Identifying Effective Chemical Disinfectants for Use in Sanitizing Greenhouses

Interim Progress Report II

Prepared for the

Alberta Professional Horticultural Growers Congress and Foundation Society

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November 22, 2007

Introduction

Alberta's greenhouse industry produces about $110 million of gross crop revenue annually. About 400 operations are located across the province comprising ca. 275 ac of production. The major crops being grown include vegetables (cucumbers, peppers, tomatoes and lettuce), bedding plants, ornamentals (cut & potted flowers and foliage plants), and forest tree seedlings. Diseases represent a significant threat to all of these crops and preventing or controlling them can mean the difference between success and failure in a greenhouse business. The first step in most disease management programs used by growers is to clean and disinfect equipment and greenhouse structures to eradicate pathogens and pests. This process is called sanitation. Sanitation of greenhouse facilities between crops is one of the most effective disease management strategies in plant production systems. Pathogens are eliminated from production surfaces by cleaning and applying disinfectants prior to establishing new plants. This allows growers to start and maintain the cropping cycle under disease-free conditions. Disinfection following crop removal is a rapid and relatively low-cost strategy in greenhouse disease management. The break period between crops is the critical point for introducing sanitation practices to a disease management program. Disinfection procedures may also be used during the growing season, if and when diseased plants are found and removed from the greenhouse.

Relatively little research work has been done in Canada on greenhouse sanitation. Surveys of vegetable greenhouses in Alberta from 2003-05 revealed that many growers, but not all, utilized disinfection as a disease management tool between crops, and that varying degrees of success were achieved. Growers want to be able to quickly and thoroughly disinfest premises between crops without doing damage to their crops, the greenhouse structure, the environment or themselves. Chemicals are the major means by which growers sanitize their greenhouses. A limited range of products is available and most have been in general use for decades, e.g. bleach and quaternary ammonia compounds. Additional research on greenhouse sanitation is sorely needed for two main reasons: 1) Many growers are unsure of which chemical disinfectant is best suited to their particular situation because there is a lack of published reports comparing the efficacy, corrosiveness, personal safety and phytotoxicity of these products, and 2) New products recently developed and in use in Europe and the U.S.A., which could be registered in Canada, are unavailable due to a lack of suitable efficacy data produced under local conditions.

Because many of the products used for greenhouse sanitation are broad-spectrum biocides and are registered for other horticultural uses, e.g. disinfecting flats, crates, totes, baskets, tools, equipment and storages, the findings from the studies described in this report will have broader application than just for greenhouse situations and may also benefit fruit, vegetable, potato and nursery crop producers.

Project Objectives

- To investigate the effectiveness of commercial and experimental disinfectants for eradicating plant pathogenic microorganisms under laboratory and simulated greenhouse conditions.
- To determine the corrosiveness or other potential harmful effects of these disinfectants to a variety of hard surfaces found in a typical greenhouse.
- To assess the sensitivity of selected greenhouse plants to an overspray of various disinfectants.

Results

Efficacy

A selection of plant pathogenic microorganisms were applied to the surfaces of small coupons of 10 materials typically found in and around greenhouses and nurseries and were grown as a biofilm, i.e. growth attached to the surface of the coupon. Infested coupons were then incubated in a solution of each of the nine chemical disinfectants under test for 30 minutes, then rinsed in sterile water, and incubated in nutrient broth. Re-growth in the nutrient broth was noted after 5 days. Experiments were done in triplicate at three rates of disinfectant, i.e. half-, full- and double-strength (½ X, 1X and 2X, respectively). The rates were based on manufacturers' label recommendations or by personal communication with the manufacturers for products where no label was available. The efficacy results at the 1X rate are shown in Tables 1-3. Overall, Chemprocide was the most effective disinfectant and Hyperox the least against *Fusarium*, *Botrytis*, *Pythium*, *Didymella* and *Erwinia* (Table 1). Copper proved to be the easiest surface to clean and rubber the most difficult (Table 2). The gray mold fungus *Botrytis cinerea* was the easiest pathogen to eradicate, while the soft rot bacterium *Erwinia carotovora* was the most difficult (Table 3).

