genus *Cryptosporiopsis* is also known to occur as endophytes in many plants, including oak, cypress, redwood, *Fagus,* fir, pine, and spruce (*Ahlich and Sieber, 1996, Kowalski et al., 1998*), and a few species of *Cryptosporiopsis* have also been reported as pathogens on kiwi (Beever and Parkes, 2007), stone fruit (Braun, 1997, Grove, et al., 1992), eucalyptus (Cortinas et al., 2004, Roux, et al., 2002), and ericaceous plants (Sigler et al., 2005).

Oomycetes are an important group of phytopathogens because several *Phytophthora* spp. have been known to cause severe root and runner rot on cranberries elsewhere in North America. Although no *Phytophthora* species was isolated directly from the cranberry root or runner samples, to our surprise, *Phytophthora cinnamomi* and a *Phytophthora* sp. (tentatively identified as *P*. *dreschleri, P*. *cryptogea* or *P*. *megasperma* by molecular markers) were isolated via lupine baiting of two soil samples (Table 1). This finding was the first report on the occurrence of *P. cinnamomi* and a *Phytophthora* sp. in cranberry beds in B.C. *P. cinnamomi* is known to cause severe root and runner rot on cranberry in Massachusetts, New Jersey and Oregon (Caruso and Wilcox, 1990). In B.C., *P*. *cinnamomi* is know to cause root rot on highbush blueberry (*Vaccinium corymbosum*) grown in the lower mainland of the Fraser Valley (Levesque et al., 1998). Among other *Phytophthora* species, *P. cryptogea* was reported on cranberry in Wisconsin and *P. megasperma* was reported on cranberries in Massachusetts, New Jersey and Washington (Polashock et al., 2005). *P. dreschleri* seems to have a wide host range including Rhododendron and numerous non-Ericaceous plants such as Douglas fir in B.C. (Levesque et al., 1998) and numerous horticultural crops such as olive, apple, cherry and rose. Several *Pythium* species, including *P. diclinum*, *P. dimorphum*, *P. helicoides*, *P. itermedium*, *P. sterilum*, *P. sylvaticum*, and *P. undulatum*, were also recovered from the cranberry beds but their distribution was limited to a few fields in Pitt Meadows and Richmond; all of them are pathogenic on various host plants (Belbahri et al., 20006, Borja et al., 1995, Ho, 1986, Hendrix and Campbell, 1974, Metzger, et al., 2007). Considering the restricted distribution and number of *Phytophthora* and *Pythium* species detected on cranberry beds surveyed, these species may not be the primary causal agent(s) of CDD. However, further field survey and soil analysis focusing chiefly on oomycetes, particularly *Phytophthora* spp., would help to understand their distribution and impact on cranberry.

Among the fungi isolated, *C*. *sporulosum*, *C*. *destructans* and *Phomopsis* sp. were the most frequently isolated in high numbers, 67, 71 and 28 isolates, respectively, from the cranberry beds with CDD symptoms. *C*. *sporulosum* and *C*. *destructans* were detected in 54 and 71% of the symptomatic fields, respectively, whereas *Phomopsis* sp. was detected in 79% of the symptomatic fields. In addition, a strong association was observed among the three pathogens where, in any given field with CDD symptoms, at least two of these pathogens were detected simultaneously. These preliminary observations strongly suggested that *C*.

sporulosum, *C*. *destructans* and *Phomopsis* sp. could be the cause for CDD, acting solely or synergistically. Although the pathogenicity on cranberry is yet to be determined, these three genera (*Coniothyrium*, *Cylindrocarpon* and *Phomopsis*) have been known to cause diseases on other plants. The *Phomopsis* sp. obtained from the cranberry samples matches the description of a *Phomopsis* sp. reported from *Vaccinium corymbosum* (blueberry) as described previously by Farr et al. (2002). It is morphologically distinct from *Phomopsis vaccinii*, the casual agent of upright dieback. Several species of *Coniothyrium* (*C*. *asterinum*, *C*. *arctostaphyli*, *C*. *fuckelii*, *C*. *phyllogenum*, *C*. *rhododendri* and *C*. *vaccinicola*) have also been reported on many host plants, including *Vaccinium* (Humphreys-Jones, 1980, Waterman, 1930). *C. sporulosum*, isolated from cranberry, was also proven to be a pathogen on hawthorn as reported recently from Italy (Montecchio and Vettorazzo, 2002). In general, *Cylindrocarpon* spp., particularly *C*. *destructans*, is known to cause considerable damages such as cankers and crown and root rot, to orchard, nursery and field crops. *Cylindrocarpon macrodidymum* and *Cylindrocarpon obtusisporum* can independently cause black foot disease of grape (Petit and Gubler, 2005).

