


Canadian Agri-Science Cluster for Horticulture 2

Progress Report April 2016

Activity 12, Apple 11

New Biological Control Agents for Postharvest Diseases of Pome Fruit

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Activity Objectives

We have identified several bacterial antagonists from Canadian soils which show efficacy against postharvest pathogens of pome fruit under controlled atmosphere and air storage. Our objectives are to test these antagonists in storage trials in British Columbia and Ontario in order to assess their potential for commercial development. This will be accomplished by determining:

1. the efficacy of the bacterial antagonists under varying storage conditions and with different fruit varieties,
2. optimal concentrations and timing of application of the antagonists,
3. the effect of the antagonists on fruit quality,
4. the performance of the antagonists alone or in combination with other chemical control methods
5. the suitability of the antagonists for commercial development

The long-term objective of this research is to enhance the quality and safety of Canadian fruit and increase Canada's competitive ability in global markets.

Research Progress to Date

British Columbia: For 2015-16, the British Columbia postharvest disease management studies were conducted using four apple cultivars, Gala, McIntosh, Spartan, and Ambrosia, in two storage environments, cold and controlled atmosphere (CA). The experiments were conducted at the BC Tree Fruit Cooperative in Winfield, BC. Three experiments were carried out to address objectives 1-4. The first experiment investigated the three isolates of *P. fluorescens*, 1-112, 2-28, and 4-6, for biological control activity against blue mold on all four apple cultivars (obj.1). The results from 2015-16 showed that *P. fluorescens* isolates were effective against blue mold (*P. expansum*) on McIntosh, Spartan, and Ambrosia apples in cold and CA storage.

The second experiment investigated the optimal concentration of the antagonists (obj. 2). *P. fluorescens* 4-6 was tested at 4 different concentrations, 10^6,10^7,10^8 and 10^9 CFU/ml, on two apple varieties, 'McIntosh' and 'Ambrosia', against blue mold, gray mold *(B. cinerea*), and mucor rot (*M. piriformis*). Results varied with apple variety and pathogen, but greater control was observed when apples were treated with higher concentrations of *P. fluorescens*. After the 15 weeks of incubation in cold storage the apples were assayed for firmness, starch and sugar content, in order to assess the effect of the antagonists on fruit quality (obj. 3). Apples treated with *P. fluorescens* isolates did not significantly differ in regards to firmness, starch and total soluble solid content in comparison to apples that were not treated with *P. fluorescens*. The efficacy of the antagonist, *P. fluorescens* 4-6 in combination with the GRAS compounds, sodium bicarbonate (SBC), salicylic acid (SA) or calcium chloride ($CaCl_2$), against the three fungal pathogens on 'McIntosh' and 'Ambrosia' apples was investigated in cold storage (obj.4). The results from 2015-16 showed that *P. fluorescens* 4-6 in combination with the three GRAS compounds was effective against mucor rot and gray mold for up to 105 days. There was no negative effect of SBC, SA and $CaCl_2$ or chemical fungicides alone on the 'McIntosh' and 'Ambrosia' apple fruit after 105 days of treatment at 4°C. To further our understanding of the mechanism of action of the antagonists each isolate of *P. fluorescens* was screened for the presence of genes for pyrrolnitrin, pyoluteorin, 2,4-diacetylphloroglucinol, hydrogen cyanide and phenazine-1-carboxylic acid synthesis using Polymerase Chain Reaction (PCR). PCR amplification of *phzCD* and *hcnBC* genes indicated the potential of isolates 1-112 and 4-6 to produce the antibiotic, phenazine-1-carboxylic acid, and of isolate 2-28 to produce hydrogen cyanide, respectively. Further studies looking at gene expression are planned using reverse transcriptase PCR. Scanning electron microscopy studies are planned *in vivo* to elucidate how the bacteria interact with the fungi on apple.

Ontario: Two soil isolates of *P. fluorescens* 4-6, and 1-112 were tested *in vivo* as potential biocontrol agents under cold storage conditions with three varieties of apple in 2015-16: Gala, McIntosh, and Empire. The experiments were conducted at the London Research and Development Centre at AAFC in Vineland Station, Ontario. In the first study of 2015-16, an isolate which exhibited biocontrol potential, *P. fluorescens* 4-6 was tested in combination with GRAS compounds, SBC, SA, and $CaCl_2$ for the control of blue mold and gray mold in 'McIntosh' and 'Gala' apples (obj.4). Physiological parameters of fruit firmness, soluble solids and titratable acidity were recorded monthly for three cultivars of apples. The results from 2015-16 showed that *P. fluorescens* 4-6 in combination with the three GRAS compounds was effective against blue mold and gray mold for up to 60 days. There was no negative effect of SBC, SA and $CaCl_2$ or chemical fungicides alone on the 'McIntosh' and 'Gala' apple fruit after 120 days of treatment at 4°C and in the subsequent shelf-life study for one week at a 20°C following the cold treatment. The chemical fungicide Scholar gave complete control of blue mold and gray mold for up to 120 days after treatment at 4°C and in the subsequent shelf-life study for one week at 20°C following the cold treatment. In the second study of 2015-16, two isolates which exhibited biocontrol potential, *P. fluorescens* 4-6 or 1-112 alone or together were tested in combination with Scholar (fludioxonil) or Mertect (thiophanate methyl) at ½ of the recommended rate or full rate for management against blue mold and gray mold in 'McIntosh' and 'Empire' apples. The results show that the biocontrol antagonists *P. fluorescens* 4-6 or 1-112 alone or together in combination with ½ rate or full rate of Scholar or Mertect gave complete control of blue mold and gray mold on 'McIntosh' and 'Empire' apples for up to 120 days.

Extension Activities

British Columbia data were presented at two regional conferences, two international conferences, a BC Tree Fruits industry meeting, as well as guest seminars at The University of British Columbia Vancouver and the University of Guelph (Canadian Society of Microbiologists award). Ontario data were presented at one international and two regional conferences.

Key Message(s)

Biological controls are a promising alternative to chemical fungicides for control of postharvest pathogens of pome fruit. Our results suggest that *P. fluorescens,* with a variety of modes of action, has potential for control of common postharvest fungal pathogens during commercial cold and controlled atmosphere storage. Its efficacy may be enhanced by use in combination with selected GRAS compounds or with lower doses of chemical fungicides.

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