Corrosion

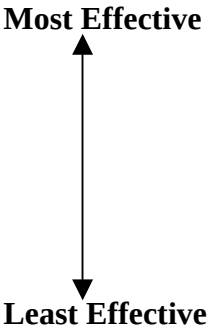
Small coupons of the 10 surface materials were incubated for 2 hours in full-strength solutions of each of the disinfectants. Gravimetric measurements were recorded before and after treatments. Coupons were thoroughly dried at 70°C overnight before weighing. This experiment, and one repeat, were completed and all data recorded. Results are shown in Table 4 and Figure 1. Overall, bleach was the most corrosive material and Chemprocide the least.

Phytotoxicity

Phytotoxicity of the disinfectants under test was measured directly on greenhouse-grown plants including vegetables, bedding plants, tree seedlings and potted flowers. Disinfectants were prepared at the manufacturers' recommended rates and sprayed directly onto plants to simulate accidental overspray. Figure 1a depicts a set of plants before treatment, and Figure 2 shows plants prior to exposure. Figure 3 shows some examples of phytotoxicity on various plants after treatment. This experiment was completed and all data recorded. Results are shown in Tables 5 and 6. Overall, ECA solution was the least phytotoxic disinfectant and Biosentry the most (Table 5). Pansy was the most sensitive plant to the disinfectants, whereas spruce proved to be the least affected (Table 6).

Table 1. Overall performance of eight disinfectants against *Fusarium, Botrytis, Pythium, Didymella* and *Erwinia* compared to tapwater.

Disinfectant	Score[1]
Chemprocide	198
Bleach	203
Biosentry 904	204
Virkon	252
ECA Water	288
OxiDate/StorOx	300
MENNO-Florades	308
Hyperox	324
Water	600



[1] The maximum score is 600 which would occur if a test product was not effective against any microorganism. The minimum score would be 150, which would occur if it was always effective.

Table 2. Overall ability of eight disinfectant products to disinfest ten hard surfaces.

Surface	Score1
Copper	208
Stainless steel	218
PVC	250
Aluminum	253
Glass	264
Polycarbonate	289
Concrete	300
Wood	320
Steel	323
Rubber	338

Easiest to Clean

Most Difficult to Clean

[1] The maximum score is 600 which would occur if the test microorganism was not cleaned away by any of the chemical treatments under test. The minimum score would be 150, which would occur if it was always cleaned away.

Table 3. Overall sensitivity of five microorganisms to eight chemical treatments.

Microorganism	Score[1]
Botrytis cinerea	404
Fusarium oxysporum	498
Didymella brioniae	511
Pythium aphanidermatum	570
Erwinia carotovora	694

Easiest to Eradicate

Most Difficult to Eradicate

[1] The maximum score is 1000, which would occur if the microorganism under test was not eradicated by any of the chemical treatments. The minimum score would be 270, which would occur if it was always eradicated.

Table 4. Corrosive potential of eight chemical disinfectants and water compared to water – average from all surfaces.

Disinfectant	Score[1]
Bleach	12.99
Virkon	7.17
Hyperox	5.30
OxiDate/StorOx	4.07
Biosentry 904	3.27
MENNO-Florades	3.13
ECA Water	2.46
Chemprocide	2.93
Water	0.10

Most Corrosive

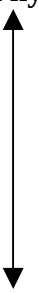
Least Corrosive

[1] This number is the sum value of the changes in weight measured for all surface material coupons after treating.

Table 5. Phytotoxicity of nine chemical disinfectants compared to water.

Disinfectant	Score[1]
Water	90
ECA Water	154
Hyperox	157
MENNO-Florades	178
Ozonated Water	179
OxiDate/StorOx	180
Chemprocide	192
Virkon	196
Bleach	235
Biosentry 904	389

Least Phytotoxic



Most Phytotoxic

[1] Ratings were done using the following scale that estimated the % leaf area with symptoms: 1 = healthy, 2 = 1%-10%, 3 = 11%-25%, 4 = 26%-50%, 5 = 50% or higher 6 = Dead. The minimum value is 90, which would occur if no symptoms of phytotoxicity were observed. The maximum value is 540, which would occur if all plants were killed within five days of exposure.