It is possible that the observed field symptoms of CDD could be the result of a combination of factors, instigated by abiotic plant-stress factors and infection by pathogens, including, perhaps, plant-parasitic nematodes. The detection of three potential phytopathogens, *C*. *sporulosum*, *C*. *destructans* and *Phomopsis* sp., consistently from CDD symptomatic beds demands for further investigation on their pathogenicity (Koch's Postulates), epidemiology and expression of typical symptoms of CDD under field conditions. Therefore, the **objective** of this study was to determine the pathogenicity and virulence of the potential pathogens, exclusively *C*. *sporulosum*, *C*. *destructans* and *Phomopsis* sp., by conducting Koch's Postulates and, thereby, confirm the pathogens that are responsible for CDD. In addition, the ability of these potential pathogens to grow at various temperatures (from 4^{o}C to 32^{o}C) and to produce propagules such as spores, sporangia, etc. *in vitro* will also be determined.

WORK IN-PROGRESS

A. Koch's Postulates (Determination of pathogenicity and virulence of potential pathogens)

Propagation of Cranberry Plants:

Disease-free cranberry (var. Stevens) cuttings (upright and runner) obtained from a healthy cranberry field in Burnaby, B.C. were surfaced sterilized with 0.1% sodium hypochlorite and washed repeated with sdH_2O to remove surface contaminants and propagated on a pasteurized peat-based growing medium in plastic flats (Figure 3). After 4-5 weeks, the established plantlets were transplanted into 1-gallon plastic pots (3 plants per pot) containing pasteurized peat-moss and

fine-sand at 1:1 ratio (pH 5-5.5) as growing medium. Plants were fertilized [(Osmocote Pro 14 (N) -11 (P) -12 (K)] at the rate of 14g/pot and watered every other day or as required and allowed to establish for 10-12 more weeks. Plants were maintained in a greenhouse at the Pacific Agri-Food Research Centre in Agassiz, B.C.

Challenges faced: 1) *Establishment of cranberry plants in greenhouse:* It was a challenging task to propagate and establish healthy, vigorous cranberry plants under greenhouse conditions. The time taken to establish disease-free plants in the greenhouse was longer than anticipated. **2**) *Fungus gnats*: fungal gnat populations were high in the greenhouse in the spring/summer-2008. They were controlled/managed by applying nematode (*Steinernema carpocapsae*)-based biological control agent to control the larvae and placing double-coated sticky yellow cards (Catch-it®) to capture the adults. **3**) *Aphids:* aphid populations were encountered frequently and controlled or kept to minimum by applying insecticides. **4**) *Unidentified Fusarium* sp.: an unidentified *Fusarium* sp. caused severe crown rot, resulted in sudden death of a few cranberry plants. It was suspected that the pathogen was spread by fungus gnats. Infected pots were immediately removed from the greenhouse and the plants in the greenhouse were treated repeatedly with copper-based fungicide at least 8-10 weeks prior to the initiation of the actual experiment.

Pathogens and Inoculum Preparation:

Cultures (Figure 2) of *Phytophthora cinnamomi*, *Coniothyrium sporulosum*, *Cylindrocarpon destructans*, *Phomopsis* sp*., Cryptosporiopsis actinidiae, Gymnopus* sp. and *Rhizoctonia* sp., previously isolated from the symptomatic cranberry plants (Fitzpatrick et al., 2007, Sabaratnam et al, 2008), were maintained on potato-dextrose-agar (PDA) medium in Petri plates at 20^{o}C for at least 7-14 days or until sufficient growth was obtained.

