CANADIAN PHYTOPATHOLOGICAL SOCIETY

MARITIME REGION MEETING 2006



Atlantic Horticulture and Food Research Centre, Agriculture and Agri-Food Canada, Kentville, Nova Scotia

November 23, 2006

Canadian Phytopathological Society Maritime Region Meeting November 23, 2006 Atlantic Horticulture and Food Research Centre, Kentville, NS

INTRODUCTION

Welcome to Kentville and to the 2006 Canadian Phytopathological Society Maritime Region Meeting. It has been a full year since we got together as a group, and I look forward to a stimulating afternoon of scientific discussion and fellowship.

There are 10 oral presentations and one poster presentation on various topics in phytopathology scheduled for the afternoon. A dinner and presentations will take place at Rosie's Restaurant and Paddy's Pub following the meeting.

I am pleased and honoured to welcome Dr. Verna Higgins, Emeritus Professor, Department of Cell and Systems Biology (formerly Botany), University of Toronto, ON, as our keynote speaker. Dr. Higgins is renowned for her work on host-pathogen interactions and has contributed to many outstanding achievements in her field of research. Her presentation is entitled: "**Understanding disease resistance: Has it helped in the field?**".

I am also pleased to welcome Dr. Bruce Gossen, current president of CPS, and would like to thank him for taking the time to attend our regional meeting. Maintaining this connection with our national society is an important aspect of our own development as a regional group.

Many thanks to all who attended the meeting. I would particularly like to thank Dr. Gordon Braun, AAFC, Kentville and his assistants for hosting this year's meeting and taking on the responsibilities of organizing the meeting, scheduling the presentations and preparing the book of abstracts. I would also like to thank the Canadian Phytopathological Society, represented by president Bruce Gossen, for sponsoring this event. I trust we will be able to get together on a regular basis in future, and on that note, we will host the 2007 meetings in Charlottetown, PE.

This booklet contains abstracts of the oral and poster presentations in the order that they were presented. All abstracts will subsequently be published in an upcoming edition of the Canadian Journal of Plant Pathology. The research work represented by these papers forms an important part of the on-going development of phytopathological research in the Maritimes.

Rick D. Peters Maritime Region Rep Canadian Phytopathological Society Canadian Phytopathological Society - Maritime Regional Meeting 2006 Scientific Program

Thursday, 23 November 2006 Cornwallis Room, Kentville Research Centre

13:00-13:10 Welcome to AFHRC Kentville: Dr. Roy Bush - Research Manager

13:10-13:20 Welcome and Introduction: Rick D. Peters, CPS Representative - Maritime region

13:20-13:30 Greetings from CPS: Dr. Bruce Gossen - CPS President

13:30-14:15 **Keynote Speaker: Dr. Verna J. Higgins**, Emeritus Professor, Department of Cell and Systems Biology (formerly Botany), University of Toronto, ON. "Understanding disease resistance: Has it helped in the field?"

Session A: Bacterial Plant Pathogens

14:15-14:30 Evaluation of products for the control/suppression of common scab (*Streptomyces scabies*) in Shepody potatoes. **Robert Coffin**, Claudia Goyer, William Hardy, Michelle Webber, Bryce Drummond, Lauren Johnson, and Steven Watts.

14:30-14:45 Molecular detection of *Clavibacter michiganensis* subsp. *insidiosus*, causal agent of bacterial wilt of alfalfa. L.J. Ward, J. Gourley and **S.H. De Boer**.

14:45-15:00 An artificial positive control for use in the real-time PCR detection of *Clavibacter michiganensis* subsp. *sepedonicus*. **D.S. Smith**, J. Gourley and S.H. De Boer.

15:00-15:30 Nutrition Break and Poster Session

A novel technique for assessing resistance to lesion expansion caused by *Sclerotinia sclerotiorum* in canola and sunflower. C. Olivier, **B.D. Gossen**, and G. Séguin-Swartz.

Session B: Fungal Plant Pathogens

15:30-15:45 Trimming the carrot canopy to reduce the incidence of Sclerotinia rot. **R. D. Peters** and K. R. Sanderson.