Table 6. Sensitivity of ten species of greenhouse vegetables, bedding plants, potted flowers and tree seedling to chemical disinfectants.

Plant	Score[1]
Pansy	327
Marigold	256
Tomato	230
Impatiens	221
Cucumber	189
Echinacea	183
Pepper	145
Lobelia	105
Dusty Miller	104
Spruce	96

Most Sensitive

Least Sensitive

[1] Ratings were done using the following scale that estimates the % leaf area with symptoms: 1 = healthy, 2 = 1%-10%, 3 = 11%-25%, 4 = 26%-50%, 5 = 50% or higher 6 = dead. The minimum value is 45 which would occur if there were never phytotoxic symptoms. The maximum value is 486 which would occur if all plants were killed within one day after exposure.

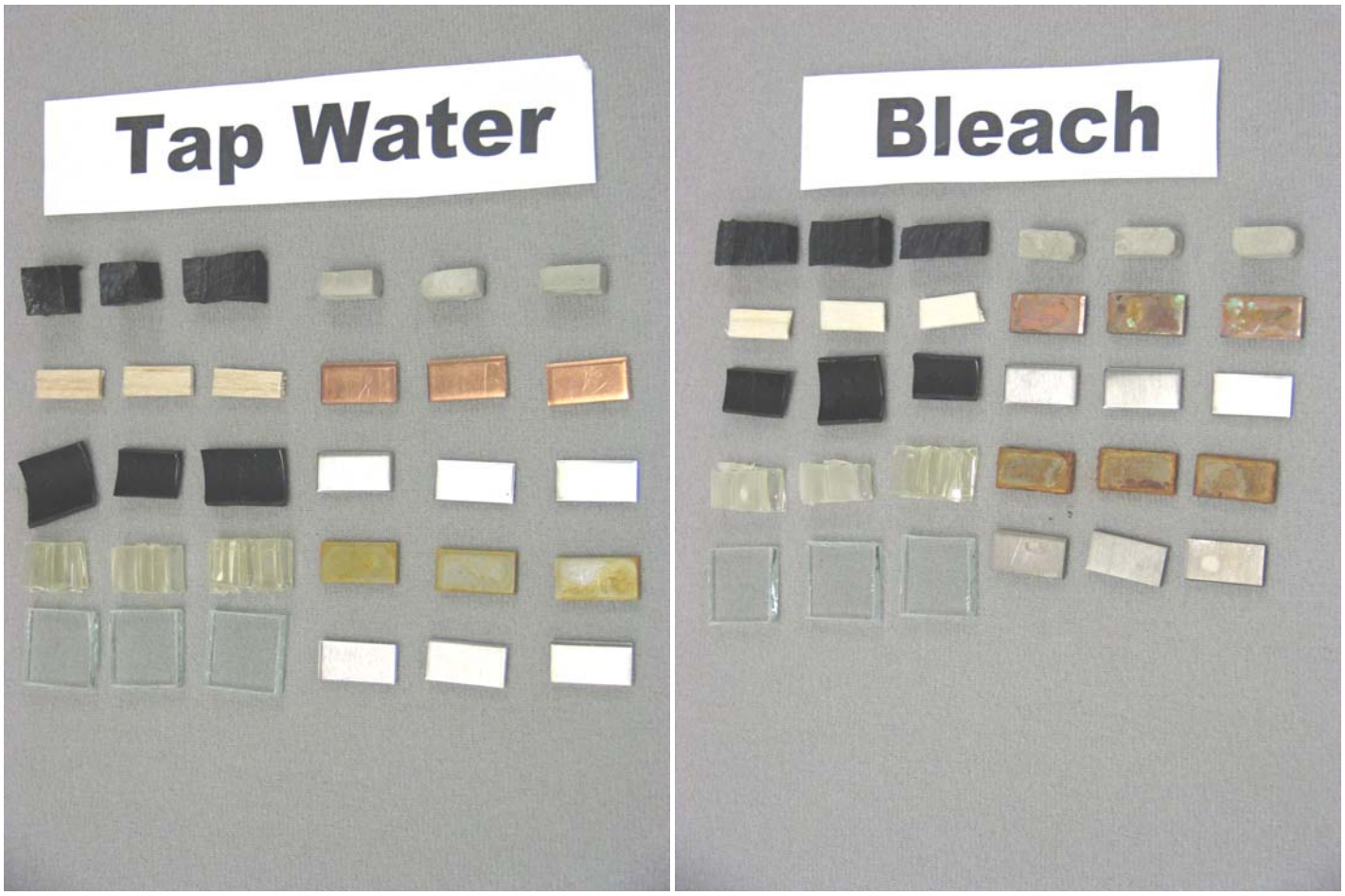


Figure 1. Coupons of ten hard surface materials after exposure to tap water and bleach. The materials tested were (top to bottom; groups of three coupons per material):

Rubber	**Concrete**
Wood	**Copper**
PVC	**Stainless Steel**
Polycarbonate	**Steel**
Glass	**Aluminum**

Figure 2. Greenhouse vegetables, bedding plants, potted flowers and tree seedlings prior to treatment with disinfectants.

Figure 3. Phytotoxicity on cucumber (upper left), spruce (upper right), bedding plant (lower left) potted flower (lower right).

Summary & Conclusions

The workplan for this project has been completed with the exception of three items:

1. Efficacy testing with ozonated water versus all pathogens
2. Efficacy testing of all disinfectants versus Tomato Mosaic Virus (ToMV)
3. Efficacy testing of all disinfectants versus *Phytophthora ramorum*

The remaining testing of ozonated water (item #1) will be completed as soon as repairs can be made to the ozone generator on loan to us from Seair Diffusion Systems. We plan to deliver the instrument to the company's manufacturing facility in Edmonton in late November, 2007 for repairs and verification that it is operating properly.

Testing disinfectants against ToMV will be completed as soon as a suitable isolate of the virus can be obtained from a greenhouse or research laboratory. We hope to find such an isolate before the end of 2007.

