Biological Control of Postharvest Fruit Pathogens in Papaya

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First of all, since the area of biological control is so vast, I will be restricting my talk to the area of biocontrol of postharvest fruit pathogens.

Fungicides are a primary means of controlling postharvest diseases. However, as a result of public concern about the presence of synthetic chemicals in our food supply and environment, several fungicides have been banned by the U. S. Environmental Protection Agency, or have been voluntarily withdrawn from the market for postharvest use (Wisniewski and Wilson 1992). The papaya industry has also experienced the temporary loss of registration of the use of Dithane fungicide. We now face an urgent need to develop new and effective methods of controlling postharvest diseases, not only for papaya, but for other commodities as well.

Sanitation and exclusion can help reduce inoculum level of pathogens, the use of non-selective chemicals (sodium carbonate, sodium bicarbonate, active chlorine, and sorbic acid), and heat treatments can lower the disease pressure on harvested commodity. Minimizing injury to the commodity during harvesting and postharvest handling, and maintaining the commodity at storage conditions that optimize host resistance, will also aid in suppressing disease development after harvest (Wisniewski and Wilson 1992). And, recently, attention has been focused on biological control of postharvest diseases as an alternative to the use of fungicides.

What is biological control? Biological control of plant disease is defined as "the decrease of inoculum or the disease-producing activity of a pathogen accomplished through one or more organisms, including the host plant, but excluding man." (Kenneth F. Baker 1987)

The area of biological control of postharvest diseases has been revolutionized by Pusey and Wilson (1984), and Wilson and Pusey's studies (1985) on the biological agent, *Bacillus subtilis*, a bacterium which was applied directly to peaches after harvest to control brown rot, *Monilinia fructicola*. Since then, there have been numerous reports of other microorganisms that control postharvest diseases of various commodities (Table 1).

Commodities that have been reported to use biocontrol agents include: apple, apricot, citrus, cherry, grape, nectarine, peach, pear, pineapple, plum, and strawberry. The microorganisms used include bacteria, yeasts, and fungi. Some of the organisms will be elaborated on later.

What are some of the characteristics of an "ideal" postharvest biocontrol agent?

The ideal postharvest biocontrol agent is (1) genetically stable, (2) effective at low concentrations (3) not fastidious in its nutritional requirements (not be too "restrictive," or requiring of "exotic" ingredients), (4) amenable to production on inexpensive growth medium with a long shelf life, (5) easy to dispense (6) able to survive adverse environmental conditions (that is, compatible to commercial handling and storage practices, including low-temperature and controlled-atmosphere storage), (7) effective against a wide range of pathogens on a variety of commodities (to make it "cost effective" and increase its market value) (8) safe to human health, and (9) nonpathogenic to the host (Wisniewski and Wilson 1992).

How does the biocontrol agent work? What are possible modes of action?

Except for the production of antibiotic zones by the biocontrol agent in petri dishes when challenged with the pathogen, the mode of action of many of the biocontrol agents is poorly understood. When antibiotic production is not a factor, the mode of action probably involves a complex syndrome of characters, including nutrient competition, site exclusion, attachment of the antagonist (biocontrol agent) to the pathogen, induced resistance in the host, and direct parasitism of the pathogen (Wisniewski and Wilson 1992).

Biological control of postharvest diseases of fruits and suggested modes of action are detailed in Table 2. Under antibiotic production, except for the fungus *Trichoderma* sp., all of the antagonists are bacteria. Under nutrient competition and or induced resistance, *Pseudomonas syringae* which controls blue mold of apple, and *Enterobacter cloacae* which controls rhizopus rot of peach, are bacteria. *Acremonium breve* is a fungus, and *Pichia* *guilliermondii* is a yeast. Note the yeast, *Pichia guilliermondii*, because I will be detailing some of the work that's being done on this biocontrol agent.

As research on biological control of postharvest disease continues, our knowledge on how the antagonists work will increase, and this knowledge should help us to develop more reliable procedures for effective application of known biocontrol agents and efficient selection of other antagonists.

As mentioned earlier, the work of Drs. Wilson and Pusey (1984, 1985) had a significant impact on the field of biological control because they applied a biological agent to control a postharvest disease. The mode of action of the bacterium, *Bacillus subtilis*, isolate B-3, is the production of an antibiotic which inhibits the pathogen, *Monilinia fructicola*, which causes brown rot of peaches and other stone fruits. In an agar culture, the bacterium produces an antibiotic which results in an inhibition zone which appears as an area of clearing among mycelia of the fungus. In their studies, *B. subtilis* isolate B-3 was applied to wounded peaches, nectarines and apricots and compared with benomyl fungicide and water. B-3 was as effective as benomyl in controlling the brown rot pathogen.