15:45-16:00 A novel technique for inoculation of field and horticulture crops with *Sclerotinia* spp. **B. D. Gossen** and M. R. McDonald.

16:00-16:15 Evaluation of products for prevention of rot in stored tubers exposed to late blight (*Phytophthora infestans*) inoculum. **Robert Coffin**, William Hardy, Bryce Drummond, Lauren

Johnson, Steven Watts, Melvin McQuillan, David Bell.

16:15-16:30 *Muscodor albus* volatiles control toxigenic fungi under CA storage conditions. **P.G. Braun**, M. Vailati, R.K. Prange and E.W.E. Bevis.

16:30-16:45 Valdensinia leaf spot of lowbush blueberry: an emerging new threat. **P.D. Hildebrand** and W.E. Renderos.

- 16:45-17:00 Bud Platt
- 17:00-17:10 Wrap-up
- 17:30- Dinner Rosie's Restaurant and Paddy's Pub.

Note to presenters; Please ensure that your presentation is given to the audio/visual coordinator at least 1 hour prior to the start of the session

Session A: Bacterial Plant Pathogens

14:15-14:30

Evaluation of products for the control/suppression of common scab (*Streptomyces scabies*) in Shepody potatoes. Robert Coffin¹, Claudia Goyer², William Hardy¹ Michelle Webber¹, Bryce Drummond¹, Lauren Johnson¹, and Steven Watts³. ¹Cavendish Farms, Kensington, PEI; ²Agriculture and Agri-Food Canada, Fredericton, N. B.; ³Engage Agro.

Replicated field plots were established on PEI to assess different products for suppression of scab. Shepody seed tubers with severe surface and pitted scab were used for trials. In 2005, seed pieces were treated with powdered sulfur (1kg.per 100kg.seed), Javex bleach (whole tuber dipped for one minute in 10% solution), "green clay" (in -furrow application at 11itre/ ha and 10 l/ha) and an in-furrow application of Polyram (metiram) fungicide (10kg/ha). Control plots did not receive any treatment. A slight suppression of scab symptoms, in daughter tubers, was noted in the (metiram) treatments. No suppression of scab occurred in the other treatments. In 2006, seed piece treatments of streptomycin sulfate 25.2% at 1kg. product /100kg of cut seed slowed plant emergence, slightly reduced tuber yields but gave a reduction in scab on daughter tubers, both in the percent of tubers infected and the severity of symptoms. Javex did not reduce the frequency of infected tubers nor severity of symptoms Seed piece treatments with oxytetracyclene (1kg product/100kg seed) caused severe retardation in plant emergence but negligible scab was evident on the daughter tubers. Common scab is caused by bacteria and antibiotics might offer a feasible method of scab suppression if plant phytotoxicity problems can be overcome.

Session A: Bacterial Plant Pathogens

14:30-14:45

Molecular detection of *Clavibacter michiganensis* **subsp.** *insidiosus*, **causal agent of bacterial wilt of alfalfa.** L.J. Ward, J. Gourley and S.H. De Boer. *Charlottetown Laboratory, Canadian Food Inspection Agency, Charlottetown, PE C1A 5T1 Canada.*

Alfalfa bacterial wilt caused by *Clavibacter michiganenesis* subsp. insidious (Cmi) is largely controlled by the use of resistant cultivars, but infected seed is of concern because it is the main means of spreading the bacterium into new production areas. Sensitivity and specificity of current seed-testing methods, based on culturing and serological identification, can be improved by molecular methods. We evaluated a conventional PCR assay using primers based on the intergenic spacer region of the rrn operon in a BIO-PCR format. The procedure involved enrichment of seed samples in a bacterial growth medium followed by DNA extraction and PCR amplification. A single artificially contaminated seed could be detected in a sample of 2500 seeds. However, the PCR method occasionally resulted in a false positive test based on detection of the 135 bp amplicon in electrophoresis gels. We also evaluated a second method that was formatted as a real time PCR Taqman assay and based on a modification of published primers and hybridization probe targeting an insertion element. The published PCR primers cross-reacted with the related bacterium Clavibacter michiganensis subsp. sepedonicus (Cms) but amplicons could be distinguished using Evagreen in a melt analysis. Inclusion of the modified hybridization probe in a Tagman assay resulted in specific detection of Cmi. Bioassays involving rootinoculation of alfalfa seedlings and subsequent verification of plant infection using the PCR Taqman assay was explored as a confirmatory test for Cmi-positive samples. Furthermore, a Cms Taqman probe was also developed and proved useful in a real time PCR test for Cms with the Cmi forward and a unique reverse primer in simplex or three-primer/two-probe multiplex assay.

