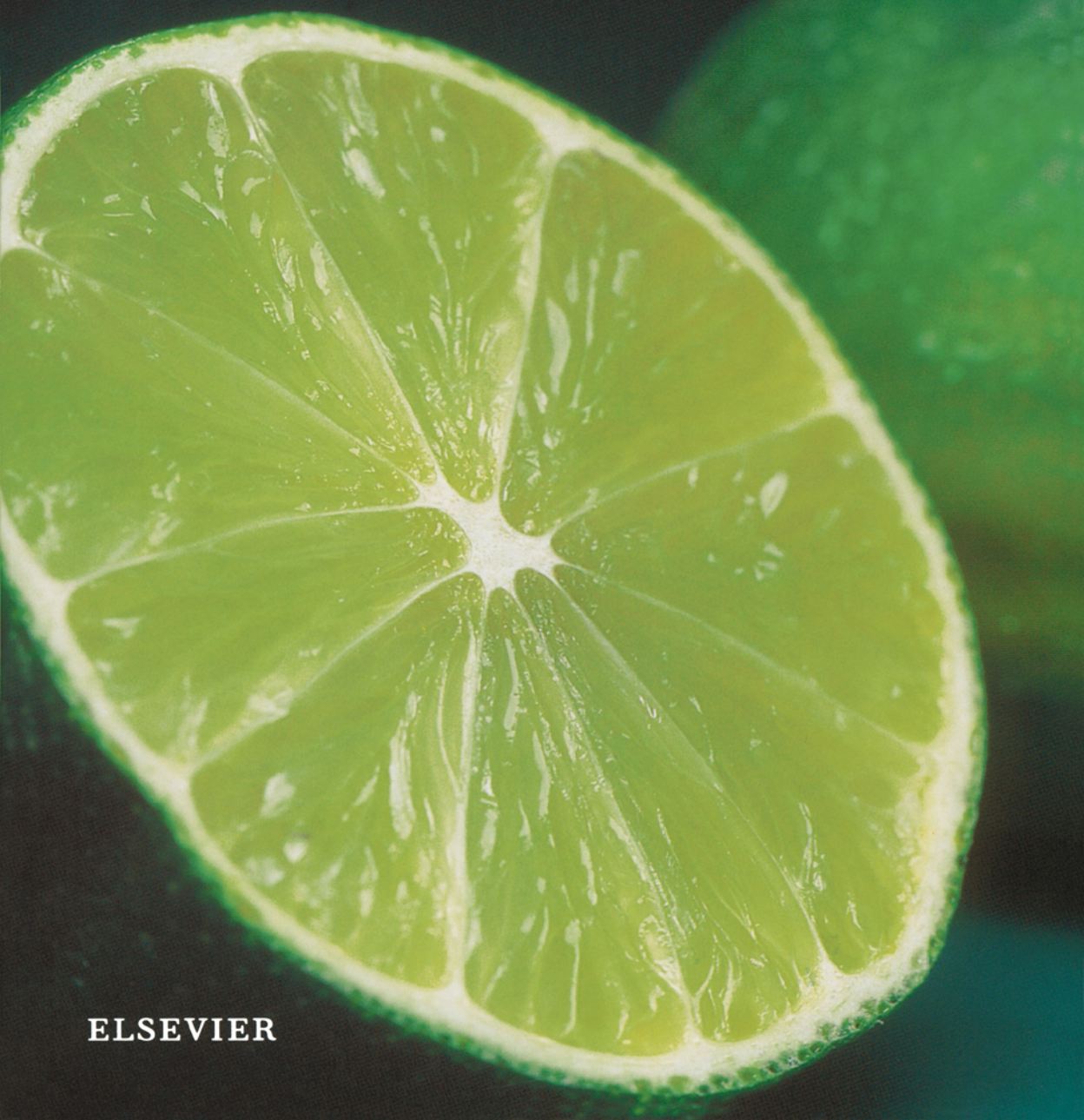
POSTHARVEST DISEASES OF FRUITS AND VEGETABLES

Development and Control

RIVKA BARKAI-GOLAN



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RIVKA BARKAI-GOLAN

Department of Postharvest Science of Fresh Produce

Institute of Technology and Storage of Agricultural Products

The Volcani Center, Bet-Dagan, Israel

2001

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Amsterdam – London – New York – Oxford – Paris – Shannon – Tokyo

ELSEVIER SCIENCE B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

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First edition 2001

Britisch Library Cataloguing in Publication data
Barkai-Golan, Rivka
Postharvest diseases of fruits and vegetables : development and control
1.Fruit - Diseases and pests 2.Vegetables - Diseases and pest
I.Title
634'.046

ISBN 0444505849

Library of Congress Cataloging in Publication Data
Barkai-Golan, Rivka.
Postharvest diseases of fruits and vegetables : development and control / Rivka Barkai-Golan --1st ed.
p cm.
ISBN 0-444-50584-9 (hardcover)
1. Fruit--Postharvest diseases and injuries. 2. Vegetables--Postharvest diseases and injuries. I. Title.

SB608.F8 B27 2001
634'.0493--dc21

2001023788

ISBN: 0-444-50584-9

♾ The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).
Printed in The Netherlands.

Dedicated to my husband, Eli, without whose loving support this book would not have been completed; and to my colleagues and students, without whose encouragement it would not have been started.

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PREFACE

This book brings together various topics concerning the diseases of harvested fruits and vegetables. A considerable part of the material on which this book is based has been gathered from my lectures given to students at the Hebrew University of Jerusalem, at the Faculty of Agriculture in Rehovot. The material has generally been based on original articles and reviews written by scientists from all over the world.

Throughout the book I have tried to impart to the reader an awareness of the great variety of research that has been constantly done in the field of postharvest pathology. Among the studies done in the field, some research has made a significant and exciting contribution to the understanding of the inter-relationships between the pathogen and the harvested fruits or vegetables. Throughout the years, with the advancement in this research, the challenge to integrate the ultimate aim - the lengthening of the postharvest life of fruits and vegetables - with an understanding of the biochemical processes that enable us to do just that, has become most prominent.

Although each chapter in the book constitutes a separate unit, the order and continuity of the chapters are relevant to a better understanding of the whole subject.

The first four chapters describe the causal agents of postharvest diseases of fruits and vegetables, their sources and their ways of penetration into the host, factors that may accelerate the development of the pathogen in the host - and those that suppress them. A list of the main pathogens of fruits and vegetables, their hosts and the diseases elicited by them are given in a separate chapter, while a detailed description of the major diseases of selected groups of fruits – subtropical and tropical fruits, pome and stone fruits, soft fruits and berries, and solanaceous vegetable fruits – is given at the end of the book, in the Summary of Postharvest Diseases of Four Groups of Fruits.

Chapters five and six focus on the attack mechanisms that the pathogen may use to invade the plant tissues and develop within them, and the defense mechanisms with which the host is equipped to stop invaders or to suppress their development. Physiological and biochemical changes following infection are described in Chapter seven. The following chapters, 8 to 12, all deal with treatments aimed at suppressing postharvest diseases. While chapter 8 describes treatments for maintaining the natural

resistance of the young fruit or vegetable, chapters 9, 10 and 11 describe chemical, physical and biological means for preventing disease initiation, inhibiting its development or reducing its severity. Increased official and public concern about the chemical residues left on the fresh produce and the resistance some pathogens have developed toward major fungicides have stimulated the search for alternative technologies for postharvest disease control. This section emphasizes the search for natural and safe chemical compounds, and the variety of alternative physical and biological control methods. It also points to the possibility of exploiting the natural defensive substances of fruits and vegetables, both constitutive and induced, to enhance host resistance during storage. This leads us to the last chapter, which introduces new approaches to the prevention of postharvest diseases, by inducing the natural host resistance by means of chemical, physical and biological elicitors. At present, with the developments in molecular genetics, we are standing at the dawn of a new era in which genetic crop modification may be of central importance among the variety of methods used to increase host resistance against postharvest diseases. Approaches to enhancing fruit resistance by creating disease-resistant transgenic plants have been discussed.

This book is intended for teachers and students who focus on postharvest pathology and plant pathology programs, for scientists in research institutes, companies and organizations dealing with fruit and vegetable preservation technologies and with extension and training in the areas of harvesting, handling, packaging and storage, and for all those striving to improve the quality of harvested fruits and vegetables and to lengthen their postharvest life.

I would like to thank the many students who expressed the desire to put the material of the lectures on paper and, therefore, encouraged me to write this book. My thanks also go to the members of the Department of Postharvest Science of Fresh Produce at the Volcani Center, who advised me and aided by reading of the text or parts of it, and to all the scientists who approved the inclusion of the illustrations of their research in this book.

A special thanks to colleagues who provided me with several photos which appear in this book: Dr. Nehemia Aharoni (Photos 1, 2) and Dr. Samir Droby (Photos 7, 8), and to the Atomic Energy Board, Pelindaba, South Africa for Photos 3, 4, 5, 6.

Rivka Barkai-Golan
Beit Dagan – Rehovot

CHAPTER 1

INTRODUCTION

We often witness the phenomenon that just harvested fresh fruits or vegetables ripen and are ready to be marketed, but the markets are overstocked and the demand is low. To achieve prolonged and constant marketing of produce that is gathered in large quantities over a relatively short period of time - the prime time for each fruit and vegetable - the produce must be stored for weeks or months prior to marketing. Fresh fruits and vegetables destined for export also require long periods of storage and shipment prior to arrival. In the course of storage and shipment part of the fresh produce, which is rich in moisture and nutrients and therefore may serve as a suitable substrate for the development of microorganisms, may rot and become unfit for sale. The evolution of decay as a result of microorganism attack during storage is one of the main causes for the deterioration of the fresh produce and can, in itself, become a limiting factor in the process of prolonging the life of the harvested fruits and vegetables. The economic loss incurred through storage diseases might exceed that caused by field diseases because of the large investments in the overall treatments and processes the product undergoes from harvest until it reaches the customer, and which include harvesting, sorting, packing, shipping and storing.

Even in societies with access to the most advanced technologies for handling fresh fruits and vegetables, such as computerized sorting and grading, improved packing materials and methods, and advanced regular or modified atmosphere storage, losses caused by postharvest diseases remain substantial. In fact, international agencies that monitor world food resources have acknowledged that one of the most feasible options for meeting future needs is the reduction of postharvest losses (Eckert and Ogawa, 1988).

Fresh fruits and vegetables supply essential nutrients, such as vitamins and minerals, and are a major source of complex carbohydrates, antioxidants and anticarcinogenic substances which are important to human health and well being (Arul, 1994). Being aware of the advantages of fresh fruits and vegetables, the consumer prefers the wholesomeness of the fresh produce over processed products. However, at

the same time, the demand of the consumer for produce free of pathogens, and also free of chemical residues remaining from chemical treatments for disease control, has been growing during recent years. Furthermore, fungal rots have been associated with mycotoxin contamination of fruits (Paster et al., 1995) and their possible interaction with the human pathogen, *Salmonella*, has also been suggested (Wells and Butterfield, 1999). In other words, the safety of fresh produce has become a major public concern.

The aim of adequate storage is, therefore, to help the harvested fruits and vegetables to arrive at their destination fresh, disease free and safe to the consumer - despite the complex of treatments they have to undergo prior to or during storage, and despite the long period between their harvest and their reaching the consumer. All the means and methods with the power to aid in preserving the quality of the harvested produce and in protecting it from decay agents during storage and shelf-life, are aimed at this objective.

To understand how decay can be prevented or delayed we must, as stage one, be familiar with the decaying agents, their nature, their origin and their time and mode of penetrating into the host, as well as with the factors that might affect their postharvest development. At a later stage we should also be familiar with suitable means for preventing disease initiation or for arresting pathogen development within the host tissues and, on the other hand, with the possibility of maintaining or enhancing host resistance to infection.

CHAPTER 2

POSTHARVEST DISEASE INITIATION

A. THE PATHOGENS

Upon harvest, ripe fruits and vegetables become subject to attacks of various microorganisms incapable of attacking earlier in the course of growth in the field. These are largely weak pathogens, fungi and bacteria, typical of the harvested and stored fruits and vegetables.

Disease resistance, which was embodied in the plant organ designated for storage during its developmental stages on the plant, weakens as a result of separation from the parent plant. In addition, picked fruits and vegetables are rich in moisture and nutrients, which suit the development of pathogens. Upon ripening the fruits and vegetables often become more susceptible to injury and, therefore, more susceptible to the attack of those microorganisms that require an injury or damaged tissue to facilitate their penetration (Eckert, 1975). Moreover, during the prolonged storage of fruits and vegetables a series of physiological processes occurs which leads to the senescencing of the vegetal tissues and, in parallel, to their increased susceptibility to weak pathogens that attack senescencing vegetal tissues.

B. THE ORIGIN OF THE PATHOGENS

Fungi and bacteria responsible for in-storage decay often originate in the field or the orchard. When penetration into the host takes place in the field, the pathogen, which is then in its early or quiescent stages of infection, will get to the storeroom within the host tissue without eliciting any symptoms of decay. Yet, even when preharvest infection has not taken place, there are always fungal spores and bacterial cells, which are typical components of the airborne microorganism population, on the fruit and the vegetable during their growth. This cargo of spores and cells is transferred to storage with the harvested fruits and vegetables.

Examination of the fungal spore population on the surface of stems, leaves, flower parts, fruits and other plant organs, after harvest, reveals

the presence of many important airborne fungi such as species of *Cladosporium, Alternaria, Stemphylium, Penicillium, Aspergillus, Rhizopus, Mucor, Botrytis, Fusarium* and others. Many of the airborne fungi are among the most important decay agents which affect harvested fruits and vegetables and, given the right conditions, develop and cause decay.

Many postharvest pathogens perpetuate on crop debris in the field and can, under suitable conditions, develop and produce abundant new spores. These fungal spores are easily carried by air currents, winds and rain, or dispersed by insects, to the flowers and the young fruits, at various stages of development, and form a potential source of infection.

Soil, irrigation water and plant debris form an important source of infection of various vegetables. Soil-residing fungi and bacteria can attack the bulb, tuber, root and other vegetal parts, while these are still attached to the parent plant, through a tight contact with the soil, by lifting of soil particles by winds, rains or irrigation, through growth, or by arriving in storage with soil residues attached to the vegetable. Some soil microorganisms, such as species of the fungi *Botrytis, Sclerotinia* and *Fusarium* or the bacterium *Erwinia carotovora*, are among the main decay agents in stored vegetables. Host infection can occur preharvest, during harvest or during any of the postharvest handling stages. Harvesting instruments, containers, packing houses with their installations, the hands of the packers or selectors, the atmosphere of the storage rooms - are all bountiful sources of fungal spores.

Despite the diversity of microorganisms that the fruits and vegetables carry with them into storage, only a few species can naturally attack them, while other species that reside on the surface, at times in large quantities, will not penetrate and will not cause any decay. The development of disease during storage depends, primarily, on the existence of the appropriate microorganisms alongside a given host. However, in order for the fungal spores or bacterial cells that have reached the suitable host to be capable of infecting, they have to encounter the appropriate conditions for germination on the surface of the host, to penetrate into the host tissues and to develop there.

C. SPORE GERMINATION

Spore germination is a preliminary stage to fungal penetration into the host. The right environmental temperature, available water or

moisture and, sometimes, the presence of nutrients transferred from the host into the water, are the most important environmental factors that aid spore germination.

Temperature. Most of the storage decay fungi are mesophiles - preferring moderate temperatures. The optimal temperature for the germination of their spores is around 20-25°C, although the temperature range allowing germination can be much wider. The further from the optimal temperature, the longer the time until the initiation of spore germination - a phenomenon that affects the prolongation of the incubation period of the disease. Shifting the temperature away from the optimum reduces the rate of germination and retards germ-tube elongation. A sufficiently low temperature can inhibit spore germination altogether and thus constitute a disease-limiting factor.

Water. Water or moisture is essential for fungal spore germination, although spores of some fungi are capable of germination if there is very high relative humidity of the surrounding air (Roberts and Boothroyd, 1984). Spores of most fruit and vegetable pathogens can germinate in pure water or water with low nutrient concentrations, transferred from the host surface to the water by osmosis or supplied to the spores by injured and battered cells in the wound region, the typical court of infection for many postharvest pathogens.

Nutrient additives. Spores of *Penicillium digitatum*, the green mold fungus which attacks only citrus fruit, germinate to a minor extent in pure water, whereas the addition of fruit juice greatly accelerates germination (Pelser and Eckert, 1977). Testing the effect of the juice components on spore germination revealed that of the sugars within the juice (glucose, sucrose and fructose), glucose is the best stimulant. A greatly enhanced germination is stimulated by the ascorbic acid, whereas the citric acid has no stimulating effect. The combination of glucose and ascorbic acid results in a germination rate quite close to that stimulated by the whole juice.

Penicillium spore germination is also stimulated by the addition of oil derived from the rind of orange, lemon, grapefruit or other citrus fruits (French et al., 1978). Fig. 1 displays the stimulating effect of various concentrations of oil produced from an orange rind on the germination rate of *P. digitatum* conidia. In the presence of 250 ppm oil, 15% of the spore population had germinated after 24 hours at 19°C, while no germination occurred in the control spores (water only). Ten days later, the germination rate amounted to 70% with the oil, in comparison with approximately 10% in the control. A similar effect occurred on

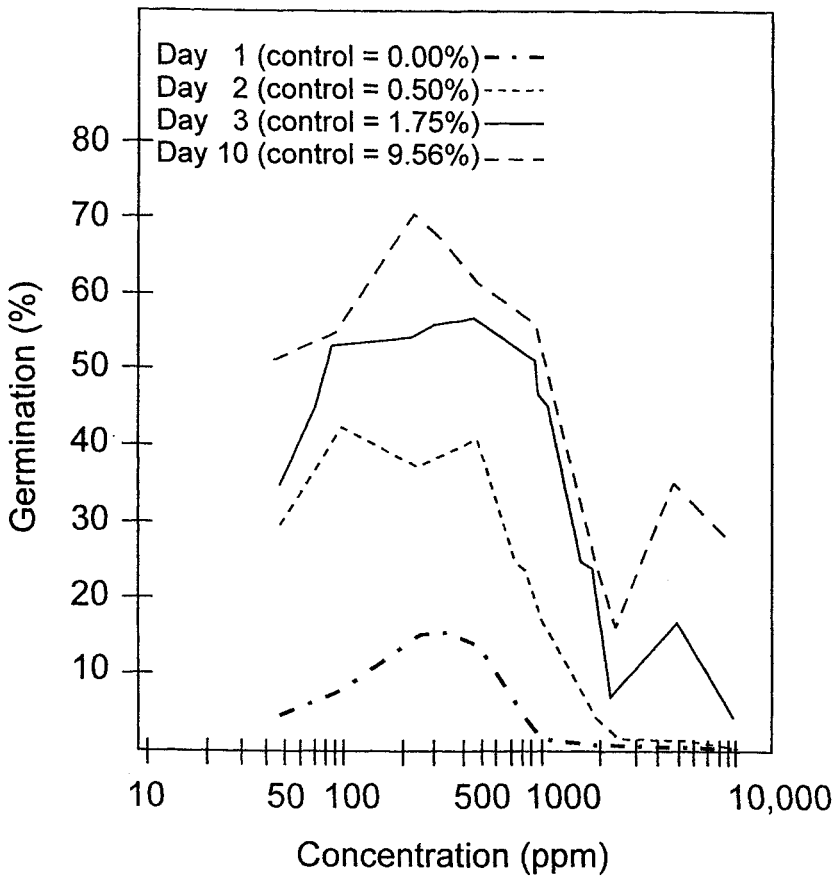


Fig. 1. Effect of orange oil suspension in 1% water agar on germination of conidia of *Penicillium digitatum*. (Reproduced from French et al., 1978 with permission of the American Phytopathological Society).

Penicillium italicum conidia also. Examination of the various oil component activities revealed that nonanal and citral were the most effective compounds. Moreover, a mixture of these two compounds had a synergetic effect, leading to high germination rates in the conidia population (French et al., 1978). It appeared that oils produced from different citrus fruits might differ in their compositions, hence the relative differences among the stimulating or suppressing activities of oils produced from different sources.

Since little or no germination of *P. digitatum* occurred on water agar alone, it was assumed that substrate nutrition was the determinant of

spore germination and an important factor in host specificity of the pathogen to citrus fruits. However, when *P. digitatum* spores on water agar were exposed to several wounded oranges in closed containers, germination did take place in the absence of substrate nutrients (Eckert and Ratnayake, 1994). Searching for the reason for this phenomenon, Eckert and Ratnayake (1994) found that a mixture of volatiles evaporating from the abrasions of wounded oranges were capable of accelerating or inducing germination of *P. digitatum* spores on water agar as well as within an injury of the rind. The major components of this mixture were the terpenes, limonene, ∝-pinene, β-myrcene, and sabinene, accompanied by acetaldehyde, ethanol, ethylene and CO_2, as identified by gas chromatography (Fig. 2). At a concentration typical of the natural mixture surrounding wounded oranges (1 x concentration), 45% of the spores germinated on water agar. Germination was reduced by both higher and lower concentrations of the mixture of volatiles (Fig. 3).

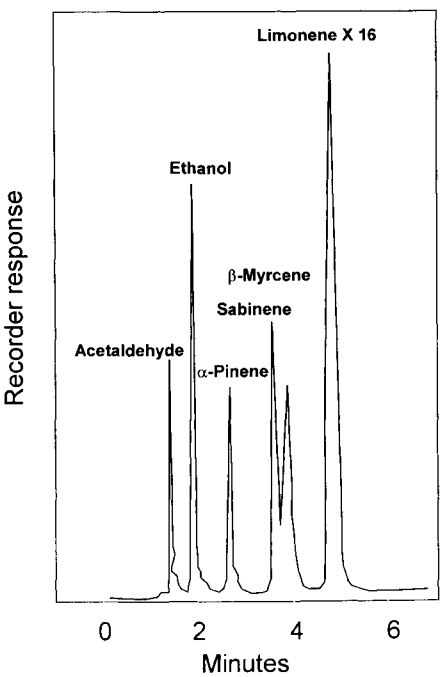


Fig. 2. Gas chromatogram of volatile organic compounds in the atmosphere surrounding wounded oranges. Limonene peak shown was attenuated 16 times. (Reproduced from Eckert and Ratnayake, 1994 with permission of the American Phytopathological Society).

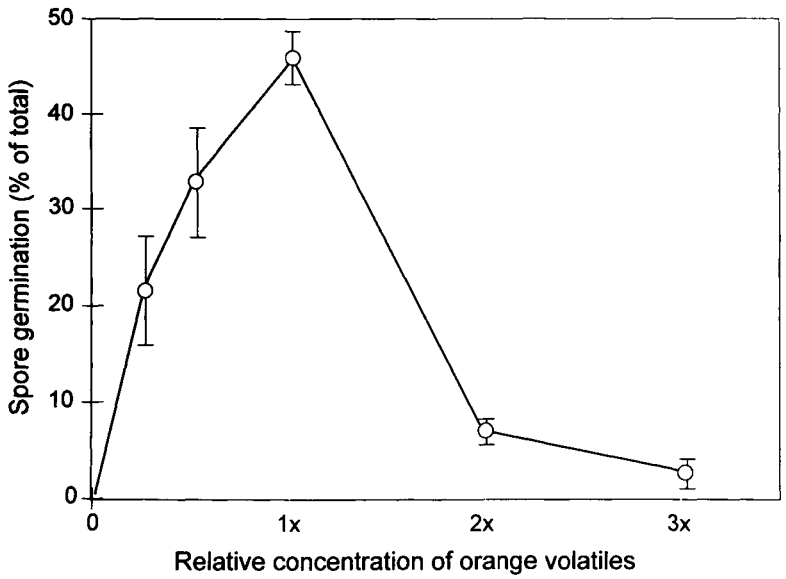


Fig. 3. Effect of concentration of the synthetic mixture of volatile compounds on the germination of *Penicillium digitatum* spores. 1x is the concentration typical of the natural mixture measured surrounding wounded oranges. Bars indicate standard errors. (Reproduced from Eckert and Ratnayake,1994 with permission of the American Phytopathological Society).

The fact that the average quantity of volatile compounds emanating from wounded fruits was 75 times greater than that from non-injured fruit may explain the lower germination of *P. digitatum* spores on water agar exposed to sound citrus fruits. The terpenes alone, in several concentrations and combinations, failed to stimulate spore germination significantly above its level on water agar. On the other hand, a synthetic mixture of limonene, the major terpene in the wounded fruit atmosphere, with acetaldehyde, ethanol and CO_2, at concentrations similar to those measured in the atmosphere around wounded oranges, stimulated spore germination on water agar to the same degree as the natural mixture of volatiles.

It is interesting to note that volatile compounds evolved from diced peels of various *Citrus* spp. (oranges, lemons, grapefruits, tangerines, kumquats) have also induced stimulation of germination of *P. digitatum* spores. Furthermore, lemons infected with *Phytophthora citrophthora, Geotrichum candidum* or *P. digitatum* also emanated volatiles that

induced germination of *P. digitatum* spores on water agar (Eckert et al., 1992; Eckert and Ratnayake, 1994).

Ripening apricots also release ethanol and acetaldehyde which, at low concentrations, stimulate germ tube growth of *Monilinia fructicola, in vitro* (Cruickshank and Wade, 1992). Volatile compounds that stimulate spore germination or fungal development were suggested by French (1985) to act by altering membrane permeability or regulating metabolism. At higher concentrations, however, some volatiles, such as acetaldehyde, were found to be fungitoxic (Prasad, 1975) and have been evaluated as fumigants to control postharvest diseases of various crops (see the chapter on Chemical Control - Natural Chemical Compounds).

Germination of various fungal spores may be stimulated by solutes that diffuse from within the fruit or other plant organs into the water film over the infection site. Spore germination of *Colletotrichum musae*, the cause of banana anthracnose, is very poor in pure water. However, on the surface of banana fruits, both spore germination and the formation of appresoria (specific resting spores responsible for fruit infection) were accelerated (Swinburne, 1976). This stimulation was attributed to the permeation of anthranilic acid from the inner tissues to the fruit surface. Fungal spores can rapidly degrade this acid to 2,3-dihydroxybenzoic acid, which is responsible for the accelerated germination. This acid is involved in the active transport of iron in various microorganisms and reduces the iron level around the spores on the surface of the banana (Harper and Swinburne, 1979; Harper et al., 1980). This phenomenon explains why siderophores, which are chelating agents with a high affinity for this metal, that are formed on the banana surface by bacteria, under conditions of iron deficiency (Neilands, 1981), stimulate and accelerate germination of *Colletotrichum* spores and the formation of appresoria (McCracken and Swinburne, 1979).

Spores of *Rhizopus stolonifer*, which causes a watery soft rot in many fruits and vegetables, also need nutrient additives in the water in order to germinate and infect carrots (Menke et al., 1964). Spores of *Botrytis cinerea*, the causal agent of gray mold, though capable of germination in pure water to some degree, cannot infect strawberries or cabbage leaves without external nutrients (Jarvis, 1962; Yoder and Whalen, 1975). Early studies with *B. cinerea* showed that distilled water on the surfaces of leaves and petals contains more electrolytes than distilled water on glass slides, and that spores of *Botrytis* usually germinate better in water containing electrolytes (Brown, W., 1922a). Several studies indicated that grape berry exudates stimulated *B. cinerea* spore germination and

that stimulation increased during the last month of fruit ripening. This period corresponds with the large increase in sugar content of the exudates, along with the increased susceptibility of grapes to infection (Kosuge and Hewitt, 1964; Padgett and Morrison, 1990).

Various fungi, which penetrate through wounds, encounter the moisture required for germination of their spores in fresh wounds in the cuticle and epidermis of the host. The fractured cells in the injured area also supply the nutrients required for the germination and infection phases. Pathogens which penetrate through the host lenticels can feed on the nutrients secreted from the cells adjoining the lenticels, especially after injury following tissue senescence (Eckert, 1978).

Atmospheric gases. Additional environmental factors, such as oxygen (O_2) and carbon dioxide (CO_2) levels in the atmosphere in which the fruits or vegetables are stored, can also affect germination. Reducing the O_2 level in the air to below 21% or increasing the CO_2 level to more than 0.03% can inhibit fungal spore germination, although various species react differently to the depressing levels. Generally, an atmosphere of 15-20% CO_2 directly inhibits spore germination of *Rhizopus stolonifer, Botrytis cinerea,* and *Cladosporium herbarum,* with inhibition rising as the gas level increases. However, such an atmosphere does not affect spores of *Alternaria alternata*; inhibition of these spores requires that the gas level be increased to more than 32%. On the other hand, spore germination of *Fusarium roseum* accelerated under the effect of high concentrations of CO_2, but when the concentration was over 32% the germination of these spores, too, was inhibited (Wells and Uota, 1970). (See Fig. 21a).

Exposure of spores to low oxygen levels, too, might damage their germination ability, but a significant delay normally occurs only with the reduction of oxygen level to 1% and below (See Fig. 21b). The capacity of high CO_2 levels and low O_2 levels, or a combination of the two, to inhibit spore germination is used for creating a modified or controlled atmosphere suitable for the prolongation of the postharvest life of harvested fruits and vegetables (See the chapter on Means for Maintaining Host Resistance – Modified and Controlled Atmospheres).

Spores encountering conditions suitable for germinating, including available water or moisture combined with the required nutrients, adequate temperature and other environmental conditions, will swell, develop germ-tubes and be ready for the next stage: penetrating into the host.

D. PATHOGEN PENETRATION INTO THE HOST

Postharvest pathogens can be divided, according to the timing of their penetration into the host, into those that penetrate the fruits or vegetables while still in the field but develop in their tissues only after harvest, during storage or marketing, and those that initiate penetration during or after harvest.

1. INFIELD PENETRATION AND QUIESCENT INFECTIONS

Harvesting and Picking after Pathogen Penetration

Late blight of potatoes, caused by *Phytophthora infestans*, is an example of decay originating in tuber infection in the field. The infection is caused by the zoospores found in the soil or that fall onto the tubers from infected foliage during harvest. Following germination the zoospores penetrate into the tubers through the "eyes", lenticels, growth cracks, wounds, or via the point of attachment to the plant (the stolon) (Lapwood, 1977). Tubers that were infected a few days prior to harvest or during the harvest itself are brought to storage carrying the disease in its early developmental stages, with no visible symptoms of decay. These tubers will decay while stored under high humidity and at a temperature over 5°C.

In a warm and damp climate, the blight fungus can also attack the tomato fruit at its various ripening stages, in the field. The attack usually takes place at the edge of the fruit stalk scar, although, given prolonged humidity, the fungus can also penetrate directly through the skin (Eggert, 1970). During epidemics the entire crop may rot in the field. However, when the disease is less severe, fruits with no visible symptoms or with slight blemishes might be picked, and the fungus will continue to develop during storage.

The brown rot, which develops in citrus fruits during storage, originates in preliminary infections initiated in the orchard by *Phytophthora citrophtora* and other *Phytophthora* species. During the rainy season, the fungal zoospores descend on the lower fruits on the tree and penetrate them directly (Feld et al., 1979). The fungus can develop in the orchard, but when zoospore infection occurs a few days prior to picking, when the external symptoms are not yet visible, the fruit will later rot and might comprise a serious problem during storage or shipping.

Penetration of the various *Phytophthora* species into the host, in the field or in the orchard, is not linked with a typical quiescent infection wherein the pathogen development ceases following its penetration into the tissues or at an earlier stage. The principle concerning this fungus is that the harvest ends its development within the host while the latter is still on the parent plant, thus causing the decay to occur after harvest or during storage.

In many vegetables, such as carrot, cucumber, lettuce, celery, cabbage, cauliflower and others, a soft watery decay caused by *Sclerotinia sclerotiorum* is common. This fungus, common in the soil, and especially in heavy soil, might attack the vegetable when still in the field or during harvest and continue to develop in its tissues throughout storage or marketing.

Quiescent or Latent Infections

The fungi that penetrate into the host in the field also include pathogens that cause latent or quiescent infection. These pathogens reach fruits or vegetables that are still on the parent plant. However, during one of the phases between their reaching the host and the development of progressive disease, their growth is arrested until after the harvest, when physiological and biochemical changes occurring within the host will enable their renewed growth. Verhoeff (1974) has described such arrested infections as "latent infections". However, since this term has been used for describing different phenomena in various areas, it was later suggested by Swinburne (1983) to leave the term "latent infection" for wider uses and adopt "quiescent infection" for cases in which the pathogen growth is temporarily inhibited. In some instances we find that the inactive state is termed 'latent' if not visible to the eye and 'quiescent' if visible (Smilanick, 1994), while in others the two terms are frequently used to describe the same phenomenon. Jarvis (1994) believes that the latent state of the pathogen, whether involving spores that have landed on the host surface, spores that have commenced germinating, or primary hyphal development within the host tissues, is linked to a dynamic balance among the host, the pathogen and the environment. The physiological and biochemical changes occurring in the host tissues after harvest and during storage might affect the pathogen, the host and their interrelationships and lead to the activation of the latent pathogen.

The gray mold, which is the main cause of decay in harvested strawberries, is an example of disease originating in a quiescent infection with *Botrytis cinerea* that is acquired in the field. The *Botrytis* spores,

with which the strawberry field air is filled during bloom, can germinate in a drop of water on the petal or other parts of the flower, and later penetrate through the senescenced parts of the flower, into the edge of the receptacle of the strawberry where they develop a dormant mycelium. During ripening and storage, as the resistance of the fruit to the pathogen decreases, the preliminary mycelium enters an active stage, and the decay develops (Powelson, 1960; Jarvis, 1962). In grapes, too, where the gray mold fungus is one of the most important decay agents during storage, the origin of the disease is usually in infections that occur in the vineyard at the time of bloom, and which remain dormant until the fruit is stored (McClellan and Hewitt, 1973). The development of the gray mold at the stem-end region of the harvested tomato is also the result of *Botrytis* penetration into the young fruit through the flower parts (Lavi-Meir et al., 1989).

The brown rot, caused by *Monilinia fructicola* in stone fruits (Tate and Corbin, 1978; Wade and Cruickshank, 1992) and the decay caused by *Nectria galligena* in apples (Swinburne, 1983), also originate in the infection of young fruit in the orchard. The *Nectria* fungus forms a preliminary mycelium within the young tissues, which is the dormant stage of the disease, while the interrupted stage in the development of the brown rot caused by *Monilinia* can occur during spore penetration through the stomata or even at the pre-germination stage.

The stem-end rots in citrus fruits, caused by *Diplodia natalensis* and *Phomopsis citri*, develop from quiescent infections located in the stem-end button (calyx and disc) (Brown and Wilson, 1968). In moist conditions a large quantity of *Diplodia* and *Phomopsis* picnidia can be found on the bark of citrus trees and on dry branches. The spores that break loose from the picnidia are spread by rain splash onto the flowers and young fruit, where a preliminary infection occurs at the stem-end region. A further development of the disease is hindered by healing of the wound and the formation of protective barriers of tightly packed cells by the host tissue (see the chapter on Host Protection and Defense Mechanisms - Wound Healing and Host Barriers). After the fruit is picked, the senescencing processes in the button region lead to the separation of the button from the fruit and the formation of an opening that enables the fungi to penetrate into the fruit and initiate the stem-end decay.

Another quiescent infection is anthracnose, caused by *Colletotrichum gloeosporioides* in many tropical and subtropical fruits, such as mango, papaya, avocado and various citrus fruits (Baker, R.E.D., 1938; Brown,

G.E., 1975; Dickman and Alvarez, 1983; Binyamini and Schiffmann-Nadel, 1972; Spalding and Reeder, 1986b), or by *Colletotrichum musae* in banana fruits (Simmonds, 1941). The fungal conidia are found in large quantities on the surface of the fruits during their development on the tree. In the presence of free water upon the fruit, the spores germinate and form an appressorium at the tip of the germ tube. The appressoria function as resting spores on the fruit, since they resist environmental conditions much better than the conidia; they remain vital for long periods while embedded in the natural wax of the fruit or bound to its surface. The binding can occur through a mucous secretion of the fruit, as with *C. musae* (Simmonds, 1941). Yet, the appressoria are infection bodies capable of germinating, under suitable conditions, and forming germinating tubes which penetrate into the tissues; they may even develop fine "infecting hyphae" that penetrate under the cuticle or the external layers of the epidermis. The appressoria themselves, or the "infecting hyphae" which they form, comprise the quiescent stage of fungal infection (Muirhead, 1981b; Prusky et al., 1990). Following picking, when the fruit commences ripening, the quiescent infection transforms into an active stage and initiates the development of the typical anthracnose. In papaya, the penetration of the germ tubes occurs through the stomata of the young, unripe fruit, while the appressoria can remain dormant for a long period over the cuticle or in "caves" beneath the stomata (Stanghellini and Aragaki, 1966).

Assumptions and explanations raised in connection with the establishment of quiescent infections in unripe fruits were summarized and classified into four categories by Simmonds (1963). Verhoeff (1974), in his review of latent infections, had grouped these theories into three groups (combining the nutritional and energetic requirements of the pathogen into one group):

(1) The shortage in the young fruit of adequate substrate to meet with nutritional and energetic requirements of the pathogen;
(2) The incapability of the pathogen to produce cell-wall degrading enzymes in the young fruit;
(3) The presence of preformed antifungal compounds in the young fruit that inhibit pathogen growth or enzymatic activity. Swinburne (1983), in a later review, added the fourth category of theory:
(4) The accumulation of phytoalexins – antifungal compounds induced by the host tissue as a result of actual or attempted fungal infection.

Following the description by Prusky and Keen (1995) of preformed compounds that can be further induced in the host tissues, the third

theory can be broadened to include: "the presence of preformed antifungal compounds in the young fruit and their further inducement in the host tissues".

Nutritional and energetic requirements of the pathogen. The first theory claims that young unripe fruit does not provide the pathogen with the nutrition and energy required for its development. Since, during ripening, a conversion of insoluble carbohydrates to soluble sugars occurs, it is no wonder that the resistance to rotting of the young fruit has been attributed, in several cases at least, to the sugar content of the tissues. This theory complies with the results of experiments conducted on apples, in which an artificial increase of the sugar level, by addition of sugar-regulated compounds (such as 2,4-dinitrophenol) to the fruit, had accelerated the decay caused by *Botryosphaeria ribis* (Sitterly and Shay, 1960). Yet, since these compounds also accelerated the onset of the climacteric peak of the fruit respiration, their stimulating effect cannot be attributed to the sugar level alone. In addition, the accelerated onset of susceptibility of apple fruits to the pathogen could also be attributed to the reduction in the toxicity of antifungal compounds in the presence of sugars (Sitterly and Shay, 1960). The fact that fungi which normally attack ripe fruit may also develop on extracts prepared from unripe fruit, attests to a lack of a clear link between resistance and the shortage of available nutrients in the young fruit (Swinburne, 1983).

Activation of pathogen enzymes. The second theory suggests that the unripe fruit does not supply the pathogen with compounds that induce the formation and activity of cell-wall degrading pectolytic enzymes. In addition, in cases whereby the fungus produces cell-wall degrading enzymes, cross-linking of the cell-wall pectic compounds might block the access of these enzymes to the sites within the cell wall. Furthermore, enzymes may be inactivated by inhibitors present in higher quantities in immature fruits than in mature fruits (see the chapter on Host Protection and Defense Mechanisms - Inhibitors of Cell-Wall Degrading Enzymes).

The presence or induction of antifungal compounds in the host. The third and fourth theories point at a relation between the presence or formation of anti-fungal compounds in the young tissues and the creation of quiescent infections. These antifungal compounds may be: (a) preformed compounds, the existence of which is not pathogen related; (b) preformed compounds further induced by the host, either in the same tissue it was originally formed or in a new tissue; (c) phytoalexins (postformed compounds) induced by the host as a counterattack against the pathogen.

A correlation between preformed antifungal compounds and resistance to postharvest disease in the young fruit is found in various host-pathogen systems. The presence of phenolic compounds in the young banana fruits was reported as early as 90 years ago (Cook and Taubenhaus, 1911); particular significance was attributed to the tannins since they are often found in higher concentrations in unripe than in ripe fruits. Examples of such antifungal compounds are the phenolic compounds in apples, which inhibit pathogen development in the young fruit (Ndubizu, 1976) and the 3,4-dihydroxybenzaldehyde compound that has proven fungistatic activity in the green banana fruit (Mulvena et al., 1969).

Another example of a preformed compound is the glycoalkaloid tomatine. Its presence at high concentrations in the young fruit peel has been related to the quiescent infection of *B. cinerea* in the green tomato fruit (Verhoeff and Liem, 1975). In this case, however, the fungus would not actively grow as the fruit ripened when the tomatine level significantly decreases.

In unripe avocado fruit, a link was established between the presence of a diene and of monoene antifungal compounds in the fruit rind and the quiescent infection of *C. gloeosporioides* in such a fruit (Prusky et al., 1982). The diene, which is the major compound, inhibits spore germination and the growth of *Colletotrichum* mycelium in concentrations lower than those present in the young fruit rind (Fig. 4).

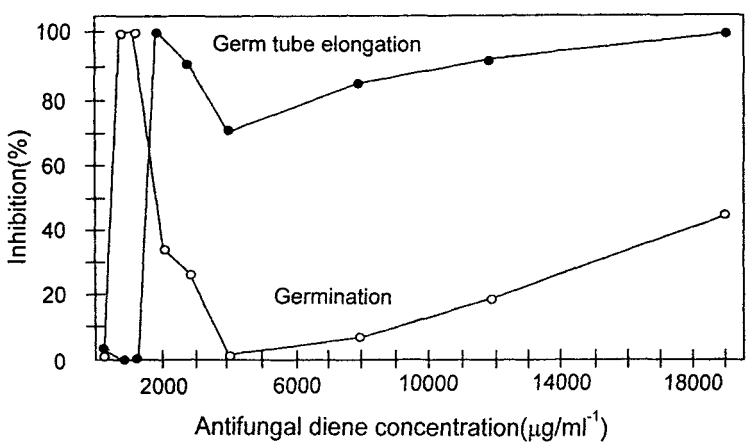


Fig. 4. Effect of the purified antifungal diene from avocado peel on conidial germination and germ tube elongation on *Colletotrichum gloeosporioides*. (Reproduced from Prusky et al., 1982 with permission of the American Phytopathological Society).

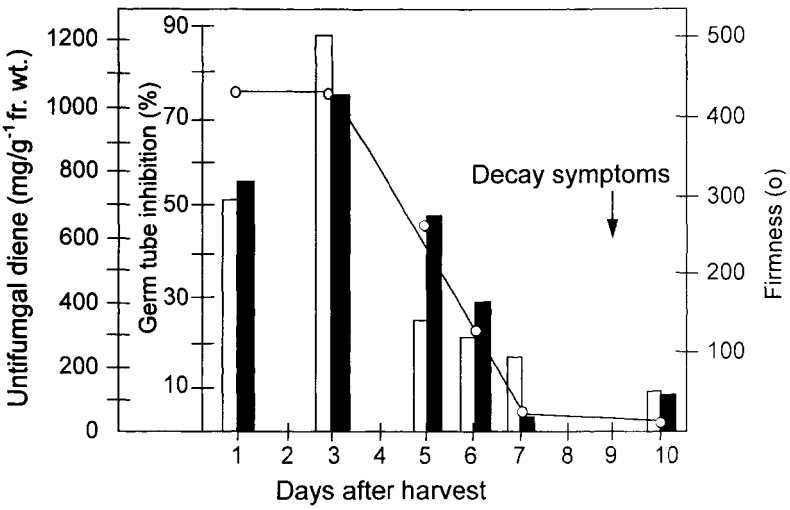


Fig. 5. Fruit firmness (○), antifungal activity (■), and concentration of the antifungal diene (□) in crude extracts from peel of avocado cultivar Fuerte at different stages after harvest. The arrow denotes the first visible decay symptoms. (Reproduced from Prusky et al., 1982 with permission of the American Phytopathological Society).

During ripening, the diene concentration decreases tenfold to subfungitoxic levels and enables the continuation of the mycelium growth and the development of the disease (Fig. 5). The reduction in the concentration of the diene results, probably, from lipoxygenase enzymatic activity that increases as ripening progresses and the fruit softens (Prusky et al., 1985b).

The dormant state of *Alternaria alternata* in young mango fruits has been attributed to the presence of two antifungal resorcinols in the unripe fruit rind (Droby et al., 1986).

The effect of the resorcinols on the germination rate of *A. alternata* spores and on the prolongation of the germ tubes is given in Fig. 6. The concentration of the resorcinols in the rind of various mango varieties decreases during the ripening process, with a parallel quiescent fungal infection (Fig. 7).

The presence of the phenolic compound, chlorogenic acid in young apple fruits has been related to their resistance to *Gloeosporium perennans*. The weak point of this hypothesis is the fact that the concentrations needed for

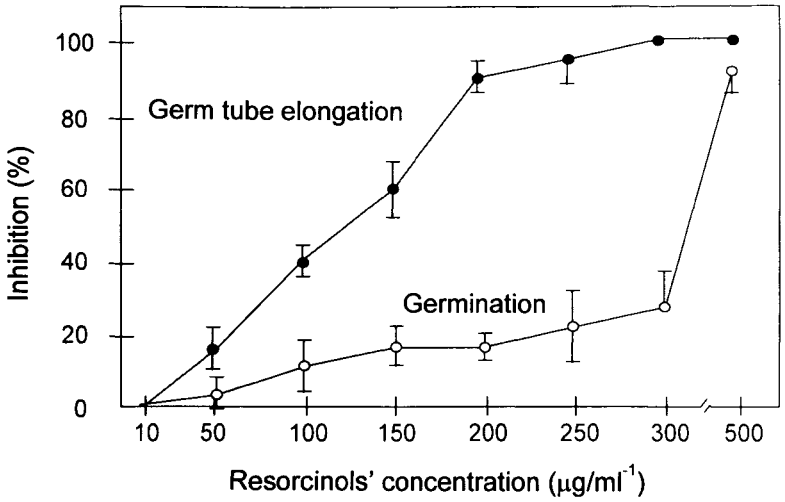


Fig. 6. Effect of 5-substituted resorcinols on spore germination (○) and germ-tube elongation (●) of *Alternaria alternata*. Vertical bars indicate standard error (Reproduced from Droby et al., 1986 with permission of Academic Press).

fungal suppression are greater than those naturally present in the fruit (Swinburne, 1983).

The resistance of Bramley seedling apples to *N. galligena* has been attributed to induced phytoalexin formation. *Nectria* invades wounds and lenticels of apple fruits before harvest, but fruit rotting does not become severe until after harvest.

The quiescence of *Nectria* in apples has been attributed to the formation of benzoic acid in the necrotic tissue, following fungal penetration into the fruit (Swinburne, 1978). The fungus resumes active growth when the fruit ripens and the benzoic acid is decomposed (Swinburne, 1983).

Some of the preformed compounds, such as the antifungal diene in unripe avocado fruits, can be further induced in the host tissues. Induction of higher concentrations of this compound was found in response to challenge inoculation of unripe, but not of ripe fruit with *C. gloeosporioides*.

The preformed antifungal substances naturally residing within the host, the inducible preformed substances, and the phytoalexins formed as a result of infection, and their role in host resistance, will be discussed below in the chapter on Host Protection and Defense Mechanisms.

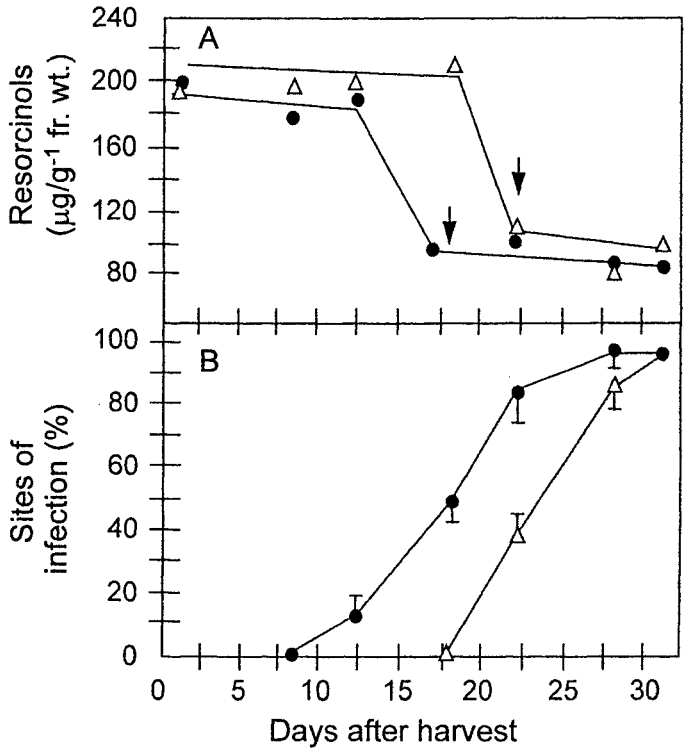


Fig. 7. (A) - Time courses of change in the concentration of 5-substituted resorcinols in peel from Tommy Atkins (●) and Haden (Δ) mango fruits. Arrows denote symptom appearance of disease expressed on 50% of inoculated sites. (B) - Percentage of *Alternaria alternata* infection sites of over 2 mm in diameter in relation to time after harvest. Vertical bars indicate standard error. (Reproduced from Droby et al., 1986 with permission of Academic Press).

Suggestions have been made of additional causes that might prolong the dormant phase of a fungus within the fruit. It was thus found that the quiescent period of *M. fructicola*, the causal agent of brown rot in peach fruits, lengthened with increasing thickness of the fruit cuticle and cell walls (Adaskaveg et al., 1991). As a matter of fact, peach cultivars with a thick cuticle and denser epidermis were found to be more resistant to the brown mold than those with a thin epidermis. This resistance has been expressed both in delayed penetration of the pathogen into the fruit and in prolonged incubation phases within the host (Adaskaveg et al., 1991).

2. PENETRATION THROUGH NATURAL INLETS

Some pathogenic fungi and bacteria that cannot normally penetrate the sound host directly, without the presence of a wound in its surface, can penetrate through natural openings such as stomata and lenticels. The penetration of germ tubes of *Colletotrichum gloeosporioides* spores into young papaya fruits (Stanghellini and Aragaki, 1966) and the penetration of *Monilinia fructicola* spore germ tubes into young stone fruits (Tate and Corbin, 1978) can take place through the stomata while the fruit is still in the orchard.

The fungus *Dothiorella gregaria* (perfect state: *Botryosphaeria ribis*) penetrates the lenticels of avocado fruits and forms hyphae that remain dormant until the harvested fruit has aged (Horne and Palmer, 1935). Penetration through lenticels has also been described for *Alternaria alternata* spores in mango and persimmon fruits (Prusky et al., 1981; Prusky et al., 1983). Penetration through lenticels is typical of *Gloeosporium album* and *Gloeosporium perennans* in apples that grow in humid areas. In the warm humid season the fungal development in the lenticels is limited, with their growth being halted until, during storage, the fruit reaches the required ripening stage (Moreau et al., 1966). *Penicillium expansum*, the cause of apple blue mold, and which is considered a typical "wound pathogen", can also penetrate the fruit through its lenticels (Baker and Heald, 1934). This phenomenon is most notable if the lenticels are bruised or when the fruit ages.

The lenticels in potato tubers are liable to develop bacterial soft-rot during storage. During harvest, most of the lenticels are already infested with cells of *Erwinia cartovora.* The bacteria remain inactive within the lenticels until the development of conditions enhancing the tuber sensitivity to decay, such as mechanical pressure, the presence of free water, or a low oxygen pressure within the tuber (Lund and Wyatt, 1972; Perombelon and Lowe, 1975). Penetration via lenticels is also typical of *Helminthosporium solani*, the causal agent of silver scurf of potato tubers (Burke, 1938), although the fungus can penetrate directly through the skin of these tubers.

An open calyx tube, typical of some fruits, can also become a natural penetration point for storage pathogens. This is what enables the penetration of *Alternaria alternata* into Delicious apple fruits (Ceponis et al., 1969) and Na'ama tomato fruits (Barkai-Golan, unpublished), both of which are characterized by open calyces. In apple fruits, the fungus that has penetrated through an open sinus in the calyx region, can develop a

spore-bearing mycelium within the fruit core, without causing the external rind to decay (Miller, P.M., 1959). Core rot of apples, that results from infection through the sinus between the calyx and the core cavity, may be attributed to various pathogens, including *Aspergillus niger* and *Fusarium* spp. (Miller, P.M., 1959); *Botrytis cinerea, Mucor piriformis, Pleospora herbarum, Penicillium funiculosum* and *P. expansum* (Combrink et al., 1985; Spotts et al., 1988); *Phomopsis mali* (Rosenberger and Burr, 1982) and *Trichothecium roseum* (Raina et al., 1971).

3. PENETRATION DURING AND AFTER HARVEST

Penetration via Wounds

In contrast to pathogens that attack the fruit and vegetable in the field, most of the storage pathogens are incapable of penetrating directly through the cuticle or epidermis of the host, but require a wound or an injury to facilitate their penetration. Therefore, the fungi and bacteria that develop during storage are often called "wound pathogens". The wound can vary in nature. Growth cracks present on harvested fruits and vegetables are natural avenues of infection. The actual harvesting is accompanied by mechanical injuries that enable the weak pathogens to penetrate. Careless separation of the fruit or vegetable from the parent plant might result in an injury liable to be attacked by the pathogen. The extent of injury caused by mechanical harvesting is far greater than that caused by a manual operation (Fuchs et al., 1984). Each scratch, incision, blow or other mechanical injury inflicted on the fruit or vegetable during each of the handling processes - harvesting, gathering, transporting, sorting, packing and storing - might present adequate penetration points for the storage pathogens. A likely penetration point is the stem-end separation area, where damage often occurs during fruit picking. In this regard, the separation area is no different from any other injury. It often happens that simultaneously with the injury, a large amount of fungal spores and bacterial cells arrive at the injured area, some of which will use the injury site to penetrate and infect the host.

Penetration through wounds is characteristic of *Penicillium digitatum* and *Penicillium italicum* conidia in citrus fruits. Yet it has turned out that the depth of the injury, combined with the atmospheric humidity conditions during storage, can determine the fate and extent of the infection (Schiffmann-Nadel and Littauer, 1956; Kavanagh and

Wood, 1967). Artificial inoculation into the fruit pulp always results in the fruit being infected, but inserting the fungus into a superficial scratch in the rind ends with infection only when the fruit is simultaneously placed under very high humidity. Maximal infection takes place when the relative humidity reaches 100%. In addition, the location of the injury is important. Inserting the fungus into the oil glands of the citrus fruit rind leads to higher infection rates than its insertion into the rind between the glands.

Wound infection is also characteristic of *Rhizopus stolonifer*, that causes watery soft rot in many species of fruits and vegetables; of *Alternaria alternata* that causes a dark, rather dry decay in a large number of fruits and vegetables stored for a prolonged period; of *Geotrichum candidum* which causes the sour rot in citrus, melons and tomato fruits; and of various *Aspergillus, Cladosporium* and *Trichotecium* species, and other storage fungi, in various fruits and vegetables.

Even pathogens that do not require an injury to facilitate their penetration - such as various *Erwinia* species that penetrate potato tubers through the lenticels, or *Monilinia fructicola* and *Penicillium expansum,* which are capable of penetrating deciduous fruits through the lenticels - will easily penetrate via wounds. In very much the same way the *Botrytis cinerea* fungus, which is capable of penetrating into young strawberry fruits through the flower parts, or into grape berries via the style-ends (McClellan and Hewitt, 1973), will penetrate into the host through an injury. Moreover, blows or pressure on apples or potato tubers increase the blue mold rot (*P. expansum*) or the bacterial soft rot (*Erwinia carotovora*), respectively, because of an injury inflicted on the cells around the lenticels - injury that eases the penetration of pathogens via the lenticels.

Penetration Following Physiological Damage

Physiological damage caused by low temperatures, heat, oxygen shortage or any other environmental stress, increases the fruit or vegetable sensitivity and exposes it to storage fungi. The physiological damage can be externally expressed through tissue browning and splitting, thus forming locations vulnerable to invasion of wound pathogens. Yet extreme environmental conditions might enhance sensitivity to an attack without any visible external signs of damage. A tomato fruit exposed to chilling temperatures or heat treatments is liable to be attacked by *Botrytis cinerea* even when there are no visible

symptoms of damage (Lavy-Meir et al., 1989). Exposure to extreme temperatures also partially broke the resistance of previously resistant tomato genotypes (*nor* and *rin* fruits and their hybrids) both to *B. cinerea* (Lavy-Meir et al., 1989) and to *Alternaria alternata* (Barkai-Golan and Kopeliovitch, 1989). *Alternaria* rot develops typically also in zucchini, following chilling injury, whereas cucumbers and melons exposed to excessively low temperatures are sensitive to various *Penicillium* and *Cladosporium* species, respectively (Snowdon, 1992). *A. alternata* and *Stemphylium botryosum* also tend to attack apple fruits following the development of sun scald lesions, while *Alternaria* may also be associated with other physiological disorders on apples such as bitter pit or soft scald (Snowdon, 1990). *Cladosporium herbarum* is another weak pathogen that may be associated with scald and other physiological disorders in some cultivars of apples (Dennis, 1983a).

Penetration Following a Primary Pathogen

Several pathogens will enter the host following a primary pathogen "breakthrough". Sometimes, nature deploys a sequence of pathogens. Various bacteria enter potato tubers infected with *Phytophthora infestans*: with the development of the secondary bacteria, the primary decay, which is hard in nature, turns into a soft decay as a result of the bacterial enzymatic activity on the tuber cell walls. *Penicillium expansum* can enter the apple fruits following their infection by the fungi *Mucor, Gloeosporium* and *Phytophthora* (Snowdon, 1990). Soft rot bacteria can enter tomato fruits following the sour rot caused by *Geotrichum candidum* (Barkai-Golan, unpublished) and the penetration of various *Fusarium* species into the melon is also, in many cases, secondary and occurs following infection by primary pathogens.

A typical sequence of primary and secondary pathogens can attack apples. The pink mold rot fungus (*Trichothecium roseum*) typically develops within the cracks caused to the fruit by the Scab fungus (*Venturia inaequalis*). *Venturia* penetrates the fruit while it is in the orchard, and can develop there while the fruit is still on the tree. However, although the fungus is still in its early stages of development within the fruit during harvest, the disease will develop during storage, and is liable to be followed by the invasion of *Trichotecium*. Similarly, the fungus may also gain entry at the infection site of the black rot fungus, *Botryoshaeria obtusa* (Snowdon, 1990).

Studying the types of wounds associated with the development of Rhizopus soft rot on papaya fruits, Nishijima et al. (1990) showed that

Rhizopus stolonifer was consistently capable of infecting the fruit through existing lesions caused by *Colletotrichum* and *Phomopsis* species. Furthermore, the reduction in the incidence of Rhizopus rot following fungicidal field sprays was related to the reduced incidence of field-initiated diseases whose lesions serve as routes of infection for *R. stolonifer*.

Penetration due to Tissue Senescence

Tissue senescence during prolonged storage also reduces disease resistance. Thus, at the end of the storage period, the sensitivity of melon to the blue-green mold caused by various species of *Penicillium* and to the pink mold caused by *Trichothecium roseum* is increased (Barkai-Golan, unpublished). A senescencing onion that has commenced sprouting often harbors base decay, caused by various species of *Fusarium* (Marlatt, 1958).

Generally, the rate of decay during storage increases with the duration of storage as tissue senescence progresses. Increasing the tissue sensitivity to diseases during storage also contributes to contact-infection of a healthy product by an infected one covered with spore-bearing mycelium.

Contact-Infection

Fruits or vegetables that were spared a pathogen invasion via any of the means of penetration might still be infected during actual storage, through contact with infected produce. Contact-infection is a significant factor in the spreading of white watery rot (*Sclerotinia* spp.) and bacterial soft rot (*Erwinia* spp.) in lettuce, cabbage, celery, carrot or squash during storage. The development of *Botrytis* in stored strawberries, which it turns into "mummies" covered with a gray layer of spore-bearing mycelium, causes a "chain" contact-infection and jeopardizes the entire basketful of fruit. Similarly, one strawberry or tomato, or a single grape berry infected by *Rhizopus* constitutes a focus from which the decay can spread within the container when it is transferred from refrigeration to shelf conditions. In fact, contact-infection by *Botrytis* or *Rhizopus* is typical of many fruits and vegetables, and may account for the major losses caused by these pathogens during long-term storage. In citrus fruits, contact-infection by the green and blue mold rots (*Penicillium digitatum* and *P. italicum*) is very common; it often occurs during shipment and can, under certain conditions, disqualify the entire shipment.

CHAPTER 3

EACH FRUIT OR VEGETABLE AND ITS CHARACTERISTIC PATHOGENS

A. HOST-PATHOGEN COMBINATIONS IN POSTHARVEST DISEASES

The surface of the fruit or vegetable is covered with fungal spores that they have acquired from the air during their development on the parent plant, or with which they have come in contact during picking or any of the subsequent stages of handling the harvested produce. However, not every fungal spore or bacterial cell that reaches the harvested product can develop and cause decay, even when conditions suitable for penetration and development are present.

Harvested fruits and vegetables are naturally attacked by a relatively small group of pathogens: approximately forty species. Moreover, each fruit or vegetable has its own typical pathogens out of this particular group.

Fruits of tropical and subtropical origin, such as mango, papaya, avocado, citrus fruits, etc., are typically attacked by the fungus *Colletotrichum gloeosporioides*, which causes anthracnose. This pathogen penetrates into the fruit while still in the orchard, but the anthracnose symptoms in the rind break out only as the fruit ripens after harvest. In a similar manner, *Gloeosporium musae* attacks the unripe banana fruits in the orchard, to initiate a quiescent infection, which becomes active only during storage.

Several fungi, such as *Diplodia natalensis, Phomopsis citri* or *Dothiorella gregaria* invade the cut stem of tropical and subtropical fruit, and induce the characteristic stem-end rot after harvest. *D. natalensis, Alternaria citri* and *P. citri*, the causal agents of postharvest stem-end rot of citrus fruit, all tend to lodge underneath the 'button' of the fruit, usually until after harvest, when the fungi progress from the button into the fruit and initiate stem-end rot.

During and after harvest, the citrus fruit is typically attacked by *Penicillium digitatum* and *P. italicum*, the causal agents of green and

blue mold, respectively, and *Geotrichum candidum*, the causal agent of sour rot: these are typical wound pathogens. *P. digitatum* is an example of a specific fungus that attacks only citrus fruits. On the other hand, *P. italicum,* which is also a significant citrus pathogen, can attack other fruits and vegetables, whereas *Penicillium expansum*, a significant apple and pear pathogen, does not naturally attack citrus fruits at all.

The typical apple and pear pathogens include, among others, *P. expansum*, *Botrytis cinerea, Gloeosporium* spp., *Alternaria alternata* and *Stemphylium botryosum*, whereas the main pathogens of stone fruits, such as peaches, apricots, nectarines and plums, are *Monilinia fructicola* and *Rhizopus stolonifer.* Furthermore, while *Penicillium* spp. are responsible for major losses in harvested citrus or apple fruits, they are of little or no importance in most other crops.

The harvested strawberry, too, has its own typical fungal pathogens. Out of the many airborne fungi found on the strawberry surface in the field or after harvest, two would normally develop during storage: the gray mold fungus (*B. cinerea*) and the soft watery rot fungus (*R. stolonifer*). In Great Britain, *Mucor* was found replacing *Rhizopus* as an additional pathogen to *Botrytis* in stored strawberries (Dennis and Mountford, 1975).

Of the entire range of fungi that can be isolated from the surface of harvested tomatoes, *A. alternata* is the major storage decay agent; at times over 80% of the overall decay in the stored fruit is caused by this particular fungus (Barkai-Golan, 1973). The main causal agents of soft watery rot in harvested tomatoes are *R. stolonifer*, *G. candidum* and *Erwinia* spp.

In much the same way, a celery head transferred to storage carries an abundance of air- and soil-borne fungi and bacteria. Here, too, the major decays that develop in the stored celery are generated by a small number of typical microorganisms: the fungi *Sclerotinia sclerotiorum* and *B. cinerea*, and the bacterium *Erwinia cartovora.*

Each picked fruit and vegetable, therefore, has its own group of characteristic pathogens to which it is susceptible and for which it serves as a suitable host.

B. THE MAIN PATHOGENS OF HARVESTED FRUITS AND VEGETABLES

The main pathogens of harvested fruits and vegetables, those that start their penetration in the field on the parent plant, and those that penetrate during or after the harvest, are listed in Table 1. Parallel to the pathogens are the disease symptoms and the primary hosts. The fungi are presented in alphabetical order followed by the bacteria.

Summaries of the postharvest diseases of four specific groups of fruits, along with notes on their control, are presented in a separate chapter at the end of the book.

