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Microbial Control of Postharvest Disease and Spoilage

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I. INTRODUCTION

Although several different strategies have been used to control the development of decay and spoilage in fresh fruits and vegetables, the use of proper postharvest temperatures is so important that all other control practices might be considered supplements (Sommer, 1982). For certain fresh fruits and vegetables, synthetic chemicals, including pesticides and preservatives, have been used widely to control decay and spoilage. Pesticides, defined as economic poisons in most state and federal laws (Ware, 1988), however, have become a major concern of many consumers. The search for alternatives to chemicals for protecting fruits and vegetables has led to renewed interest in traditional disease control methods. These methods, which include heat treatment and controlled atmosphere storage, have been largely abandoned since the development of effective fungicides (Ben-Yehoshua, 1985; Brecht, 1980; Buick and Damoglou, 1987; Daniels et al., 1985).

Biological control, which exploits the activities of one microorganism to control the development of a second microorganism, although not a new concept in pest and disease control, has only recently attracted interest as an alternative to the application of fungicides for control of postharvest diseases. The evolution of biological control from its primitive origins in food preservation (pickling, etc.) to application of microorganisms to fresh fruits and vegetables for control of microbial spoilage and decays has accelerated in the past few decades. Various reviews provide details on the advancement of biological control of postharvest diseases of fruits (Janisiewicz, 1988b, 1998; Korsten et al., 1994; Wilson et al., 1991).

Microorganisms have been beneficial in preserving food, including fruits and vegetables, for centuries (Hanlin and Evancho, 1992; Rose, 1982). Fruits and vegetables became preserved because colonization by beneficial organisms caused physical and chemical changes that prevented spoilage organisms from growing. More recently, many of the "beneficials" have been found to inhibit various bacteria of human health concern, such as *Salmonella* spp., *Clostridium botulinum*, *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Halin and Evancho, 1992). For example, lactic acid bacteria, which are used to make various fermented dairy products, pickled vegetables and various preserved animal feeds have been suggested as a control of food-borne pathogens (Daeschel, 1991; El-Gazzar et al., 1992; Gould, 1992; Lewus et al., 1991; Ray, 1992a; Vaughan et al., 1994; Kim, 1993). Lactic acid bacteria also have been suggested as additives to extend the storage life of pea tempeh (Ashenafi and Busse, 1991) and to ensure the microbiological safety of ready-to-use vegetables (Vescovo, 1995).

The use of antagonistic microorganisms to control postharvest diseases of various temperate, subtropical, and tropical fruits has been investigated intensively for more than a decade. The agents used to control postharvest pathogens belong to various taxonomic groups including bacteria, yeasts, and filamentous fungi. In 1995 a bacterium, *Pseudomonas syringae*, and a yeast, *Candida oleophila*, were registered by the U.S. Environmental Protection Agency for postharvest application to apples, pears, and citrus fruits. These antagonists are commercially available as BioSave 11 and BioSave 110, (dry and wet formulations, respectively, of *P. syringae*, EcoScience, Orlando, FL) (Janisiewicz and Jeffers, 1997), and Aspire (*C. oleophila*, Ecogen Inc. Langhore, PA). Reports from various laboratories indicate that several additional biocontrol agents will be registered for use on fruit in the near future. The progress made in the biocontrol of fruit decay suggests that devoting similar resources and effort to the biocontrol of vegetable spoilage and decay should lead to successful commercial products.

II. POTENTIAL FOR BIOLOGICAL CONTROL OF VARIOUS INFECTIONS

The route by which pathogenic or spoilage bacteria and fungi infect vegetables influences both the application and function of biological control agents. Most postharvest pathogens and spoilage agents infect the host through natural openings and wounds, while certain fungal pathogens can also penetrate the intact surface (see Chap. 20 for discussion of fungi and Chap. 21 for bacteria). Infections leading to postharvest decay can be separated into three categories; wound infections, incipient infections, and latent or quiescent infections (Bruton, 1994).

A. Wound Infections

Certain decay pathogens lack the ability to penetrate the intact surface of plants. These pathogens usually first colonize damaged tissues including wounds, frost/freeze injury, or certain types of lesions and then grow into surrounding tissues. If internalized in a fruit or vegetable, many of these pathogens can directly infect various types of cells.

The wound invaders, as a group, are relatively easily controlled by application of an organism that rapidly colonizes damaged tissues and, as a result, either physically or chemically excludes the pathogen from colonizing that site. Early models for this phenom-

enon include colonization of roots of certain crops by *Agrobacterium radiobacter* strain K84, which protects the plant against crown gall caused by *Agrobacterium tumefaciens* (Kerr, 1980), and the application of *Peniophora gigantea* (now *Phlebia gigantea*) to tree stumps to protect against infection by *Fomes annosus* (now *Heterobasidion annosum*) (Rishbeth, 1963). Thus, it is not surprising that the recently registered biocontrol products are aimed at wound-invading pathogens.

Biocontrol agents that exclude decay pathogens from wounds usually are most effective when applied to fresh wounds. For example, neck rot of onion and garlic, caused by *Botrytis* spp., is primarily initiated when the pathogen infects leaves after the plants have been topped, a cultural practice applied when 50% of the leaves are mature. Applying antagonists to freshly topped plants may reduce the colonization of the wounds by *Botrytis* spp., resulting in less disease after harvest (Kohl et al., 1991). Similarly, applying biocontrol agents to citrus or pome fruits immediately after harvest ensures that harvest-related wounds are inoculated. The ensuing rapid development of the antagonist excludes decay pathogens from the wound.

