INVESTIGATION OF THE CAUSAL AGENTS ASSOCIATED WITH CRANBERRY DIEBACK DISORDER (CDD) IN BRITISH COLUMBIA

Phase III (Objective 1c): Confirmation of pathogenicity of the causal agent(s) of CDD and synergistic impact of the causal agent(s) and plant-parasitic nematode on cranberry and CDD severity.

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Investigation of cranberry dieback disorder (CDD), including the factors responsible for the observed symptoms in cranberry fields, has been conducted with the Overall Objectives as outlined below (for details please refer Research Proposals submitted to the B.C. Cranberry Marketing Commission Research Committee in 2007, 2008, 2009 & 2010).

Overall Objectives as proposed in 2007:

1. Determine the causal agent(s) of CDD. Probable agents include the insect cranberry girdler (*Chrysoteuchia toparia*), plant pathogens, including those in the genus *Phytophthora*, and plant parasitic nematodes. Other causal agents may be implicated. The role of probable causal agents will be assessed through the following approaches:
   a. Determine the spatial distribution of probable causal agents in relation to areas of cranberry decline (Year 1 - 2007 Project, completed).
   b. Koch’s Postulates to assess pathogenicity and virulence of potential plant pathogens (fungi and oomycetes) associated with symptomatic plants, using greenhouse-grown cranberry plants in greenhouse (Year 2 & 3 - 2008 & 2009 Projects, completed).
   c. Determine the effects of plant pathogens responsible for cranberry decline on growth and symptoms of cranberry in growers’ field or research-plot (Year 4 - 2010 & 2011 Projects).

2. Once the causal agent(s) have been identified and their impact and epidemiology are characterized, develop effective management strategies for cranberry dieback disorder.

Previous studies conducted in 2007, 2008 and 2009 indicated that CDD is probably a complex phenomenon caused by a combination of factors, chiefly phytopathogenic fungi and environmental plant stress factors (refer Appendix “A” for the summary of previous research conducted in 2007, 2008, 2009 & 2010). Field survey and Koch’s postulates (i.e. plant-pathogen interaction studies) conducted in the greenhouse in 2008/2009 identified *Phomopsis* sp. alone or in combination with *Coniothyrium* sp., *Cryptosporiopsis* sp. and *Cylindrocarpon* sp, as the most likely candidates to cause CDD like symptoms under greenhouse conditions; among those, *Phomopsis* sp. I (isolate 07 CB 04) expressed the most severe symptoms on cranberries. Although the greenhouse studies identified the probable causal agents of CDD, in order to confirm the causal agents, the pathogenicity and the expression of typical CDD symptoms caused by the potential pathogens on cranberry must be demonstrated under field conditions. Therefore, the objective of this study was to establish a cranberry field research plot in 2010 (Objective 1c) at the Pacific Agri-Food Research Centre, AAFC, in Agassiz to:

1) confirm the causal agents of CDD by carrying out plant-pathogen interaction (inoculation) study via Koch’s Postulates,
2) demonstrate the symptoms of CDD as per 2007 finding, and
3) assess the interaction between CDD causal agent(s) and plant-parasitic nematode and their synergistic impact on cranberry.
METHODS and RESULTS

Propagation of cranberry transplants in greenhouse (2010):

In April-May 2010, disease-free cranberry (var. Stevens) cuttings of uprights obtained from a healthy cranberry field in Delta, B.C., were surface sterilized with a solution of 0.1% sodium hypochlorite (NaOCl) and washed repeatedly with sterile distilled water (sdH2O) to remove surface contaminants. The cuttings were propagated on a pasteurized peat-based growing medium (75% peat: 25% perlite) in 36-celled transplant trays. After 8-10 weeks, the plantlets were transplanted into 1-gallon pots; each pot contained at least 3-4 healthy plantlets. Approximately, 450 plants in 120 pots had been maintained in the greenhouse at the Pacific Agri-Food Research Centre (PARC), AAFC, in Agassiz and used in the following studies as required. The remainder of the plants were maintained in the greenhouse and used in the 2011 studies (Sabaratnam et al., Research Report, November 30, 2010).