TABLE 1
The main pathogens of harvested fruits and vegetables *

Pathogen	Disease	Primary Hosts
Acremonium spp.	crown rot	banana
Alternaria alternata (Fr.) Keissler	fruit rot, dark spot, sooty mold	stone and pome fruits, grapes, papaya, tomato, pepper, eggplant, cucumber, melon, watermelon, squash, cabbage, cauliflower, broccoli, corn, pea, bean, carrot, potato, sweet potato, onion
Alternaria alternata (Fr.) Keissler	stem-end rot; black spot	avocado, mango, papaya persimmon
Alternaria citri Ell. & Pierce	stem-end rot	citrus fruits

* The main sources of the information in this Table are: Barkai-Golan, 1981; Dennis, 1983; Bartz and Eckert, 1987; Snowdon, 1990, 1992.

Pathogen	Disease	Primary Hosts
Aspergillus niger v. Tieghem	black rot	date, grape, tomato, melon, corn, onion, garlic
Botryodiplodia theobromae Pat. (see also *Diplodia natalensis*)	crown rot finger rot stalk rot stem-end rot	banana citrus fruits, avocado, mango
Botrytis allii Munn Perfect state: *Botryotinia* sp.	neck rot	onion
Botrytis cinerea Pers. ex Fr. Perfect state: *Botryotinia fuckeliana* (de Bary) Whetzel	gray mold	strawberry, raspberry, cherry, grape, pome and stone fruits, persimmon, citrus fruits, tomato, pepper, eggplant, cucumber, squash, melon, pumpkin, artichoke, cabbage, cauliflower, lettuce, broccoli, pea, bean, carrot, onion, potato, sweet potato
Cladosporium herbarum (Pers.) Link	olive-green mold, sooty mold	date, grape, pome and stone fruits, papaya, fig, tomato, pepper, melon
Ceratocystis paradoxa (Dade) C. Moreau, Perfect state: *Thielaviopsis paradoxa* (de Seynes) Höhnel.	black rot, stalk rot, crown rot, soft rot	banana, pineapple
Colletotrichum gloeosporioides (Penz.) Sacc. Perfect state: *Glomerella cingulata* (Stonem.) Spauld & v. Schrenk	anthracnose	avocado, mango, papaya, guava, citrus fruits

Pathogen	Disease	Primary Hosts
Colletotrichum gloeosporioides (Penz.) Sacc. (syn. *Gloeosporium fructigenum* Berk.) Perfect state: *Glomerella cingulata* (see above)	bitter rot	pome and stone fruits
Colletotrichum musae (Berk. & Curt.) v. Arx (syn. *Gloeosporium musarum* Cke. & Massee)	anthracnose crown rot	banana
Diplodia natalensis P.E.	stem-end rot, stalk rot, finger rot, crown rot	citrus fruits, avocado, mango, banana
Dothiorella gregaria Sacc. Perfect state: *Botryosphaeria ribis* Gross. & Duggar	stem-end rot	avocado, mango, citrus fruits
Fusarium pallidoroseum (Cooke) Sacc.	crown rot	banana
Fusarium sambucinum Fuckel Perfect state: *Giberella pulicaris* (Fr.:Fr.) Sacc.	dry rot	potato
Fusarium verticilloides (Sacc.) Nernberg (=*F. moniliforme* Sheldon) Perfect state: *Gibberella fujikuroi* (Saw.) Ito.	black heart brown rot	banana, pineapple

Pathogen	Disease	Primary Hosts
Fusarium spp.	dry or soft rot	tomato, pepper, eggplant, melon, squash, pumpkin, watermelon, cabbage, celery, artichoke, asparagus, corn, carrot, potato, sweet potato, onion, garlic
Geotrichum candidum Link	sour rot	citrus fruits, melon, tomato
Gloeosporium album Osterw. Perfect state: *Pezicula alba* Guthrie	lenticel rot	pome fruits
Gloeosporium perennans Zeller & Childs Perfect state: *Pezicula malicorticis* (Jackson) Nannf.	lenticel rot	pome fruits
Helminthosporium solani Dur. & Mont.	silver scurf	potato
Monilinia fructicola (Wint.) Honey	brown rot	stone fruits mainly, but also pome fruits
Monilinia fructigena (Aderh. & Ruhl.) Honey	brown rot	stone and pome fruits
Monilinia laxa (Aderh. & Ruhl.) Honey	brown rot	stone fruits mainly, but also pome fruits
Mucor hiemalis Wehmer	watery soft rot	tomato, strawberry, raspberry, melon, corn
Mucor piriformis Fischer	watery soft rot	tomato, strawberry, raspberry
Penicillium digitatum Sacc.	green mold	citrus fruits (exclusively)
Penicillium expansum (Link) Thom	blue mold	pome fruits mainly, but also stone fruits

Pathogen	Disease	Primary Hosts
Penicillium italicum Wehmer	blue mold	citrus fruits mainly
Penicillium spp.	blue mold	tomato, cucumber, melon
Phoma caricae-papayae (Tarr) Punith. Perfect state: *Mycosphaerella caricae* H. & P. Sydow	stem-end rot, dry black rot	papaya
Phomopsis citri Fawc. Perfect state: *Diaporthe citri* Wolf	stem-end rot	citrus fruits
Phytophthora citrophthora (Smith & Smith) Leon.	brown rot	citrus fruits
Phytophthora infestans (Mont.) de Bary	late blight	potato, tomato
Pythium spp.	soft water-soaked lesion, cottony leak	tomato, pepper, eggplant
Rhizopus stolonifer (Ehr. ex Fr.) Lind	watery soft rot	stone and pome fruits, grape, avocado, papaya, strawberry, raspberry, cherry, tomato, pepper, eggplant, carrot, melon, pumpkin, squash, pea, bean, sweet potato
Sclerotinia sclerotiorum (Lib.) de Bary	watery white rot, cottony rot	citrus fruits, cabbage, cauliflower, lettuce, celery, broccoli, artichoke, pea, bean, carrot, eggplant, melon, cucumber, pumpkin, squash, onion, garlic

Pathogen	Disease	Primary Hosts
Stemphylium botryosum Wallr. Perfect state: *Pleospora herbarum* (Pers.) Rabenh.	black lesion, dark spots	pome fruits, papaya, grape, tomato, lettuce
Stemphylium radicinum (Meier, Drechsler & Eddy) Neerg.	black lesion black rot	carrot
Trichoderma viride Pers. ex S.F. Gray	stem-end and fruit rot, green-yellow mold	citrus fruits
Trichothecium roseum Link	pink mold	stone and pome fruits, banana, avocado, tomato, melon
Verticillium theobromae (Turc.) Mason & Hughes	crown rot, cigar-end rot	banana
Erwinia carotovora ssp. carotovora (Jones) Dye *Erwinia* spp.	bacterial soft rot	tomato, pepper, melon, squash, pumpkin, cucumber, cabbage, cauliflower, lettuce, celery, broccoli, spinach, asparagus, pea, bean, potato, sweet potato, onion
Pseudomonas syringae (Kleb.) Kleb. *Pseudomonas* spp.	spots or bacterial soft rot	tomato, cucumber, melon, squash, asparagus, cabbage, cauliflower, lettuce, celery, broccoli, spinach, pea, bean, onion
Pseudomonas syringae pv. *syringae* van Hall	black pit	citrus fruits

CHAPTER 4

FACTORS AFFECTING DISEASE DEVELOPMENT

A. PREHARVEST FACTORS, HARVESTING AND HANDLING

The first preharvest factor which may affect postharvest quality is the cultivar, since different cultivars may vary greatly in their susceptibility to diseases. In fact, one of the aims of plant breeding and genetic engineering is to incorporate resistance genes in new varieties of crop plants. Differences in cultivar characteristics can markedly affect the keeping quality of the fresh produce. Thus, melons with a thick skin and raspberries with a firm texture are better able, than others, to withstand the rigors of harvesting and handling and should, therefore, have longer storage lives (Snowdon, 1990). Variability in postharvest decay among apple cultivars has been related to differences in the wounding resistance of their skins, a feature which may be of major importance for decay pathogens that depend on a wound to initiate infection (Spotts et al., 1999).

Another preharvest factor is the health of the planting material. Various pathogens may contaminate the planting material and cause disease in the field or in storage. Hence the importance of obtaining clean seeds and of the establishment of seed certification schemes (Jeffs, 1986).

Environmental conditions during growth, such as unfavorable high or low temperature, wind, rain or hail, can all affect the crop and determine not only the yield but also the quality of the stored commodity (Sharples, 1984). High temperature was found to increase *Botrytis cinerea* infection of tomatoes via the flowers because it increased the rate of flower development and senescence (Eden et al., 1996). Environmental conditions may also affect the pathogens directly. Many pathogens persist in the soil or survive on plant debris in the field, from which winds and rain may be directly responsible for their dispersal to potential hosts. Other pathogens, such as *Phytophthora* spp. which infect potato tubers or citrus fruits, are actually dependent on rainwater for germination of their spores and initiation of infection (Lapwood, 1977; Feld et al., 1979). In fact, the percentage of brown rot caused by *Phytophthora parasitica* in orange orchards was directly related to the

amount of rainfall in the principle infection period (Timmer and Fucik, 1975).

Cultural practices, such as pruning of fruit trees and destruction of crop debris, can markedly affect the survival of pathogenic microorganisms (Palti, 1981). Application of preharvest fungicides can directly reduce the level of infestation. However, preharvest chemical sprays, with the same chemical that is designated for postharvest application, can enhance the production of new fungal strains resistant to that fungicide (Eckert and Ogawa, 1988). Cultural practices can also be changed in order to reduce the inoculum level through sanitation, or to produce conditions less favorable for disease by modifying the canopy microclimate (Legard et al., 1997). Plant spacing within the row may also affect the incidence of rot. Legard et al. (2000) found that wider within-row plant spacing reduced Botrytis rot in strawberries compared with narrower spacing. This may be due to the increased number of target hosts available to intercept inoculum in the latter case, or to the fact that more fruit may escape timely harvesting and thus contribute to increased levels of inoculum. Increased plant density could also reduce the efficacy of fungicide applications by reducing plant coverage (Legard et al., 2000). Soil solarization, applied in hot countries by covering the soil with clear plastic film to heat the soil to a lethal temperature (Katan, J., 1987), or steam sterilization and fumigation applied in cooler countries, are practices which help to cleanse the soil by destroying harmful organisms. The sequence of crop rotation in the field can reduce the source of infection and thus influence the quality of the harvested commodity by affecting the health of the subsequent crop (Palti, 1981).

Field nutrition, too, can have an impact on the development of storage decay. Thus, the rapid development of bacterial soft rot in tomato fruits depends, to a great extent, on the application of nitric fertilizer to the plant while in the field (Bartz et al., 1979), and the resistance of pears to postharvest decay increases after the trees have been given nitrogen and calcium (Sugar et al., 1992). Despite all these data, the linking relationship between stimulating nutrients within the host and its susceptibility to the pathogen is quite unclear.

Harvesting by hand is the predominant method for fruits and vegetables destined for the fresh produce market (Kader et al., 1985). With proper training, pickers can select for the optimal maturity stage of a given commodity and can keep damage to a minimum. Mechanized harvesting, even when used correctly, can cause substantial damage to the commodity, which may serve as suitable areas of penetration for

'wound pathogens'. It is, therefore, confined mainly to less vulnerable commodities, such as carrots or potatoes, or to crops which are intended for immediate processing (Snowdon, 1990). The harvesting date may be of great significance for fruits intended for prolonged storage. However, despite the use of various criteria for determining the appropriate stage of maturity for harvesting (such as color, size, shape, flesh firmness and content of starch, sugar, juice or oil), the prediction of an optimal harvest date is often imprecise (Kader et al., 1985). Furthermore, unfavorable weather conditions, such as rain prior to harvesting, are liable to increase the risk of infection, even in fruits suitable for harvesting. The time of harvesting during the day may also affect the keeping quality of the produce. For most crops the cool hours of the night, or the early morning, can be advantageous.

The risk of damage and subsequent infection accompanies the fresh produce along its way from harvesting and handling in the field, through transport from the field to the packinghouse, during the packinghouse operations and through the cooling processes - before and/or after packing - until its arrival in the storage room, and even onwards to its final destination.

B. INOCULUM LEVEL

For a pathological disease to occur, a meeting between a vital pathogen and its suitable host should take place. However, when such a meeting does occur a successful infection may be dependent on the level of the inoculum (generally comprising fungal spores or bacterial cells) available. Gauman (1946) and his followers have claimed that a minimum number of pathogen spores is necessary to establish certain diseases, even under favorable conditions. Such a theory denies, for many fungi, the possibility of a single spore infection and has not been accepted by other investigators (van der Plank, 1975). Infection studies with fungal spore suspensions on different hosts showed that increasing the spore concentrations frequently leads to higher rates of infection and lesion formation. Eden et al. (1996) found that infection of tomato flower parts by *Botrytis cinerea* increased as a function of inoculum concentration. This finding demonstrated the practical importance of reducing the inoculum level on the crop in order to minimize infection. For postharvest pathogens, which depend on a wound to enable them to penetrate into the host, it has generally been accepted that disease

development is related to both the pathogen spore load on the fruit or vegetable surface and the availability of wounds for penetration. In fact, the control of postharvest infections initiated by 'wound pathogens' is based primarily on the reduction of the inoculum level that may reach the host, along with continuing efforts to reduce pre- and postharvest injury. Regular sanitation in the field, in the packinghouse and in the storerooms, will all contribute to the reduction of the spore load on the harvested produce, while careful handling and prevention of mechanical damage will help to reduce the number of entry points for the pathogen.

Discussing postharvest diseases of pome and stone fruits, Edney (1983) states that the incidence of rotting is influenced by the number of viable spores at potential infection sites when the fruit is at the stage of ripening suitable for infection to develop. However, the amount of inoculum present is closely related to weather conditions during the growing season, particularly when the spores are dispersed by rain as in the cases of *Gloeosporium* and *Phytophthora* species.

An interaction between wounding and inoculum level has been described for the brown rot fungus, *Monilinia fructicola* on stone fruits (Hong et al., 1998). In the presence of a high level of fungal inoculum on the fruit surface, penetration of the unwounded fruit can take place through the stomata or directly through the peel. However, as the spore load on the fruit decreases below a certain level (5 or 50 spores per mm^2 for nectarines or peaches, respectively) the significance of wounds as entry points increases. With plums, on the other hand, brown rot can develop only on wounded fruit, even at an inoculum level of 10^6 spores/ml.

Studying surface colonization and lesion formation by various inoculum densities of single conidia of *Botrytis cinerea* on grapes, Coertze and Holz (1999) found that individual conidia readily infected cold-stored berries, which are highly susceptible to infection, and that the number of lesions tended to increase at high inoculum concentrations. However, these conidia did not induce disease symptoms on fresh, resistant berries, irrespective of the inoculum density. These results suggested that infection was not always governed by conidial density on berry surfaces, but rather by the level of host resistance.

The inoculum level of the pathogen may determine the success of biological control of postharvest diseases with antagonistic microorganisms (see the chapter on Biological Control). For various host-pathogen interactions, the efficacy of the antagonistic microorganism in reducing decay has frequently been affected by the

inoculum levels of both the pathogen and the antagonistic microorganism. At a high fungal spore concentration, low concentrations of the antagonist were not sufficient to reduce the incidence of infection. However, when wounds were inoculated with low spore concentrations, the percentage of infection could be effectively reduced even by low concentrations of the antagonist (Chalutz et al., 1991). Reducing the spore load of the pathogen on the fruit may, therefore, contribute to improved biological control.

C. STORAGE CONDITIONS

The actual pathogen penetration does not in itself ensure that the disease will develop. For the fungi or bacteria that have penetrated the host to become established and to continue their development, they have to encounter tissues under conditions suitable for their settling and developing. These conditions include appropriate temperature and relative humidity, the presence of available nutrients, suitable pH level, and other environmental conditions.

1. TEMPERATURE

The spore germinating and mycelial growing temperatures constitute basic or even limiting factors in the development of the disease. The optimal temperature for growth of most storage fungi is 20-25°C, though some species prefer higher or, more rarely, lower temperatures. The optimum can vary according to fungal species and at times even to its strain or isolate. However, the optimum for growth is not necessarily identical to the optimum for germination. The further from the optimal temperature for the fungus the longer the time required for initiation of germination and mycelial growth, and the longer the duration of the incubation period of the disease (the time until the appearance of decay symptoms). In parallel to the prolongation of its incubation period, the subsequent development rate of the pathogen is reduced. Fig. 8 displays the retardation of the gray mold development process in tomatoes infected with *Botrytis cinerea* spores, as a consequence of shifting the temperature away from the optimum (17-20°C).

The minimal growing temperature can differ among the various storage fungi (Table 2). However, it should be noted that different isolates of the same fungus, or the same isolate under different growing

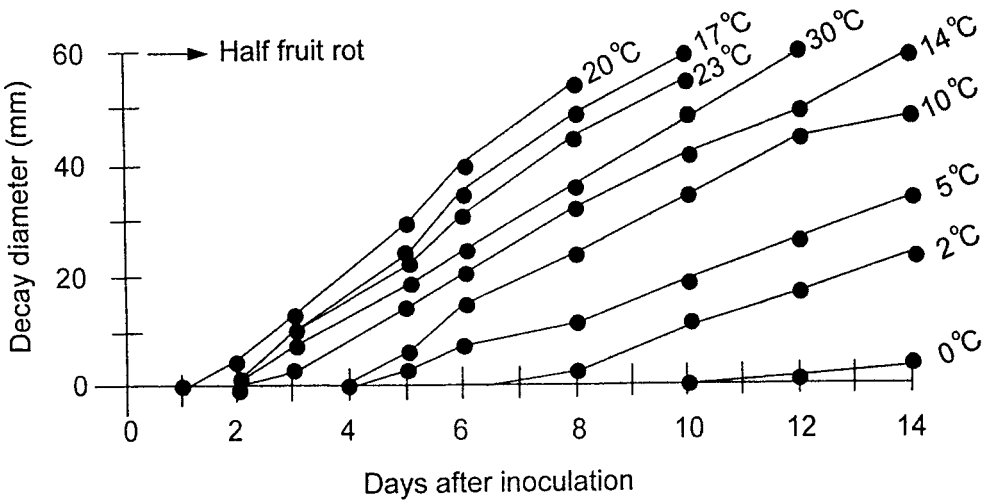


Fig. 8. Rate of development of Botrytis rot in inoculated tomato fruit at different temperatures. (Barkai-Golan, unpublished).

conditions, can have different minimal temperatures, which explains why the differing minimal temperatures are cited in the literature for a given fungal species. For some fungi, the minimal developmental temperature is below 0°C (Sommer, 1985). That is why *Botrytis cinerea,* whose minimum is -2°C, continues to develop in cabbage, celery, lettuce and other vegetables stored at 0°C, and why various isolates of *Penicillium expansum* and *Alternaria alternata,* whose minimum is -3°C, continue their development in certain apples stored at 0°C or below.

On the other hand, some pathogens, such as species of *Colletotrichum* or *Aspergillus niger* have a mycelium susceptible to low temperatures, and their growth minima are 9°C and 11°C, respectively. Nevertheless, the *Colletotrichum* fungus, which is characteristic of tropical and subtropical fruits, can easily develop in these fruits during storage since, because of their susceptibility to low temperatures, they are stored at relatively high temperatures. *Rhizopus stolonifer* spores are also susceptible to low temperatures and do not develop at temperatures under 5°C; though a certain percentage of the spores can germinate at 2°C, their germ tubes cannot continue their growth at such a temperature (Dennis and Cohen, 1976).

TABLE 2

Minimal temperatures for postharvest decay fungi *

Fungus	Minimal Temperature °C
Alternaria alternata	-3
Aspergillus niger	11
Botrytis cinerea	-2
Cladosporium herbarum	-4
Colletotrichum gloeosporiodes	9
Colletotrichum musae	9
Diplodia natalensis	-2
Geotrichum candidum	2
Monilinia fructicola	1
Penicillium digitatum	3
Penicillium expansum	-3
Penicillium italicum	0
Phomopsis citri	-2
Rhizopus stolonifer	2; 5**

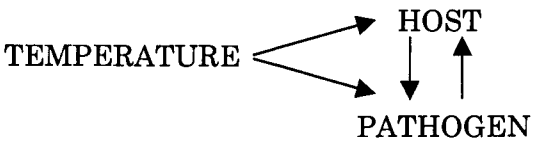
* Reproduced from Sommer (1985) with permission of the Canadian Journal of Plant Pathology.

** Reproduced from Dennis and Cohen (1976).

As to *Rhizopus*, significant differences were found between dormant and germinating spores in their susceptibility to low temperatures. Dormant spores in the pre-germinating phase were not damaged by the chilling temperatures, but when they entered the primary germination stages, their susceptibility to chill increased, and at 0°C they perished within a few days (Matsumoto and Sommer, 1968); in some fungi it was found that a young mycelium within a tiny decay spot was more susceptible to low temperatures than a mature mycelium within a developed decay spot.

One should bear in mind that the environmental temperature affects both the host and the pathogen simultaneously. Exposing the fruit or vegetable to temperatures that cause tissue damage does not inhibit decay development, but even enhances the infection rate of those pathogens that penetrate through a wound or a damaged tissue (Segall, 1967).

The interrelations among the environmental temperature, the pathogen and the host can be depicted as a triangle displaying the effect of the temperature on each of the others, which have their own mutual interrelations:



In fact, a significant proportion of the decay that occurs in the markets, particularly that of tropical fruits, results from overexposure to damaging temperatures. For some tropical fruits and vegetables, storage below 10°C is sufficient to cause chilling injuries, even when external physiological symptoms are not visible.

The use of low temperatures to depress decay by maintaining host resistance will be described in a separate chapter (See: Means for Maintaining Host Resistance - Cold Storage).

2. RELATIVE HUMIDITY AND MOISTURE

The high relative humidity (RH) required for the protection of fruits and vegetables from dehydration and weight loss might stimulate pathogen development during storage. The danger of decay is enhanced by the condensation of mist over the fresh fruit or vegetable surface. Similarly, wrapping the fruit or vegetables in sealed plastic film with low permeability to water vapor often causes enhanced decay when these films are not punctured and ventilated. Rodov et al. (1995c) found that the RH within an airtight polymeric wrap can be reduced by adding a hygroscopic substance such as sodium chloride. This reduction results in a reduced decay of peppers wrapped in a polyethylene film and stored at 8°C. Without the insertion of the hygroscopic substance, the relative humidity within the wrapping might approach saturation, with condensed water drops on the fruit and on the wrapping inner surface.

Leafy vegetables and, especially, leafy herbs, characterized by rapid dehydration following harvest, store better under high RH and subsequently decay less because of the reduced accumulation of dead tissues, which may provide nutrients for decay organisms (Yoder and Whalen, 1975). Packing such crops in polyethylene-lined crates has the advantage of preventing dehydration and preserving turgidity. The main limitation to the use of polymeric film packaging is the water condensation

within the package, which results from extreme changes in the storage and shipping temperatures. Perforating the lining is of primary importance, since the holes allow limited vapor emission, with acceptable weight loss (with no visible shriveling), prevent ethylene accumulation in the herbal atmosphere, and protect the leaves from decay (Aharoni, 1994).

Many fruits and vegetables are more susceptible to the pathogen when their tissues are in a turgid state through being under high RH. In many cases, the increased decay rate should be attributed to moisture held within the wounds, lenticels or stomata under these conditions. Fungal spores use this moisture when germinating, prior to their penetration into the tissues. Crops such as apples and pears, with well developed cuticle and epidermal layers, tolerate relatively lower RH levels, which helps to prevent storage decay due to the inhibition of fungal spore germination (Spotts and Peters, 1982b). However, when Anjou pears were wounded prior to inoculation, spores of some of the important pathogens, such as *Botrytis cinerea* and *Penicillium expansum,* did germinate and cause decay at 97% RH in cold storage (-1.1°C). It is well known that accumulated water drops on the surface of citrus fruits stimulate the development of the green mold (*Penicillium digitatum*). Therefore, slightly drying the fruit might reduce its susceptibility to decay (Eckert, 1978).

The water content of host tissues (water status) is a major contributing factor in the development of soft rot bacteria. In various vegetables, such as bell peppers and Chinese cabbage, high water status has frequently been associated with high potentials for bacterial soft rot (Bartz and Eckert, 1987). Accumulation of free water on potatoes stored in a relative humidity of over 95% and at a low temperature increases the rate of soft rot caused by bacterial penetration through the lenticels. Ventilated storage rooms and dried-up tubers can, therefore, reduce the bacterial decay (Perombelon and Lowe, 1975). On the other hand, *Botrytis* and *Rhizopus* easily attack carrots that have commenced withering and have lost over 8% of their water volume (Thorne, 1972). The increased susceptibility of the carrot under these conditions is attributed to the cell separation and enlargement of the intercellular spaces, which accompany the dehydration.

Studies conducted by van den Berg and Lentz (1973, 1978) found that the decay rates in cabbages, Chinese cabbages, cauliflowers, carrots, celery and other vegetables, in cold storage with relative humidity of 98-100%, were no higher than those under lower relative humidity, and at times were even lower. Under hyper-humidity, the firmness and freshness of vegetables were preserved much longer, and the pectolytic action of the

fungi pathogenic to carrots (especially that of *Botrytis cinerea* and *Sclerotinia sclerotiorum*) was weaker when the relative humidity was close to saturation than when it was at the 90-95% level (van den Berg and Lentz, 1968). Since these enzymes play an important part in the development of the pathogen within the host tissues, the pectolytic activity levels of the pathogens under the various humidity conditions might constitute a significant factor in the development of decay.

3. THE STOREROOM ATMOSPHERE

The gas composition of the storeroom atmosphere directly affects the development of fungal mycelium in the plant tissue, as it also affects spore germination on fruit and vegetable surfaces. Yet, like the other environmental conditions, the storeroom atmosphere, too, simultaneously affects the host, the pathogen and their interrelations. Since the atmospheric gas composition can significantly affect the ripening and senescence processes of the host, and since the physiological state of the host can affect its susceptibility to disease, the atmospheric gas composition indirectly affects decay development in the tissue (Barkai-Golan, 1990). The direct and indirect effects of the atmospheric gases (the levels of O_2, CO_2 and their combinations) on the development of pathogens, and the use of a suitable atmosphere composition for inhibiting disease in stored fruits and vegetables is discussed in the chapter on Means for Maintaining Host Resistance - Modified and Controlled Atmosphere.

D. CONDITIONS PERTAINING TO THE HOST TISSUES

1. ACIDITY LEVEL (pH)

The low pH that characterizes many fruits (below pH5) is probably an important factor in their general resistance to bacterial decay agents, but it furthers the postharvest development of various fungi. In fact, fungi cause most of the decays in harvested fruits, whereas bacteria are important mainly in vegetables. Unlike the fruit, the various other plant organs such as roots, tubers, stems or leaves, where the pH level is generally higher and ranges between 4.5 and 7.0, might often be attacked by soft-rot bacteria (Lund, 1983; Bartz and Eckert, 1987). Exceptions to that rule are some "fruit-vegetables", such as tomatoes, bell peppers and cucumbers, in which bacterial decay is quite common. The pH of soft rot lesions on tomato fruits is higher ($pH \geq 5.0$) than that of surrounding healthy tissue ($pH = 4.3 - 4.5$), suggesting that the bacteria are capable of

buffering their environment in a range suitable for their growth and the activity of their pectolytic enzymes (Bartz and Eckert, 1987).

2. GROWTH STIMULI

Certain compounds in the host tissues might, on certain occasions, affect the host susceptibility to infection by stimulating pathogen growth. Generally, spores of *Penicillium digitatum* do not germinate in water on the surface of citrus fruit until the peel is injured (Smoot and Melvin, 1961). However, when the peel is wounded, during harvesting or subsequent handling and processing, *P. digitatum* spores germinate and their germ tubes may penetrate the fruit, to initiate infection and, eventually, to develop the typical green mold symptoms. Ascorbic acid and a number of terpene compounds in citrus fruits, much like their stimulating effect on the germination of *P. digitatum* spores, can also stimulate mycelial growth of this fungus, which is specific to citrus fruits (Pelser and Eckert, 1977; French et al., 1978). Thus, the presence of citrus juice with the appropriate acidity level can determine both the germination rate and the germ tube growth and, in turn, the rate of fungal development and the incubation period of the disease (Pelser and Eckert, 1977) (Fig. 9).

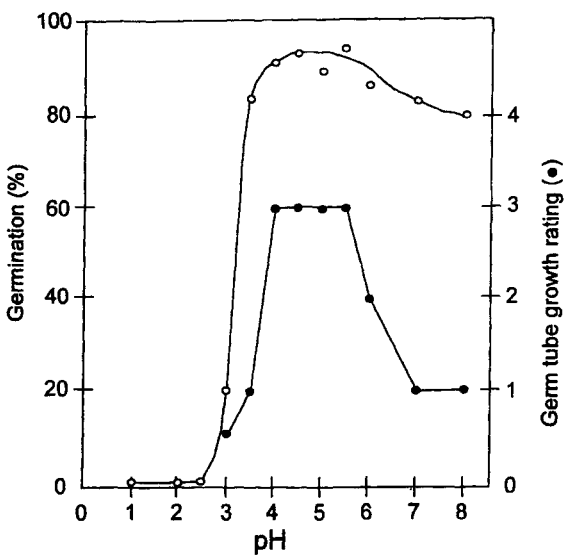


Fig. 9. Conidia germination and germ tube growth of *Penicillium digitatim* in 1% (v/v) orange juice dialysate at several pH values (Reproduced from Pelser and Eckert, 1977 with permission of the American Phytopathological Society).

Eckert and Ratnayake (1994) found that a mixture of limonene, acetaldehyde, ethanol, CO_2 and other volatile compounds emanating from wounded oranges induced germination of *P. digitatum* conidia on water agar. Fungal conidia that accumulate in water condensate on citrus fruits can also be induced to germinate by volatile compounds emanating from adjacent wounds. On the other hand, washing wounded lemon fruit peel (epicarp) was found to greatly suppress *P. digitatum* infection, when the fruit was inoculated with fungal spores, so that only 2% of the fruit showed green mold symptoms. However, when a comparable amount of isolated lemon peel oil was topically applied to the washed wounds, 92% of the inoculated wound sites did develop complete green mold symptoms (Arimoto et al., 1995).

In the light of the finding that peel oil extracts applied to wounded epicarps can restore disease development potential to the pathogen, Arimoto et al. (1995) investigated the possibility that some components of the lemon peel oil might be essential for fungal development in the peel tissues. These studies indicated that lemon epicarp oil, with the limonene removed, promoted the production of green mold symptoms on only 28% of the wounds, suggesting that limonene was one facilitator of green mold formation on wounded fruits. However, another promoting factor was isolated from the oil and was identified as prangolarin by spectrometric analysis. Prangolarin by itself enhanced *P. digitatum* development on wounded epicarps of lemons, resulting in the development of green mold symptoms because of the production of masses of conidiophores and conidia (Arimoto et al., 1995).

In potato tubers a close connection was found between the reducing sugar content and the susceptibility of the tuber to bacterial soft rot during storage at various temperatures (Otazu and Secor, 1981). A correlation was also found between the sugar contents of nectarine and plum fruits and their susceptibility to *Botrytis cinerea* infection (Fourie and Holz, 1998); this finding will be discussed below in regard to enhanced susceptibility to decay during the ripening stage. Edlich et al. (1989) found that certain sugars taken up by *B. cinerea* could stimulate fungal growth and enhance its infection capability, but suggested that the stimulation was due to the active oxygen formed rather than to a nutritional effect. Pollen exudates from weeds commonly found in stone fruit orchards have also been found to be stimulators of *B. cinerea* growth (Fourie and Holz, 1998). When added to the fungus conidia during the 4 weeks prior to the picking-ripe stage the exudates significantly increased the aggressiveness of the pathogen on plum and nectarine fruits. The

investigators believe that weed pollens with high sugar contents are likely to lead to further stimulation of the pathogen on fruit in the orchard (Fourie and Holz, 1998).

3. THE FRUIT RIPENING STAGE

The susceptibility of harvested fruits and vegetables to decay agents depends mainly on their ripening stage at the time of picking; it increases as ripening progresses. Many types of fruits are more susceptible to injuries as they ripen and, therefore, become more susceptible to pathogen attack (Eckert, 1975). However, in general, the susceptibility of the fruit to pathogen invasion increases regardless of its susceptibility to injury. Various tissue characteristics, such as the acidity level, the turgor state of the tissues, or nutrient availability, change throughout the senescencing and ripening stages and might, separately or in combination, enhance the susceptibility to disease. Other factors affecting the impact of the ripening stage on disease susceptibility involve the enhanced virulence of the pathogen, on the one hand, and weakened host resistance and protection, on the other hand. These factors include:

a. increased availability of compounds that induce formation of pectolytic enzymes by the pathogen;
b. enhanced susceptibility of the host cell walls to the pectolytic activity of the pathogen;
c. reduced concentrations of compounds toxic to the pathogen or of those that inhibit its enzymatic activity within the host tissues;
d. changes in nutrient availability during ripening.

One of the primary factors enhancing the susceptibility of the fruit to infection is the enhanced susceptibility of the plant cell walls to the activity of pectolytic enzymes produced and secreted by the pathogen. The pectic substances that comprise the cell wall of the young fruit are present in the form of insoluble protopectin. The insolubility of protopectin stems from both its high molecular weight and its close links with the cell wall cellulose, which comprise bridges of neutral polysaccharides and perhaps proteins as well (Eckert, 1978). At this stage, the cell walls withstand the pectolytic activity, which constitutes a most significant attack mechanism for many pathogens. As the fruit ripens, the links connecting the pectic substances to the cell wall break, so that the increased solubility of the pectic substances increases, and the tissue starts to soften (Eckert, 1978; Paull et al., 1999). At this stage,

the fruit can provide pectic compounds that induce the formation of pectolytic enzymes and their activity, and the plant tissue becomes more susceptible to the pectolytic enzymes secreted by the pathogen. Recent studies with papapya cell walls suggested that, in addition to pectin hydrolyisis, the modification of another cell-wall compound – the hemicellulose – is also involved in fruit softening (Paull et al., 1999).

Another factor that changes during the ripening process is the ability of the tissues to produce toxic compounds, which inhibit the pathogen growth; this diminishes as the tissues ripen. These compounds include: tomatine, which can be found in high concentrations in the green tomato skin (Verhoeff and Liem, 1975); tannins in young bananas (Green and Morales, 1967; van Buren, 1970); monoene and diene compounds in young avocado skin; resorcinols in the skin of mangoes in their early developmental stages (Prusky and Keen, 1993); and various toxic compounds found in the oil glands of young citrus fruit rind (Rodov et al., 1995b). Polyphenols and tannins, highly concentrated in the younger fruits, have been described both as germination and growth inhibitors of microorganisms, and as suppressors of enzymatic activity.

Regarding the nutrient availability changes during the advanced development or ripening of the fruit, a direct relationship was found between the sugar contents in nectarines and plums and the late-season susceptibility of these fruits to *B. cinerea* infection (Fourie and Holz, 1998). During their early developmental stages, the young unwounded fruits are resistant to the pathogen. It is not until the early phase of rapid cell enlargement of the nectarine and the last phase of rapid cell enlargement of the plum that the fruit becomes susceptible to infection, and disease development occurs (Fourie and Holz, 1995).

Studying the sugar content in exudates of immature plum and nectarine fruits, Fourie and Holz (1998) found that fructose, glucose and sorbitol were the only sugars present, whereas sucrose was first detected during fruit maturation, which occurs approximately two weeks before harvest. The sugars were exuded at low concentrations by immature fruits but their concentrations increased as the fruit ripened. In nectarines there was a pronounced increase in the sugar concentrations, especially that of sucrose, near the picking-ripe stage. Fungal growth was found to increase when the reducing sugars or sucrose were added to culture media in excess of 0.27 or 0.14 mM and when sorbitol was supplied in excess of 0.66 mM. With the progress in fruit maturity during the last two weeks before harvest, total sugars in plum exudates approached these values, whereas in nectarine exudates they far

exceeded them. Fourie and Holz (1998), therefore, concluded that the stimulatory effect of fruit exudates on *B. cinerea* growth coincided with the period of rapid sugar release from the fruit and the exhibition of fruit susceptibility to decay.

4. EFFECTS OF ETHYLENE

A strong link between the ripening stage of a fruit and its sensitivity to decay may explain why conditions or chemical substances that stimulate ripening, generally also enhance decay. A classic example of conditions that stimulate ripening is the exposure of various citrus fruit cultivars to low concentrations (50 ppm) of ethylene for degreening. Ethylene treatments are applied commercially at the beginning of the citrus fruit picking season, to degreen the fruits that have reached maturity but have not yet developed the desired color. However, this economically important procedure, which enhances chlorophyll decomposition and exposes the yellow, orange or red color in the fruit peel, is accompanied by enhanced sensitivity of the fruit to decay (Brown, G.E. and Lee, 1993). It was found that along with the enhancement of ripening, ethylene also stimulates senescence and disruption of the stem-end 'button', thus activating the quiescent infection of *Diplodia natalensis* at this location (Brown, G.E. and Wilson, 1968; Brown, G.E. and Burns, 1998) and leading to increased incidence of stem-end rot (McCornack, 1972).

An increase in rot incidence generally follows the use of ethylene at concentrations above those needed for adequate degreening. *In vitro* studies showed that the pathogen growth was stimulated by exposure to high ethylene concentrations (Brown, G.E. and Lee, 1993). However, the increased growth rate of the pathogen in response to high ethylene is not the only factor responsible for enhanced susceptibility of the fruit to disease. Following the finding of the correlation between ethylene treatment and the activities of the enzymes, polygalacturonase (PG) and cellulase (Cx) during citrus abscission (Riov, 1974; Ratner et al., 1969), it was recently found that the increase in disease incidence caused by the use of high concentrations of ethylene may be related to the activity of the abscission enzymes (Brown, G.E. and Burns, 1998). Enzymes formed during abscission degrade pectic substances in the middle lamella and lead to cell separation in the abscission zone. The activity of these enzymes in oranges was found to increase rapidly on exposure to high ethylene concentrations (0.055 ml l^{-1}) and a larger number of cells were degraded within the abscission layer by these enzymes than by those

exposed to lower, more typical degreening concentrations (0.002 ml l^{-1}). Furthermore, the addition of commercial or partially purified preparations of the abscission enzymes to abscission areas of debuttoned oranges, before inoculation with *D. natalensis,* caused a significant increase in stem-end rot development.

Another way to study the relationship between ethylene-dependent abscission enzymes and the susceptibility of the fruit to stem-end rot was the use of metabolic inhibitors that reduce enzyme activity during high-ethylene treatment (Brown, G.E. and Burns, 1998). When citrus fruits were dipped before degreening in 2,4 dichlorophenoxyacetic acid (2,4-D), which is known to reduce PG and Cx activity (Riov, 1974) or silver thiosulfate, which is an inhibitor of ethylene action (Veen, 1983), they showed enhanced resistance to stem-end rot when inoculated after degreening (Fig. 10).

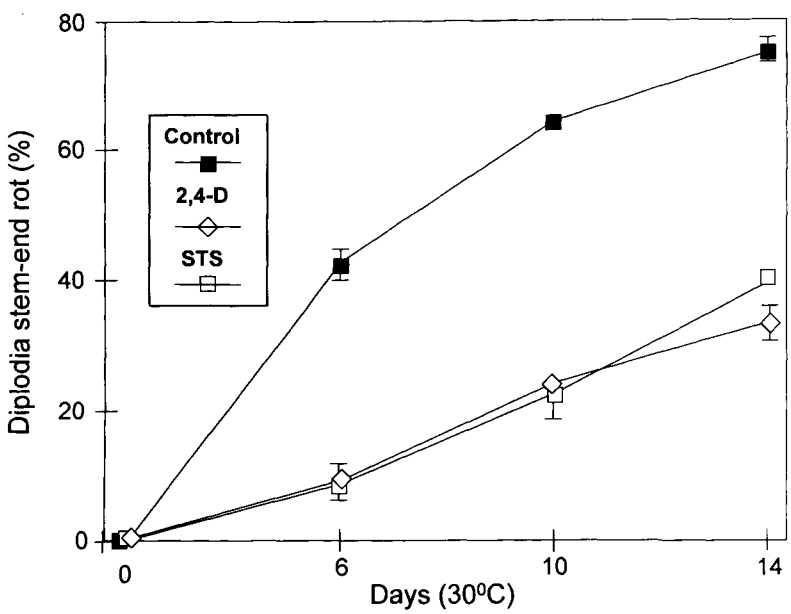


Fig. 10. Incidence of stem-end rot in Valencia oranges dipped in water (control), 2,4-dichloro-phenoxyacetic acid (2,4-D), or silver thiosulfate (STS) before degreening with 0.055 ml l^{-1} of ethylene for 42h. Debuttoned fruit were inoculated with *Diplodia natalensis* mycelium after ethylene treatment. Bars represent standard deviation. (Reproduced from Brown and Burns, 1998 with permission of Elsevier Science).

Similarly, the addition of cycloheximide, which is a protein-synthesis inhibitor (Riov, 1974), to the abscission zone of debuttoned fruits during early stages of degreening with high ethylene concentrations, also suppressed disease development. In other words, the inhibition of the enzymatic activity led to reduced fruit susceptibility to stem-end rot development.

The correlation between ethylene application to citrus fruit and the enhancement of fruit susceptibility to the green mold rot (*Penicillium digitatum*) has been attributed to the reduction in the antifungal activity of the peel: ethylene treatment stimulates the natural reduction in the amount of the antifungal citral and thus stimulates decay development (Ben-Yehoshua et al., 1995). Brown, G.E. and Barmore (1977) showed that anthracnose development in tangerines infected with *Colletotrichum gloeosporioides* occurred only when the fruits had been exposed to ethylene immediately after inoculation. In this case, disease induction by ethylene probably resulted from the stimulation of the infection hyphae production by the appressoria found on the fruit exposed to ethylene, and their penetration into the epidermis cells.

Flaishman and Kolattukudy (1994) reported that ethylene treatment at concentrations much lower than those produced during ripening of climacteric fruits, was capable of inducing both conidia germination and appressoria formation in *Colletotrichum musae* and *C. gloeosporioides*, and that endogenic ethylene, produced in climacteric fruits during ripening, can serve as a signal for the termination of the appressoria latency on the fruit. The induction of appressoria formation does not take place in *Colletotrichum* species attacking non-climacteric fruits, such as citrus fruits. When spores of this fungus come into contact with citrus fruits, which normally do not produce a considerable amount of ethylene after harvest, appressoria will not be produced in large quantities, and the fungus will not cause a serious decay. However, when such a fruit is exposed to ethylene during degreening, an abundance of spores is produced and a massive infection of the fruit takes place, as was previously reported for ethylene-treated citrus fruits (Brown, G.E., 1975).

In a later study, however, Prusky et al. (1996) differentiated between the effect of ethylene on the climacteric process and ripening of avocado fruits, on the one hand, and, on the other hand, the process of initiation of lesion growth from quiescent appressoria of *C. gloeosporioides*. They found that ethylene treatment at 45 $\mu l\ l^{-1}$, applied to immature avocado fruits,

enhanced appressorium formation and induced an early climacteric, but did not trigger earlier lesion development: progress in lesion development in ethylene-treated fruits was similar to that in untreated fruits (Fig. 11). It was concluded that exposure of avocado fruits to exogenous ethylene did induce multiple appressorium formation but did not activate disease development. The activation of appressoria during avocado ripening may rather be related to the decline in the antifungal diene in the peel, to subfungitoxic concentrations (Prusky et al., 1982).

Ethylene application also stimulates *Botrytis cinerea* development in strawberries (El-Kazzaz et al., 1983), and enhances *B. cinerea* and *Alternaria alternata* development in tomatoes (Barkai-Golan et al., 1989a; Segall et al., 1974). For strawberries, which are non-climacteric fruits and are, therefore, regarded as independent of ethylene for ripening, it was found that storage life was extended by reducing the ethylene level (Wills and Kim, 1995).

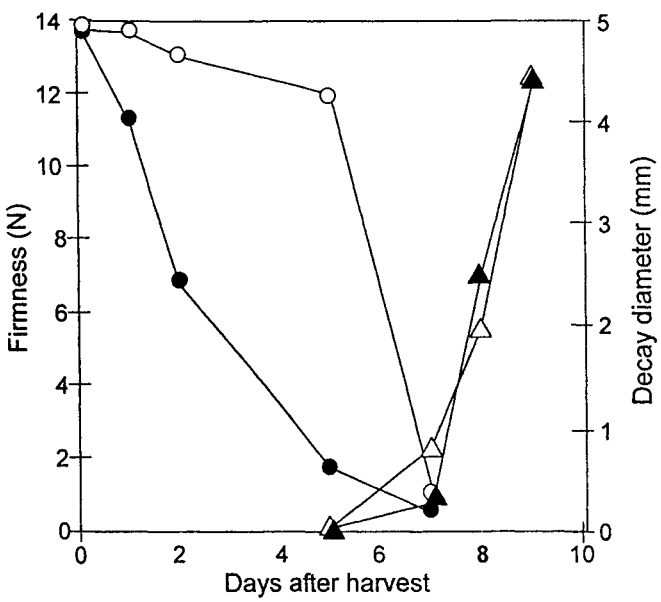


Fig. 11. Effect of ethylene on decay development caused by *Colletotrichum gloeosporioides* (▲) and changes in fruit firmness (●) in overmature inoculated avocado fruits (cv. Reed) as compared to decay (Δ) and firmness (○) in air treated fruit. Fruit firmness was determined by a penetrometer and expressed by N(=Newton, a parameter of fruit ripening). (Reproduced from Prusky et al., 1996 with permission of the American Phytopathological Society).

Relatively high levels of ethylene, that were found to accumulate in strawberry punnets overwrapped with polyethylene during marketing, were suggested to be the contributing cause to enhanced postharvest loss. The addition of an absorbent containing potassium permanganate was found to be an effective means of reducing ethylene in strawberry punnets during marketing, at ambient or reduced temperatures, with a corresponding increase in storage life. The potassium permanganate-treated fruit was less susceptible to *B. cinerea*, which is the main cause for deterioration at 20°C (Wills and Kim, 1995). With regard to the effect of ethylene on *Botrytis* in tomatoes, this gas was found directly to stimulate germ-tube elongation of fungal spores when introduced into tomato fruit tissue; but when the fruit is exposed to ethylene prior to its inoculation by the fungus, the gas may stimulate the pathogen development indirectly because of the stimulation of fruit ripening (Barkai-Golan et al., 1989a). Indirect stimulation of fungal growth occurs in normal tomato fruits at the mature-green stage, on which exogenous ethylene can normally act, but will not occur in non-ripening tomato mutants (*nor*) in which ripening cannot be induced by ethylene application (Barkai-Golan et al., 1989b). Exposure to ethylene also resulted in the stimulation of ripening in young mango fruits. Along with fruit ripening, the concentration of the antifungal resorcinol compounds was decreased and the sensitivity of the fruit to *A. alternata* was enhanced (Droby et al., 1986).

In contrast to cases in which exposure to ethylene stimulates disease development, in other cases it does not affect disease development, and may even enhance host resistance to pathogen attack. Induction of resistance to decay by ethylene was reported for sweet potatoes infected by *Ceratocystis fimbriata* (Stahmann et al., 1966), and tangerines exposed to ethylene for 3 days prior to their inoculation with *C. gloeosporioides* (Brown, G.E. and Barmore, 1977).

E. HOST-PATHOGEN INTERACTIONS

A pathogen that has reached a suitable host and also finds conditions suitable for germination, for penetration into the host, and for establishment within its tissues, will develop and cause a disease only if it succeeds in withstanding the protective means of the host. In other words: the struggle between the pathogen attack capability and the

defense capability of the host will dictate the success or failure of the infection. The attack mechanisms of the pathogen and the defense mechanisms of the host are summed up in Table 3.

TABLE 3

Attack mechanisms of the pathogen and defense mechanisms of the host

Attack mechanisms of the pathogen:
1. The ability to produce cuticle- and cell wall-degrading enzymes.
2. The ability to produce toxins causing cell death.
3. The ability to detoxify resistance compounds in the host.
4. The ability to develop on the fruit and vegetable surfaces, and within their tissues.
Defense mechanisms of the host:
1. The ability of the intact cuticle to provide a barrier to fungal penetration; its ability to prevent diffusion of cellular solutions, so limiting water and nutrient availability on the plant surface.
2. The resistance of the young fruit and vegetable cell wall to degradation by the pathogen enzymes.
3. The presence of preformed (constitutive) compounds inhibiting pathogen growth and enzymatic activity, and the ability to further induce, or enhance their production.
4. The ability to produce low molecular-weight secondary metabolites with antimicrobial activities, phytoalexins, in response to fungal penetration or other stress conditions.
5. Wound healing and the formation of barriers against the progress of the pathogen and its enzymatic activity; reinforcement of plant cell walls by formation of glycoproteins, callose, lignin and other phenolic polymers; the formation of papillae and plugging of intercellular spaces.

6. The hypersensitive response, which is thought to function by isolating and killing microorganisms in a small region of the plant tissue by localized cell death.
7. The induction of host resistance by the formation of pathogenesis-related proteins, such as chitinase and β-1,3 glucanase, in pathological situations or in response to other stresses.
8. The production of active oxygen in response to pathogens and elicitors; this oxygen has an antimicrobial effect and plays a role in various defense mechanisms.

Attack mechanisms of the pathogen and defense mechanisms of the host will be discussed separately in the following chapters.

CHAPTER 5

ATTACK MECHANISMS OF THE PATHOGEN

A. ENZYMATIC ACTIVITY

Many plant pathogens produce extracellular products that may influence their growth, be determinant factors in their pathogenic capability, or contribute to their pathogenicity or virulence. Such products may include enzymes capable of hydrolyzing the cuticle layer of the epidermic plant cells (Kolattukudy, 1985), cell wall degrading enzymes, toxins, hormones, siderophores, DNA and signaling molecules (Salmond, 1994), or factors capable of degrading host chemical defense agents (Staples and Mayer, 1995). We will concentrate on the secretion of extracellular enzymes and toxins, the two major attack means with which the pathogen may be equipped, and the ability of the pathogen to detoxify host defense compounds.

Cutinase

The enzyme cutinase, which hydrolyzes the primary alcohol ester linkages of the polymer, cutin (Kolattukudy, 1985), has been found in many plant pathogenic fungi, including *Botrytis cinerea* (Shishiyama et al., 1970). However, the importance of this enzyme for penetration of the cuticle seems to be a matter of debate (Stahl and Schafer, 1992). Many lines of evidence suggest that cuticular penetration of *Colletotrichum gloeosporioides* into the host is associated with the induction of extracellular cutinase production, and that insertion of the cutinase gene into this pathogen facilitates infection of an intact host (Dickman et al., 1989). Following the finding that cutinase secretion was also directed towards the region that penetrates the host, the infection peg of an appressorium produced by *Colletotrichum* spores (Podilia et al., 1995), it was suggested that the inhibition of this enzyme by active inhibitors could result in delay in the penetration of germinating appressoria into the host (Prusky, 1996).

Secretion of extracellular cutinase has been reported for *Aspergillus flavus* (Guo et al., 1996) when grown on purified cutin as the sole carbon source. Pretreatment of corn kernels with *A. flavus* culture filtrate or with bacterial cutinase promoted increased levels of aflatoxin production

typical of this fungus in wounded kernels. Furthermore, cutinase activity was strongly inhibited by an inhibitor specific to fungal cutinase, supporting the suggestion that cutinase may have a role in the pathogenicity of *A. flavus*.

In a recent study, the involvement of phenolic acids, which are found in the peach fruit surface, in disease resistance to *Monilinia fructicola*, the fungal cause of brown rot, has been related to their effect on cutinase production by the fungus (Bostock et al., 1999). Chlorogenic and caffeic acids, the major phenolic acids in the epidermis and subtending cell layers of peach fruits, were found in higher concentrations in a peach genotype with a high level of resistance; they declined as the fruit matured, with a corresponding increase in disease susceptibility. These phenolic compounds, however, did not show any suppressive effects on fungal development. On the other hand, in cultures amended with either of these phenolic acids, the cutinase activity of the fungus was markedly reduced. It was, therefore, suggested that the relationship between the high concentration of the phenolic acid in the immature peach fruit and the resistance of this fruit to infection may be because they can interfere with the capability of the pathogen to degrade the host polymers. (See also the chapter on Host Protection and Defense Mechanisms – Preformed Inhibitory Compounds.)

Pectolytic and Cellulolytic Enzymes

The pectic substances, which constitute an important component of the primary cell wall of plants, and form the major component in the middle lamella (Bateman and Basham, 1976), are responsible for the tight link between the cells and the integrity of the plant tissue. These substances are composed mainly of a high-molecular weight polymer comprising linear chains of D-galacturonic acid (1,4-α-galacturonic acid) which underwent various degrees of methylation, combined with side chains of neutral sugars (Bateman and Basham, 1976). Non-methylated chains are termed pectic acid; chains with about 75% or more of the units methylated are termed pectin, while chains with smaller percentages of the galacturonic acid units methylated are referred to as pectinic acid. The development of disease within the harvested fruit or vegetable is dependent, to a great extent, on the ability of the pathogen to secrete pectolytic enzymes, which are capable of decomposing the non-soluble pectic compounds and so causing cell separation and tissue disintegration. This process leads to enhanced permeability of the plasma membranes of attacked cells and to cell death, and facilitates the

diffusion of nutrients which can serve as a medium for pathogen development (Mount, 1978).

Two groups of pectolytic enzymes take part in the disintegration of the pectic substances. The first group includes pectin methylesterase (PME), which breaks the ester bonds and separates the methyl groups from the carboxylic groups of the pectin or pectinic acid, to yield pectic acid and methanol. Enzymes of this group do not break the pectin chain. The second group includes pectolytic enzymes that break the 1,4-glycoside bonds between the galacturonic acid subunits. The glycoside bonds may be broken by the hydrolytic activity of polygalacturonases (PG), which act on pectic acid chains, or by that of pectin methylgalacturonases (PMG), which act on chains of pectin or pectinic acid. The glycosidic bonds may also be broken by the action of pectin or pectate lyase (PL), also called transeliminase (PTE). These enzymes cleave the 1-4-glycoside linkages by unsaturating the ring between the fourth and the fifth carbon atoms in the subunits of the chain (Bateman and Millar, 1966). Thus, both the hydrolytic enzymes and the lyases attack the galacturonide links of the polymeric chain and cause it to break, but they differ from one another in their mode of action: the polygalacturonases act hydrolytically, while the lyases function in a lytic way by transelimination.

According to the point of attack in the pectic compound, the polygalacturonases and the lyases are classified as endo-enzymes, that break the galacturonide connections at random while producing oligogalacturonides, or as exo-enzymes, which attack only the terminal linkages. Disintegration and softening of the tissues (maceration), resulting in the formation of soft to watery rot caused by species of *Rhizopus, Penicillium, Geotrichum* and *Sclerotinia*, and by the bacterium *Erwinia carotovora* in many fruits and vegetables during storage, is generally related to the extracellular activity of endo-PG (Rombouts and Pilnik, 1972). However, the involvement of exo-PG in *Penicillium digitatum* pathogenesis in citrus fruits has been reported (Barash and Angel, 1970; Barmore and Brown, 1979). Enzymes from the endo-pectin-lyase group have been described as the main cause of the maceration of citrus fruits infected by *Penicillium italicum* or *P. digitatum* (Bush and Codner, 1970). However, in more recent studies the enzymes causing maceration of citrus fruit peel by these fungi were identified by Barmore and Brown (1979, 1980) as endo-PG and exo-PG, respectively.

The virulence of a pathogen has been related to the total PG activity or to specific PG isozymes. The production of PG isozymes may be influenced by various factors, such as growth conditions of the pathogen, nutrients and fungal strains (Cleveland and Cotty, 1991; Tobias et al., 1993). Recently an increase in total PG activity and in that of PG isozymes has also been correlated with reactivation of latent infections and the beginning of tissue maceration by the reactivated pathogen (Zhang et al., 1997).

Endo-pectate lyase, which attacks the pectic acid at random, is produced by various pectolytic bacteria and is probably the major cause of cell wall decomposition of potatoes infected by these bacteria (Hall and Wood, 1973), but enzymes of the endo-PG group may also be involved in tuber maceration (Beraha et al., 1974). The involvement of pectate lyase (PL) in fungal decay has been described for anthracnose (*Colletotrichum gloeosporioides*) in stored avocados (Wattad et al., 1994); PL was found to cause softening in unripe avocado fruits, whereas the addition of antibodies to this enzyme inhibited tissue maceration.

Saprophytic fungi and bacteria, which are not included in the postharvest pathogens' category, may also produce pectolytic enzymes on various substrates; some of these are capable of macerating discs of potato tuber or of other plant organs (Ishii, 1977). Therefore, the fact that a given microorganism can produce pectolytic enzymes *in vitro* cannot serve, by itself, as evidence of pathogenic capability. To determine the capability of a pathogen to decompose the pectic compounds of plant cell walls, its pectolytic activity should be examined in the host tissues themselves.

Simmonds (1963) suggested that the failure of the pathogen to produce adequate levels of pectolytic enzymes in the host may be related to the establishment of infections that remain quiescent until the ripening process leads to suitable changes in the cell wall structure. An inadequate level of pectolytic activity in the plant tissue may be caused by several factors (Swinburne, 1983): (1) Pectolytic enzymes are not all constitutive and they may need inducers for their production (Bateman and Basham, 1976); the inducing substrates could be supplied to the pathogen only as the fruit ripens. (2) Even if such enzymes were produced, access to the labile sites within the wall could be blocked by cation cross-linking. (3) Enzymes may be inactivated by inhibitors present at higher levels in the immature fruit than in the mature one

(see the chapter on Host Protection and Defense Mechanisms – Inhibitors of Cell Wall Degrading Enzymes).

Many fungi are capable of utilizing cellulose for their growth, when it is present as the sole source of carbon in the culture medium, thanks to their ability to produce and secrete cellulases. Cellulose, which is a linear polymer of D-glucose, the configuration being that of a 1,4-β-glucoside, is the principal polysaccharide of plants, being an important component of the plant cell wall, mainly of the secondary wall. Cellulose is by far the largest reservoir of biologically utilizable carbon on the surface of the earth, and its decomposition, in which fungi probably play a major role, is of great ecological importance. Most fungal cellulases are induced enzymes, the inducing substrate being cellulose in the medium. However, various microorganisms, such as the fungi, *R. stolonifer, Penicillium digitatum* and *P. italicum*, produce and secrete cellulase independently of the presence of cellulose (Spalding, 1963; Barkai-Golan and Karadavid, 1992).

Although plant tissue decomposition and cell death during disease development are strongly connected with the activity of extracellular pectolytic enzymes, the pathogen cellulases, as well as hemicellulases (the enzymes responsible for breakdown of hemicelluloses - plant cell wall constituents, particularly polysaccharides and polyuronides), may also play a role in this complex process. The description of the involvement of cellulases in pathogenesis has generally addressed the late stages, the saprophytic stages of pathogen development (Bateman and Basham, 1976). However, studies of the Cx-cellulase (the enzyme splitting the polymeric chain of cellulose to form cellobiose residues) of *P. digitatum* and *P. italicum* in citrus fruits, revealed high levels of enzymatic activity during the incubation period of the disease, before the appearance of disease symptoms; moreover, a correlation was found between cellulase activity of the pathogen in the fruit and the severity of the disease symptoms (Barkai-Golan and Karadavid, 1992). These findings led to the suggestion that the cellulolytic enzymes of the two *Penicillia* may play an active role in the early stages of pathogenesis, including the penetration of the fungus into the tissue. In tomato genotypes a correlation was drawn between the level of cellulolytic activity in the fruit prior to infection and the extent of cellulolytic activity of *Alternaria alternata* in infected fruit tissues. Thus, the cellulolytic activity of the pathogen in the ripening normal fruit was higher than those in the non-ripening *nor* mutant and its hybrid at each stage of

maturity. In fact, the enzymatic levels recorded for the infected mutant and hybrid fruits at their mature stage were similar to those recorded for the infected ripening normal fruit at its mature-green stage (Barkai-Golan and Kopeliovitch, 1989).

B. TOXIN PRODUCTION

Some pathogenic fungi secrete toxic compounds into the plant tissues, inflicting damage on them. These compounds are frequently called 'phytotoxins'; however, this term may be misleading since it is sometimes used to designate toxins originating in higher plants.

Toxins are pathogen-produced metabolites, most of which are low-molecular-weight compounds that cause histological and physiological changes in the host (Knoche and Duvick, 1987). They may be the primary cause of the disease induced by the pathogen or they may constitute only a part of the disease process (Scheffer, 1983; Walton and Panaccione, 1993). Toxins that have an injurious effect only on plant species or cultivars that serve as hosts of the toxin producing pathogen are termed host-specific or host-selective toxins. Conversely, toxins that affect a wider range of plant species, including both host and non-hosts of the pathogen, are termed non-host-specific or non-host-selective toxins (Rudolph, 1976; Mitchell, 1984). These toxins are not necessarily related to disease initiation. Pathogen-produced toxins can also be classified, according to Yoder (1980), as bearers of a 'pathogenicity factor', a factor essential for a pathogen to cause disease, and those bearing a 'virulence factor', that only enhances the extent of disease. Toxins that are considered as virulence factors turn out to be the non-host-specific toxins, whereas those considered as pathogenicity factors are generally host-specific toxins (Mitchell, 1984).

Host-Specific Toxins

Host-specific toxins were for a long time considered to be the only agents of specificity in plant/microbe interaction (Walton and Panaccione, 1993). They represent widely differing chemical categories, including peptides, glycosides, esters and other compounds (Macko, 1983), which function as a chemical interface between pathogens and their host plants. However, in order to study the biosynthesis and the role of the toxins in pathogenesis, structural information is needed. In

other words: the structure of a toxin yields clues about the biosynthetic process and the mechanism of action.

Among the microorganisms producing host-specific toxins, we find pathogens that survive saprophytically when they lose their capacity to produce the toxin (Macko 1983; Scheffer 1983). These include various species of *Helminthosporium* and *Alternaria*, as well as other fungal species not related to postharvest diseases. Among the *Helminthosporium* species we find: *H. sacchari*, the causal fungus of eyespot disease of sugarcane, which is the producer of HS-toxins; *H. victoriae*, which attacks oats and produces HV-toxin; *H. maydis*, the causal fungus of specific blighting on leaves of sensitive corn, which is the producer of T-toxin; and *H. carbonum*, which attacks corn and produces HC-toxin. The corn fungus *Phyllosticta maydis* produces PM-toxin, a host-specific toxin similar to but not identical to T-toxin. The pathogenic species of *Alternaria* in this class were identified mainly on the basis of their specific host. They include: *Alternaria alternata* f. sp. *kikuchiana* (*Alternaria kikuchiana*), the causal fungus of black spot disease of the Japanese pear, which produces AK-toxin; *Alternaria alternata* f. sp. *lycopersici*, the causal fungus of stem canker disease of the tomato, which produces AL-toxin; *A. alternata* f. sp. mali (*Alternaria mali*), which attacks leaves, shoots and fruits of apples and produces AM-toxin; *A. alternata* f. sp. *citri* (*Alternaria citri*), which attacks rough lemon fruits and tangerines and produces AC-toxins; and *A. alternata* f. sp. *fragariae* (*Alternaria fragariae*), which attacks strawberries and produces AF-toxin. A toxin released from germinating spores of *Alternaria tenuissima*, causing leaf spot of pigeon pea in the field, has also been found to have host-specific properties (Nutsugah et al., 1994). Each of these fungi has its specific toxin that may be responsible for the selective pathogenicity of the fungus.

According to Nishimura and Kohomoto (1983), the host range of the pathogenic strains of *Alternaria* is limited to cultivars that are susceptible to their host-specific toxins, while other cultivars are highly resistant. However, as reviewed by Knoche and Duvick (1987), such a definition might be too sharp, since AM-, AK-, and AF-toxins were able to exert a slight effect on hosts other than their specific hosts. Most of the *Alternaria* species or subspecies are similar morphologically and are classified today in the extended group of *Alternaria alternata*, being considered as pathotypes of this fungal species. Although these fungi are generally not included among the common postharvest pathogens, they

belong to the same group that includes the weak pathogens which attack various harvested fruits and vegetables via wounds, following physiological damage, or through aging tissues, without being selective to a specific host.