Biocontrol agents that colonize wounds may also be valuable in controlling decay and spoilage in semiprocessed produce. Freshly washed and then cut vegetables provide an empty niche for microbial colonization and are an excellent substrate for growth of a variety of microorganisms (King and Bolin, 1989). Microbial load on vegetables does not, per se, reduce the wholesomeness of the product (Bracket and Splittstoesser, 1992). However, if a significant portion of the microbial population on the product is likely to affect plant or human health or the organoleptic qualities of the product, then the wholesomeness and postharvest life of the product can be compromised. Examples from the food industry indicate that antagonistic microorganisms can prevent the development of undesirable microbes on fresh-cut produce (see reviews by Dillion and Board, 1994; Dillion and Cook, 1994). This approach may be feasible on whole vegetables (Vescovo et al., 1995).

B. Incipient Infections

Incipient infections are those that are just beginning to exist or come to notice, and according to Bruton (1994), may occur at any time before or after harvest. Many incipient infections pass unnoticed through the culling process during harvest, packaging, and retail display. These infections usually remain active and, if not completely arrested by the application of fungicides, heat, drying, or refrigeration, eventually cause decay. Incipient lesions on vegetables at the time of packaging may lead to the development of an active decay during transport and storage, which can lead to formation of nests of decaying vegetables. Biological control of diseases developing from incipient infections is likely to be difficult, since the pathogen has established an intimate relationship with the host. These pathogens, however, are active and do not produce resistant structures, making them vulnerable to antagonists that produce lytic enzymes and antifungal compounds.

C. Latent or Quiescent Infections

Latent infections, which may be confused with the incipient ones discussed above, occur when pathogen development is temporarily arrested. The latent or quiescent infection, which often becomes established on immature fruits and vegetables, has been defined in various ways by different authors (Agrios, 1988; Hayward, 1974; Swinburne, 1983). Verhoef (1974) defined latency as a quiescent or dormant parasitic relationship which, after

a time, changes to an active one, and Swinburne (1983) as a period between spore landing and production of new spores. To avoid further confusion, we use the term *quiescent infection*, as suggested by Swinburne (1983), to differentiate the quiescent parasitic relationship from a latent period. Stages in the development of latent infections are discussed in Chapter 20.

Biocontrol of pathogens that often enter into quiescent parasitic relationships with their host presents a similar challenge as do incipient infections. Initial pathogen populations can be reduced or their activities greatly inhibited. For example, hyperparasitic biocontrol agents can be applied to the soil where pathogen resting structures are located (Sundheim and Tronsmo, 1988). Typical examples of a hyperparasitic relationship are parasitism of powdery mildew mycelium on aerial plant surfaces (cucumbers) by *Ampelomyces quisqualis* (Sundheim, 1982) or of sclerotia of *Rhizoctonia solani* in the soil by *Verticillium biguttatum* (Velvis and Jager, 1983). Certain bacteria, such as *Pseudomonas syringae* pv. *glycinae* cause of bacterial blight of soybean, are parasitized by *Bdellovibrio bacteriovorus*, a small, comma-shaped prokaryote (Scherff, 1973). In greenhouse studies, applications of this hyperparasite to soybean plants effectively inhibited the development of local and systemic lesions. In the field, however, use of *B. bacteriovorus* for control of bacterial blight presents significant implementation problems. The mechanism of control is unusual: *B. bacteriovorus* penetrates its host by a rapid drilling action, and, once inside, destroys the cell. Efficacy in the greenhouse required a 9:1 or 99:1 antagonist-to-pathogen ratio. Therefore, sizable pathogen populations would be required to maintain effective populations of the hyperparasite. Alternatively, *B. bacteriovorus* preparations may have to be reapplied to maintain critical population levels.

Another way to implement biocontrol of pathogens that enter into significant quiescent relationships with their hosts is to eliminate the food base (e.g., flower petals, senescing leaves, etc.) required by the pathogens prior to entry into immature plant organs. The application of biocontrol agents that rapidly colonize the food base provides a measure of control. Additionally, biocontrol agents can be applied to the plant organ to stimulate or maintain host resistance factors that often are responsible for the change of an initial infection from active to quiescent (see Chap. 24 for discussion of host resistance).

D. Considerations in Developing a Biocontrol Program

An advantage of using biological control for postharvest diseases is that the target and the environment are usually well defined. A biocontrol agent can be applied to the entire exposed surface of a vegetable. The probability of contact between the agent and wounds or exposed pathogen structures is quite high. Moreover, agents can be selected based on their adaptation to the expected storage environment. However, the investment in time, effort, and funds required to develop a biocontrol program must be justified by factors other than the suitability of the disease. First, the economic impact of the disease must be sufficient to compel growers to apply a postharvest treatment. Second, the crops or pathogens involved must be of such economic importance as to generate the sales necessary for a company to realize profits from sales of the biocontrol agent. Third, competing chemical products or future prospects for developing and registering new chemical products should not close the market for the biocontrol agent. Fourth, the biocontrol agent must be competitive in terms of cost and efficacy with available alternative control measures.

In selecting a biocontrol agent, one must realize that the silver-bullet approach (one antagonist against one pathogen) (Spurr and Knudsen, 1985) is not always ideal. Vegeta-

bles can be affected by a spectrum of pathogens that cause diseases of major and minor importance. Controlling only one disease may increase the importance of another disease that is currently of only minor concern (iatrogenic diseases). This problem can be addressed by developing mixtures of mutually compatible antagonists that will have a broader range of activity than individual antagonists (Janisiewicz, 1988a, 1996, 1998; Schisler et al., 1997).