Inoculum of the soilborne pathogens, *P*. *cinnamomi*, *C*. *destructans*, *Gymnopus* sp. and *Rhizoctonia* sp., was individually spawned on sterile barley grain supplemented with clarified V8 juice. Portions of 300 g barley grain were washed thoroughly in tap water to remove debris and drained to remove the excess water. Each portion of barley was supplemented with 100 mL of 20% clarified V8 juice, autoclaved for 15 min under wet cycle and allowed to cool down to room temperature. V8 supplemented barley medium (V8-barley) was, then, seeded with 20-30 mycelial plugs taken from the actively growing margins of the pathogen grown on PDA, and incubated in the dark at 20^{o}C until the V8-barley was fully colonized by the pathogen. Inoculum of the foliar pathogens, *C. actinidiae*, *C. sporulosum*, and *Phomopsis* sp., was prepared as spore-suspension in sdH2O from the sporulating cultures of the pathogens grown either on PDA or V8 medium. The final inoculum concentration of each pathogen was adjusted to 1 x 10^{6} spores/mL sdH2O before inoculation.

Koch's Postulates (Pathogenicity and Virulence of Suspected Potential Pathogens):

An experiment was initiated in a greenhouse facility at the Pacific Agri-Food Research Centre, AAFC, in Agassiz to identify the causal agent(s) of CDD via Koch's Postulates. The experiment included 13 treatments; each treatment had eight replicates with a sample size of three plants per replicate, arranged in a complete randomized design. The treatments included were cranberry plants challenged with 1) *P. cinnamomi*, 2) *C. destructans*, 3) *Gymnopus* sp., 4) *Rhizoctonia* sp., 5) *C. actinidiae*, 6) *C. sporulosum*, 7) *Phomopsis* sp., 8) *C. destructans* + *C. sporulosum* + *Phomopsis* sp., 9) *C. destructans* + *C. sporulosum*, 10) *C. destructans* + *Phomopsis* sp., 11) *C. sporulosum* + *Phomopsis* sp., 12) *C. destructans* + *C. actinidiae* and uninoculated plants as 13) healthy control. Due to constraints in generating inoculum of all 7 pathogens at the same time (growth-rate and duration of sporulation of each pathogen vary from each other), inoculation of cranberry plants with each pathogen was carried out as soon as the inoculum of each pathogen was generated. Pathogens, *P. cinnamomi*, *C. destructans* and *Rhizoctonia* sp., were applied to the potting medium as a root treatment. Equal volumes of the inoculum, i.e. 60 mL inoculum (V8-barley pre-colonized with the pathogen), per pot, was evenly dispensed into 3 holes (approximately 2 cm diameter and 10 cm depth) made around the periphery of the plants, and covered with a layer of peat-sand medium. Inoculation of *Gymnopus* sp. was done by placing the same amount of V8-barley inoculum, as above, on the surface of the potting medium and covered with a layer of sterile (i.e. autoclaved) cranberry cuttings (runner/upright) to enhance its establishment in the pots (Figure 4) Foliar pathogens, *C. actinidiae*, *C. sporulosum*, and *Phomopsis* sp. were inoculated as foliar spray, at a rate of 1×10^6 spores per mL, until runoff. Plants that received the combinations of foliar pathogens as treatments, a 24-h interval was kept after the application of each pathogen. Soon after the inoculation of a foliar pathogen, plants were kept in a humid chamber (Figure 5) for at least 8 h to maintain high humidity to help fungal spore germination and the infection process. The inoculation process for all 13 treatments was completed on August 28, 2008 (Figure 6).

WORKPLAN

WINTER 2008 & JANUARY-DECEMBER 2009

A. Koch's Postulates

Inoculated plants will be maintained in the greenhouse at the Pacific Agri-Food Research Centre, AAFC, in Agassiz for the next 3-6 months or even longer until visible symptoms of CDD are expressed; expression of disease symptoms is largely dependent on the biology and epidemiology of the pathogen, nature of plant-pathogen interaction and environmental conditions. Plants will be monitored periodically and data will be collected on symptoms, disease incidence and

disease severity. At the end of the experiment, pathogens from the symptomatic plant tissues will be reisolated and compared with the fungal pathogens previously isolated from the fields. This information will ensure the pathogenicity of each pathogen and conclusions will be drawn to confirm the causal agent(s) of CDD.

