Charles L. Wilson
USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV

P. Lawrence Pusey
USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA

Potential for Biological Control of Postharvest Plant Diseases

Postharvest diseases of fruits and vegetables are a major expense in food production. Losses are difficult to estimate reliably, but according to a 1965 U.S. Department of Agriculture survey, postharvest losses in fruits, nuts, and vegetables amounted to about 23% of the harvested crop (22). Losses in underdeveloped countries run even higher because of poor storage and food-handling technologies. Postharvest losses in tropical Africa and in India have been put at 30% (13). Such losses are greater in export than in domestic shipments and often prevent the export of certain food commodities, such as peaches and papayas, in the United States.

Harvested food has a higher value than the same crop in the field. A harvested food commodity carries the cumulative cost of soil preparation, planting, fertilization, watering, pest and weed control, harvesting, storage, distribution, and sales. Therefore, a 20% loss of a high-value commodity has a tremendous impact on the total food production budget. The high dollar value of the harvested crop presents unique opportunities for disease control. With harvested food, one can afford to contemplate and use control procedures that would be cost-prohibitive in the field. Cost-effective postharvest treatments

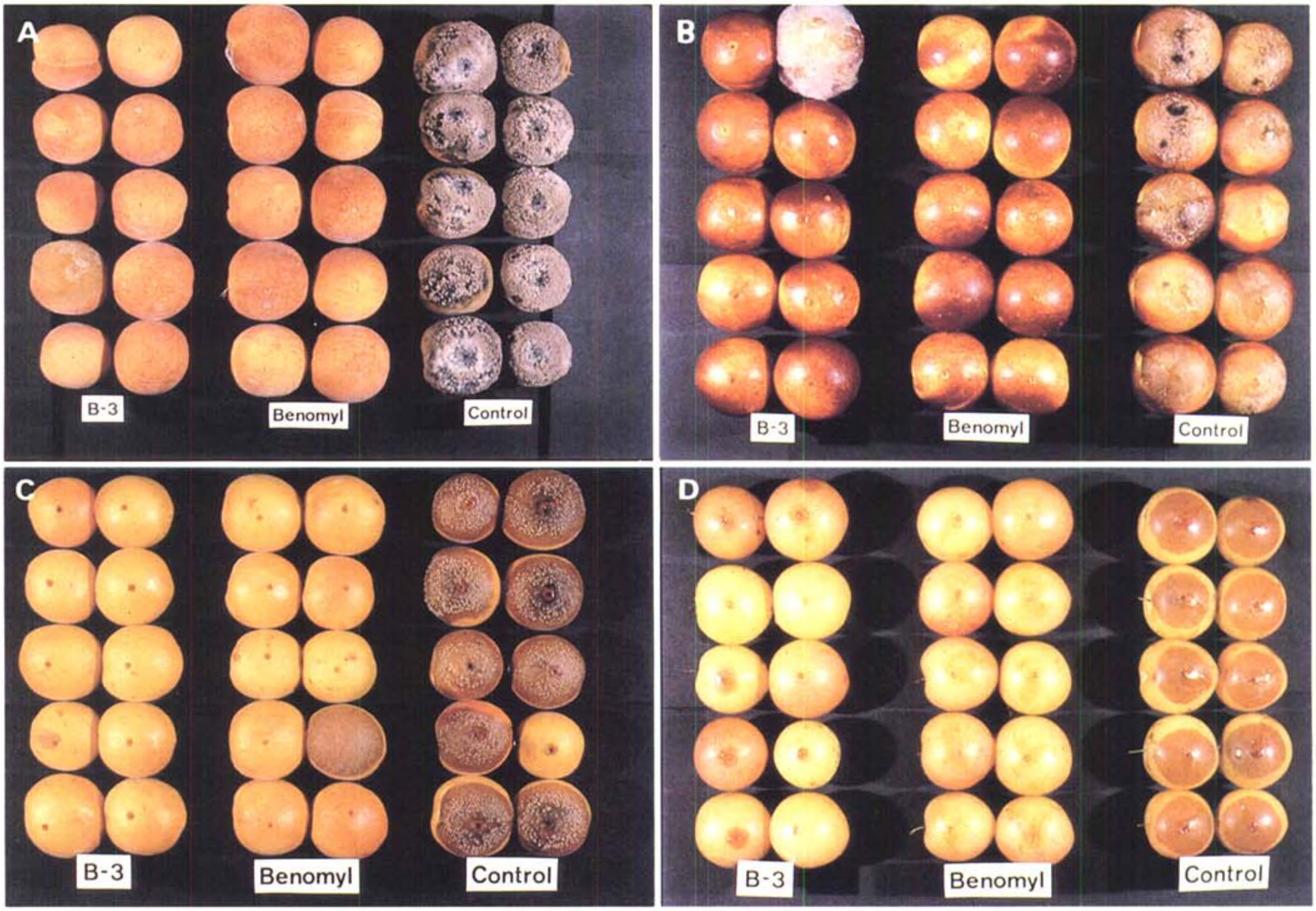


Fig. 1. Wounded (A) peaches, (B) nectarines, (C) apricots, and (D) plums 5, 3, 3, and 2 days, respectively, after treatment with *Bacillus subtilis* (B-3), benomyl, or water (control) and subsequent challenge with spore inoculum of *Monilinia fructicola*. Fungal mycelium on some fruit treated with B-3 or benomyl is that of naturally occurring *Rhizopus* sp.

include refrigeration and controlled-atmosphere storage to control rot organisms (7,18) and various fungicides and waxes (5,6).

Ethylene dibromide contamination has heightened public awareness of pesticide residues in our food. Consumers and scientists are looking at other sources of pesticide contamination, which will probably lead to more restrictions in the use of pesticides on harvested food. Also, resistance in microorganisms to pesticides applied after harvest has occurred rather frequently (3). These factors contribute to a weakening of the arsenal of weapons against the microorganisms responsible for most of our postharvest losses. Other than controlled storage, the major methods developed to control postharvest diseases are chemicals, heat, and irradiation (6); of these, only chemical treatments have found widespread application. The urgent need for new and more effective means of controlling postharvest diseases is obvious.