Nishimura and Kohomoto (1983) related the origin of the host-specific toxins of *A. alternata* to the wild strains of *A. alternata*. Since the transition from a non-specific *Alternaria* type to a host-specific type is associated with the production of host-specific toxins, it might be assumed that a wild population of the non-host-specific *A. alternata* initially contained toxin producers which have apparently arisen by mutation. Mutation to toxin production probably affected an extremely minute proportion of the field population of *A. alternata* before the introduction of susceptible plants. The introduction of new susceptible host genotypes might have led to multiplication of the toxin-producing mutants (Nishimura and Kohomoto, 1983). Monocultures of the susceptible plants act as a selection medium and increase the proportion of toxic mutants. If, on the other hand, there is no chance to meet with uniformly genetically susceptible genotypes, the property of toxin production will not have a chance to appear and will soon be lost.

Non-Host-Specific Toxins

In contrast to the host-specific toxins, many fungi produce non-specific toxins, which are toxic to hosts and non-hosts of the pathogen, and may attack many different plants (Rudolph, 1976). A wide range of non-host-selective toxins of fungal and bacterial origin have been described and identified. Some of them seem to be necessary for successful infection of the host, whereas others play only a secondary role. Also, many non-selective toxins are metabolites that are toxic in bioassays, although this property may be unrelated to disease development. For many of the non-specific toxins, the toxicity to plants is just another aspect of pathogenesis, acting in addition to penetration mechanisms, enzymatic activity, recognition capability and other factors. In spite of the damage caused by the non-specific toxins to the plant tissues, the relationship between their synthesis and disease inducement has not yet been proven. However, even if these toxins are not necessary for disease initiation, they may enhance the pathogen virulence by causing phytotoxic phenomena in the attacked tissues (Mitchell, 1984). Rudolph (1976) lists several criteria to be investigated before deciding upon the pathogenic role of a phytotoxic compound. Among these are:

(1) The inducement of disease symptoms by the isolated toxin;
(2) Demonstration of the presence of the toxin within the diseased host;
(3) Demonstration of a correlation between the rate of toxin production *in vitro* and the pathogenicity of the isolate within the host;
(4) A correlation between disease susceptibility and toxin sensitivity. Although this is the main criterion for host-specific toxins, it may also be applicable to some non-host-specific toxins at the cultivar level, e.g., tenuazonic acid from *Alternaria alternata* or the raw toxin solution from *Fusarium moniliforme;*
(5) Indications of the role of toxins during pathogenesis can be obtained when their activity *in vivo* is inhibited within the host or by specific environmental conditions, and these manipulations result in suppression of disease development.

Examples of non-host-specific fungal toxins that have a role in disease development, or have been suggested to be involved in disease, are produced by fungi that may be responsible for postharvest diseases. They include: fumaric acid, produced by *Rhizopus* species; oxalic acid, produced by *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*; ophiobolins produced by *Helminthosporium* species; fusaric acid produced by *Fusarium oxysporum*; malformin produced by *Aspergillus niger*; colletotrichins produced by *Colletotrichum* species; and tentoxin produced by *A. alternata f. tenuis* (Macko, 1983; Scheffer, 1983). In fact, most *Alternaria* species may produce non-host-specific toxins, which are not prerequisite for infection.

A. alternata, one of the most common postharvest pathogens of various fruits and vegetables (Barkai-Golan, 1981), has been reported to produce several toxic compounds in harvested fruits. Stinson et al. (1981) reported that the major toxin produced in tomato fruits was tenuazonic acid, with a maximal concentration in tomato tissue of 13.9mg/100g; alternariol, alternariol monomethyl ether, and altenuene were produced in much smaller amounts. The major toxins recorded in apples were alternariol and alternariol monomethyl ether, with maximum concentrations in tissue of 5.8 and 0.23mg/100g, respectively. Inoculation of oranges and lemons with the same fungal isolate showed that the predominant toxins in these hosts were tenuazonic acid, alternariol monomethyl ether and alternariol, with concentrations in tissue of only 2.66, 1,31 and 1.15mg/100g, respectively. Working with another strain of

A. alternata, Ozcelik et al. (1990) found that concentrations as high as 120.6mg of alternariol and 63.7mg of alternariol methyl ether per 100g tissue were recorded in inoculated tomatoes after 4 weeks at 15°C. The highest in-tissue concentration of altenuene (19mg/100g) was found after 3 weeks at 25°C. However, tenuazonic acid was not detected in inoculated tomatoes, regardless of the storage temperature or type of packaging. Inoculations of Red Delicious apples indicated that alternariol was also the predominant toxin in this fruit, although its in-tissue concentrations were lower than those produced in tomatoes (e.g., 49.8mg/100g at 25°C); relatively low concentrations were recorded for the other two toxins, while tenuazonic acid was not detected in infected apples (Ozcelik et al., 1990). Alternariol, alternariol methyl ether and tenuazonic acid were recorded in *A. alternata*-infected peppers (Bottalico et al., 1989).

In addition to their phytotoxic effects, non-specific toxins may also function as mycotoxins – toxic to both humans and animals – as antibiotic substances, as plant growth regulators, etc. Examples of mycotoxins that also function as phytotoxins (Scheffer, 1983) are: aflatoxins, produced by *Aspergillus flavus* and related fungi; citrinin, patulin and penicillic acid, which are produced mainly by species of *Penicillium* and *Aspergillus*; moniliformin and fumonisins produced by strains of *Fusarium moniliforme*; and trichothecenes produced by various fungi, such as species of *Fusarium, Cephalosporium, Myrothecium, Trichoderma* and *Stachybotrys*. In addition to their mycotoxic effects on humans and animals and their toxic effects on plants and plant organs, the trichothecenes may also have insecticidal, antifungal, antibacterial, antiviral, and antileukemic effects (Macko, 1983).

Of special interest to postharvest pathology is the production of patulin by *Penicillium expansum* in pome and stone fruits. The amount of patulin produced by *P. expansum* may vary greatly according to the strain involved. Sommer et al. (1974) found that patulin production by different strains of *P. expansum* in Golden Delicious apples ranged from 2 to 100 μg/per gram of tissue. On the other hand, various apple and pear cultivars may differ in their sensitivity to the same fungal strains. The storage temperature is another factor affecting patulin production in a given fruit cultivar. Paster et al. (1995) demonstrated that while more patulin was produced in Starking apples than in Spadona pears held at 0-17°C, higher toxin levels were produced in pears than in apples held at

25°C. However, in spite of the differences in the amounts of toxin produced, no significant differences in pathogenicity were recorded among fungal strains, as determined by lesion diameter on each of the fruits. In other words, no correlation was found between patulin production and disease development. Even strains that did not produce patulin at 0° and 25°C infected the fruits to the same degree as strains capable of growth and patulin production at these temperatures. Furthermore, patulin production was totally inhibited when inoculated apples were held in a 3%CO_2/2%O_2 atmosphere (25°C), although the level of infection under the controlled atmosphere conditions was fairly high, reaching 70% of that of the control.

Non-host-specific toxins are generally acknowledged to be an element of virulence for many bacteria as well. Most of the known bacterial toxins are produced by various pathovars of *Pseudomonas syringae* (Scheffer, 1983; Gross, 1991). These toxins constitute a family of structurally diverse compounds, usually peptide in nature, that, in some cases, display wide-spectrum antibiotic activity. Progress has been made in identifying gene clusters associated with toxin production, and there is some evidence that toxin genes may be activated in response to specific plant signals (Gross, 1991).

C. DETOXIFICATION OF HOST DEFENSE COMPOUNDS BY PATHOGENS

In addition to the activities of cell-wall-degrading enzymes and toxins of the pathogen, the ability to degrade plant chemical defense compounds, such as the tomato α-tomatine, has also been discussed as a potential pathogenicity determinant (Staples and Mayer, 1995).

The saponin, α-tomatine is a steroidal glyco-alkaloid that occurs in many plant species and provides a preformed barrier to fungal invasion (Verhoeff and Liem, 1975; Osbourn, 1996). It is found in all parts of tomato plants but is especially plentiful in the peel of green tomato fruits (see the chapter on Host Protection and Defense Mechanisms – Preformed Inhibitory Compounds). The toxicity of α-tomatine to fungi is due to its ability to interact with membrane sterols, and thus cause membrane leakage (Fenwick et al., 1992; Quidde et al., 1998).

Tomato pathogens have developed two primary strategies to overcome this chemical defense (Osbourn et al., 1994): either they are resistant to

α-tomatine by virtue of their membrane composition or they are capable of detoxifying α-tomatine. Detoxification of α-tomatine has been reported for various fungi, such as *Fusarium oxysporum* f.sp. *lycopersici* (Ford et al., 1977), *Alternaria solani* (Schlösser, 1975), *Alternaria alternata* (Sandrock and VanEtten, 1997), *Septoria lycopersici* (Arneson and Durbin, 1967), and *Botrytis cinerea* (Verhoeff and Liem, 1975).

In a recent study, Quidde et al. (1998) showed that *B. cinerea* metabolizes α-tomatine by removal of the terminal D-xylose, to yield β_1-tomatine, and not by deglycosylating α-tomatine completely, as was previously suggested (Verhoeff and Liem, 1975). It was concluded that the tomatinase of *B. cinerea* is a β-xylosidase. Using a tomatinase-deficient strain of *B. cinerea*, Quidde et al. (1998) found that β_1-tomatine is far less toxic to axenic culture than α-tomatine, confirming that the removal of the xylose moiety represent a detoxification process. Most *B. cinerea* strains tested, originating from various host plants, were found capable of detoxifying α-tomatine in this way. It was thus suggested that α-tomatine degradation has a role in the interaction between *B. cinerea* and tomato.

CHAPTER 6

HOST PROTECTION AND DEFENSE MECHANISMS

A. THE CUTICLE AS A BARRIER AGAINST INVASION

The cuticle provides a barrier to pathogen penetration. Wounding and treatments that disrupt or dissolve the cuticle result in more rapid infection by various pathogens (Elad and Evensen, 1995). The cuticle may, however, function not only as a physical barrier but also as a chemical barrier, since it may contain substances antagonistic to fungi (Martin, 1964). The cuticle thickness has been correlated with the resistance of tomato fruit to *Botrytis cinerea* (Rijkenberg et al., 1980), or of peach cultivars to *Monilinia fructicola* (Adaskaveg et al., 1989, 1991). Biggs and Northover (1989) showed that the cuticle and cell-wall thicknesses were correlated with longer incubation periods of the brown rot in cherry fruits, accompanied by a lower incidence of infection. Elad and Evenson (1995) listed several ways by which a thicker cuticle might enhance host resistance: (a) by being more resistant to cracking and thus to penetration of wound pathogens (Coley-Smith et al., 1980); (b) by providing mechanical resistance to penetration (Howard et al., 1991); (c) by presenting a greater quantity of substrate to be degraded by fungal enzymes, and preventing diffusion of cellular solutions, thus limiting the access of water and nutrients to the microenvironment where they are required for spore germination and the infection process (Martin, 1964).

Studies with various genotypes of peaches clearly showed that genotypes resistant to brown rot caused by *Monilinia fructicola* had a thicker cuticle and denser epidermis than susceptible genotypes. Their resistance was correlated with both a delay in fungal penetration and a longer incubation period for infection initiation, and resulted in the extension of the quiescent period of the pathogen (Adaskaveg et al., 1989, 1991).

B. INHIBITORS OF CELL WALL-DEGRADING ENZYMES

Pathogen development within the host is correlated in many cases with the activity of cell wall-degrading enzymes, which are responsible

for cell death and the liberation of nutrients, which then become available to the pathogen. Liberation of nutrients results in a stimulation of pathogen growth and accelerated disease development. This may explain the great importance attributed to compounds that are capable of suppressing or preventing enzymatic activity.

Trials with various pathogens have emphasized the point that sugars in the culture medium, which serve as available nutrients for the pathogen and stimulate its growth, may inhibit pectolytic and cellulolytic enzyme production and activity (Spalding et al., 1973; Biehn and Dimond, 1973; Bahkali, 1995). It was found that the presence of glucose in the culture medium, either as a sole source of carbon or in addition to malic or citric acid, inhibited pectin lyase activity of *Penicillium expansum*, while resulting in enhanced fungal growth (Spalding and Abdul-Baki, 1973)

Polyphenols and tannins have been described by Byrde et al. (1973) as inhibitors of polygalacturonase (PG) activity of *Sclerotinia fructicola* (*Monilinia fructicola*) in apples and other pathogen/host combinations, although they have no effect on the pathogen itself. Decay development in many fruits and vegetables may be the result of the delicate balance between active production of enzymes and their inhibition by polyphenols, mainly in their oxidized condition, as a result of polyphenol-oxidase activity (Dennis, 1987).

Another class of inhibitors of cell wall-degrading enzymes comprises the PG-inhibitory proteins present in both infected and uninfected plant tissue (Albersheim and Anderson, 1971; Abu-Goukh et al., 1983). A PG-inhibiting protein from a given plant source may act on pectic enzymes produced by different fungal species, both pathogenic and non-pathogenic, or on various PG isozymes from the same fungus. Research carried out with pepper fruits has shown that cell wall proteins of the host inhibited pectolytic enzyme production by *Glomerella cinigulata*, whereas the pectolytic activity of *Botrytis cinerea* was much less affected by these proteins (Brown, A.E. and Adikaram, 1983). The fact that *B. cinerea* can rot an immature pepper fruit whereas *G. cingulata* can attack only the ripened fruit, suggested that protein inhibitors might play a role in the quiescent infections of pepper fruits by *Glomerella*.

Purified pear inhibitory proteins were found to inhibit various fungal PGs, including that of *B. cinerea*, but did not affect endogenous PG activity in pear fruits (Abu-Goukh and Labavitch, 1983). In a later study, Stotz et al. (1993) reported on the molecular characterization of the

PG-inhibiting protein from pears, a step that may lead to the expression of inhibitory proteins in transgenic plants and contribute to the inhibition of decay development. Following the finding that ripening tomato fruits from transgenic plants expressing the pear inhibitory protein gene were more resistant to *B. cinerea* infection than the control fruits (Powell et al., 1994), PG-inhibiting proteins have been considered as pathogen infection resistance factors.

A PG-inhibiting protein purified from mature apple fruits (Yao et al., 1995) showed differential inhibitory activity against five PG isozymes purified from *B. cinerea* grown in liquid culture. However, inhibition was not detected against PG extracted from apple fruits inoculated with the same fungus; in contrast to the several isozymes isolated from fungal culture, only one PG was isolated from apple fruits inoculated with *B. cinerea.* The absence of multiple PG isozymes in inoculated fruits may suggest that the PG-inhibiting protein is involved in limiting the production of most PG isozymes during fungal infection. In other words, although the apple fruits did not show inhibitory activity against the PG isozyme isolated from inoculated fruits when assayed *in vitro*, this does not exclude the possibility that an apple inhibitor may contribute to the general resistance mechanism of the fruit when fighting fungal infection. Its application has even been considered as a possible alternative method for controlling postharvest diseases (Yao et al., 1995). A new protein inhibitor that may be involved in the inhibition of enzymes necessary for microbial development was isolated from cabbages (Lorito et al., 1994); it significantly inhibited the growth of *B. cinerea* by blocking chitin synthesis, so causing cytoplasmic leakage. Several studies supported the theory that natural protein compounds within the plant tissue may act as inhibitors of pathogen enzymes, and that these inhibitors may be responsible for the low levels of PG and PL found in infected tissue (Fielding, 1981; Barmore and Nguyen, 1985; Bugbee, 1993).

Recent studies show a close correlation between the changes in the level of epicatechin in the peel of avocado fruits and the inhibition of pectolytic enzyme activity of *Colletotrichum gloeosporioides.* The epicatechin was found in unripe fruits at concentrations much higher than those required for *in vitro* inhibition of purified PG and pectate lyase (PL) produced by the fungus (Wattad et al., 1994). This led to the suggestion that epicatechin may contribute to the quiescence of the fungus by inhibiting the activity of pathogenicity factors. The importance of pectate lyase as a pathogenicity factor during the activation of

quiescent infections has been demonstrated in two experiments: (a) antibodies produced against PL of *C. gloeosporioides* incubated with fungal spores, inhibited anthracnose development in ripe fruits; and (b) a nonsecreting PL mutant was found to be non-pathogenic to susceptible avocado, indicating that even when concentrations of antifungal compounds were reduced, PL production was required for infection (Wattad et al., 1995).

C. PREFORMED INHIBITORY COMPOUNDS

Phenolic compounds have long been implicated in disease resistance in many horticultural crops (Ndubizu, 1976). Some occur constitutively and are considered to function as preformed or passive inhibitors, while others are formed in response to the ingress of pathogens, and their appearance is considered as part of an active defense response (Kurosaki et al., 1986a; Nicholson and Hammerschmidt, 1992).

Activity of Preformed Compounds

Preformed resistance involves the presence in healthy tissues of biologically active, low-molecular-weight compounds whose activity affords protection against infection (Ingham, 1973). These are regarded as constitutive antimicrobial barriers (Schönbeck and Schlösser, 1976; Prusky, 1997). Phenolic compounds contribute to resistance through their antimicrobial properties, which elicit direct effects on the pathogen, or by affecting pathogenicity factors of the pathogen. However, they may also enhance resistance by contributing to the healing of wounds via lignification of cell walls around wound zones. Evidence strongly suggests that esterification of phenols to cell-wall materials is a common aspect of the expression of resistance (Friend, 1981).

The antifungal properties of phenolic compounds and their derivatives, and the fact that these compounds are frequently found in the young fruit at concentrations higher than in the ripe fruit, led to the hypothesis that these compounds play an important role in the maintenance of latency in the unripe fruit.

In vitro assays have shown that the phenolic compounds, chlorogenic acid and ferulic acid directly inhibited *Fusarium oxysporum* and *Sclerotinia sclerotiorium*, respectively. Benzoic acid derivatives have been shown to be the best inhibitors of some of the major postharvest pathogens, such as *Alternaria* spp., *Botrytis cinerea, Penicillium*

digitatum, S. sclerotiorium and *F. oxysporum* (Lattanzio et al., 1995). The principal phenols in the peach fruit epidermis and subtending cell layers are chlorogenic and caffeic acids. The concentrations of these phenols decline as fruits mature, with a corresponding increase in fruit susceptibility to the brown rot fungus *Monilinia fructicola*. The levels of chlorogenic and caffeic acids are higher in peaches with a high level of resistance than in fruits of similar maturity, of more susceptible genotypes (Bostock et al., 1999). Studying the direct effects of chlorogenic and caffeic acids on *M. fructicola* growth indicated that fungal spore germination or mycelial growth were not inhibited by concentrations similar to or exceeding those that occur in the tissue of immature, resistant fruit. Examination of the effects of phenolic compounds on other factors that may be involved in pathogenicity of the fungus showed that the presence of either of these phenolic acids in culture during fungal growth resulted in a sharp decrease in cutinase activity. It was further proposed that the high concentration of chlorogenic acid present in immature fruit and in fruit from highly resistant genotypes may contribute to the brown rot-resistance of the tissue by interference with the production of factors involved in the degradation of cutin, rather than by direct toxicity to the pathogen (Bostock et al., 1999).

The saponin, α-tomatine, present in high concentrations in the peel of green tomatoes, is inhibitory to *B. cinerea* and other fungal pathogens, and its involvement in the development of quiescent infection has been suggested (Verhoeff and Liem, 1975). In this case, although no further development of fungal lesions occurred after ripening, tomatine was not detected in mature fruits (Table 4).

TABLE 4
Tomatine content of tomato fruits*

Fruit tissue	Tomatine content	
	% of dry weight	mg/100 g fresh weight
Green fruits (2-3 cm diameter)	0.5	43
Skin of green fruits (2-3 cm in diameter)	0.95	82
Red, ripe fruits	none	none

* Reproduced from Verhoeff and Liem, 1975 with permission of Blackwell Wissenschafts-Verlag GmbH.

However, because of the marked antifungal activity of tomatine and other saponins, these preformed compounds are believed to be involved in host resistance towards saponin-sensitive fungi. Tomatine presumably owes its toxic properties to its ability to bind to 3β-hydroxy sterols in fungal membranes (Steel and Drysdale, 1988). Most tomato pathogens, on the other hand, can specifically degrade tomatine and detoxify its effect through the activity of tomatinase (Osbourn et al., 1994).

In a recent study, Sandrock and VanEtten (1998) examined 23 strains of fungi for their sensitivity to α-tomatine and to its two breakdown products, β_2-tomatine and tomatidine. The two saprophytes and the five non-pathogens of tomato included in this study were found to be sensitive to α-tomatine, while all the other fungi tested, except for two tomato pathogens, were determined to be tolerant to α-tomatine. All the tomato pathogens tested except *Phytophthora infestans* and *Pythium aphanidermatum* were able to degrade α-tomatine. These included *Alternaria alternata*, the common postharvest pathogen of tomato fruit. A strong correlation was found between tolerance to α-tomatine, ability to degrade this compound, and pathogenicity to tomato. However, β_2-tomatine and tomatidine, which were less toxic to most pathogens because of their inability to complex with membrane-bound 3β-hydroxy sterols (Steel and Drysdale, 1988), were inhibitory to some of the non-pathogens of tomato, suggesting that tomato pathogens may have multiple tolerance mechanisms to α-tomatine (Sandrock and VanEtten, 1998).

Tannins in young banana fruits (Green and Morales, 1967) and benzylisothiocyanate in unripe papaya fruits (Patil et al., 1973) are additional examples of in-fruit toxic compounds. Since the concentration of these compounds decreases with fruit ripening, it has been considered that they have a role in resistance to decay in young fruits.

Antifungal substances isolated from unripe avocado fruit peel include monoene and diene compounds, of which diene is the more important (Prusky and Keen, 1993). Resistance of unripe avocado fruits to postharvest pathogens has been suggested to be closely related to the presence in the peel of the antifungal diene compound (1-acetoxy-2 hydroxy-4-oxo-heneicosa 12,15) (Prusky and Keen, 1993). This compound inhibits spore germination and mycelial growth of *Colletotrichum gloeosporioides*, at concentrations lower than those present in the peel (see Fig. 3). The concentration of diene diminishes considerably as the fruit ripens and, in parallel, the dormant fungus in the peel starts its development (Prusky et al., 1982) (see Fig. 4). The decrease in the

antifungal compound in the ripe fruit, which tends to decay, has been attributed to the enzymatic activity of lipoxygenase in the fruit. Several findings supported the suggestion that this enzyme is the cause for diene decomposition and increased sensitivity of the ripe fruit to decay (Prusky and Keen, 1993):

(a) The enzyme activity increased considerably during ripening;

(b) Partially purified lipoxygenase oxidized diene under *in vitro* conditions;

(c) Treatment with tocopherol acetate, an antioxidant compound that non-specifically suppresses lipoxygenase, or with an enzyme-specific inhibitor, delayed the reduction in diene concentration and inhibited the appearance of disease symptoms elicited by *C. gloeosporioides*.

The activity of lipoxygenase in ripe avocado fruits is affected by the activity of the epicatechin, a natural antioxidant present in the fruit peel. The concentration of this compound decreases during ripening thus allowing the activity of lipoxygenase to increase. A considerable reduction in the epicatechin concentration in sensitive avocado cultivars occurs along with the reduction in fruit firmness, and disease symptoms are expressed only when the concentration is reduced to the lowest levels (Prusky and Keen, 1993). Thus, epicatechin appears to play a key role in fruit susceptibility during ripening, by indirectly controlling the level of the antifungal compound.

A mixture of resorcinols, found in the peel of unripe mango fruits, showed antifungal activity against *A. alternata,* the causal agent of the black spots in harvested fruit. The presence of this mixture was related to the latent stage of the fungus in young mango fruits (Droby et al., 1986): during ripening, the resorcinol concentrations in the peel are reduced, the fruit loses its resistance to the fungus and the quiescent *Alternaria* infections become active. Similarly to the preformed compounds in young avocados, the preformed resorcinols in unripe mangoes can also be further induced in the fruit. The fact that the resorcinol concentrations in Keit mango, which is disease-sensitive, do not decrease to non-toxic levels during ripening also supports the assumption that the resorcinols mixture has a role in disease suppression. Large quantities of resorcinols generally occur in the fruit peel, whereas the fruit flesh contains only very small quantities.

Several natural antifungal compounds have been isolated from lemon peel and have been identified. Among these are citral, limetin, 5-geranoxy-7-methoxycoumarin and isopimpenellin. Of these, only the citral, which is a monoterpene aldehyde, has been found in the flavedo in concentrations sufficient to inhibit decay development. By adding citral to a green lemon, previously inoculated with *Penicillium digitatum,* it was possible to prevent decay development under shelf-life conditions, but high concentrations of citral damaged the peel (Ben-Yehoshua et al., 1992). The presence of antifungal compounds, mainly citral, in young lemon fruits, may explain why citrus fruits, in their developmental stages on the tree, are resistant to decay, in spite of their constant contact with *Penicillium* spores and the injuries that inevitably occur in the grove and provide points for penetration.

The antifungal activity of citral has been exhibited against various fungi (Onawunmi, 1989). Introducing *P. digitatum* spores into the oil glands of the peel of young lemon fruits revealed that citral is the main factor within the glands responsible for the inhibition of the pathogen development (Rodov et al., 1995b). However, during long-term storage of lemon fruits - during which fruit senescence progresses - the citral concentration within the glands declines, resulting in decreased antifungal activity of the peel and increased incidence of decay. The changes in citral concentration in the lemon peel may thus determine the fruit sensitivity to postharvest decay. In parallel with citral decline, the flavedo of yellow lemons exhibited an increased level of neryl-acetate, which is a monoterpene ester which exhibits no inhibitory activity against *P. digitatum*, and which, in low concentrations (of less than 500 ppm) may even stimulate development of the pathogen. The increase in the level of monoterpenes in the peel may explain, at least in part, the stimulatory effect on *P. digitatum* and *P. italicum* development, of the etheric oil derived from stored citrus fruit, a phenomenon recorded earlier by French et al. (1978). The resistance of citrus peel to inoculations applied between the oil glands, found in early studies by Schiffmann-Nadel and Littauer (1956), was attributed to another factor, which does not change with fruit ripening and may be related to its chemical composition or its anatomic structure (Rodov et al., 1995b).

Inducible Preformed Compounds

Over the last two decades several studies have indicated that preformed antifungal compounds, which are normally present in healthy plant tissues, can be further induced in the host in response to pathogen

attack or presence, as well as to other stresses. Induction of existing preformed compounds can take place in the tissue in which they are already present, or in a different tissue (Prusky and Keen, 1995).

An example of a preformed compound being induced in the same tissue where fungitoxic concentrations are naturally accumulated is the antifungal diene in the peel of unripe avocado fruits. Prusky et al. (1990) found that inoculation of unharvested or freshly harvested avocado fruits with *C. gloeosporioides*, but not with the stem-end fungus *Diplodia natalensis*, resulted in a temporarily enhanced level of this compound. The response to this challenge doubled the amount of the preformed diene after 1 day, and the effect persisted for 3 days, suggesting persistence of the elicitor (Prusky et al., 1990). A significant increase in the concentration of the antifungal diene, with longer persistence of the elicitors but without symptom expression, has been induced by a nonpathogenic mutant of *Colletotrichum magna* (Prusky et al., 1994). The inducement of preformed antifungal compounds by two different *Colletotrichum* species suggests that nonspecific eliciting factors are probably present in the hyphae of both species (Prusky, 1996). Wounding is an abiotic factor that may enhance diene concentration. However, whereas wounding of freshly harvested fruit resulted in a temporarily enhanced diene accumulation in the fruit, inducement did not occur in fruits 3-4 days after harvest (Prusky et al., 1990). Gamma irradiation is another abiotic factor capable of inducing diene accumulation. Following irradiation at 5 or 20 krad, the diene concentration was doubled, but a day later its level was lower than in the untreated controls. This phenomenon was probably due to the fact that irradiation also enhanced fruit ripening and, in turn, the decline in the diene concentration (Prusky and Keen, 1995).

An inducement of the antifungal diene also followed a high-CO_2 application. Exposing freshly harvested avocado fruits to 30% CO_2 resulted in increased concentration of the diene upon removal from the controlled atmosphere storage. The concentration of diene then decreased but this decrease was followed by a second increase which correlated with suppressed decay development (Prusky et al., 1991). The increased concentration of the antifungal diene in the fruit could result from direct synthesis or from inhibition of its breakdown during fruit ripening. Several findings have pointed to the importance of lipoxygenase activity in the breakdown of the diene, and to its being affected by the activity of epicatechin in the fruit peel. In the flesh of avocado fruits both diene and monoene antifungal compounds are among the compounds that

accumulate within special oil cells named idioblasts (Kobiler et al., 1994). It is not clear whether induction of the antifungal diene involves *de novo* synthesis or a release of the compound from the idioblasts. However, the fact that about 85% of all antifungal compounds within the fruit mesocarp are located in idioblasts suggests that their synthesis might occur in these specific cells (Kobiler et al., 1994).

Inducement of preformed antifungal compounds has also been described in mango fruits. A mixture of resorcinolic compounds normally occurs in fungitoxic concentrations (154–232 $\mu g\ ml^{-1}$ fresh weight) in the peel of unripe mangoes, whereas only very low concentrations are present in the flesh of the fruits (Droby et al., 1986). Peeling of unripe fruits and exposing them to atmospheric conditions for 48 h enhanced the concentrations of resorcinols in the outer layers of the flesh to fungitoxic levels (Droby et al., 1987). This enhancement was accompanied by an increase in fruit resistance to fungal attack. Fruit peeling resulted in browning of the flesh accompanied by enhanced activity of phenylalanine ammonia lyase (PAL). There are indications that PAL activity is connected with production of phytoalexins and other compounds involved in the defense mechanisms of the plant (Kuć, 1972). However, it was further found that cycloheximide application inhibited both the enhancement of PAL activity and the browning of the tissue, but did not interfere with the production of resorcinols and the resistance of the fruit to *Alternaria alternata* (Droby et al., 1987). These results confirm the assumption that the resorcinols that accumulate in the fruit flesh following peeling are responsible for the decay resistance of the peeled fruit.

Exposure of the fruit to a controlled atmosphere containing up to 75% CO_2 was found to enhance the level of resorcinols in the peel itself, where they are normally present; this enhancement was accompanied by decay retardation, as indicated by a delay in the appearance of the symptoms of *A. alternata* infection (Prusky and Keen, 1995). These results showed that it is possible to induce the preformed resorcinols both in the tissue in which they are already present, and in a different tissue.

In carrot roots, high concentrations of the antifungal polyacetylene compound, falcarindiol, were recorded. This compound is found in extracellular oil droplets within the root periderm and the pericyclic areas (Garrod and Lewis, 1979). The high concentrations of the antifungal compound were suggested to result from the continuous contact of the carrots with organisms in the rhizosphere or with various pathogens. One of the important antifungal compounds in carrot roots is

the polyacetylenic compound, falcarinol. Heat-killed conidia of *Botrytis cinerea* applied to freshly cut slices of carrot root further induced the accumulation of falcarinol and, in parallel, increased the resistance of the tissue to living cells of the pathogen (Harding and Heale, 1980).

The fact that specific factors may enhance the levels of some preformed compounds, and lead to the prevention of fungal attack, has a physiological importance in fruit resistance. Furthermore, understanding the mechanisms behind these phenomena may enable us to modulate them to control the levels of the natural preformed inhibitory compounds, as a means of disease control.

D. PHYTOALEXINS – INDUCED INHIBITORY COMPOUNDS

Phytoalexins are low-molecular-weight toxic compounds produced in the host tissue in response to initial infection by microorganisms, or to an attempt at infection. In other words, in order to overcome an attack by the pathogen, the host is induced by the pathogen to produce antifungal compounds that would prevent pathogen development. However, the accumulation of phytoalexins does not depend on infection only. Such compounds may be elicited by fungal, bacterial or viral metabolites, by mechanical damage, by plant constituents released after injury, by a wide diversity of chemical compounds, or by low temperature, irradiation and other stress conditions. Phytoalexins are thus considered to be general stress-response compounds, produced after biotic or abiotic stress. The most available evidence on the role of phytoalexins shows that disruption of cell membranes is a central factor in their toxicity (Smith, 1996), and that the mechanism is consistent with the lipophilic properties of most phytoalexins (Arnoldi and Merlini, 1990).

Earlier studies by Muller and Borger (1940) already provided strong evidence that resistance of potato to *Phytophthora infestans* is based on the production of fungitoxic compounds by the host. A terpenoid compound, rishitin, produced in potato tubers following infection by *P. infestans*, was first isolated by Tomiyama et al. (1968) from resistant potatoes that had been inoculated with the fungus. It accumulates rapidly in the tuber and reaches levels much higher than those required to prevent fungal development. The relationship between the accumulation of rishitin in the tuber and its resistance to late blight may

point to its role in resistance development (Kuć, 1976). It was clarified that the rapidity and magnitude of phytoalexin accumulation were important in disease resistance, and that potato tubers could be protected against disease caused by a compatible race of *P. infestans* by inoculation with an incompatible race. Rishitin has also been found to be induced in potato tuber discs 24 h after inoculation with *Fusarium sambucinum*, which causes dry rot in stored potatoes (Ray and Hammerschmidt, 1998). Other sesquiterpenoids that have been found in potatoes may also play a role in tuber disease resistance; they include rishitinol (Katsui et al., 1972), lubimin (Katsui et al., 1974; Stoessl et al., 1974), oxylubimin (Katsui et al., 1974), solavetivone (Coxon et al., 1974), and others. The terpenoid phytuberin was found to be constitutively present in tuber tissues at low levels, but it was further induced after inoculation with *F. sambucinum*. The phytoalexins, phytuberol and lubimin appeared in potato discs by 48 h after inoculation, while solavetivone was produced in very low quantities. At least eight additional terpenoid compounds were induced in potato tubers in response to inoculation with pathogenic strains of *F. sambucinum,* and they appeared 48-70 h after inoculation. Successful infection was necessary for terpenoid accumulation. No inducement of terpenoid accumulation occurred following inoculation of tuber discs with non-pathogenic strains of *F. sambucinum* (Ray and Hammerschmidt, 1998).

Rishitin and solavetivone were recently isolated from potato tuber slices inoculated with *Erwinia carotovora.* These sesquiterpenoids suppressed mycelial growth of the potato pathogen *P. infestans* on a defined medium (Engström et al., 1999). A similar effect, however, was recorded for the naturally occurring plant sesquiterpenoids abscisic acid, cedrol and farnesol, although these compounds are found in healthy plant tissue and are not associated with post-infection responses. At concentrations of 50 or 100 $\mu g\ ml^{-1}$, rishitin and cedrol showed the strongest inhibitory effects on *P. infestans*, while solavetivone, abscisic acid and farnesol were somewhat less inhibitory. *P. infestans* has frequently been used for testing the activity of potato phytoalexins, the criteria for inhibition being the rates of spore germination, germ-tube elongation, mycelial growth and the inhibition of fungal glucanases (Ishizaka et al., 1969; Hohl et al., 1980). The effects of these sesquiterpenoids - phytoalexins as well as non-phytoalexins - were found to be much lower than the effect of the fungicide metalaxyl. In general, phytoalexins are not considered to be as potent as antibiotic compounds (Kuć 1995; Smith 1996).

Several phytoalexinic compounds, such as umbelliferone, scopoletin and esculetin, are produced in sweet potato roots infected by the fungus *Ceratocystis fimbriata*, and it was noted that these compounds accumulate more rapidly in roots resistant to this fungus than in sensitive roots (Minamikawa et al., 1963).

The resistance of celery petioles to pathogens has been attributed over the years to psoralens, linear furanocoumarins which are considered to be phytoalexins (Afek et al., 1995a, b; Beier and Oertli, 1983). These compounds, which have deleterious effects on the skin of humans, were previously thought to be mycotoxins produced by *Sclerotinia sclerotiorum* in celery (Ashwood-Smith et al., 1985). However, mechanical damage during harvesting and storage, and other elicitors, such as low temperatures and UV, have also been shown to induce furanocoumarin production (Beier and Oertli, 1983; Chaudhary et al., 1985). The psoralen content of celery increases during storage (Chaudhary et al., 1985), and the levels of various psoralens found in older celery stalks are higher than those in younger stalks (Aharoni et al., 1996). Furthermore, infection of celery with *S. sclerotiorum* or *Botrytis cinerea*, the main fungal pathogens of stored celery, was found to stimulate psoralen production during storage (Austad and Kalvi, 1983). Recent studies with celery (Afek et al., 1995b) indicate that (+) marmesin, the precursor of linear furanocoumarins in this crop, and not psoralens, is the major compound involved in celery resistance to pathogens; it has at least 100 times greater antifungal activity than psoralens. Also, increased susceptibility of stored celery to pathogens is accompanied by a decrease in (+) marmesin concentration and a corresponding increase in total psoralen concentration. However, treatment of celery prior to storage with gibberellic acid (GA_3), a naturally occurring phytohormone in juvenile plant tissue, resulted in decay suppression during 1 month of storage at 2°C (see the chapter on Means for Maintaining Host Resistance - Growth Regulators), although GA_3 does not have any effect on fungal growth *in vitro* (Barkai-Golan and Aharoni, 1980). It was suggested that the phytohormone retards celery decay during storage by slowing down the conversion of (+) marmesin to psoralens, thereby maintaining the high level of (+) marmesin and low levels of psoralens and, thus increasing celery resistance to storage pathogens (Afek et al., 1995b).

Another phytoalexin found in celery tissue is columbianetin, which probably also plays a more important role than psoralens in celery

resistance to decay (Afek et al., 1995c). This supposition is derived from the following facts: (a) columbianetin exhibits stronger activity than psoralens against *Alternaria alternata, B. cinerea* and *S. sclerotiorum*, the main postharvest pathogens of celery; (b) the concentration of psoralens in celery is much lower than that required to inhibit the growth of celery pathogens, while the concentration of columbianetin in the tissue is close to that required for their suppression; (c) increased sensitivity of celery to pathogens during storage occurred in parallel with the decrease in the concentration of columbianetin, and with the increase in that of psoralen (Fig. 12).

The phytoalexin capsidiol is a sesquiterpenoid compound produced by pepper fruits in response to infection with a range of fungi (Stoessl et al., 1972). Pepper fruits inoculated with *B. cinerea* and *Phytophthora capsici* contain only small quantities of capsidiol, whereas fruits inoculated with saprophytic species or with weak pathogens may produce higher concentrations of the phytoalexin, which inhibit spore germination and mycelial growth. Inoculating peppers with *Fusarium*

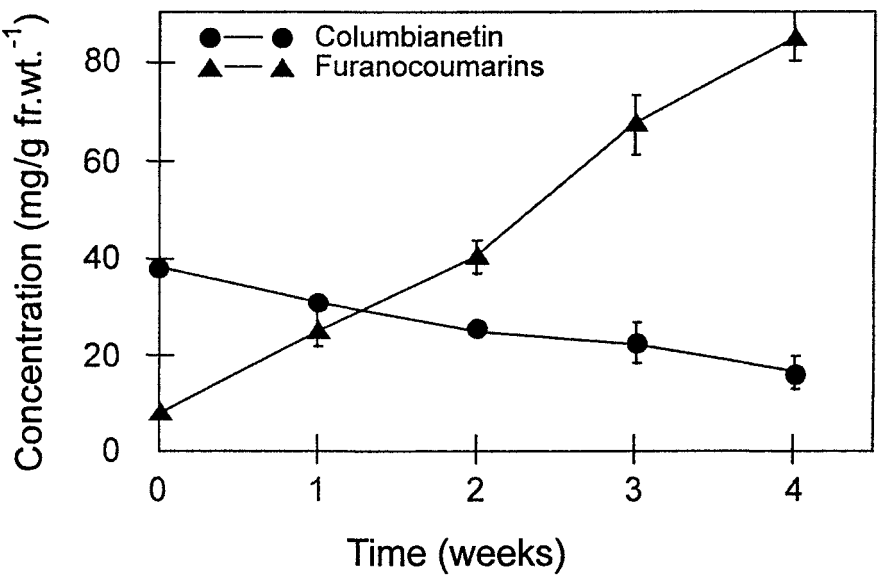


Fig. 12. Concentration of columbianetin and total furanocoumarins in celery during 4 weeks of storage at 2°C. Vertical bars indicate standard error. (Reproduced from Afek et al., 1995 with permission of Elsevier Science).

species results in increased capsidiol concentration 6 to 12 h after inoculation. In such cases, the capsidiol accumulation in the tissue is rapid, whereas in other cases it is rapidly oxidized to capsenone, which is characterized by a much weaker toxic effect than that of the capsidiol. When unripe pepper fruits were inoculated with *Glomerella cingulata*, the cause of anthracnose, a phytoalexin was readily identified in tissue extracts (Adikaram et al., 1982). This compound, possibly related to capsidiol but much less water soluble, has been named capsicannol (Swinburne, 1983).

The resistance of unripe banana to anthracnose incited by *Colletotrichum musae* has been attributed to the accumulation of five fungitoxic phytoalexin compounds that were not present in healthy tissue. As the fruit ripened these compounds diminished and, at a progressive stage of disease development, no phytoalexins were detected (Brown, A.E. and Swinburne, 1980). Elicitors composed of a glucan-like fraction of the cell walls of hyphae and conidia of *C. musae* elicited both necrosis and the accumulation of the two major phytoalexins found with the live inoculum.

Benzoic acid is a phytoalexin produced in apples as a result of infection by *Nectria galligena* and other pathogens. Fruit resistance to this pathogen at the beginning of a long storage period, was attributed to the formation of this phytoalexin (Swinburne, 1973). *Nectria* penetrates apples via wounds or lenticels prior to picking, but its development in the fruit is very limited. Benzoic acid is the compound isolated from the limited infected area; its production is correlated with the activity of protease produced by the pathogen (Swinburne, 1975).

The accumulation of this antifungal compound in the necrotic area inhibits the progress of *Nectria* within the fruit. Benzoic acid has proved to be toxic only as the undissociated molecule and it is expressed only at low pH values, such as can be found in unripe apples where the initial development of the fungus was indeed halted. With ripening and the decline in fruit tissue acidity, in conjunction with increasing sugar levels (Sitterly and Shay, 1960), the benzoic acid is decomposed by the pathogen, ultimately to CO_2, and the fungus can resume active growth (Swinburne, 1983). The elicitor of benzoic acid synthesis was found to be a protease produced by the pathogen (Swinburne, 1975). This protease is a non-specific elicitor and a number of proteases from several sources may elicit the same response. On the other hand, *Penicillium expansum,*

B. cinerea, Sclerotinia fructigena, and *Aspergillus niger,* which do not produce protease in the infected tissue and do not induce the accumulation of benzoic acid, can rot immature fruit (Swinburne, 1975).

Inoculating lemon fruits with *Penicillium digitatum,* the pathogen specific to citrus fruits, results in the accumulation of the phytoalexin scoparone (6,7-dimethyloxycoumarin). The induced compound has a greater toxic effect than that of the preformed antifungal compounds naturally found in the fruit tissue, such as citral and limetin, as indicated by the inhibition of *P. digitatum* spore germination (Ben-Yehoshua et al., 1992). A considerable increase in the scoparone level was found in lemons which had been preheated at 36°C for 3 days, and then inoculated with *P. digitatum* spores. The increased concentration of scoparone in the fruit was in good correlation with the enhanced antifungal activity of the fruit extract. This finding led to the supposition that scoparone plays an important role in the increased infection resistance of heated fruit. It is worth mentioning that various citrus fruits (lemon, orange, grapefruit, etc.) differ from one another in their ability to produce scoparone in response to fungal infection (Ben-Yehoshua et al., 1992).

Scoparone production can also be induced in the peel of various citrus fruits by ultraviolet (UV) illumination (Rodov et al., 1992). In UV-irradiated (254 nm) kumquat fruits the accumulation of scoparone reached its peak (530 $\mu g\ g^{-1}$ dry weight of the flavedo tissue) 11 days after illumination. However, its level then declined rapidly, returning to the minimal trace levels characteristic of the untreated fruit, 1 month after treatment (Fig. 13). Increasing the radiation dose and raising the storage temperature (from 2 to 17°C) enhanced scoparone production.

A correlation has been drawn between the level of phytoalexin accumulated in the flavedo of irradiated fruits and the increased antifungal activity of the flavedo. It has also been found that irradiating kumquat fruits prior to their inoculation with *P. digitatum* resulted in decreased incidence of green mold decay, whereas irradiating fruits which had previously been inoculated with *P. digitatum* failed to prevent decay development (Fig. 14). The finding that decay reduction was achieved when irradiation was applied to the fruit prior to its inoculation, and therefore without any direct exposure of the pathogen to the radiation, led to the suggestion that disease inhibition stems from increased resistance of the fruit to infection and not from the direct fungicidal effect of UV on the pathogen (Rodov et al., 1992).

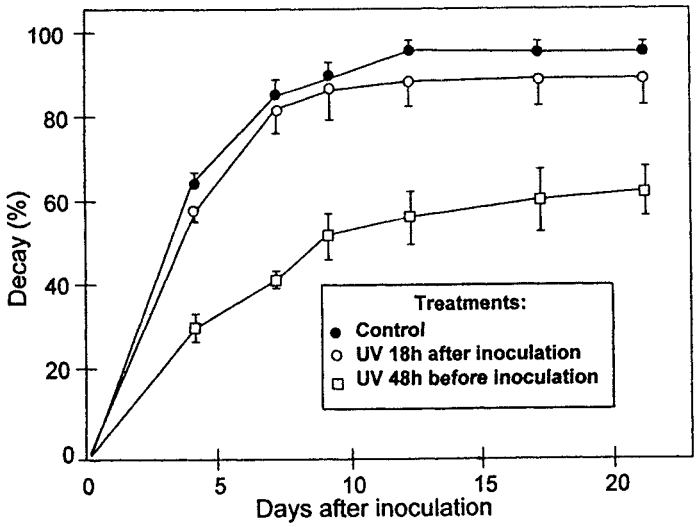


Fig. 13. Effect of ultraviolet (UV) treatment on decay development in kumquat fruit inoculated with dry spores of *Penicillium digitatum*. Control: non-illuminated fruit. UV dose: $1.5x10^3 J\ m^{-2}$. Bars indicate standard deviations. (Reproduced from Rodov et al., 1992 with permission of the American Society for Horticultural Science).

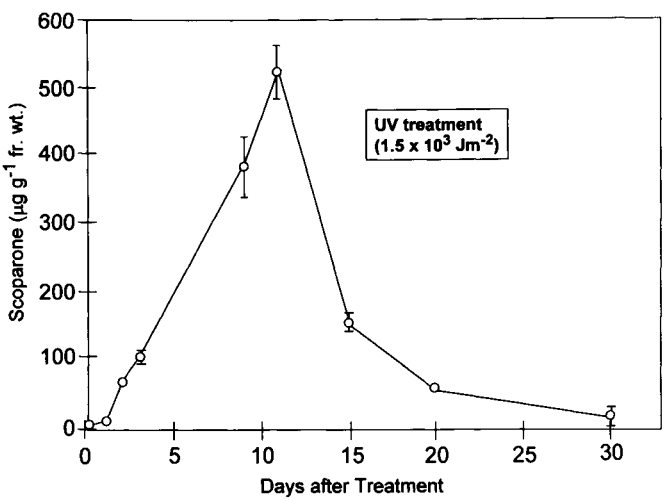


Fig. 14. Time course of scoparone accumulation and depletion in UV-treated kumquat fruit. Bars indicate standard deviations. (Reproduced from Rodov et al., 1992 with permission of the American Society for Horticultural Science).

Several studies with citrus fruits have also described gamma irradiation as a stress factor leading to the induction of antifungal phytoalexinic compounds in the treated fruit tissues. (See the chapter on Physical Means – Ionizing Radiation).

Biosynthesis of toxic compounds as a result of wounding or other stress conditions, is a ubiquitous phenomenon in various plant tissues. An example of such a synthesis is the production of the toxic compound 6-methoxymellein in carrot roots in response to wounding or to ethylene application (Chalutz et al., 1969; Coxon et al., 1973); the application of *B. cinerea* conidia and other fungal spores to the wounded area was found to stimulate the formation of this compound (Coxon et al., 1973). A similar result is also achieved by the application of fungal-produced pectinase, in spite of the fact that this enzyme does not affect cell vitality (Kurosaki et al., 1986b). This toxic compound probably has an important role in the resistance of fresh carrots to infection. Carrots that have been stored for a long period at a low temperature lose the ability to produce this compound and, in parallel, their susceptibility to pathogens increases. Enhanced resistance of carrots can also be induced by application of dead spores; carrot discs treated with *B. cinerea* spores which had previously been killed by heating developed a marked resistance to living spores of the fungus, which was much greater than that of the control discs. The increased resistance of the tissue to active fungal spores, as evaluated by the inhibition of germ-tube elongation on the treated carrot, is shown in Fig. 15. The most effective inhibitors found in the tissues after the induction of resistance, as well as in the control tissues, were methoxymellein, p-hydroxybenzoic acid, and polyacetylene falcarinol (Harding and Heale, 1980).

In spite of the demonstration that many plant organs produce low-molecular-weight antibiotic compounds in response to infection, there remain substantial questions as to the role of phytoalexins in disease interactions. The lack of clear answers to questions such as whether phytoalexin accumulates in suitable concentrations at the right place and the right time, has led to the conclusion that phytoalexin synthesis is but one of a vast array of phenomena that, taken together, contribute to the expression of resistance (Nicholson and Hammerschmidt, 1992).

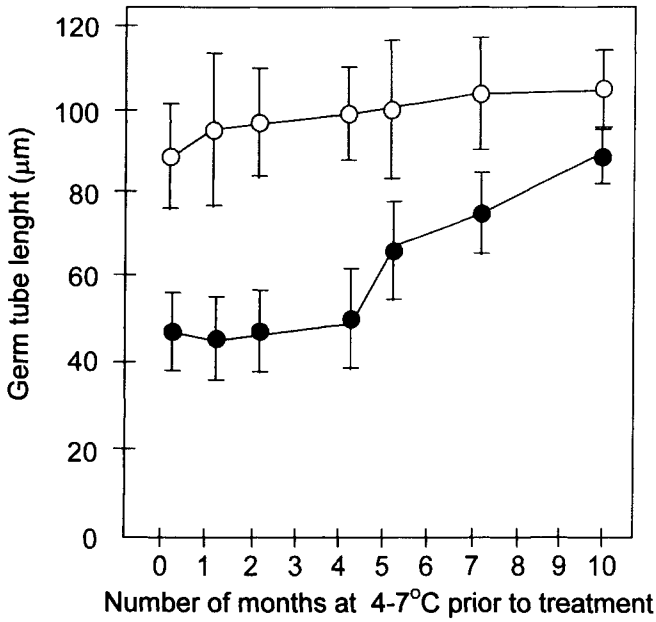


Fig. 15. Assessment of resistance induction in carrot root slices 16 h after treatment with heat-killed conidia of *Botrytis cinerea*. The criterion: germ-tube elongation on the surface of treated (●) and untreated (○) carrot slices. Vertical bars indicate standard error. (Reproduced from Harding and Heale, 1980 with permission of Academic Press).

E. WOUND HEALING AND HOST BARRIERS

Pathogen penetration through a wound, cut, scratch or other injury to the fruit or other plant organs, is a major way for initiating postharvest disease. However, a fresh fruit, which escaped infection because of a lack of appropriate spores or the absence of conditions suitable for germination, may, in many cases, become more resistant to later infection. In some instances, the reduced sensitivity of a wound to infection may be related to its natural drying. In other cases, some biochemical changes which take place at the wound area may lead to its protection.

Mechanically injured fruit is characterized by enhanced respiration and ethylene evolution ("wound ethylene"). The injured cells lose their vitality, whereas living cells around the wound are exposed to stress

conditions and their metabolic activity is enhanced. These cells may repair the damage sustained by the injured tissue. In response to injury, the host tissue is capable of forming protective "barriers" composed of tightly packed cells, but such barrier formation takes place only when the injured tissue is still capable of cell division, or the cells surrounding the wound can produce and deposit lignin and suberin in their walls (Eckert, 1978). These compounds protect the host from pathogen penetration or from the action of cell-wall degrading enzymes secreted by the pathogen. As a result of wounding, the production of antimicrobial polyphenolic compounds can also contribute to wound protection. Phytoalexins are other toxic compounds that can be formed at the wound area following inducement by initial infection.

Ray and Hammerschmidt (1998) found that inoculation of potato tubers with *Fusarium sambucinum*, the fungal pathogen of potato dry rot, resulted in an increase in phenolic acids suggesting that phenolic acid biosynthesis was induced. Following such inducement, free phenolic acids are removed as they are converted into lignin or are cross-linked to cell walls. Lignin production is mediated by peroxidase, which is strongly induced in the infected tuber in a number of isoforms. While a lignin barrier may inhibit the advance of the fungus, microscopy has indicated that lignin barriers are repeatedly breached or circumvented as dry rot progresses. This may suggest that lignin is not in itself an effective defense against *F. sambucinum* or, alternatively, that it cannot be induced quickly enough to defend the tissue (Ray and Hammerschmidt, 1998). Furthermore, the phenolic acid and total peroxidase contents are not higher in tubers of the resistant line than in more susceptible genotypes, suggesting that neither is critical to tuber infection resistance.

Suberization of cells at the wound area, a process that heals the wound with a layer of phenolic and aliphatic compounds (Mohan and Kolattukudy, 1990), is an important anti-infection defense mechanism of the wounded tuber. When superficially wounded tubers are stored at high relative humidity and moderate temperature, suberin is formed in the walls and intercellular spaces of the living cells surrounding the injury. Under optimal environmental conditions this takes place within 24 h after wounding (Fox et al., 1971). The suberized periderm which forms after several days is composed of tightly packed cells, the walls of which are impregnated with suberin and contain only a small amount of pectin (Fox et al., 1971). On the other hand, storing tubers at too low

temperatures, which do not promote suberin formation by the cells surrounding the wound, facilitates the development of gangrene caused by *Phoma* infection in stored potatoes. This is because temperatures which prevent the formation of suberinic barriers by the host are still suitable for the development of the fungus (Boyd, 1972).

Suberin also serves as a defensive layer on the lenticels of potato tubers. Lenticels of potatoes are normally covered with a suberin layer that prevents the entry of soft rot bacteria into the cortex of the tuber. Removal of this layer renders the lenticels susceptible to bacterial invasion (Fox et al., 1971; Perombelon and Lowe, 1975).

Kolattukudy (1987) described suberin as a complex biopolyester comprising a phenolic (aromatic or lignin-like) domain attached to the cell wall, and an aliphatic (lipid, hydrophobic) domain, which is probably attached to the phenolic domain. Soluble waxes are imbedded within the suberin matrix and provide resistance to water vapor loss but have not been indicated to play a role in disease resistance (Lulai and Corsini, 1998). However, rapid suberization of wounded tubers is critical in avoiding both bacterial soft rot (*Erwinia carotovora* subsp. *carotovora*) and fungal dry rot (*F. sambucinum*). Lulai and Corsini (1998) emphasized the differential development of potato tuber resistance to bacteria and then to fungal penetration during suberization. They related this phenomenon to the differential deposition of the two major suberin components (the phenolic and the aliphatic domains) during wound healing (18°C at 98% RH). It was found that the initiation of suberin phenolic deposition at the wound site preceded that of suberin aliphatic deposition. Total resistance to *Erwinia* infection occurred after completion of the phenolic deposition on the outer wall of the first layer of cells (2-3 days). However, the phenolic suberin deposition offered no resistance to *Fusarium* infection. Resistance to fungal infection began to develop only after deposition of the suberin aliphatic domain was initiated. Total resistance to fungal infection was attained after completion of deposition of the suberin aliphatic domain within the first layer of suberized cells (5-7 days). This suberin domain was not required for tuber resistance to bacterial soft rot infection.

Curing sweet potatoes for several days at high relative humidity (85-90%) and high temperatures (26-32°C) facilitates the formation of suberized periderm, which protects harvest injuries against attack by *Rhizopus stolonifer* (Clark, 1992). In fact, curing is a standard prestorage treatment to prevent decay in various varieties of sweet potatoes. Similarly, holding carrots at high relative humidity and 22-26°C for 2

days prior to removal to cold storage (5°C) results in the suberization of cells at the wound area, and the formation of a barrier against *Botrytis cinerea* and other pathogens. Such a treatment considerably reduces the incidence of decay at the end of prolonged storage (Heale and Sharman, 1977).

Studies with cultured carrot cells indicated that phenolic compounds with low molecular weight, which are a link in lignin biosynthesis, and free radicals produced during its polymerization, may take part in resistance inducement by damaging fungal cell membranes, fungal enzymes or toxins (Kurosaki et al., 1986a; Vance et al., 1980). Accumulation of phenolic compounds and callose deposition in cell walls of young tomato fruits, following inoculation with *B. cinerea*, were found to arrest fungal development, thus retarding or preventing decay (Glazener, 1982). The mechanism by which phenolic compounds accumulate in the host is not yet clear, but research carried out with wheat leaves suggested that the chitin in the fungal cell walls acts as a stimulator to lignification in the leaves (Pearce and Ride, 1982).

Curing, involving the use of warmth and high humidity to harden wounds and promote the development of resistance to infection, was long ago recommended for control of *Penicillium* decay in sweet oranges (Tindale and Fish, 1931). Since then, the efficiency of curing in protecting against infection by various wound pathogens has been described for several citrus fruits. It was thus found that holding wounded citrus fruits for several days at 30-36°C and high relative humidity (90-96%) markedly suppressed decay development during storage (Baudoin and Eckert, 1985; Brown, G.E., 1973; Hopkins and Loucks, 1948). The resistance of the fruits developed under these conditions has been attributed to the deposit of lignin or lignin-like material by the living cells of the flavedo (the external, colored layer of the peel) adjacent to the injury, leading to the formation of a barrier which prevents fungal penetration and restricts its growth (Brown, G.E. and Barmore, 1983). In addition, lignification of cell walls at the site of infection protects them against the activity of cell-wall-degrading pectolytic enzymes produced by the fungus, and prevents degradation of the middle lamella of cell walls (Ismail and Brown, 1979; Vance et al., 1980).

The combination of moderate temperatures with high relative humidity was found to be suitable for rapid production of lignin at the wounded area, while such a temperature was too high for *Penicillium digitatum* development (Brown, G.E., 1973), but lowering the temperature to 27°C allowed rapid fungal development, which preceded

lignin formation. Thus, holding the fruit at this temperature facilitated decay development. On the other hand, lowering the relative humidity below 75% dried up the cells around the wound, and such injured cells were incapable of lignin production (Brown, G.E., 1973). In an earlier study, the infection resistance of unripe papaya fruits was similarly attributed to the ability of the tissue to produce lignin, which prevented fungal progress within the tissue (Stanghellini and Aragaki, 1966).

Decay reduction has also been achieved through the curing and lignification of superficial wounds in the peel of citrus fruits, by using plastic film to seal-package individual fruits for 1-3 days at high relative humidity and moderate or high temperatures (32-36°C) prior to storage (Brown, G.E. and Barmore, 1983; Eckert et al., 1984; Golomb et al., 1984). Healing of wounds under these conditions was attributed to the water-saturated atmosphere formed within the plastic wrap. The water-saturated atmosphere, when applied together with high temperatures, in addition to retarding tissue aging and preserving the integrity of all membranes, may also stimulate the biosynthesis of lignin by the living cells surrounding the wound. Under these conditions, lignification results in a considerable reduction in decay incidence, without affecting the taste of the fruit (Ben-Yehoshua et al., 1987).

Wrapping various citrus fruits (lemon, orange, grapefruit and pomelo) in sealed plastic film protects them from damage caused by the high temperature; the optimal temperature and duration of curing can be determined for each fruit/fungus system (Ben-Yehoshua et al., 1988). (Figs. 16, 17).

In more recent studies Stange and Eckert (1994) indicated that improved decay control in lemons could be achieved by dipping the fruits in a surfactant solution, prior to curing at 32°C in a water-saturated atmosphere. The enhanced decay control was probably due to the increased mortality of *P. digitatum* conidia and germ tubes. In other studies with citrus fruits, however, Stange et al. (1993) came to the conclusion that the evidence for the involvement of lignin in wound protection in citrus fruits was based on histochemical tests which were not entirely specific for lignin; they suggested that the resistance of the wound to infection had to be attributed to the deposition of induced antifungal gum materials, rather than lignin, in the resistant wound. In support of this hypothesis, Stange et al. (1993) presented the finding that extracts from hardened, resistant wounds contained several induced, low-molecular-weight aldehydes, some of which had marked antifungal activity. The resistance of the wound to infection may be associated

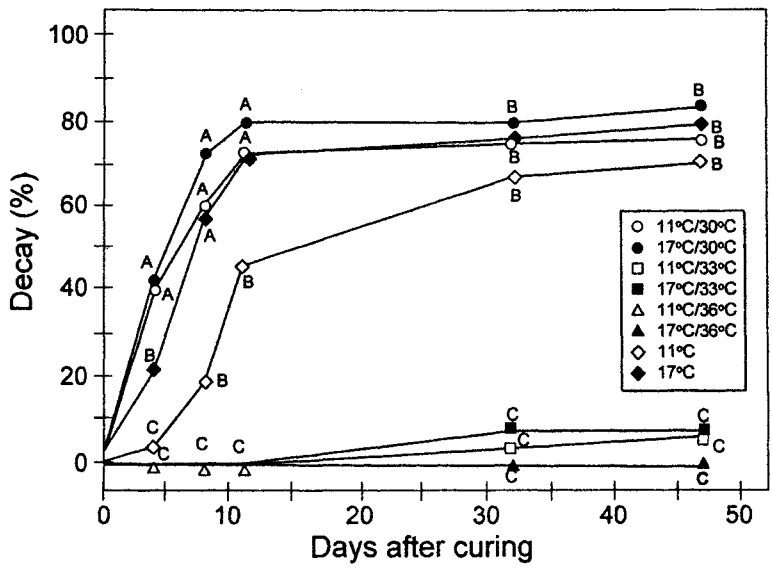


Fig. 16. Effect of 72 h curing at various temperatures on percent decay of inoculated pummelo held at 11° and 17°C. (Reproduced from Ben-Yehoshua et al, 1988).

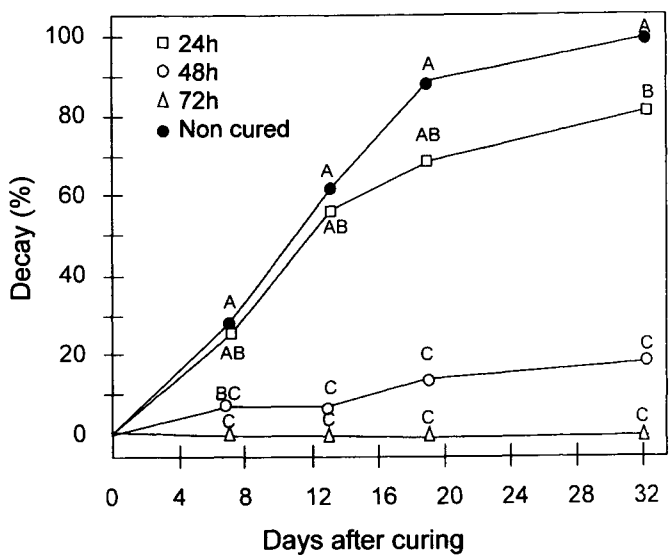


Fig. 17. Effect of duration of the curing period at 36°C on percent decay of inoculated pummelo at 17°C. (Reproduced from Ben-Yehoshua et al. 1988).

directly with the inducement of these compounds at the wound site. It was further found that dipping the fruits in the non-toxic compound, potassium phosphonate, increased the resistance of the wound to *P. digitatum* infection (Wild, 1993). This was only noticeable when at least 24 h was allowed between dipping the freshly wounded fruits and inoculating them with *Penicillium* spores. Since this compound has no direct effect on *Penicillium* growth *in vitro*, it was concluded that the potassium phosphonate was eliciting enhanced production of these antifungal compounds in the host, and this conclusion was further supported by the finding that cycloheximide, which is a protein synthesis inhibitor, negated the effect of the phosphonate (Wild, 1993).

Studies with young apricot fruits indicated that the mechanical barrier formed in response to fungal penetration may also prevent reactivation of a quiescent infection. The cell-wall suberization of living cells surrounding the infection point of *Monilinia fructicola* in the young fruit and the accompanying accumulation of phenolic compounds, have been hypothesized to be the reason for the non-activation of the quiescent fungus growth (Wade and Cruickshank, 1992). When the fruit ripens, however, viable hyphae of *M. fructicola* may escape from the arrested lesions and cause fruit decay. Curing effects and decreased incidence of *Botrytis cinerea* can be obtained in kiwifruit by prolonging the time between harvesting and cool storage (Pennycook and Manning, 1992). In determining the critical conditions required during curing of kiwifruit in order to minimize Botrytis rot during storage, Bautista-Baños et al. (1997) concluded that temperatures between 10 and 20°C, together with relative humidity (RH) higher than 92% for a three-day curing period, should provide adequate disease control without lowering fruit quality during cold storage (0°C); increased temperature or decreased RH generally resulted in increased fruit weight loss.

