Biological Control of Postharvest Diseases: A Promising Alternative to the Use of Synthetic Fungicides

S. Droby,[1] E. Chalutz,[1]
C.L. Wilson[2] and M.E. Wisniewski[2]

[1]Institute for Technology and Storage of Agricultural Products
ARO, The Volcani Center, Bet Dagan 50250, Israel
[2]USDA-ARS, Appalachian Fruit Research Station
Kearneysville, WV 25430, USA

Postharvest diseases of fruits and vegetables cause serious losses of fresh produce world-wide. Most rot pathogens are controlled by various methods such as refrigeration, controlled atmosphere and fungicides. Biological control strategies are emerging as promising alternatives to the use of synthetic fungicides. Several antagonistic microorganisms have been found that can effectively inhibit postharvest diseases of fruits and vegetables.

INTRODUCTION

Fresh fruits and vegetables are susceptible to attack by several pathogenic fungi and bacteria after harvest because they are rich in water and nutrients and have lost most of the intrinsic resistance that protects them during their development while attached to the plant. Currently, the main method used to control postharvest decay of fruits and vegetables involves the use of potent fungicides. Public and scientific concern about the presence of synthetic chemicals in our food supply and in the environment has been increasing in the past decade. As a direct result of these mounting concerns, real or perceived, several fungicides (*e.g.* captan, benomyl, thiophanate) have been banned by the U.S. Environmental Protection Agency or voluntarily withdrawn by the manufacturer from some or all postharvest uses. These actions have the potential of greatly diminishing our ability to control postharvest diseases of many commodities. In addition, loss of effectiveness of several fungicides has been attributed to the selection and proliferation of fungicide-resistant biotypes in pathogen populations (Eckert, 1990). Therefore, there is clearly an urgent need to develop new and effective methods of controlling postharvest diseases that pose no harm to human health and the environment and which are accepted as safe by the general public. Considerable effort has been made to evaluate the potential of biological control as an alternative to chemical treatments. A recent review of the literature (Wilson and Wisniewski, 1989) outlined a long list of antagonists which have demonstrated effective control of postharvest pathogens in laboratory or semi-commercial tests.

BIOLOGICAL CONTROL STRATEGIES

Manipulation of epiphytic microflora

Do naturally occurring microbial populations on the surface of fruits act to suppress disease development? Our knowledge in this area is meager and we have to draw primarily from studies of epiphytic populations on leaf surfaces and assume that similar events may occur on fruits.

It has been commonly observed that washed commodities develop rot more than unwashed commodities. Chalutz and Wilson (1990) found that citrus fruits that were washed, dried and stored, rotted much more rapidly than unwashed fruit. They also discovered that bacteria and yeasts predominated when undiluted washings from citrus fruit had been plated out; it was only after the washings were diluted that fungal rot pathogens appeared. This suggested that there may be an epiphytic microbial population on citrus fruit that is naturally suppressive to rot pathogens.

Recent results (Droby, Chalutz, Wilson and Wisniewski, unpublished data) suggest the possibility of reducing the development of postharvest decay of grapefruit by a preharvest spray of a single antagonist (*Pichia guilliermondii*) on grapefruit trees (Fig. 1). The preharvest introduction of *P. guilliermondii* also changed the composition of epiphytic microbial populations on the fruit (Fig. 2).

Introduction of single antagonists

A considerable effort has been made to explore antagonistic microorganisms for the control of postharvest diseases of fruits and vegetables (Wilson and Wisniewski, 1989). The efficacy of various antagonists has been high under laboratory conditions and marginal in the field.

The postharvest environment provides a special milieu for developing biological control systems because more standardized and controllable environmental conditions exist in storage than in the field. Also the concentrated nature of harvested commodities makes it easier to target effectively the application of antagonistic microorganisms than under conditions prevailing in the soil, field or orchard.