What about biological control of postharvest pathogens? Three factors indicate this may be an exceptionally productive area to explore. First, one of the main reasons for the failure of biocontrol procedures has been our inability to control environmental conditions. Under storage conditions for harvested food, exact environmental conditions can be established and maintained. Second, targeting biocontrol agents to the effective site is often difficult. Harvested food does not present that problem because the areas for application are much more limited than those on whole plants. Third, rather elaborate control procedures that may not be economically feasible under field conditions are cost-effective for harvested food.

Table 1. Successful biological control of postharvest diseases

Crop	Disease	Antagonist	Reference
Birch	Decay	*Trichoderma* sp.	Shields and Atwell (16)
Citrus	Green mold	*Trichoderma* sp.	de Matos (4)
Pine	Decay	*Trichoderma* sp.	Lindgren and Harvey (12)
Pineapple	Penicillium rot	Attenuated strains of *Penicillium* sp.	Lim and Rohrbach (11)
Potato	Soft rot	*Pseudomonas putida*	Colyer and Mount (1)
Stone fruits	Brown rot	*Bacillus subtilis*	Pusey and Wilson (14)
Strawberry	Botrytis rot	*Trichoderma* sp.	Tronsmo and Dennis (20)

Some Successful Attempts

With all this going for postharvest biocontrol, what has actually been done? Surprisingly little. In their recent book, Cook and Baker (2) list only two examples of biocontrol of postharvest diseases. One is the work of A. Tronsmo and associates in Norway with *Trichoderma* applied to blossoms to protect against fruit rots during storage. The other is Rishbeth's work (15) on the biocontrol of *Heterobasidion annosum* applied to cut stumps on a crop "residue." We might add the early work to control decay in pine logs in the South (12) and birch rot in Canada (16). Log preservation can correctly be considered postharvest disease control, but treating stumps with antagonists to protect standing trees (15) is questionable. Cook and Baker (2) state that "Although antagonists applied to control pathogens after harvest may seem only remotely practical or feasible, nevertheless the first successful biological control with an introduced antagonist used this approach [15]."

Despite being limited, work in the area of biological control of postharvest diseases has been encouraging. The few attempts have met with considerable success (Table 1), perhaps confirming our thesis that a number of factors may favor biocontrol in the postharvest environment. Lim and Rohrbach (11) were able to reduce the incidence of disease in pineapple fruit caused by *Penicillium funiculosum* by spraying the fruit with nonpathogenic strains of the pathogen. Tronsmo and Dennis (20) reduced the *Botrytis* responsible for preharvest and postharvest rotting of strawberries by spraying with *Trichoderma* species. The sprays were applied at the early flower stage, then at 14-day intervals, with the final spray 14 days after the first harvest. The antagonist performed as well as dichlofluanid applied according to the same schedule. Isolates of *Trichoderma* adapted to grow at low temperatures appeared to be more effective. Perhaps the antagonists could be used at harvest to prolong the shipping and marketing periods. Tronsmo and Ystaas (21) also demonstrated biocontrol of dry rot on apple caused by *Botrytis cinerea* and evaluated the treatment in the postharvest environment.