How does all of this relate to the Papaya Industry?

Except for studies on the control of phytophthora root rot of papaya by microorganisms in soil by Dr. Wen Ko in 1971 and 1982, the area of biological control of pathogens of papaya has been ignored. Our laboratory became involved in the area of biological control of pathogens of papaya about 5 years ago. More specifically, we worked on biological control of Phytophthora fruit rot of papaya.

Papaya fruits and leaves were washed in distilled water, then the filtered "washes" were plated out on agar which were "seeded" with spores of *Phytophthora palmivora* or *Colletotrichum gloeosporioides*. "Clear" areas in the mycelial area indicated that microorganisms in the "washes" were inhibiting fungal growth. Plates "seeded" with *Colletotrichum gloeosporioides*, showed the inhibition effects from the washes more clearly than plates seeded with *Phytophthora palmivora*.

We isolated an unidentified bacterium, designated as Wa-60, which produces an antibiotic compound in media. Wa-60 was streaked on agar medium, incubated for 2-3 days, then challenged with spores of *Phytophthora palmivora* or *Colletotrichum gloeosporioides*. Zones of inhibition were pronounced on potato dextrose agar challenged with spores of *Colletotrichum gloeosporioides*.

Wa-60 also inhibited germination of *Phytophthora palmivora* zoospores in in vitro tests, and symptom development on papaya fruit. Inoculation tests on papaya fruits were conducted in which assay discs were dipped in cell-free broth extracts of Wa-60, placed on papaya fruit, then challenged (inoculated) with zoospores of *P. palmivora*. Fruits were held in humidity chambers consisting of plastic vinyl bins containing a layer of water on the bottom of the bins. The result of the inoculation tests on papaya fruit was the absence of phytophthora symptoms where discs were treated with cell-free extracts of Wa-60, compared to phytophthora symptoms on areas with water control discs.

How can biological control agents be used commercially?

Attempts are being made to commercialize some of the biocontrol agents. As part of this process, patents have been issued or are pending on some of these microorganisms (Table 3). The bacterial biocontrol agent, *Bacillus subtilis*, which has a patent, was incorporated into a fruit wax and was treated on peaches on a commercial packing line (Pusey et al. 1986, 1988).

The yeast biocontrol agent, *Pichia guilliermondii*, which controls gray mold of apple and green mold of citrus, also has commercial potential. McLaughlin et al. (1990) demonstrated that the addition of 2% calcium chloride to the yeast suspension, increased the ability of the yeast to control gray mold on apple. Hofstein et al. (1991) showed that the biocontrol activity of *Pichia guilliermondii* was enhanced with the addition of 10% of the normal rate of thiabendazole fungicide. In addition, a USDA-ARS researcher, Dr. Raymond McGuire, found that adding this yeast to fruit coatings inhibited green mold of grapefruit, and extended the shelf life of grapefruit for up to two months (Stanley 1993). At a commercial packing house, grapefruit were washed and inspected for defects, then the wax and yeast mixture was sprayed on the fruit surface. Fruit not treated with the yeast became decayed with Penicillium mold, while fruit coated with the wax and yeast remained healthy. Of special note: the yeast was originally discovered on lemons and has been patented by Dr. Charles Wilson. The fruit coating used in Dr. McGuire's research is called Nature Seal, which is an "edible" coating that is produced commercially.

These reports suggest that biocontrol procedures can be integrated into commercial postharvest operations.

With all of these antagonists reported to control postharvest pathogens, what's preventing their successful commercialization?

Three primary barriers have been (1) the relative ineffectiveness of antagonists (biocontrol agents) compared to chemical control procedures; (2) the procedural processes for governmental clearances that have yet to be streamlined; and (3) a lack of economic incentives. With regard to the latter, a huge investment of time and money is required to establish whether an antagonist has commercial potential.

