Canadian Agri-Science Cluster for Horticulture 2

Progress Report April 2015

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

In British Columbia three strains of *Pseudomonas fluorescens* 4-6, 1-112 and 2-28, isolated from the rhizosphere of pulse crops in Western Canada were examined as potential biocontrol agents under commercial cold and Controlled Atmosphere (CA) storage conditions with four varieties of apple: Gala, McIntosh, Spartan and Ambrosia. All three isolates inhibited the growth of *B. cinerea*, *P. expansum*, and *M. piriformis* on plates. Lesion diameters of apples inoculated with each of the three pathogens and biocontrol strains were determined in commercial cold storage or after CA storage and

compared with the fungicide Scholar® (fludioxonil) and the biocontrol agent Bio-Save® (*P. syringae*).

Overall, the efficacy of *P. fluorescens* strains varied with pathogen, storage environment and apple variety in year 2 storage trials. Disease control by *P. fluorescens* isolates on the 4 varieties of apples was comparable to Bio-Save® but less effective than the fungicide Scholar®. In general, strain 4-6 provided the best disease control on the four varieties of apples in the two storage environments tested. Significant differences in physiological fruit quality parameters, firmness, titratable acidity, starch and sugar content, were observed between apple varieties before and after commercial storage and may contribute to their differing susceptibility to pathogens and also may influence the efficacy of the biocontrol agent. The antagonists generally showed higher efficacy at the highest concentration tested of 1 x 10^9 CFU/ml.

Mechanism of action studies showed that all three *P. fluorescens* strains produced proteinases, enzymes that degrade proteins, but were negative for the production of cell wall-degrading enzymes. All three strains of *P. fluorescens* inhibited conidial germination and mycelial growth of *B. cinerea*, *M. piriformis*, and *P. expansum*, and produced volatile compounds that are inhibitory to the three fungal pathogens, *B. cinerea, M. piriformis* and *P. expansum.* Potential mechanism of actions of *P. fluorescens* may include competition for nutrients, competition for space, production of inhibitory metabolites, targeting conidial germination and mycelial growth, suggesting that development of resistance by fungal pathogens to these biocontrol agents is unlikely.

In Ontario in year 1, the three soil isolates of *Pseudomonas fluorescens* 4-6, 1-112 and 2-28, were examined as potential biocontrol agents under different cold storage conditions with five varieties of apple: Honey Crisp, Gala, McIntosh, Ambrosia and Empire. All three isolates inhibited the growth of the postharvest pathogens, *B. cinerea* and *P. expansum* on plates. Disease incidence of apples inoculated with each of the two pathogens and biocontrol strains were determined after every 28 days in cold storage and compared with the fungicide Scholar® (fludioxonil), the biocontrol agent Bio-Save® (P. syringae) and Bio-Save® in combination with Mertect® (thiabendazole). On all five varieties of apples in cold storage, *P. fluorescens* isolate 4-6 was the most effective at inhibiting the two fungal pathogens, *B. cinerea*, and *P. expansum,* isolate 1-112 was next most effective and *P. fluorescens* isolate 2-28 was the least effective among the three *P. fluorescens* biocontrol strains tested. Isolates 4-6 and 1-112 were as effective as the formulated commercial biocontrol product, Bio-Save, but less effective than the chemical control, Scholar, which consistently gave 100% control of blue mold and gray mold. There was no direct correlation between the physiological parameters of fruit firmness, soluble solids and titratable acidity and postharvest disease control by the antagonists in different cultivars of apples. Following CA storage, the three antogonists, *Pseudomonas fluorescens* 4-6, 1-112 and 2-28, were tested against blue mold and gray mold on two apple cultivars, McIntosh and Empire apples, but none of the apples that were stored in CA storage could be infected. Pathogens were demonstrated to be viable as shown by the growth of inoculum in our spot tests on Petri dishes. Three concentrations of antagonist, 1 x 10^7 CFU/ml; 1 x 10^8 CFU/ml; 1 x 10^9 CFU/ml were tested against blue mold and gray mold on two apple cultivars, McIntosh and Empire apples and

the two higher concentrations were the most effective. All three experiments from year 1 were repeated in year 2 and the data are being analyzed. CA storage experiments are underway and will be completed in June 2015.
Early Outcomes (if any) or Challenges Studies in British Columbia and Ontario show similar outcomes for efficacy of the three *Pseudomonas fluorescens* against three postharvest fungal pathogens with control similar to that of a commercial biocontrol product already on the market, but less effective than that of the synthetic fungicide, Scholar.
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.

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Growing Forward 2
A federal-provincial-territorial initiative