MODE OF ACTION OF BIOCONTROL AGENTS

Antibiosis

The production of antibiotic substances by micoorganisms in nature is a well known phenomenon and its role has been discussed in numerous reviews and books. Antibiotic-producing microorganisms may also have a role in protecting fruits and vegetables against diseases. Several antagonists of postharvest pathogens exhibit their biocontrol activity through secretion of antibiotic compounds. *Bacillus subtilis* produces a potent antibiotic substance (iturin) in culture, active against several important pathogens of fruits and vegetables. *Pseudomonas cepacia* also produces an antibiotic, pyrrolnitrin, and controls a number of fruit pathogens.

Competition for nutrients

Special emphasis has been placed on isolating antagonistic microorganisms that heavily colonize the wound site and compete with the pathogen for nutrients and space. The yeast *P. guilliermondii* has been investigated in this connection (Droby *et al.*, 1989). The yeast antagonist *P. guilliermondii* multiplies very rapidly at the wound site under a wide range of temperature, humidity and nutrient conditions. The yeast cells are able to increase in number by two orders of magnitude within 24 h. By the end of this period the pathogen spores have only just started to germinate and grow.

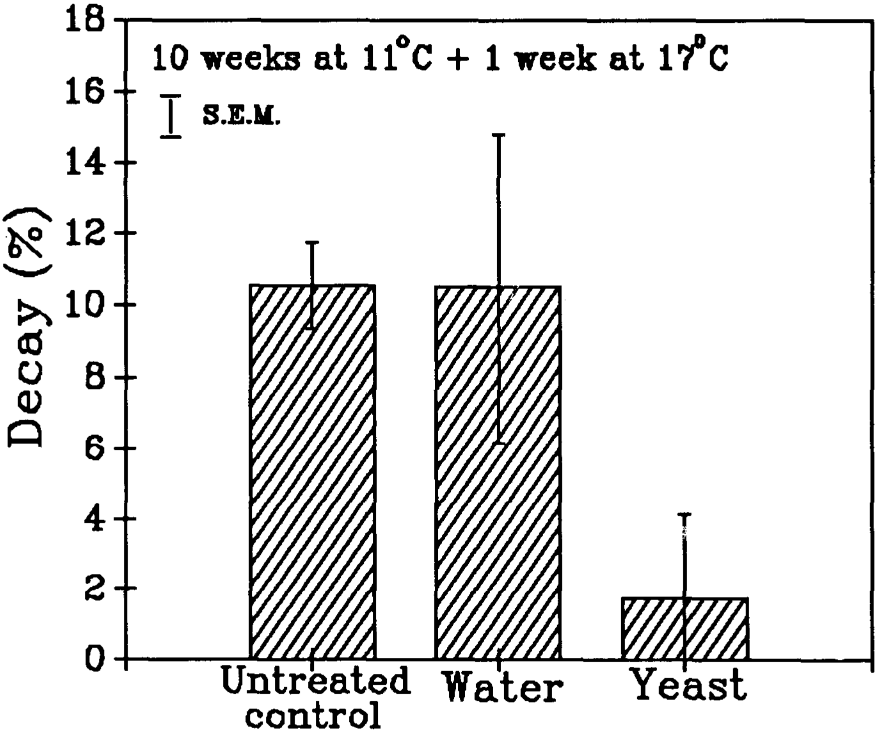

FIG. 1. Effect of preharvest treatment of *Pichia guilliermondii* on the development of postharvest decay of grapefruit.