Establishment of cranberry microplots for field experiments (2010):

In 2010, a research field plot consisting of 48 microplots (each 0.5 m² area) had been established at the PARC, in Agassiz (Figure 1). In spring 2010, trenches were dug, and 100 L pots with extra drainage holes were lined up in the trenches, backfilled with a sandy soil, and minirhizotron access tubes (see below for details) were installed. Each microplot was then fumigated with Dazomet (Basamid). In August 2010, after fumigant had adequately dissipated, a layer of approx. 6 cm pasteurized peat was placed over the fumigated sandy soil and then each microplot was planted with 2-3 cranberry transplants that were raised in the greenhouse. Plants have been maintained through fall/winter 2010 and 2011 growing season as per best cranberry management practices (Sabaratnam et al., Research Report, November 30, 2010).

Plant-pathogen interaction (inoculation) study via Koch’s postulates (2011/2012):

In spring 2011, a factorial fungal pathogens x plant parasitic nematode interaction experiment was initiated to 1) confirm the causal agent(s) of CDD, 2) demonstrate the typical CDD symptoms, as observed in 2007 field survey and 3) assess the impact of the fungal pathogens and plant parasitic nematode, Paratrichodorus renifer, on CDD. A total of 14 treatments, each with 3 replicates, were included in the experiment. The treatments contained the potential fungal pathogens, as identified from the greenhouse experiment in 2008/2009, Phomopsis spp. (I, II & III), Coniothyrium sporulosum, Cryptспорiopsis actinidiae, Cylindrocarpon destructans, in the presence and absence of the plant parasitic nematode, P. renifer. The 14 treatments were:

1) Uninoculated healthy control  
2) Phomopsis sp. I (07 CB 4)  
3) Phomopsis sp. II (07 CB Ph2)  
4) *Phomopsis sp. III (07 CB 9)  
5) Coniothyrium sporulosum (07 CB 3)  
6) Cryptспорiopsis actinidiae (07 CB 7)  
7) Cylindrocarpon destructans (07 CB 8)  
8) Plant parasitic nematode, Paratrichodorus renifer  
9) Phomopsis sp. I + Paratrichodorus renifer  
10) Phomopsis sp. II + Paratrichodorus renifer  
11) *Phomopsis sp. III (07 CB 9)  
12) Coniothyrium sporulosum + Paratrichodorus renifer  
13) Cryptспорiopsis actinidiae + Paratrichodorus renifer  
14) Cylindrocarpon destructans + Paratrichodorus renifer
On June 6, 2011, approximately 150 *P. renifer* in 200 ml sandy soil were introduced to half of the randomly selected microplots; a trowel was used to cut two slits about 20 cm deep in the soil midway between the cranberry crown and the edge of the pot, 100 ml infested soil was added to each slit, and then the slit was covered over with peat. The non-inoculated pots received 200 ml of the same soil that had been heat-treated to eliminate nematodes. Subsequently, on June 15-22, 2011, plants in the presence and absence of *P. renifer* were inoculated with the pathogens to form a factorial (fungal pathogen x nematode) experimental design. The inoculum of each pathogen was prepared as conidial spore suspension at a concentration of $1 \times 10^6$ spores/ml from a culture of the pathogen grown on a microbiological medium. Cranberry plants in the microplot were inoculated with the pathogens using two different methods. Firstly, in each microplot, 5 runners were randomly selected and peripherally wounded by rubbing with a sterile needle near the base of each runner. Twist ties were left attached to the wounded areas as visible tags to identify the wounded areas. Approximately, 40 ml of the inoculum was spayed over the entire foliage (canopy), including wounded tissues, until runoff. The soil-borne, root pathogen, *C. destructans*, was introduce to the soil-peat growing medium around the root zone by dispensing 40 ml of the conidial spore suspension, but wounding of the runners and foliar spay with sdH2O were carried out to mimic the other treatments. The healthy control treatment were also subjected to similar wounding and foliar spay with 40 ml sdH2O until runoff. A third *Phomopsis* sp. III (07 CB 9) was included to the 6 remaining microplots, four with the nematode and two without nematode, that were not randomized and, therefore, not included in the factorial experimental design. To assess the impact of plant parasitic nematode on cranberry, 5 cm diam. X 100 cm long clear polyacrylic “minirhizotron” access tubes were inserted into, both *P. renifer*-inoculated and non-inoculated microplots. The minirhizotron access tubes will allow for insertion of a camera that will enable us to quantify the root growth and thereby assess the impacts of nematodes on root performance before effects are manifest above-ground (foliar symptoms). Microplots were maintained and observed periodically for visible symptoms. On September 28, 2011, microplots were assessed for treatment effects. Each microplot was rated for disease incidence/severity (i.e. CDD symptoms) as percentage of symptomatic foliage (dead/dying plants) and canopy density (foliage coverage) on a scale of 0-5, where 1 = poor coverage, less than 20%, and 5 = full coverage, over 80%. The canopy density was considered as a parameter for estimating the impact of CDD severity on cranberry.