In addition to the greenhouse study, an *in vitro* pathogenicity experiment will be conducted in 2009 under controlled environmental conditions. Disease-free runner and upright segments of cranberry var. Stevens will be individually challenged with 1) *C. destructans, 2*) *C. sporulosum, 3*) *Phomopsis* sp., 4) *C. actinidiae, 5) Gymnopus* sp., 6) *S*. *conigenus* and 7) *Rhizoctonia* sp. and compared with an uninoculated healthy control. Tissue samples from each treatment will be taken periodically and analysed microscopically for tissue infection, cell death and pith browning death and compared with the symptoms, browning of pith and blackening of peripheral tissues of runner, of CDD.

B. Determination of *In Vitro* Growth Rate of Potential pathogens

Pathogens differ from each other in their ability to grow and produce infectious propagules such as spores, sporangia, etc. under different environmental conditions. Soil/air temperature and moisture regimes have a large influence on the spread and survival of the pathogen in nature. Although, the *in vitro* growth-rate of a pathogen under laboratory conditions may not mimic the actual growth/survival of the pathogen in its natural environment, this information will help us understand the activity and possible infection period of the pathogen and contribute towards developing better management practices to control/manage pathogens in the field.

The pathogens *Phytophthora cinnamomi*, *Coniothyrium sporulosum*, *Cylindrocarpon destructans*, *Phomopsis* sp*., Cryptosporiopsis actinidiae, Gymnopus* sp. and *Rhizoctonia* sp., are included in this study. The growth rate of each pathogen will be assessed at 4, 8, 12, 16, 20, 24, 28 and 32^{o}C in a growth chamber. Pathogens will be maintained on PDA as described previously and a mycelial plug (6 mm diameter) from the actively growing margin of the culture will be placed on the centre of freshly prepared PDA in 9 cm Petri plate. For each temperature-growth assessment, two similarly prepared plates per pathogen will be used. Plates will be incubated at the desired temperature for at least 10 days or until the mycelial growth reaches the edge of the plate. Growth measurements will be taken as colony diameter (at least 2 readings per colony) at every 24-h interval and the mean diameter will be calculated. The growth rate of each pathogen will be expressed as growth of hyphae (mm) per hour (i.e. mean diameter/24 h). In addition to growth-rate estimation, each pathogen will also be examined for its ability to produce infectious/over wintering propagules such as asexual/sexual spores, sporangia etc., at above mentioned temperatures. This information will help to understand the most favourable environmental conditions that are

conducive for the survival and spread of the pathogen and lead to effective management strategies to minimize the impact of the pathogens.

These experiments are in-progress and the results will be summarized and presented in spring/summer 2009.

ANTICIPATED FUNDS for 2009

1). Completion of Koch's Postulates (greenhouse study) to confirm the casual agent(s) of Cranberry Dieback Disorder.

2). Completion of temperature dependent growth-rate and spore production study to assess the infection period, spread and survival of the potential pathogens responsible for Cranberry Dieback Disorder.

FUTURE STUDIES

Phase III (Year 2009/2010): Based on the observations from the greenhouse and laboratory studies, a field trial will be proposed in 2009/2010 to further confirm the casual agent(s) of CDD and assess the degree of damage (dieback severity) and expression of typical CDD symptoms by the potential pathogen(s) under field conditions.

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REFERENCES

Ahlich, K. and Sieber, T.N. 1996. The profusion of dark septate endophytic fungi in non-ectomycorrhizal fine roots of forest trees and shrubs. New Phytologist 132:259-270.

Beever, R.E. and Parkes, S.L. 2007. Vegetative compatibility groups in the fungus *Cryptosporiopsis actinidiae*. New Zealand Journal of Crop and Horticultural Science 35:67-72.

Belbahri, L., Calmin, G., Sanchez-Hernandez, E., Oszako, T. and Lefort, F. 2006. *Pythium sterilum* sp. nov. isolated from Poland, Spain, and France: its morphology and molecular phylogenetic position. FEMS Microbial Letter 225:209-214.

Borja, I., Sharma, P., Krekling, T. and Lonneborg, A. 1995. Cytopathological response in roots of *Picea abies* seedlings infected with *Pythium dimorphum*. Phytopathology 85:495-501.