There are also challenges in the development of fruit biocontrol agents: (1) limitations of the biocontrol agents, (2) adaptability to commercial processing and storage practices, (3) determining effect of a biocontrol agent on other microorganisms on fruit, (4) determining modes of action, (5) economic feasibility (cost, market potential, range of activity, patent potential), (6) potential pathogenicity to humans or other commodities, (7) public acceptance, and (8) potential for pathogens developing resistance to biocontrol agents (Janisiewicz 1988, 1991; Wilson and Wisniewski 1989; Wilson et al. 1991; Wisniewski and Wilson 1992).

This brings us to the ultimate challenge for biocontrol researchers: Develop biocontrol agents that are as effective as fungicides and are safer for humans and the environment.

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Reference to company and/or product names is only for purposes of information and does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others which may also be suitable.

Table 1. Reports of postharvest biological control (Wisniewski and Wilson 1992).

Biocontrol agent	Commodity	Disease	Reference year
Bacteria			
Pseudomonas syringae	Apple	Blue mold	1987
P. cepacia	Apple	Blue mold	1988
	Apple	Gray mold	1988
	Apple	Mucor rot	1987
	Pear	Blue mold	1988
	Pear	Gray mold	1988
P. gladioli	Pear	Gray mold	1989
Bacillus subtilis	Citrus	Green mold	1984
	Citrus	Sour rot	1984
	Citrus	Stem end rot	1984
	Nectarine	Brown rot	1984
	Peach	Brown rot	1984
	Apricot	Brown rot	1984
	Plum	Brown rot	1984
	Cherry	Brown rot	1986
Enterobacter cloacae	Peach	Rhizopus rot	1987
E. aerogenes	Cherry	Alternaria rot	1986
Yeasts			
Pichia guilliermondii	Apple	Blue mold	1990
	Apple	Gray mold	1988, 1990
	Citrus	Green mold	1989, 1990
	Citrus	Blue mold	1990
	Citrus	Sour rot	1990
	Grape	Gray mold	1988
	Grape	Rhizopus rot	1988
Cryptococcus spp.	Apple	Blue mold	1991
C. laurentii	Apple	Gray mold	1990
	Pear	Mucor rot	1990
C. flavus, C. albidus	Apple	Gray mold	1991
	Pear	Mucor rot	1990
Fungi			
Acremonium breve	Apple	Gray mold	1988
Trichoderma sp.	Citrus	Sour rot	1983
	Strawberry	Gray mold	1977
T. harzianum	Grape	Gray mold	1984
Attenuated strains of *Penicillium* sp.	Pineapple	Penicillium rot	1980

Table 2. Biological control of postharvest diseases of fruits and suggested modes of action (Wilson and Wisniewski 1989).

Commodity	Disease	Antagonist	
Antibiotic production			
Apple	Blue mold	*Pseudomonas cepacia*	
	Mucor rot		
Apricot	Brown rot	*Bacillus subtilis*	
Cherry	Brown rot	"	
	Alternaria rot	*Enterobacter aerogenes*	
Citrus	Stem end rot	*B. subtilis*	
	Sour rot	"	
	Green mold	"	
	Sour rot	*Trichoderma* sp.	
Nectarine	Brown rot	*B. subtilis*	
Peach	Brown rot	*B. subtilis*	
Pear	Blue mold	*P. cepacia*	
	Gray mold	"	
Plum	Brown rot	*B. subtilis*	
Nutrient competition (N) and/or induced host resistance (HR)			
Apple	Blue mold	*P. syringae*	(HR)
	Gray mold	*Acremonium breve*	(HR)
	Gray mold	*Debaryomyces hansenii* (=*Pichia guilliermondii*)	(N + HR)
Citrus	Green mold	"	"
	Blue mold	"	"
	Sour rot	"	"
Grapes	Gray mold	"	(N)
	Rhizopus rot	"	(N)
Peach	Rhizopus rot	*E. cloacae*	(N)

Table 3. Issued or pending patents for biocontrol microorganisms (Wilson et al. 1991).

Biocontrol agent	Commodity	Disease	Reference
Bacteria			
Bacillus subtilis	Stone fruit	Brown rot	Pusey & Wilson 1988
Pseudomonas cepacia	Pome fruit	Botrytis rot Penicillium rot	Janisiewicz & Roitman 1988
Fungi			
Acremonium breve	Pome fruit	Botrytis rot	Janisiewicz, 1988
Yeasts			
Pichia guilliermondii	Citrus	Various rots	Wilson & Chalutz 1989
	Stone fruit		
	Pome fruit	Chalutz & Wilson 1990	
Hanseniaspora uvarum			