III. UTILIZING NATURAL MICROBIAL COMMUNITIES FOR BIOCONTROL

Microbial populations on above and below ground parts (phyllosphere and rhizoplane/rhizosphere, respectively) of vegetables, which vary greatly both quantitatively and qualitatively, are usually capable of multiplying to high numbers in the absence of major damage to the plant. Plant surfaces have populations of resident microorganisms that are well adapted to that niche (see Chap. 21). By contrast, contaminants (casuals)—which may come from soil, water, dust or other natural sources—usually do not survive well on the plant. Often, the most effective antagonists of plant pathogens can be found among resident microorganisms on plants.

The potential for a resident microorganism to be an effective biocontrol agent depends, to a great extent, on its ability to colonize food sources on the plant without causing damage to living plant cells. Reviews on the growth and colonization of plants by resident populations of microorganisms are available (see Chap. 21). Knowing the type of organisms residing on various vegetables and different plant parts, and factors affecting their growth, can be very helpful in selecting potential antagonists. The chemical composition, naturally occurring antimicrobial compounds, plant maturity, vegetable type (habitat provided), type of surface damage, environmental conditions [temperature, relative humidity (RH), pH, oxidation-reduction potential (Eh), atmospheric composition], and practices used in growing and handling vegetables after harvest profoundly affect the establishment of microbes on plant surfaces (see Chap. 21).

Fluorescent and nonfluorescent pseudomonads and *Erwinia* spp. are among the major groups of bacteria found on vegetable surfaces (Dennis, 1987). Low numbers of lactic acid bacteria are also found frequently on the surface of various vegetables. Soil contaminants on vegetable surfaces comprise mainly spore-forming and coryneform bacteria (Brackett and Splittstoesser, 1992). Certain strains of *Pseudomonas* spp. can be used to control postharvest diseases of pome, stone, and citrus fruits (Janisiewicz, 1987; Janisiewicz and Marchi, 1992; Smilanick and Denis-Arrue, 1992; Smilanick et al., 1993), and lactic acid bacteria are known for their antimicrobial activity in food preservation (Daeschel, 1991; Dillion and Cook, 1994; El-Gazzar et al., 1992; Gould, 1992; Lewus et al., 1991; Ray, 1992a). However, certain strains of *Pseudomonas* spp. are pectolytic and cause soft rots of vegetables such as lettuce and crucifers (Lund, 1983). Additionally, certain strains of lactic acid bacteria cause decays in tomatoes (Conn et al., 1995). Therefore, while both microorganisms should be explored for their potential to control postharvest decay on vegetables, the studies must include analysis of a candidate agent's ability to cause spoilage or decay.

The most frequently isolated filamentous fungi from plant surfaces were *Aureobasidium pullulans*, followed by *Fusarium* spp., *Alternaria tenuis*, *Epicoccum nigrum*, *Mucor* spp., *Chaetomium fimeti*, *Rhizopus nigricans*, and *Phoma* spp. *A. pullulans* and *E. nigrum* have been reported to provide good protection against postharvest pathogens of apple

(Falconi and Mendgen, 1994). Yeasts are also common on fresh vegetables (Miller, 1979; Deak and Beuchat, 1996). The yeast genera commonly isolated from cabbage (Geeson, 1979), tomatoes and bell pepper (Golden et al., 1987), and corn (Deak et al., 1987) are nonfermenting *Cryptococcus* and *Rhodotorula* and fermenting *Candida* and *Kloeckera*. Representatives from each of these yeast genera have been shown to reduce postharvest decay of fruits and may be also effective against decay on vegetables (Chand-Goyal and Spotts, 1995; McLaughlin et al., 1992; Roberts, 1990).

The microbial ecosystem on vegetables is dynamic, changing with various field injuries and plant maturation as well as with harvest, handling, storage, and marketing (Geopfert, 1980; Dennis, 1987). Populations of microorganisms on plants generally increase greatly during storage, but this increase is not firmly linked with loss of product quality or safety (Brackett and Splittstoesser, 1992). Sound vegetables may have very high populations of resident microorganisms or contaminants from soil and other sources (Brackett and Splittstoesser, 1992). However, in developed countries these microbial populations usually pose few health hazards. On the other hand, vegetables may harbor human pathogens or parasites such as *Listeria*, *Salmonella*, *Shigella*, or *Aeromonas* spp.; viruses; amoebas; or nematodes (Splittstoesser and Corlett, 1980, Beuchat, 1995) (see Chap. 21).

Changes in the sources of vegetables and the way vegetables are marketed will impact the potential for use of biocontrol agents on vegetables. For example, food safety issues confound the importation of vegetables from developing into developed countries. The use of biocontrol agents on such crops is likely to require different handling steps than use of the same agents on domestic crops. The demand for packaged vegetables is increasing, mainly because of high consumer appeal, convenient handling, and the potential for longer maintenance of high quality. The main determinants of vegetable quality are physiological conditions and microbial spoilage. When the physiological deterioration of vegetables is slowed, the quality is maintained longer and the period of natural resistance to postharvest pathogens is extended. This lengthens shelf life and also extends the growth period of a variety of microorganisms including those causing spoilage and foodborne illnesses.