Braun, P.G. 1997. Distribution and severity of anthracnose canker and European canker of apple in Kings County, Nova Scotia. Canadian Journal of Plant Pathology 19:78-82.

British Columbia Cranberry Growers Association.2007. http://www.bccranberrygrowers.com

Caruso, F.L. and Ramsdell, D.C. 1995. Compendium of blueberry and cranberry diseases. APS Press.

Caruso, F.L. and Wilcox, W.F. 1990. *Phytophthora cinnamomi* as a cause of root rot and dieback in cranberry in Massachusetts. Plant Disease 74:664-667.

Conyers, M.K. and Davey, B.G. 1998. Observations on some routine methods for soil pH determination. Soil Science 145: 29-36.

Cortinas, M.N., Koch, N., Thain, J., Wingfield, B.D. and Wingfield M.J. 2004. First record of the Eucalyptus stem canker pathogen, *Coniothyrium zuluense* from Hawaii. Australian Plant Pathology 33:309-312.

Farr, D.F., Castlebury, L.A. and Rossman, A. 2002. Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the eastern United States. Mycologia 94:494-504.

Farr, D.F., Castlebury, L.A., Rossman, A. and Putnam, M.L. 2002b. A new species of *Phomopsis* causing twig dieback of *Vaccinium vitis-idaea* (lingonberry). Mycological Research 106:745-752.

Fitzpatrick, S., Sabaratnam, S., Forge, T., Cook, M., Dicarlo, A., Koch, C. and Fonda, A. (2007). Investigation of cranberry dieback disorder in British Columbia. Research Report submitted to British Columbia Cranberry marketing Commission, November 2, 2007.

Gardes, M. and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118.

Grove, G.G., Dugan, F.M. and Boal, R.J. 1992. Perennial canker of apple: seasonal host susceptibility, spore production, and Perennation of *Cryptosporiopsis perennans* in infected fruit in eastern Washington. Plant Disease 76:1109-1114.

Hendrix, F.F. and Campbell, W.A. 1974. Taxonomy of *Pythium sylvaticum* and related fungi. Mycologia 66:1049-1053.

Ho, H.H. 1986. *Pythium dimorphum* from rhododendron. Mycopathologia 93:141-145.

Humphreys-Jones, D.R. 1980. Leaf and shoot death (*Coniothyrium fuckelii*) on *Thuya orientalis* cv. Aurea Nana. Plant Pathology 29:199-200.

Kowalski, T., Halmschlager, E. and Schrader, K. 1998. *Cryptosporiopsis melanigena* sp. nov., a root-inhabiting fungus of *Quercus robur* and *Q. petraea*. Mycological Research 102:347-354.

Levesque, C.A., Harlton, C.E. and de Cock, A.W.A. M. 1998. Identification of some oomycetes by reverse dot blot hybridization. Phytopathology 88:213-222.

McManus, P., Vanathakumar, A. and Best, V. 2004. Cranberry stem gall. University of Wisconsin Extension Factsheet A3795.

Metzger, R., Belbahri, L., Calmin, G., Badin, N. and Lefort, F. 2007. First report of *Pythium intermedium* causing root rot of *Rosa canina* rootstock in France. Plant Disease 91:1055.

Montecchio, L., Causin, R. and Vettorazzo, M. 2002. A twig canker on English hawthorn caused by *Coniothyrium sporulosum* in Italy. Plant Disease 86:1403.

Oudemans, P.V., Caruso, F.L. and Stretch, A. W. 1998. Cranberry fruit rot in the Northeast: a complex disease. Plant Disease 82:1176-1184.

Petit, E. and Gubler, W.D. 2005. Characterization of *Cylindrocarpon* species, the cause of black foot disease of grapevine in California. Plant Disease 89:1051-1059.

Polashock, J.J., Vaiciunas, J. and Oudemans, P.V. 2005. Identification of a new *Phytophthora* species causing root and runner rot of cranberry in New Jersey. Phytopathology 95:1237-1243.

Roux, J., Wingfield, M.J. and Cibrian, D. 2002. First report of Coniothyrium canker of Eucalyptus in Mexico. New Disease Reports.