F. ACTIVE OXYGEN

Active oxygen, produced by plant cells during interactions with potential pathogens and in response to elicitors, has recently been suggested to be involved in pathogenesis. "Active oxygen species", including superoxide, hydrogen peroxide and hydroxyl radical, can affect many cellular processes involved in plant-pathogen interactions (Baker and Orlandi, 1995). The direct antimicrobial effect of active oxygen

species has not yet been clarified, but they are considered to play a role in various defense mechanisms, including lignin production, lipid peroxidation, phytoalexin production and hypersensitive responses. However, active oxygen can be difficult to monitor in plant cells because many of the "active oxygen species" are short lived and are subject to cellular antioxidant mechanisms such as superoxide dismutases, peroxidases, catalase and other factors (Baker and Orlandi, 1995).

A first report on the production of active oxygen in potato tubers undergoing a hypersensitive response was given by Doke (1983), who demonstrated that O_2 production occurred in potato tissues upon inoculation with an incompatible race of *Phytophthora infestans* (i.e., a race causing a hypersensitive response), but not after inoculations with a compatible race (i.e., a disease-causing race). Following this early report the connection between active oxygen species and phytoalexin synthesis has been found in several host-pathogen interactions (Devlin and Gustine, 1992).

In a recent study Beno-Moualem and Prusky (2000) found that the level of reactive oxygen species in freshly harvested unripe avocado fruit, which is resistant to infection, was higher than that in the susceptible ripe fruit. Moreover, inoculation of resistant fruits with *Colletotrichum gloeosporioides* further increased their reactive oxygen production, whereas inoculation of susceptible fruits had no such effect. It was also indicated that reactive oxygen production could be induced by avocado cell cultures treated with cell-wall elicitor of *C. gloeosporioides*.

When isolated avocado pericarp tissue was treated with H_2O_2 (1 mM) the reactive oxygen production was enhanced and, in parallel, a rapid increase in the levels of epicatechin was detected. Epicatechin appears to play a key role in fruit susceptibility during ripening, by indirectly controlling the levels of the antifungal compounds naturally present in the peel of unripe fruits (Prusky et al., 1982); phenylalanine ammonia lyase (PAL), one of the enzymes involved in epicatechin synthesis, also increased, in parallel with the enhanced production of reactive oxygen species. On the other hand, pre-incubation of avocado tissue in the presence of protein kinase inhibitors inhibited the release of H_2O_2 from avocado cell cultures and PAL activity was no longer induced. Beno-Moualem and Prusky (2000) suggested that the inducement of reactive oxygen species by fungal infection of unripe fruits may modulate fruit resistance and lead to the inhibition of fungal development and the continuation of the quiescent stage of the pathogen.

G. PATHOGENESIS-RELATED PROTEINS

Several **glucanohydrolases** found in plants, such as chitinase and β-1,3-glucanase, have received considerable attention as they are considered to play a major role in constitutive and inducible resistance against pathogens (El Ghaouth, 1994). These enzymes are low-molecular-weight proteins, frequently referred to as pathogenesis-related (PR) proteins. They hydrolyze the major components of fungal cell walls which results in the inhibition of fungal growth (Schlumbaum et al., 1986).

The chitinases, which are ubiquitous enzymes of bacteria, fungi, plants and animals, hydrolyze the β-1,4-linkage between the N-acetylglucosamine residues of chitin, a polysaccharide of the cell wall of many fungi (Neuhaus, 1999). The glucanases, which are abundant, highly regulated enzymes, widely distributed in seed-plant species, are able to catalyze endo-type hydrolytic cleavage of glucosidic linkages in β-1,3-glucans (Leubner-Metzger and Meins, 1999).

The chitinases and β-1,3-glucanases are stimulated by infection and in response to elicitors (Bowles, 1990). Chitosan, a β-1,4-glucosamine polymer found as a natural constituent in cell walls of many fungi, is capable of both directly interfering with fungal growth and eliciting defense mechanisms in the plant tissue (El Ghaouth, 1994). Postharvest treatment with this elicitor has been found to activate antifungal hydrolases in several fruits: treatment of strawberries, bell peppers and tomato fruits with chitosan induced the production of hydrolases, which remained elevated for up to 14 days after treatment and reduced lesion development by *Botrytis cinerea* (El Ghaouth and Arul, 1992; El Ghaouth et al., 1997). When applied as a stem scar treatment to bell peppers, chitosan stimulated the activities of chitinase, chitosanase and β-1,3-glucanase. Being capable of degrading fungal cell walls, these antifungal hydrolases are considered to play a major role in disease resistance (Schlumbaum et al., 1986; El Ghaouth, 1994).

In chitosan-treated bell peppers the production of lytic enzymes was followed by a substantial reduction of chitin content of the cell walls of invading fungal hyphae, expressed as a reduction of chitin labeling in the walls (El Ghaouth and Arul, 1992). The hypothesis of fungal infection suppression was also supported by the finding that invading fungal hyphae were mainly restricted to the epidermal cells ruptured during wounding (El Ghaouth et al., 1994). It was thus suggested that the deliberate stimulation and activation of PR proteins in the fruit tissue might lead to disease suppression by enhancing host resistance to infection.

Peroxidases are another group of PR proteins whose activity has been correlated with plant resistance against pathogens. Plant peroxidases, which are glycoproteins that catalyze the oxidation by peroxide of many organic and inorganic substrates, have been implicated in a wide range of physiological processes, such as ethylene biosynthesis, auxin metabolism, respiration, lignin formation, suberization, growth and senescence. Findings of correlations between deposition of cell wall strengthening materials, such as lignin, suberin and extensin, and peroxidase activities are consistent with a role for this enzyme in defense through wall-strengthening processes (Chitoor et al., 1999). The importance of peroxidase lies in the fact that the host cell wall constitutes one of the first lines of defense against pathogens, and peroxidase is a key enzyme in the wall-building processes. Such processes include the accumulation of lignin and phenolic compounds, which has been correlated with enhanced resistance in various host-pathogen interactions, and suberization, which leads to enhanced resistance by healing wounds with a layer of phenolic and aliphatic compounds (Mohan and Kolattukudy, 1990). However, the resistance against pathogens may also be related to the highly reactive oxygen species such as H_2O_2 or oxygenase, which are likely to be toxic to pathogens and which are formed by peroxidase activity during the deposition of cell wall compounds (Goodman and Novacky, 1994).

In a recent study Ray and Hammerschmidt (1998) found that the activity of peroxidase in potato tubers increased greatly following infection with *Fusarium sambucinum*, the cause of potato dry rot. Such an infection induces several isoforms of peroxidase whose role has not been identified. Lignin levels also increased but the lignified zones could be breached, allowing the infection to develop further into the tuber. The increased peroxidase levels in infected tubers were expected to increase their potential to synthesize lignin and so enhance their resistance to infection. However, infection of tubers of transgenic potatoes following introduction of a foreign peroxidase gene from cucumbers, which was associated with an induced resistance response, had no effect on disease caused by several potato pathogens (Ray et al., 1998). These results indicated that the incorporation of a putative defense-associated peroxidase and the formation of transgenic potato plants does not necessarily result in the enhancement of resistance. It has been suggested, however, that understanding the function of peroxidase and determining the availability of the substrates needed for the enzyme activity are important for using transgenic studies to evaluate the role of peroxidase in defense responses (Ray et al., 1998, Chitoor et al., 1999).

CHAPTER 7

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES FOLLOWING INFECTION

Postharvest infection of fruits and other plant organs may induce a number of alterations in their physiological and biochemical processes or in the host tissue constituents, as a result of host-pathogen interactions. Process changes may include acceleration of ethylene evolution, stimulation of the respiratory enzymes, enhanced pectolytic activity, altered protein synthesis or polyamine-synthesis enzyme activity, etc.; and tissue changes may include increased cell-wall soluble pectin, and changed organic acid and sugar contents. The infection, or sometimes an attempted infection, may also induce the accumulation of low-molecular-weight defense chemicals – the phytoalexins – or enhance the production of preformed antimicrobial compounds in the host. The latter changes are dealt with in the chapter on Host Protection and Defense Mechanisms.

A. CHANGES IN FRUIT RESPIRATION AND ETHYLENE EVOLUTION

Studies carried out over the last few decades have found enhanced respiratory activity and increased ethylene production following injury or exposure to other stresses, such as low temperature, gamma irradiation, etc. Invasion by pathogens, including fungi, bacteria and viruses, seems to be no different in this respect from other plant stresses.

Both respiration and ethylene evolution are important indicators of the physiological state of the fruit, and their enhancement following infection highlights its effects on the postharvest ripening and senescence processes. Stimulation of respiration and ethylene evolution processes in citrus fruits infected by *Penicillium digitatum*, was first recorded at the beginning of the 1940s (Biale, 1940; Biale and Shepherd, 1941; Miller et al., 1940), and subsequent studies of various citrus fruits inoculated with divers postharvest fungi confirmed that this was a general post-infection phenomenon (Schiffmann-Nadel, 1974). Similarly to the fungi, pathogenic bacteria such as *Pseudomonas syringae* can also enhance respiratory

activity and ethylene evolution in citrus fruits (Cohen et al., 1978). When we compare the effects of different fungi on a certain fruit, e.g., lemon (Schiffmann-Nadel, 1974), we see that the greatest enhancements, both of ethylene evolution and respiration, are caused by *P. digitatum,* followed by *P. italicum, Diplodia natalensis* and *Geotrichum candidum.* Much less enhancement is caused by the fungi *Fusarium moniliforme* and *Alternaria citri* (Figs. 18, 19). In general, a correlation was found between respiration and ethylene levels, on the one hand, and the rate of fungal development on the fruit, on the other hand. Thus, fungi with short incubation periods and rapid growth cause greater increases in the levels of ethylene and CO_2 evolution than those characterized by slow development.

Acceleration of the respiratory activity, ethylene evolution, or both, has also been found in infected climacteric fruits, e.g., peaches infected by *Monilinia fructicola* (Hall, R., 1967); tomatoes infected by Rhizopus *stolonifer, Botrytis cinerea* and *G. candidum* (Barkai-Golan and Kopeliovitch, 1983; Barkai-Golan et al., 1989b); and bananas, mangoes

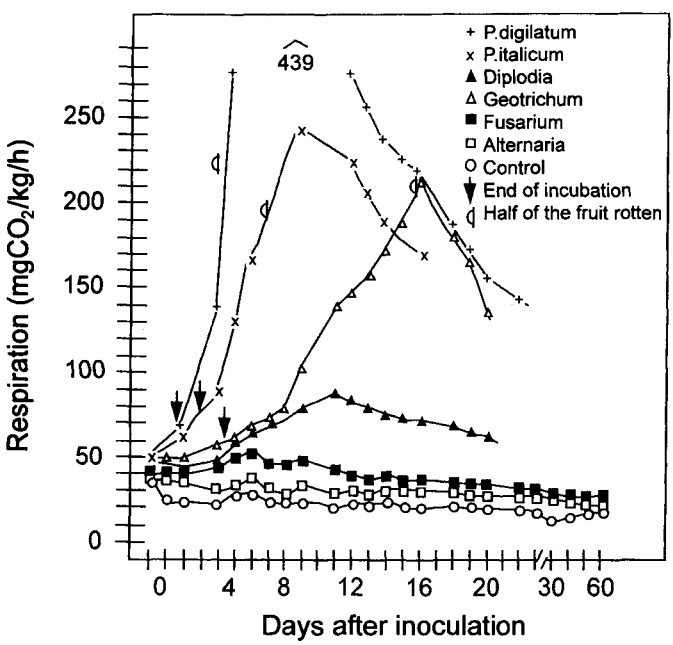


Fig. 18. Respiration rate of green lemon fruit inoculated with postharvest fungi. (Reproduced from Schiffmann-Nadel, 1974).

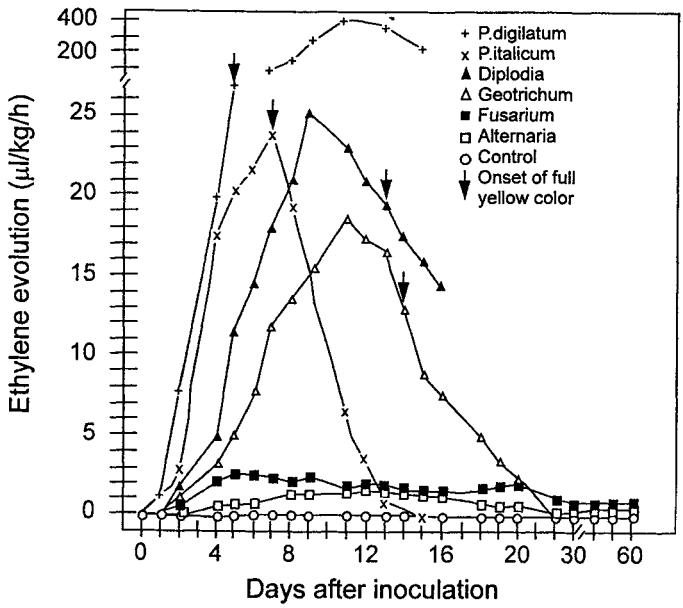


Fig. 19. Ethylene evolution from green lemon fruit inoculated with postharvest fungi. (Reproduced from Schiffmann-Nadel, 1974).

and apples infected by *Penicillium expansum* and species of *Alternaria, Trichothecium, Colletotrichum, Diplodia* and *Pestalotia* (Schiffmann-Nadel et al., 1985).

Similarly to non-climacteric fruits, correlations between the rates of fungal growth on the fruit and the enhancement of physiological processes was again recorded. Inoculating avocado fruits with *Fusarium solani* accelerated fruit respiration and ethylene evolution without enhancing the peak levels of these two physiological processes (Zauberman and Schiffmann-Nadel, 1974); i.e., in spite of the acceleration of these processes, no change in their general patterns had occurred (Figs. 20A, 20B). Inoculating the fruits with other *Fusarium* spp., characterized by slow growth rates, only slightly accelerated these two processes.

Inoculating tomato fruits at their pre-climacteric stage (mature-green fruit) with *B. cinerea,* typically stimulated ethylene evolution, while inoculations at the post-climacteric stage (mature fruit) elicited new ethylene peaks (Barkai-Golan et al., 1989b) (Fig. 21).

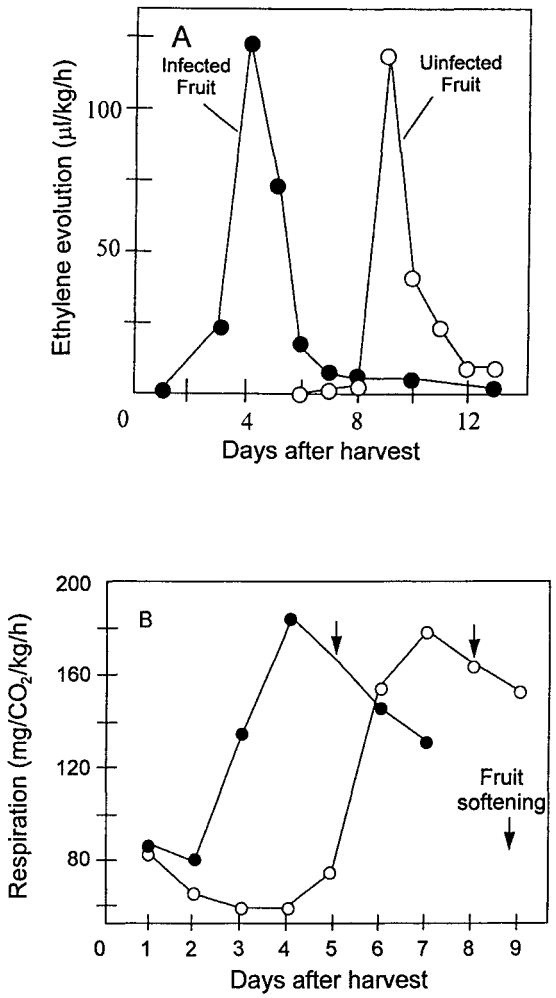


Fig. 20. Ethylene evolution (A) and respiration rates (B) of avocado fruit infected by *Fusarium solani* in comparison to uninfected fruit. (Reproduced from Zauberman and Schiffmann-Nadel, 1974 with permission of the American Phytopathological Society).

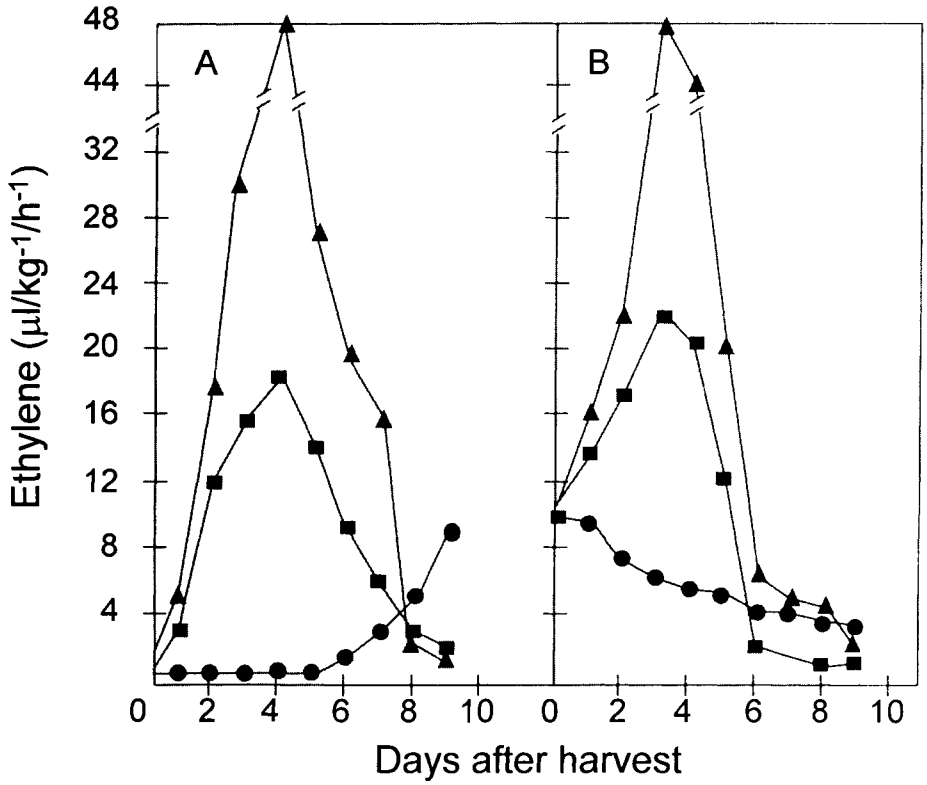


Fig. 21. Ethylene evolution from normal 'Faculta 38' tomato fruit, at its nature-green (A) and mature (B) stages of maturity, following inoculation with *Botrytis cinerea* (▲), or following treatment prior to inoculation (■) as compared to uninfected fruit (●). (Reproduced from Barkai-Golan et al., 1989 with permission of Blackwell Wissenschafts-Verlag GmbH).

Ethylene evolution was stimulated, not only in the normal ripening tomato fruit, but also in the non-ripening *nor* mutant fruit, which did not show any rise in the ethylene level when uninfected (Fig. 22). Thus, fungal infection of the non-ripening mutant tomatoes induced thylene synthesis by tissues which normally lack an active ripening system and do not even respond to exogenic ethylene treatment (Tigchelaar et al., 1978). Earlier and higher ethylene peaks were detected in fruits infected with *B. cinerea* than in those infected with *G. candidum*, corresponding to the faster growth of the former (Barkai-Golan et al., 1989b).

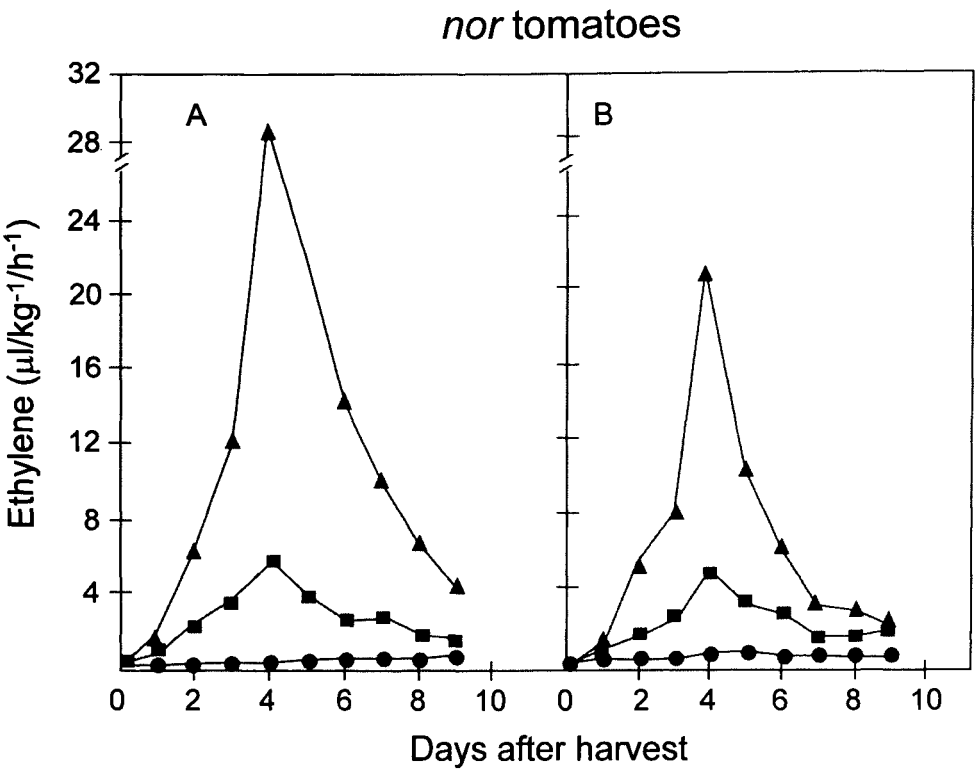


Fig. 22. Ethylene evolution from *nor* mutant tomato fruit, at its mature-green (A) and mature (B) stages of maturity, following inoculation with *Botrytis cinerea* (▲), or following treatment prior to inoculation (■) as compared to uninfected fruit (●). (Reproduced from Barkai-Golan et al., 1989 with permission of Blackwell Wissenschafts-Verlag GmbH).

Ethylene synthesis is also stimulated by mechanical wounding, such as occurs while introducing the inoculum into the host. However, comparison between rates of ethylene production elicited by wounds of certain dimensions and those elicited by fungal lesions of similar size, indicates that ethylene production in fungus-infected fruits is considerably greater than that in wounded ones. These results may suggest that, in addition to the mechanical stress produced during pathogenesis, which is responsible for the 'wound-ethylene', another factor is involved in the synthesis of 'infection-ethylene'. This 'biological factor' seems to differ for different fungi (Barkai-Golan et al., 1989b).

B. ETHYLENE SOURCE IN INFECTED TISSUE

The marked increase in ethylene evolution during infection stimulated the questions: What is the source of ethylene in the infected fruit? Is ethylene produced by the attacking pathogen or by the attacked host?

It is known that various fungi do produce ethylene *in vitro* as a metabolic product during their development (Ilag and Curtis, 1968). However, with the exception of a few species, such as *Aspergillus clavatus, A. flavus, Penicillium digitatum* and *P. corylophilum*, which produce considerable levels of ethylene, the production of ethylene by most species is low, reaching 0.5–1.0 ppm/g dry weight. Determination of ethylene production *in vitro* may be complicated due to the fact that different strains or even different isolates, of the same species, may differ in their capacity to produce ethylene. Moreover, when no ethylene is detected for a given fungus in culture, it is still possible that the pathogen can synthesize it in the fruit, because of the special substrate supplied by the living tissue. However, the healthy tissue is also capable of producing and emitting ethylene but, in contrast to climacteric fruits, in which ethylene evolution may reach high peaks during ripening, most plant tissues produce very low levels of this gas when healthy. The increased ethylene produced by the diseased fruit may, therefore, be from the damaged host tissue, from the activity of the pathogen within the host, or both.

Examination of the source of ethylene in apples infected by the brown rot fungus, *Sclerotinia fructigena* (Hislop et al., 1973), and in tomatoes infected by *Rhizopus stolonifer* or *Botrytis cinerea* (Barkai-Golan and Kopeliovitch, 1983; Barkai-Golan et al., 1989b) showed that the rotted tissues themselves, which contained an abundance of actively growing mycelium, contributed only slightly or not at all to the production of ethylene by the infected tissue. On the other hand, the healthy fruit tissue at the periphery of the rot, where only a few hyphae could be seen penetrating the healthy tissue, were producing considerably higher levels of ethylene (Table 5). The induction of ethylene production by the host tissues in response to infection has also been exhibited by the non-ripening, non-ethylene-producing *nor* tomato mutant at various stages of maturity (Table 5). Moreover, none of the fungi mentioned produce ethylene *in vitro*. These results suggested that the ethylene recorded in the various host/pathogen systems is produced by the host in response to fungal infection.

TABLE 5

Ethylene production by fruit discs (3 mm^3) of *Rhizopus*-infected 'Rutgars' tomatoes and non-ripening *nor* tomatoes*

Tested tissue	Ethylene Production (μl kg^{-1} h^{-1})		
	'Rutgars' mature-green	*Nor* mature-green	Mature
a	1.2	0.4	0.3
b	8.1	5.8	0.5
c	30.2	19.9	11.2
d	2.0	0.0	0.3
e	0.0	0.2	0.0

a – healthy pulp; b – healthy pulp 1-5 cm from rotted area; c – healthy pulp at the periphery of rot; d – rotten pulp at the periphery of the healthy pulp; e – rotten pulp from the center of the lesion.

* Reproduced from Barkai-Golan and Kopeliovitch (1983) with permission of the Academic Press.

Barkai-Golan et al. (1989b) studied the source of ethylene in tomatoes inoculated with *B. cinerea* or *Geotrichum candidum* by using aminoxyacetic acid (AOA), a potent inhibitor of plant-originating ethylene (Yang, 1985). AOA application at the site of inoculation with *B. cinerea* was found to inhibit ethylene production by 55-60% in the normal tomato fruits and by about 80% in the non-ripening *nor* mutant fruits (see Figs. 21 and 22). Since AOA acts as a specific inhibitor of ethylene biosynthesis by higher plants, these results suggest that ethylene production in both normal and mutant fruits is of plant origin, and is induced by the pathogen. The inability of AOA to arrest the production of infection-ethylene totally may be due to the short lag time between its application and the fungal inoculation, which prevents sufficient uptake by the tissue.

The source of ethylene in the infected tissue was also studied in citrus fruit inoculated with *P. digitatum* (Achilea et al., 1985a, b) which is one of the fungi capable of producing high levels of ethylene *in vitro* (Ilag and Curtis, 1968). Inoculation of citrus fruit with the fungus, similarly to other stresses, enhances the production of both ethylene and the compound ACC (1-aminocylopropane-1- carboxylic acid), which is the precursor of ethylene production in higher plants (Yang and Hoffman, 1984). Achilea et al.

(1985a, b) studied *P. digitatum* ethylene production in several ways:

(a) By comparing an isolate of *P. digitatum* producing very high levels of ethylene with an isolate producing very low levels of the gas.

(b) By adding ACC, which is typically connected with the production of ethylene of plant origin, or glutamic acid, which is probably the source of fungal ethylene (Chou and Yang, 1973).

(c) By adding AVG (aminoethoxy vinyl glycine), which inhibits plant ethylene production (Lieberman et al., 1974) and only slightly affects its production by *P. digitatum* (Chalutz and Lieberman, 1978).

(d) By the addition of Cu^2 ions, which are toxic to the fungus but stimulate ethylene production in higher plants (Abeles and Abeles, 1972).

The application of this complex of methods to the study of the origin of the ethylene produced in infected hosts clearly proved that the high level of ethylene produced in the infected area itself is derived wholly or in part from the fungus, which is capable of producing it both in culture and in the fruit. The production of this ethylene was not affected by the presence of ACC, but was considerably enhanced by the addition of glutamic acid. The addition of AVG inhibited its production only slightly, whereas the addition of $CuSO_4$ resulted in a marked inhibition of ethylene synthesis. On the other hand, the ethylene produced by the healthy part of the fruit, at the periphery of the rot, must be of plant origin, since it was stimulated by ACC and suppressed by AVG application.

C. PECTOLYTIC ACTIVITY AND ITS SOURCE IN INFECTED TISSUE

An increase in polygalacturonase (PG) activity in infected tissue during disease development has generally been accepted as being of fungal origin (Wood, 1967). However, studies of the origin of pectolytic activity in various host/pathogen systems demonstrated that the fungus not only 'contributes' its enzymes to the host, but may also induce enzyme production by the host itself, as a response to infection (Barkai-Golan et al., 1986). It was thus found that PG production by avocado fruits following infection with *Colletotrichum gloeosporioides* was induced by the fungus, and that softening of the mesocarp during anthracnose development was primarily due to the induction of the fruit PG (Barash and Khazzam, 1970).

The origin of PG in *Rhizopus stolonifer*-infected tomatoes has been studied in various tomato fruits (Barkai-Golan et al., 1986): (a) a non ripening *nor* tomato mutant, which contains no PG during any stages of development; (b) a green normal fruit, which does not contain PG while still unripe; and (c) a ripe red fruit, which contained PG of plant origin. Analysis by gel electrophoresis of *Rhizopus* PG, after *in vitro* growth, revealed the production of at least seven molecular forms of this enzyme. In each of the fruits tested, *Rhizopus* infection resulted in the accumulation of these typical PG isozymes, which are all of fungal origin; however, in the infected ripe tomato fruit, both fruit and fungal enzymes were found. An analysis by gel electrophoresis enabled differentiation between the isozyme bands of the tomato and those of the fungus which migrated in the gel faster than those of the fruit PG. It could not, however, provide a clear differentiation between the slower-migrating fungal isozymes and the fruit isozymes (Barkai-Golan et al., 1986). The differentiation between these isozymes was completed by immunological analysis with antiserum against specific forms of tomato enzymes; this analysis demonstrated that the infected fruit contained a higher level of tomato enzyme than the uninfected fruit of similar maturity. It was concluded that infection by *R. stolonifer* enhanced PG production in the normally ripening fruit or, in other words, that the infection advanced the ripening process in genetically competent tomato tissue. The infection did not, however, induce PG in the non-ripening *nor* fruits, which lack an active ripening system and are unable to produce PG naturally (Barkai-Golan et al., 1986). Clarification of the origin of PG in the infected tissue also suggests that the pectolytic enzymes of the mature tomato fruit can take an active part in fungal pathogenicity or contribute to disease development.

D. STIMULATION OF FRUIT SOFTENING AND CHANGES IN THE PECTIC COMPOUND CONTENTS

Examination of avocado fruits infected by *Fusarium solani* has revealed that their softening starts earlier than in uninfected fruits, and that it occurs during the incubation period of the pathogen, before the appearance of decay symptoms (Zauberman and Schiffmann-Nadel, 1974). Furthermore, softening does not begin at the point of infection and progress outwards from there, but occurs simultaneously in the whole fruit. This finding raised the suggestion that the advance in fruit

softening is not related to the local effect of the fungus but to its ability to induce a softening mechanism similar to that of the healthy fruit. The inducement of this mechanism may be directly related to the enhanced ethylene emission typical of infected fruit, and to the ability of the 'infection ethylene', similarly to exogenic ethylene, to enhance fruit softening and lead to earlier senescence.

Earlier studies of apples infected with various pathogens had already shown a considerable reduction in the insoluble pectin in their tissues, particularly in those cases where the pathogen was responsible for fruit soft rot (Cole and Wood, 1961). Studies with *F. solani*-inoculated avocados indicated that fungal infection affected the rates of changes in the pectic substances: the increase in the soluble pectin and the reduction in the insoluble protopectin are more rapid in the infected than the uninfected fruit, even though the final values of each of the fractions are similar in the two cases. Since fruit softening starts with the increase in the soluble pectin and the decrease in the protopectin, it is clear that it occurs earlier in the infected fruit (Zauberman and Schiffmann-Nadel, 1974).

Cell disintegration and tissue maceration were followed by an increase in soluble pectin in grapefruit peel treated with endo-PG produced by *Penicillium italicum* (Barmore and Brown, 1980). The increase in soluble pectin was recorded after 3-5 h incubation of the enzyme with host tissue, as presented in Table 6:

TABLE 6

Maceration of grapefruit peel mesocarp by endo-polygalacturonase from fruits infected with *Penicillium italicum*[1]

Treatment time (h)	Maceration index[2]	Pectin solubilization[3] (mg/g of peel)
1	1	0.47
3	3	0.88
5	5	1.02

[1] Reproduced from Barmore and Brown (1980) with permission of the American Phytopathological Society.

[2] 0 = no maceration; 5 = complete maceration

[3] Soluble pectin content resulting from the action of endo-polygalacturonase

E. CHANGES IN BIOCHEMICAL CONSTITUENTS OF INFECTED TISSUES

In various host/pathogen systems, fruit infection results in the decrease or total disappearance of the sugar content of the fruit. Such systems include: lemon fruits infected by *Phytophthora citrophthora* (Cohen and Schiffmann-Nadel, 1972); pineapples infected by *Ceratocystis paradoxa* (Adisa, 1985a); other tropical fruits, such as guava, papaya and banana, infected by various pathogens (Ghosh et al., 1964; Odebode and Sansui, 1996); watermelons infected by *Alternaria cucumerina* strains (Chopra et al., 1974); and cucumbers infected by *Pythium aphanidermatum* (McCombs and Winstead, 1964). This common phenomenon has generally been related to the stimulation of respiration in the infected tissue. An interesting phenomenon was recorded in banana fruits: whereas the presence of glucose, sucrose, fructose, maltose and raffinose were recorded in healthy fruits, only sucrose appeared during storage of bananas infected with *Botryodiplodia theobromae* (Odebode and Sansui, 1996). On the other hand, inoculation of pineapples with the fungus *Curvularia verruculosa* resulted in an increased level of sugars, probably because of the activity of cell-wall-degrading enzymes of the fungus (Adisa, 1985a).

Inoculation of citrus fruits with *Penicillium digitatum* considerably affected the D-galacturonic acid content of the peel (Achilea et al., 1985a). During the infection process the fungus produces exo-polygalacturonase, which hydrolyzes the cell wall pectin into monomers of galacturonic acid (Barmore and Brown, 1979), which is responsible for cell disintegration and tissue maceration.

Fungal infection may result in the reduction of the organic acid level. Reductions in ascorbic acid were recorded in lemons infected by *Phytophthora citrophthora*, in bananas infected by various storage pathogens, and in chili fruits infected by *Colletotrichum gloeosporioides* (Cohen and Schiffmann-Nadel, 1972; Khodke and Gahukar, 1995; Odebode and Sansui, 1996). The absence of citric acid from pineapples was recorded in fruit infected by *Ceratocystis* and *Curvularia* (Adisa, 1985a). It has been suggested that the pathogenic fungi might use organic acids for their respiration, thus leading to their decreased level or disappearance from the tissue.

Several cases have been reported of changes in the protein level in the plant tissue during disease development: a reduction in the protein

content along with an increase in the free amino acids. However, in other cases infection had no effect on the protein content of the tissue, or even resulted in their increase (Adisa, 1985a; Khodke and Gahukar, 1995). Several amino acids, such as histidine, arginine, aspartic acid, glycine, alanine and leucine, increased in infected fruits, whereas the levels of lysine, threonine, glutamic acid, proline, cystein, valine, methionine and others all decreased following infection (Khodke and Gahukar, 1995).

Polyamines, which are responsible for the control of nucleic acid synthesis and function (Slogum et al., 1984), play an important role in plant growth and development (Galston, 1983). The relationship between disease development and polyamine content in the fruit has been studied in *Rhizopus stolonifer*-infected tomato fruits (Bakanashvili et al., 1987). Marked reductions in the activities of the enzymes ornithine decarboxylase and arginine decarboxylase were recorded in the healthy tomato fruit during fruit development and towards ripening, but, as a result of the changes in the activities of these enzymes, the content of the polyamine putrescine, as well as those of the polyamines permidine and spermine which derive from it, were markedly reduced, and reached their lowest levels during ripening (Bakanashvili et al., 1987). Inoculation of preclimacteric tomato fruits with *R. stolonifer* was found to induce, in addition to fruit ripening, a reduction in the activity of the enzymes ornithine and arginine decarboxylase and, consequently, to reduce the polyamine level in the fruit. Since treatment with exogenic ethylene elicited phenomena similar to those associated with fungal infection or fruit ripening, it has been suggested that the ethylene induced by pathogen infection may be responsible for the altered polyamine content of the *Rhizopus*-infected tomato fruits.

Numerous biochemical changes occurred in potato tubers inoculated with *Fusarium sambucinum*, the causal agent of dry rot in stored tubers (Ray and Hammerschmidt, 1998). Peroxidase activity, which is very low in the uninfected tuber, increased up to 500-fold after inoculation with the fungus. High levels were recorded in tissue just ahead of the advancing hyphae. Smaller increases were recorded in tubers following wounding alone. Lignin content increased in tubers following inoculation, the highest concentrations being detected 40 h after inoculation, when the infected surface was often partly or entirely decayed. As disease progressed, the lignified zone increased in depth until it comprised five or six cell layers. Here again, responses to *F. sambucinum* infection were

stronger than responses to wounding alone, and the lignified zone in wounded, non-infected tissue was much narrower than that in tissue adjacent to the infection (Ray and Hammerschmidt, 1998). The inducement of peroxidase activity and lignin formation is included among the defense responses of the host to infection (see the chapter on Host Protection and Defense Mechanisms).

Polyphenol oxidase activity, which is involved in quinone formation, browning and other reactions in potato tubers, increased following inoculation with *F. sambucinum* and wounding, but to a lesser extent than peroxidase activity. The greatest polyphenol oxidase activity was found in tissues adjacent to the diseased zone; it seemed to be associated with tuber resistance and was highest in the more resistant potato varieties than in the susceptible ones (Ray and Hammerschmidt, 1988). Furthermore, resistant tubers also browned more quickly than the susceptible ones following infection or wounding.

Phenolic compounds were found to increase following potato tuber inoculation or wounding alone in both susceptible and moderately resistant tubers, suggesting that phenolic biosynthesis was induced. Examination of extracts of infected tuber discs revealed the presence of a number of phenolic compounds. Constant amounts of ferulic acid were detected, whereas chlorogenic acid, which is a phytoalexinic compound induced in apple fruit infected by *Nectria galligena* (Swinburne, 1983), accumulated to maximal levels at 48 h and then decreased. These results indicate that free phenolic acids may be induced by infection or wounding, and are then removed as they are converted into lignin or cross-linked to cell walls (Ray and Hammerschmidt, 1998). For discussion of the inducement of phytoalexins in tuber tissue in response to infection, see the chapter on Host Protection and Defense Mechanisms.

CHAPTER 8

MEANS FOR MAINTAINING HOST RESISTANCE

It was previously pointed out that fruits become more susceptible to invasion by postharvest pathogens as they ripen (see the chapter on Factors Affecting Disease Development - The Fruit Ripening Stage). Treatments and conditions that lead to delayed ripening and senescence can, therefore, indirectly suppress postharvest disease development. These include low-temperature storage, low-O_2 and high-CO_2 atmospheres, ethylene removal from the atmosphere, growth regulators, calcium application; these as well as other treatments or strategies may contribute to maintaining the natural resistance typical of the young fruit or vegetable.

A. COLD STORAGE

Storage at low temperature is the main method for reducing deterioration of harvested fruits and vegetables. The importance of cold storage in decay suppression is so great that all other control methods are frequently considered as supplements to refrigeration (Eckert and Sommer, 1967).

Low temperatures affect both the host and the pathogen simultaneously. They prevent moisture loss from the host tissues and consequent shriveling; they retard metabolic activity and delay physiological changes that lead to ripening and senescence. Since fruits and vegetables become generally more susceptible to pathogens as they mature and approach senescence, the retardation in the physiological activity of the host is accompanied by a delay in decay development after harvest. As with the host, the metabolic activity of the pathogen is also directly influenced by the environmental temperature, and both its growth ability and enzymatic activity can be greatly retarded by low temperatures. Low temperatures can thus delay postharvest disease development in two ways: (a) indirectly, by inhibition of ripening and senescence of the host and extension of the period during which it maintains its resistance to disease; and (b) directly, by inhibition of pathogen development by subjecting it to a temperature unfavorable for its growth.

We have seen that the minimum temperature for growth of some fungal species is around 0°C, while other species are capable of growth, although at a very slow rate, even at temperatures as low as -2°C or less (see Table 2). For these pathogens, storing the commodity at 0°C would not arrest their growth nor prevent disease development, but would only delay the appearance of the disease or, in other words, would prolong its incubation period. We have also seen that the closer the storage temperature approaches to the minimum for growth of the pathogen, the longer is the incubation period of the disease and the slower the progress of decay (see Fig. 8). It should be emphasized, however, that even if the pathogen is not eradicated at 0°C or at temperatures close to it, it is very significant that its rate of growth under these conditions is very slow; this enables us to understand our general desire to lower the temperature of the storage room atmosphere as much as possible. However, the possibility of lowering the storage temperature is limited by the sensitivity of the fruit or vegetable to chilling injury. This sensitivity varies among fruit and vegetable species, and even among cultivars of the same species, or depends on the state of maturity of a given cultivar.

Storing cold-sensitive fruits and vegetables at temperatures below their resistance threshold results in the development of chilling injuries – physiological injuries caused by low, but above-freezing, temperatures. The severity of the damage depends on the cultivar sensitivity, on the temperature and on the duration of exposure to it (Ryall and Lipton, 1979). Chilling injuries are generally associated with the destruction of groups of cells, which leads to the formation of sunken areas and to external or internal browning. The development of chilling injuries in cold-sensitive crops has been hypothesized to derive from changes in lipids, which leading, in turn, to changes in the permeability of cell membranes and the accumulation of toxic intermediate compounds, which may cause cell destruction or death (Lyons, 1973).

Following chilling injury, the sensitivity of the fruit or vegetable to decay increases considerably, and decay incidence might reach higher levels than those in fruits held at higher temperatures even though the latter are unable to reduce decay. The increased decay in the chill-injured host may result from the easy penetration of the pathogen through damaged tissues (see the chapter on Postharvest Disease Initiation – Pathogen Penetration into the Host). Such increased decay in cold-sensitive fruits was exhibited in Navel oranges which had to undergo cold-sterilization against insect infestation, under Japanese quarantine

regulations which require all imported South African citrus fruit to be cold sterilized (12 days at 0.5°C) in order to destroy all forms of insect infestation. The resulting incidence of core rot caused by *Alternaria citri* and *Fusarium* spp. may become unacceptably high in cold-sensitive oranges stored at 4.5°C after the cold treatment (Pelser and Grange, 1995).

The guiding principle for choosing storage temperature is the use of the lowest temperature that does not harm the host. The storage temperature for fruits and vegetables should, therefore, be just above their injury threshold (Hardenburg et al., 1986). Recommended temperatures and relative humidities for storing fruits and vegetables are summed up in Tables 7 and 8.

TABLE 7

Recommended storage conditions for fruits [1, 2]

Commodity	Temperature (°C)	Relative humidity (%)
Apple		
Bramley's Seedling	3 to 4	90 – 95
Cox's Orange Pippin	3 to 3.5	90 – 95
Crispin (Mutsu)	1.5-2	90 – 95
Discovery	3.5	90 – 95
Golden Delicious	1.5 to 2	90 – 95
Granny Smith	-1 to 0	90 – 95
Jonathan	3 to 3.5	90 – 95
McIntosh	1.5 to 3.5	90 – 95
Red Delicious	3.5 to 4	90 – 95
Rome Beauty	1 to 2	90 – 95
Spartan	0 to 0.5	90 – 95

[1] Reproduced with permission from A Colour Atlas of Postharvest Diseases and Disorders of Fruits and Vegetables, Vol. I, by Anna S. Snowdon, published by Manson Publishing, London, 1990. It also includes data from the Agricultural Research Organization, Bet Dagan, Israel.

[2] The leading lines in determining these data are the temperatures suitable for achieving the longest storage duration followed by appropriate 'shelf life'.

Commodity	Temperature (°C)	Relative humidity (%)
Apricot	-1 to 0	90 – 95
Avocado		
unripe		
Booth 1 and 8, Taylor	4.5	85 – 90
Ettinger	5.5	85 – 90
Fuchs, Pollick, Waldin	10 to 13	85 – 90
Fuerte, Hass	5.5 to 8	85 – 90
Lula	4.5	85 – 90
ripe		
Fuerte, Hass	2 to 5	85 – 90
Banana		
green		
Cavendish	13	85 – 90
Gros Michel	13	85 – 90
Lacatan	13 to 15	85 – 90
Poyo Robusta, Valery	13	85 – 90
Colored	13 to 16	85 – 90
Blueberry	0	90 – 95
Carambola	5 to 10	90
Cherry		
Sour	0 to 1	90 – 95
Sweet	-1 to 0	90 – 95
Citrus Fruits		
Clementine	4 to 5	90
Grapefruit		
California and Arizona	14 to 15	90
Florida and Texas	10 to 15	90
Israeli	10 to 11	90
South African	11	90
Lemon	10 to14	90
Lime	9 to 10	90
Mandarin	4 to 8	90
Minneola	4 to 9	90

Commodity	Temperature (°C)	Relative humidity (%)
Orange		
Castellana, Spanish	1 to 2	90
Jaffa, Shamouti	8	90
Navel		
Australian	4.5 to 5.5	90
Israeli	6 to 8	90
Moroccan, Spanish	2 to 3	90
Valencia		
California	2 to 7	90
Cyprus	2 to 3	90
Florida and Texas	0 to 1	90
Israeli	2 to 5	90
Morrocan	2 to 3	90
South African	4.5	90
Spanish	2	90
Pomelo	10 to 15	90
Satsuma	4 to 5	90
Tangelo	4 to 5	90
Tangerine		
California and Arizona	4.5 to 7	90
Florida and Texas	0 to 4.5	90
Cranberry	2 to 4	90 – 95
Date	0	85 – 90
Feijoa	4	90
Fig	0	90
Gooseberry	-0.5 to 0	90 – 95
Grape	-1 to - 0.5	90 – 95
Guava	5 to 10	90
Kiwifruit	-0.5 to 0	90 – 95
Litchi	5 to 10	90 – 95
Loquat	0	90 – 95

Commodity	Temperature (°C)	Relative humidity (%)
Mango		
Alphonso, Indian	7 to 9	90
USA	13	90
Bangalore, Safeda	5.5 to 7	90
Haden, Keitt	12 to 14	90
Zill	10	90
Nectarine	-1 to 0	90 – 95
Papaya		
green	10	90
turning	7	90
Passion Fruit	7 to 10	85 – 90
Peach	-1 to 0	90 – 95
Pear	-1 to 0	90 – 95
Persimmon	-1 to 0	90 – 95
Pineapple		
mature green	10 to 13	90
ripe	7	90
Plantain		
green	10	85 – 90
colored	11 to 15.5	85 – 90
Plum	-0.5 to 0	90 – 95
Pomegranate	5 to 6	90
Rambutan	10 to 12	95
Raspberry	0	90 – 95
Strawberry	0	90 – 95
Tamarillo	3 to 4	90

TABLE 8

Recommended conditions for vegetables [1, 2]

Commodity	Temperature (°C)	Relative humidity (%)
Artichoke		
Globe	0 to 1	95 – 100
Jerusalem	-0.5 to 0	95 – 98
Asparagus (Asperge)	0 to 2	95 – 100
Bean		
Common	4 to 8	95 – 100
Faba	0 to 1	95
Beet	0 to 1	95 – 100
Broccoli	0 to 1	95 – 100
Brussels sprout	0 to 1	95 – 100
Cabbage		
White cabbage	0 to 1	95 – 100
Chinese cabbage	0 to 1	95 – 100
Carrot	0 to 1	95 – 100
Cassava	0 to 2	85 – 90
Cauliflower	0 to 1	95 – 98
Celeriac	0 to 1	95 – 98
Celery	0 to 1	95 – 100
Chicory	0 to 1	95 – 100
Chilli	7 to 10	90 – 95
Cucumber	10 to 12	90 – 95

[1] Reproduced with permission from A Colour Atlas of Postharvest Diseases and Disorders of Fruits and Vegetables, Vol. 2, by Anna S. Snowdon, published by Manson Publishing, London, 1992. It also includes data from N. Aharoni (1992).

[2] The leading lines in determining these data are the temperatures suitable for achieving the longest storage duration followed by appropriate 'shelf life'.

Commodity	Temperature (°C)	Relative humidity (%)
Eggplant	8 to 12	90 – 95
Garlic		
fresh	0 to 1	60 – 70
dry	0 to 1	60 – 70
Ginger	12 to 14	70
Herbs (fresh):		
Basil	12	95 – 98
Chervil	0 to 1	95 – 98
Chives	0 to 1	95 – 98
Coriander	0 to 1	95 – 98
Dill	0 to 1	95 – 98
Endive	0 to 1	95 – 98
Fennel	0 to 1	95 – 98
Marjoram	1 to 3	95 – 98
Melissa	1 to 3	95 – 98
Mint	1 to 3	95 – 98
Oregano	1 to 3	95 – 98
Parsley	0 to 1	95 – 98
Parsnip	0 to 1	95 – 98
Rosemary	1 to 3	95 – 98
Sage	1 to 3	95 – 98
Sorrel	0 to 1	95 – 98
Tarragon	0 to 1	95 – 98
Thyme	1 to 3	95 – 98
Watercress	0 to 1	95 – 98
Z'atar	1 to 3	95 – 98
Horseradish	0 to 1	95 – 100
Kohlrabi	0 to 1	95 – 100
Leek	0 to 1	95 – 100
Lettuce	0 to 1	95 – 100

Commodity	Temperature (°C)	Relative humidity (%)
Melon		
Galia	5 to 7	90 – 95
Ogen	6 to 7	90 – 95
Cantaloupe	4 to 5	90 – 95
Honeydew	10 to 15	90 – 95
USA	5	90 – 95
Onion		
green	0 to 1	95 – 100
dry	-1 to 0	70
Pea	0 to 1	95
Pepper	7 to 10	95
Potato		
culinary	10 to 14	90 – 95
seed	4 to 5	90 – 95
Pumpkin	10 to 13	60 – 70
Radish	0 to 1	95 – 100
Rhubarb	0 to 1	95 – 100
Shallot	0 to 1	95 – 100
Spinach	0 to 1	95 – 100
Squash		
summer	8 to 10	90 – 95
winter	10 to 13	60 – 70
Sweet corn	0 to 1	95 – 98
Sweet Potato	12 to 14	85 – 90
Tomato		
mature-green	12 to 15	90
turning	10 to 12	90
ripe	8 to 10	90
Turnip	0 to 1	90 – 95
Watermelon	10 to 14	85 – 90
Zucchini	7 to 10	90 - 95

The effect of refrigeration as a means for disease suppression is manifested mainly in systems in which the host is tolerant of near-freezing temperatures, while the pathogen requires higher temperatures for growth. *Rhizopus stolonifer* strains or isolates that do not develop at temperatures lower than 5°C (Dennis and Cohen, 1976) do not raise a serious problem for strawberries or carrots, which can be stored or shipped at temperatures below 5°C. For these vegetables, as well as for most fruits of temperate origin, such as certain cultivars of apples and grapes, the optimal temperature, which will preserve the required quality of the commodity for the maximum duration, is near 0°C. Therefore storing strawberries at around 1°C retards decay both directly by retarding fungal growth (mainly of *Botrytis cinerea*) and indirectly by delaying senescence, as exhibited in the maintenance of fruit and calyx freshness.

On the other hand, fruits of tropical origin, such as bananas, pineapples, lemons, grapefruits and tomatoes, are already liable to suffer chilling injury after several hours at 10-15°C, and fruits of subtropical origin, such as avocado cultivars, melon cultivars and various citrus fruits, are sensitive to temperatures below 10°C. Therefore, the fungus *Geotrichum candidum*, although it is sensitive to low temperatures, will continue to develop and cause the sour rot in stored lemons, since this fruit has to be stored at 13-14°C because of its sensitivity to prolonged chilling. Similarly, cold storage is also unable to suppress *Penicillium* spp. in various cold-sensitive citrus fruits; the storage temperature suitable for these commodities allow fungal development. Tomato fruits stored at temperatures lower than 8-12°C are extremely sensitive to attack by microorganisms, such as *Alternaria alternata* and *R. stolonifer*, even before the appearance of external chilling injury symptoms. The optimal temperature for tomatoes is closely related to the stage of ripening: mature-green fruits are more sensitive to chilling and should not be stored at temperatures below 12°C; those at a more advanced stage of ripening may be stored at 8-10°C without their quality suffering. Similarly, sweet peppers are injured below 7°C and become susceptible to *Alternaria* and *Botrytis* decay (McColloch and Wright, 1966) and sweet potatoes stored below 10°C are predisposed to *Alternaria*, *Botrytis*, *Mucor* and *Penicillium* decay (Lauritzen, 1931). Direct control of the pathogen in these cold-sensitive commodities is not possible, because of the relatively high temperatures required for their storage; decay in these cases is suppressed mainly indirectly by delaying the ripening and senescence processes.

When low-temperature storage is used for decay suppression, it is necessary to take into consideration not only the storage temperature but also the temperature from which the commodity was removed to cold storage, and the shelf-life temperature to which it will be transferred. Maximum storage life prolongation demands the rapid removal of the 'field heat' from the commodity. Holding the commodity at a high temperature prior to refrigeration, or delaying its removal to cold storage, will permit germination of many of the spores which infest wounds and will contribute to pathogen establishment in the tissues, thus shortening the way to decay initiation. In addition, we should remember that low temperatures during storage are generally not lethal to the pathogenic fungi and bacteria, but merely retard their development. When the commodity is transferred to higher temperature conditions to complete its ripening or for marketing, the pathogen resumes growth and develops rapidly into decay lesions. Thus, growth of *B. cinerea*, the gray mold fungus in strawberries, which is markedly suppressed during cold storage at 0-2°C, will develop rapidly upon removal of the fruit to shelf-life conditions. On the other hand, the exposure of germinating sporangiospores of *R. stolonifer* to temperatures close to freezing, causes their death and prevents decay development during shelf life. It is no wonder, therefore, that the incidence of *Rhizopus* rot in peaches stored at 0°C for one week prior to transfer to ambient temperatures was lower than that in fruits which were held continuously for a week at room temperature to ripen before marketing (Pierson, 1966).

Chilling Injury Retardation

In order to store fresh commodities, which are sensitive to chilling, at temperatures lower than their critical chilling range, their tolerance to chilling conditions should be enhanced or the appearance of chilling injury symptoms should be delayed. Various postharvest techniques for preventing or alleviating chilling injury have been reported during the recent decades (Wang, 1993).

One approach is to condition fruits at temperatures above the critical chilling level, to increase their tolerance to chilling during subsequent low-temperature storage. This method has been found effective in alleviating chilling injury in several citrus fruits. Preconditioning limes at 7-20°C for 1 week prior to storage at 1.5°C, or exposure of lemons to 5 or 15°C for 1 week prior to storage at 0-2.2°C, reduced chilling injury during cold storage (Houck et al., 1990; Spalding and Reeder, 1983).

Low-temperature conditioning was similarly effective for grapefruits: preconditioning grapefruits at 10-16°C for 7 days or at 17°C for 6 days resulted in reduced chilling injury and decay development during cold storage of this chilling-sensitive fruit (Chalutz et al., 1985; Hatton and Cubbedge, 1982). Exposure of papaya fruits to 12.5°C for 4 days markedly reduced chilling sensitivity at storage temperatures below 7.5°C (Chen and Paull, 1986).

For certain commodities, a double-step, or 'stepwise', temperature conditioning has been found more effective in reducing chilling injury symptoms than a single temperature conditioning. The advantage of this technique was exhibited in mangoes by Thomas and Oke (1983), who found that preclimacteric mangoes exposed to 20°C for 1 day and then to 15°C for 2 days were more tolerant of storage at 10°C than those treated with a single temperature conditioning. Marangoni et al. (1990) showed that tomatoes can also be acclimated to cold storage by gradual cooling: this included exposure to 12°C for 4 days, to 8°C for 4 days and to 5°C for 7 days.

Another way to prevent or retard chilling injury is by the application of intermittent warming. Studies with various horticultural crops have shown that periodic interruption of cold storage by exposure of fruits to short periods of warming can reduce chilling injury symptoms and maintain fruit quality for longer durations (Wang, 1993). The optimum storage conditions with intermittent warming may vary greatly according to the cultivar, fruit maturity stage and growing conditions. For each fruit, however, interruption of chilling exposure with warm treatments must be performed before chilling injury has developed beyond a reversible stage. Wang (1993) emphasized the importance of correct timing and duration for intermittent warming treatments to be effective; if the critical time at chilling temperature has been exceeded and chilling injury progressed beyond recovery, the high temperature would only accelerate the degrading processes and the appearance of injury symptoms. On the other hand, warming treatments applied too early or too frequently result in excessively soft tissues, which are vulnerable to invasion by postharvest pathogens.

Intermittent warming was found to be effective in reducing chilling injury in various cold-sensitive citrus fruit cultivars. Early studies by Brooks and McColloch (1936) had already shown that removal of grapefruits from 2°C storage for 1 day after 1 week and again after 2 weeks markedly reduced the development of chilling injury symptoms, including pitting, scald and watery breakdown. Hatton et al. (1981)

found that intermittent warming treatments in grapefruit made it possible to reduce the recommended dosage of the fungicide benomyl by 50%, from 600 to 300 ppm, while still maintaining good decay control. The advantage of intermittent warming in enhancing fruit resistance to chilling injury has been proven for Olinda oranges: 2 weeks of intermittent warming to 15°C every 3 weeks, during storage at 3°C, delayed the onset of chilling injury by 15 weeks and greatly suppressed the incidence of damage during the subsequent 10 weeks of storage (Schirra and Cohen, 1999). Experiments with lemons have shown that intermediate heating to 13°C for 7 days after every 21 days enables them to be stored at 2°C, a temperature much lower than the optimal storage temperature for the fruit. Under these conditions, the percentage of peel pitting, characteristic of lemon fruits stored at 2°C, was markedly reduced and decay was suppressed (Artés et al., 1993; Cohen et al., 1983). The technique of storing lemons at 2°C with intermittent warming at 13°C has been adopted as a commercial method for long-term storage of these fruits (Cohen, 1988).

Mature-green tomato fruits are susceptible to chilling injury at storage temperatures below 12°C. Chilling injury symptoms include surface pitting, uneven ripening or failure to ripen, and altered flavor. These symptoms are accompanied by increased disease development (Hobson, 1981; Cheng and Shewfelt, 1988). Intermittent warming treatments have proved to be effective in reducing chilling injury in tomatoes, to an extent which depends on the cultivar and the time-temperature regime. Artés and Escriche (1994) found that storing the fruit at 9°C (but not at 6°C), with cycles of intermittent warming at 20°C for 1 day every 6 days, was effective in reducing chilling injury symptoms in two tomato cultivars, while fruits stored at a continuous 9°C were susceptible to *Alternaria* decay, which typically developed on the pitted area. Intermittent warming was more beneficial than intermittent cooling; pitting developed at 2°C in the intermittently cooled fruits (Artés et al., 1998).

Chilling injury is not limited to fruits of tropical or subtropical origin. Cultivars of peaches, which were recommended for storage under refrigerated conditions, may develop chilling injury symptoms, including breakdown or woolly breakdown, discoloration, low juice content, and failure to ripen or to ripen normally. Intermittent warming at 20°C or at higher temperatures, for 1-3 days every 2 weeks, during storage at 0 or 1°C, gave good control of chilling injury in peaches (Ben-Arie et al., 1970; Buescher and Furmanski, 1978); while intermittent warming at 18°C for

2 days every 3 or 4 weeks reduced chilling injury symptoms in stored nectarines (Anderson and Penney, 1975).

The time taken for an irreversible chilling injury to occur is usually very short for perishable produce with short storage life, such as cucumber or zucchini squash. Therefore, intermittent warming of these commodities must be applied earlier and more frequently (Cabrera and Saltveit, 1990; Wang, 1993).

B. MODIFIED AND CONTROLLED ATMOSPHERES

A controlled atmosphere (CA) or modified atmosphere (MA) around the produce is created by alterations in the concentrations of the respiratory gases in the storage atmosphere; these alterations include elevation of carbon dioxide (CO_2) level, the reduction of oxygen (O_2) tension, or both . Whereas the term 'CA storage' generally implies precise control of O_2 and CO_2 concentrations in the atmosphere, the term 'MA storage' is broader and may indicate any synthetic atmosphere, arising intentionally or unintentionally, in which the composition of its constituent gases cannot be closely controlled.

Keeping fruits and vegetables in modified atmospheres is an old technique. Wang (1990), in his review of physiological and biochemical effects of controlled atmosphere on fruits and vegetables, mentions an ancient Chinese poem that describes the fondness for lychees of an empress in the 8th century. She demanded that freshly picked lychees be transported, on horseback, from southern China to her palace, a distance of about 1,000 km, and the fruit carriers discovered that lychees would keep well if the fruits were sealed inside the hollow centers of bamboo stems, along with some fresh leaves. Wang (1990) adds that while the poem did not explain the reason for this, we know today that the atmosphere produced within the bamboo stems by the continued respiration of the fruits and the fresh leaves, was probably the reason for the freshness of those lychees. During the last few decades, understanding of the physiological and biochemical processes in the fruit associated with alterations in the CO_2 and O_2 contents of the atmosphere has considerably increased. Alterations in the levels of the atmospheric gases were primarily aimed at suppressing the respiration and other metabolic reactions of harvested fruits and vegetables, and so retarding the ripening and senescence processes. During storage, many volatile compounds are evolved from the stored fruits and vegetables, and these

accumulate in the storage atmosphere. Of these compounds, ethylene is apparently the most important. Since the accumulation of ethylene above certain levels may hasten the ripening and enhance senescence of many fruits and vegetables, its removal from the atmosphere may help to suppress the physiological processes related to ripening and senescence.

However, for many fruits and vegetables, the factor limiting the extension of their useful life is the development of postharvest diseases. Many studies have indicated that modifications of the storage atmosphere, apart from their effects on the physiological processes of the host, can also retard postharvest disease development during storage (El Goorani and Sommer, 198l; Barkai-Golan, 1990). The effect of low O_2 levels or high CO_2 levels on postharvest disease development can be direct - by suppressing various stages of the pathogen growth, and its enzymatic activity - or indirect - by maintaining the resistance of the host to infection by keeping it in a superior physiological condition.

1. CONTROLLED ATMOSPHERE

Direct Effects on the Pathogen

Oxygen is required for normal respiration and growth of the pathogen. Suppression of growth by low O_2 is most likely due to effects of electron transport on the cytochrome system, although other oxidative enzyme systems present in cells may also be suppressed by low oxygen (Sommer, 1985). In most fungi, no growth occurs in the absence of molecular O_2, but the reduction in the level of O_2 required to inhibit the various stages of fungal growth varies considerably among species. In general, however, lowering the level of O_2 from 21 to 5% has little or no effect on fungal growth. In order to obtain appreciable reduction of spore germination, mycelial growth and sporulation in many fungal species, O_2 concentrations of less than 1% are required (Wells and Uota, 1970), although spore sensitivity to low O_2 does not necessarily match hyphal sensitivity. Wells and Uota (1970) showed that spore germination of *Rhizopus stolonifer* and *Cladosporium herbarum* in 1% O_2 was about 50% of that in air, and they found that the rate decreased gradually as the O_2 concentration decreased from 1 to 0.25%. Spore germination of *Alternaria alternata, Botrytis cinerea* and *Fusarium roseum,* on the other hand, decreased significantly only when the O_2 concentration was 0.25% or less (Fig. 23A). All cultures resumed normal growth when returned to air. Mycelial growth (as indicated by mycelial dry weight) of these fungi was inhibited by more than 50% when the O_2 concentration was 4%.