Packaging also affects microbial populations on vegetables by changing environmental conditions (see Chap. 9). The selection of microbes adapted for growth on vegetable surfaces during managed atmosphere storage could provide a biological control that is part of a strategy to reduce the risk from undesirable microbes. Precolonization of vegetables by well-adapted biocontrol agents may prevent growth or even survival of foodborne pathogens, thus significantly reducing the risk of illnesses.

IV. EXAMPLES OF THE APPLICATION OF BIOLOGICAL CONTROL FOR CONTROLLING DISEASES OF VEGETABLES

A. Underground Vegetables

A cold-tolerant isolate of *Trichoderma harzianum* was used to control licorice and crater rots on carrots harvested from fields in Norway that were heavily infested with the pathogens *Mycocentrospora acerina* and *Rhizoctonia carotae*, respectively (Tronsmo, 1989). Immersion of freshly harvested carrots in 10^7 conidia/mL of *T. harzianum* for 5 min led to a 47% or 75% increase in marketable carrots following storage at 0 to 0.5°C for 6 or 8.5 months, respectively. Interestingly, the antagonist used did not reliably grow at a 0 to 0.5°C (Tronsmo, 1989, 1993). Sesan (1993) tested 10 fungi for biocontrol potential

against watery soft rot and black rot of carrots caused by *Sclerotinia sclerotiorum* and *Alternaria radicina*, respectively. Only *Trichoderma viride* inhibited growth of both *S. sclerotiorum* and *A. radicina* in dual culture tests in vitro.

Numerous diseases, of which 15 can be of major economic importance, can affect the quality of potato tubers (*Solanum tuberosum*). Pre- or postharvest applications of biocontrol agents have successfully controlled several of these diseases.

The severity of brown rot (bacterial wilt) caused by *Ralstonia solancearum* (synonym: *Pseudomonas solancearum*) (Martin and French, 1985) was reduced by dipping tuber seed pieces in a suspension of different bacteria (Kempe and Sequeira, 1983; McLaughlin et al., 1990). Ciampi-Panno et al. (1989) treated seed pieces with a selected bacterial antagonist coated with $CaCO_3$. This calcium amendment improved the level of control provided by the biocontrol agent. The application of *R. solancearum* and pectolytic strains of *Pseudomonas fluorescens* to seed pieces also caused a significant reduction in disease severity. Certain of these treatments may have induced systemic resistance (see Chap. 24). McLaughlin et al. (1990) described an interaction between bacteria and root-knot nematodes whereby avirulent strains of *R. solancearum* suppressed nematode activities in the roots, which increased the level of control of brown rot. Avirulent *R. solancearum* (PSSOL) is commercially available for the control of *R. solanacearum* on vegetables (Natural Plant Protection, Route d'-Artix, Nogueres, France).

Bacterial soft rot of potato tubers was reduced by application of two different strains of fluorescent *Pseudomonas* spp. in laboratory tests (Burr and Schroth, 1977). Gross (1988) reported that coapplication of *Pseudomonas* spp. strains was no better than use of a single strain for suppressing the soft rot pathogen. Preplant treatment of potato seed pieces and postharvest treatment of potato tubers with *Pseudomonas putida* reduced soft rot by 50% and 75%, respectively (Colyer and Mount, 1984). The greater reduction in soft rot with postharvest treatments may have resulted from a greater colonization of infection courts on intact tubers by *P. putida*. Both antibiosis and induced resistance were offered as possible control mechanisms. The inability of *P. putida* to provide complete control of soft rot may be related to incomplete colonization, particularly in seed pieces. Furthermore, pectolytic bacteria, resistant to antibiotics produced by *P. putida*, were found in soft rot lesions on *P. putida*–treated tubers. The occurrence of these resistant populations is one of the limitations of commercializing this antagonist. Burr et al. (1978) were also able to increase yield and control soft rot by treating seed pieces with *P. putida* and *P. fluorescens*.

Recent interest in plant growth-promoting rhizobacteria (PGPR) has prompted several investigations on use of these bacteria for biological control of specific diseases. Growth promotion by these bacteria may result through suppression of deleterious root-colonizing microorganisms (Suslow and Schroth, 1982). Kloepper (1983) suggested that PGPR might be useful in the management of potato blackleg and soft rot diseases. He also confirmed that PGPR causes shifts in populations of root zone microorganisms. Burr and Caeser (1984) found plant growth–promoting fluorescent pseudomonads able to decrease soft rot incidence in potato tubers. Xu and Gross (1986), developed a procedure to screen fluorescent pseudomonads for siderophore and antibiotic production, which would be effective in suppressing soft rot pathogens. Strains of *P. putida* and *P. fluorescens* that produce both inhibitory siderophores and antibiotics were most effective against *E. c. atroseptica* in greenhouse trials. PGPR are inhibitory to *E. carotovora* in vitro (Kloepper and Schroth, 1981), which is related to siderophore production (Kloepper et al., 1980). Bacteriophages (viruses of bacteria) have been suggested for control of *E. c.* subsp. *caroto-*

vora (Eayre et al., 1995). These agents, which were isolated from freshwater lakes, had distinct host ranges. Between one and six host strains were susceptible to a given phage. A mixture of four phages protected potato tubers against 16 of the 23 serogroups of *E. c.* subsp. *carotovora* tested.

Ring rot caused by *Clavibacter michiganensis* subsp. *sepedonicus* (*Corynebacterium sepedonicum*) was successfully suppressed in greenhouse-grown potatoes by application of fluorescent pseudomonads (Cruz et al., 1992). Treatments with different combinations of strains of *P. aureofaciens* and *P. fluorescens* were no more effective than applications of a single strain. Although field experiments were still needed to determine the feasibility of such a biocontrol system, the authors felt that it showed promise for commercial potato production but doubted that it could replace the zero tolerance applied to seed stocks.