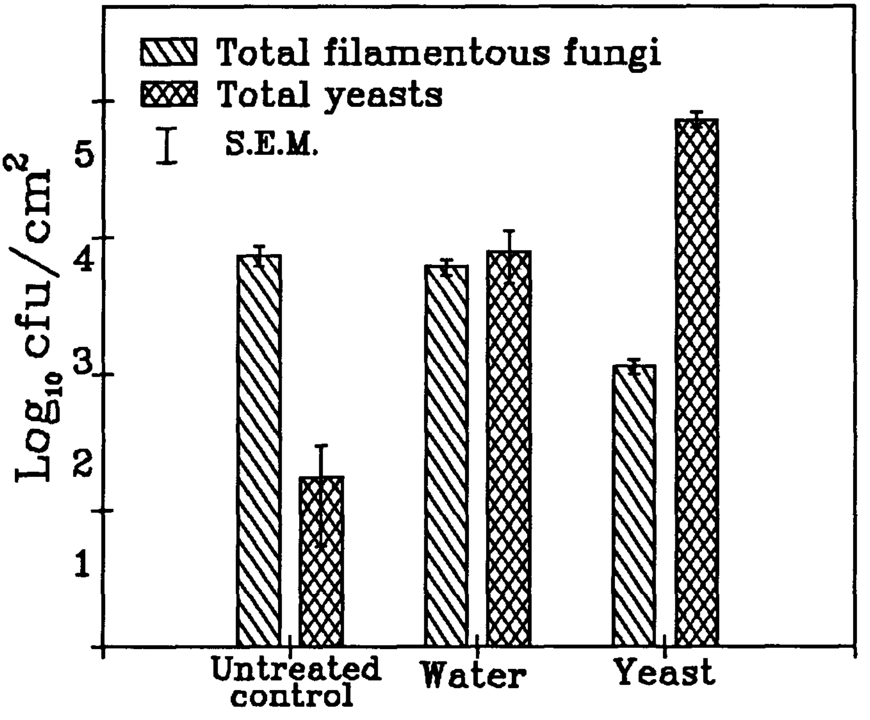

FIG. 2. Effect of preharvest treatment of *Pichia guilliermondii* on the epiphytic microbial populations.

Several lines of evidence support the assumption that inhibition of pathogen development by *P. guilliermondii* involves competition for nutrients (Droby *et al.*, 1989). For example, the efficacy of the yeast can be markedly reduced by the addition of nutrients to spore suspensions used for inoculation. Effective competition with the pathogen for nutrients has been demonstrated with this yeast in culture (Droby *et al.*, 1989). Similarly, *Enterobacter cloacae,* a bacterium, inhibited germination of *Rhizopus stolonifer* through nutrient competition (Wisniewski *et al.*, 1989).

Induction of host defense mechanisms

During the interaction of some biocontrol agents with the host tissue, processes common to the wound healing response have been noted. For example, as a result of application of a cell suspension of the yeast antagonist *P. guilliermondii,* to grapefruit peel wounds, ethylene production increased as well as the activity of phenylalanine ammonia-lyase. The involvement of these processes in citrus defence mechanisms against postharvest pathogens has been demonstrated in the past.

Direct interactions

The yeast antagonist *P. guilliermondii* may also affect the pathogen directly, thereby reducing fungal infectivity. Recent studies indicate that the antagonist cells attach firmly to the fungal mycelium. The possible production of glucanases or chitinases by the antagonist during such interaction is indicated by the apparent degradation of mycelial cell walls at the point of attachment. It is also possible that such attachment facilitates a more efficient depletion of nutrients from the area adjacent to the mycelium or serves as a mechanical barrier to nutrient uptake.

LARGE-SCALE TESTING OF BIOCONTROL AGENTS

Following successful testing of the biocontrol agent under laboratory conditions, antagonistic activity must be expressed under natural conditions which simulate full-scale field trials. The most important prerequisite for conducting large-scale or field tests is an understanding of the mode of action of the antagonist to enable the development of more reliable procedures for effective application of the antagonist. Pusey *et al.* (1988) conducted a pilot test employing *Bacillus subtilis* under simulated commercial conditions for the control of brown rot of peaches. They were able effectively to incorporate the antagonist into a wax that is normally used in the packingline. The yeast antagonist *P. guilliermondii* has also been subjected to a series of large-scale tests under packinghouse conditions for the control of postharvest diseases of citrus fruit.

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