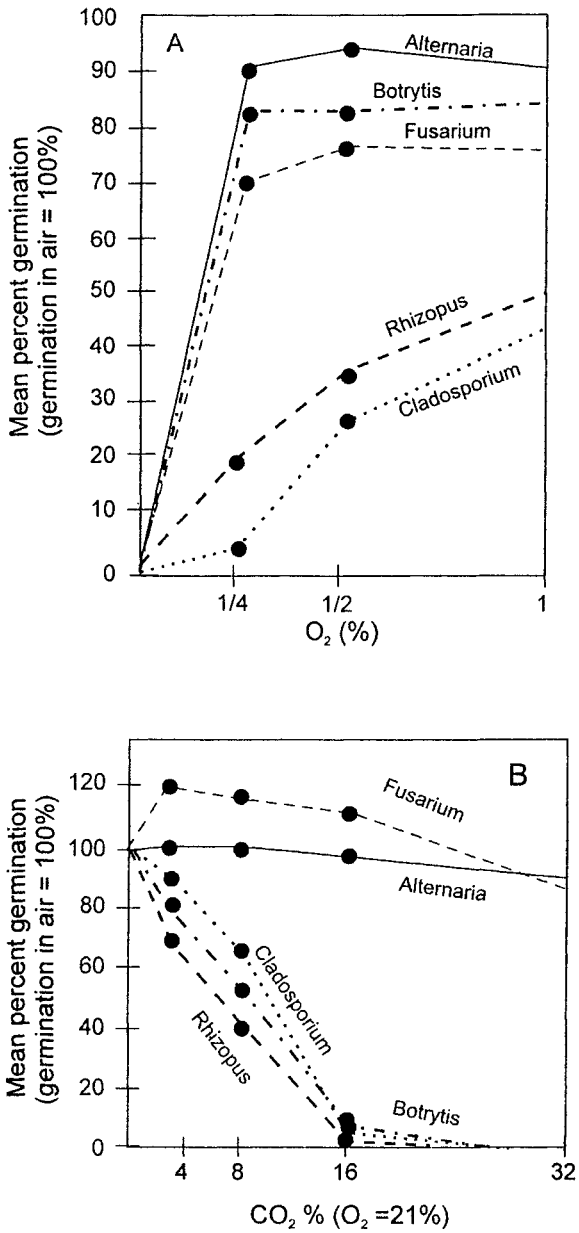


Fig. 23. Germination of *Alternaria alternata, Botrytis cinerea, Cladosporium herbarum, Fusarium roseum, and Rhizopus stolonifer* in atmospheres containing different concentrations of oxygen (A) and different concentrations of CO_2 (B). Data are mean percents of controls germinated in air. (Reproduced from Wells and Uota, 1970 with permission of the American Phytopathological Society).

For *R. Stolonifer,* however, a 50% inhibition was caused only at 2% O_2 and some growth was still recorded at 0% (Follstad, 1966, Wells and Uota, 1970). Differences between the sensitivity of spores and sporangia to low O_2 were recorded for *R. stolonifer*: whereas fungal sporangia remained viable after 72 h of anoxia, only a few of the sensitive sporangiospores survived under these conditions (Bussel et al., 1969).

The response of fungal sporulation to low O_2 may also differ with the species: *B. cinerea* produces an abundance of aerial mycelium when grown in 1% O_2, but no spores develop in this level of O_2; sporulation of *A. alternata* and *C. herbarum*, on the other hand, was found even in 0.25% O_2, although mycelial growth was markedly reduced (Follstad, 1966). Lowering of O_2 concentrations also suppressed sclerotium formation in species that naturally produce them during their life cycle. It was thus found that sclerotium production by *Sclerotinia minor* was more sensitive to low O_2 than was radial growth and at 1% O_2 no sclerotia were produced, although some growth was recorded (Imolehin and Grogan, 1980).

Low oxygen tension of 1-3%, a range tolerated by many agricultural commodities in storage, also reduces growth of various decay-causing bacteria, such as *Erwinia carotovora, E. atroseptica* and *Pseudomonas fluorescens*, although some growth was recorded even at 0% O_2 (Wells, 1974).

Carbon dioxide is essential for the growth of many aerobic microorganisms, since it is fixed in lactic, fumaric, citric and other acids of the Krebs cycle. However, although these microorganisms can fix CO_2 for their use, they cannot use it as a sole source of carbon for metabolism. High concentrations of CO_2 may directly suppress fungal growth by retarding various metabolic functions, so causing lowered respiration (Sommer, 1985). The early studies of Brown, W. (1922b) and Brooks et al. (1932) had already demonstrated the inhibitory effect of high-CO_2 atmospheres on mycelial growth and spore germination of *B. cinerea, R. stolonifer, Mucor* spp. and other fungi. The retarding effect of CO_2 on fungal growth is greater in the early phases of growth and at low storage temperatures (Brown, W. 1922b).

Similarly to O_2 concentration, that of CO_2 required to inhibit spore germination and mycelial growth varies with the species (Wells and Uota, 1970) (Fig. 23B): spore germination of *R. stolonifer, C. herbarum,* and *B. cinerea* was inhibited by over 90% at 16% CO_2, but levels as high

as 32% had no effect on *A. alternata* spore germination. Similarly, growth of *A. alternata, B. cinerea* and *C. herbarum* were inhibited by 50% in an atmosphere of 20% CO_2, whereas growth of *F. roseum* was inhibited by 50% only when the CO_2 level was elevated to 45%. Growth of colonies of the two bacteria, *E. carotovora* and *E. atroseptica* was also inhibited by high CO_2 concentrations. On the other hand, in the absence of CO_2 no growth of these fungi occurred at any O_2 concentration (Wells, 1974).

Combining low O_2 with high CO_2 concentrations does not necessarily result in additive effects. For certain fungi, such as *Sclerotinia sclerotiorum,* growth retardation induced by low O_2 concentrations is not enhanced by elevating the CO_2 content of the atmosphere. On the other hand, certain fungi, such as *A. alternata* and *Penicillium expansum* have been found to grow more slowly in CAs composed of low O_2 (2.3%) and high CO_2 (5%) than in air, although neither low O_2 nor high CO_2 alone caused any significant growth retardation (El-Goorani and Sommer, 1981).

In contrast to the fact that CA usually retards the growth of the pathogen, it has been found that atmospheres low in O_2 stimulated the fungus, *Geotrichum candidum.* Furthermore, a combination of low O_2 concentrations with 3% CO_2 increased the stimulatory effect of the low O_2 alone (Wells and Spalding, 1975). CO_2, at concentrations higher than 0.03%, has been found to stimulate the growth of several pathogenic fungi even when the O_2 level was sufficiently low to limit fungal development, and the CO_2 concentrations were not in themselves inhibitory (Wells and Uota, 1970). The growth stimulation by CO_2 under these conditions has been attributed to fixation of CO_2 into acids of the Krebs cycle, by fungal cells, and utilization of the derived energy for fungal growth.

Several investigations have addressed the effects of CA on the enzymatic activity of the pathogen. Holding *R. stolonifer* cultures in a low-O_2 atmosphere retarded their growth and also repressed fungal enzymatic activities. Pectin methyl-esterase (PME), polygalacturonase (PG) and cellulase (Cx) activities were directly related to fungal growth on potato-pectin culture filtrates, with the highest activities found in the normally aerated cultures (Wells, 1968). Similarly, a controlled atmosphere of 5% CO_2 and 3% O_2, which may be suitable for storing various apple cultivars, markedly reduced mycelial growth as well as the enzymatic activities of PG and polymethylgalacturonase (PMG) produced by *Gloeosporium album* and *G. perennans* (Edney, 1964).

Indirect Effects on Pathogen Development

It is well known that susceptibility of fruits to postharvest decay organisms generally increases with ripening. A controlled atmosphere may retard ripening and senescence processes of fruits and vegetables, through the suppression of respiration and other metabolic functions. Low O_2 levels and high CO_2 levels may also affect ripening by retarding or inhibiting the production and activity of ethylene, whose accumulation at certain levels in the storage atmosphere may result in the initiation of ripening or the enhancement of tissue senescence in fruits (Yang and Hoffman, 1984).

A study carried out by Aharoni et al. (1986) showed that ethylene played a primary role in the initiation of broccoli inflorescence senescence, and that the senescence-retarding effects of a CO_2-enriched atmosphere were related to its ability to block ethylene action. Furthermore, the marked decrease in rot development observed in broccoli florets inoculated with *Botrytis cinerea* spores and stored in a CO_2-enriched atmosphere, was completely nullified by the addition of 10 ppm ethylene to the atmosphere (Table 9).

Removal of ethylene from long-term CA storage also delayed ripening in certain cultivars of apples, as expressed by improved firmness retention. This was especially apparent in fruit picked in its preclimacteric state (Knee, 1990). Along with the prolongation of the physiological life of apples, these conditions also led to decay suppression during storage (Bompeix, 1978; Sams and Conway, 1985).

TABLE 9

Effect of CO_2 and ethylene on decay development in broccoli florets inoculated with *Botrytis cinerea* spores[1]

Treatment	Index of decay[2] (after 120 h of incubation)
Air	4.0
CO_2 (10%)	2.6
C_2H_4 (10 ml l^{-1})	4.2
$CO_2 + C_2H_4$	4.2

[1] From Aharoni, N., et al., (1986)

[2] 1, no decay; 5, maximum decay development

The ability of modified or controlled atmospheres to extend the physiological life of fruits have been closely related to their action on the host cell walls. As fruits ripen the pectic substances of the middle lamella, which are initially laid down by the cell in a relatively insoluble form, protopectin, become more soluble, and the tissue begins to soften. The increased solubility of the pectic substances and the softening render the tissue more vulnerable to maceration by pectolytic enzymes released by the pathogen. Storing the fruit in CA may retard changes in the pectic constituents, with longer retention of a firm texture, which is less vulnerable to pathogen attack (Smock, 1979).

Under CA storage, when ripening and senescence are retarded, the fruit may retain its ability to produce antifungal compounds, normally characteristic only of young tissues. Such effects lead to disease inhibition and delay the transformation from quiescent to active infections, by prolonging the pathogen latency (Barkai-Golan, 1990). An exception to the association between ripeness and disease development is the bacterial soft rot of tomatoes caused by *Erwinia carotovora*, which develops even more rapidly in mature-green fruits than in mature ones (Parsons and Spalding, 1972). This phenomenon may explain why CA conditions, which delay ripening and reduce decay by *Rhizopus* and *Alternaria* in mature-green tomatoes do not result in bacterial soft rot control.

In general, atmospheres containing about 2.5% O_2, a level which is commonly maintained in CA storage, are most likely to suppress decay indirectly, by acting upon host resistance, rather than by acting directly on the pathogen, since most pathogenic fungi grow under these conditions. In order to suppress the main pathogens directly, O_2 should be lowered to 1% or less. However, excessively low O_2 concentrations result in anaerobic respiration and the accumulation of alcohols and aldehydes in the tissues, which leads to the development of off-flavors and irreparable damage to the tissues (Sommer, 1982). Similarly, elevating the CO_2 level of the atmosphere above 5% suppresses fruit respiration, but has almost no direct effect on fungal growth. In order to inhibit fungal growth significantly, the level of CO_2 must be increased to about 10% or more (Wells and Uota, 1970) (see Fig. 23B). However, only a few fruits can tolerate very high CO_2 levels for extended storage or transit periods. Most fruits are injured under these conditions and develop off-flavors.

Evaluation of CA Efficiency

Apples are an example of fruits for which CA is aimed primarily at retardation of ripening (Blanpied, 1990). Low O_2 and high CO_2, in combination with a low temperature, delay the climacteric of the fruit more than low temperature alone. Striking benefits were obtained with such combinations, especially in apple cultivars that suffer from chilling injury at temperatures near 0°C, and those which have a short storage life (Salunkhe and Desai, 1982; Sommer, 1985). A considerable improvement in the storage quality of several apple cultivars occurs following a reduction of the O_2 concentration in the atmosphere to 1-1.5%, immediately after harvest (Couey and Williams, 1982). Later findings showed the advantage of CA in which O_2 at less than 1% was combined with ethylene removal. Under these conditions apple fruits remained firmer and their resistance to pathogens, such as *Penicillium expansum* and *Monilinia fructicola,* was increased (Stow and Geng, 1990). Studying the effect of decreased O_2 concentrations combined with ethylene removal on Cox's Orange Pippin apples, Johnson et al. (1993) found that fruits stored in 0.75% O_2 were firmer and showed lower incidence of *Penicillium* and *Monilinia* rots than those stored in 1.0 or 1.25% O_2. This was achieved through the reductions both in primary infection and in secondary infection by contact between infected and sound fruit. The development of *Botrytis* and *Nectria* rots, however, was unaffected by the lower oxygen concentrations.

Low O_2 storage (1%) also considerably reduced *Botrytis* development in Kiwifruits at 0°C, along with the delay of the fruit softening and ethylene production, which are typical with infected fruit (Niklis et al., 1992).

Sitton and Patterson (1992) found that CA storage of several apple cultivars with CO_2 concentrations above 2.8% was more effective than low-O_2 oxygen atmospheres, in reducing decay development incited by *Botrytis cinerea* and *P. expansum.* However, the response to CO_2 treatment depended on the physiological age of the fruit. Apples that had been stored in air at 0°C for 8 months, prior to the CO_2 treatment showed increased skin discoloration, whereas younger apples, even under higher CO_2 did not show skin darkening. It was suggested that high-CO_2 CAs could be beneficially used to control postharvest disease in freshly harvested apples (Sitton and Patterson, 1992). High CO_2 (25%) was also found beneficial for cherry fruits by totally preventing decay development during both refrigeration and shelf life (Brash et al., 1992).

Avocados benefit greatly from CA storage. Early studies had already shown that the climacteric respiration peak of avocado fruits was delayed

by decreasing the O_2 concentration from 21% to 2.5% or raising the CO_2 level from 0.03% to 10% (Biale, 1946; Young et al., 1962). Storing Florida avocados for 45-60 days at 10°C in an atmosphere of 2% O_2 and 10% CO_2 resulted in a remarkable reduction in anthracnose (*Colletotrichum gloeosporioides*), the main cause of spoilage during storage and marketing, and at the same time prevented chilling injury, which is generally accompanied by increased susceptibility to fungal attack (Spalding and Reeder, 1975). Since such concentrations of O_2 and CO_2 have only a slight inhibitory effect on the growth of *C. gloeosporioides*, decay control in this atmosphere was attributed to maintenance of the fruits in the firm mature-green stage and thus to the maintenance of the quiescence of the fungus. In contrast to avocados, CA was only slightly, or not at all beneficial to papayas, for which, similarly, decay is the chief limiting factor in storage extension (Spalding and Reeder, 1974).

Atmospheres of 10-20% CO_2 or more reduce the incidence of decay caused by *B. cinerea* and *Rhizopus stolonifer* in several cultivars of strawberries, as exhibited, not only during cold storage but also after removal of the fruit to shelf-life conditions, without causing objectionable off-flavors (Harris and Harvey, 1973). Under these conditions decay suppression may be attributed both to the direct effect of the CA on the pathogens and to its effect on the physiological deterioration of the fruit. The resistance of the fruit to *Botrytis* and *Rhizopus* decay in a CO_2-enriched atmosphere was hypothesized to be the result of the high levels of acetaldehyde and ethyl acetate produced by fruit under such conditions (Shaw, 1969). An atmosphere enriched with CO_2, at 10, 15 or 20%, retarded storage decay of blueberries by 1-2 days after the berries had been removed from the CA and held at 21°C, whereas an atmosphere of 2% O_2 was ineffective on its own in suppressing decay, and did not enhance the effect of the CO_2 atmosphere (Ceponis and Cappellini, 1985). Decay suppression by high CO_2 (20%) has also been recorded in muskmelons after 14 days at 5°C followed by shelf-life conditions (Stewart, 1979), while CA (10-20% CO_2 + 10% O_2) along with ethylene absorbance prolonged the storage life of Galia melons, through the maintenance of tissue firmness and decay suppression (Aharoni et al., 1993a).

CA had considerable benefits over air for the long-term refrigerated storage of white cabbage (Geeson and Browne, 1980): storing cabbage in an atmosphere of 5-6% CO_2 + 3% O_2 at 0-2°C resulted in the retardation of general senescence effects, such as yellowing, toughening and loss of flavor, and in the inhibition of physiological disorders and the repression of decay development during prolonged storage.

One of the beneficial effects of CA storage is its ability to reduce chilling injury in certain sensitive crops. Several studies indicated that prestorage treatments of grapefruits with high concentrations of CO_2 were effective in inhibiting the development of pitting in cold storage (Hatton and Cubbedge, 1982). However, no reduction in chilling injury was induced by CA in other citrus fruits, such as limes and lemons (Spalding and Reeder, 1983; McDonald, 1986). Intermittent exposure of unripe avocado fruits to 20% CO_2 reduced chilling injury during storage at 4°C (Marcellin and Chaves, 1983). An atmosphere of 2% O_2 and 10% CO_2 prevented the development of anthracnose (*Colletotrichum gloeosporioides*) at 7.5°C and reduced chilling injury at 4.4°C in Fuchs avocados (Spalding and Reeder, 1975). Low-oxygen atmospheres were found to delay the development of chilling injury symptoms while reducing their severity, in peaches stored at 5°C (Ke et al., 1991), and to ameliorate chilling-induced pitting in zucchini squash (Mencarelli et al., 1983; Wang and Ji, 1989).

2. CONTROLLED ATMOSPHERE WITH CARBON MONOXIDE

The addition of CO to the atmosphere results in the suppression of various fungi sensitive to the gas, such as *Penicillium digitatum, P. italicum* and *Monilinia fructicola*. The effectiveness of this fungistatic gas is pathogen dependent and is greatly enhanced in combination with a low-O_2 atmosphere (2.3%). The addition of high CO_2 to this atmosphere further enhanced fungal growth inhibition, because of the additive effects of CO and CO_2 (El Goorani and Sommer, 1979, 1981).

The possibility of using CO atmosphere for decay suppression during storage has been studied for various commodities. Kader et al. (1978) showed that the addition of CO (5 or 10%) to a low-O_2 atmosphere (4%) reduced the incidence and severity of the gray mold decay in *Botrytis cinerea*-inoculated tomatoes at their mature-green or pink stage. When added to air, CO increased the CO_2 and ethylene production rates and, in parallel, hastened the ripening of mature-green tomatoes, while the addition of CO to a low-O_2 atmosphere had no or very little effect on these physiological responses. Studying the effects of various atmospheres on the severity of watery soft rot of celery caused by *Sclerotinia sclerotiorium*, Reyes (1988) found that whereas a low-O_2 CA (1.5%), with or without high CO_2 (16%), reduced decay development only slightly, a combination of 7.5% CO + 1.5% O_2 suppressed disease significantly without causing undesirable effects on celery quality. Applying mixtures of O_2 and CO to *B. cinerea*-inoculated apples inhibited

lesion development, and the inhibition was closely related to the decrease in the O_2 level from 8% to 2% (Sommer et al., 1981). The development of the CO-sensitive *Monilinia fructicola* in peaches was completely inhibited in cold storage by the addition of CO to a low-O_2 atmosphere (4%); however, a normal rate of rot development was resumed once the fruits were transferred to air at 20°C (Kader et al., 1982).

In spite of the efficiency of CO combined with low-O_2 atmospheres in decay suppression, the use of this gas is limited, mainly because of its high toxicity to humans and the fear of inhalation of the poisonous gas by workers. The use of CO is additionally limited by its tendency to mimic ethylene effects, as has been found in tomatoes, strawberries, sweet cherries and peaches (Barkai-Golan, 1990).

3. MODIFIED ATMOSPHERE PACKAGING

Sealing certain commodities in polymeric film packages promotes the extension of their storage life as a result of the formation of a modified atmosphere (MA) within the package. This practice is referred to as modified atmosphere packaging (MAP). The method is based on the alteration in the composition of the gases around the commodity within the sealed package. Elevation in the CO_2 level along with the decrease in the O_2 level in the package result in a decreased respiration rate of the stored fruits or vegetables, and the extension of their physiological life. The levels of the gases accumulated in the sealed package are determined by the respiration rate of the commodity, the storage temperature, the permeability of the film material to the atmospheric gases, the ratio of the produce weight or surface area to the area of semi-permeable film, among other factors (Smock, 1979).

In looking for suitable wrapping for strawberries exported from Israel to Europe by sea, it was found that polyethylene or polyvinyl chloride (PVC) film enabled fruit to be stored at 0-2°C for 10 days, followed by two additional days at 20°C (Barkai-Golan, 1990). The prolongation of the storage life was evident in the firmness and the fresh appearance of the fruit and the calyx, and the considerable reduction of decay, caused mainly by *Botrytis cinerea.* The incidence of decay was correlated with the level of CO_2 accumulated in the atmosphere around the fruit. During cold storage the level of CO_2 ranged between 1.5 and 2.5%, but the low temperature controlled decay. Following transfer to 20°C, CO_2 levels rose up to 7.5-12%; under these conditions, the suppressive effect of CO_2 on decay development is attributed mainly to its effect of delaying the

senescence processes of the fruit and maintaining host resistance. Decay during prolonged storage can be almost prevented by application of fungicidal sprays during the flowering season preceding polymeric film packaging (Aharoni and Barkai-Golan, 1987).

A reduction in the incidence of brown rot caused by *Monilinia fructicola* was recorded in sweet cherries packaged in polyethylene bags and stored at 0.5°C for 42 days. Disease reduction under these conditions was attributed to the modified atmosphere of 5.1% O_2 and 11.4% CO_2 produced within the package during storage (Spotts et al., 1998). A further reduction in disease incidence was achieved when a preharvest fungicide treatment had been applied prior to packaging, or when the fruit was treated with a postharvest antagonistic yeast (see the chapter on Biological Control). The disease-suppression effect of CO_2 in sweet cherries was regarded as fungistatic, since decay developed normally when the fruit was returned to air (De Vries-Paterson et al., 1991). In a study with d'Anjou pear fruits, the combination of MAP (2-6% O_2 and 0.5-1% CO_2) with an antagonistic yeast as a biological control agent was found to reduce the blue mold (*Penicillium expansum*) better than the modified atmosphere without the yeast or the yeast application followed by storage in air (Miller and Sigar, 1997).

MAP has been found to improve the storability of perishable commodities such as fresh herbs, broccoli and green onions. Packaging broccoli heads in sealed polyethylene bags inhibited both senescence and decay development in the flower buds, and kept the produce green and fresh during prolonged cold storage (0.5°C) followed by 2 days of shelf-life conditions (20°C) (Aharoni et al., 1986). The extension of the useful life of broccoli was attributed to the high respiration rate of this vegetable, which resulted in CO_2 accumulation from 2-3% in cold storage to 6-8% after transfer to shelf-life conditions (20°C); O_2 concentrations decreased to about 6% at shelf life. Decay suppression under these conditions was attributed to the delay in host senescence by the CO_2, because of the ability of the gas to curb the action of the endogenic ethylene, which plays a primary role in the control of aging of broccoli. The improvement of storability of broccoli heads in sealed PVC film is illustrated in Photo 1.

Green onions were found to resist a relatively high concentration of CO_2 (more than 15% for a short period) produced in sealed microperforated film (Aharoni et al., 1996b). The elevated level of CO_2, combined with the decreased level of O_2 inside the packaging reduced growth, yellowing and decay of the green leaves (Photo 2).

Photo 1. Delay of aging and decay in broccoli packaged in sealed polyethylene bag during storage and after bag opening.

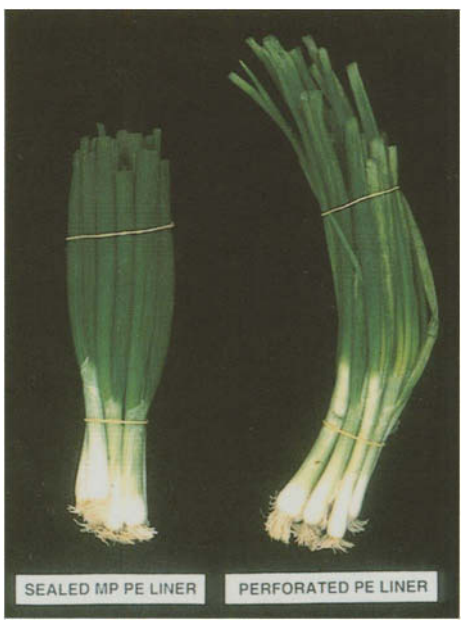


Photo 2. The effect of modified atmosphere packaging with a microperforated film on green onion.

The retardation of yellowing and decay in CA storage has been reported for various leafy vegetables, for which CO_2 and O_2 concentrations are controlled during storage (Lipton, 1987). MAP has proved effective for extending the postharvest lives of green herbs, including dill, parsley, coriander, chives, chervil, watercress and sorrel. Such an atmosphere was achieved by packaging fresh herbs in non-perforated polyethylene-lined cartons (Aharoni et al., 1989). Retardation of senescence processes in these yellowing-susceptible herbs was again attributed to the high CO_2 levels accumulated within the package and the ethylene-antagonistic action. However, only little commercial success was reported for the use of this procedure with green herbs, because of the difficulty in controlling the O_2 and CO_2 variations within the package. The drastic decrease in the O_2 level, combined with the increased CO_2 level, may lead to anaerobic respiration, fermentation and development of an off-flavor (Cantwell and Reid, 1993).

In order to obtain beneficial fungistatic concentrations of CO_2 within polymeric packages of sweet corn, retail packages were stored at 2°C within additional plastic liners (Rodov et al., 2000). The MA generated in these packages by the corn respiration complied with the recommended range of 5-10 kPa CO_2, and inhibited mold development. However, when the produce is transferred to shelf life, the outer layer should be opened to counterbalance the enhancement of the corn respiration at the higher temperature and to prevent O_2 depletion and off-flavor development.

Further advances in modified atmosphere packaging involve the development of 'active' or 'smart' films which, in addition to their ability to generate the conventional modified atmosphere around the commodity, are also capable of actively improving the atmosphere (Church, 1994). These properties have been imparted by introducing into the package, or into the packaging materials, compounds capable of altering the atmosphere composition by absorbing atmospheric gases, vapors, wetness and by-products. Ethylene, which is produced by fruits and vegetables during storage, may accumulate in the package and stimulate their ripening and senescence processes that may lead to shortening their postharvest life. By the use of ethylene-scavenging materials, the smart packaging is capable of retarding these processes, so providing a significant shelf-life extension. As a matter of fact, packaging broccoli in such a film doubled the shelf life of the fresh produce (Church, 1994). The shelf life of banana fruits sealed in bags made of polyethylene treated with the anti-ethylene compound, 1-methylcylclopropane, was extended from 16 days in non-treated polyethylene bags up to 58 days in

the anti-ethylene treated bags. Ethylene-mediated ripening changes were markedly delayed, suggesting that MAP, in combination with the potent antagonist of ethylene action, may serve as a technology for long-distance transport of green bananas without refrigeration (Jiang et al., 1999).

For food products other than fruits and vegetables, developments in 'smart' packaging include films impregnated with anti-microbial compounds that actively retard the development of harmful microorganisms during storage. Church (1994) indicates that changes in the gas composition within the package during storage may provide an indirect indication of the condition of the product. Therefore, labels which change color in response to the concentrations of CO_2, O_2 and other gases in the atmosphere could serve as indicators to detect damage in the packaged product.

With the development of polymeric films suitable for the providing the advantages of MAP, and the increased choice of packaging materials, the number of products that can benefit has increased. Today, thanks to the introduction of the 'smart packages', the in-package atmosphere offers the potential to extend the technology to a wider range of products and markets.

4. HYPOBARIC PRESSURE

Hypobaric or low-pressure (LP) storage, similarly to modified or controlled atmosphere storage, is designed to delay ripening and senescence processes in fruits and vegetables, and to extend their postharvest life. In this procedure, the commodity is maintained in a vacuum-tight and refrigerated container, held at subatmospheric pressure and continually ventilated with humidified air at relative humidity (RH) of 80-100% (Burg, 1990; Dilley et al., 1982). With the reduction in the atmospheric pressure beneath 760 mm Hg, the oxygen level in the atmosphere is reduced. Under continuously ventilated partial pressure, CO_2, ethylene and various volatile by-products of metabolism rapidly diffuse out of the commodity and are flushed from the storage chamber. As a consequence of the low partial pressure of O_2 and the low levels of ethylene in the atmosphere, ripening and senescence of fresh fruits and vegetables are delayed and storage life is extended.

Following the disclosure of hypobaric storage technology by Burg and Burg in 1966, extensive investigations have been conducted with a wide range of fruits and vegetables (Laugheed et al., 1978). Although the high-RH conditions under LP storage appear ideal for fungal growth and decay development, several studies report on the beneficial effects of the

combination of these conditions on decay control. Hypobaric pressure has direct fungistatic effects on fungal spore germination and mycelium growth of various storage fungi. Apelbaum and Barkai-Golan (1977) showed that the degree of inhibition increased with the reduction in pressure below 150 mm Hg. Reducing the pressure from 760 to 100 mm Hg inhibited spore germination of *Penicillium digitatum*, while inhibition of spore germination of *Botrytis cinerea* and *Alternaria alternata* occurred only at 50 mm Hg. Such a level had no effect on germination of *Geotrichum candidum* spores. Reducing the pressure to 25 mm Hg totally prevented spore germination of the first three fungi mentioned but had almost no effect on the germination of *Geotrichum* spores (Fig. 24). Colony growth and the consequent fungal sporulation of the four fungi were markedly reduced at 50 mm Hg, while at 25 mm, *Geotrichum* was the only fungus exhibiting some growth. Transfer of inhibited cultures from hypobaric to atmospheric pressure resulted in renewed growth, suggesting that there was no irreversible damage to the fungi.

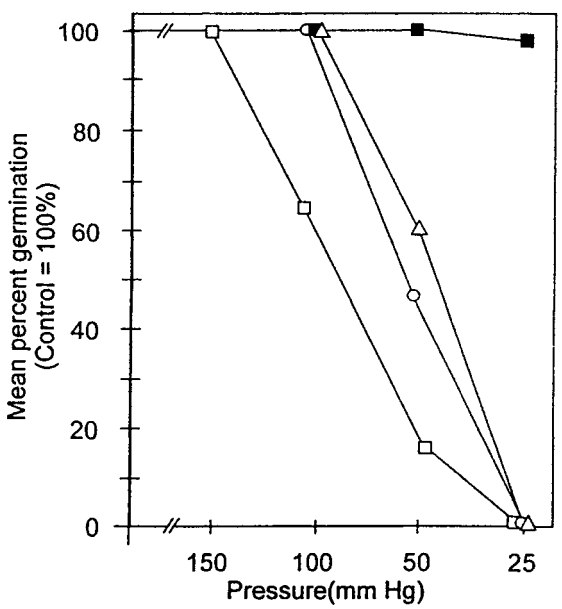


Fig. 24. Hypobaric pressure effects on spore germination of *Penicillium digitatum* (□), *Botrytis cinerea* (○), *Alternaria alternata* (Δ) and *Geotrichum candidum* var. *citri-curantii* (■). (Reproduced from Apelbaum and Barkai-Golan, 1977 with permission of the American Phytopathological Society).

The reduction in fungal growth under hypobaric pressure can be attributed to the reduction in the partial O_2 tension, which decreases gradually with the decrease in the total pressure to 100 or 50 mm Hg. However, growth retardation may be due, at least in part, to other factors, such as reduction in the CO_2 partial pressure or the accelerated escape of volatile compounds from the tissues (Apelbaum and Barkai-Golan, 1977).

Beneficial effects of hypobaric storage have been reported for various fruits. Storing apples under LP conditions resulted in delayed softening, control of physiological disorders and reduced decay development, which resulted in extended shelf life after removal from storage (Dilley et al., 1982; Laugheed et al., 1978). Several Florida cultivars of mango which ripened in air at shelf-life temperature, showed less anthracnose (*Colletotrichum gloeosporioides*) and stem-end rot (*Diplodia natalensis*) when they had previously been stored under LP at 13°C. The reduction in decay coincided with a retardation in fruit ripening, permitting a prolonged storage at 13°C (Spalding and Reeder, 1977). Similarly, exposure of papayas to LP at 10°C during shipment, inhibited both ripening and disease development as compared with fruit stored in refrigerated containers at normal atmospheric pressure (Alvarez, 1980). Studies on LP storage of avocados suggested that atmospheres both low in O_2 and high in CO_2 are required for successful suppression of anthracnose development and for increasing the percentage of acceptable fruit retained after softening in air under normal pressure (Spalding and Reeder, 1976). Studies with various 'fruit-vegetables', such as tomatoes, peppers and cucumbers, found a high incidence of decay after storage at subatmospheric pressure; in order to suppress decay, the combination of LP with postharvest fungicidal treatments was required (Bangerth, 1974).

C. GROWTH REGULATORS

Plant growth regulators, or plant hormones, may – similarly to low temperatures – suppress decay development indirectly, by retarding ripening and senescence processes in fruits and vegetables, and maintaining the natural resistance of young tissue.

Prolonged storage of citrus fruits generally results in the development of stem-end fungi, mainly *Diplodia natalensis, Phomopsis citri, Alternaria citri* and *Fusarium* spp. Applying the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) to citrus fruits prior to storage

delayed aging of the stem-end button, which is the usual point of attack by the stem-end fungi, resulting in considerable reduction of stem-end rot (Schiffmann-Nadel et al., 1972). The pathogenic fungi lodge in the button area in a quiescent state, and the delayed dropping of the button from the fruit which results from 2,4-D treatment delays their transition to the active state, so retarding decay development. The effectiveness of 2,4-D in preserving green buttons and suppressing decay development, has been proven both by using preharvest spraying in the orchards and by postharvest treatment by dipping the fruits in aqueous solutions of 2,4-D or of waxes containing 2,4-D (Stewart, 1949; Schiffmann-Nadel et al., 1972). Most lemons grown in California are treated with 2,4-D before storage to delay senescence of the button, and a wax formation containing 2,4-D is applied to various citrus cultivars in South Africa and Israel, to control stem-end rot during long-distance shipment and prolonged storage (Ben-Arie and Lurie, 1986).

Gibberelins, which are hormones found naturally in plant tissue, may also function in a similar way. The increased susceptibility of citrus fruits to *Penicillium digitatum* and *P. italicum*, as they become physiologically older, may be due to the reduction in resistance to fungal entry as well as to an improvement of the rind as a medium for the pathogen growth. Gibberelic acid (GA_3) application delayed the senescence of the rind of Navel oranges, as exhibited by softening, the appearance of intercellular spaces, weakened or broken cell walls and increased fruit resistance to infection (Coggins and Lewis, 1965).

A more recent study (Ben-Yehoshua et al., 1995) indicated that dipping lemon fruits in GA_3 (50 or 100 ppm) or in 2,4-D (200 ppm) prior to storage retards the decrease in the antifungal citral compound in the fruit rind (the flavedo), suppresses the antifungal activity of the rind and results in reduced decay during storage. The inhibition of the decomposition of the antifungal compounds in the rind is the basis of the mode of action of the growth regulators in decreasing decay in the harvested lemon fruit.

Pre-harvest sprays with GA_3 (20 mg ml^{-1}) applied during the development of persimmon fruits were effective in reducing decay caused by the black spot disease (*Alternaria alternata*), which typically appears in the high humidity environment beneath the calyx in stored persimmon fruits (Perez et al., 1995). As a result of GA_3 treatment, the calyx of the fruit remained erect until harvest and fruit softening was inhibited, and in parallel, the fruit area covered with the black spot was markedly reduced during 3 months of storage at 0°C. The plant hormone had no

effect on fungal development *in vitro,* or *in vivo* on inoculated fruits, and its effect on decay development was attributed to the enhancement of fruit resistance. A pre-harvest combination of GA_3 and iprodione treatments may further reduce the percentage of fruits rendered unmarketable by the black spot disease (Perez et al., 1995).

Retardation of senescence by growth regulators has also been reported for leafy vegetables. Fresh herbs are prone to accelerated senescence because of their high rate of metabolism, which is further increased following harvesting and handling procedures. Their accelerated senescence is expressed in decreased levels of RNA, proteins, fats, carbohydrates, organic acids and vitamins, and increased enzymatic activity of RNase, protease, lypase and other hydrolytic enzymes, as well as those associated with fat oxidation. These changes are accompanied by chloroplast destruction and chlorophyll decomposition, resulting in a rapid yellowing of the leaves (Aharoni, 1994). During harvesting, grading and packaging, when the leaves lose water because of evaporation, and suffer from mechanical injury, the senescence process is further enhanced and is accompanied by increased ethylene and respiration levels. The endogenous ethylene continues to accelerate chlorophyll destruction and leaf senescence (Meir et al., 1992). Along with their aging, the leaves lose their natural resistance to pathogens and tend to rot. In many cases, the senescence process is controlled by plant hormones; it is accompanied by decreases in the levels of growth-stimulating hormones – gibberelin, cytokinin and auxin, and increases in those of growth-inhibiting hormones – ethylene and abscissic acid (ABA). It is not surprising that application of synthetic hormones – gibberelin, cytokinin and a very low concentration of auxin – was very efficient in delaying leaf senescence, while application of ABA or ethylene accelerated it (Aharoni and Richmond, 1978). Delay of senescence by gibberelin and cytokinin is attributed to their antagonistic effects on ethylene and to their ability to neutralize the stimulation of chlorophyll destruction caused by ethylene (Aharoni, 1989).

The delay of aging conferred by GA_3 has also been found in several fresh herbs which tend to yellow, such as chives, dill, chervil, coriander and parsley (Aharoni, 1994). In a laboratory experiment with chives, a dip in GA_3 solution (10 ppm) was found to prevent the accelerated respiration caused by the cutting stress, and then to prevent the climacteric respiration associated with leaf senescence. A marked effect on respiration was achieved by spraying chives in the greenhouse, close to harvesting. Such a treatment delays chlorophyll destruction, retards

the climacteric respiration and maintains the level of climacteric ethylene (Aharoni, 1994) (Fig. 25). Along with the delay in the senescence process, there was a reduction in leaf decay during storage.

Experiments conducted in Israel with Romaine lettuce showed that a single spray in the field with GA_3 (25 ppm), several hours prior to harvest, delayed deterioration during storage by delaying leaf yellowing. In parallel, there was a reduction in the rate of decay caused mainly by *Sclerotinia sclerotiorum* and soft rot bacteria (N. Aharoni and R. Barkai-Golan, unpublished data).

The addition of GA_3 (10 ppm) to a disinfecting solution containing thiabenolazole (TBZ) and chlorine resulted in considerable delay in celery senescence as expressed by the delayed yellowing of the petioles and leaflets. Along with the maintenance of celery freshness, fungal soft rots caused by *S. sclerotiorum* and *Botrytis cinerea*, and bacterial soft rot caused by *Erwinia carotovora* were reduced (Barkai-Golan and Aharoni, 1980). It is interesting to note that the decrease in decay following GA_3 treatment is greater than the decrease caused by the chemicals alone, and that is manifested mainly in the prevention of decay progress along the petiole during storage. TBZ itself exhibits activity similar to that of the plant hormones, and it has a synergistic effect with GA_3 in the delay of senescence processes in celery leaves and petioles. No direct effect of

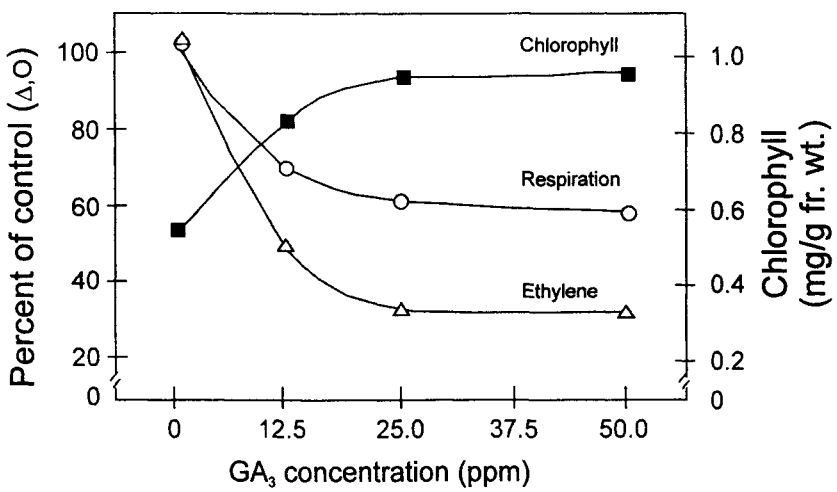


Fig. 25. Effect of GA_3 concentrations applied to chives immediately before harvest, on levels of chlorophyll, respiration (CO_2 emanation) and ethylene production rates after 7 days at 20°C in darkness (Reproduced from Aharoni, 1994).

GA_3 on fungal and bacterial growth *in vitro* has been recorded, and its effect on the development of pathogens has been attributed to maintaining the decay resistance of the fresh celery heads.

Dipping harvested celery heads in GA_3 solution with TBZ and chlorine served for many years in Israel as a standard treatment in celery intended for export. A recent study showed that spraying the fields with GA_3 before harvest delayed the natural decrease in the antifungal compound marmesin (Afek et al., 1995a). Maintenance of the high level of marmesin in the petiole tissues after harvest was suggested to be the mode of action by which the plant hormones inhibit decay development in stored celery.

Delay of deterioration by the synthetic auxin, h-naphthalene acetic acid (NAA) was studied in eggplants (Temkin-Gorodeiski et al., 1993). Eggplant fruits deteriorate during prolonged storage, mainly because of accelerated senescence of the calyx, which precedes deterioration of the fruit. Dipping the calyx in NAA solution (200 ppm) maintained calyx freshness, which is an important aspect of the appearance of the fruit, but it did not prevent decay development. The addition of the fungicide prochloraz (900 ppm) retarded both calyx senescence and decay development, caused mainly by *A. alternata*, and kept the fruit firm. Dipping the whole fruit gave even better results and the residual level of prochloraz in such fruits was less than 0.34 mg l^{-1}, which is lower than the maximal level approved by the authorities in Europe. A triple combination of NAA, fungicide solution (Prochloraz, 900 mg l^{-1} active ingredient) and bulk packaging in polyethylene-lined bags with water-absorbent materials, prevented water condensation and maintained eggplant quality along with reduced incidence of decayed fruit during storage (Fallik et al., 1994b). The higher levels of CO_2 obtained within the bags (1.2%) may have partially contributed indirectly to the inhibition of decay development, by delaying fruit senescence. Such a combination could considerably expand the export potential of high-quality fruit (Fallik et al., 1994b).

The use of auxins is limited since, in high concentrations, these compounds might accelerate ethylene production and thus enhance the aging process. In general, plant growth regulators may be of special importance mainly for fruits and vegetables that cannot be stored at low temperatures because of their cold sensitivity, as well as in developing countries where cold storage cannot be applied.

D. CALCIUM APPLICATION

Another means of suppressing storage disease by maintaining or enhancing the natural resistance of fruits and vegetables to pathogens, is to increase the calcium content of the various plant organs (Conway et al., 1994b). Calcium is an essential element which influences the growth and fruiting of plants and contributes to preserving the structural integrity and functionality of membranes and the cell wall during fruit ripening and senescence.

Calcium treatments applied to improve fruit and vegetable quality have primarily addressed the association of low calcium content in plant tissue with the development of physiological disorders. Calcium treatment may reduce storage disorders, such as bitter pit and internal breakdown in apples (Bangerth et al., 1972; Reid and Padfield, 1975) or the internal brown spot in potato tubers (Tzeng et al., 1986). However, many reports have indicated that an increase in tissue calcium content also led to reductions in fungal and bacterial decay. It was thus found that pre-harvest calcium sprays reduced the rate of storage losses caused by *Gloeosporium* spp. in apples (Sharples and Johnson, 1977), or *Botrytis* and *Geotrichum* rots in stored grapes (Miceli et al., 1999), while postharvest calcium treatments reduced the rate of the blue mold disease caused by *Penicillium expansum* in this fruit (Conway and Sams, 1983). Similarly, bacterial soft rot caused by *Erwinia carotovora* pv. *atroseptica* in potato tubers decreased as tissue calcium increased (McGuire and Kelman, 1984).

Various methods for increasing the calcium concentration in storage organs have been investigated. Applying Ca to harvested fruit seems to be the best method for increasing the calcium content in apples; active infiltration procedures with $CaCl_2$ solution, such as vacuum or pressure that force solutions into the fruits, were more effective in reducing decay following inoculation with *P. expansum*, than dipping into the solution (Conway et al., 1994a). Vacuum infiltration of $Ca(NO_3)_2$ solutions also increased potato tuber calcium and was efficient in reducing soft rot in tubers inoculated with *E. carotovora* pv. *atroseptica*, although an increase in the calcium content of potato tubers could have been achieved by calcium fertilization during the growth period (McGuire and Kelman, 1984).

Calcium enters the tissues through lenticels (Betts and Bramlage, 1977), but cracks in the cuticle and epidermis may also provide important points of entry. The extent of cracking may, therefore, play a

significant role in calcium intake and affect the efficiency of the treatment. However, the amount of calcium taken in during treatment also depends on the growing conditions and environmental factors, and can vary with the cultivar and with the state of maturity of a given cultivar. Experiments with 'Golden Delicious' apples showed that calcium absorption in fruits picked too early (2 weeks before the prime harvest period) and treated with $CaCl_2$ solution was low and did not inhibit decay. However, fruit picked 2 weeks after prime harvest absorbed large quantities of calcium that markedly reduced decay development, but caused severe calcium injury. The optimum calcium treatment at prime harvest reduced decay 40% with no injury (Conway and Sams, 1985).

Most of the calcium that penetrates into the host tissue seems to accumulate in the middle lamella region of the cell wall. The calcium-induced resistance of storage organs (fruits or tubers) to postharvest pathogens has been attributed to an interaction between the cell wall pectins and Ca ions. By binding to pectins in the cell wall, Ca ions contribute to maintaining the structural integrity of the cell wall. Pectins are chains of polygalacturonic acid residues into which rhamnose is inserted. The rhamnose insertion causes kinks in the chain, which allow the attachment of Ca ions. The cations form bonds between adjacent pectic acids or between pectic acids and other polysaccharides, forming cross bridges which make the cell walls less accessible to the action of pectolytic enzymes of the pathogen (Preston, 1979). When apples were infiltrated with Ca, or potato tubers were fertilized with high Ca or infiltrated with Ca, the quantity of Ca bound to the cell walls increased (Conway et al., 1987; McGuire and Kelman, 1984), and cell walls became less vulnerable to the pectolytic enzymes of the pathogen. The reduction in decay caused by *P. expansum* in apple fruits has thus been correlated, at least in part, to the improved structural integrity of the tissues imparted by an increase in the Ca content of the cell walls (Conway et al., 1987).

Analysis of the pectic fractions of grape berries cv. Sauvignon, known to be resistant to *Botrytis cinerea*, showed that the content of host cell-wall pectins decreased following infection with this pathogen. At the same time, the proportions of water and a chelator-soluble protein increased. The changes in the pectin composition of the berries were markedly less pronounced when the berries were infiltrated with calcium prior to infection (Chardonnet et al., 1997). It was suggested that dimethylated proteins produced by *B. cinerea* are probably protected by a rapid calcium chelator.

Applying $CaCl_2$ solution to developing table grapes cv. Italia during the season increased calcium content in the whole berries and in the peel, resulting in reduced decay incidence during cold storage and the subsequent shelf life (Miceli et al., 1999). In this case, calcium-treated berries infected by *B. cinerea* showed an increase in the cellulose content of cell walls, but no changes in the water-soluble pectin or hemicellulose contents. However, a higher protopectin content was recorded in the cell walls of Ca-treated berries after infection, indicating the involvement of Ca ions in the stabilization of the cell-wall structure that led to increased resistance to the pathogen (Miceli et al., 1999).

Although prolongation of storage life as a result of calcium application is thought to be due mainly to the role of calcium in ameliorating physiological disorders and thus indirectly reducing pathogen activity, direct effects of calcium on the pathogen have also been recognized. Calcium interfered with spore germination and germ tube elongation of *P. expansum* and *B. cinerea* (Conway et al., 1994a). At low concentrations, calcium may also directly inhibit polygalacturonase activity. Conway et al. (1994a), who studied the commercial potential of Ca for maintaining the quality of apples in storage, found that the Ca content of fruits from trees that had been sprayed with $CaCl_2$ solutions throughout the growing season was significantly increased but not enough to be expected to retard pathogens infecting wounded fruits. Pressure-infiltrating fruits after harvest with high $CaCl_2$ concentrations (4-8%) resulted in a tissue Ca level that would potentially maintain fruit firmness and retard decay, but it was suggested that proper sanitation, to reduce fungal infection that could arise from the pressure-infiltration procedure, should also be implemented before this technique could be seriously considered for commercial use. Furthermore, such concentrations stimulate pectate lyase activity; in order to inhibit this activity, higher calcium concentrations would be required (Conway et al., 1994a).

Biggs (1999), who studied the potential role of preharvest calcium supplementation of apples in reducing pathological diseases, found that calcium salts directly suppress bitter rot caused by *Colletotrichum gloeosporioides* and *C. acutatum*. Calcium chloride and calcium propionate at 1000μg of calcium per milliliter had no effect on conidial germination, but markedly inhibited germ-tube growth of these pathogens. These two salts, as well as calcium silicate, also inhibited mycelial growth: fungal dry weight in liquid culture media was reduced. When calcium salts were applied to wounded apples prior to inoculation

with *Colletotrichum* spp., fruits treated with calcium chloride and calcium propionate exhibited delayed formation of acervuli (aggregated conidial structure of *Colletotrichum*) by the fungi, and markedly reduced lesion development. Reduced rates of infection were demonstrated in field trials, when fruits treated with weakly diluted applications of calcium salts were inoculated with conidia of either *C. gloeosporioides* or *C. acutatum*.

Biggs et al. (1997) demonstrated the direct effect of 18 calcium salts (at 600 mg calcium per liter) on the colony growth of *Monilinia fructicola*, the cause of brown rot, mainly on stone fruits. All the salts, except calcium formate, calcium pantothenate and dibasic calcium phosphate, reduced fungal growth on amended potato-dextrose agar after 7 days at 20°C. Calcium propionate was the most inhibitory compound, reducing growth by 90%. Calcium hydroxide, calcium oxide, calcium silicate and calcium pyrophosphate reduced growth by approximately 65% compared with the control, and were not significantly different from each other. In general, salts that were inhibitory on amended agar medium were also inhibitory in a liquid culture (potato-dextrose broth). However, the effect of calcium salts on fungal growth in the liquid culture, but not on the agar medium, was correlated with the incidence and severity of disease in detached non-wounded fruit inoculated by spraying with *Monilinia* spore suspension. It was suggested that calcium salts are more likely to remain in continuous contact with fungal mycelium in the liquid culture system and that future tests should utilize growth in a liquid medium for preliminary determination of toxicity levels (Biggs et al., 1997). All the salts examined, except dibasic calcium phosphate and calcium tartrate, inhibited polygalacturonase (PG) activity of *M. fructicola*. The greatest inhibition of PG was caused by calcium propionate (85%), followed by calcium sulfate, tribaric calcium phosphate, calcium gluconate and calcium succinate. These four salts reduced PG activity by approximately 75%. Regarding these results, it is interesting to note that disease incidence and severity following calcium treatment were correlated with PG activity. The ability of calcium salts to reduce fungal growth and PG activity directly suggested the possibility that the effects of calcium on the incidence of brown rot and lesion diameter may result partly from suppressed pathogen activity.

Calcium propionate, which proved to be the most active salt inhibitor of fungal PG, has been extensively used as a food additive, and is known as an inhibitor of molds and bacteria in stored grain (Raeker et al., 1992). Furthermore, a substance such as calcium propionate, that has no

activity against yeasts, could be used to supplement biological control by yeasts (Biggs et al., 1997). As a matter of fact, calcium salts were actually found to improve the efficacy of yeast biocontrol against various postharvest pathogens (McLaughlin et al., 1990; Droby et al., 1997).

Combined Treatments

Calcium application can be combined with postharvest fungicides, heat treatment or biological control, allowing a lower concentration or level of either of the components while maintaining the effectiveness of the treatment in decay suppression. For combined treatments comprising heating or biological control with Ca application, see the chapter on Physical Means - Heat Treatments, or that on Biological Control - Combined Treatments and Integration into Postharvest Strategies.

New combinations which have recently been described (Saftner et al., 1997) include $CaCl_2$ application plus polyamine biosynthesis inhibitors. Three different polyamine biosynthesis inhibitors have each been found to reduce the *in vitro* growth of *B. cinerea* and *P. expansum,* either alone or in combination with $CaCl_2$, whereas $CaCl_2$ applied alone had no effect on fungal growth. However, experiments *in vivo* showed that pressure infiltration of each of the polyamine inhibitors or of $CaCl_2$ solution into apples, reduced soft rot development by the two pathogens. A combination of Ca with a polyamine inhibitor enhanced the decay reducing effect of either of the separate treatments. It was, therefore, concluded that a combination of specific polyamine inhibitors with $CaCl_2$ pressure infiltration is capable of effective decay suppression, although fruits treated with such a combination were less firm than fruits treated with Ca alone (Saftner et al., 1997).

CHAPTER 9

CHEMICAL CONTROL

Chemical substances intended for controlling postharvest diseases may be fungicides and bactericides (lethal to fungi and bacteria), or fungistats and bacteristats (inhibiting fungal and bacterial development). To serve its purpose, the chemical should come into contact with the pathogen; the minimal effective concentration is pathogen dependent. Chemical treatments can be applied under various strategies, with various timings: (1) preharvest application to prevent infection in the field; (2) sanitation procedure to reduce the level of inoculum in the environment to which injured fruits or vegetable are liable to be exposed; (3) postharvest application to prevent infection through wounds and to eradicate or attenuate established infections, so as to prevent their development and spread during storage. In order to choose the appropriate strategy for decay control, we have to understand the mode of infection of the pathogen, its time of infection, and the environmental factors that may affect disease development.

A. PREHARVEST CHEMICAL TREATMENTS

The possibility of controlling well established pathogens by postharvest disinfection is very small since most fungicides are unable to penetrate deeply into the tissues, and effective concentrations of the fungicide would not reach deep-seated infections. The effective way to reduce infections initiated in the field, including quiescent infections, is the application of broad-spectrum protective fungicides to the developing fruit on the plant, in order to prevent spore germination or infection establishment in the lenticels or in floral remnants of the fruit. In practice, preharvest fungicides are combined with other preharvest treatments applied against pests in the field.

A classic example of an effective preharvest treatment is the preventive spraying of citrus fruit in the grove with fixed copper compounds, to inhibit incipient infections of brown rot (*Phytophthora citrophthora*) in the fruit peel. In this case, the preventive sprays are essential for disease control, since penetration of the fungal zoospores

into the fruit occurs on the tree. Furthermore, the success of the preventive sprays depends on the time of infection: since germination of the zoospores depends on water, the sprays should be applied prior to the rains (Timmer and Fucik, 1975). Oranges are sprayed with benomyl before harvest, to prevent the development of stem-end rot, which arises from infections of *Diplodia natalensis* and *Phomopsis citri* initiated on the tree in the button of the fruit where they remain quiescent until the buttons start to separate from the fruit (Brown, G.E. and Albrigo, 1972).

Protective sprays in the plantation have been widely used to prevent anthracnose (*Colletotrichum gloeosporioides*) in various tropical and sub-tropical fruits, since this fungus penetrates the young fruit on the tree, and establishes a quiescent infection. The developing fruits are sprayed to prevent spore germination and subsequent formation of appressoria and infective hyphae, which are the quiescent stages of the fungus. Plantation spraying every 7-14 days successfully prevents anthracnose on mangoes (Prusky et al., 1983), avocados (Darvis, 1982; Muirhead, 1981a), papayas (Alvarez et al., 1977) and bananas (Griffee and Burden, 1974; Slabaugh and Grove, 1982). In addition to the reducing the incidence of anthracnose, preharvest spraying of papaya fruits reduces the infectivity of *Phytophthora* and *Alternaria* and the inoculum levels of the stem-end fungi, *Botryodiplodia, Mycosphaerella (Ascochyta),* and other fungi. These sprays reduce the 'infection pressure' on the fruit at the time of harvest and enable hot water treatment, applied after harvest, to complete the control of fruit decay (Aragaki et al., 1981). Furthermore, field sprays of mancozeb on papaya were also found to reduce postharvest Rhizopus soft rot, probably by reducing field-initiated fruit diseases caused by *Colletotrichum* and *Phomopsis* species; lesions caused by these fungi may serve as courts of infection for *Rhizopus stolonifer*, which requires 'wounds' to penetrate the host (Nishijima et al., 1990).

The gray mold (*Botrytis cinerea*), which is the major decay in stored strawberries, results in part from fungal penetration through the aging floral parts at the base of the fruit, and the formation of quiescent infections in the young, developing fruits while still in the field. Upon harvest, no initial infections are visible, but the fungus begins active development during marketing, producing typical rot 'nests'. Therefore, a good control should both reduce the spore contamination or the inoculum level on the flowers, and prevent the formation of quiescent infections in the young fruits (Sommer et al., 1973). Fungicidal sprays with the systemic benzimidazole compounds, during the flowering period,

successfully controlled both preharvest and postharvest diseases in the early 1970s (Jordan, 1973). Following the emergence of benzimidazole-resistant *B. cinerea* strains because of the very specific site of action of the chemicals (Dekker, 1977), dicarboximide fungicides (including iprodione and vinclozolin), which have a different mode of action, were adopted for preharvest application to soft fruits. Sprays with iprodione several times during the flowering season have provided good control of Botrytis rot during subsequent storage (Aharoni and Barkai-Golan, 1987; Dennis, 1983a). In this case, the use of preharvest treatments not only matches the mode of fungal penetration, but is probably the only way to apply chemicals for decay control because of the great sensitivity of the fruit to wetting. However, dicarboximide-resistant strains have also been found on soft fruits (Hunter et al., 1987). Furthermore, similarly to the benzimidazole compounds, the dicarboximides are also ineffective against *Mucor* and *Rhizopus* species, and their use may result in increased incidence of postharvest diseases caused by these fungi (Davis and Dennis, 1979). Following the discovery of dicarboximide-tolerant *B. cinerea* strains in strawberry fields and in cucumber greenhouses, control strategies, including the use of dicarboximides in combination with other fungicides or in rotation with an unrelated fungicide, were developed (Katan, 1982, Katan and Ovadia, 1985; Creemers, 1992).

Field treatments are generally less effective than postharvest treatments in controlling wound infections, since only part of the field-sprayed fungicides will remain attached to the product to protect wounds that occur later. In addition, fungicides that do remain on the surface of the produce may be removed by washing and waxing (Eckert, 1978). However, several investigations have indicated that in some cases field sprays may also be effective in reducing wound decay or lenticel decay, because of the sedimentation of the fungicide in the infection site and its maintenance in appropriate levels. It was thus possible to reduce wound infection in *Rhizopus*-inoculated peaches by sprays of dicloran applied in the orchard 1 week before harvest, while orchard sprays with benomyl reduced lenticel infection by *Gloeosporium* spp. in apples, and orchard sprays with benomyl, thiabendazole and carbendazim in the 1970s controlled the blue mold (*Penicillium expansum*) and the gray mold (*B. cinerea*) in stored pears (Eckert and Ogawa, 1988). Similarly, sprays of benomyl and thiabendazole, applied in the grove 30 days before harvest, markedly reduced the green mold (*Penicillium digitatum*) in stored oranges (Brown and Albrigo, 1972; Gutter and Yanko, 1971).

Although a postharvest treatment is usually more effective in cases where wound infection is involved, preharvest sprays may be a suitable control means when considerable harvest injury is involved and handling practices make postharvest treatment difficult to apply soon after harvest. Thus, orchard sprays may be the best means for reducing decay in peaches that will be subjected to controlled ripening after harvest, and in oranges that will be subjected to degreening, since both of these practices often increase decay by wound pathogens (Eckert and Ogawa, 1985).

When dealing with preharvest sprays, it is important to emphasize the need for careful selection of the fungicides. With the emergence of fungal strains resistant to chemicals, we frequently need to replace a chemical, previously proven to be effective, with another chemical or with two or several different compounds. Furthermore, there is a significant risk that residues from preharvest treatments will encourage the buildup of fungicide-resistant strains of the pathogens, and these will not allow us to benefit from postharvest treatment with the same fungicide or with related fungicides with similar chemical structures (Eckert and Ogawa, 1988).

B. SANITATION

Fruits and vegetables that have been injured during harvest or shipping, and have succeeded in avoiding infection by wound pathogens, are still liable to come into contact with the pathogens during packing or storage. Since disease development requires the presence of a given pathogen along with an available wound for penetration, a reduction in either of these factors will lead to the suppression of disease development.

Wounding can be minimized by careful harvesting, sorting, packaging and transportation, including preventing the fruit from falling at all stages. Regarding the avoidance of wounds one should remember that physiological injuries caused by cold, heat, oxygen deficiency, and other environmental stresses, also predispose the commodity to attack by wound pathogens. A general reduction in wound formation should also take such factors into consideration, even when no external symptoms can be distinguished.

The level of inoculum may be reduced by careful and strict sanitation procedures. The air of the packinghouse permanently carries an

abundance of pathogenic spores, which may originate from infected fruits and vegetables covered with spores, or from plant remnants in the packinghouse or its surroundings, which serve as substrates for many pathogenic fungi. During the citrus packing season, spores of *Penicillium digitatum* and *P. italicum* form the most conspicuous components of the spore population in packing houses and their vicinity. Fruit containers, the equipment in the packinghouse, as well as the workers' hands and tools, all bear pathogenic fungal spores during this season. Fresh fruits arriving at the packinghouse may, therefore, come into contact with pathogenic spores from any of these sources (Barkai-Golan, 1966).

Many fruits and vegetables have to be cleaned and washed on arrival at the packinghouse, in order to remove soil particles, dust or other contaminants. Water that does not contain disinfectants becomes heavily contaminated by fungal spores and bacterial cells and may infect products treated with non-recirculated water. Chlorine is the principal disinfectant used to sanitize wash water in packinghouses. Solutions of hypochlorous acid and its salts (sodium or calcium hypochlorite) are the most effective and economical agents available for destroying microorganisms in water and they have been applied widely to reduce bacterial contamination of the water used to wash or to hydrocool fruits and vegetables (Eckert and Sommer, 1967). Elemental chlorine (Cl_2) reacts with water to form hypochlorous acid (HOCl), hydrogen ion, and chloride ion:

$$Cl_2+H_2O \rightleftharpoons HOCl + Cl^-+H^+$$

Hypochlorous acid is a weak acid (pk_a=7.46), which dissociates to hypochlorite ion (OCl^-) and H^+:

$$HOCl \rightleftharpoons OCl^-+H^+$$

Hypochlorous acid and OCl^- are in equilibrium in the solution, but the antimicrobial effectiveness of the chlorine is correlated with the concentration of the hypochlorous acid in the solution (Bartz and Eckert, 1987). The relative concentration of the hypochlorous acid and its antimicrobial activity depend on the pH of the solution (Fig. 26).

If the initial concentration of hypochlorous acid is not sufficient to kill microbes on contact, the hypochlorous acid formed by conversion from hypochlorite ion should become involved, provided the exposure period is long enough and the pH is not too high to inhibit the

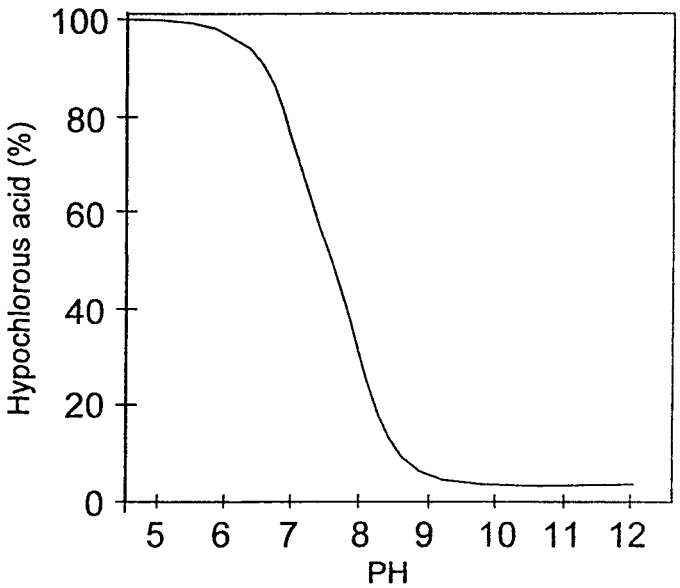


Fig. 26. Relationship between pH and the relative concentration of undissociated hypochlorous acid (HOCl) in a solution of chlorine. (Reproduced from Bartz and Eckert, 1987 with permission of Marcel Dekker Inc.).

conversion (Robbs et al., 1995). Examining the effect of chlorine solutions on cells of the bacterium, *Erwinia caratovora* subsp. *carotovora* and the fungus, *Geotrichum candidum* suspended in water, Robbs et al. (1995) showed that the chlorine solution toxicity was correlated with free chlorine concentrations, the pH of the solution and the oxidation-reduction potential of the solutions. The oxidation-reduction potential was directly correlated with $\log_{10}$ of the chlorine concentration at each pH. It was also found that cells of *E. carotovora* subsp. *carotovora* were 50 times more sensitive to chlorine than were conidia of *G. candidum*, with populations of 10^7 cells or 10^7 conidia per milliliter, respectively. Populations of *Erwinia* were reduced below detectable levels (> 10^2 cells ml^{-1}) by approximately 0.5, 0.5 or 0.75 mg l^{-1} of free chlorine at pH 6.0, 7.0 or 8.0, respectively. With conidia of *Geotrichum*, 25, 25 and more than 3 mg l^{-1}, respectively, were required to produce similar levels of efficacy.