Treating planting material with *Bacillus subtilis* proved effective in reducing seed-piece decay caused by *Macrophomina phaseolina*, which also causes charcoal rot of tubers (Thirumalachar and O'Brien, 1977). The bacterial antagonist reduced the frequency of charcoal rot at harvest and may have potential as a supplement to cultural practices presently used to control the disease.

Biological control of black scurf (*Rhizoctonia* canker) caused by *Thanatephorus cucumeris* (sclerotial state: *Rhizoctonia solani*) is difficult because the subterranean parts of a potato plant, such as sprouts and stolons, are susceptible during the entire growing season (Van den Boogert and Jager, 1984). Both seed and soil-borne inocula contribute to the disease. A common mycoparasite, *Verticillium biguttatum*, was found to kill sclerotia of *R. solani* when placed on inert material (perlite) or in soil (Velvis and Jager, 1983). In their preliminary field experiments, *Verticillium* did not reduce stem symptoms but was able to reduce sclerotium formation on newly formed potato tubers. The reduction in severity of black scurf at harvest resulting from the applications of the antagonist was comparable to and in some cases even superior to that produced by soil treatment with chemical disinfectants. Jager et al. (1979) reported *Gliocladium roseum* to be the most prevalent mycoparasite on sclerotia of *R. solani* in Dutch potato fields. Aluko (1968) succeeded in killing sclerotia of *R. solani* on potato tubers by application of *Gliocladium virens*. However, the treatment was effective only if performed before storage of potato tubers. *G. virens* disappeared quickly after treated tubers were planted, whereas *V. biguttatum* continued to be active on sclerotia even in the soil. Antagonistic activities of *V. biguttatum* decreased at temperatures below 15°C and was absent at 10°C. Jager et al. (1979), and Velvis and Jager (1983) reported that *Hormiactis fimicola* reduced the viability of sclerotia and was effective at temperatures below the minimum for growth of *V. biguttatum*. Therefore mixing these two fungi to improve biological control efficacy seems logical, since their growth temperature ranges are complementary. However, in the soil, *H. fimicola* inhibited the colonization of sclerotia by *V. biguttatum*. This resulted in a distinct loss of antagonistic potential and the control achieved by mixed inocula was similar to that provided by *V. biguttatum* alone. Isolates of another *R. solani* antagonist, *Azotobacter chroococcum*, was evaluated in combination with *V. biguttatum* and proved effective in controlling *R. solani* infestations of potato plants (Meshram, 1984). By combining *A. chroococcum* and *V. biguttatum*, effective protection of sprouts, stems, and stolons was obtained. Inoculating seed tubers with three isolates of *V. biguttatum* separately or mixed was also successful in reducing the sclerotia formed on new tubers (Jager and Velvis, 1986). Inocula of 6×10^5 or 3×10^6 spores per milliliter were equally effective in controlling *R. solani*. In soils with large populations of *R. solani*, the efficacy of biological control

was not satisfactory, although significant reductions in disease were obtained (Jager and Velvis, 1985).

Large populations of *R. solani* have been controlled by an integration of biological control with reduced application of chemicals (Jager and Velvis, 1986). Beagle-Ristaino and Papavizas (1985) tested several fungal antagonists in greenhouse and field tests. Fermentor biomass preparations of *T. viride* and *G. virens* applied as dusts to seed potatoes infested with sclerotia of *R. solani*, reduced disease incidence and severity in the field. Both soil- and tuber-borne inocula were effectively reduced. *T. viride* is a mycoparasite of hyphae, while the production of one or more toxic substances may also have been involved in the loss of viability of sclerotia (Beagle-Ristaino and Papavizas, 1985). Aluko and Hering (1970) detected gliotoxin and viridin on potatoes treated with *G. virens* and then placed in storage. The combination of antagonist dusts with fungicides and the mass production of these antagonists on a relatively inexpensive medium could result in practical introduction of this biocontrol agent into agricultural production systems (Beagle-Ristaino and Papavizas, 1985). Several biocontrol products are commercially available for control of *R. solani* on vegetables. They are formulated from *Trichoderma* sp., *T. harzianum* strain KRL-AG2, and *Streptomyces griseoviridis* strain K61.