Efforts to test disinfectants against *Phytophthora ramorum* have been discontinued. This quarantine pathogen is under extremely tight control by the Canadian Food Inspection Agency (CFIA) and it was not possible for us to obtain a culture of the sudden oak death (SOD) pathogen in any form. We made a formal written request to the agency, including an offer to do our testing at the level 3 biocontainmant facility at the Lethbridge Research Centre, but all of our overtures were denied. Relevant correspondence documenting our efforts are given in Appendix 1. It should also be noted that numerous telephone communications were made to the CFIA that are not documented. We have contacted Dr. Saad Masari, a research scientist with the CFIA in Sidney, B.C., who is working on SOD, and he has informed us that he intends to conduct some efficacy trials with disinfectants amongst a number of other studies that he will be doing. We hope to be able to obtain and include a summary of the results from his work with the final report for this project, if it is available before the conclusion of our study. The outstanding studies related to items 1 and 2 will be completed by March, 2008, and a final results report will be prepared at that time.

Appendix 1. Correspondence with the Canadian Food Inspection Agency regarding efforts to obtain cultures of *Phytophthora ramorum* for disinfection studies.

Note: The following memos are listed in chrononological order with the most recent ones at the beginning.

Michael Harding/AAFRD

01/25/2007 08:45 AM

To: brieresc@inspection.gc.ca

Cc: ron.howard@gov.ab.ca

Subject: *Phytophthora ramorum*

Hello Stephan,
We would like to test various hard surface disinfectants for efficacy against *P. ramorum*, similar to the study on fungicide efficacy described by Dr. James (see below). The work we propose is all in vitro, on hard surfaces such as plastic, glass, wood, concrete and metal (no plant material). Additionally we have arranged for space in a Bio-containment level III facility at the AAFC facility in Lethbridge Alberta, or alternatively, at the provincial BSE laboratory in Edmonton.