**Results:** The first sign of visible symptoms were noticed in mid-August 2011 in the microplots plots treated with *Phomopsis* sp. (I) (isolate 07 CB 4) and recorded on September 28, 2011. The symptoms included uprights losing (dropping) lower leaves and upper leaves turning copper/brown colour, thinning of vines due to dropping of leaves, affected runners are desiccated and, in particular, runners become brittle and easily break away from the plant during handling (Figure 2). The symptoms observed in the microplots were similar to the symptoms observed in fields affected by CDD during the survey conducted in 2007 (*Fitzpatrick et al., Research Report 2007*). However, the extent of the damage observed in the fields was much greater than the symptoms manifested in the microplots, perhaps due to exposure of cranberry to environmental and plant stress factors, e.g. water/drought stress, in the field. The observed symptoms were visible in five of the six replicated microplots treated with *Phomopsis* sp. I and grown with or without *P. renifer*. Neither the uninoculated control nor treatments of other pathogens and *P. renifer* expressed any visible symptoms of CDD as of September 28, 2011 (Figure 3). Two of the microplots treated with *Phomopsis* sp. III (07 CB 9) were observed with 1 or 2 dying uprights that may not be related to the treatment. When closely observed, the affected plants from the treatment, *Phomopsis* sp. (I), had charcoal-black discoloration on the runners (Figure 4), similar to the symptoms observed on cranberry plants affected with CDD in fields in 2007 (*Fitzpatrick et al., Research Report 2007*). Representative plant samples, both symptomatic and non-symptomatic, were collected from each treatment and brought to the laboratory for close observation and re-isolation of the pathogens to fulfill Koch’s postulates (work in-progress).
The microplots treated with *Phomopsis* sp. (I) had the highest mean percentage disease incidence/severity that was statistically significant compared to the other treatments (Figure 5). The mean canopy densities (coverage) of the microplots treated with *Phomopsis* sp. (I) were 30% lower than the canopy density of all other treatments (Figure 6) although it was not significantly different from the other treatments. However, it is important to note that the estimated canopy densities of the microplots treated with *Phomopsis* sp. (I) also included the symptomatic blighted leaves still attached to the plants. Furthermore, none of the treatments, other than *Phomopsis* sp. (I), expressed visible symptoms as of September 28, 2011. Therefore, it is apparent that *Phomopsis* sp. (I) was responsible for the observed symptoms and cause of CDD observed in fields. The microplots will be maintained through winter 2011/2012 and similar data will be collected in spring and summer 2012.

**Identification of the probable causal agents to species using PCR-based molecular tools 2010/2011:**

The identification of the probable causal agents, *Phomopsis* sp. (4 isolates), *Coniothyrium sporulosum*, *Cryptosporiopsis actinidiae*, and *Cylindrocarpon destructans*, were confirmed by their colony, fruiting body and spore morphology on potato-dextrose-agar (PDA) medium and PCR-based amplification of the genomic DNA with universal fungal primers, ITS1 & ITS4, followed by DNA sequencing and analysis. All the isolates, except genus *Phomopsis*, were confirmed to their species with 100 percent similarity to GenBank database (Sabaratnam et al., Research Report, November 30, 2010).

**Work in-progress:** The identity of the *Phomopsis* isolates to their species was not successful due to unavailability of matching descriptions or gene sequence database in the GenBank. The only closest match was *Phomopsis vaccinii* with 93% similarity. It is important to note that the isolates of *Phomopsis* spp. obtained from CDD symptomatic plant samples were morphologically different from *Phomopsis vaccinii*, the causal agent of upright dieback, a common disease of cranberry grown in B.C. A representative culture of *Phomopsis vaccinii* from cranberry twigs deposited at the Canadian Cultures of Fungal Cultures in Ottawa has been ordered for comparison with the *Phomopsis* isolates obtained from the CDD samples. In addition, representative cultures of the *Phomopsis* isolates have been submitted to the Mycology Identification Services, AAFC, in Ottawa for identification (work in-progress). Knowing the full identify of the *Phomopsis* isolates to their species is crucial for their reference, literature review, understanding their biology and epidemiology and to develop appropriate disease management strategies.