De Matos (4) evaluated a number of antagonists for biocontrol of green mold on citrus caused by *Penicillium digitatum* and was able to reduce mold incidence from 35 to 8% by inoculating the antagonist *Trichoderma viride* with the pathogen into the lemon peel. Colyer and Mount (1) recently showed that dipping seed pieces of the potato cultivar Superior in a suspension of the antagonistic bacterium *Pseudomonas putida* before planting reduced postharvest development of soft rot by 50%.

We took a slightly different approach toward postharvest disease control (14). We treated the commodity with an antagonist after, rather than before, harvest. We treated peaches, nectarines, apricots, and plums with suspensions of antagonistic bacteria. One, *Bacillus subtilis* (B-3), gave excellent control of brown rot caused by *Monilinia fructicola* in storage (Fig. 1). No fruit treated with 10^8 colony-forming units per milliliter of suspension developed brown rot, although other fungi caused decay after 9 days. A pilot test is being conducted with this procedure to determine whether or not it can be adapted commercially.

Storage Environment vs. Natural Environment

Antagonistic microorganisms for epiphytic biocontrol often are effective in petri dishes or in the greenhouse but fail in the field. Leben et al (9) indicate that destruction by ultraviolet rays and desiccation are major reasons for such failures. Storage conditions for food commodities do not present the same hazards for antagonists. This should greatly expand the range of antagonistic organisms that might be useful in postharvest biocontrol.

Refrigeration is a major way of manipulating the storage environment to control postharvest rot diseases. The cardinal temperatures for growth and reproduction of antagonists should correspond to those of the target pathogen (2). Once we gain a better understanding of how antagonists behave in different storage environments, we can manipulate these environments to favor the activities of the antagonists. Through selection, it has been possible to choose antagonists that adapt to low temperatures and consequently are effective biocontrol agents (19). Thus, biocontrol can be achieved or enhanced in storage environments by manipulating both the antagonist and the environmental parameters.

As food commodities mature in storage, they become "leaky." Nutrients that favor the growth of microorganisms become bountiful on food surfaces. This nutritional milieu favors development of rot pathogens but may also favor development of antagonists or parasites of the pathogens. Competition for nutrients in a particular biological niche is one form of biocontrol (2). We need to know more about the nutritional composition of the surfaces of stored foods and how this influences microbial behavior.

Microecosystem at Wound Site

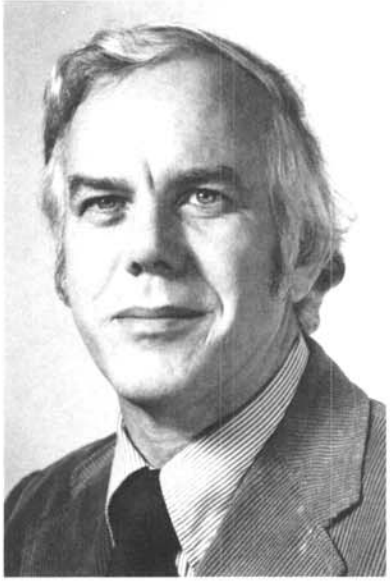
Many rot organisms require wounds for infection. The wound site is a special ecological niche in which nutrients abound for microbial growth (Fig. 2). Plant tissues generally respond to wounding by laying down protective barriers or wound periderm (4). Such responses generally delay the advance of rot microorganisms. We need to know more about host defense responses to wounding and the microecosystem at the wound site.

A new wound generally represents a "fresh" food supply for microorganisms. It is reasonable to assume that if antagonists well adapted to wounds occupy these sites first, other pathogenic microorganisms could be warded off. This has been our strategy in applying *B. subtilis* for the biocontrol of brown rot of peaches (Figs. 1 and 3) (14). Application of biphenol to control fruit rot in citrus is another example of this "firstest with the mostest" strategy for disease control at the wound site (5).

The normal ecological succession of microorganisms at wound sites on stored food surfaces needs investigation. Several studies have been made on successions of wound microorganisms in trees (17). Such studies may suggest ways of manipulating the succession to disfavor the rotting organism. Through nutritional manipulations, Hulme and Shields (8) were able to favor the development of *Trichoderma* species over rotting organisms in trees. It would be worthwhile to explore naturally occurring antagonists of parasites at wound sites. Cook and Baker (2) recommend that where disease does not occur or is less severe, a natural biocontrol system should be suspected.

Genetically Engineered Agents

Frequently, microorganisms are shown to be good antagonists in vitro (Fig. 4) but not in vivo. Also, we know that some epiphytic microorganisms are well adapted to an infection site but do not possess antagonistic or parasitic qualities. Genetic engineering may offer us the promise of joining the desired characteristics of both organisms into one that will be adapted ecologically and behave



Charles L. Wilson

Dr. Wilson is research leader of the Pathology Unit at the USDA-ARS Appalachian Fruit Research Station, Kearneysville, West Virginia. He received his B.A. degree from the University of Virginia and his M.S. and Ph.D. degrees from West Virginia University. From 1958 to 1968 he was a professor at the University of Arkansas. He was recently elected a fellow and honored by the Washington Academy of Sciences "for pioneering research in understanding and manipulating plant diseases."