Sabaratnam, S., Fitzpatrick, S., Forge, T. and Dicarlo, A. (2008). Cranberry dieback Disorder: a new and emerging threat to cranberry production in British Columbia. Manuscript submitted to Acta Horticulturae.

Schilder, A.M.C., Erincik, O., Castlebury, L., Rossman, A. and Ellis, M.A. 2005. Characterization of *Phomopsis* spp. infecting grapevines in the Great Lakes region of North America. Plant Disease 89:755-762.

Sigler, L., Allan, T., Lim, S.R., Berch, S. and Berbee, M. 2005. Two new *Cryptosporiopsis* species from roots of ericaceous hosts in western North America. Studies in Mycology 53:53-62.

Waterman, A. M. 1930. Diseases of rose caused by species of *Coniothyrium* in the United States. Journal of Agricultural Research 40:805-827.

Table 1. Potential plant pathogens isolated from cranberry plant and soil samples collected from 32 cranberry beds, belonging to 24 farms, in the lower mainland of the Fraser Valley region of B.C.

Organism isolated	Number of farms	Farm location [v]
Oomycetes		
Phytophthora cinnamomi [w, y]	1	R
Phytophthora sp. [y]	1	P
Pythium spp.	4	P, R
Fungi		
Allantophomopsis sp.[w]	6	R
Alternaria sp.	6	D, P, R
Botrytis sp.[w]	1	R
Colletotrichum acutatum [w]	2	R
Coniothyrium sporulosum [z]	13	D, P, R
Cryptosporiopsis actinidiae	3	R
Curvularia sp.	1	R
Cylindrocarpon destructans [z]	17	D, P, R
Epicoccum sp.	5	R
Fusarium spp.	17	B, D, P, R
Gymnopus sp.	1	R
Humicola sp.[x]	3	D, R
Papulospora spp.[x]	4	B, D
Penicillium spp.	4	B, D, P, R
Pestalotia sp.[w]	14	B, D, P, R
Phomopsis vaccinii [w]	3	R, D
Phomopsis sp.[z]	19	B, D, P, R
Phyllosticta sp.[x]	1	R
Rhizoctonia sp.	7	R
Sirococcus conigenus	1	R
Viruses		
Blueberry Scorch Virus	4	R

[v] Farm location: B = Burnaby, D = Delta, P = Pitt Meadows, R = Richmond.
[w] Pathogen that is already known to cause disease(s) on cranberry.
[x] Genus that was identified tentatively, but not confirmed.
[y] *Phytophthora* sp. was isolated by lupine baiting of soil.
[z] Pathogen that was most frequently and consistently isolated from CDD beds.

Figure 1. Typical symptoms of *Cranberry Dieback Disorder*. Early symptoms; falling of lower leaves and remaining upper leaves turning copper to burgundy-brown colour on uprights (A and B), and thinning of uprights (C). Late symptoms; advancing edges of dying vines (E) and patchy appearance of small to large grey-black areas with dying vines (E). Severely affected dieback areas are dry and brittle, and blackened, dead canes become apparent when pulled away from the ground (F). Dark, blackened areas are noticeable on runners (G and H), and transverse sections of affected runners often show brown to dark-brown discolouration of pith tissue (I).