In addition, the hypochlorous acid attacks organic matter, and its concentration in the solution should always be appropriately determined (Bartz and Eckert, 1987). Although a few ppm active chlorine may be

sufficient to destroy bacterial cells suspended in clean water within seconds, 50-500 ppm at pH 7.5-8.5 is frequently recommended for commercial conditions, necessitating killing bacteria in packinghouse water containing a considerable amount of soil and organic matter. Sulfamic acid and other amines have been added to the water to form N-chloramines, in order to stabilize the concentration of the active chlorine in the solution. Surfactants are sometimes added to the water to improve the wetting of vegetable surfaces and to enhance the cleaning capability of the chlorinated water (Bartz and Kelman, 1984). The use of chlorine dioxide (ClO_2) as a disinfectant has been evaluated for water containing high levels of organic matter. This compound may be effective for packinghouse water systems since its efficiency is not significantly influenced by pH in the presence of organic matter and it does not react with amines or ammonia, while reacting slowly with organic matter (Bartz and Eckert, 1987).

Because of its instability in the presence of organic matter, chlorine is not effective in killing microorganisms embedded within injured tissue or in natural openings of the host, such as stomata and lenticels. The effectiveness of chlorine lies, therefore, in its ability to reduce the level of the inoculum or to eliminate most of the waterborne pathogenic microorganisms that might inoculate the product during the treatment. In other words, the main contribution of chlorine solutions to decay control is the prevention of the buildup of the pathogenic microbial population in the water used for washing and hydrocooling the harvested produce. The effectiveness of chlorine on the human pathogen *Escherichia coli*, which is responsible for food poisoning outbreaks linked to the consumption of contaminated vegetables, has recently been studied. Dipping inoculated Cos lettuce leaves or inoculated broccoli florets in hypochlorite solutions was found to reduce *E. coli* counts significantly more than water alone, but did not eliminate the bacterium population. The reduction in *E. coli* cells was dependent on the time of exposure and the concentration of free chlorine (Behrsing et al., 2000).

Immersing fruits and vegetables in appropriate disinfectants may actually remove most of the fungal spores from their surfaces. However, a product that has undergone disinfection may be rapidly reinfested with pathogenic spores, either directly by the continuous fall of airborne spores on their surfaces, or by contact with infested equipment in the packinghouse. An appropriate sanitation system should therefore ensure both the removal of pathogen sources from every corner and the

disinfection of the atmosphere and the equipment in the packinghouse and storage rooms.

Removal of pathogen sources may be achieved through the immediate disposal of every rotted fruit or vegetable, or by immersing it in a disinfectant solution in a special container, which would eradicate fungal spores and prevent their dispersion. For disinfecting packinghouses or store rooms, including their equipment, it is possible to use one of the following disinfectants: formaldehyde, isopropyl alcohol, quadronic ammonium compounds, captan or other chemicals. A standard commercial practice in fruit packinghouses is to disinfect the atmosphere with formaldehyde, boxes and other equipment with quaternary ammonium compounds and the surface of the fruit with a solution of active chlorine (Eckert, 1990). Steam may also be used to sanitize citrus boxes (Klotz and DeWolfe, 1952).

C. POSTHARVEST CHEMICAL TREATMENTS

Since open wounds, created during harvesting, handling and packaging, are the major sites of invasion by postharvest wound pathogens, the protection of wounds by chemicals will considerably decrease decay in storage. Among the various types of 'wounds' we should also include injuries created in severing the crop from the plant or cuts created deliberately during handling procedures, such as stem cuts in banana hands or petiole cuts in celery intended for export. Other potential sites of infection are the natural openings in the host surface, such as lenticels and stomata, whose sensitivity to infection is increased by wounding or after washing the commodity in water. An efficient disinfection process should reach the pathogenic microorganisms accumulated in all these sites.

Disinfection should be applied as close as possible to the time of exposure to infection, since a fungal spore located in the wound may, under appropriate conditions, germinate in several hours and initiate its establishment in the tissue. The allowable time between inoculation (harvesting) and the chemical application, if the treatment is not to lose its effectiveness, depends on several factors: the rate of spore germination and mycelial growth of the pathogen, the level of host resistance, the prevailing environmental conditions, and the penetrability of the tissue to the chemical.

In some instances, however, several hours delay between the picking and the chemical treatment will not reduce the effectiveness of the treatment, and may even increase it. The increased efficiency of the delayed treatment may be related to the fact that germinating spores are more sensitive to the treatment than dormant spores (Eckert, 1978). Since, in practice, a few hours will generally elapse between harvest and treatment, application of the chemical should take place as soon as possible after harvest in order to avoid further development of the germinating spores in the host tissue. In the case of quiescent infections, such as anthracnose (*Colletotrichum gloeosporioides*) in tropical and sub-tropical fruits, and lenticel rotting (*Gloeosporium* spp.) in apples, in which the pathogen remains confined to the tissue beneath the peel, because of host resistance, it is permissible to delay the systemic fungicide application until several weeks after inoculation (Eckert, 1978).

During the last 50 years, more than 30 organic compounds have been introduced for controlling decay by postharvest application. The selection of the appropriate compound depends on: (a) the sensitivity of the pathogen to the chemical substance; (b) the ability of the substance to penetrate through surface barriers into the infection site; and (c) the tolerance of the host, as expressed both by injury and other phytotoxic effects, and by any adverse effect upon the quality of the product (Eckert and Ogawa, 1985). The 'first generation' of postharvest fungicides includes diphenyl, sodium *ortho*-phenylphenate, dicloran and *sec*-butylamine. These compounds are effective in preventing decay caused by wound pathogens, such as species of *Penicillium* and *Rhizopus*, but they have little effect on quiescent infections, or other infections situated within the host tissue (Eckert, 1977).

Biphenyl (diphenyl) is a fungistat that has been used extensively by citrus exporters for more than five decades. It played a most important role in the development of distant markets for citrus fruits and of the world trade in these fruits. The fungistat may be impregnated into paper wraps on each individual fruit, or into paper sheets placed beneath and above the fruits within the container. It sublimes slowly into the atmosphere and protects the fruits during the entire period of shipping to distant markets. The main function of biphenyl is the inhibition of sporulation of *Penicillium* spp. (*P. digitatum* and *P. italicum*) on decaying fruits. Because of this action, biphenyl prevents contact infection of adjacent sound fruits by fungal spores which normally cover the surface of decayed fruit. However, this compound is only weakly

effective against stem-end rot fungi, such as *Diplodia* and *Phomopsis*, and does not protect the fruit against initiation of infection by either *Penicillium* spp. or stem-end pathogens. Biphenyl is not active against *Geotrichum, Alternaria* or *Phytophthora* (Eckert and Eaks, 1989).

Although biphenyl is still sometimes used in export shipments, mainly by impregnation into paper sheets covering the fruit in the container, its commercial utilization is discouraged by consumer resistance to the characteristic odor of the treated fruit and by undesired residues on the fruit.

Sodium *ortho*-phenylphenate (SOPP) is an example of a broad-spectrum fungicide, effective against many postharvest pathogens. It is used mostly against fungal pathogens but is also known for its antibacterial properties. The undissociated phenol (*ortho*-phenylphenol) penetrates with comparative ease into the surfaces of various fruits and vegetables, causing phytotoxicity and leaving considerable residues on the fruit. However, a solution of SOPP containing excess alkali to suppress hydrolysis of the salt, with a pH around 11.5, is both effective and quite safe for treatment of several fresh fruits and vegetables. These include citrus fruits, apples, pears, peaches, tomatoes, peppers, cucumbers, carrots and sweet potatoes.

Although the intact cuticle of the fruit is not permeable to aqueous solutions of the fungicide, the crop is generally rinsed lightly with fresh water to remove most of the fungicide from the surface of the product, leaving a significant residue at the injured site. The fungicide has, therefore, a double function: (1) it eradicates fungal spores and bacteria cells infesting the surface of the treated commodity; and (2) through accumulation in the wound site, it prevents infection via wounds during storage and marketing (Eckert, 1978).

The intensive and continuous use of biphenyl and SOPP has led to the development of *Penicillium digitatum* and *P. italicum* strains resistant to these chemicals (Davé et al., 1980; Eckert and Wild, 1983; Houck, 1977). Furthermore, *Penicillium* isolates that are resistant to SOPP show cross-resistance to biphenyl, which is structurally related to it.

***Sec*-butylamine** (amino-butane) was developed as a postharvest fungicide for citrus fruits in the mid 1960s (Eckert and Kolbezen, 1970). It has a relatively narrow spectrum of antifungal activity and its main function is the prevention of wound infection by *Penicillium digitatum* and *P. italicum*; it does not, however, suppress their sporulation on

decaying fruit. The compound has some activity against stem-end rot but has no effect on *Geotrichum, Alternaria* or *Phytophthora.* Since *sec*-butylamine has low phytotoxicity and toxicity to mammals, the fruit does not have to be rinsed after treatment (Eckert and Ogawa, 1985).

Sec-butylamine can be applied to the harvested fruit as a salt solution to protect oranges during the period of ethylene degreening; or it can be added to wax formulations applied to lemons before storage. The fungicide may also be volatilized and applied as a fumigation treatment which inhibits the development of *Penicillium* after the injuries on the surface of the fruit become alkaline by absorption of the amine vapor. In addition, residues of neutral *sec*-butylamine salts persist in the injuries following the fumigation treatment (Eckert and Kolbezen, 1970).

Captan N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide), which is a bicarboximide fungicide, has been proven effective as a postharvest dip against decay development in various fruits and vegetables, such as strawberries, peaches, cherries, pears, figs and potatoes (Eckert and Sommer, 1967). However, captan is not well suited to postharvest treatment because, when applied as a wettable powder suspension at effective concentrations, it may leave visible residues of powder on the surface of the fruit.

Dicloran (2,6-dichloro-4-nitroaniline, DCNA, botran) is effective against several postharvest fungi. It is particularly efficient in controlling soft-watery rot caused by *Rhizopus stolonifer* in stone fruits and sweet potatoes, for which *Rhizopus* is the main postharvest pathogen. The capability of dicloran to penetrate to a depth of 11 mm into peaches (Ravetto and Ogawa, 1972) explains the inhibitory action of dicloran treatment against established lesions of *Rhizopus* on peaches. However, this treatment is less effective against other decays of stone fruits, such as the brown rot caused by *Monilinia fructicola,* and the blue mold caused by *Penicillium expansum* (Daines, 1970), and dicloran-resistant strains of *R. stolonifer* have also been reported (Webster et al., 1968). The effectiveness of dicloran against postharvest pathogens depends on the level of the fungicide persisting on the fruit after treatment, and the optimum deposit of dicloran for brown rot control is much greater than that required for adequate control of Rhizopus rot.

The effect of dicloran against *M. fructicola* can be greatly improved by heating the fungicide suspension or combining it with another fungicide,

such as benomyl or iprodione, which are active against *Monilinia* (Eckert and Ogawa, 1988).

Sulfur dioxide (SO_2) is applied as a postharvest fumigation to grapes in order to eradicate spores of *Botrytis cinerea*, to inhibit very superficial infections on the fruit and, above all, to prevent the contact spread or 'nesting' of the fungus on grapes during storage. The tolerance of grapes to SO_2 is higher than that of all other fruits and vegetables; however, even for grapes an effective combination of concentration and time has to be carefully chosen in order to compromise between effective decay control and minimum fruit injury as exhibited by bleaching and stem drying (Eckert and Ogawa, 1988). In a recent study Coertze and Holz (1999) found that exposure of grapes to SO_2, similarly to prolonged cold storage, reduced the resistance of the berry to infection by individual conidia of *B. cinerea*, and could result in an increased rate of lesions. Individual conidia did not induce disease on the fresh, resistant berries.

A common fumigation procedure consists of an initial application of 0.5% SO_2 (v/v) for 20 min, immediately after harvest, followed by low-concentration (0.1-0.2%) fumigation for 30-60 min every 7-10 days during the storage period. Another procedure involves applications of reduced levels of sulfur dioxide (0.2% SO_2) three times weekly, which results in less residual bisulfite in the grapes, with less bleaching than the traditional high dosage treatment (Marois et al., 1986). In California, Australia, South Africa, Chile and Israel, the exposure of grapes to SO_2 during export shipments is carried out by adding a bisulfite or metabisulfite salt to the packaging material (Nelson, 1985; Guelfat-Reich and Safran, 1973). The SO_2 is released into the atmosphere around the grapes and prevents infection with *B. cinerea* when in contact with infected berries. Liquid or solid SO_2 formulations which have been developed (Combrink and Truter, 1979; Guelfat-Reich et al., 1975; Kokkalos, 1986), release the SO_2 after the package has been closed, and maintain efficient concentrations (5-10 ppm) of SO_2 in the atmosphere, which prevent contact-infection during prolonged storage. Studies of the efficacy of a range of SO_2-generating pads in stored grapes showed that some properly regulated pads were capable of controlling Botrytis rot in table grapes even when moderate levels of initial inoculum were present (Mustonen, 1992).

SO_2 fumigation at 1600 ppm for 20-30 min or 3200 ppm for 5 min gave almost complete control of Botrytis storage rot of kiwifruit in New Zealand (Cheah et al., 1993). Absorption of SO_2 by the fruit gradually

increased as the duration of fumigation increased from 5 to 30 min; and total SO_2 residues were proportional to exposure time and treatment rate, and declined sharply after fumigation. No SO_2 injury was observed on the fruit at the concentration-time combinations tested.

The possibility of using SO_2 solutions against the green mold in citrus fruits has been examined in *Penicillium digitatum*-inoculated lemons (Smilanick et al., 1995). Immersion of inoculated fruits in 2% SO_2 solutions reduced green mold incidence without injuring the fruit, but heating of the solution was needed to attain acceptable efficacy (see Combined Applications in the chapter on Physical Means - Heat Treatments).

The benzimidazole compounds – **thiabendazole** (TBZ), **benomyl**, **carbendazim** (methyl-2-benzimidazole carbamate – MBC) and **thiophanate-methyl** – were introduced as postharvest fungicides in the late 1960s. The two systemic fungicides, TBZ and benomyl, have undoubtedly added a new dimension to the control of postharvest diseases. These compounds, whose action is associated with the inhibition of mitosis (Davidse, 1988), are active against a broad spectrum of pathogenic fungi. They have been used all over the world to control: decay of citrus fruits caused by the two wound molds, *Penicillium digitatum* and *P. italicum* and by the stem-end fungi, *Diplodia natalensis* and *Phomopsis citri*; the brown rot caused by *Monilinia fructicola* in stone fruits; blue mold (*Penicillium expansum*), gray mold (*Botrytis cinerea*) and lenticel rot (*Gloeosporium* spp.) in apples; anthracnose (*Colletotrichum gloeosporioides*) in banana, papaya, mango and other tropical fruits (Eckert, 1977, 1990); and black rot (*Ceratocystis paradoxa*) in pineapple (Eckert and Ogawa, 1985).

The strong inhibitory effects of the thiabendazole compounds, especially benomyl, have been attributed to their systemic property and their ability to penetrate the wax and the cuticle of the host surface to reach and inhibit the pathogen beneath them (Ben-Arie, 1975; Phillips, 1975). This property enables the fungicides to act also against quiescent infections, such as anthracnose in tropical fruits, or stem-end rot at the button of citrus fruits. Both TBZ and benomyl act as antisporulants of *Penicillium* on decaying citrus fruit and, therefore, inhibit infection of adjacent fruits during storage and shipment. Benomyl at equivalent concentrations is usually more effective in controlling fruit decay than the other benzimidazole fungicides. This is in accordance with its ability to penetrate into the cuticle of the fruit and the stem button. In providing

control of banana crown rot caused by several fungi (including *Colletotrichum, Fusarium, Verticillium, Botryodiplodia* and *Cephalosporium*) and anthracnose (*Colletotrichum*), carbendanzim and thiophanate-methyl were superior to TBZ, but both were slightly inferior to benomyl (Eckert, 1990).

The benzimidazole compounds are insoluble in water but are fairly soluble in dilute acids and alkalis. They are generally applied to fruits as water suspensions or in wax emulsions, but may also be applied as solutions in a hydrocarbon solvent used in the formulation of fruit coating (Eckert, 1975). TBZ is stable under postharvest application, whereas benomyl is unstable and decomposes slowly in water to methyl-2-benzimidazole carbamate (MBC). MBC is only slightly less active than benomyl and probably plays a major role in disease control. However, its penetration ability into the tissue is very small (Eckert, 1978).

One of the limitations of the benzimidazole compounds is that they are not active against a number of important postharvest pathogens, such as *Rhizopus, Mucor, Phytophthora, Alternaria, Geotrichum* and soft rot bacteria (Eckert and Ogawa, 1988). Because of its lack of activity against these pathogens, benzimidazole cannot be used alone to control total decay in storage. Furthermore, the resistance of these pathogens towards the benzimidazole compounds may result in changes in the normal balance that naturally exists within the pathogenic population of each product. Thus, diseases of relatively little importance, incited by *Alternaria* and *Geotrichum* in citrus fruit or by *Alternaria* in white cabbage and in zucchini squashes, may become a limiting factor in storage of these products (Albrigo and Brown, 1977; Temkin-Gorodeiski and Katchanski, 1974; Wale and Epton, 1981). Furthermore, of the two species of *Penicillium* which attack citrus fruits, *P. digitatum* has proven to be more sensitive to the benzimidazoles; continuous treatment of citrus fruits with these fungicides resulted in a remarkable increase in decay by *P. italicum,* the less sensitive fungus of the two (Gutter, 1975).

A very serious problem arising from long and continuous treatment with the benzimidazole compounds, is the development of fungal strains resistant to these fungicides, as a result of the heavy selection pressure exerted by the chemicals on the pathogen populations, (Georgopoulus, 1977). Common examples are the resistant strains of *P. digitatum* and *P. italicum* in citrus fruit, of *P. expansum* in pome and stone fruits, and of *B. cinerea* in various crops, that have frequently been reported within the natural sensitive population of these species. Recently the appearance

of thiabendazole-resistant *Colletotrichum musae* isolates has been reported in banana plantations that have commonly received pre- and postharvest thiabendazole treatments (de Lapeyre de Bellaire and Dubois, 1997). The ability of the benzimidazoles to persist in the fruit throughout the storage period ensures continuous contact with the pathogen, which exerts continuous pressure for selection of benzimidazole-resistant strains with various degrees of resistance. In addition, pathogens which develop resistance toward one benzimidazole compound, show a 'cross-resistance' to other compounds with similar chemical compositions and structures (Eckert and Ogawa, 1985).

The emergence of fungal strains resistant to benzimidazoles, and the resulting decrease in the efficacy of the postharvest benzimidazole treatments, raise questions about the future use of these compounds in the control of Penicillium rots in citrus fruits and apples and of Botrytis rot in various fruits and vegetables. The risk of the emergence of such resistant strains significantly increases when these compounds are applied as both pre- and postharvest treatments in certain crops.

In order to broaden the scope of benzimidazole activity, as well as to solve the problem of the emergence of resistant strains, their combination with a fungicide of a different chemical structure and a different mode of action, was suggested. It is thus possible to use a combination of benomyl and prochloraz in order to control both *Penicillium* and *Alternaria* in pears stored at 0.5°C (Sitton and Pierson, 1983). A combination of benomyl and dicloran is effective in controlling the two main decays in stone fruits: the brown rot caused by *Monilinia*, and the watery rot caused by *Rhizopus* (Wade and Gipps, 1973). Similarly, a combination of TBZ and iprodione controls both TBZ-resistant and TBZ-sensitive *Botrytis* strains in stored celery (Barkai-Golan et al., 1993a), and is effective against both *Botrytis* and *Alternaria* in white cabbage, although it is ineffective against bacterial soft rots in this crop (Geeson and Browne, 1979). Thiabendazole and dicloran dust, applied to white cabbage in alternate years, was recommended by Brown and collaborators (Brown, A.C. et al., 1975) to minimize the development of benzimidazole-resistant strains of *B. cinerea* in storage. For solving the problems associated with the emergence of benzimidazole-resistant strains of *P. expansum* in pears, TBZ was combined with captan, and this combination was alternated with another fungicide (imazalil, etaconazole or iprodione). Such a

strategy considerably decreased the incidence of *P. expansum* strains resistant to TBZ during storage (Prusky et al., 1985a).

Following the development of benomyl-resistant strains of *B. cinerea*, one of the most important pre- and postharvest pathogens of grapes, Chiba and Northover (1988) studied the fungitoxicity of four synthesized alkyl isocyanate homologues of benomyl towards both benomyl-resistant and benomyl-sensitive isolates of the fungus. On the basis of spore germination as a criterion for fungal response, the benomyl-resistant isolates were found to be more sensitive than the benomyl-sensitive isolates to ethyl isocyanate and propyl isocyanate. This response was inverse to the relative response of sensitive and resistant isolates to benomyl and constituted an example of 'negative cross resistance' (Chiba and Northover, 1988). When the germ-tube length of *Botrytis* germinating spores was used as a criterion, the four isocyanate homologues of benomyl – methyl, ethyl, propyl and hexyl – were found to be as effective against sensitive isolates as benomyl, but more effective against the resistant isolates. The hexyl isocyanate homologue was less effective than the other three compounds. Studies with wounded apples clearly demonstrated that, while benomyl gave better protection against sensitive isolates of *B. cinerea*, the ethyl isocyanate homologue of benomyl was effective against both benomyl-sensitive and benomyl-resistant isolates.

Iprodione and **vinclozolin**, which are dicarboximide fungicides, have been represented as alternative treatments to TBZ in many commodities, such as cucumbers, tomatoes, strawberries, eggplants and grapes (Lorenz, 1988). These fungicides are characterized by a different mechanism of action from that of the benzimidazoles, although this mechanism is not yet clear. Iprodione retards the development of *Botrytis, Penicillium, Monilinia* and *Rhizopus* and it is, therefore, capable of suppressing the main storage diseases of stone and pome fruits (Bompeix and Morgat, 1977; Heaton, 1980). However, the emergence of *Penicillium expansum* strains resistant to both iprodione and vinclozolin, may prevent their use for decay control on these fruits (Rosenberger and Meyer, 1981). In contrast to TBZ, iprodione is also effective against *Alternaria*, and considerably reduces the incidence of the black spots caused by this fungus on mangoes (Prusky et al., 1983) and on potato tubers in Israel (Droby et al., 1984). However, iprodione-tolerant isolates of *Alternaria alternata* pv. *citri* have already been found in Mineola tangelo orchards in Israel and Florida, where the

fungicide had previously been applied successfully for several years (Solel et al., 1996). A combination of iprodione and TBZ was found to be advantageous in controlling the gray mold decay of celery caused by a heterogenic spore population of *B. cinerea*, which comprised spores sensitive to TBZ and/or to iprodione, along with spores resistant to these fungicides (Barkai-Golan et al., 1993a).

Imazalil is a systemic fungicide, which was introduced as a postharvest treatment in the early 1970s. It belongs to a group of fungicides that act by inhibiting the biosynthesis of ergosterol, an essential component in the membrane of fungal cells, and it was the first ergosterol biosynthesis inhibitor to be used as a postharvest fungicide.

The fungicide is very efficient in controlling *Penicillium digitatum* and *P. italicum* in citrus fruits, including isolates that are resistant to TBZ, benomyl, SOPP and *sec*-butylamine (Harding, 1976; Eckert and Ogawa, 1985). Thanks to these properties, imazalil has become the most popular postharvest fungicide for controlling decay in citrus fruits. As a result of the enhanced resistance of *P. digitatum* and *P. italicum* to TBZ, imazalil was registered in the United States in 1984 as a postharvest treatment of citrus fruits and has been commercially used in several citrus-producing areas of the world. Water solutions of imazalil act on the two Penicillia, as both protectants and anti-sporulants (Laville et al., 1977). When applied in a water-wax formulation, its effectiveness is reduced and the concentration of the fungicide must be doubled to achieve an effect similar to that in water solutions (Brown, G.E., 1984). It is generally applied one or more times at relatively high dosages (2 to 4 g l^{-1}) in a wax formulation that covers the surface of the fruit. Following its application, substantial antifungal residues of imazalil persist for the postharvest life of the fruit. These conditions produce intense pressure for selection of imazalil-resistant biotypes in the *P. digitatum* population and several years after imazalil was accepted commercially for citrus decay control, biotypes of *P. digitatum* with reduced sensitivity to the fungicide were reported and their potential impact on decay control has been demonstrated (Eckert et al., 1994).

Wild (1994) reported on differential sensitivity of *P. digitatum* isolates to imazalil: while isolates obtained from Australian citrus fruits were defined as sensitive to the fungicide, those imported from the USA were much more tolerant, and an imazalil concentration of 0.1 mg ml^{-1} in the growth media was established as suitable for differentiating imazalil-tolerant strains.

The relative fitness of imazalil-resistant and imazalil-sensitive wild biotypes of *P. digitatum* was evaluated by Holmes and Eckert (1995) in Californian citrus groves and packinghouses. Both resistant and sensitive biotypes of the fungus were found to be stable with respect to their responses to imazalil over several disease cycles in non-treated lemon fruit and over several generations on culture medium. According to Holmes and Eckert (1995), the fact that imazalil-resistant biotypes have reached high frequency in packinghouses with a history of heavy imazalil application, could have indicated that the resistant biotypes have an advantage in relative fitness over the sensitive wild biotype in imazalil-treated fruit. On the other hand, the absence of resistant biotypes from groves where imazalil has never been applied, despite the regular introduction of resistant spores on picking boxes, suggested that resistant biotypes may be less fit than sensitive biotypes of *P. digitatum* in non-treated fruit. Upon studying the competition between sensitive and resistant biotypes of *P. digitatum* in resistant/sensitive mixtures (1:1), it was found that both on non-treated fruit and in culture medium without imazalil, resistant biotypes were generally less competitive than sensitive biotypes. Holmes and Eckert (1995) considered that such results should encourage an evaluation of resistance-management programs involving the rotation of imazalil with a non-selective fungicide. However, the continued intensive use of imazalil could adversely affect this strategy by the development of increased fitness among fungicide-resistant fungi (Holmes and Eckert, 1995).

In most packinghouses in California, citrus fruits are routinely treated with sodium *o*-phenylphenate, imazalil and TBZ, to control *Penicillium* decay. A recent study on the sensitivity of *Penicillium* spp. to postharvest citrus fungicides indicated that the intensive use of the three chemically unrelated fungicides has resulted in the proliferation of triple-resistant biotypes of *P. digitatum* (Holmes and Eckert, 1999). This did not lead, however, to an increase in the level of resistance to any of the fungicides in the various isolates of *Penicillium* collected during several years. On the other hand, the proportion of isolates that were resistant to all three fungicides increased from 43% in 1948 to 74% in 1994. It was also found that while imazalil-resistant biotypes of *P. digitatum* were frequently isolated in packinghouses, resistant *P. italicum* was rare. It was suggested that a fundamental difference in the biology of *P. italicum* and *P. digitatum* might be involved. The difference could result from smaller populations of *P. italicum* available for selection in the packinghouse, greater imazalil sensitivity of resistant mutants, and the lower parasitic

fitness and reproductive capacity of this species compared with *P. digitatum* (Holmes and Eckert, 1999).

The antifungal spectrum of imazalil is similar to that of the benzimidazoles, but it is also active against *Alternaria.* The fungicide was also found active against stem-end rots caused by *Diplodia natalensis* and *Phomopsis citri,* although it was less effective than benomyl (Brown, G.E., 1984); no activity was recorded against sour rot caused by *Geotrichum candidum.* Postharvest treatments with imazalil have also been effective against benzimidazole-resistant isolates of *Colletotrichum, Diplodia* and *Phomopsis* from mango fruits (Spalding, 1982). However, good control of both stem-end rot and anthracnose and considerably more fruit acceptable for marketing were recorded when imazalil was applied in hot water at 53°C (Spalding and Reeder, 1986b). Imazalil inhibits both spore germination and mycelial growth of *Alternaria* and suppresses decay development caused by this fungus in apples, pears, persimmons, tomatoes and bell peppers (Miller et al., 1984; Prusky and Ben-Arie, 1981; Spalding, 1980). Postharvest treatment of tomatoes also controls the gray mold caused by *Botrytis cinerea* during storage (Manji and Ogawa, 1985). Imazalil is also effective against the black rot incited by *Ceratocystis paradoxa* in pineapple, when applied within 6 to 12 hours after harvest (Eckert, 1990).

Prochloraz, which also acts by inhibiting the synthesis of ergosterol, is an imidazole with a spectrum of activity similar to that of imazalil. It eradicates initial infections by the two *Penicillium* species in citrus fruit, including strains resistant to benomyl and TBZ. Its efficacy in suppressing the blue and green molds is just as good as that of benomyl or imazalil (Tuset et al., 1981), and similarly to these fungicides, it also has a marked antisporulant activity. This fungicide was also effective in controlling anthracnose and stem-end rot on papayas (Muirhead, 1981a), and virtually eliminated anthracnose from mango lots with up to 72% diseased fruit in the untreated control (Knights, 1986). Prochloraz was found to be efficacious against both Fusarium rots and soft rots caused by *Geotrichum* and *Rhizopus* in muskmelon fruits (Wade and Morris, 1983).

Etaconazole is a triazole with a mode of action similar to that of imazalil and prochloraz. It controls initial infections of the two species of *Penicillium* in citrus fruits, including infections by benzimidazole-resistant isolates, and it is also characterized by its ability to suppress

sporulation of these fungi on diseased fruits and protect them from infections through new wounds sustained after treatment (Brown, G.E., 1983). The fungicide is markedly effective against sour rot (*Geotrichum candidum*) but provides only moderate protection against the stem-end fungi, *Alternaria citri, Phomopsis citri* and *Diplodia natalensis.* The ability of etaconazole to control the major pathogens of harvested citrus fruits gives this fungicide a very important role as a postharvest fungicide for these fruits. Trials with Fuerta avocados have shown that etaconazole controls stem-end rot but is not effective against anthracnose (Muirhead et al., 1982). However, both anthracnose and stem-end rot in mangoes have been controlled by this compound during storage and ripening of the fruit (Spalding, 1982).

Guazatine is a fungicide with a wide spectrum of activity, capable of eradicating incipient infections by the two Penicillia, including benzimidazole-resistant isolates, as well as infections by *Geotrichum* in citrus fruit (Brown, G.E., 1983). The fungicide is also efficient in controlling *Geotrichum* and *Alternaria* in melons (Wade and Morris, 1983).

Metalaxyl (ridomil), which is an acylalanine fungicide, acts as a strong inhibitor of the various developmental stages of *Phytophthora* spp. (Bruck et al., 1980; Farih et al., 1981). In addition to the inhibition of the mycelial growth of the fungus, the fungicide generally inhibits the formation of sporangia, chlamidospores and oospores at low concentrations. This systemic fungicide uniquely arrests incipient infections of *Phytophthora* in citrus fruits and prevents contact spread of brown rot during prolonged storage (Cohen, 1981, 1982). Application of metalaxyl, in a water-wax formulation, to *Phytophthora citrophthora*-inoculated Shamouti oranges, followed by the standard packinghouse procedure (including washing, immersion in a 0.5% sodium *ortho* phenylphenate for 3 min at 36°C, washing in tap water and drying), has shown that the fungicide considerably delays or prevents the development of brown rot in storage. The antifungal effect on *Phytophthora* is much more pronounced than that of TBZ or imazalil, but it has no influence on the development of other postharvest pathogens. However, the combination of metalaxyl with etaconazole, in a water-wax formulation, broadens the scope of the antifungal activity and controls Penicillium rots, sour rot (*Geotrichum*) and brown rot (*Phytophthora*) in citrus fruits (Cohen, 1981).

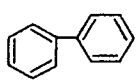
Studies carried out 10 years later (Ferrin and Kabashinna, 1991), described the development of metalaxyl-resistant isolates of *Phytophthora parasitica*, with EC_{50} values for inhibition of linear mycelial growth exceeding 700 $\mu g\ ml^{-1}$, as compared with values of 0.25-3.08 $\mu g\ ml^{-1}$ for the sensitive isolates. However, the resistances to the mycelium growth may differ from that for the formation of sporangia or chlamidospores.

Metalaxyl-resistant genotypes emerged rapidly worldwide and are responsible for a lack of *Phytophthora infestans* control in potatoes and tomatoes. A cross-resistance was reported for this class of fungicides, and the metalaxyl-resistant isolates were also resistant to **oxadixyl**, while the metalaxyl-sensitive isolates were also sensitive to oxadixyl; both of these are systemic fungicides (Sedegui et al., 1999). Resistance to metalaxyl, which is mediated by a single gene (Cooke, 1991), has evolved where this fungicide was used as a single product for disease control. A coordinated strategy of using the systemic fungicide, metalaxyl in mixtures with protective multisite fungicides, such as **chlorothalonil**, has been effective in slowing the development of resistance (Cooke, 1991; Kadish et al., 1990).

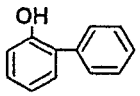
Fosetyl al (Fosetyl aluminum) is another selectively active fungicide against incipient infections of *Phytophthora* (Gaulliard and Pelossier, 1983). It is applied to harvested citrus fruits to protect against and to control *Phytophthora* spp., but it also reduces the incidence of green mold (*Penicillium digitatum*) in storage (Eckert and Ogawa, 1985).

Among the fungicides that have proven efficient in controlling citrus fruit decay, including infections by *Geotrichum candidum*, we have to mention **fenpropimorph** and **flutriafol**. These compounds considerably reduce the rate of rot, both in artificially inoculated lemons and in those under natural infection conditions in the packinghouse. Their major action is the suppression of contact-spread of the sour rot and the green and blue molds following close contact with diseased fruit in the container (Cohen, 1989).

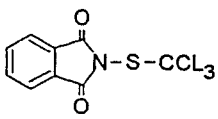
Fungicides and fungistats, used for postharvest decay control, and their chemical structures are given in Fig. 27. Detailed descriptions of chemical compounds used for the control of postharvest diseases, and their effect on disease development, are given in two comprehensive reviews by Eckert and Ogawa (1985, 1988).



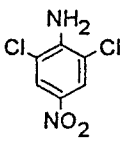
BIPHENYL



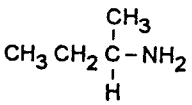
O - PHENYLPHENOL



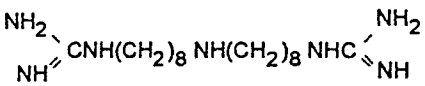
CAPTAN



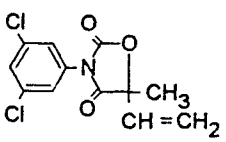
DICLORAN



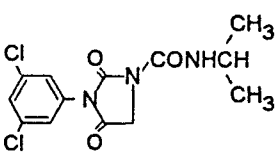
SEC - BUTYLAMINE



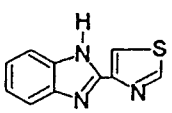
GUAZATINE



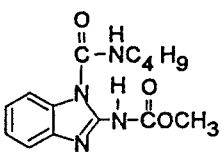
VINCLOZOLIN



IPRODIONE

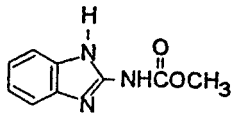


THIABENDAZOLE

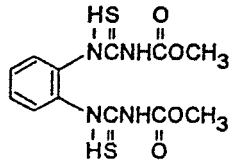


BENOMYL

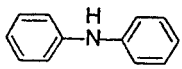
Fig. 27. Postharvest fungicides. (Reproduced from Eckert and Ogawa, 1985, 1988 with permission of the Annual Review of Phytopathology).



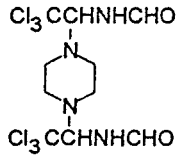
CARBENDAZIM



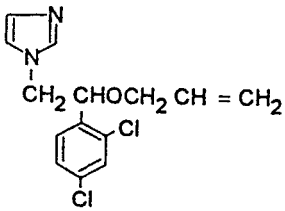
THIOPHANATE - METHYL



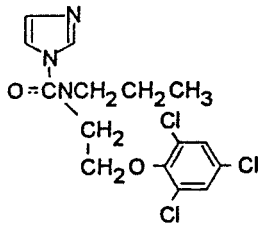
DIPHENYLAMINE



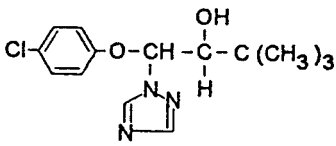
TRIFORINE



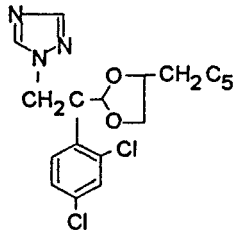
IMAZALIL



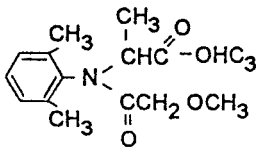
PROCHLORAZ



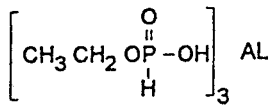
TRIADIMENOL



ETACONAZOLE



METALAXYL



FOSETYL AL

Fig. 27. Postharvest fungicides (cont.).

The Use and Development of Fungicides – Perspectives

It should be emphasized that the use of a fungicide or bactericide is not a substitute for appropriate storage conditions, since these compounds only seldom affect the physiological deterioration of the product. Moreover, chemical compounds are more effective when the fruit or vegetable is held under storage conditions which maintain the natural infection resistance of the produce, but are not good for the growth of the pathogen. However, when appropriate refrigeration or a controlled atmosphere cannot be installed, the chemical treatment may be the only means available to prolong the postharvest life of fruits and vegetables.

In the past, the selection of fungicides for development was largely based on their fungicidal performances and safety to the consumer and operator. However, there is now a greater awareness of the need to define other aspects of new fungicides early in the development process. According to Knight et al. (1997) these include, in addition to cost-effective fungicidal activity: low toxicity to humans and wildlife, low environmental impact, low residues in food, and the ability to integrate with other disease control technologies. Examples of substances with low environmental impact and low residues in the treated commodity are compounds that are 'generally recognized as safe' (GRAS) and naturally occurring plant products, as given below.

D. GENERALLY RECOGNIZED AS SAFE (GRAS) COMPOUNDS

Fungicides that leave low or non-detectable residues in the commodity are actively sought in research programs. Compounds are selected that rapidly degrade on the host surface or metabolize quickly in the tissue.

Hydrogen peroxide is such a compound; it degrades into O_2 and H_2O leaving no harmful residue, and is considered a generally-recognized-as-safe (GRAS) compound by the Food and Drug Administration of the USA. Vapor phase hydrogen peroxide treatment was found significantly to reduce the number of germinable *Botrytis cinerea* spores on grapes. It also resulted in reduced decay in non-inoculated 'Thompson Seedless' and 'Red Globe' grapes after 12 days of storage at 10°C, without affecting grape color or soluble solids content (Forney et al., 1991).

Sanosil-25, a disinfectant containing 48% hydrogen peroxide, and silver salts as stabilizing agents, inhibits spore germination and mycelial growth of *Alternaria alternata*, *Fusarium solani* and *B. cinerea*, and markedly decreases decay in melons at 5000 µl l^{-1} when incorporated into a wax treatment, without causing any phytotoxic effects (Aharoni et al., 1994). Dipping commercially harvested eggplants and red peppers in 0.5% Sanosil-25 reduced decay development by *A. alternata* and *B. cinerea* after storage and shelf life, to a commercially accepted level (Fallik et al., 1994a). On the other hand, immersion of *Penicillium digitatum*-inoculated lemons in hydrogen peroxide at 5-15% did not effectively control the green mold and caused unacceptable injury to the fruit when the immersion period was increased to 90 s (Smilanick et al., 1995).

Acetic acid and other short-chain organic acids, such as propionic acid, are commonly used by food manufacturers as antimicrobial preservatives or acidulants in a variety of food products (Davidson and Juneja, 1990). The possibility of using vaporized acetic acid against postharvest decay has been studied in various fruits (Sholberg, 1998; Sholberg and Gaunce 1995, 1996; Sholberg et al., 1996). Sholberg and Gaunce (1995) found acetic acid, applied as a vapor at low concentrations in air (2-4 mg l^{-1}), to be extremely effective in reducing or preventing decay in: various cultivars of apples and Anjou pears inoculated with *B. cinerea* and *Penicillium expansum* conidia; tomatoes, grapes and kiwifruit inoculated with *B. cinerea*; and Navel oranges inoculated with *Penicillium italicum*. The same authors (Sholberg and Gaunce, 1996) found acetic acid to be similarly effective as a postharvest fumigant on stone fruits, controlling decay by *Monilinia fructicola* and *Rhizopus stolonifer* at concentrations in air as low as 1.4 mgl^{-1}. On table grapes, fumigation with 0.27% (vol/vol) acetic acid controlled *Botrytis* and *Penicillium* decay as effectively as sulfur dioxide applied at commercial rates (Sholberg et al., 1996). This treatment has been suggested as an alternative to SO_2 fumigation, that could provide the table grape industry with many benefits. While SO_2 treatment leaves sulfite residues on the surface of the berries, acetic acid does not leave any toxic residues on grapes, and no differences from SO_2 in terms of external fruit quality and fruit composition have been recorded. Furthermore, wine grapes could also benefit from fumigation with acetic acid (Sholberg et al., 1996).

Sholberg (1998) found that fumigation with acetic acid (1.9 µl l^{-1}), and other closely related short-chain organic acids, **formic** (1.2 µl l^{-1}) and

propionic (2.5 µl l^{-1}), significantly reduced decay in eight cherry cultivars inoculated with fungal spores (10^6 spores/ml) of *M. fructicola, P. expansum* and *R. stolonifer*. However, phytotoxicity, expressed as black-end stems and pitting of the fruit surface, occurred on fumigated cherries. On the other hand, decay in *P. expansum*-inoculated pome fruits was reduced from 98% to 16, 4 and 8% by acetic, formic and propionic acids, respectively, without injury to the fruit.

Limited data are available on the effects of **peracetic acid** on postharvest fruit decay. Evaluating its effectiveness on the control of the brown rot caused by *Monilinia laxa* on stone fruits, Mari et al. (1999) indicated that the chemical acted directly on the fungal spores, its effect being related to the chemical concentrations and duration of treatment. Complete inhibition of spore germination was observed with peracetic acid at 500 µg/ml after 5 min of contact with *Monilinia* conidia. The inhibitory effect on the pathogen spores was confirmed *in vivo* in inoculated plums, for which a 1000 µg/ml treatment for 20 sec. reduced decay incidence by more than 50%. Under semi-commercial conditions decay was totally controlled when the conidia remained in contact with peracetic acid at 250 µg/ml for 5 min (Mari et al., 1999).

Bicarbonate and carbonate salts are widely used in the food industry – they are common food additives for leavening, pH control, taste and texture modifications and spoilage control (Corral et al., 1988). These compounds have a broad spectrum of activity against food-borne bacteria and yeasts, and are generally recognized as safe (GRAS) compounds for many applications, by the US Food and Drug Administration. As a matter of fact, early studies with harvested citrus fruits (Barger, 1928; Fawcett, 1936) had already shown that immersion of citrus fruits in solutions of sodium bicarbonate reduced the incidence of postharvest green mold caused by *P. digitatum*. Public concerns about pesticide residues on food crops, which have made registration of new fungicides very difficult, have led to renewed interest in these compounds and to investigations of their potential as safe alternatives for postharvest disease control of fruits and vegetables (Fallik et al., 1997; Aharoni et al., 1997; Smilanick et al., 1999). Such treatments are inexpensive, pose a minimal risk of injury to the fruit and can be a useful tool in the management of fungicide-resistant isolates (Smilanick et al., 1999).

A comparison among the inhibitory effects of various carbonate and bicarbonate salts on *P. digitatum* spore germination (Smilanick et al., 1999) showed that the effective dose (ED_{50}) concentrations of **sodium**

carbonate, potassium carbonate, sodium bicarbonate, ammonium bicarbonate and **potassium bicarbonate** were 5.0, 6.2, 14.1, 16.4 and 33.4 mM, respectively. All these compounds were fungistatic: spores removed from the solutions resumed germination when incubated in potato dextrose broth. In spite of the differences between the effects of sodium bicarbonate and sodium carbonate on spore germination, the two solutions were similarly effective in controlling the green mold in lemon and orange fruits inoculated 24 h before treatment. Therefore, the *in vitro* toxicity of the solutions does not indicate their efficacy in controlling decay. Since bicarbonate anion concentration is related to pH, this parameter was examined in combination with several salts to separate pH effects from the bicarbonate effects on *B. cinerea* growth (Palmer et al., 1997). It was found that as the pH increased from 7.0 to 8.5, colony growth on media supplemented with bicarbonates and phosphates decreased more than could be accounted for from pH alone.

Potassium bicarbonate was found to inhibit spore germination, germ-tube elongation and mycelial growth of *B. cinerea* and *A. alternata*, the main postharvest pathogens of bell pepper fruits. Its action was fungistatic rather than fungicidic: mycelial plugs of both fungi, when transferred from potassium bicarbonate-amended PDA to unamended medium, grew similarly to unamended controls (Fallik et al., 1997). At concentrations greater than 1% for *Botrytis* or 2% for *Alternaria*, mycelial mats remained white because of the inability of the fungi to sporulate. Scanning electron microscopy analysis revealed that the fungistat caused shrinkage and collapse of hyphae and spores, resulting in the prevention of normal sporulation. A 2-min pre-storage dip of red sweet peppers in 1 or 2% potassium bicarbonate reduced decay incidence to a commercially acceptable level during a storage and marketing simulation. However, a longer dipping time, especially at higher salt concentrations (3%), reduced fruit quality, as indicated by decreased firmness and further decay development (Fallik et al., 1997). Collapse of hyphal walls and shrinkage of *B. cinerea* conidia were suggested to be partly due to the reduction in fungal cell turgor pressure or the increase of the permeability of the fungal cell membrane, caused by the bicarbonate ion (Palmer et al., 1997; Fallik et al., 1997).

A direct inhibitory effect of sodium bicarbonate on *in vitro* mycelium growth was similarly recorded for *R. stolonifer, A. alternata* and *Fusarium* spp., the major pathogens of stored melons (Aharoni et al., 1997). Coating harvested melons with wax containing 2% sodium bicarbonate resulted in a marked reduction in decay incidence after

storage, while the fresh appearance of the fruit was maintained. A trial shipment of 'Galia' melons by sea transport from Israel to Europe demonstrated that sodium bicarbonate incorporated into a wax coating maintained the marketability of the fruit. It was suggested that this biocide could serve as an alternative to imazalil, which had been incorporated into the wax until recently, to control decay during prolonged storage (Aharoni et al., 1997).

Since *Helminthosporium solani*, the causal agent of silver scurf in potato tubers, developed resistance to benzimidazole compounds, postharvest losses attributed to this disease have increased and no fungicide is currently registered for its control in the United States (Olivier et al., 1998). Looking for alternative antifungal compounds for suppression of silver scurf on potatoes, Olivier et al. (1998) evaluated the effectiveness of several organic and inorganic salts on *H. solani* development; they found that **potassium sorbate, calcium propionate,** sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate and ammonium bicarbonate directly reduced radial growth of the fungus *in vitro* at 0.06-0.2 M, and that no lesions developed on *H. solani*-inoculated tubers treated with 0.2 M solutions of each of the salts during 6 weeks of incubation at 22-24°C. Postharvest applications of these compounds (at 0.2 M) on naturally infected field-grown tubers also significantly reduced disease severity and *Helminthosporium* sporulation after 15 weeks of storage at 10°C. In this case, radial growth inhibition of *H. solani* on salt-amended media was generally predictive of the capability of the salts to suppress lesion development and sporulation on potato tubers. Similarly to the effect of the salts on *P. digitatum*, the citrus pathogen (Smilanick et al., 1999), treatments with sodium and potassium carbonate were also more effective than those with the respective bicarbonate salts against *H. solani* growth (Olivier et al., 1998).

Several studies with carbonate and bicarbonate salts have confirmed that the anions are primarily responsible for pathogen suppression by inorganic salts, while the cations play only a minor role. However, ammonium bicarbonate is an exception since, in addition to the anion, ammonium too may contribute to the toxicity of the salt (DePasquale and Montville, 1990).

Chlorine (as hypochlorous acid) is an effective and economical biocide that has long been accepted as a potent disinfectant for sanitation purposes and is recognized as safe in many countries (Eckert and Ogawa, 1988). Chlorination of the washing water used in tanks and hydrocoolers,

with calcium hypochlorite or sodium hypochlorite, is a standard procedure to disinfect various fruits and vegetables in the packinghouse. Its main effect in reducing decay development is via reduction of the level of inoculum in the treated water (see the chapter on Chemical Control - Sanitation). Chlorine acts on fungal propagules by direct contact and can inactivate spores which are suspended in water or located on the surface of fruits or vegetables. It does not act on pathogens under the fruit skin or after infection has occurred. Because of the rapid drop in the biocidic activity of chlorine in the presence of organic substances (that modify the pH of the solution), it has frequently been replaced by chlorine dioxide (ClO_2), which is more stable than chlorine and is effective over a wide pH range (Bartz and Eckert, 1987). In addition, ClO_2 is not corrosive to the packinghouse equipment.

In a recent study, ClO_2 has been evaluated for its effectiveness in controlling the brown rot caused by *M. laxa* in stone fruits (Mari et al., 1999). ClO_2 was found to affect *M. laxa* conidia directly, and at 100 µg/ml for 20 s or 50 µg/ml for 1 min, totally inhibited their germination. The direct effect of the chemical on fungal spores has also been exhibited *in vivo*, when decay development in nectarines and plums, wounded and inoculated with ClO_2-treated conidia, was markedly suppressed. Under these conditions, however, the effects varied with the ClO_2 concentration, the time of exposure of the conidia, and the species of fruit tested (Mari et al., 1999).

For table grapes, that cannot be subjected to water immersion treatments without altering their quality, a modified chlorine atmosphere with a relatively long residual effect has been developed (Zoffoli et al., 1999). Chlorine gas (Cl_2) produced by a salt mixture of calcium hypochloride $[Ca(OCl)]_2$, sodium chloride (NaCl) and calcium chloride ($CaCl_2$) with citric acid, combined with cold storage (25 days at 0°) significantly reduce Botrytis rot in both inoculated and field-infected grape cultivars. Infections by conidia or mycelium of *B. cinerea* were suppressed for up to 45 days in cool storage and no deleterious effect of chlorine gas generation was detected. This procedure was suggested to be an alternative to SO_2 treatment for controlling decay in table grapes, since the latter frequently leaves residues exceeding the tolerance limits registered in the US (Zoffoli et al., 1999).

Sugar analogs have long been known to affect metabolic processes of fungi and plant cells (Moore, 1981). The compounds 2-deoxy-D-glucose and L-sorbose were found to interfere with the growth of several filamentous fungi and yeasts when used as a sole carbon source (Biely

et al., 1971). The antifungal action is thought to be due to the interference with the cell-wall-forming enzymes.

Studying the potential of various sugar analogs as antifungal agents for decay suppression, El Ghaouth et al. (1995) found that only 2-deoxy-D-glucose was effective in controlling decay in apples inoculated with *B. cinerea* and *P. expansum* (Table 10), or in peaches inoculated with *M. fructicola*. This compound inhibited *in vitro* radial growth of *P. expansum, B. cinerea, M. fructicola* and even *R. stolonifer*, which is known to be insensitive to most postharvest fungicides. At 0.1% it induced severe morphological alterations in the tested fungi, suggesting that growth inhibition is the result of direct antifungal properties of the sugar analog. It was also suggested that this sugar analog could serve as a useful additive to a biological control method, provided that the antagonist is resistant to its inhibitory action (El Ghaouth et al., 1995).

TABLE 10
Effect of sugar analogs on gray mold and blue mold of apples after 7 days at 24°C[1]

Treatment[2]	Infected fruit (%)	
	Botrytis cinerea	***Penicillium expansum***
Control	100	100
Glucose	100	100
Mannose	97.0	95.2
L-sorbose	94.3	93.1
Rafinose	98.1	100
Deoxy-D-ribose	99.3	96.4
2-deoxy-D-glucose	0 (21.3)[3]	0 (22.4)[3]
LSD_{05}	4.9	2.9

[1] Reproduced from El Ghaouth et al. (1995) with permission of the American Phytopathological Society.

[2] Wounded fruits treated with 50 µl of different sugar analogs or sterile water and 30 min later were inoculated with 30 µl conidial suspensions of *Botrytis cinerea* or *Penicillium expansum*.

[3] After 14 days' incubation.

E. NATURAL CHEMICAL COMPOUNDS

The use of synthetic fungicides has been the major commercial means of postharvest decay control for several decades. However, the chemical residues that are liable to remain on the fruit or within its tissues following fungicidal treatment, and the 1986 report from the US National Academy of Sciences (Research Council, Board of Agriculture, 1987) indicating that fungicide residues on food pose a great health risk to the consumer, led to the search for safe alternatives to synthetic fungicides. The fact that the effectiveness of synthetic fungicides has been reduced by the frequent development of resistance by the pathogens, further highlighted the need for new substances and methods for the control of storage diseases. Naturally occurring plant products are important sources of antifungal compounds with low toxicity to mammals and safe to the environment, which may serve as substitutes for synthetically produced fungicides. It was later suggested that these compounds might be developed either as products per se or used as starting points for synthesis (Knight et al., 1997).

Acetaldehyde, which is a natural volatile compound produced by various plant organs, and accumulates in fruits during ripening, has shown fungicidal properties against various postharvest pathogens. In sublethal concentration it is capable of inhibiting both spore germination and mycelial growth of common storage fungi (Aharoni and Stadelbacher, 1973; Prasad, 1975; Vaughn et al., 1993; Hamilton-Kemp et al., 1992), and the development of yeast species responsible for spoilage of concentrated fruit juices (Barkai-Golan and Aharoni, 1976). It has been reported to inactivate ribonuclease (Mauch et al., 1987) and to bind to other proteins (Perata et al., 1992), but the mechanism of aldehyde toxicity to fungal spores is still unknown.

Fumigation of apples with acetaldehyde inhibits *Penicillium expansum* development in the fruit (Stadelbacher and Prasad, 1974), while fumigation of strawberries with acetaldehyde considerably reduces the incidence of postharvest decay caused by *Rhizopus stolonifer* and *Botrytis cinerea* (Prasad and Stadelbacher, 1974; Pesis and Avissar, 1990). Treating ripe strawberries with 5000 $\mu l\ l^{-1}$ acetaldehyde for 1 h or with 1500 $\mu l\ l^{-1}$ for 4 h resulted in minimal fungal decay during storage for 4 days at 5°C following 1 day at 20°C. Such treatments also induced modest amounts of various fruit volatiles, such as ethanol, ethyl acetate and ethyl butyrate, which increased fruit aroma and improved its taste

(Pesis and Avissar, 1990). Trials carried out with table grapes under natural infection conditions (Avissar and Pesis, 1991) showed that 0.5% acetaldehyde for 24 h suppressed decay caused by *B. cinerea, R. stolonifer,* and *Aspergillus niger*, without injuring the fruit or changing its taste (Fig. 28). Furthermore, fumigating the fruit under these conditions prevents drying of the stems after removal of the grapes to shelf life. The effect of acetaldehyde on *Rhizopus* development is well exhibited after artificial inoculation of grape bunches with an inoculum rich in spores. This effect is of importance because SO_2, which is still in use for decay control in some cases, is efficient mainly in preventing *Botrytis* development, but is ineffective against *Rhizopus* (Avissar and Pesis, 1991).

The efficacy of acetaldehyde vapors and of a number of other aliphatic aldehydes, produced naturally by sweet cherry (cv. Bing), was evaluated in *P. expansum*-inoculated fruits (Mattheis and Roberts, 1993). High concentrations of acetaldehyde, **propanal** and **butanal** suppressed

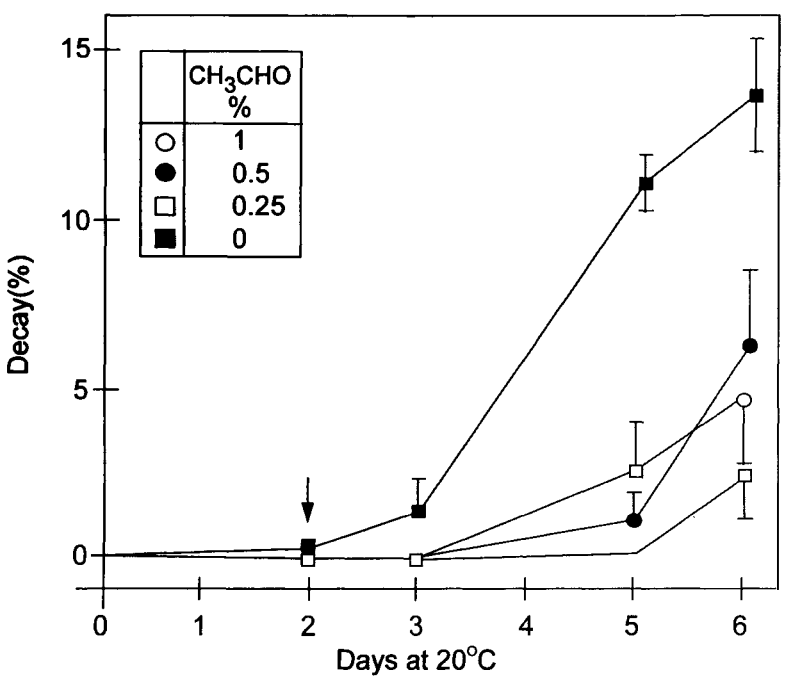


Fig. 28. Effect of acetaldehyde vapor application on the percentage of decay in 'Sultanina' grapes. Vertical bars indicate standard error. (Reproduced from Avissar and Pesis, 1991 with permission of the Association of Applied Biologists).

conidial germination but resulted in extensive stem browning and fruit phytoxicity, which increased with the aldehyde concentration. The degree of inhibition decreased with increasing aldehyde molecular weight. The incidence of *P. expansum* decay was significantly impacted by aldehyde identity and concentration and by the interaction of the two factors. Since stem quality of sweet cherries is a critical component in the evaluation of fresh fruit quality, the browning which follows aldehyde treatments will limit their commercial use. On the other hand, stem quality is less of a concern for fruits intended for processing, and for this purpose aldehyde fumigation may present an alternative to the use of synthetic fungicides.

Injuries resulting from acetaldehyde vapors have been reported for various products, such as cultivars of apples (Stadelbacher and Prasad, 1974), strawberries (Prasad and Stadelbacher, 1974), grapes (Pesis and Frenkel, 1989), lettuce (Stewart et al., 1980) and carrot tissue cultures (Perata and Alpi, 1991).

Nine out of 16 volatile compounds occurring naturally in peach and plum fruits greatly inhibited spore germination of *B. cinerea* and *Monilinia fructicola* (Wilson et al., 1987a). The volatiles most effective in inhibiting spore germination were **benzaldehyde, benzyl alcohol, γ-caprolactone** and **γ-valerolactone**. Of these, benzaldehyde was active at the lowest concentrations tested and completely inhibited germination of *B. cinerea* spores at concentrations of 25 μl l^{-1} and germination of *M. fructicola* spores at 125 μl l^{-1}. It was clearly shown that the same compounds were effective on both fungi and that the relative inhibition by these compounds was similar against each pathogen. Various volatiles (benzaldehyde, **methyl salicylate** and **ethyl benzoate**) have been recorded as growth suppressors. It is of interest to note that while benzaldehyde and methyl salicylate were fungicidal against both pathogens, another volatile, **ethyl benzoate** was fungicidal against one pathogen (*Monilinia*) and fungistatic against the other pathogen (*Botrytis*). The most effective volatiles were found to be active at concentrations that should make them potential fumigants for postharvest disease control (Wilson et al., 1987a).

Evaluation of 15 volatile odor compounds, released from raspberries and strawberries during ripening, for their ability to inhibit postharvest decay fungi, showed that five of them inhibited the growth of *Alternaria alternata, B. cinerea* and *Colletotrichum gloeosporioides* directly on the fruit at 0.4 μl ml^{-1} (Vaughn et al., 1993). Among the five compounds, benzaldehyde was the most toxic to the fungi and completely inhibited

cultures of the three fungal species at only 0.04 μl ml^{-1}, while **1-hexanol, E-2-hexenal** and **2-nonanone** inhibited them at 0.1 μl ml^{-1} and **Z-3-hexen-1-ol** did not completely suppress growth when applied at <0.4 μl ml^{-1}. Furthermore, the compound, 2-nonanone was capable of reducing decay incidence in raspberries and strawberries in enclosed containers at 10°C under natural infection conditions.

Ethanol, which occurs naturally in fruits and other food products, was tested in 1960 for control of brown rot and Rhizopus rot in peach fruits (Ogawa and Lyda, 1960) with varying degrees of success. Recently, the effects of ethanol solutions, at concentrations of 10-20%, were evaluated for the control of postharvest decay of citrus fruits (Smilanick et al., 1995) and peaches and nectarines (Margosan et al., 1997). However, heating of the solutions was required to improve decay control markedly and to obtain acceptable efficacy (see the chapter on Physical Means - Heat Treatments: Combined Applications).

Hinokitiol is a natural volatile extracted from the root or trunk of Japanese cypress (Hinoki – *Hiba arborvitae*), with outstanding antifungal properties. The effective dose of hinokitiol that reduced fungal spore germination by 50% (ED_{50}) was found to be 15-30 μg ml^{-1} for *M. fructicola, Rhizopus oryzae* and *B. cinerea.* In parallel, this volatile prevented decay of commercially harvested peaches in which more than 40% of the untreated fruit developed brown rot (*M. fructicola*) (Sholberg and Shimizu, 1991).

To evaluate the control capability of hinokitiol in eggplants and red peppers, its effect on *B. cinerea* and *A. alternata*, the main pathogens of these vegetables, has been studied (Fallik and Grinberg, 1992). Sensitivity of *Botrytis* to hinokitiol was found to be greater than that of *Alternaria*, and the germination process to be more sensitive than that of mycelial growth. Immersion of eggplants and peppers in a 750 μl l^{-1} solution of hinokitiol gave good protection against decay during storage and, especially, at shelf life without injury to the fruit. Fig. 29 shows that the effectiveness of hinokitiol (750 μl l^{-1}) in suppressing decay in eggplants after 14 days of storage at 12°C and four additional days under shelf life conditions, is greater than that of prochloraz at the common concentration (2000 μl l^{-1}) (Fallik and Grinberg, 1992).

Hinokitiol is also effective in controlling decay in Galia melons stored for 14 days at 6°C followed by an additional 6 days under shelf life conditions, simulating sea transport of the fruit from Israel to Europe

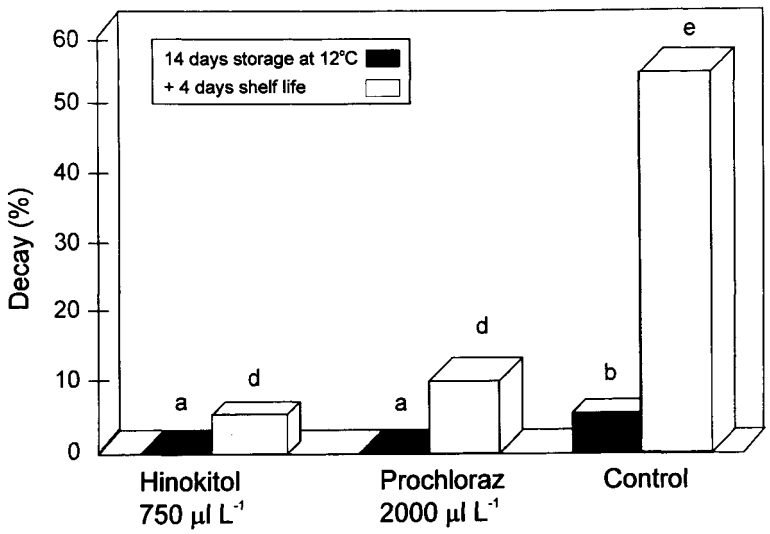


Fig. 29. Effect of hinokitiol and prochloraz on decay development in eggplant fruit after 14 days of storage (left) and an additional 4 days of shelf-life (right), compared with water dipped control. Letters represent significant differences according to Duncan's Multiple Range Test. (Reproduced from Fallik and Grinberg, 1992 with permission of Elsevier Science).

(Aharoni et al., 1993b). Today, melons are coated with wax containing imazalil (200 ppm), and under the above conditions, this leaves fungicidal residue above the level approved in some countries (0.5 ppm). Introducing hinokitiol into the wax was also found to control decay during cold storage, caused mainly by *A. alternata* and *Fusarium* spp. – without any phytotoxic effects. In order to suppress decay at shelf life also, the concentration of the hinokitiol has to be increased, but such an increase causes peel browning.