Dry rot caused by *F. solani* var. *coeruleum*, *F. sulphureum* (syn *F. sambucinum*), *F. thrichothecioides*, *F. equiseti*, *F. oxysporum*, *F. sporotrichioides*, *F. avenaceum*, and several other *Fusarium* spp. of lesser importance (Snowdon, 1992; Common Names for Plant Diseases, APSnet) is well suited to biological control. Burkhead *et al.* (1994) found effective but not consistent control of dry rot by application of *Burkholderia cepacia* (*Pseudomonas cepacia*). Slininger et al. (1994) used a two-dimensional liquid culture focusing method to select commercially promising microbial isolates for postharvest control of dry rot. Consideration of both liquid-culture growth kinetics as well as cell-product biocontrol efficacy is of great value, since the one feature is not necessarily reflective of the other, yet both are critical to economic and commercial success. Schisler and Slininger (1994) looked at the microbiota of 29 different agricultural soils. Samples were irradiated and assayed for biological suppressiveness to *Fusarium* dry rot, using a whole-tuber/infested soil assay. Over 350 isolates of bacteria, yeasts, and actinomycetes were recovered from five samples of the most suppressive soils. In the whole-tuber assay, 18 bacterial strains—identified as species of *Pseudomonas*, *Enterobacter*, and *Pantoea*—consistently suppressed dry rot. Each of the strains produced antifungal antibiotics, but antibiosis was not the only mechanism involved (Burkhead et al., 1994). When certain antagonists were used in pairs, control success involved complementary antibiotic production (Schisler et al., 1997). Optimum antagonist concentrations of 1×10^8 CFU/mL provided near maximal disease suppression in these studies. By contrast, the yeast strains tested controlled dry rot only occasionally (Schisler et al., 1995). This inconsistent performance was attributed to a lack of effective competitive ability for simple sugars at the infection sites. Only *Cryptococcus laurentii* had biological control capabilities worthy of further study, although it was not as effective as a strain of *P. fluorescens* that had been used in previous dry rot biocontrol studies. McCormack et al. (1994) found that the majority of yeasts isolated from the potato phylloplane could produce antibacterial compounds, while strains isolated from the soil or obtained from culture collections could not. Combining two or more yeasts based on differing modes of action or integrating yeast-based control measures with compounds that can control dry rot may prove effective (Schisler et al., 1997). However, the bacterial antagonists appeared to have greater potential to control the disease effectively and efficiently.

The discovery of several potential biocontrol agents for *Fusarium* dry rot by Slininger et al. (1994) is of particular importance since *Gibberella pulicaris* (anamorrph: *F. sambucinum*) can produce trichothecene toxins that have been implicated in mycotoxicoses of humans and animals (Senter et al., 1991). Furthermore, many strains of the dry rot *Fusarium* group have become resistant to thiabendazole, the only registered postharvest chemical effective against dry rot. The resistant strains are well adapted and are likely to persist (Desjardins et al., 1993).

The need for simultaneous control of all soilborne diseases of potatoes presents certain limitations and challenges because (a) the pathogens vary largely in biology and methods of control; (b) only relatively inexpensive methods of control can be used; (c) the crop must be protected for the whole season; and (d) application technology must be compatible with crop production (Elad et al., 1980). Combining biocontrol with broad-spectrum control measures such as soil solarization could improve the efficacy of the antagonist. A holistic disease-control approach, where all control options are combined in an integrated program, can be of great importance where crops are attacked simultaneously by numerous types and kinds of pathogens. The potato represents an ideal crop for this kind of disease control strategy. Surprisingly little has been done with such an approach since Elad et al. (1980) showed the potential of controlling several diseases with a multifaceted disease control strategy. Certain chemical and physical practices were combined with applications of *T. harzianum* to suppress effectively diseases caused by *R. solani*, *Verticillium dahliae*, and *Sclerotium rolfsii*. Incidence of disease caused by *R. solani* remained low throughout the season, apparently due to a shift in the biological equilibrium in the soil in favor of the antagonists. Biocontrol agents commercially available for the control of *Fusarium* specifically on potato include two formulations of *T. harzianum* strain KRL-AG2, whereas two registered for general use on vegetables are formulations of *Trichoderma* spp. and *Streptomyces griseoviridis* strain K61.

B. Flower, Leafy, and Stem Vegetables

To alleviate various problems with fungicides, Leifert et al. (1992) explored a biological control strategy for controlling gray mold (*B. cinerea*) and *Alternaria* rot in stored Dutch white cabbage. More than 100 candidate biocontrol agents were isolated from cabbage leaves to cabbage-extract-agar seeded with conidia of *B. cinerea*. Leifert et al. (1993a, b) identified five strains that gave the greatest inhibition of *B. cinerea* and *Alternaria brassicicola* on heat-treated cabbage-leaf disks. *Serratia plymuthica* strain CL43; *S. liquefaciens* strain CL80; and *P. fluorescens* strains CL42, CL66, and CL82 were used in biocontrol tests with cabbage heads in commercial cold storage (Stanley et al., 1994). The heads were dipped into either 10^7 to 10^8 CFU/mL of antagonist or the fungicide metalaxyl, which is used primarily to control water molds, then sprayed with conidia of *B. cinerea*, and stored at 4 to 6 or 1 to 3°C. Disease severity (percent weight of infected leaves) was evaluated when approximately 80% of the surface of control heads was covered with fungal growth. Two strains, CL80 and CL82, provided consistent control, whereas strain CL82 gave better control than the fungicidal treatment. Strain CL82 not only survived very well but totally dominated leaf surface microflora during the 32-week test. Populations of the other bacteria began to decline after approximately 15 weeks in storage.

Results of these tests confirmed earlier findings of a strong quantitative relationship between the concentration of the antagonist and pathogen and the effectiveness of biocontrol (Leifert et al., 1993b). Control of gray mold on cabbage heads did not correlate with

the ability of the antagonists to inhibit the pathogen in plate or disk assays, confirming limitations of the in vitro tests in selecting biocontrol agents (Leifert et al., 1993b). This system is likely to be commercialized if the strains pass the safety tests necessary for registration of microorganisms for postharvest application.