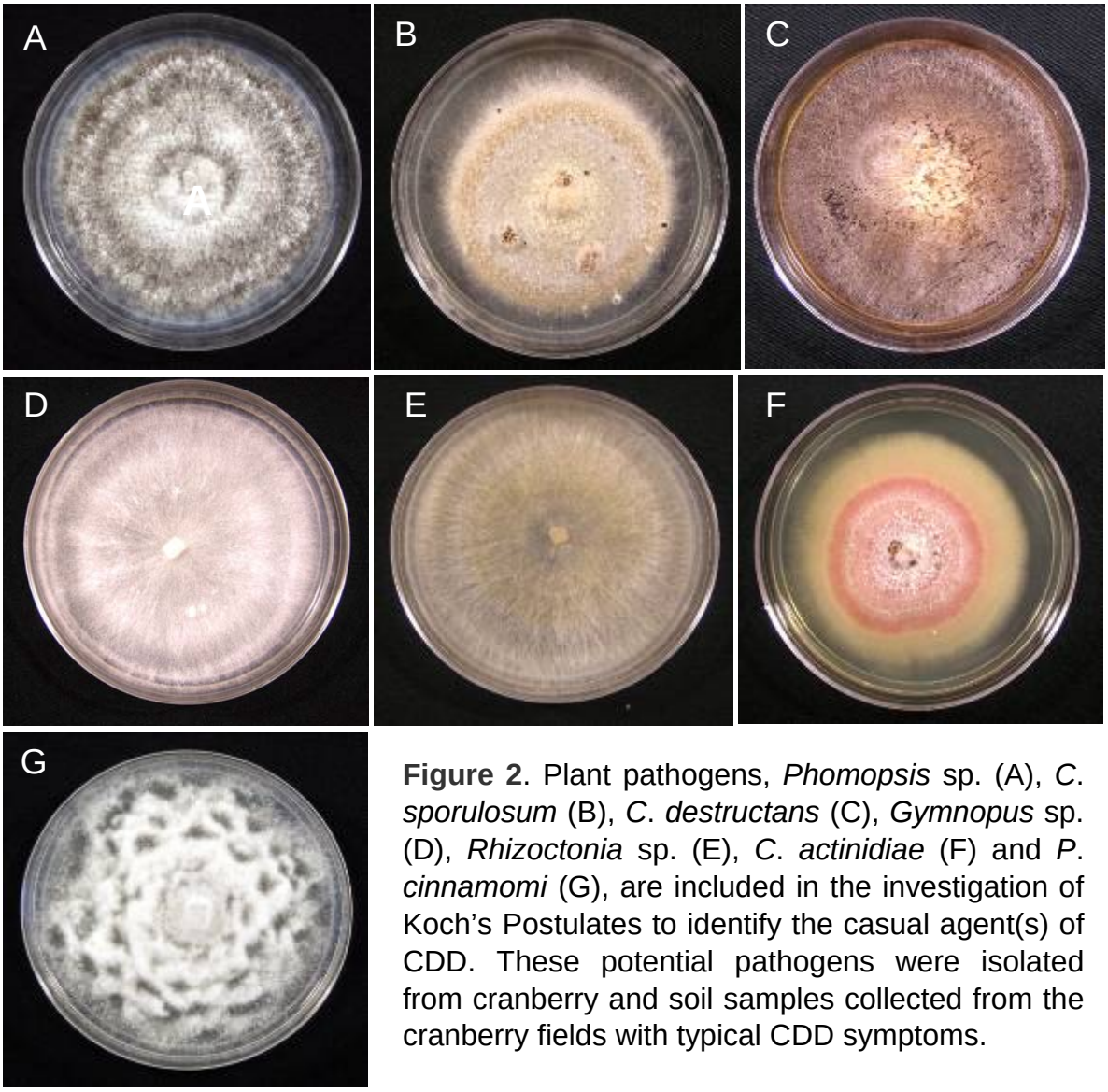

Figure 2. Plant pathogens, *Phomopsis* sp. (A), *C. sporulosum* (B), *C. destructans* (C), *Gymnopus* sp. (D), *Rhizoctonia* sp. (E), *C. actinidiae* (F) and *P. cinnamomi* (G), are included in the investigation of Koch's Postulates to identify the casual agent(s) of CDD. These potential pathogens were isolated from cranberry and soil samples collected from the cranberry fields with typical CDD symptoms.

Figure 3. Cranberry planting stock (var. Stevens) was propagated on peat-moss medium in plastic flats in the greenhouse

Figure 4. Soilborne pathogens were raised on V8-barley grain, applied to the cranberry growing medium (peat-sand, 1:1 ratio) and overlaid with sterile cranberry vine clippings to enhance the infection process.

Figure 5. Plants that were inoculated with pathogens as foliar treatment were kept in a humid chamber for at least 4-8 h to maintain high humidity to enhance germination of spores and infection process of the pathogen.

Figure 6. The experiment on Koch' Postulates (A to D)., with 13 treatments arranged in completely randomized design, has been carried out in a greenhouse facility at the Pacific Agri-Food Research Centre, AAFC, in Agassiz. (D) Healthy growth of cranberry plants at the time of inoculation with each pathogen (treatment).