We would be willing and prepared to abide by any restrictions on containment and movement of this organism. Could you please explain what approval and conditions would be necessary for us to perform our work here in Alberta, and how we might obtain an isolate? I hope to hear from you soon.

Best regards,
Mike

Michael W. Harding, Ph.D.
Research Associate
Crop Diversification Centre South
301 Horticultural Station Rd. East, Brooks Alberta,T1R 1E6
Tel: (403) 362-1338 Fax: (403) 362-1326
michael.harding@gov.ab.ca

"Delano James"
<jamesd@inspection.gc.ca>

01/24/2007 06:01 PM

To: "Michael Harding"
<Michael.Harding@gov.ab.ca>

Cc: <ron.howard@gov.ab.ca>

Subject: *Phytophthora ramorum*

Hello Mike,

Sorry for the delayed response. I was away at the beginning of the week. Now we are hosting a meeting of some senior administrators from Ottawa and around the country, so presentations, tours etc.
We are involved in a collaborative project with Forestry Canada and have several isolates of P. ramorum. Part of this research involves the evaluation of various fungicides to identify conditions for effective control. Unfortunately, to get the isolates we needed special approval and conditions. Some of the terms of receiving the isolates included containment restrictions and restrictions on movement.
You may wish to contact Stéphan C. Brière (613) 228-6698 ext.5911 brieresc@inspection.gc.ca He is the Head of the Quarantine Plant Pathology Diagnostic Laboratory, Canadian Food Inspection Agency.
Regards,
Delano

Delano James, Ph.D.
{(250)363 6650 ext 235} {jamesd@inspection.gc.ca}
{Facsimile/Télécopieur: (250) 363 6661}
Head, Research Section, Canadian Food Inspection Agency, Sidney Laboratory,
Chef, Section de Recherches, Agence canadienne d'inspection des aliments, Laboratoire de Sidney,
8801 East Saanich Road | 8801 Chemin East Saanich, Sidney, BC, V8L 1H3
Government of Canada | Gouvernement du Canada
www.inspection.gc.ca

>>> Michael Harding <Michael.Harding@gov.ab.ca> 01/22/07 2:56 pm >>>

Dr. James,

I am a research assistant working with Dr. Ron Howard at a provincial research facility in Brooks, Alberta.

We have been working on a project aimed at testing hard surface disinfectants for efficacy in greenhouses and storages and our sponsors have requested that we include P. ramorum as a test species in our work. We have already generated efficacy data against organisms such as Fusarium, Pythium, Erwinia, etc, but SOD is raising a lot of eyebrows in Alberta nurseries and production systems. The Alberta Farm Fresh Producers, and Alberta Professional Horticultural Congress Foundation & Society (our sponsors) would like to know what product(s) will be most effective should they be faced with the need for eradication of P. ramorum from greenhouse and propagation surfaces and tools.

I have arranged for Bio-Containment Level III facilities at the AAFC centre in Lethbridge Alberta, or alternatively at one of our provincial facilities in Edmonton to insure that the organism is contained. The work to be done is all in vitro, laboratory testing and we will not be infecting any living tissues. The work is simply to assess which hard surface disinfectants can most effectively eradicate P. ramorum hyphae and spores from a number of surfaces (including metal, rubber, concrete, plastic and wood).

We would like to obtain an isolate of P. ramorum to complete this work at the request of our industry but, as you may imagine, have had difficulty obtaining an isolate.

I would like to know your thoughts on this project, and whether you may be able to assist us in the completion of this work by helping us obtain an isolate of P. ramorum.

Thanks for your attention,
Best regards,
Mike

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michael.harding@gov.ab.ca

Michael Harding/AAFRD

12/15/2006 01:54 PM

To: brieresc@inspection.gc.ca

Cc: ron.howard@gov.ab.ca

Subject: Phytophthora ramorum

Good afternoon Mr. Brière,

At the request of one of our sponsors, the Alberta Professional Horticultural Growers Congress and Foundation Society, we would like to include *Phytophthora ramorum* in a study assessing the efficacy disinfectants used in greenhouse clean-up (summary is attached).