**Further confirmation of the causal agents of CDD via histology 2011/2012:**

Based on the foliar symptoms expressed by the cranberry plants challenged with various pathogens in the greenhouse and field microplots, *Phomopsis* sp. (I), isolate 07 CB 4, was identified as the primary causal agent of CDD. However, documentation of host-pathogen interactions (i.e. colonization process of the host plant tissues by the pathogen) at the cellular-level can further strengthen the confirmation of the causal agent of CDD. Healthy and vigorously growing runners and uprights from the cranberry plants grown in the greenhouse were excised, cut into 6-8 cm segments and surfaced sterilized with 2% NaOCl and washed repeatedly with sdH2O. Excess water was removed from segments and they were mounted on 9 cm Petri plates containing 2% agar-water medium. Ten µl aliquots of the spore suspension of *Phomopsis* sp. (I) were placed at the cut ends of each segment and the plates were incubated until lesions were observed at the inoculated points. Vertical segments of 0.5-1.0 cm long were removed from the advancing edges of the lesion and preserved in formalin: acetic acid: alcohol solution until processing.
Work in-progress: The infected plant segments will be subjected to dehydration and embedding processes. A series of microtome sections (vertical and longitudinal) will be taken and subjected to microscopic histology analysis. Any evidence of colonization of cranberry tissues by the pathogen, *Phomopsis* sp. I, will be recorded and photographic images will be taken wherever possible.

DISCUSSION

• Plant-pathogen interaction studies (Koch’s postulates) conducted in the greenhouse (Sabaratnam et al., *Research Report 2009*) and field microplots (2010/2011) confirmed *Phomopsis* sp. I (isolate 07 CB 4) as the causal agent of Cranberry dieback disorder.

• The symptoms (uprights losing lower leaves and upper leaves turning copper/brown colour, thinning of vines due to loss of leaves, affected plants become desiccated, charcoal-black discolouration of affected runners, and runners become brittle and easily break away from plants) observed in the microplots challenged with *Phomopsis* sp. (I) are very similar to the symptoms expressed by cranberry fields identified with CDD during the survey in 2007. This further confirmed *Phomopsis* sp. (I) as the causal agent of CDD.

• *Phomopsis* sp. (I) has not been identified to species due to insufficient data in the literature. However, based on the morphology of conidial spores, *Phomopsis* sp. (I) can be considered as a different species from *Phomopsis vaccinii*, the cause of cranberry Upright dieback. A representative culture of *Phomopsis* sp. (I) has been submitted to the National Fungal Identification Services in Ottawa for identification (results pending). Type cultures of various *Phomopsis* spp., including *Phomopsis vaccinii*, will be obtained from various sources to compare *Phomopsis* sp. (I) with other *Phomopsis* species.

• Although the microplots treated with the potential pathogens *Coniothyrium sporulosum*, *Cryptosporiopsis actinidiae* and *Cylindrocarpon destructans* did not produce any visible symptoms as of September 28, 2011, observations will be made in the spring and summer 2012 to confirm their pathogenicity on cranberry and potential contribution to CDD. It is important to note that *Phomopsis* spp., *Coniothyrium sporulosum* and *Cylindrocarpon destructans* were consistently recovered at high frequencies from nearly 80% of the symptomatic CCD fields in 2007, suggesting that these pathogens may synergistically contribute to the severity of CDD in the field (*Fitzpatrick et al., Research Report 2007* and *Sabaratnam et al., Research Report 2008*).

• In 2011, we did not observe more severe symptoms of fungal pathogens on nematode-inoculated plants than on non-inoculated plants, indicating that *P. renifer* probably does not interact synergistically with fungal pathogens to cause or exacerbate the development of CDD. Nematodes can nonetheless also have direct effects on plant health that are different in nature from fungal diseases. The direct effects of nematodes on perennial crops are usually a long-term chronic stress, manifest as multi-year declines in vigour, as nematode population densities build up. The microplot setup will enable us to observe these potential impacts of *P. renifer* through 2012 and 2013 growing seasons.
• Any environmental factors (e.g. water stress, high temperature etc.) that contribute to plant stress may predispose plants to pathogen attack, thus contribute to the severity of CDD as seen in the fields in 2007; however further research is required for assessing such impacts of plant stress factors on CDD severity.