Essential oils and plant extracts are sources of antifungal activity against a wide range of fungi (Grange and Ahmed, 1988). A rapid assay to determine antifungal activity in both plant extracts and essential oils has recently been described by Wilson et al. (1997b). Among 345 plant extracts analyzed, 13 showed high levels of activity against *B. cinerea*, which served as a test fungus. The major plants showing the highest persistent antifungal activity were garlic (*Allium* sp.) and pepper (*Capsicum* sp.). The high antifungal activity recorded for garlic extracts is in accordance

with an earlier study indicating the marked antifungal activity hidden in the tissues of this crop (Ark and Thompson, 1959). Among the 49 essential oils tested, those of palmarosa (*Cymbopogon martini*) and red thyme (*Thymus zygis*) showed the greatest inhibitory effect on *B. cinerea* spore germination at the lowest concentration. The next best inhibitors were essential oils of clove buds (*Eugenia caryophyllata*) and cinnamon leaf (*Cinnamomum zeylanicum*). The most frequently occurring constituents in essential oils showing high antifungal activity were: *D*-limonene, cineole, α-pinene, β-pinene, β-myrcene and camphor. The fungicidal activity of the individual components, singly and in combination, is being studied (Wilson et al., 1997b). Essential oil derived from another species of *Thymus, T. capitatus*, markedly reduced development of *B. cinerea* in inoculated mandarin fruit when applied as a vapor. Scanning electron microscope observations indicated a direct damaging effect of the thyme oil on fungal hyphae (Arras and Piga, 1994).

Glucosinolates, a large class of compounds produced by plants of the Cruciferae (Mari and Guizzardi, 1998), are other natural substances with potential antimicrobial activity. When cells of plant tissues that metabolize glucosinolates are damaged, these compounds come into contact with the enzyme myrosinase, which catalyzes hydrolysis. The antifungal activity of six **isothiocyanates**, produced by the enzymatic hydrolysis of glucosinolates, has been tested on several postharvest pathogens *in vitro* (Mari et al., 1993) and *in vivo* on artificially-inoculated pears (Mari et al., 1996), with encouraging results.

Chitosan is an animal-derived polymer formed by de-acetilation of chitin. It can serve as a coating for the fruit or vegetable and for regulating gas moisture and exchange around the product (Wilson et al., 1994). When applied as a coating, chitosan delayed the ripening of tomatoes and reduced decay incidence indirectly by modifying the internal atmosphere. A marked reduction of decay has been recorded in chitosan-treated bell peppers, cucumbers, strawberries and carrots (Cheah et al., 1997; Wilson et al., 1994). In litchi fruits, chitosan coating delayed browning of the pericarp, which is the main problem during prolonged storage (Zhang and Quantick, 1997). Application of a chitosan coating, in addition to reducing weight loss of the fruits, delayed changes in the content of antocyanin, flavonoid and total phenolics, delayed the increase in polyphenol oxidase activity and partially inhibited the increase in peroxidase activity, which are associated with tissue browning. This implies that a chitosan coating

may form a protective barrier on the surface of the fruit and reduce the supply of oxygen for enzymatic oxidation of phenolics (Zhang and Quantick, 1997). Application of a chitosan coating also delayed, to some extent, the increase in decay of stored litchi.

Chitosan application was also found to stimulate the formation of structural defense barriers in bell peppers and tomatoes. These included the induction of callose synthesis, thickening of host cell walls, formation of papillae and plugging of some intercellular spaces with fibrillar material, probably impregnated with antifungal phenolic-like compounds (Wilson et al., 1994).

However, chitosan also exhibits a direct fungicidal activity and can affect postharvest pathogens by suppressing their growth: it inhibited spore germination, germ-tube elongation and radial growth of *B. cinerea* and *R. stolonifer*, inducing cellular alterations and damage in culture (El Ghaouth et al., 1992). Similarly, exposure of *Sclerotinia sclerotiorum* to chitosan resulted in hyphal deformation and restricted mycelium growth (Cheah et al., 1997). Trials with chitosan-treated bell peppers indicated the damage caused to *B. cinerea* hyphae, exhibited as changes in the cell wall and by cytoplasm decomposition (El Ghaouth et al., 1994). Examination of sections from chitosan-treated tissue of bell pepper fruits by transmission electron microscope revealed that chitosan prevented the disintegration of host cell walls by the pectolytic enzymes of *B. cinerea* (El Ghaouth et al., 1997). This was indicated by the preservation of pectic binding sites and the regular intense cellulose labeling over host cell walls pressed against fungal cells. In addition to reducing the production of polygalacturonase by the pathogen, chitosan also caused severe cytological damage to invading hyphae. These phenomena may explain, at least in part, the limited ability of *Botrytis* to colonize the fruit tissues in the presence of chitosan (El Ghaouth et al., 1997). In parallel, chitosan may also induce the formation of antifungal hydrolases, such as chitinase and β-1,3-gluconase, which may lead to the reduction in the chitin content of fungal cell walls and the stimulation of various structural defense barriers in fruits such as bell peppers and tomatoes. Because of these characteristics, chitosan has been hypothesized to be an elicitor of resistance in harvested crops (see the chapter on Novel Approaches for Enhancing Host Resistance – Induced Resistance).

Gel derived from **Aloe vera** plants has been found to have antifungal activity against four common postharvest pathogens: *Penicillium digitatum, P. expansum, B. cinerea* and *A. alternata.* The natural gel

suppressed both germination and mycelial growth, with *P. digitatum* and *A. alternata* being the most sensitive species. The antifungal potential of the gel in decay suppression was exhibited on *P. digitatum*-inoculated grapefruit, and was expressed in a delay in lesion development as well as a significant reduction in infection incidence at shelf life conditions (Saks and Barkai-Golan, 1995).

Latex, present in some fruits, is another natural fungicide or a source of fungicides, which is regarded as both safe and effective against various diseases of banana, papaya and other fruits (Adikaram et al., 1996). Papaya latex is a complex mixture of sugars with several enzymes, notably proteases, glucosidases, chitinases and lipases. The water-soluble fraction of papaya latex can completely digest the conidia of many fungi, including important postharvest pathogens (Indrakeerthi and Adikaram, 1996). A small cystein-rich protein, hevein, was isolated from the latex of the rubber tree (*Hevea brasiliensis*). It showed a strong antifungal activity *in vitro* against several fungi such as *B. cinerea* and species of *Fusarium* and *Trichoderma* (van Parijs et al., 1991). Hevein, which is a chitin-binding protein, might interfere with fungal growth by binding or cross-linking newly synthesized chitin chains. Alternatively, the delicate balance between chitin synthesis and selective hydrolysis of preformed chitin chains in hyphal walls might be interrupted (Cabib, 1987; van Parijs et al., 1991).

Antibiotic compounds secreted by antagonistic bacteria are also among the natural compounds which may suppress postharvest pathogen development. Such compounds are the **iturins**, antimicrobial substances produced by *Bacillus subtilis* strains or isolates. They have a wide antifungal spectrum and were found effectively to control *M. fructicola* development in cold-stored stone fruits (Pusey, 1991). **Pyrrolnitrin** is a natural antibiotic compound produced by *Pseudomonas cepacia*. This compound was found to delay *B. cinerea* development in strawberries, although no reduction of the total incidence of decay was recorded (Takeda et al., 1990).

F. LECTINS

Lectins are a class of sugar-binding proteins that are widely distributed in nature and their occurrence in plants has been known

since the end of the 20th century. However, the role of plant lectins is still not well defined and understood. One of the theories proposed hypothesizes that lectins act as recognition determinants in the formation of symbiotic relations between leguminous plants and nitrogen-fixing bacteria, a process of great importance in agriculture and in the nitrogen cycle of terrestrial life. According to a second hypothesis, these lectins can play a role in the defense of plants against various animals, as well as against phytopathogenic fungi (Sharon, 1997).

Interactions of fungal hyphae with lectins were first demonstrated by Mirelman et al. (1975), who found that wheat germ agglutinin (WGA), which is specific to chitin oligosaccharides, binds to hyphal tips and hyphal septa of *Trichoderma viride* – a fungus with a chitinous hyphal surface. This interaction resulted in the inhibition of hyphal growth and spore germination. Based on these findings it was suggested that WGA has a role in the protection of wheat seedlings against chitin-containing fungi.

To assess whether the binding of lectins to fungal surfaces and their inhibiting effects on fungal growth are general phenomena, Barkai-Golan et al. (1978) investigated the interaction of several lectins, characterized by differing sugar specificities, with various fungi belonging to different taxonomic groups. WGA was found to bind to young hyphal walls of all the fungi examined except for the chitinless *Phytopthora citrophthora* (Table 11). Soybean agglutinin (SBA), specific for D-galactose and N-acetyl-D-galactose, and peanut agglutinin (PNA), specific for D-galactose, were found to bind those fungi that contain galactose residues in their chitinous cell walls. Among these were several species of *Penicillium* and *Aspergillus*. Other fungi, such as *Geotrichum candidum, Botrytis cinerea* and *Fusarium moniliforme*, exhibited binding with SBA alone. The abilities of SBA and PNA to bind to fungal surfaces of *Penicillium* and *Aspergillus* species is in good agreement with the presence of galactose reported in some species of these genera, such as *Penicillium digitatum* and *P. italicum* (Grisaro et al., 1968) or *Aspergillus niger* (Bardalaye and Hordin, 1976). The binding of fungi to concanavalin A (Con A) is more difficult to account for since this lectin reacts only poorly with β-linked glucans and with chitin (Sharon and Lis, 1989). It is possible, however, that a positive reaction with Con A indicates the presence of small quantities of α-linked D-glucose (or D-mannose) residues on the fungal surface (Barkai-Golan et al., 1978).

TABLE 11
Binding of different lectins to fungal cell walls[1]

Fungus species and taxonomic group	Chemical category[2]	Lectin[3]			
		WGA	SBA	PNA	ConA
Phycomycetes					
Zygomycetes	Chitosan-chitin				
Rhizopus stolonifer Ehr.		+	–	–	+
Mucor sp.		+	–	–	+
Oomycetes	Cellulose-β-glucan				
Phytophthora citrophthora (Sm. et Sm.) Leon.		–	±	–	+
Ascomycetes	Chitin-β-glucan				
Pleospora herbarum (Pers.) Raben.		+	–	–	–[4]
Sclerotinia sclerotiorum (Lib.) de By.		+	±	–	+
Basidiomycetes	Chitin-β-glucan				
Rhizoctonia solani Kuhn		+	–	–	–
Sclerotium rolfsii Sacc.		+	+	+	+
Deuteromycetes	Chitin-β-glucan				
Penicillium italicum Wehm.		+	±	±	+
P. expansum Link		+	±	±	+
P. citrinum Thom		+	±	±	+
P. digitatum Sacc.		+	±	±	±

+ = marked binding; ± = moderate to weak binding; – = no binding

[1] Reproduced from Barkai-Golan et al. (1978) with permission of Springer-Verlag GmbH & Co. KG.

[2] As proposed by Bartnicki-Garcia, 1968

[3] WGA = wheat germ agglutininm; SBA = soybean agglutinin; PNA = peanut agglutinin; ConA = concanvalin A

[4] Binding confined to thin hyaline hyphae

Fungus species and taxonomic group	Chemical category[2]	Lectin[3]			
		WGA	SBA	PNA	ConA
Aspergillus niger v. Tiegh.	Chitin-β-glucan	+	±	±	+
A. flavus Link		+	±	±	+
A. ochraceus Wilhelm		+	+	+	+
Geotrichum candidum Link		+	+	–	+
Botrytis cinerea Pers.		+	±	–	+
Fusarium moniliforme Sheld.		+	±	–	+
Trichoderma viride Pers. ex Fries		+	–	–	+
Diplodia natalensis P.E.		+	–	–	±
Stemphylium botryosum Wall.		+	–	–	–
Alternaria tenuis Nees		+	–	–	–
Cladosporium herbarum (Pres.) Link		+	–	–	–

The binding of PNA and SBA occurred typically along the hyphal walls, with no pronounced binding at the tips and septa, indicating that both lectins bind preferentially to relatively mature regions of the hyphae. The binding pattern of WGA, on the other hand, emphasized that this lectin binds to regions of the hyphae in which chitin is actively synthesized (Barkai-Golan et al., 1978).

The three lectins were also found to inhibit the incorporation of acetate, N-acetyl-glucoseamine and galactose into young hyphae of *Aspergillus ocharaceus*, indicating the interference with normal fungal activity and growth (Barkai-Golan et al., 1978).

These results suggest that lectins may be useful in studies of chemical composition of hyphal wall surfaces. Lectins with different sugar specificities have actually been efficient in studying hyphal cell walls of mycobionts isolated from different lichens (Galun et al., 1976) and were a useful aid for the study of cell wall composition of yeasts of different taxonomic groups (Barkai-Golan and Sharon, 1978). However, the findings also support the suggestion that one role of lectins in plants is protection against fungal pathogens.

Additional support for the antifungal capacity of lectins was contributed by an extensive study with 11 purified lectins, representing

all the major groups of lectin specificity. A growth disruption during germination of spores was reported for three fungal species, including the postharvest pathogen *Botryodiplodia theobromae* (Brambl and Gade, 1985). According to a subsequent report, however, the antifungal activity of WGA was most likely due to contaminating chitinases in the lectin preparations used, which are known as potent inhibitors of fungal growth (Schlumbaum et al., 1986). However, this criticism does not invalidate the results obtained with lectins such as peanut and soybean agglutinins, which are specific for sugars other than oligomers of N-acetylglucosamine (Sharon and Lis, 1989). Furthermore, a chitinase-free lectin obtained from stinging nettle (*Urtica diocia*), also specific for chitin, was demonstrated to inhibit the growth of several phytopathogenic fungi (Broekaert et al., 1989). The nettle lectin probably acts by interfering with chitin synthesis by the fungi.

It should be mentioned, however, that although plant lectins have shown antifungal activity towards various plant pathogenic fungi, no protective role of these lectins has been demonstrated *in vivo*.

CHAPTER 10

PHYSICAL MEANS

There is increasing public awareness that many of the chemical treatments applied to fresh horticultural products to control decay are potentially harmful to consumers. This fact, along with the possible consequence that a number of chemicals may be withdrawn from use, has revived and increased interest in physical treatments that could serve as alternatives to fungicides. Cold storage and controlled or modified atmospheres are physical means discussed in the chapter on Means for Maintaining Host Resistance. The present chapter will discuss other physical means: heating, ionizing radiation and ultraviolet illumination.

A. HEAT TREATMENTS

Heat treatment may be applied to the commodity by means of hot water dips and sprays, hot vapor or dry air, or infrared or microwave radiation. However, practical systems have used mainly hot water or vapor (Couey, 1989). While hot water was originally used for fungal control and was extended to removal of insects from fresh commodities, vapor heating, developed for insect control may also serve to reduce fungal decay. Thus, the vapor heat treatment used for disinfestation of Carabao mangoes in the Philippines also significantly reduced the incidence of anthracnose and stem-end rot in fruit, although the onset of decay was not delayed by the treatment (Esquerra and Lizada, 1990). Similarly, the development of green mold on grapefruit, caused by *Penicillium digitatum* was inhibited by the 300-min treatment with moist forced air at 46°C used to provide quarantine security against the Mexican fruit fly (Shellie and Skaria, 1998). Hot humid air has also been useful in controlling decay in crops that would have been injured in hot water. For example, postharvest decay of strawberries caused by *Botrytis cinerea* and *Rhizopus stolonifer* was controlled by exposure of the fruit to humid air at 44°C for 40-60 min (Couey and Follstad, 1966).

The possibility of using hot water dips to control decay in citrus fruits

had already been reported in the 1920s (Fawcett, 1922), and during the following decades, hot water treatments were tested on and applied to various fruits and vegetables, although the intensive use of chemicals pushed them aside, along with other means of control. Today, with the trend toward less reliance on chemical treatments, the interest in postharvest use of heat treatment has revived. A list of commodities heat-treated to control postharvest decay is presented in the reviews by Barkai-Golan and Phillips (1991) and Coates and Johnson (1993).

Short-term and Long-term Heat Treatments

Short-term heating, where the fruit or vegetable is dipped in hot water at temperatures above 40°C (generally 44-55°C) for a short time (from a few minutes to 1 h), has been the main heat treatment studied over the years. The principle is in the use of temperatures that are high enough to inactivate the pathogen without causing significant changes to the host. Early studies had already confirmed that fruits and vegetables commonly tolerate such temperatures for 5-10 min, and that even shorter exposures to these temperatures is sufficient to control many of the postharvest pathogens (Smith, W.L., Jr. et al., 1964).

Recent studies reveal increased interest in long-term heat treatments, in which the commodity is exposed to temperatures lower than those mentioned above (usually 38-46°C), but for a longer duration (12 h to 4 days) (Fallik et al., 1996; Klein et al., 1997). Both short-term and long-term heating, aimed at suppressing storage decay, could act directly by inactivating the pathogen, or indirectly via physiological and biochemical changes in the host, which enhance the resistance of the tissues to the pathogen.

The Effect of Heat on the Pathogen

Genetic differences among fungi are expressed in differences in their sensitivities to high temperatures and, therefore, in differences among levels which kill them or inhibit spore germination and hyphal growth (Sommer et al., 1967) (Fig. 30). For a given species, spore inactivation increases with both temperature and duration of treatment; conidia of *Alternaria alternata* may be inactivated equally by treatment for 2 min at 48°C or for 4 min at 46°C (Barkai-Golan, 1973) (Fig. 31).

Spore sensitivity to heat is also dependent on their physiological state. Germinated fungal spores are markedly more sensitive to heat than non-germinated spores. It was found that water at 42°C does not affect dormant conidia of *A. alternata* but does inactivate many germinating

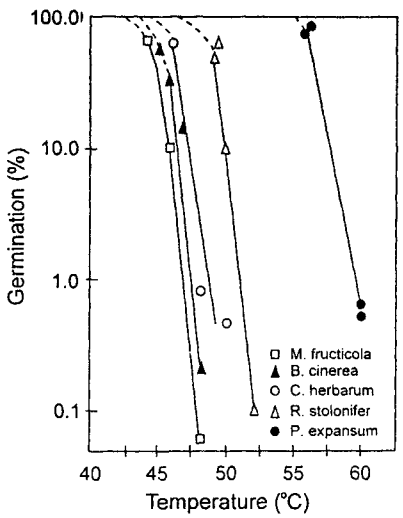


Fig. 30. Survival of spores of *Molinilia fructicola, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer* and *Penicillium expansum* after 4 min heat at the indicated temperatures. Reproduced from Sommer et al., 1967 with permission of the American Phytopathological Society.

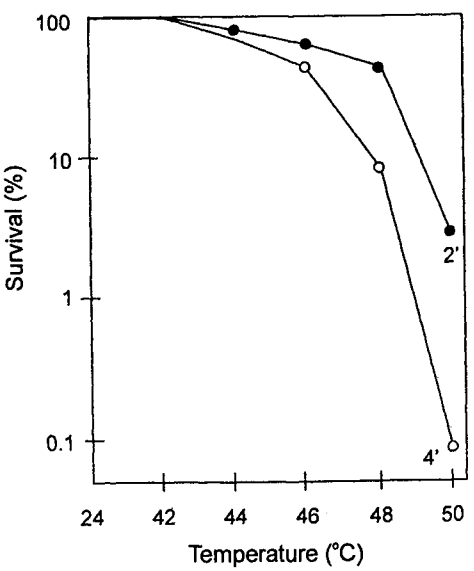


Fig. 31. Heat response curve for *Alternaria alternata* spores (2 and 4 min heat treatment). Reproduced from Barkai-Golan, 1973 with permission of the Mediterranean Phytopathological Union).

conidia (Barkai-Golan, 1973) (Fig. 32). Similarly, the LD_{50} temperature for sporangiospores of *Rhizopus stolonifer* exposed to hot water for 4 min, was 49°C, whereas that for germinating spores was only 39°C (Eckert and Sommer, 1967).

The moisture content of spores before or during exposure to heat can considerably influence heat transfer and spore survival (Edney and Burchill, 1967). Comparisons between the heat sensitivities of moist and dehydrated conidia of *Penicillium digitatum* revealed that exposure to 70°C for 30 min killed 90% of the moist spore population but only 10% of the dry spore population. Dry spores that survived this treatment were capable of infecting citrus fruit, although the onset of symptoms was delayed 24 hours. The resistance of the dry spores to high temperatures may explain their ability to survive from season to season without losing their pathogenicity to citrus fruit (Barkai-Golan, 1972).

Possible mechanisms of pathogen control by heating include: pectic enzyme inactivation or denaturation of other proteins; lipid liberation; destruction of hormones; depletion of food reserves; or metabolic injury, with or without accumulation of toxic intermediates. More than one of these mechanisms may act simultaneously (Barkai-Golan and Phillips, 1991).

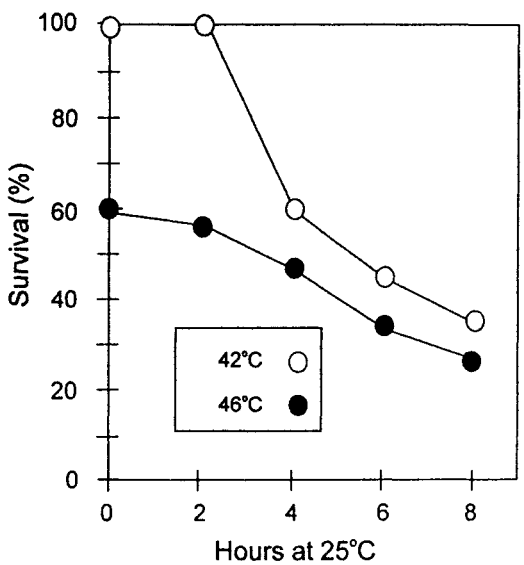


Fig. 32. Sensitivity to heat (2 min at 42°C or 46°C) of freshly harvested and germinating spores of *Alternaria alternata*. (Reproduced from Barkai-Golan, 1973 with permission of the Mediterranean Phytopathological Union).

Ultrastructural changes in heat-treated, non-germinated spores of *Monilinia fructicola* were seen as progressive destruction of the mitochondria, disruption of vacuolar membranes and formation of gaps in the conidial cytoplasm (Margosan and Phillips, 1990). The site most sensitive to heat in dormant conidia of *M. fructicola* may be the mitochondria, probably in the inner membrane. In germinating conidia, exposure to heat results in changes in the nuclei, in the cell wall, or both (Baker and Smith, 1970).

As can be seen from the *in vitro* studies (see Fig. 28), *Botrytis cinerea* is very sensitive to high temperatures, and heat treatments have found to control it on various fruits and vegetables, including apples (Fallik et al., 1995; Klein et al., 1997), sweet peppers (Fallik et al., 1996), tomatoes (Fallik et al., 1993) and strawberries (Garcia et al., 1996).

Short-term, hot water dips (50°C for 3 min) completely inhibited, or significantly reduced, decay development in artificially inoculated or naturally infected sweet red peppers (Fallik et al., 1996). Higher temperatures or longer exposures to 50°C resulted in heat damage. The mode of action of hot water dips on decay development appears to be interaction with fungal pathogens, as was exhibited by the inhibition of spore germination and germ-tube elongation of *B. cinerea* and *A. alternata*, the two main fungi responsible for postharvest decay of peppers.

Long-term, hot water immersion (38°C for 3 days) of *Botrytis*-inoculated tomatoes totally prevented gray mold decay under shelf-life conditions, with no damage to the fruit (Fallik et al., 1993). It was shown that heat treatments at 38°C directly suppressed spore germination of *B. cinerea* within 24 h following their exposure to heat stress. Longer heat treatments inhibited hyphal growth and prevented expansion of the colony. The direct effect of long-term heat treatment has been exhibited also for the more heat-resistant fungus, *Penicillium expansum* (Fallik et al., 1995). Heating to 38, 42 or 46°C directly arrested spore germination and mycelial growth in culture, the necessary duration of exposure being in inverse proportion to the temperature. However, the direct effect is not the only way in which the long-term heating (38°C, 96 h) inhibits decay development in *P. expansum*-inoculated apples: a similar inhibition of spore germination also occurs when the spores are incubated in peel extracts derived from fruits heated under the same conditions without any direct exposure of the spores to the heat. In this case, decay inhibition is the result of the indirect effect of heat on the pathogen, via the heat-treated host.

The Effect of Heat on the Host

Heat can affect many of the processes within the tissues, such as the rate of fruit ripening, the development of fruit color, sugar metabolism, electrolyte leakage, ethylene production, respiration rate, pectic enzyme activity, volatile production and susceptibility to pathogens (Barkai-Golan and Phillips, 1991). Decay control together with ripening inhibition was reported for plastic-wrapped nectarines treated by moist air heating (Anthony et al., 1989). The inhibition of ripening by heat may be mediated via its effect on the ripening hormone, ethylene, since heat treatment has been found to inhibit ethylene synthesis within hours, in both apples and tomatoes (Biggs et al., 1988; Klein, 1989). Elevated temperatures can lead to the accumulation of endogenous ACC (1-aminocyclopropane -1- carboxylic acid), the precursor of ethylene synthesis, in apple and tomato tissue, concomitantly with the decrease in ethylene. However, raising the temperature higher or extending the exposure of the fruit to it will cause the disappearance of ACC as well (Klein, 1989; Atta Aly, 1992). The inhibition of ethylene formation is reversed when the fruits are removed from heat; this recovery requires protein synthesis, and both mRNA and protein of ACC oxidase were found to accumulate during recovery from heat treatment (Lurie et al., 1996). A relationship between the inhibition of both ACC synthase and ACC oxidase was detected in mango fruits during heat treatment (Ketsa et al., 1999): following heating, ACC oxidase recovered full activity while ACC synthase recovered only partially, but sufficiently to allow the previously heated fruits to achieve an ethylene peak. During the heating period, not only is endogenous ethylene production inhibited, but the fruits also do not respond to exogenous ethylene (Yang et al., 1990).

Heated fruits often soften more slowly than non-heated fruits. Plums, pears, avocados, and tomatoes have all been found to soften more slowly when held at temperatures between 30 and 40°C than at 20°C. The rate of softening increased when the heated fruits were returned to 20°C, but it was still less than that of non-heated fruits (Klein and Lurie, 1991). Holding apples at 38°C for 3-4 days prior to removal to prolonged cold storage (0°C) resulted in maintenance of firmness even after 6 months of storage at 6°C, and 10 shelf-life days at 20°C. The retardation of softening was related to the high level of insoluble pectin in the heated fruit as compared with that in the non-heated fruit, because of the inhibition of the synthesis of cell-wall degrading enzymes.

A heat treatment can change the climacteric respiration peak as well as advancing or delaying it after treatment; the extent to which it does so

depends on the temperature and the length of exposure. The response of a particular fruit or vegetable is determined by a combination of factors: the physiological age of the commodity, the time and temperature of exposure, whether the commodity is removed from heat to storage or to ripening temperature, and whether the heat treatment causes damage (Lurie, 1998).

Decay Suppression through Increased Host Resistance

Along with the retardation of the ripening process which follows heat treatments, heating may lead to the maintenance of fruit quality during prolonged storage. In many cases no damage is caused to the fruit and, in some fruits, enhanced resistance to pathogens is induced in parallel with the maintenance of fruit firmness. In this respect, heat treatment could be included among the means that suppress decay by maintaining host resistance.

Spotts and Chen (1987) described decay suppression in injured pears, obtained through enhanced resistance to postharvest diseases which followed heating at 37°C; decay caused by *Mucor* and *Phialophora* was reduced when the fruits were inoculated after heating – that is to say, without the direct exposure of the pathogen to heat. In this case, the heating was found to increase the resistance of the wound to fungal infection. The mechanism by which heat treatment causes changes in fruit ripening and induces host resistance may be tied to changes in gene expression and protein synthesis (Sachs and Ho, 1986). Various studies, however, related the increased infection resistance of the host, following heating, to the accumulation and enhanced activity of antifungal compounds.

1. Enhanced antifungal activity of the fruit

Heating green lemon fruits (36°C, 3 days) was found to inhibit the reduction of the antifungal citral in the rind, which occurs naturally during fruit ripening (Rodov et al., 1995b). This was suggested to be the reason why heating the fruit prolongs the antifungal activity of the rind and elicits a considerable reduction in decay development (Ben-Yehoshua et al., 1995). The hypothesis is, therefore, that decay suppression following heat treatments is related, at least in part, to the enhanced activity of the antifungal compounds located in the peel (preformed antifungal compounds).

Heat stress can also induce the synthesis of the enzyme, phenylalanine ammonia lyase (PAL) in citrus fruit (Golomb et al., 1984). This is one of

the key enzymes in the production of various antifungal compounds. PAL has also been suggested to be involved in the enhanced resistance of kiwifruits to infection, after curing; Ippolito et al. (1994) found that the gray mold rot in kiwifruits which had been inoculated with *Botrytis cinerea* at the pedicel scars or on artificial wounds, was significantly decreased after curing at 15°C and 95-98% RH for 48 h, before storage at 0°C. The activity of PAL at the sites of infection was enhanced in cured fruits and, under blue light, an accumulation of phenolic compounds was recorded.

The accumulation of a phytoalexinic compound in heat-treated citrus fruits was reported by Kim et al. (1991). Heat treatments applied to lemon fruits inoculated with *Penicillium digitatum* resulted in the accumulation of the phytoalexin scoparone (6, 7-dimethoxy- coumarin) in the peel during heating and several days later. The increase in the scoparone concentration in the fruit was directly correlated to the increase in the antifungal activity of the fruit extracts, as exhibited in their ability to retard spore germination and germ-tube elongation. From these results arose the hypothesis that the phytoalexins have an important role in enhancing the disease resistance of the heated fruits (Kim et al., 1991).

2. Induction of heat shock proteins

Various varieties of fruits and vegetables may differ in their tolerance to high temperatures. In general, tropical fruits, such as mangoes and papayas, are more heat tolerant than fruits from temperate zones (Couey, 1989). Exposing the host to too high a temperature may result in heat injury - expressed in changes in color and texture, increased water loss and enhanced susceptibility to infesting microorganisms.

The exposure of plant tissues to thermal stress was found to result in the rapid induction of a small set of specific proteins called heat shock proteins (HSPs) (Key et al., 1981). A correlation was found between the development of enhanced fruit thermotolerance and the synthesis of HSPs, as well as between the loss of thermotolerance and the disappearance of HSPs (Vierling, 1991). The development of thermotolerance depends on the temperature level: to initiate HSP synthesis, the temperature should be high enough (35-40°C), but at higher temperatures (>42°C), however, HSP synthesis is attenuated and commodities are likely to suffer heat damage (Ferguson et al., 1994). The induction of HSPs of differing molecular weights has been described in various fruits, such as papaya, plum, apple and tomato, or in pear fruit

cell culture (Lurie, 1998). By enhancing host thermotolerance, the HSP synthesis which follows heat treatment may contribute to the possibility of using higher, more effective heat exposures for heat-sensitive fruits as well.

Induction of Resistance to Chilling Injury

Immersing citrus fruits in hot water for short periods was found to reduce their sensitivity to cold-storage temperatures, thus reducing both chilling injury and decay incidence during storage (Rodov et al., 1995a; Wild and Hood, 1989). The addition of fungicides, such as thiabendazole or imazalil, to the hot water (53°C, 2 min) can enhance the tolerance of the fruit to low temperatures (McDonald et al., 1991; Wild and Hood, 1989), but even when this combination does not reduce chilling injury, it enhances the decay control imparted by the hot water.

Electron microscopic examination of grapefruits which have been immersed in hot water has revealed structural changes, and less cracking in the fruit cuticle than in those of fruits which developed chilling injury. Wrapping fruits which had previously been immersed in hot water, in sealed plastic film enhanced their tolerance to chilling, but was not essential for the success of the treatment (Rodov et al., 1995a).

Similarly to the short-term heat treatments (involving high temperatures), long-term heat treatments (involving lower temperatures) can also induce resistance to chilling injury in cold-sensitive fruits. Mangoes, which are a tropical crop, are subject to chilling injury when stored below 10°C (Couey, 1986). The symptoms of injury include rind discoloration, pitting, uneven ripening, poor color and flavor, and increased susceptibility to decay. However, when the fruits were kept at 38°C for 24 or 48 h before storage at 5°C for 11 days, chilling injury symptoms were reduced, and the reduction was enhanced with increased duration of the high-temperature treatment (McCollum et al., 1993). The most pronounced effect was reduction of the rind pitting and discoloration that were apparent at the time of transfer from 5 to 21°C, and which persisted during ripening.

Chilling injury is also characteristic of tomato fruits stored at temperatures lower than 10-12°C. However, tomatoes exposed to 36-40°C for 3 days did not develop chilling injury, and ripened normally following storage at 2°C for 3 weeks (Klein and Lurie, 1991; Sabehat et al., 1996). The resistance to low-temperature injury was found to be contingent on the presence of HSP (Sabehat et al., 1996), but it may not be due solely to the presence of HSP. Heat treatment may cause damage to the cell

membrane and is known to cause a temporary increase in membrane leakage (Lurie and Klein, 1990; Salveit, 1991). The ability of heat treatments to enhance resistance to low-temperature injury is an example how one stress – heat stress – may protect the plant tissue against another stress – low-temperature stress (Lurie et al., 1994). One should remember that the defense of the fruit against chilling injury may result, in addition to the repair of the physiological damage, in a reduction in the decay that typically develops in chilled tissues. Thus, following heat treatment, it is possible to store sensitive fruits at low temperatures without inducing chilling injury and subsequently increased decay development.

Efficiency of Heat Treatments

Heat is delivered to the commodity by means of contact with hot air or water. The water content of the air greatly influences heat transfer, and greater pathogen inactivation is usually achieved by treatment with moist heated air than with dry air at the same temperature (Teitel et al., 1989). The reason for this may be the more vigorous physiological activity of moist spores than of dry spores, or the fact that moist air transfers heat more effectively than dry air. When dry air is applied, condensation does not form on the target commodity, and the rate of heat transfer depends largely on the temperature of the air passing over the surface of the commodity and the heat conductivity of the commodity. When the air is saturated (vapor heat), condensation forms on surfaces that are cooler than the air and heat is transferred rapidly to the surface (Edney and Burchill, 1967).

The complex structure of a given host may greatly influence the rate of heat transfer. A grape berry, for example, transfers heat faster than the tissues of an apple. Also, heat transfer from tissue to tissue can vary greatly within the leaf, stem, root or fruit. Furthermore, heat transfer may differ among different tissues of the fruit itself. The colored outer layer (flavedo) of the citrus rind, which may have few and small intercellular spaces, can transfer heat faster than the underlying white spongy tissue (albedo) (Barkai-Golan and Phillips, 1991).

Since heat treatments may act directly on the spore population infesting the host surface, a short exposure to heat may sometimes be sufficient to reduce decay incidence markedly. However, heat treatment is also based on the gradual penetration of heat into the host tissues; thus the extent of pathogen progress within the tissues may determine the success or failure of the treatment. Immersion of lemons in hot water

(46-49°C) for 4 min arrests development of *Phytophthora citrophthora*, the causal agent of brown rot, only if the fungus has not yet penetrated the outer layer of the rind. Furthermore, factors affecting the rate of fungal development and its progress in the fruits, prior to harvesting, such as the temperature prevailing in citrus groves during the rainy season, may also determine the efficacy of heat treatment in arresting decay (Schiffmann-Nadel and Cohen, 1966).

The increased water loss that often follows hot water treatment can be reduced by applying wax, with or without fungicides (Wells, 1972), or by wrapping the produce in plastic film before or after heat treatment (Anthony et al., 1989; Teitel et al., 1989). Such a wrapping may protect the fruit, not only from water loss but also from recontamination and discoloration (Barkai-Golan and Phillips, 1991). The fruit may also be protected from heat injury by preconditioning. Lemons that were slightly wilted or had been kept for 2-8 days at 15.5°C prior to heating, were more tolerant to heat than those exposed to the high temperature immediately after picking (Houck, 1967). On the other hand, the benefits of heat treatment may be augmented by enhanced pathogen sensitivity to high temperatures. Alternaria rot was controlled more effectively in tomatoes heat-treated 8 h after inoculation than in those treated immediately after inoculation (Barkai-Golan, 1973); the spores that had germinated during that period, as well as the germ-tubes or the young hyphae, were more sensitive to heat than non-germinated spores.

The efficacy of postharvest hot water dipping in decay suppression has been reported for many host-pathogen combinations. Among these are: *P. citrophthora* and *Penicillium digitatum* in citrus fruits; *Colletotrichum gloeosporioides* in mangoes; various fungi in papayas and melons; *Monilinia fructicola* in plums; *M. fructicola* and *Rhizopus stolonifer* in peaches and nectarines; and *Gloeosporium* spp. and *Penicillium expansum* in apples. The efficacy of moist hot air treatment (43°C, 30 min) in decay control was reported for species of *Botrytis, Rhizopus, Alternaria* and *Cladosporium* in strawberries (Barkai-Golan and Phillips, 1991). Although hot water treatment of strawberries has generally resulted in fruit injury, a recent study with strawberries of cv. Tudela showed that dipping the fruit at 44 or 46°C for 15 min delayed *B. cinerea* development, with good retention of firmness and no development of off-color or off-flavor (Garcia et al., 1996). Of all the effective heat treatments, the one most commonly used commercially is that applied on mangoes. In this treatment, the fruits are immersed for 15 min in hot water (50-55°C) prior to storage, to prevent anthracnose development

(Spalding and Reeder, 1986b). The traditional hot water treatment (48°C, 20 min) has been used successfully in Hawaii to control *C. gloeosporioides*, stem-end rots and incipient *Phytophthora* infection in papaya (Aragaki et al., 1981). However, several problems, such as delayed color development and increase in *Stemphylium* rot, arose from the hot water application (Glazener and Couey, 1984).

Following the withdrawal of ethylene dibromide as a pesticide against fruit flies on papaya in 1984, the double hot water dip (42°C for 30 min followed by 49°C for 20 min) has been adopted for fruit disinfestation in papaya shipments to fruit fly-free zones. The double-dip procedure also provides postharvest disease control when coupled with field fungicide sprays (Alvarez and Nishijima, 1987). However, various papaya cultivars are sensitive to heat, and the fruit becomes increasingly susceptible to heat injury as it ripens. A single hot water dip at 49°C for 15 min was reported by Nishijima (1995) to be the optimum treatment for disease control with minimum detrimental impact on fruit quality.

The basic problem in short-term treatments is the high temperature needed for decay suppression. Such a temperature is often near the level injurious to the commodity and it must, therefore, be carefully measured and controlled. Furthermore, temperatures too high for a given host may increase the host susceptibility to pathogens, even when no visible damage develops (Phillips and Harris, 1979).

Combined Applications

Over the years, combined heat-plus-chemical treatments have been developed in order to achieve decay control by using lower temperatures and shortened exposure time on the one hand, and reduced fungicide concentration, on the other. The improvement may be due to the more effective infiltration of the fungicides into the wound sites on the fruit, that are exploited by the fungus (Brown, G.E., 1984).

Hot water containing fungicides has been found more effective than either of the separate treatments alone in controlling *Rhizopus stolonifer* infection in peaches, plums and nectarines (Wells and Harvey, 1970). The addition of thiabendazole, benomyl, captan or botran (dicloran) to water heated to 52°C enabled the immersion time to be reduced from 15 to 0.5 min, without affecting decay control. Hot suspensions of benomyl or botran were similarly more effective than unheated suspensions, in controlling brown rot (*Monilinia fructicola*) in peaches (Smith, W.L., 1971). Combinations of fungicides at a quarter of the recommended rates with 1.5-2-min dips in hot water at 51.5 and 54.5°C were equally or more

effective in controlling brown rot on sweet cherries, peaches and nectarines, than unheated water treatments containing higher concentrations (Jones and Burton, 1973).

In heat-sensitive mango cultivars, the addition of fungicides to the hot water enabled the temperature needed for controlling anthracnose (*Colletotrichum gloeosporioides*) to be reduced below the level which causes heat injury (Spalding and Reeder, 1986b). Such a reduction obviates the need for pedantic temperature control during treatment. The mechanism of improved control with heated fungicide mixes may be related in part to the direct effect of heat or to increased chemical activity; however, improved control may also be attributed to increased penetration of the fungicides into the host tissues (Wells and Harvey, 1970). Trials with mature guavas similarly indicated that the incidence of rots, including *Colletotrichum* and *Pestalotia* spp., was greatly reduced by a 5-min dip in benomyl (0.5-2.0g l^{-1}) heated to 48-50°C, compared with the effect of each treatment alone (Wills et al., 1982).

The fungicidal activity of imazalil, which is registered for postharvest application to citrus fruit, to reduce both the incidence and the sporulation of *Penicillium digitatum*, was considerably increased when the fungicide was applied in hot water. Studies with Redblush grapefruits (Schirra et al., 1995) showed that dipping the fruits for 3 min in 1500 ppm imazalil solution at 50°C considerably increased the effect of the chemical, compared with its use in cold water, on fruits stored for 16 weeks at 8°C followed by shelf-life conditions.

The chilling injury index under these conditions was threefold lower than in fruits dipped in water at 20°C. Smilanick et al. (1997) found that imazalil effectiveness on citrus fruit was substantially improved when the fruits were passed through an aqueous solution of the chemical heated to only 37.8°C, as compared with the current commercial practice, in some areas, of spraying wax containing imazalil at ambient temperatures. The improvement in imazalil efficacy in this case was due not only to the combination of the fungicide with heating, but also to its application in water, which is known to reduce the green mold more effectively than application in wax (Eckert et al., 1994). In addition, heating the fungicide solutions also accelerates the accumulation of fungicide residues in the fruit (Schirra et al., 1995), and the immersion of lemon or orange fruits for 30 s in a 350-400 μg ml^{-1} fungicidal solution, instead of spraying the fungicide on the fruit, deposited sufficient residues (2-4 μg g^{-1}) to control *P. digitatum* sporulation on the fruit. No rind injury was observed following these procedures (Smilanick et al., 1997).

Immersion of *P. digitatum*-inoculated lemons in 2% sulfur dioxide solutions reduced green mold incidence, without injury to the fruit; however, heating of the solutions was needed to attain acceptable efficacy (Smilanick et al., 1995). Heated solutions of sulfur dioxide were also superior to hot water alone for the control of green mold. Green mold incidence was reduced to less than 10% by sulfur dioxide treatment applied for 10 min at 30°C followed by two fresh water rinses, and no decay was recorded after storage for 1 week at 20°C, following 10-min treatments at 40 or 47°C (Fig. 33). No injury was observed after these treatments except in the case of immersion in sulfur dioxide solution heated to 47°C.

Decay of lemon fruits inoculated with *P. digitatum* was effectively controlled by heated solutions of ethanol in water, at concentrations of 10 to 20%, and control was superior to that of water alone at 32, 38 or 44°C (Smilanick et al., 1995). At 50°C the green mold was reduced to less than

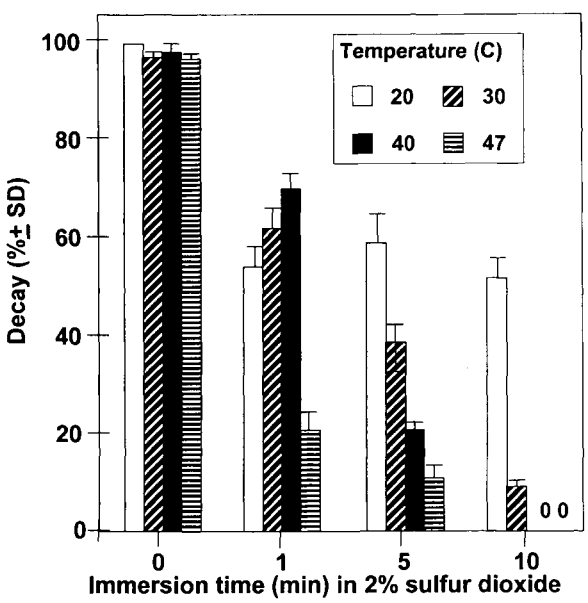


Fig. 33. Incidence of postharvest green mold in *Penicillium digitatum*-inoculated lemons followed 5h later by immersion for 1 to 10 min in 2% sulfur dioxide solutions at various temperatures followed by two fresh water rinses and storage for 1 week at 20°C. (Reproduced from Smilanick et al., 1995 with permission of the American Phytopathological Society).

5% even when low ethanol concentrations (2.5-5%) were used. Spore mortality of *M. fructicola* and *R. stolonifer*, the main postharvest pathogens of peaches and nectarines, occurred much more quickly in heated ethanol than in heated water (Margosan et al., 1997): a 10% ethanol solution at 46 or 50°C greatly reduced the LT95 (95% lethality) of both fungi (Table 12).

TABLE 12

Estimated time (seconds) to kill 95% of the spores of *Monilinia fructicola* or *Rhizopus stolonifer* in water, alone or containing 10% ethanol, at 46 or 50°C*

	M. fructicola		*R. stolonifer*	
Treatment	**46°C**	**50°C**	**46°C**	**50°C**
Water	734.0a	206.3a	>1000.0a	410.3a
10% ethanol	57.4b	7.0b	94.4b	46.3b

* Values followed by unlike letters are significantly different ($P \leq 0.05$). Reproduced from Margosan et al., 1997 with permission of the American Phytopathological Society.

When peaches and nectarines, infected with *M. fructicola* spores, were immersed in 10% ethanol at 46 or 50°C, or 20% ethanol at 46°C the incidence of decayed fruit was 83, 25 or 12%, respectively, i.e., similar or a better control was achieved than that following a 1-min dip in the fungicide, iprodione (1000 μg ml^{-1}). No injury to the fruits occurred and no off-flavors were detected in the fruits. However, unlike fungicides, the hot ethanol treatments did not deposit persistent antifungal residues, so there was a lack of continuous protection of the fruits from recontamination.

The increases in spore mortality and decay control – occurring when hot water and ethanol were combined – may result from their affecting the same sites in the spores. Since low concentrations of ethanol can lower the temperature at which phospholipids undergo a phase change (Rowe, 1983), the increases in spore mortality and decay control following the addition of ethanol may have resulted from a lowering of the phase-change temperature of the mitochondrial membranes of the spores under these conditions (Margosan et al., 1997).

Non-pesticide chemicals can also improve the effectiveness of heat treatment. The addition of sucrose to hot water (52°C) reduced the external discoloration of peaches and nectarines, which usually appears under shelf-life conditions, following heat treatment. In fact, the addition of sucrose enabled heat to be used to control decay in these fruits (Barkai-Golan and Phillips, 1991). The action of sucrose is not yet clear, but it is possible that the sugar slows hydration of the fruit surface exposed to the hot water, or protects this surface in some other way.

Enhanced efficiency of heat treatment in maintaining fruit firmness and reducing storage diseases can be achieved by combining heat and calcium treatments after harvest. Whereas immersing apples in water at 45°C reduced *Gloeosporium* decay, but increased tissue breakdown, the addition of $CaCl_2$ to the hot water controlled tissue breakdown and suppressed decay development (Sharples and Johnson, 1976). Later studies with combined treatments showed that long-term heating (38°C, 4 days) of *Penicillium expansum*- or *Botrytis cinerea*-inoculated Golden Delicious apples followed by pressure infiltration of 2 or 4% $CaCl_2$ solution, elicited accumulative or synergistic effects in retarding both softening and decay development during cold storage and shelf-life conditions (Conway et al., 1994c; Sams et al., 1993).

In another study with Golden Delicious apples, Klein et al. (1997) found that infections resulting from pre-storage inoculations of the fruit with *B. cinerea* spores were controlled only by a subsequent 4-day exposure to 38°C, and that neither 42°C for 1 day nor Ca treatment were effective in preventing decay during storage. Combining Ca infiltration (2% $CaCl_2$) with 38°C heat treatment had no advantage over heating alone but resulted in increased decay development. It was suggested that the pressure infiltration with $CaCl_2$ solution may have carried some *B. cinerea* spores further into the wound beyond the "zone of protection" afforded by the heat treatment (Klein et al., 1997). Furthermore, holding apples at 38°C for 4 days before Ca infiltration, decreased the amount of Ca taken in. The decreased intake of Ca may have been the result of a flow of epicuticular wax during heating, resulting in the sealing of surface cracks through which $CaCl_2$ solution might have entered the fruit (Roy et al., 1994).

A combination of heat with ionizing radiation has been found to be more effective than either means separately, in reducing various host-pathogen interactions, including oranges infected by *Penicillium digitatum* (Barkai-Golan et al., 1969), nectarines infected by *Monilinia fructicola* (Sommer et al., 1967), mangoes infected by *Colletotrichum*

gloeosporioides (Spalding and Reeder, 1986b), and papayas infected by various postharvest pathogens (Brodrick et al., 1976). Heat and irradiation act synergistically to inactivate spore germination (Barkai-Golan et al., 1969, 1977a; Sommer et al., 1967), shortening the time of exposure needed for either treatment applied alone. The efficiency of the combined treatment is influenced by the sequence and is generally greater when heat precedes irradiation. The interval between heating and irradiation also affects the synergism; for good results, radiation should be applied within 24 h of the hot water treatment (Barkai-Golan et al., 1969; Spalding and Reeder, 1986b).

A combination of heating and rinsing of fruits and vegetables has recently been achieved by the development of a fast hot water spray machine which simultaneously cleans the commodity and reduces the pathogen population on its surface (Fallik et al., 1999; 2000); the device is designed to be a part of a sorting line, and it exposes the commodity to hot water (50-70°C) for 10-60 s. This method significantly reduced decay incidence on sweet bell peppers and enabled fruit firmness to be maintained during prolonged storage and marketing. The optimal treatment conditions for this fruit were 55°C for about 12 s, while 59±1°C for 15 s was optimal for Galia melons. The low percentage of decayed fruits, together with the high level of cleanliness, were achieved by the combination of the hot water rinse with brushing that removes dirt, dust and fungal spores from the fruit, calyx and skin. The improved keeping quality of the rinsed and disinfected fruit has also been attributed to the melting of the wax layer, which seals small, almost invisible, cracks in the epidermis (Fallik et al., 1999; 2000).

B. IONIZING RADIATION

The possibility of utilizing ionizing radiation for extending the shelf life of fresh fruits and vegetables has been studied since the 1950s. These studies were mainly aimed at three directions: (a) control of postharvest diseases; (b) delay of the ripening and senescence processes; and (c) control of insect infestation for quarantine purposes. Most of the studies were carried out with CO^{60} gamma rays. Here, we will focus on irradiation as a physical means for decay control and postharvest life extension of fruits and vegetables. This subject has been separately reviewed (Barkai-Golan, 1992).

Radiation Effects on the Pathogen

Ionizing radiation may directly harm the genetic material of the living cell, leading to mutagenesis and eventually to cell death. The nuclear DNA, which plays a central role in the cell, is the most important target molecule in microorganism irradiation, although radiation lesions in other components of the cell may also contribute to cell injury or even result in cell death (Grecz et al., 1983). Several factors affect the response of a pathogen to radiation, the first being the genetic resistance; fungal species may vary widely in their resistance to irradiation (Fig. 34). In general, multicellular spores, such as *Alternaria* or *Stemphylium* spores, or bicellular spores, such as those of the *Cladosporium* or *Diplodia*, are

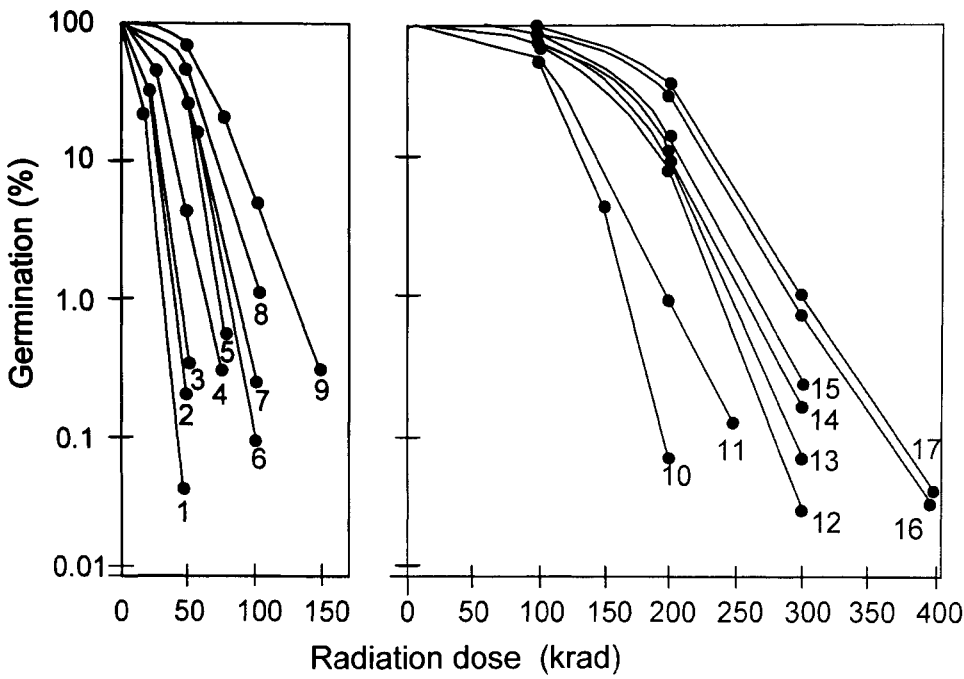


Fig. 34. Dose-response curves or inactivation of postharvest fungi by gamma radiation. 1 – *Trichothecium roseum; 2 – Trichoderma viride; 3 – Phomopsis citri; 4 – Penicillium italicum; 5 – P. expansum; 6 – Aspergillus niger; 7 – P. digitatum; 8 – Geotrichum candidum; 9 – Monilinia fructicola; 10 – Botrytis cinerea; 11 – Diplodia natalensis; 12 – Stemphylium botryosum;13 – Rhizopus stolonifer; 14 – Alternaria citri; 15 – A. alternata; 16 – Cladosporium herbarum; 17 – Candida* sp. (Reproduced from Barkai-Golan, 1992 with permission of Springer-Verlag GmbH & Co. KG).

more resistant to gamma radiation than the unicellular spores of other fungal species (Sommer et al., 1964b). The multicellular or bicellular spore presumably exhibits resistance when one of the cells escapes injury and remains capable of forming a colony.

For a given fungal species, the inhibiting effect of radiation increases with the radiation dose; however, for a certain dose, the rate of irradiation application may affect both spore survival and mycelial growth. It has generally been found that higher rates of irradiation increased the efficacy of the radiation, enabling the dose levels needed for pathogen inactivation to be reduced (Beraha, 1964). The greater radiobiological effect of a rapidly applied dose has been attributed to the lack of, or fewer opportunities for repair.

The number of fungal spores or mycelial cells in the inoculum exposed to radiation may affect the radiation dose required for their inactivation. Increased spore density in the inoculum has generally necessitated an elevated radiation dose. In oranges inoculated with *Penicillium digitatum* and *P. italicum*, the reduction in spore concentration resulted in the prolongation of the incubation period of the disease (Barkai-Golan, 1992).

The presence of oxygen in the atmosphere at the radiation site is an important factor in enhancing the effectiveness of a given dose. It was thus found that the dose required to reduce survival of *Rhizopus stolonifer* spores to 1% was much less in the presence of oxygen than that under anoxia (Sommer et al., 1964a). The increased antifungal effect was attributed, among other factors, to the formation of peroxides, which caused cell injury.

Another factor affecting the radiation sensitivity of microorganisms is the water content of their cells. This may be the reason why vegetative cells are more sensitive to radiation than spores (Barkai-Golan, 1992). This is true for both fungi and spore-bearing bacteria. The high water content of bacterial vegetative cells may favor the production, within the cytoplasm, of a variety of harmful radicals that enhance the effects of radiation injury (Grecz et al., 1983).

The timing of irradiation may also affect pathogen sensitivity: extending the time lag between inoculation and irradiation may increase dose requirements for pathogen suppression (Zegota, 1987). Since many of the postharvest pathogens enter the host via wounds incurred at harvest, the time lag between harvest and irradiation would parallel the time between inoculation and irradiation. During this period, the infection may be initiated and the size of population exposed to irradiation may increase. For quiescent fungi, an extension of the time interval between harvest and

treatment may result in renewal of fungal development because of the progress of fruit ripening (Prusky et al., 1982; Droby et al., 1986). Under such conditions, decay cannot be suppressed by irradiation.

Since radiation effects are associated with the size of the target population, one may presume that all environmental factors encouraging fungal growth, such as appropriate storage temperature or high humidity, will indirectly affect the dose required for pathogen control. Several studies indicate that the radiation dose required for pathogen suppression within host tissues is higher than under *in vitro* conditions. This is believed to be the consequence of the chemical protective effect afforded by the tissues when in contact with the pathogen.

Irradiation may induce various degrees of injury and result in the formation of mutants among the survivors. Although mutants usually appear to be less pathogenic than the parent organism, mutants of wider virulence have also been observed in various plant pathogens (Sommer and Fortlage, 1966). An irradiated pathogen has the capacity to repair radiation damage under post-irradiation conditions. Environments found to be encouraging for recovery are characterized by their ability to slow down metabolism, such as sub-optimal temperatures, or starvation or anaerobic conditions (Barkai-Golan, 1992).

Radiation effects on the pathogen may also be associated with the ability of the host tissue to induce antifungal phytoalexins in response to irradiation. Riov (1971) and Riov et al. (1971) reported on the accumulation of the stress metabolites, scopoletin and scopolin in the peel of mature grapefruits irradiated at 1-4 kGy, and of scoparone following irradiation at 3 and 4 kGy. These compounds were formed in the radiosensitive flavedo tissue of the peel in correlation with increased ethylene production, enhanced phenylalanine ammonia lyase activity (PAL), and the accumulation of phenolic compounds, which lead to cell death and peel pitting (Riov, 1975). More than 15 years after the first isolation of scoparone from irradiated grapefruits, and following isolation of this compound from other citrus cultivars (Valencia oranges and Eureka lemons), Dubery et al. (1988) showed its antifungal activity. Another irradiation-induced non-coumarin metabolite was extracted from damaged regions of citrus peel and was identified as 4-(3-methyl-2-butenoxy) isonitrosoacetophenone. This compound, which did not occur in extracts from non-irradiated fruits, has also been reported to have antifungal properties (Dubery et al., 1988).

It should be mentioned, however, that in contrast to the radiation-induced phytoalexins in citrus fruits, El-Sayed (1978) reported that the

phytoalexins, rishitin and lubimin formed in potato tubers and rishitin formed in tomato fruits, in response to infection, were reduced after gamma irradiation at doses required for potato tuber sprout inhibition (100 Gy) and for tomato fruit shelf-life extension (3 kGy).

The formation of antifungal compounds in the host in response to radiation may be one factor in the complex of radiation effects on the pathogen, although its importance in disease suppression has not yet been evaluated.

Radiation Effects on the Disease

An important advantage of gamma radiation over most chemical treatments, which derives from its short wavelength, is its ability to penetrate into the tissues. This enables irradiation to reach not only microorganisms in wounds, but also those located within the host, as quiescent or active infections. Thus, we may also refer to irradiation as a therapeutic means, effective after infection has already started. However, the use of irradiation for decay suppression is basically determined by the tolerance of the host to radiation, rather than the fungicidal dose required for pathogen suppression; different host species, and even different cultivars of a given species, may differ in their tolerance to radiation. Furthermore, dose tolerance may be influenced by the state of fruit ripeness at the time of treatment and by the subsequent storage conditions (Maxie and Abdel-Kader, 1966).

Early studies in the 1950s and 1960s had already established that fruits and vegetables are usually susceptible to radiation doses that are lethal to the common postharvest pathogens. However, the same studies also indicated that extension of the postharvest life of several commodities could be achieved by using sublethal doses, which could temporarily inhibit fungal growth and, therefore, prolong the incubation period of the disease. Such a prolongation could be sufficient to control decay in fruits with a short postharvest life, for which even a few additional lesion-free days are valuable.

The best example of such a fruit is the strawberry, for which a dose of 2 kGy may be sufficient to prevent decay for several days, without causing fruit injury or changes in the ascorbic acid content (Maxie et al., 1964; Barkai-Golan et al., 1971). However, one has to keep in mind that different cultivars of strawberries may respond differently to irradiation. Irradiating naturally infected strawberries of cv. Lassen with a 2-kGy dose, Barkai-Golan et al. (1971) found that the incubation period of gray mold at 15°C was extended from 3 days to 10 days, and that the subsequent

growth rate of the surviving fungal population was markedly reduced. Such a delay in the initiation of *Botrytis* infection may also indirectly reduce the levels of contact infection and "nesting" during storage.

A report from South Africa (Du Venage, 1985) indicates that irradiation of strawberries under commercial conditions prolonged the summer shelf life of the fruit from 3-12 days at ambient temperatures up to 50 days at 2°C. However, for most commodities, even doses which are sublethal for the pathogen but are capable of retarding or temporarily halting its growth, may inflict radiation damage on the host.

Radiation doses required for direct suppression of postharvest pathogens are generally above the tolerance level of the fruit and result in radiation damage (Barkai-Golan, 1992). Decay control by sublethal irradiation doses, which are beneath the damage threshold of the commodity, may be possible in two instances: a) when decay suppression is caused indirectly by delaying the ripening and senescence processes of the commodity; and b) when irradiation is combined with other physical or chemical treatments.

Decay Suppression via Delay of Ripening and Senescence

For several fruits a reduction in the incidence of fungal diseases has been recorded after exposure to relatively low radiation doses, that are incapable of directly suppressing, or even temporarily retarding, pathogen growth but are sufficient to delay the ripening and senescence of the commodity. Since the susceptibility of fruits and vegetables to postharvest infection increases as the ripening and senescence processes progress (see the chapter on Factors Affecting Disease Development – The Fruit Ripening Stage), one should not be surprised that radiation doses capable of retarding these processes may contribute towards disease suppression by maintaining the natural resistance to infection characteristic of younger tissues. It was thus found that doses of 50-850 Gy are capable of inhibiting the ripening of mango, papaya, banana and other tropical and subtropical fruits (Thomas, 1985; Barkai-Golan, 1992). The success of the treatment in each of these fruits depends upon the balance between the dose needed to retard ripening and the dose tolerance of the fruit. Alabastro et al. (1978) found that irradiation of mature green Carabao mangoes with doses of up to 220 Gy delayed the appearance of anthracnose and stem-end rots by 3 to 6 days without any adverse effect on fruit appearance. The suppression of decay by such low doses suggests that no direct fungicidal effect on the pathogens is involved. Similarly, doses of 50-370 Gy on Cavendish bananas were

sufficient to reduce *Colletotrichum* decay significantly, but not *Thielaviopsis* decay. The reduction of anthracnose was probably the result of ripening inhibition, while higher doses caused darkening of the peel, that increased with the dose (Alabastro et al., 1978).

Irradiation is mostly effective in delaying senescence in potatoes and onions, the delay being typically expressed by the inhibition of sprouting during storage (photos 3 and 4). Because of its action on the meiosis in the meristematic area of these crops, irradiation may delay sprouting at doses as low as 50-150 Gy or even less (Thomas, 1983, 1984). Gamma irradiation at 60 Gy has similarly been found effective in preventing sprouting and rooting of fresh ginger rhizomes held at ambient temperatures (Mukherjee et al., 1995).

However, in contrast to fruits, in which ripening and senescence delay is accompanied by decay suppression, potato or onion irradiation at sprout-inhibiting doses may result in enhanced susceptibility to storage pathogens. In order to prevent significantly increased decay in irradiated onions and garlic, healthy, good-quality bulbs should be used; they should be irradiated during the dormancy period; and good storage management should be practiced (Thomas, 1984). El-Sayed (1978) reported that the phytoalexins, rishitin and lubimin formed in potato tubers, and rishitin formed in tomato fruits, in response to infection were reduced after gamma irradiation. In parallel, increased incidence of rotted tubers and fruits was frequently reported after exposure to the radiation doses required for potato sprout inhibition (100 Gy) and for tomato fruit shelf-life extension (3 kGy). Post-irradiation treatment of tubers and fruits with phytoalexins produced by members of the Solanaceae, contributed to the control of microbial spoilage (El-Sayed, 1978).

Irradiating carrots at a sprout-inhibiting dose (120 Gy) generally results in increased decay during prolonged storage (6 months at 2°C and 95-100% relative humidity). However, irradiation seems to contribute to decay suppression for short-term storage of carrots (Skou, 1977). Electron irradiation (β rays) applied only to the top ends of carrots prevented sprouting for 6 months, without increasing the incidence of rot (Skou, 1977).

Combined Treatments

Treatments that combine radiation with other control means, physical, chemical or biological, were developed in an attempt to reduce the radiation doses required for disease control to beneath the damage threshold of the fruit or vegetable. Among the combined treatments, special attention has been drawn to heat-plus-radiation combinations.