Lettuce (*Lactuca sativa*) is unique among the major vegetables in that it is used almost exclusively as a fresh raw product (Snowdon, 1992). In a greenhouse environment, biological control of diseases may be feasible. Adams (1989) reported the exploitation of natural organisms in the soil that could destroy sclerotia of *Sclerotinia minor* and *S. sclerotiorum*, which cause watery soft rot (also lettuce drop). Application of *Coniothyrium minitans* to the soil decreased disease and increased lettuce yield in the glasshouse (Budge and Whipps, 1991), whereas the *Trichoderma* spp. tested were ineffective. *C. minitans* survived in the field for a year and even spread to adjacent plots. Adams and Ayers (1982) applied a mycoparasite *Sporidesmium sclerotivorum* to field plots and were able to control lettuce drop for four consecutive crops. They found that the antagonist became established in the field plots and infected and destroyed sclerotia produced on the diseased lettuce. Newhook (1951) showed that various bacteria gave protection against *B. cinerea* (gray mold rot) when applied to the leaves of wounded lettuce seedlings. The mode of action was attributed to antibiotics and an increase in pH in lettuce tissue from 6.1 up to 7.8 to 8.4 at which level growth of *B. cinerea* and activity of any pectinase it produced were minimal.

Budge and Whipps (1991) illustrated the potential effective control of pink rot of celery (*Apium graveolens*) caused by *S. minor* or *S. sclerotiorum* by applications of *C. minitans* in field and glasshouse studies. However, levels of control diminished as factors favoring disease development and time after the incorporation of *C. minitans* in the soil increased. They concluded that repeated applications might be necessary to achieve significant, rapid and continued reduction in sclerotium numbers. They also suggested integration of biological control with a chemical spray when disease development is likely.

C. Fruity Vegetables

The postharvest environment of stored or packaged tomato fruit (*Lycopersicon esculentum*) lends itself to implementing biological control systems. The antagonist can be applied on the packingline, during washing or by dipping or incorporating into a wax.

Moline (1991) reported effective in vivo control of *E. c. carotovora* when natural antagonists were screened in preliminary tests. Although the disease was significantly reduced, decay could not be prevented. Isolates identified as *Erwinia cypripendii* and *Pantoea herbicola* (*E. herbicola*) were the most promising, while other potential antagonists—*P. cepacia*, *Pichia guilliermondii*, and *B. subtilis*—proved less effective. Members of the nonpectolytic *Erwinia* spp. show great promise as biocontrol agents and should be further exploited for control of bacterial soft rots.

Gray mold rot (*B. cinerea*) and *Alternaria* rot (*A. alternata*) were effectively controlled by applications of *P. guilliermondii* to wounded tomatoes after harvest (Chalutz et al., 1988). Postharvest fungicide treatments have been used successfully for control of *B. cinerea*, however, repeated use of certain fungicides has resulted in the development of strains of pathogens that have practical field resistance to the fungicides (Locke and Fletcher, 1988). Two different formulations containing *T. harzianum* are commercially available for use on tomato. Thus far only limited control of *Alternaria* rot has been reported with postharvest fungicide dip treatments (Spalding and King, 1981). Strains of

A. alternata are also known to produce toxins (Snowdon, 1992), but it is unknown if the toxins are produced in tomato fruit. Effective control strategies for these diseases necessitate careful quality control, particularly with fruit destined for processing.

Biological control has also been evaluated against certain other preharvest tomato diseases, which can profoundly affect the postharvest quality of fruit. In order to reduce postharvest losses, potential antagonists can be applied preharvest to seed or seedlings as field foliage sprays or incorporated into the irrigation system.

Soil rot (or fruit rot) is of particular importance on tomatoes and can cause heavy losses on fresh market fruit. The fungus *Thanatephorus cucumeris*, sclerotial state *Rhizoctonia solani* may be transmitted in seed and is very common in soil. The disease is especially common in tomatoes intended for processing, since the fruit of these crops are more likely to contact the soil and have a longer residence time in the field. The decay can spread postharvest by direct contact of lesions with healthy fruit. However, more severe losses are associated with the secondary development of bacterial soft rot, sour rot, or *Rhizopus* rot. Strashnov et al. (1985) reported significant control of fruit rot when *T. harzianum* was added to soil or was applied as a coating of tomato fruits. A concentration of 10^9 conidia/mL was used as a fruit dressing but the concentration on the skin decreased from 4.5×10^4 to 6×10^3 conidia per square centimeter over a 2-week period of storage. When applied to the soil, antagonist populations remained high for the duration of the growing season. Direct parasitism and competition for space and nutrients were postulated as possible modes of action. Several biocontrol products commercially available for control of *R. solani* include formulations of *T. harzianum* KRL-AG2, *Trichoderma* spp., and *P. cepacia*.

The cucumber (*Cucumis sativus*) is usually eaten raw or pickled. The fruit is harvested physiologically immature. Anthracnose caused by *Colletotrichum gloeosporioides* f.sp. *cucurbitae* (often known as *C. lagenarium* or *C. orbiculare*) can be especially virulent on cucumbers and is of major concern in most countries. Strict hygiene is necessary in the field or greenhouse. Disease severity can be reduced by inoculating young plants with necrosis causing pathogens so that they acquire resistance against various pathogens (Hammerschmidt et al., 1982; Metraux and Boller, 1986). The cucumber has been used as a model to study induced systemic resistance (Wei et al., 1991) (See Chap. 24). Ninety four strains of plant growth promoting rhizobacteria (PGPR) were screened for induction of systemic resistance in cucumber against the anthracnose pathogen. Six PGPR strains significantly reduced lesion size after challenge inoculation by *C. orbiculare* and four of these six strains produced HCN in vitro. Leben and Daft (1965) applied an epiphytic bacterium to seedlings in the greenhouse, which subsequently controlled anthracnose after challenge inoculation. The mode of action was attributed to production of inhibitory substances, which were either antifungal antibiotics or acids.