We have been in contact with AAFC, Lethbridge obtaining permission to use their Level III containment facility if level III containment is required. Alternatively, we also have access to a BL-III containment facility in Edmonton through Alberta Agriculture, Food and Rural Development if the Lethbridge facility is not available.

I would like to know if it will be possible to obtain a sample of *P. ramorum* to be included in our study in Alberta. And if so, what steps are needed to secure a permit and an isolate.

I've attached a summary of our proposed work. Please note that any mention of *in vivo* or *in planta* work refers to work done with viruses or other obligate biotrophs that cannot be cultured. We will **not** be doing any work with *P. ramorum* on plants or in greenhouses. All work will be contained in the laboratory on/in growth media and on artificial surfaces. Also note that *P. ramorum* is not listed in Table 2 as it was not included in our proposal. However, the sponsor (APHGCFS) requested its inclusion at the time they approved sponsorship. We feel that the industry's concern regarding this pathogen is significant and we would like to meet their needs as best we can. We also feel that data from this study will be a valuable springboard for work (by others) aimed at managing sudden oak death in greenhouse nurseries.

I look forward to hearing from you.
Best regards,
Mike

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"Bourchier, Robert"
<BourchierR@AGR.GC.CA>

12/13/2006 03:45 PM

To: "Michael Harding"
<Michael.Harding@gov.ab.ca>

Cc: "Goettel, Mark" <GoettelM@agr.gc.ca>, Floate, Kevin" <FloateK@agr.gc.ca>, "De Clerck-Floate, Rosemarie" <FloateR@agr.gc.ca>, "Baines, Danica" <BainesD@agr.gc.ca>

Subject: Pathogen Containment

Hi Mike:

Thanks for the answers to my questions. The committee that oversees operation of the containment facility of Lethbridge has reviewed your request. The next steps are:

1. Make contact with Stephan Brière or alternate at CFIA and get an official letter indicating the CFIA containment requirements for your proposed work on SOD.

2. Please send a copy of the CFIA letter, accompanied by a letter from your group requesting to use the IMCF containment facility for the work, to me as chair of the IMCF Committee.

Assuming our facility meets the CFIA requirements and the work does not affect existing studies, I will then forward this request to the appropriate AAFC director
requesting a decision for the work to proceed.

Sorry for the nature of this process, but because of the pathogen in question is not agricultural or within our sphere of responsibility
we must seek departmental approval for the work to be done here. There may also be some sort of bench fees, depending on departmental policy for external work.

Regards
Rob

Rob Bourchier, Ph.D., Research Scientist /Chercheur Scientifique
Biological Control / Lutte biologique
Agriculture and Agri-Food Canada-Lethbridge Research Centre
5403 - 1st Avenue S., Lethbridge, Alberta CANADA T1J 4B1
403-317-2298 Fax 403-382-3156
bourchierr@agr.gc.ca

-----Original Message-----
From: Michael Harding [mailto:Michael.Harding@gov.ab.ca]
Sent: Monday, December 11, 2006 1:53 PM
To: Bourchier, Robert
Cc: ron.howard@gov.ab.ca
Subject: RE: Fw: Pathogen Containment

Hi Rob,
We will be working with mainly mycelial cultures (no spores). However, as the agar plates mature to 2-3 weeks there may be development of sporangia with zoospores and chlamydospores. Agar plates will be stored sealed with parafilm. In broth cultures we will be working exclusively with mycelia.

Because I have not previously worked with this particular organism, I don't know the precise details on the timing of sporulation and which (if any) spores are produced on standard agar media. I would suggest that the possibility for sporulation will be significant, but because all plates are sealed, the risk of spread is very low. Additionally please note that aerial dispersal by this organism is speculated based on symptoms in the field. It is not a point of record. I think that the risk associated with release of this organism is high, but the probability of release is very low because we are not working with infected plants, and cultures are confined in sealed plates at all times (or in a containment cabinet when plates are open).