• Temperature requirements for the growth (activity) of Phomopsis sp. (I) as measured in vitro indicated that the pathogen is active between 12°C to 32°C with the optimum growth at 24°C (Sabaratnam et al., Research Report 2009). Although the environmental conditions are different or may vary under field conditions the extent of damage and severity of CDD can be enhanced by dry, summer conditions that are conducive to the pathogen.

• Preliminary in vitro efficacy experiments conducted in 2010 identified Bravo, Copper oxychloride, Funginex and Topaz and few other fungicides (Captan, Senator, Lance, Pristine and Switch) as effective fungicides to prevent initial infection and subsequent development of the pathogens (Sabaratnam et al., Research Report 2010). We propose that the fungicides identified in this study should be tested in the field for their efficacy to control CDD and, thus, generate data that would support the registration of the fungicides via PMRA fungicide-registration process.

• Although Phomopsis sp. (I) has been confirmed as the major pathogen responsible for CDD it is important to understand the epidemiology of the disease (i.e. disease cycle) to develop appropriate management strategies. Epidemiological studies involving development of the disease, time of production of infectious propagules (spores) by the pathogen, time and mode of dispersal of spores, time of infection of cranberry plants by the pathogen, plus the environmental conditions that are favourable for the disease cycle are essential components in the development of effective management strategies for CDD.

• Current disease management practices such as removal of both symptomatic and surrounding healthy vines from the infected area, sanding the infected area, and application of fungicides (Bravo, Topaz, Funginex and Copper) that are already registered on cranberry can eliminate/minimize the impact of CDD.

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Figure 1: The layout and aerial view of the research field plot, consisting of 48 microplots (each 0.5 m² area) at the PAPC in Agassiz. Note the minirhizotron access tubes in the microplots that allow in situ assessment of plant parasitic nematode, *P. renifer*, and cranberry root interaction.

Figure 2: The symptoms expressed by the cranberry plants in microplots challenged with *Phomopsis* sp. (I), in the presence and absence of *P. renifer*. The observed symptoms in the microplots are similar to the symptoms expressed by the CDD suspected fields surveyed in 2007 (*Research Reports, 2007 & 2008*).
Figure 4: Symptomatic plants infected with *Phomopsis* sp. (I), showing charcoal-black discolouration on runners which are brittle and easily break away from the plant, similar to those observed in the fields with CDD surveyed in 2007 (Fitzpatrick et al., Research Report, November 30, 2007).

Figure 3: An aerial view of the representative cranberry microplots of the control treatment +/- *P. renifer* (A), *C. destructans* +/- *P. renifer* (B), *C. sporulosum* +/- *P. renifer* (C), *C. actinidiae* +/- *P. renifer* (D), *Phomopsis* sp. I +/- *P. renifer* (E), *Phomopsis* sp. II +/- *P. renifer* (F), and *Phomopsis* sp. III +/- *P. renifer* (X). Foliar blight symptom of copper-brown colouration was visible in the microplots that had been challenged with *Phomopsis* sp. I.

Figure 4: Symptomatic plants infected with *Phomopsis* sp. (I), showing charcoal-black discolouration on runners which are brittle and easily break away from the plant, similar to those observed in the fields with CDD surveyed in 2007 (Fitzpatrick et al., Research Report, November 30, 2007).
Figure 5: Mean percentage incidence of CDD expressed by cranberry plants in the microplots that received the control treatment +/- *P. renifer* (A), *C. destructans* +/- *P. renifer* (B), *C. sporulosum* +/- *P. renifer* (C), *C. actinidiae* +/- *P. renifer* (D), *Phomopsis* sp. I +/- *P. renifer* (E), *Phomopsis* sp. II +/- *P. renifer* (F), and *Phomopsis* sp. III +/- *P. renifer* (X). The mean percentage disease symptoms expressed by *Phomopsis* sp. I treatment (E) was significantly different from all the other treatments. Although treatments B, D and X showed less than 1% of foliar discolouration on 1 or 2 uprights it was not related to the respective treatment effect, rather due to an unknown factor, mechanical or environmental damage.