Session A: Bacterial Plant Pathogens

14:45-15:00

An artificial positive control for use in the real-time PCR detection of *Clavibacter*

michiganensis subsp. *sepedonicus*. D.S. Smith, J. Gourley and S.H. De Boer. *Canadian Food Inspection Agency, Charlottetown Laboratory, Charlottetown, PE C1A 5T1 Canada.*

Bacterial ring rot of potato, caused by *Clavibacter michiganensis* subsp. sepedonicus (Cms) is an economically important disease and its early detection is essential to prevent its spread. PCR is emerging as the method of choice for many diagnostic applications because of its high sensitivity and is useful for detecting Cms. A reaction control (RC), however, is ideally required in any PCR test to validate negative results. A previously published real-time TaqMan PCR method for Cms detection was modified to incorporate an artificial construct that functioned as an internal RC. The construct, consisting of an invertase gene fragment flanked by sequences complementary to the Cms primers, was designed to generate an amplicon that differed from the Cms-specific amplicon but would confirm the absence of PCR inhibitory substances in template DNA. The RC construct was cloned into plasmid vector pCRII-TOPO and propagated in Escherichia coli strain DHa-T1. Partial sequencing of the resulting plasmid, pCmsC4, confirmed the presence of the RC insert. The RC amplicon did not react with the Cms-specific TaqMan probe, but could be detected with the inclusion of SYBR Green in the reaction mix. The Cms-specific and the RC amplicons could be readily distinguished by their respective postreaction melting temperatures in the presence of SYBR Green. The addition of 100 copies of pCmsC4 to the reaction did not adversely affect the limit of Cms detection in the TaqMan assay. Using this method, 28 of 28 known positive samples were correctly identified as positive, while 50 of 50 known negative samples tested negative for Cms. Based on the RC performance, 3 of the 50 negative extracts appeared to contain inhibitors. Preliminary validation data indicated that this modified TaqMan method has promising potential for investigating possible bacterial ring rot outbreaks and for confirming or resolving inconclusive results generated by other methods.

Poster Session 15:00-15:30

A novel technique for assessing resistance to lesion expansion caused by *Sclerotinia sclerotiorum* in canola and sunflower. C. Olivier, B.D. Gossen, and G. Séguin-Swartz. *Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, S7N 0X2.*

A novel inoculation technique was designed to assess the sclerotinia stem rot reaction of three canola (*Brassica napus* L.) lines ('Westar', 'Ebony' and AAFC line 14678) in controlled environmental conditions and in field tests. The main stem of a canola plant at early flower was clipped off and an Eppendorf tube containing a PDA culture of *S. sclerotiorum* (Lib.) de Bary was inserted on the cut stem. Lesion length was measured 7 days after inoculation and at harvest time in field nurseries. The Eppendorf inoculation technique was compared with inoculation using infested petals under controlled environmental conditions and with natural infection in field nurseries. In the growth room, lesion length resulting from the Eppendorf inoculation was positively correlated with lesion length obtained with infested petals. In field trials, the correlation with natural inoculation was generally significant under wet weather conditions (conducive for epidemic development), but not significant under dry weather conditions, where there was little or no natural infection. The Eppendorf technique was also assessed in sunflower (*Helianthus annuus* L.) in a field trial by inoculating cut petioles at heading. In sunflower, the Eppendorf inoculation technique produced high incidence of stem rot, which often resulted in complete collapse of the plant, under severe drought conditions.