My contact at CFIA is Stephan Brière (613) 228-6698 ext. 5911. I left a message with him last week and am waiting to hear back. I also have contact information for Delano James (250) 363-6650 ext. 235 but have not made contact at this time.

We would need a cabinet to work with agar plates, to prepare inoculum and work with treatment plates. It would be convenient to have access to a rotary shaker. All other materials we would bring (blender & cups, plates, pipettes, media, etc). All experiments are done at room temperature.

Thanks,
Mike

"Bourchier, Robert"
<BourchierR@AGR.GC.CA>

12/11/2006 11:38 AM

To: "Michael Harding"
<Michael.Harding@gov.ab.ca>

Cc: "De Clerck-Floate, Rosemarie" <FloateR@AGR.GC.CA>, <ron.howard@gov.ab.ca>, "Goettel, Mark" <GoettelM@AGR.GC.CA>, "Baines, Danica" <BainesD@AGR.GC.CA>

Subject: Pathogen Containment

Mike:

A couple of additional questions:

In your experimental procedure what stage (zoospores, sporangia, chlamydospores or all three) of the SOD will be present with your procedure, in the plates, or in the broth?

What equipment would be required?

Who have you been in contact with at CFIA?

Thanks
Rob

Rob Bourchier, Ph.D., Research Scientist /Chercheur Scientifique
Biological Control / Lutte biologique
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bourchierr@agr.gc.ca

Sent: Monday, December 11, 2006 10:34 AM

To: Bourchier, Robert

Cc: De Clerck-Floate, Rosemarie; ron.howard@gov.ab.ca

Subject: Re: Fw: Pathogen Containment

Good Morning Rob,

I'm writing at the request of Dr. Ron Howard, who had been asked to provide a description and background of some proposed BL-III containment experiments at the Lethbridge Research Centre's IMCF.

Last year Dr. Howard and I received funding from the Alberta Professional Horticultural Growers Congress and Foundation Society to study the effectiveness of registered and experimental chemical disinfectants at eradicating fungal, bacterial and viral plant pathogens on a variety of surface materials. One of the requests made by the APHGCFS was the inclusion of *Phytophthora ramorum*, the cause of sudden oak death (SOD). This disease is causing major problems in California, and has spread to the Pacific Northwest, and into British Columbia. This disease of great concern to Alberta plant and tree nurseries. It is a quarantined pest in Canada at this time and strictly regulated by CFIA. The quarantine is designed to keep our province(s) free of the disease to maintain the value of international trade of Alberta-grown plant and tree stocks and prevent the deterioration of shelterbelts and landscapes.

The pathogen produces motile zoospores that can move short distances in water and moist soil. It also produces sporangia that can be aerially dispersed and thick-walled chlamydospores that can be long-lived. Infected plant material is considered to be highly infectious and the main source of spread of the disease. Secondarily, aerially dispersed sporangia are believed to contribute to the epidemiology of SOD. A fact-sheet with more detail is attached. This factsheet outlines the species of trees and plants susceptible to this disease.

Since we will not be working with infected plants, and we will be maintaining contained cultures of the fungus, the risk of spread of infectious material is minimal. The level three containment will be an added measure of security that will hopefully satisfy the CFIA and allow the disinfectant efficacy testing to be performed in Alberta.

Our experiments are all in a miniaturized system performed in a laboratory using 12-well tissue culture plates. We do not have a greenhouse component to our efficacy tests. We would need to culture the pathogen on agar plates, amplify inoculum in broth cultures, incubate inoculated surfaces (in 12-well plates) and treat with various disinfectants. The treated plates are then sampled for surviving inoculum by secondary incubation in broth. The process takes approximately 5-days and would need to be repeated at least three times. We would like to commence our experiments in February, 2007 and complete the work by March, 2007.

Culture plates and liquid inoculum are immediately autoclaved and disposed as biological waste. We have been sterilizing and re-using our 12-well tissue culture plates using dry heat, but these could also be autoclaved and discarded a after each use if necessary.

Please let me know if you need any additional information. Thanks for your assistance in this work.

Best regards,
Mike

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