Figure 5: Mean canopy density of the microplots that received the control treatment +/- *P. renifer* (A), *C. destructans* +/- *P. renifer* (B), *C. sporulosum* +/- *P. renifer* (C), *C. actinidiae* +/- *P. renifer* (D), *Phomopsis* sp. I +/- *P. renifer* (E), *Phomopsis* sp. II +/- *P. renifer* (F), and *Phomopsis* sp. III +/- *P. renifer* (X). Although the mean canopy density of *Phomopsis* sp. I treatment (E) was not significantly different from the canopy densities of all the other treatments a 10-30% reduction in the canopy density was observed in the replicate microplots treated with *Phomopsis* sp. I. A scale of 1 to 5 was used in the estimation of canopy density, where 1 = weak coverage, <20% and 5 = healthy coverage, >80%.
REFERENCES


Field Survey & Analyses of Cranberry & Soil Samples (2007 Study):

A comprehensive field and grower survey in spring/summer 2007 excluded insect pests, particularly cranberry girdler, weevil or Dearness scale damage, herbicide injury and poor field conditions as major causes of cranberry dieback disorder (CDD). To identify the possible causal agent(s), systematic field observation, sampling and laboratory analyses of root, runner and upright tissues and soils from 32 affected cranberry beds belonging to 24 farms were carried out in spring and summer 2007. Several previously recorded pathogens, namely Allantophomopsis sp., Coleophoma sp., Colletotrichum acutatum, Pestalotia sp., Phomopsis vaccinii, Phyllosticta vaccinii, Alternaria sp. and Botrytis cinerea, responsible for fruit rots and foliar diseases of cranberry, were occasionally encountered during microscopic examination and culturing of tissue samples. Based on the symptoms, frequency of occurrence and consistency with CDD symptomatic fields, these pathogens were ruled out as the causal agents of CDD. Pathogens, 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. P. cinnamomi can cause substantial root and runner rot under wet and warm soil conditions. Analysis of plant tissue samples for phytopathogenic bacteria (e.g. Pseudomonas syringae) and viruses did not confirm their presence on CDD symptomatic plant samples. A virus similar to Blueberry scorch virus (BlScV) was detected on samples collected from six fields; this virus on cranberry has now been considered as a saprophytic endophyte that does not cause any disease on cranberry and not related to BIScV (personal communication, Bernardy, M., 2009). Among the several other potential fungal pathogens isolated from the tissue samples, Coniothyrium sporulosum, Cylindrocarpon destructans and a Phomopsis sp. (Figure 4), 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. Analysis of soil samples also revealed the presence of plant-parasitic nematodes, particularly Paratrichodorus spp. and Hemicycliophora spp. in many cranberry fields. However, based on their distribution and population densities, these nematodes may not be considered as the primary cause of CDD but can contribute to root damage and, thus, weakening of 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.

Cranberry growers were advised on the findings; survey information reports were prepared for individual fields that were included in the survey and handed over to the respective growers.

Determination of pathogenicity of potential pathogens, responsible for cranberry dieback disorder (2008/2009 Study):

In 2008/2009, a greenhouse study was conducted to determine the pathogenicity (Koch’s Postulates) and virulence of potential pathogens, isolated from the CDD symptomatic plants, and, thereby, confirm the causal agent(s) of CDD. Four-month-old cranberry plants (var. Stevens), propagated from disease-free stem (upright) cuttings and grown on peat-sand medium in 1 gallon pots, were 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 were included in this study. The plants were maintained at the Pacific Agri-Food Research Centre, AAFC, in Agassiz over a 14-month period and monitored periodically until the plants expressed visible symptoms. Evidently, plants inoculated
with *Phomopsis* sp. or in combination with *C. sporulosum* or *C. destructans* or *C. sporulosum* and *C. destructans* (a total of four treatments) expressed severe symptoms of leaf browning and blight, leaf dryness and defoliation followed by plant death. None of the other nine treatments expressed noticeable foliar symptoms or signs of plant stress. Reisolation of *Phomopsis* sp. from the symptomatic plant tissues of all the four treatments confirmed the pathogenicity of *Phomopsis* sp. on cranberry and, thus, fulfilled Koch's postulates. However, the pathogenicity, degree of damage and expression of typical field symptoms of CDD by *Phomopsis* sp. can only be confirmed via field-inoculation studies. In addition, studies on disease epidemiology and weather parameters for infection, development and spread of *Phomopsis* sp. and other suspected pathogens are essential for developing effective control/management strategies to combat CDD. Although pathogens, *C. sporulosum* and *C. destructans*, did not produce any noticeable symptoms within the observed experimental period their pathogenicity to cranberry and synergistic contribution to CDD cannot be ruled out. Particularly, *C. sporulosum* had recently been encountered and recovered from stem and crown cankers on mature blueberry plants and cranberry transplants. Since both *Phomopsis* sp. and *C. sporulosum* have never been reported to cause any diseases on cranberry further studies focussing on the histology of infection process of cranberry tissues, degree of damage to plant tissues and expression of typical field symptoms have become essential for confirming the causal agents of CDD.