Symptoms of cottony leak (or *Pythium* fruit rot) of cucumber caused by *Pythium* spp. were delayed when fruits were immersed in sterile filtrates of *Acrophialophora nainiana* and *Stachybotrys atra* prior to inoculation (Sharma et al., 1981). Smith et al. (1993) later showed that *Bacillus cereus* could significantly reduce rot of cucumber caused by *Pythium aphanidermatum*, one of the causal agents. Culture filtrates or concentrations of less than 10^8 cells per milliliter were ineffective. They speculated that an extracellular product was being produced that suppressed rotting of cucumber fruits. However, commercial use of this bacterium may encounter serious obstacles, as some of its strains cause food-borne infections in humans (Ray, 1992b).

Lewis and Papavisas (1980) reported partial control of the soil rot of cucumber

caused by *T. cucumeris*, sclerotial state *R. solani*, by adding *Trichoderma* spp. and other fungi to the soil. Integration of the antagonist applications with mechanical plowing of the soil reduced the severity of *R. solani* rot of cucumber fruits. Biocontrol agents for general control of *R. solani* in vegetables have been described above.

Spraying flowers of snap beans, *Phaseolus vulgaris*, with *Trichoderma hamatum* proved effective in controlling gray mold of the pod caused by *Botrytis fabae* or *Botryotinia fuckeliana*, conidial state *B. cinerea*. Inhibitory volatiles have been suggested as possible modes of action. Since the disease cycles of white mold (*S. sclerotiorum*) is very similar to gray mold, any biocontrol study of the latter must consider its effect on the former. Nelson and Powelson (1988) speculated that since *B. cinerea* and *S. sclerotiorum* depend on senescent flowers for infection of healthy bean pods, both can be suppressed by the same antagonists as was shown in their preliminary field trials. A formulation of *T. harzianum* is commercially available, primarily as a spray application on soybean (*Glycine max*).

There have been numerous attempts at biological control of white mold in which certain naturally occurring organisms were added to the soil to parasitize sclerotia, either before or after the crop was planted. The most effective fungi included isolates of *Drechslera* sp., *Epicoccum purpurascens*, *A. alternata*, *Fusarium graminearum*, *Fusarium heterosporum*, and *Myrothecium verrucaria* (Inglis and Boland, 1990). Although these fungi could not provide consistent control in field tests, the first two were effective in a number of field plots. Furthermore, in certain trials, the combination of *A. alternata* with the fungicide benomyl suppressed diseases more than either one individually.

V. APPLICATION OF BIOCONTROL AGENTS AFTER HARVEST

The most effective ways to apply biocontrol agents to vegetables are not well understood. Agents have been applied as drenches, product dips, and spray applications. Tissue wraps may effectively deliver biocontrol agents. Eckert and Kolbezen (1962) applied a chemical to wrap-pack tomatoes to contain the spread of sour rot, rhizopus rot, buckeye rot, and watery soft rot. Incorporation of biocontrol agents in edible coatings may work with certain vegetables such as rutabagas or tomatoes. There is also incentive for more creative approaches; for example, fruit flies, which are important in the dissemination of *Rhizopus stolonifer*, *Mucor pyriformis*, *G. candidum*, and *B. cinerea* could deposit antagonists directly into potential infection courts (Butler and Braker, 1963; Janisiewicz et al., 1998, Louis et al., 1996; Michailides and Spotts, 1990). Biocontrol agents can also be applied in combination with other control measures, such as chemical control. This approach has been successfully used to control pre- and postharvest diseases of fruits (Korsten et al., 1997; Pusey, 1986). The use of these combination sprays may reduce the amount of chemical needed or increase the level of control above that realized by chemical control alone (Korsten et al., 1997). Alternatively, reduced concentrations of chemicals can be combined with antagonists in postharvest applications (Pusey et al., 1986). Antagonists can also be combined with heat treatment for control of postharvest fruit diseases (Conway et al., 1998). In many instances, biological control practices alone have effectively controlled postharvest diseases. However, a combination of practices based on the principles of sanitation, a hazard analysis critical control point (HACCP) system (Pierson and Corlett Jr., 1992) (see Chap. 23), and biocontrol should provide superior disease control along with assurances of wholesome, low-risk fresh vegetable products.

VI. CONCLUDING REMARKS

As the vegetable industry faces a new era of expansion, particularly in minimally processed ready-to-use and ready-to-eat vegetables (see Chap. 29), there are also new challenges and opportunities for biological control. Development of biological control on vegetables should address both microorganisms affecting vegetable quality and those of health concerns. Control of plant and human pathogens simultaneously will require in-depth studies of microbial interactions on vegetables. A strong emphasis on research into the microbial ecology of vegetables can provide the essential knowledge needed to develop models of microbial colonization in various situations and will greatly accelerate progress in developing reliable biological control methods.

Microbial growth on vegetables should be viewed in the context not only of factors affecting growth directly but also of those affecting it indirectly, such as production, storing, processing, and delivery of vegetables. The rapid increase in microbial populations on vegetables after harvest indicates that this system is dynamic and therefore should be prone to manipulation. The successful introduction of living microbes into fruits and vegetables that result in impressive reduction of diseases is strongly suggestive that certain natural inhabitants of plants can be found that prevent development of both postharvest decay pathogens as well as microorganisms of health concern. It appears that the time is right to move aggressively to make use of our largely untapped natural microbial resources.

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