15:30-15:45

Trimming the carrot canopy to reduce the incidence of Sclerotinia rot. R.D. Peters and K.R. Sanderson. *Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, 440 University Ave., Charlottetown, PE C1A 4N6, Canada.*

Previous research has indicated that mowing the carrot canopy can prevent the establishment of conditions that are conducive to the development of Sclerotinia rot, caused by *Sclerotinia sclerotiorum*. To further develop this concept as a tool for plant disease management, a prototype carrot foliage trimmer (CFT) was designed and manufactured at the Harrington Research Farm in Prince Edward Island. The trimmer unit was designed to trim four adjacent carrot rows using rotary saw blades that could be adjusted for width to define the severity of cut. The CFT also has a series of lifter bars which lift older foliage lying on the soil surface prior to cutting with the blades. In this way, carrot canopies are opened to allow sunlight to penetrate and foliage to dry, and older senescing tissues that are most susceptible to infection are also removed. Initial evaluation of the CFT in 2006 indicated that mowing at row closure significantly (P=0.05) reduced the incidence of Sclerotinia rot in foliage and harvested carrots placed in storage. The CFT provides an environment-friendly option to control a disease for which there are currently no adequate control measures.

15:45-16:00

A novel technique for inoculation of field and horticulture crops with *Sclerotinia* **spp.** B.D. Gossen and M.R. McDonald. *Agriculture and Agri-Food Canada, Saskatoon Research Centre,* 107 Science Place, Saskatoon, SK, Canada S7N 0X2 and University of Guelph, Guelph, ON, Canada N1G 2W1.

Sclerotinia sclerotiorum (Lib.) de Bary causes severe injury on a wide range of field and horticultural crops. Infection of healthy tissues occurs via mycelium, which generally originate from sclerotia or from senescent tissue such as flower petals infected by ascospores. A novel inoculation technique was designed to create artificial epidemics in field or greenhouse conditions that simulate infection from either source. Sheets of a coarse grade of filter paper were cut to fit into shallow aluminium trays and then steam sterilized. Plugs (about 4 mm²) from 1-wk-old colonies of S. sclerotiorum grown on PDA were placed on the filter paper at 5-cm centres. The paper was saturated with ¹/₂ strength sterile potato dextrose broth, and the trays were covered with transparent cling-wrap. The filter paper was incubated at 22 °C until mycelium covered the surface, then dried in a laminar flow hood overnight and chopped to produce small pieces intended to simulated the size and texture of infected flower petals. The pathogen survives on the simulated petals for extended periods (> 3 mo) when stored under dry conditions at -18 °C. In 2005, simulated petals were applied to a small-plot trial of lettuce grown on muck soil at the Bradford Marsh, ON, and the trial was expanded in 2006 to include S. minor Jagger. In both years, inoculation increased disease incidence relative to the control, and produced disease levels sufficient for studies of fungicide efficacy.

16:00-16:15

Evaluation of products for prevention of rot in stored tubers exposed to late blight (*Phytophthora infestans*) inoculum. Robert Coffin¹, William Hardy¹, Bryce Drummond¹, Lauren Johnson¹, Steven Watts², Melvin McQuillan³, and David Bell⁴. ¹Cavendish Farms, Kensington, PEI; ²Engage Agro; ³Agr. Storage Man. Inc.; ⁴UAP

The late blight pathogen can colonize potato tubers if spores contact healthy tubers during mechanical harvest operations. An experiment was conducted to assess the effect of Javex bleach, Oxidate and two sources of phosphorous acid (Phostrak from Omex, K-fight from UAP) on potato tubers exposed to late blight inoculum. Shepody and Russet Burbank tubers were scratched with a wire brush to break the integrity of the skin. Tubers were tumbled in plastic bags with late blight infected leaves (sporulating). Tubers were immediately sprayed with 6.5 litres of spray solution per tonne of potatoes. The Javex was a 10% solution, Oxidate was a 1% solution and the phosphorous acid products at 400 mls of product plus water per tonne of potatoes. Check treatments were comprised of scraped and non- scraped tubers; some being exposed and non-exposed to late blight. No rot occurred in the scraped and non- scraped tubers that were not exposed to late blight. For scraped tubers exposed to late blight, after three weeks of storage at 10-12 Celsius, all tubers in the "check", Javex and Oxidate treatments were exhibiting dry and wet rot. Rot developed most quickly in the Javex and check treatments and more slowly in the Oxidate. Despite the physical damage (scraping) and inoculum (late blight), no rot occurred in the tubers treated with the products containing phosphorous acid.