*In vitro* growth-rates of potential pathogens measured on potato-dextrose-agar medium in the laboratory, indicated that most pathogens, importanty *Phomopsis* sp., *C. sporulosum* and *C. destructans*, were able to grow at temperatures between 12°C to 24°C or 28°C. However, the optimum growth rate of *Phomopsis* sp., *C. sporulosum* and *C. destructans* was measured at 24°C, 20°C and 20°C, respectively. Understanding the interaction among the biology of the pathogen(s), environmental conditions (e.g. temperature and soil and air moisture regimes, photoperiod etc.) that favour the growth, development and reproduction (production of infectious propagules) of the pathogen, and physiology and growing conditions of the crop will determine the efficacy of control/management measures.

For details, please refer the Sabaratnam et al., Research Report, 2009.

**Establishment and maintenance of research field-plots (microplots) at PARC (2010 study):**

A research field plot, containing 48 microplots has been established at the PARC, AAFC, in Agassiz. In 2011, a field experiment using the microplots will be designed to carry out Koch's Postulates for the probable causal agents, *Phomopsis* sp., *Coniothyrium* sp. *Cryptosporiopsis* sp. and *Cylindrocarpon* sp., and, thereby, confirm the causal agent(s) and demonstrate the typical CDD symptoms, as observed in 2007 field survey. In addition, field experiments will be conducted to understand the interactions among CDD causal agent(s) and plant-parasitic nematodes and their synergistic impact on cranberry. If applicable, micro-beds will be used in the subsequent years to evaluate the efficacy of potential fungicides, as selected from the *in vitro* studies, to control the causal agent(s) and, thereby, propose fungicide screening trials in the field and facilitate PMRA fungicide registration process.

**Screening of potential fungicides for efficacy to control pathogens (2010 study):** Fourteen fungicides belonging to 10 different functional chemical groups were evaluated under laboratory conditions for their efficacy to prevent the germination of spores and prevent or suppress the mycelial growth of the probable causal agents, *Phomopsis* sp. *Coniothyrium sporulosum* and *Cryptosporiopsis actinidiae* (foliar pathogens). Since *Cylindrocarpon destructans* is a soil-borne, root pathogen it was not included in the experiment. Fungicides, Bravo, Copper oxychloride, Funginex and Topaz, that have already been registered on cranberry, and other
potential fungicides, particularly those registered on highbush blueberry (Vaccinium corymbosum L.), were included in the evaluation.

Based on the in vitro assessment, it was evident that the fungicides Bravo (chlorothalonil, Group M), Funginex (triforine, Group 3), Topaz (propiconazole, Group 3) and Copper oxychloride (Group M), that have already been registered for cranberry diseases, demonstrated efficacy to prevent/suppress both spore germination and mycelial growth of Phomopsis sp., C. sporulosum and C. actinidiae. Germination of Phomopsis and C. sporulosum spores was completely inhibited by Bravo, Funginex and Topaz. Bravo and Funginex also prevented C. actinidiae spore germination by 100%. Although Copper oxychloride demonstrated 100% efficacy in preventing spore germination of C. sporulosum and C. actinidiae only 66% efficacy was obtained for Phomopsis sp. Fungicide, Topaz prevented the mycelial growth of all three pathogens by 100%, whereas, Bravo, Copper oxychloride and Funginex exhibited 90-100% efficacy to prevent/suppress mycelial growth of the three pathogens. Apart from the four fungicides, several other fungicides were also demonstrated efficacy to prevent/suppress spore germination and mycelial growth of the fungal pathogens.

For details, please refer Sabaratnam et al., Research Report 2010.