P. Lawrence Pusey

Dr. Pusey is a research plant pathologist with the USDA at the Southeastern Fruit and Tree Nut Research Laboratory in Byron, Georgia. He received a B.S. degree in biology from Ball State University and an M.S. degree in botany from Kent State University. His Ph.D. degree in plant pathology was earned at Ohio State University in 1980. His work at Ohio State University focused on the potential use of fungal viruses or transmissible double-stranded ribonucleic acid in the biological control of Dutch elm disease. He first became involved in research on postharvest biocontrol of fruit diseases while a postdoctoral associate at the Appalachian Fruit Research Station in Kearneysville, West Virginia.

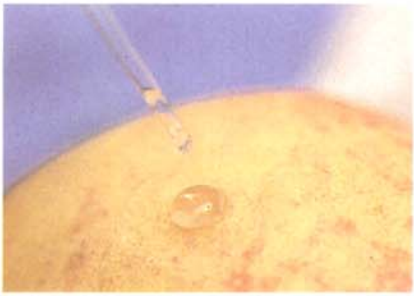


Fig. 2. Application of an antagonist to a wound on a peach.

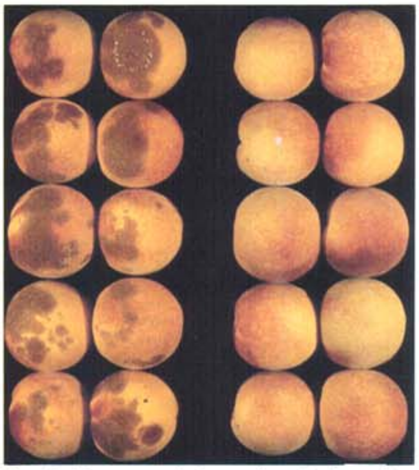


Fig. 3. Nonwounded peaches 3 days after dip treatment with (right) *Bacillus subtilis* or (left) water, then inoculation with *Monilinia fructicola*.

Fig. 4. In vitro inhibition of *Monilinia fructicola* by *Bacillus subtilis*.

antagonistically or parasitically to a particular pathogen. It is also conceivable that genes involved in the mode of action of an antagonist could be incorporated into the host plant itself.

Postharvest Applications of Biocontrol Agents

Most applications of antagonists to control postharvest diseases have been made before harvest. Postharvest applications have not been fully exploited. We need to study procedures during the processing of food for opportunities to apply antagonistic or parasitic microorganisms. The practice of coating peaches with wax at the end of the processing line in commercial packinghouses proved to be such an opportunity for us. We effectively mixed our antagonistic bacterium in commercial wax formulations and applied it without disrupting normal processing procedures or adding new ones.

Many chemicals are used in food processing primarily as pesticides and antioxidants. It is important to know how any potential biocontrol agent will interact with existing chemical treatments. Also, once a biocontrol procedure has been developed, its application will need to be integrated into existing pest control programs. When an antagonist that is resistant to a certain fungicide is applied with the fungicide, the antagonist, because of its resistance, can be more dominant and effective. Antagonists and parasitic organisms may also reduce the amount of fungicide needed for effective control. Procedures that include both chemical and biological control can be developed. The possibility exists of developing preparations of biocontrol agents that could be applied on food products in the grocery. Such "stay fresh" preparations could be used to extend the life of food while it is being marketed.

Public Acceptance of "Contamination"

The introduction of "exogenous" microorganisms into the food chain for biocontrol will require a number of safety procedures. The pathological and allergenic potential of the organisms to man will have to be thoroughly investigated. Also, the effect of these agents on the nutrients of the food should be determined.

Once biocontrol procedures have been developed for postharvest diseases, they will still need to gain public acceptance. Resistance to the "contamination" of food with "exogenous" microorganisms can be anticipated. However, the "wholesomeness" of these procedures vs. chemical control should be acceptable. Since man is accustomed to accommodating a great variety of microorganisms in and on his food, no major obstacles are anticipated in this area. The Japanese actually grow *B. subtilis* as a food (10). At present, the public has no problem with the addition of "exogenous" microorganisms into such food products as yogurt and acidophilus milk.

We envision biological control procedures being developed for a number of postharvest diseases. It is surprising to us that more research has not been done in this area, particularly since postharvest biological control has a number of advantages over biological control in the field.

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