16:15-16:30

Muscodor albus volatiles control toxigenic fungi under CA storage conditions. P.G. Braun, M. Vailati, R.K. Prange and E.W.E. Bevis. *Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada. 32 Main St., Kentville, NS, B4N 1J5, Canada.*

Muscodor albus, a biofumigant fungus, has great potential in managing plant pathogens in postharvest storage. It has been shown to produce over 20 volatile compounds with fungicidal and fungistatic properties. However, M. albus is a warm climate endophyte and its biofumigant activity is significantly inhibited at temperatures below 10°C. Seven ochratoxin-producing fungi, Aspergillus carbonarius, A. flavus, A. niger, A. ochraceus, Penicillium verrucosum, Fusarium culmorum, and F. graminearum were completely controlled by exposure to volatiles from 2 g/L M. albus-colonized rye grain in sealed glass jars for 24 h at 20°C. Two major volatiles of M. albus, isobutyric acid (IBA) and 2-methyl-1-butanol (2-MB) at 50 µL/L and 100 µL/L, respectively, gave differential control of the seven fungi when applied individual at 20°C. When the fungi were exposed to both IBA and 2-MB together an average of 94% of the spores were killed. In a factorial experiment with controlled atmospheres (CA) at 3°C with 72 h exposures to five concentrations of IBA and 2-MB combinations, CA atmospheres had no significant effect. At the highest volatile concentration of 50 µL/L IBA and 100 µL/L 2-MB all seven fungi were completely controlled at 3°C, regardless of atmosphere. Major volatiles of *M. albus* may have significant potential for managing plant pathogens in either air or CA storage. However, combinations of volatiles may be required to provide a broader spectrum of control than individual volatiles.

16:30-16:45

Valdensinia leaf spot of lowbush blueberry: an emerging new threat. P.D. Hildebrand and W.E. Renderos. *Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, 32 Main St. Kentville, NS B4N 1J5, Canada.*

An unusual leaf spot of lowbush blueberry was first observed in 1997 in two fields of Nova Scotia. The disease caused defoliation of blueberry stems, but was confined to an area of about $1m^2$. However, by 2004, the disease was more common and in 2005 and 2006, severe defoliation occurred in numerous fields resulting in estimated yield reductions of >60% in some cases. The causal organism was identified as Valdensia heterodoxa Peyr., the anamorph of the ascomycete Valdensinia *heterodoxa* Peyr. Symptoms on blueberry appear as circular, brown (sometimes zonate) lesions variable in size up to 1 cm in diameter. A single lesion frequently results in leaf abscission. Defoliated cropping stems may recover by producing new leaf shoots, but sprout stems produce new vegetative shoots in leaf axils where flower buds normally occur resulting in reduced yields the following year. The disease usually begins in moist, shaded areas of fields during early June as small foci (10 cm diam) encompassing a few stems. Large stellate conidia (up to 600 um diam) are produced on abscised leaves which are forcibly propelled upward landing on healthy leaves of adjacent stems. During wet weather, disease foci expand and coalesce to eventually encompass large areas. The disease subsides during dry weather, but may resume in late summer with the return of more frequent rain. Fungicide applications in June 2006 showed that Pristine WGTM (boscalid and pyraclostrobin) reduced disease, whereas chlorothalonil, propiconazole and captan were less effective. This is a first report of V. heterodoxa causing economic losses to lowbush blueberry. We Valdensinia this disease. propose the name leaf spot for

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