Hot water dips and gamma radiation were found to react synergistically on spore inactivation of various postharvest fungi (Barkai-Golan et al., 1977a) permitting the use of a lower level of each of the treatments than when it was applied separately. In most cases the combined treatment is more effective when the hot water dip preceeds the irradiation (Sommer et al., 1967) (Fig. 35); the exposure of spores to high temperatures sensitizes them to radiation. As well as conferring the advantage of the synergistic effect of the two physical means, the combined treatment markedly delays decay development in many fruits, such as citrus, papaya, mango, plum and nectarine (Barkai-Golan, 1992).

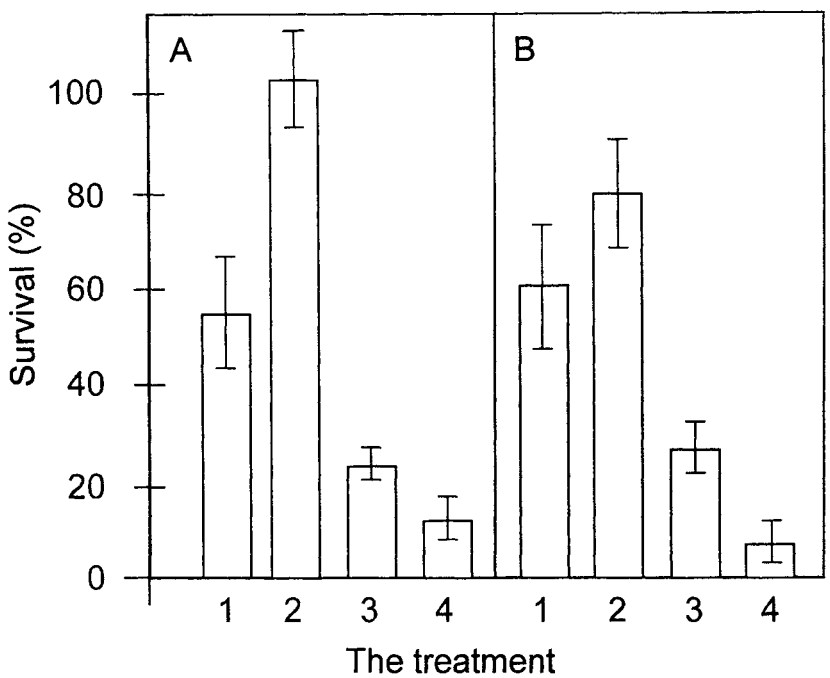


Fig. 35. Survival of conidia after heat treatment and irradiation. 1 = irradiation alone, 2 = heat only, 3 = irradiation followed by heat, 4 = heat followed by irradiation: (A) *Botrytis cinerea* subjected to 44°C for 4 min or a 75-krad dose, or both; (B) *Penicillium expansum* subjected to 56°C for 4 min or a 20-krad dose, or both. Vertical lines indicate standard deviations (Reproduced from Sommer et al., 1967 with permission of the American Phytopathological Society).

In *Penicillium digitatum*-inoculated citrus fruit, a combined treatment of hot water (52°C, 5 min) and gamma irradiation (0.5 kGy) delayed the appearance of green rot by 33-40 days (Fig. 36) (Barkai-Golan et al., 1969). Combining irradiation (0.75 kGy) with the conventional hot water treatment (50°C, 10 min) prolonged the shelf life of papayas by 9 days beyond the prolongation achieved by heating alone, facilitating the distribution of South African fruits within that country and making large-scale export by sea possible (Brodrick and Thomas, 1978). Hot water (55°C, 5 min) plus irradiation (0.75 kGy) also act synergistically in controlling anthracnose (*Colletotrichum gloeosporioides*) in mangoes. This treatment is commercially used for treating mangoes in South Africa, both for decay control and for quarantine control of the mango seed weevil (*Sternochetus mangiferae*) (Brodrick and Thomas, 1978).

A study on South African cultivars of plums and nectarines has shown that a hot water dip (46°C, 10 min) enabled the suppression of postharvest

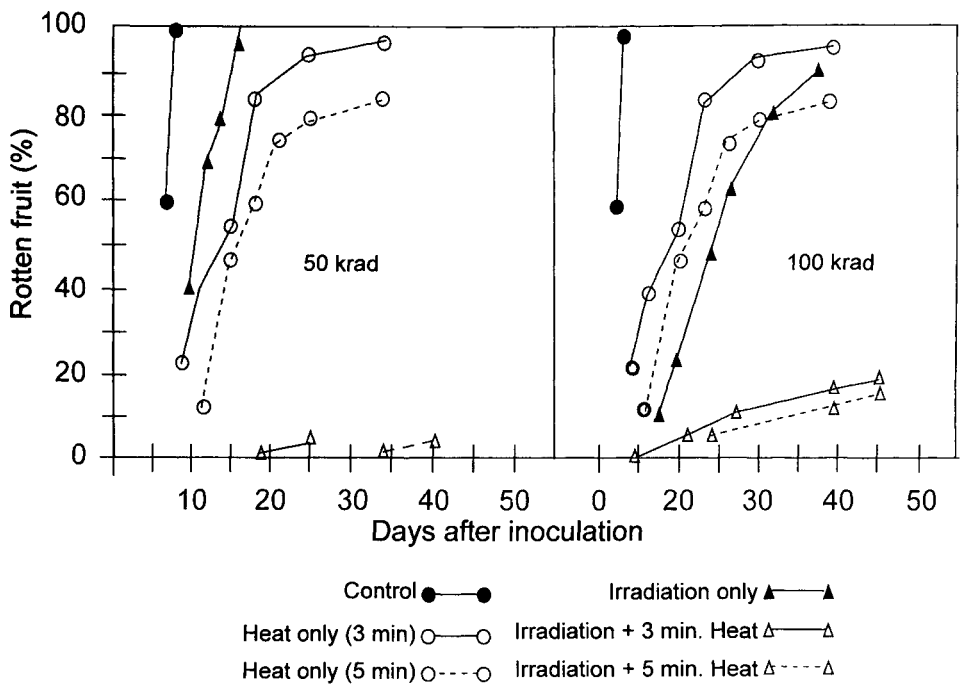


Fig. 36. Effects of combined treatments of heat and gamma radiation upon inactivation of *Penicillium digitatum* in inoculated Shamouti oranges. (Reproduced from Barkai-Golan et al., 1969 with permission of the American Phytopathological Society).

Photo 3. Sprouting inhibition of potatoes by gamma irradiation.

Photo 4. Sprouting inhibition of onions by gamma irradiation.

Photo 5. Decay inhibition in strawberries by gamma irradiation.

Photo 6. Effect of gamma irradiation combined with heating on mango fruits as compared to heating alone.

pathogens but resulted in fruit damage (Brodrick et al., 1985); whereas irradiation alone (2 kGy) controlled fungal development but resulted in fruit softening. A combination of mild heat treatment (42°C, 10 min) with low-dose irradiation (0.75-1.5 kGy) effectively controlled development of *Monilinia fructicola, Rhizopus stolonifer* and *Botrytis cinerea*, with no significant changes in fruit texture, aroma or taste.

Treating tomatoes with the combination of heating (50°C, 2 min) and low-dose irradiation (0.5 kGy) totally eliminated Alternaria rot under shelf-life conditions, but each of these treatments resulted in accelerated softening of the fruit (Barkai-Golan et al., 1993b). Gamma irradiation without hot water treatment reduced the incidence of bacterial soft rot caused by *Erwinia* and *Pseudomonas* species (Spalding and Reeder, 1986a). In this case, the combination of heating and irradiation provided no better rot control than irradiation alone.

By combining irradiation with fungicidal or fungistatic treatment it was possible to reduce both the irradiation dose and the concentration of the chemical compound. Moreover, since the suppressive effects of irradiation and the chemical treatments may differ greatly among fungal species, their combination might broaden the operational range against a wide variety of microorganisms (Barkai-Golan, 1992).

Several studies have shown advantages in combining low-dose radiation, mild heat treatment and chemicals, compared with the double-component combined treatments, in controlling storage decay. Such an advantage was recorded in apples for which the association of heating (50°C, 10 min), irradiation (1.5 kGy) and benomyl (250 ppm), in this sequence, inhibited development of the blue mold (*Penicillium expansum*) under shelf-life conditions (Roy, 1975). For *P. digitatum*-inoculated Shamouti oranges, the combination of radiation (200 Gy), diphenyl (15 mg per fruit) and hot water dip (52°C, 5 min) extended the incubation period of the green mold beyond the extension caused by heat and radiation or by diphenyl and radiation (Barkai-Golan et al., 1977a). For Kensington Pride mangoes, a hot benomyl dip prior to low-dose irradiation (0.3-1.2 kGy) resulted in an additive effect which markedly improved the partial control achieved by irradiation alone (Johnson et al., 1990).

A combination of gamma and ultraviolet radiation was found to be effective against fungi sensitive to low gamma doses, such as *Phytophthora* and *Colletotrichum*. In such cases, the combined treatment facilitates the reduction of the ionizing radiation dose needed for fungal inactivation (Moy et al., 1978).

The feasibility of a combination process involving gamma irradiation, packing in closed polyethylene bags and biological control of rot-causing fungi was evaluated as a means of extending the shelf life of fresh ginger rhizomes at ambient temperatures (25-30°C) in India (Mukherjee et al., 1995). The recommended procedure consisted of dipping rhizomes, which had previously been washed and dried, in a spore suspension of a *Trichoderma* sp. isolated from sclerotia of *Sclerotium rolfsii* (the major pathogen during extended storage) along with other antagonistic microorganisms. The next steps included air-drying, packing in chosen low-density polyethylene bags and irradiation at 60 Gy. The treated ginger rhizomes remained in good marketable condition for up to 2 months at ambient temperatures without sprouting (thanks to irradiation at a sprout-inhibiting dose), showed suppressed decay development (thanks to the protection of the rhizomes or the cut surface of sliced rhizomes by the antagonistic *Trichoderma* isolate) and reduced weight loss (thanks to storage in closed polyethylene bags).

Radiation Approval

Following extensive studies in recent decades of the "wholesomeness" of irradiated food, the list of commodities approved for radiation by health authorities in various countries has been considerably lengthened. An important event took place in 1986 when gamma irradiation up to a dosage of 1 kGy was approved by the Food and Drug Administration of the United States, for treatment of fruits and vegetables. This has accelerated both research and development in the use of ionizing radiation as a physical means for postharvest life extension.

C. ULTRAVIOLET ILLUMINATION

Ultraviolet (UV) illumination is known to damage plant DNA and to affect several physiological processes (Stapleton, 1992). However, a special interest has recently been drawn to the ability of low doses of UV-C light (wavelength of 190-280 nm) to induce disease resistance in a wide range of fruits and vegetables.

A number of lines of evidence from various fruits and vegetables (Wilson et al., 1994) indicate that the effect of UV-C is not solely due to its germicidal activity: (1) tissue inoculated after UV treatment was more resistant to invasion by the pathogen; (2) the UV effect was not always correlated with increased UV doses. In addition, UV treatments have

also been found to retard ripening of several harvested commodities, such as tomato and peach (Liu et al., 1993; Lu et al., 1991), thus leading indirectly to reduction in their susceptibility to infection.

Optimum UV-C doses for induced resistance in the various commodities fall within narrow ranges, specific for each commodity. Also the maximum protection provided by UV treatments occurs at varying times after treatment and depends on the commodity. Studies with UV-treated grapefruits showed that fruits picked at different times during the growing season responded differently to UV treatments. The temperature at which the fruit was stored following treatment was another factor affecting resistance development (Droby et al., 1993a). Maximum resistance of peaches against *Monilinia fructicola* and tomatoes against *Rhizopus stolonifer* was observed 48-72 h after UV treatment, whereas sweet potatoes exhibited maximum protection against *Diplodia tubericola* 1-7 days after treatment (Wilson et al., 1997a).

Ultraviolet Effects

In UV-illuminated apples and peaches the induced resistance to decay has been attributed to the inhibition of ripening and the maintenance of the natural resistance of the young fruits to infection (Lu et al., 1991). Studying the changes found in UV-treated citrus fruits indicated that induced resistance occurs in concomitance with the induced activity of the enzymes phenylalanine ammonia lyase (PAL) and peroxidase (Chalutz et al., 1992; Droby et al., 1993a). These findings led to the hypothesis that induced activity of the two enzymes plays a role in the enhanced resistance to decay that follows UV treatment.

Several investigations indicated the ability of UV illumination to induce phytoalexin production in various host tissues. UV illumination (254 nm) of citrus fruits was found to induce the phytoalexin scoparon (Rodov et al., 1995b; Rodov et al., 1992), the production of which is enhanced both by increasing the UV dose and by raising the storage temperature (Rodov et al., 1992). Its accumulation in kumquat fruit reached its peak 11 days after illumination, but the amount declined rapidly, returning to trace levels, typical of non-illuminated fruit, 1 month after treatment. The accumulation of the phytoalexin was correlated with an increase in the antifungal activity of the flavedo which led to improved resistance of the fruit to infection by *Penicillium digitatum*. Such a defense against infection was achieved when

illumination was applied before inoculation with *P. digitatum*, without direct contact of the pathogen with the UV light. Moreover, illumination of previously inoculated fruit failed to prevent decay development (see Fig. 14).

The experiments with kumquats clarified that the fruit/pathogen interaction depends on the rate of fungal growth against resistance development. Inoculation of the fruits before UV treatment gives an advantage to the pathogen, whereas illumination applied 2 days before inoculation was sufficient to improve fruit resistance. Host resistance is thus dependent on the existence of a lag period between illumination and inoculation, during which phytoalexin can be accumulated and can suppress pathogen development (Rodov et al., 1992). Induced resistance in UV-treated grapefruits was found to reach its maximum level 24-28 h after the exposure to UV light, after which the level decreased (Droby et al., 1993a). Resistance was affected by the temperature at which the fruit was stored 24 h after UV treatment and before *P. digitatum* infection. Fungal development in the UV-treated fruit was characterized by sporadic mycelium and a marked inhibition of sporulation. All these findings in citrus fruits emphasize the fact that decay suppression following UV treatment is not connected with its germicidal properties alone, but also with its ability to induce biochemical and physiological changes in the tissues. This is especially marked when low doses of UV are involved.

Further studies with UV-illuminated grapefruits (Porat et al., 1999) indicated that UV-treatment induced the accumulation of chitinase protein, while the combination of UV and wounding induced both chitinase and β-1,3-endoglucanase. These two enzymes, which are included among the 'pathogenesis-related' (PR) proteins, are believed to be involved in plant defense mechanisms against fungal infection (El Ghaouth, 1994).

UV illumination of carrots was found to induce the accumulation of the antifungal phytoalexin, 6-methoxymellein, and to enhance root resistance to infection by *Botrytis cinerea* and *Sclerotinia sclerotiorum* (Mercier et al., 1993). The direct relationship between the accumulation of methoxymellein and the development of resistance to infection by *B. cinerea*, has previously been exhibited in freshly cut slices of carrot roots treated with heat-killed conidia of *B. cinerea* (Harding and Heale, 1980).

A recent study on table grapes (Nigro et al., 1998) found a significantly lower incidence of infected berries and a reduced diameter of *B. cinerea* lesions among artificially inoculated berries treated with UV-C doses of

0.125-0.5 kJ m^{-2}. These results were obtained both for berries wounded and inoculated just after the UV treatment, and for berries inoculated 24-48 h after the treatment. Similarly to the effect of UV-C light on citrus fruit (Rodov et al., 1992), an interaction between the UV-C dose and the time of inoculation was also found in grapes. A significantly lower level of disease was found, however, in berries inoculated 24-48 h after illumination than in those inoculated just after the treatment. To check the possible influence of UV-C illumination on the wound-healing processes, berries were wounded before illumination and inoculated at different times (hours) later. Such berries showed lower infection levels than those wounded after the UV treatment. These results could be due to a wound-type response since, during the period between irradiation and inoculation, the wounded berries were kept at 15°C under high-RH, conditions reported to be suitable for the induction of wound-healing processes in several fruits and vegetables (Ben-Yehoshua et al., 1988; Ippolito et al., 1994). However, a disease reduction recorded in grape berries illuminated after the inoculation of freshly cut wounds led to the suggestion that the effect of UV-C light was independent of a wound-healing reaction and could be attributed to UV-C-induced resistance alone (Nigro et al., 1998). Induced resistance was exhibited within 24-48 h of irradiation, and increasing the time until inoculation resulted in increased disease, probably because of a decline in the UV-C-induced resistance. These results suggest a temporary effect of UV-C treatments, as was also found in citrus fruits inoculated with *P. digitatum* (Droby et al., 1993a).

CHAPTER 11

BIOLOGICAL CONTROL

Increased official and public concern about the presence of fungicide residues in foods, and the development by pathogens of resistance to major fungicides, are two important reasons for the enhanced interest in the possibility of using biological control as an alternative, non-chemical means of decay suppression.

The term "biological control" or "biocontrol" refers to the use of naturally found microorganisms which antagonize the postharvest pathogens we wish to suppress. Antagonism between microorganisms is a ubiquitous phenomenon involving fungi (including yeasts) and bacteria which naturally inhabit the soil and the surfaces of various plant organs (Blakeman and Fokkema, 1982; Fokkema and van den Heuvel, 1986; Andrews, 1992). It is assumed that biocontrol of plant diseases occurs naturally on aerial plant surfaces and may be one of the main reasons why crops are protected to some extent during their cultivation (Droby et al., 1996).

One of the approaches to the isolation of antagonistic microorganisms for controlling postharvest diseases is through the promotion and management of natural epiphytic antagonists, already present on fruit and vegetable surfaces. Preharvest pesticide application and various postharvest treatments, such as fungicide and wax sprays, washes and dips, can greatly affect the resident microflora, both qualitatively and quantitatively. It has been commonly observed that the common practice of postharvest washing of harvested fruits and vegetables to obtain clean produce, may affect the microbial populations by removal of naturally occurring microorganisms. Fungicides may change the epiphytic microflora by affecting microorganisms other than the pathogens against which they are directed. These changes can, in turn, affect the pathogen resistance of the host. Such practices could possibly be modified to promote beneficial antagonistic microflora (Wilson and Wisniewski, 1989). To manipulate epiphytic microbial populations of fruits and vegetables effectively, in order to control decay, information is needed on their ecology. Spurr et al. (1991) suggested that emphasis be placed on studying the impact of the environment on the microflora and that of preharvest, harvest and postharvest activities on disease development.

In accordance with the results of these steps, models to guide management should be constructed.

Essentially, a great amount of research over the years has been focused on developing biological control procedures based mainly on artificially introduced selective antagonists against postharvest pathogens. Considerable success has been achieved in this field, and a large amount of information on the subject has been accumulated (Wilson and Wisniewski, 1994).

A. ISOLATION AND SELECTION OF ANTAGONISTS

The production of antibiotics by the antagonist was long considered to be the only mechanism of antagonism. In accordance with this hypothesis, screening of antagonistic microorganisms has generally been carried out *in vitro*, based on encouraging the selection of organisms that produce growth-free antibiotic zones in Petri dishes when challenged with the pathogen. However, such screening is likely to overlook many of the microorganisms which may serve as potential antagonists *in vivo*, in spite of failing to produce antibiotic compounds in culture (Wilson and Wisniewski, 1989). Recent screening procedures, therefore, emphasize the isolation of antagonists which do not necessarily produce antibiotic compounds during their life cycle (Droby et al., 1992; Wilson et al., 1993).

Most investigators have usually preferred to study antagonistic microorganisms which occur naturally on fruit and vegetable surfaces and, in fact, these have proved to be a reliable source of such microorganisms. Much attention has been given to naturally occurring yeast species, which do not rely on the production of antibiotic substances. They can colonize a wound for long periods, produce extracellular polysaccharides that enhance their survival, can proliferate rapidly by using available nutrients and are minimally affected by pesticides (Janisiewicz, 1988).

Looking for alternative non-chemical methods to control postharvest diseases of avocados in South Africa, Korsten et al. (1995) evaluated the inhibitory effects of 33 bacteria isolated from avocado leaf and fruit surfaces, against the major fungal pathogens of avocado fruits. These pathogens included *Colletotrichum gloeosporioides, Dothiorella aromatica* and species of *Thyronectria, Phomopsis, Pestalotiopsis* and *Fusarium*. The antagonistic bacteria included various *Bacillus* species, which comprise a major component of the microflora tested, and species

of *Corynebacterium, Erwinia, Pseudomonas, Staphylococcus, Vibrio* and *Xanthomonas*. Of these, *B. subtilis* was the most effective antagonist. The search for antagonistic fungi against *Colletotrichum musae*, the causal agent of anthracnose of banana, revealed that almost all the fungi isolated from banana fruit surfaces had at least some antagonistic effect against the pathogen. The greatest antagonistic activity was recorded for *Alternaria tenuissima* and *Acremonium strictum*, that effectively inhibited *Colletotrichum* growth, both *in vitro* and on banana fruits (Ragazzi and Turco, 1997). Searching for bacteria antagonistic to *Botrytis cinerea*, the main pathogen of strawberry fruits, Moline et al. (1999) showed that most of the 52 bacterial isolates that were obtained from the surface of 'organically grown' strawberry fruits had some ability to inhibit the growth of the fungus. Eleven of these, which were found capable of interfering with conidial germination or fungal growth *in vitro*, were selected for additional screening tests on *B. cinerea*-inoculated strawberry fruit. These isolates were identified as strains of *Pseudomonas putida* and *Chryseobacterium indologenes*. Three of the best isolates (two *Pseudomonas* and one *Chryseobacterium*) were also found to reduce the incidence of gray mold rot on fruits under field conditions (Moline et al., 1999).

Wilson et al. (1993) developed a method for the isolation and screening of yeast antagonists. The method is based on the application of washings to fruit wounds, which are then challenged with the fungal spores; following a few days of incubation, microorganisms are isolated from the non-infected wounds. Under these conditions, the predominant microorganisms on the culture medium were of yeast species. Tests for antagonism were performed with pure cultures under *in vivo* conditions.

Lorito et al. (1993) attempted to isolate selective biocontrol agents by utilizing media with purified polymers, such as chitin, in order to detect chitinase activity exhibited by the antagonistic microorganisms. Isolation based on chitinase activity of the antagonist offers some level of selection for properties potentially important to fungal control, but effective microorganisms which do not produce chitinases could be missed by the screening process. Some level of selection could also be achieved by using media made of pasteurized or autoclaved homogenates of the pathogens (Valois et al., 1996). This method may select only the microorganisms that can live on specific cellular content. A simple selection method for isolating bacterial antagonists to *B. cinerea*, which is both a pre- and a postharvest pathogen of strawberries, was recently developed by Moline et al. (1999); it is based on the ability of selected antagonistic bacteria to

grow on insoluble material consisting primarily of cell-wall material obtained following the extraction of freeze-dried mycelium.

In order to act as effective biocontrol agents and to qualify for development for commercial use on harvested crops, the antagonists should meet certain criteria. Moreover, determining the features needed for an antagonist to serve as a successful biocontrol agent is the first step before selecting potential antagonists. Wilson and Wisniewski (1989) listed the desirable characteristics of a potential antagonist: (1) genetically stable; (2) effective at low concentrations; (3) simple nutrient requirements; (4) capable of surviving adverse environmental conditions; (5) effective against a wide range of pathogens and on various fruits and vegetables; (6) resistant to pesticides; (7) a non-producer of metabolites deleterious to human health; and (8) non-pathogenic to the host. Various criteria connected with commercial development aspects, such as being easy to dispense and compatible with commercial handling practices, have also been mentioned. These desired features have guided many scientists in their biocontrol studies.

In selecting an antagonist suitable for postharvest application, we need to look for those that are well adapted to survival and growth in wounds or on the produce surface under storage conditions, and that have an "adaptive advantage" over specific pathogens (Wilson and Wisniewski, 1989). For example, *Rhizopus stolonifer* is more sensitive to low temperatures than many microorganisms; therefore, an antagonist well adapted to low temperatures might prove advantageous against this pathogen. Another example is the advantage of a *Candida oleophila* strain in reducing the level of *Penicillium expansum* infection in nectarines under storage conditions: the effectiveness of the antagonistic yeast was not reduced by controlled atmosphere (CA) storage or by application of a commercial fungicide, therefore, it can be applied under CA conditions which are important for maintaining fruit quality, and in combination with dicloran which will, at the same time, prevent infection by other pathogens, such as *Rhizopus* (Lurie et al., 1995).

For satisfactory control of postharvest pathogens of pome cultivars, the antagonist should be able to function under cold-storage conditions. Thus, the capability of several epiphytic bacteria isolated from apple leaves to control postharvest fungi on cold-stored apples, or that of a new strain of the yeast, *Candida sake* to control *P. expansum*, *Botrytis cinerea*, and *R. stolonifer* under various cold-storage conditions, is of importance when considering commercial application (Vinas et al., 1996; Sobiczewski et al., 1996). Since low temperatures and, in some cases,

controlled atmospheres are applied for prolonged storage for other crops as well, Arul (1994) pointed out the importance of testing the effectiveness of antagonists under the intended storage conditions rather than at ambient temperatures or in air.

The need for biocontrol agents with resistance to the chemicals commonly used in fruit and vegetable production has been emphasized by Spotts and Sanderson (1994). This feature is important since biocontrol agents applied postharvest would contact residues of fungicides which had been applied within a few days before harvest or those applied postharvest to control decay.

While selecting antagonistic isolates, Sobiczewski et al. (1996) emphasized microorganisms that are capable of inhibiting several postharvest pathogens of the host. By screening of the antagonistic effects of 107 isolates of epiphytic bacteria originating from apple leaves, they found that only six of them satisfied this need and were capable of inhibiting both *B. cinerea* and *P. expansum*, the two main postharvest apple pathogens.

In a recent study, Schena et al. (1999) found that isolates of the common yeast-like fungus, *Aureobasidium pullulans* at high concentrations (10^8 and 10^7 cells ml^{-1}), were able to control *Penicillium digitatum* on grapefruit, *B. cinerea, R. stolonifer* and *Aspergillus niger* on table grapes, and *B. cinerea* and *R. stolonifer* on cherry tomatoes. Less decay control was exhibited at lower concentrations. The ability of *A. pullulans* isolates markedly to suppress postharvest decay caused by a range of pathogens on various fruits, and its ability to survive and increase its population under a variety of field conditions and during cold storage, suggested that this widespread and well adapted saprophytic fungus be considered as a biocontrol agent against postharvest pathogens (Leibinger et al., 1997; Schena et al., 1999). It is interesting to note that some of the *A. pullulans* isolates significantly reduced decay caused by four different postharvest pathogens that could not be controlled with a single chemical fungicide. Furthermore, the resistance of *A. pullulans* to some commonly used fungicides (Lima et al., 1997) suggested the possible integrated use of this antagonist with chemical fungicides, to control postharvest rots.

However, *A. pullulans* is characterized by extreme morphological and cultural variability and genetic instability (Bulat and Mironenko, 1992), whereas genetic stability has been mentioned as an important feature required for a successful biocontrol agent by Wilson and Wisniewski (1989). Schena et al. (1999) have recently confirmed the high genetic

variability of *A. pullulans,* by obtaining 41 isolates from the surfaces of fruits and vegetables cultivated in Italy. They found that only a few isolates had the same genetic patterns and showed similar biocontrol activities.

Research in the past decade has mainly focused on microorganisms that are antagonistic to 'wound pathogens'. Since infection of wounds by fungal spores is very rapid, rapid colonization and growth at the wound site is a key characteristic of a successful antagonist (Droby et al., 1996). The features required for effective biocontrol agents should, therefore, include: the ability of the antagonist to colonize wounds; a rapid rate of growth in surface wounds; effective utilization of the nutrients present in the wound; and the capability to survive and develop at the infection sites better than the pathogen and to do so under a wider range of temperature, pH and osmotic conditions (Droby et al., 1996).

An important point in the selection of biological control antagonists is the necessity to avoid strains or isolates that may injure either the plants they are intended to protect or other plants of economic importance. Hence, screening tests to prevent the use of harmful microorganisms should be a part of any effort to develop biocontrol antagonists (Smilanick et al., 1996). *Pseudomonas syringae* strains, for instance, can occupy wounds on the peel of citrus fruit and reduce the incidence of postharvest rots initiated by wound pathogens. However, some *P. syringae* pv. *syringae* strains are pathogens of citrus and other plants and, therefore, strains should not be approved for biological control until their risk of virulence to many hosts has been determined (Smilanick et al., 1996).

B. INTRODUCTION OF ANTAGONISTS FOR DISEASE CONTROL

To identify antagonists as promising agents, a screening system should simulate natural inoculation, and the inoculum should be applied in the proper infection courts at the proper time (Smilanick, 1994). The selection of the inoculation method used for *in vivo* screening is, therefore, critical to a successful strategy. A biocontrol agent has generally been introduced to the wound site prior to the arrival of the pathogen or shortly thereafter (Smilanick, 1994). Applying the antagonists after inoculation of the pathogen involves eradicative action. The synthetic fungicide imazalil, for instance, controlled green mold

decay in lemons effectively if applied 24 h after inoculation, while the antagonistic bacterium *Pseudomonas cepacia* was effective only if applied within 12 h after inoculation (Smilanick and Denis-Arrue, 1992). The antagonistic yeast *Debaryomyces hansenii* was effective against wound pathogens in citrus fruits if applied 3 h after the fruit was inoculated with the pathogen, but was ineffective 7 h after inoculation (Chalutz and Wilson, 1990).

The time of application of the antagonist was found to be an important factor in the control of *Lasiodiplodia theobromae* in banana fruits by *Trichoderma viride* and other *Trichoderma* spp.: the highest reduction in the infection rate was achieved when the antagonistic fungus was applied 4 h prior to inoculation with the pathogen. There was less disease reduction following simultaneous application, and the least disease inhibition occurred when the *Trichoderma* was applied 4 h after inoculation (Mortuza and Ilag, 1999). These results demonstrated that the antagonistic *Trichoderma* species were not effective against infections already established in the fruits and indicated that the nature of the biocontrol activity is protective.

Another factor affecting the effectiveness of the antagonist is the presence of moisture in the wound. The biocontrol of *Botrytis cinerea* on apples was more effective when the antagonistic yeast *Candida oleophila* was applied to fresh wounds rather than to 1-day-old wounds (Mercier and Wilson, 1995). Introducing *C. oleophila* to wounds in apple fruit prior to inoculation with *B. cinerea* significantly reduced the percentage of gray mold of the fruit after 14 days at 18°C. However, as the fruit surface dries, moisture rapidly becomes a limiting factor for yeast growth, therefore, the application of the antagonist should follow the occurrence of wounding as closely as possible (Mercier and Wilson, 1995). It is likely that the poorer establishment of *C. oleophila* on dry wounds prevented it from multiplying to population levels that would be inhibitory to *B. cinerea*, although a dry environment could also interfere with the mode of antagonistic action.

Zehavi et al. (2000) inoculated berries with *B. cinerea, Aspergillus niger* and *Rhizopus stolonifer* in order to study the activity of epiphytic microorganisms isolated from table and wine grapes, against these pathogens. They concluded that wound inoculation is ideal for initial screening of antagonists but that this situation differs from that in the field. On the other hand, dipping grape bunches in an antagonist suspension and then spraying them with the pathogen spore suspension provides a closer simulation of a field biological control system, where the

fungi are sparse, most berries are not wounded and the antagonist, applied as a spray, covers the fruit.

Reductions in the percentage of decay, or in its rate of development in storage have been reported, following both field sprays with the antagonistic organism suspension and a postharvest application.

Preharvest application

Since the infection of fruits by postharvest pathogens often occurs in the field prior to harvest (see the chapter on Postharvest Disease Initiation – Pathogen Penetration into the Host), it is no wonder that preharvest application of the antagonist may sometimes be advantageous in controlling postharvest diseases. For this approach, successful biocontrol strains should be able to tolerate not only low nutrient availability but also UV-B radiation and climatic changes (Schena et al., 1999).

Field sprays of strawberry flowers with antagonistic non-pathogenic *Trichoderma* isolates resulted in a decreased incidence of gray rot (*B. cinerea*) during storage (Tronsmo and Dennis, 1977). Similarly, application of epiphytic isolates of the yeast-like fungus *Aureobasidium pullulans*, one of the most widespread saprophytes in the phylosphere, to strawberries grown under plastic tunnels, markedly reduced storage decay by both *B. cinerea* and *R. stolonifer* (Lima et al., 1997). The antagonists were more effective when applied at the flowering stage than at fruit maturity. Under these conditions, *A. pullulans* showed significantly higher activity against Botrytis rot than the fungicide vinclozolin.

An isolate of *A. pullulans* was found to reduce gray mold in table grapes significantly when applied several times in the field, and its effect was not significantly different from that of the chemical control (iprodione) (Schena et al., 1999). It is worth noting that populations of this antagonist rapidly increased on grape berries under field conditions, and that they increased further during cold storage (0°C). Moreover, this antagonist was able to survive and increase its population when transferred to a new environment characterized by high temperature and low relative humidity. Considering its high biocontrol efficacy and its ability to survive and control gray mold in diverse environmental conditions, this cosmopolitan yeast-like fungus was considered as a potential biocontrol agent, especially where the use of chemical protection is restricted (Schena et al., 1999).

Application of the two epiphytic fungal antagonists, *A. pullulans* and *Epicoccum purpurescens,* obtained from healthy peach blossoms, to cherry blossoms, reduced the number of quiescent infections of *Monilinia fructicola* in green cherries (Wittig et al., 1997). Selected strains of *A. pullulans, Rhodotorula glutinis* and *Bacillus subtilis,* isolated from leaf and fruit surfaces, were found to suppress postharvest decay development by *P. expansum, B. cinerea* and *Pezicula malicorticis,* when applied in combined mixtures to apple trees in the field (Leibinger et al., 1997). A combination of two strains of *A. pullulans* and one strain of *R. glutinis* suppressed rotting of apples to the same extent as the commonly used fungicide euparen. It was further noted that applications before harvest are also of great interest because European regulations governing integrated pest management do not allow postharvest treatments of apples (Leibinger et al., 1997). Application of cell suspensions of the yeasts *R. glutinis* and two *Cryptococcus* species to pear fruits in the field, 3 weeks prior to harvest, was found to maintain high population levels through harvest. The three yeast species provided significant postharvest control of blue mold (*P. expansum*), gray mold (*B. cinerea*) and side rots (*Cladosporium herbarum, Alternaria alternata* and *Phialophora malorum*). The most consistent decay control, however, was provided by *Cryptococcus infirmo-miniatus,* although the decay incidence and type of decay observed varied among years and among pear cultivars (Benbow and Sugar, 1999).

A reduction in pathogen development following postharvest application of the antagonist has been recorded for pineapple fruits on which field sprays with non-pathogenic strains of *Penicillium funiculosum* suppressed decay caused by pathogenic strains of this fungus during storage (Lim and Rohrbach, 1980).

A significant reduction in the incidence of green mold rot (*Penicillium digitatum*) in stored grapefruits was also achieved by spraying the fruits, prior to picking, with yeast cells of *Pichia guilliermondii* (Droby et al., 1992). Such a treatment seems to reduce the potential inoculum level of the pathogenic spores which are naturally located on the fruit prior to harvesting.

Postharvest application

Postharvest application of the antagonist is often done by postharvest sprays or by bringing wounds on the fruit peel in contact with it. Spraying with suspensions of *Trichoderma harzianum, T. viride,*

Gliocladium roseum and *Paecilomyces variotii,* resulted in a partial control of *Botrytis* in strawberry fruits and of *Alternaria* in lemon fruits (Pratella and Mari, 1993). In the case of Alternaria rot in lemons, biological control by the fungus *Paecilomyces* was more effective than the conventional treatment with iprodion; and in the case of Fusarium rot in potato tubers, control by *T. harzianum* was more effective than the conventional benomyl treatment (Figs. 37, 38). However, some *Trichoderma* strains have been found to be pathogenic to harvested fruits, which may limit its possible use to only a few strains. Moreover, spraying strawberries with this antagonistic fungus has no effect on the quiescent infections of *Botrytis*, which comprise an important proportion of total storage infection.

A significant reduction in storage decay was achieved by bringing several yeast species in direct contact with wounds in the peel of harvested fruit. This procedure resulted in the suppression of the main wound pathogens in citrus fruit, including *P. digitatum, P. italicum* and

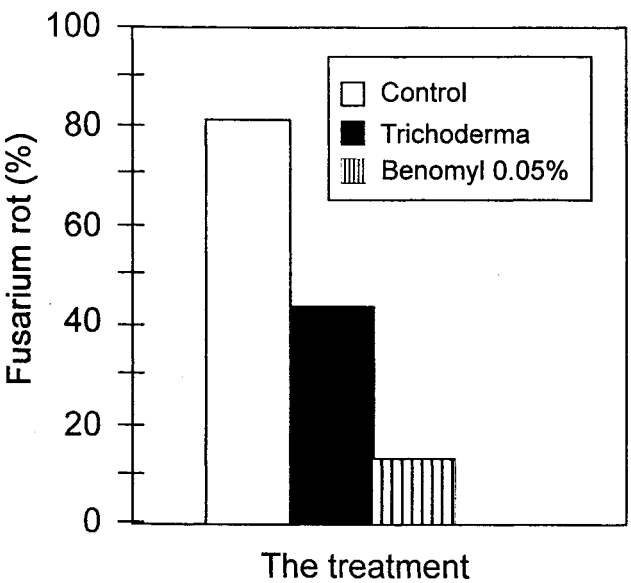


Fig. 37. The antifungal activity of *Trichoderma harzianum* compared with conventional fungicide, benomyl, on potato tubers. Statistical differences (at p= 0.05) are indicated by different letters through the application of the Duncan's Multiple Range Test. (Reproduced from Pratella and Mari, 1993 with permission of Elsevier Science).

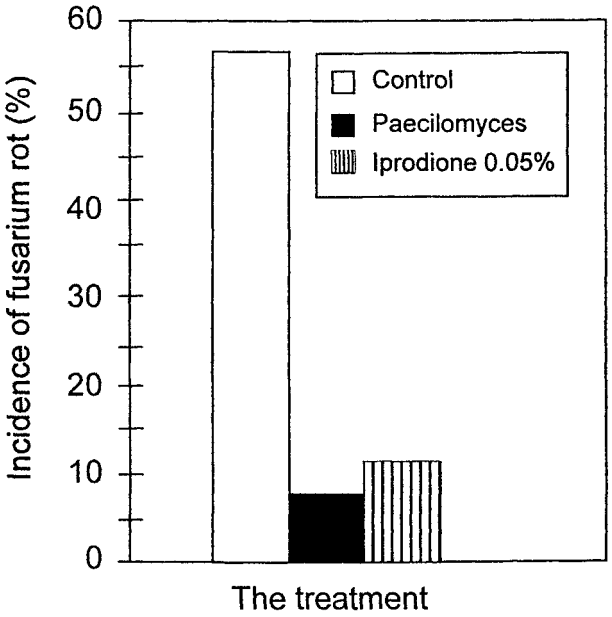


Fig. 38. The antifungal activity of *Paecilomyces variotii* compared with a conventional fungicide, iprodione, on lemon fruit. Statistical differences (at p= 0.05) are indicated by different letters through the application of the Duncan's Multiple Range Test. (Reproduced from Pratella and Mari, 1993 with permission of Elsevier Science).

Geotrichum candidum (Chalutz and Wilson, 1990); of *B. cinerea* in apples (Gullino et al., 1992; Mercier and Wilson, 1995; Roberts, 1990; Wisniewski et al., 1988); of *B. cinerea, P. expansum* and *Phialophora malorum* in pears (Chand-Goyal and Spotts, 1996a; Sugar and Spotts, 1999); of *P. expansum* in apples and nectarines (Wilson et al., 1993; Lurie et al., 1995), and *B. cinerea, R. stolonifer* and *A. alternata* in tomatoes (Chalutz et al., 1991). However, not all the pathogens react similarly to a given antagonist. For instance, under natural infection conditions, dipping grapes in a cell suspension of yeasts of the genera *Kloeckera* and *Candida*, was effective in controlling *Rhizopus* decay but had no effect on *Aspergillus* decay caused by *A. niger* in storage (McLaughlin et al., 1992).

Experiments focused on *B. cinerea* indicated that the efficacy of the antagonist was affected both by the concentration of yeast cells in the wound and by the number of pathogen spores used for inoculation. When wound inoculation was done with a high concentration of *Botrytis* spores

(10^6 spores ml^{-1}), a reduced percentage of infection could be achieved only by the highest yeast concentration (10^9 cells ml^{-1}). On the other hand, when wounds were inoculated with low concentrations of spores, decay suppression could be achieved by each of the antagonist concentrations tested, including the lowest concentration (Chalutz et al., 1991) (Fig. 39).

A similar trend was shown for *Trichoderma* species antagonistic to various fungal pathogens: the best activity was generally observed at high concentrations of the antagonist and the lower inoculum levels of the pathogen (Elad et al., 1982; Mortuza and Ilag, 1999).

The marked antagonistic effects attributed to various yeast species raised the question of the antagonistic capacity of industrial yeasts commonly used in food processing. Screening of industrial yeasts for control of *P. digitatum* in lemons showed that four out of 150 isolates completely controlled the green mold development. These included one isolate of *Saccharomyces cerevisiae* and three *Kluyveromyces* isolates. None of these isolates was found to produce antibiotics (Cheah and Tran, 1995).

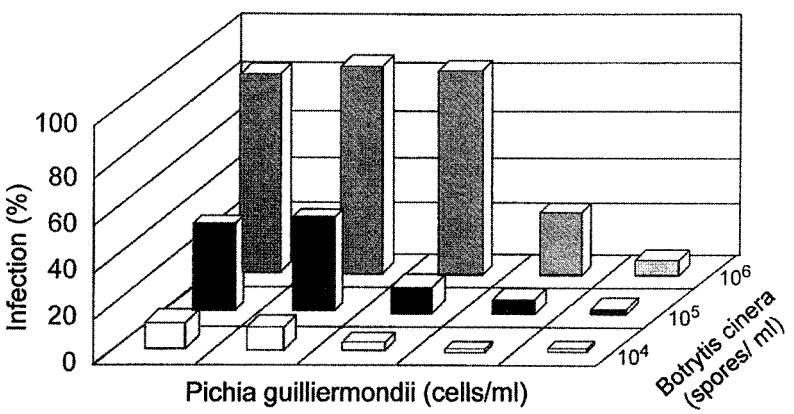


Fig. 39. The relationship between concentration of the pathogen spore suspension and the antagonist cells in the inhibition of *Botrytis cinerea* decay of tomato by *Pichia guilliermondii* (isolate US-7). Values in the same column (spore concentration) followed by different letters are significantly different (p= 0.05) according to Duncan's Multiple Range Test. (Reproduced from Chalutz et al., 1991).

In fact, postharvest decay control in pear fruits by the yeasts, *Rhodotorula* and *Cryptococcus* has been demonstrated following both field sprays (Benbow and Sugar, 1999) and postharvest application (Chand-Goyal and Spotts, 1996a). Similarly, the efficiency of yeasts of the genera *Pichia* and *Hanseniaspora* in controlling *Rhizopus* and *Botrytis* decay in grapes has been expressed following both sprays in the vineyard prior to harvest, and dipping of harvested fruit in the antagonist yeast cell suspension (Ben Arie et al., 1991).

C. MODE OF ACTION OF THE ANTAGONIST

Elucidation of the mechanisms by which antagonists inhibit postharvest pathogens is important for the development of a more reliable procedure for the effective application of known antagonists and for providing a rationale for the selection of more effective antagonists (Wilson and Wisniewski, 1989). Several modes of action have been suggested to explain the biocontrol activity of antagonistic microorganisms (Droby et al., 1992; Droby and Chalutz, 1994; Wilson et al., 1994):

(a) the secretion of antibiotic compounds;
(b) competition for nutrients at the wound site;
(c) direct effect of the antagonist or enzymes secreted by it, on the pathogen;
(d) induction of host defense mechanisms.

(a) The secretion of antibiotic compounds by the antagonist. Examples of microorganisms which inhibit the pathogen via the production of antibiotics are the bacteria, *Bacillus subtilis* and *Pseudomonas cepacia.* The early study of Gutter and Littauer (1953) found *B. subtilis* able to inhibit the development in culture of the main pathogens of harvested citrus fruits. More than 30 years later, it was found that this antagonist was effective against fungal development in citrus fruit (Singh and Deverall, 1984) and against *Monilinia fructicola* in peaches and cherries (Pusey and Wilson, 1984; Utkhede and Sholberg, 1986). Analytical tests of the antibiotic substance secreted by an active isolate of *B. subtilis* indicated the presence of iturins, which are cyclic peptides made of seven α acids and one β acid. They are characterized by a wide antifungal spectrum including fungi pathogenic to man and plants (Pusey, 1991; Gueldner et al., 1988). It is interesting to note that

the inhibition of *Monilinia* development within the fruit has been achieved by the use of both cell-free filtrates of the bacterium and of the living cells separated from the liquid medium on which they grew, although autoclaved cells offered no protection (Pusey, 1991). These facts indicated that living cells of the *Bacillus* may act on the fruit either by producing antifungal metabolites or by antagonizing the pathogen in another way (such as competition for nutrients).

The bacterium, *P. cepacia* inhibits *Botrytis cinerea* and *Penicillium expansum* growth in apples via the antibiotic compound, pyrrolnitrin (Janisiewicz and Roitman, 1988). Trials performed with strawberries have shown that dipping the fruit in pyrrolnitrin alone delayed gray mold development by several days, but had no effect on the extent of decay (Takeda et al., 1990) (Fig. 40).

Experiments with *Penicillium digitatum*-inoculated lemon fruit (Smilanick and Denis-Arrue, 1992) showed that *P. cepacia* was the most effective of several *Pseudomonas* species in controlling green mold decay. The bacterium developed rapidly in the wound area, did not cause any

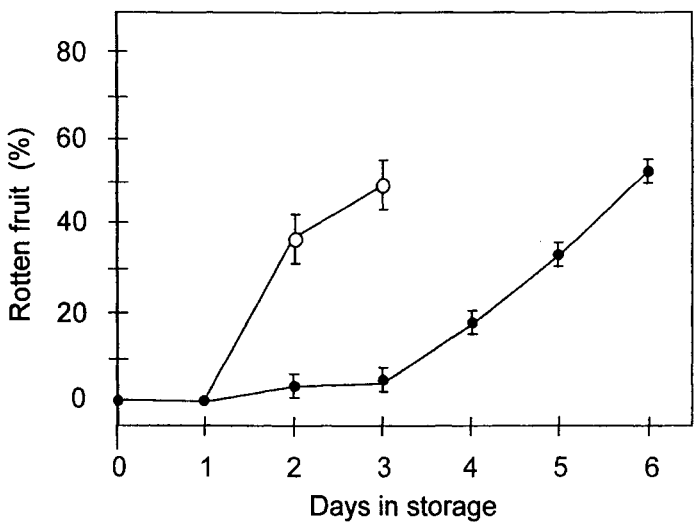


Fig. 40. Effect of pyrrolnitrin on rot development on 'Tribute' strawberry fruit stored at room temperature. Fruit were dipped in water (O) or 250 mg pyrrolnitrin/liter (●) and stored at 18°C. Bars indicate LSD, p=0.05. (Reproduced from Takeda et al., 1990 with permission of the American Society for Horticultural Science).

visible injury and prevented green mold development when it was applied within 12 h of inoculation. Laboratory tests confirmed that fungal inhibition *in vitro* was caused by the presence of pyrrolnitrin in the culture medium. However, experiments also showed that when the fruit was inoculated with pyrrolnitrin-resistant *P. digitatum* mutants, inhibition of decay development was still recorded, even though the spores of the resistant isolate were capable of germination in the presence of the antibiotic substance. This result suggests that the antibiotic substances produced by the *Pseudomonas* are not the only means by which the bacterium functions, and that the presence of *Pseudomonas* cells in the wound also involved a similar antifungal effect. Furthermore, another species of *Pseudomonas* – *P. fluorescence* – which had no inhibiting effect on the *Penicillium* growth in culture, was capable of significantly reducing the growth rate of the green mold in the harvested fruit.

Various antibiotic compounds which control human diseases have been tested against plant diseases: chlortetracycline, cycloheximide, fungicidin, griseofulvin, mycostatin and streptomycin, were found to be effective in controlling serious postharvest diseases. These include bacterial soft rot caused by *Erwinia carotovora,* gray rot caused by *B. cinerea* and brown rot caused by *M. fructicola* (Goodman, 1959). However, none of these compounds is used commercially to control postharvest diseases since, in addition to their high cost, their application to fresh fruits and vegetables would endanger their medicinal effectiveness when needed by humans (Pusey, 1991). The possibility of rapid development of pathogen resistance towards antibiotic substances may be another obstacle in the practical use of antibiotic-producing microorganisms for decay control.

As the list of microorganisms effective in suppressing pathogen development without being capable of producing antibiotic compounds grows, much research has been focused on understanding and defining their mode of action in the fruit.

(b) Competition for nutrients between the antagonist and the pathogen. A fresh wound is a good source of nutrients for invading microorganisms. A delicate balance apparently exists at the wound site between the propagules of the antagonist and the pathogen, which affects the interaction between them and will determine whether or not the wound becomes the site of infection. As was previously mentioned, to compete successfully with the pathogen at the wound site, the antagonist should be better adapted than the pathogen to various environmental and

nutritional conditions: it should grow more rapidly than the pathogen, while using low concentrations of nutrients, and should be able to survive under conditions that are unfavorable to the pathogen (Droby et al., 1991).

One can assume that the antagonist, which grows rapidly in the wound by utilizing nutrients located there, will deprive the pathogen of available nutrients and inhibit spore germination and germ-tube elongation, which are the stages prior to pathogen establishment in the tissues. Thus, it was found that the rapid development of the antagonistic yeast, *Pichia guilliermondii* on grapefruit peel at a broad range of temperatures and at various levels of relative humidity, enables it to populate the wound within 24 h, while *P. digitatum* spores are still at their initial stages of germination (Droby et al., 1992). Similarly, the development of the yeast cells in wounds of tomato peel preceded the development of *Rhizopus stolonifer, B. cinerea* or *Alternaria alternata*, which had also been introduced to the same wound (Chalutz et al., 1991).

Several findings support the hypothesis that the main mechanism by which *P. guilliermondii* inhibits *B. cinerea* and other postharvest pathogens, is competition for nutrients at the wound site (Chalutz et al., 1991): (1) the addition of nutrients to the wound during inoculation markedly reduced the antagonistic effect of the yeast, and the degree of reduction depended on the concentration of nutrients added; (2) a marked reduction in the growth rate of the pathogen occurred only under conditions of limited nutrition, when the antagonist cells were cultured with the pathogen on a poor synthetic medium, and there was no growth inhibition on a rich medium (PDA); (3) the rapid rate of growth of the yeast at the wound site during the critical first 24 h of incubation. The non-specific nature of the antagonistic yeast and its ability to inhibit several wound pathogens of diverse hosts, such as citrus, apple, grape and tomato, support the hypothesis that competition for nutrients is the major mode of action of *Pichia* in decay suppression.

A similar mode of action was suggested also for non-pathogenic yeasts of the genus *Cryptococcus* which have been isolated from a natural epiphytic population on leaves and fruits of apples and pears (Roberts, 1991). These yeasts, which effectively prevented or reduced postharvest diseases of apples, pears and cherries, were able rapidly to colonize, to prosper and to survive in wounds for long periods under a broad range of temperatures (0-20°C). Other features of these yeasts, which could be important for the storage of apples, are their ability to survive in CO_2-enriched controlled atmospheres and their resistance to various fungicides, such as benomyl, sodium *ortho*phenylphenate and rovral.

However, the antagonistic capacity of *Cryptococcus* spp. depended on their concentration as well as on the storage temperature (Roberts, 1991).

Biocontrol of the gray mold on apples by two isolates of another yeast, *Metschnikowia pulcherrima*, was found to be reduced or totally suppressed by the addition of several nitrates. This finding supports the hypothesis that competition for nutrients plays a role in the biocontrol capability of the yeast against *B. cinerea*. Under culture conditions, however, both isolates of the antagonist inhibited spore germination and mycelial growth of the pathogen, regardless of restrictive nutrient conditions (Piano et al., 1997).

Non-pathogenic species of *Erwinia*, such as *Erwinia cypripedii*, showed an antagonistic activity against various isolates of *Erwinia carotovora*, the causal agent of soft rot in many vegetables (Moline, 1991). Dipping carrot disks in a cell suspension of the antagonistic *Erwinia*, followed by inoculating them with active pathogenic isolates of *E. carotovora*, prevented the tissue maceration typically caused by the pathogen; whereas dipping the disks in a cell suspension previously heated, to inactivate the antagonistic cells, canceled the antagonistic effects. The antagonistic *Erwinia* also significantly reduced the incidence of soft rot caused by *E. carotovora*, in tomatoes and peppers, after it was introduced to a fresh wound in the fruit (Moline, 1991). Since the antagonistic strain of *Erwinia* did not exhibit any antibiotic activity, its inhibitory effect on disease development was hypothesized to be the result of competition for substrate in the wound site, between the pathogenic and the antagonistic *Erwinia* spp. This hypothesis is supported by the finding that various bacteria serve as biocontrol agents in the soil, through their ability to bind iron, thus limiting the pathogen growth in the micro-environment (Neilands, 1981; Leong, 1986).

(c) Direct effect of the antagonist or its enzymes, on the pathogen. Several electron microscope observations (Wisniewski et al., 1988, 1991) showed that the antagonist yeast cells may injure the pathogen directly. Co-culturing *P. guilliermondii* with the pathogenic fungi, *B. cinerea* or *P. expansum* demonstrated the ability of the yeast to attach fungal hyphae closely (Photo 7). In many instances, individual yeast cells appeared to be embedded within depressions in the hyphal wall and in some areas extensive pitting and holes were observed in the fungal hyphae (Photo 8). In contrast, co-culturing *B. cinerea* with a non-antagonistic yeast elicited only a loose attachment to the fungus, with no pitting in the hyphae.

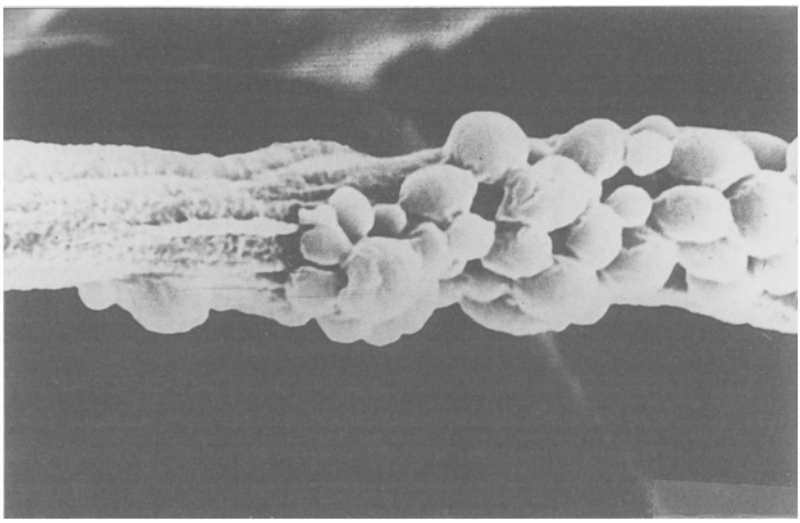


Photo 7. Coculture of *Botrytis cinerea* and the antagonistic yeast *Pichia guilliermondii*: tenacious attachment of the yeast cells to fungal hyphae. (From Wisniewski, Biles and Droby, 1991).

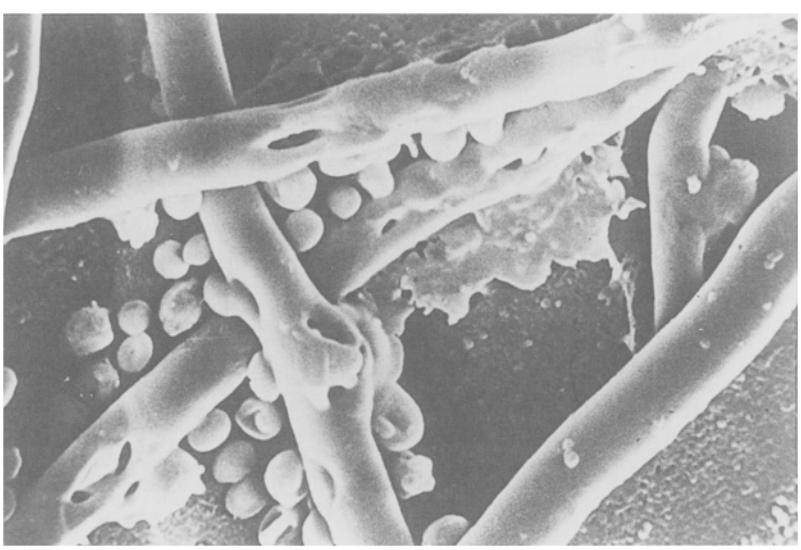


Photo 8. Coculture of *Penicillium expansum* and the antagonistic yeast *Pichia guilliermondii*: formation of pitting and holes in the fungal hyphae. (From Wisniewski, Biles and Droby, 1991).

Apart from the effects of direct contact of the antagonistic yeast with the pathogen hyphae, filtrates of the antagonist cells can produce higher levels of gluconase than filtrates of the non-antagonistic yeast, and the gluconase activity is the cause of cell wall degradation at the sites of attachment. These findings raised the suggestion that the firm attachment to the fungus, in conjunction with the enhanced activity of cell-wall degrading enzymes, may have an important role in the biological activity of *P. guilliermondii*, and that its efficacy is not dependent only on its competition with the pathogen for nutrients (Wisniewski et al., 1991). However, there is still the possibility that the firm attachment enhances the utilization of nutrients by the antagonist, at the site of invasion, and thereby blocks access to the available nutrients by the pathogen (Wisniewski et al., 1988).

Various studies with species of *Trichoderma*, which are known as effective biocontrol agents of several important plant pathogenic fungi, highlighted the role of lytic enzymes, including glucanases, chitinases and proteinases, in the capability of the antagonist to attack the pathogen and thus to reduce disease incidence (Chet et al., 1993; Goldman et al., 1994, Lorito et al., 1993; Mortuza and Ilag, 1999). *Trichoderma harzianum* isolates were found to produce chitinases and glucanases when grown on live mycelium of *Sclerotium rolfsii* and *Rhizoctonia solani* in soil (Elad et al., 1982). The chitinolytic system of *T. harzianum* is made up of six distinct chitinolytic enzymes: two β-1,4-*N*-acetylglucosaminidases (exoenzymes) and four endochitinases (Haran et al., 1996). Both the levels and the expression patterns of these enzymes are specifically affected by the pathogenic fungus attacked by the *Trichoderma*. The parasitic interaction with *R. solani* involved the expression of both the endochitinase activities and the exotype activity. During the mycoparasitic interaction with *S. rolfsii*, however, only the exotype activities of two β-1,*N*-acetylglucosaminidases were detected. It was suggested that the differential expression of *T. harzianum* chitinases might influence the overall antagonistic ability of the fungus against a specific pathogen.

When 15 *Trichoderma* isolates were tested for their antagonistic ability against *Lasiodiplodia theobromae*, the greatest inhibition in dual culture was exhibited by *T. harzianum*, followed by *T. viride* (Mortuza and Ilag, 1999). For each species, however, inhibition increased with increasing density of spores in the inoculum. All the *Trichoderma* species reduced spore germination and inhibited germ-tube elongation of *L. theobromae*. Microscopic investigation demonstrated direct parasitism

and coiling of *T. harzianum* and *T. viride* around the hyphae of *L. theobromae*. In addition to the direct parasitism, and deformation of some cells of the pathogenic fungus, granulation of the cytoplasm and disintegration of the pathogen hyphal walls of *L. theobromae* also occurred without intimate contact between the hyphae. It was, therefore, suggested that the two *Trichoderma* species could produce antifungal metabolites that contributed to their antagonistic activity. In fact, culture filtrates of the antagonists were found to be more suppressive of the conidia of the pathogen than spore suspensions, suggesting the involvement of certain metabolites in the antagonistic effect of *Trichoderma* species on *L. theobromae*. Both antibiosis and direct parasitism have also been suggested to be the modes of action of *T. harzianum* in reducing the incidence of postharvest stem-end rot (*Botryodiplodia theobromae*), anthracnose (*Colletotrichum gloeosporioides*) and brown spot (*Gliocephalotrichum microchlamydosporum*) on rambutan fruits (Sivakumar et al., 2000).

An isolate of *T. harzianum*, which is used commercially prior to harvest, to control *B. cinerea* on grapes and greenhouse crops, was found to retard spore germination and germ-tube elongation of the pathogen. This retardation was associated with a limiting of disease severity, probably because of reduced penetration of *B. cinerea* germ tubes into the host. However, the efficient antagonistic isolate was also found to reduce the activity of hydrolytic enzymes of the pathogen (Kapat et al. 1998). Levels of cutin esterase, pectin methyl esterase, exopolygalacturonase, endopolygalacturonase and pectate lyase were all reduced when *B. cinerea* was grown with the active isolate of *T. harzianum*, either in a liquid culture or on the surface of bean leaves. The antagonist had no effect on the carboxymethyl cellulase activity of the pathogen. When different isolates of *T. harzianum* were used, a correlation was found between the enzyme inhibition capacity of the antagonist and its disease suppression efficacy, supporting the hypothesis that the inhibition of the activity of pathogenic enzymes of *B. cinerea* is a mechanism by which the biocontrol agent affects disease (Kapat et al., 1998).

(d) Induction of the host defense mechanism. One of the explanations offered for the mode of action of the antagonistic yeast, *P. guilliermondii* lay in its ability to stimulate wound healing or to induce other defense mechanisms in the host (Droby et al., 1992). Experiments with *Pichia*-inoculated citrus fruit showed that the yeast stimulated ethylene production and raised the levels of the enzyme phenylalanine

ammonia lyase (PAL). The involvement of these processes in the enhanced defense mechanisms in citrus fruit has previously been described by G.E. Brown and Barmore (1977) in tangerines inoculated with *C. gloeosporioides*.

Candida famata (isolate F35) was found to be one of the most active yeasts tested against *P. digitatum* in citrus fruits: it was able to reduce decay by 90-100% in artificially wounded fruits (Arras, 1996). Scanning electron microscope observations revealed a rapid colonization of the fungal mycelium at the wound sites, with numerous yeast cells strongly attached to the hyphae, exhibiting lytic activity and rapid alterations. Furthermore, when the yeast was inoculated into artificial wounds, either alone or with the pathogen, it stimulated the fruit to produce the phytoalexins, scoparone and scopoletin at the wound site. The concentrations of the phytoalexins depended significantly on the time lag between inoculation with the antagonist yeast and inoculation with the pathogen. Four days after inoculating the fruit with the yeast alone, scoparone concentration reached 124μg g^{1} fresh weight, 12 times higher than that in the non-inoculated wound. The concentration reached only 47μg g^{-1} when the antagonist was inoculated together with the pathogen, and only 37μg g^{-1} when the pathogen alone was introduced into the wounds. Since scoparone inhibits spore germination of *P. digitatum* at 46 μg g^{-1} and germ tube elongation at 29μg g^{1} (Kim et al., 1991), it was concluded that the phytoalexin had already reached fungitoxic concentrations within a few days of the application of the antagonistic yeast (Arras, 1996).

Increased resistance to infection can result not only from enhanced production of phytoalexins but also from preformed inhibitory compounds. It was thus found that a non-pathogenic mutant of *Colletotrichum magna* was capable of enhancing the preformed antifungal diene compound in avocado fruit peel and could, therefore, protect the fruit against infection by *C. gloeosporioides* (Prusky et al., 1994).

Recently, Ippolito et al. (2000) related the capability of the antagonistic yeast-like fungus, *Aureobasidium pullulans* to control decay in apple fruits inoculated with *B. cinerea* and *P. expansum* to its ability to enhance the activities of the enzymes, β-1,3-gluconase, chitinase and peroxidase in the treated fruits, in addition to its capacity to overcome the pathogen in competition for nutrients and space. These enzymes are all considered to be involved in host defense mechanisms. Chitinase and β-1,3-gluconase are capable of hydrolyzing fungal cells and so inhibiting

fungal growth (Schlumbaum et al., 1986), while peroxidases are involved in the formation of structural barriers against pathogen invasion (El Ghaouth et al., 1998). *A. pullulans* could thus induce further decay control through a multiple-component mode of action against the pathogen (Ippolito et al., 2000).

D. ANTAGONIST MIXTURES TO IMPROVE DISEASE BIOCONTROL

The development of biological control has made much progress during the last decade. Today, with several biocontrol treatments approved for commercial application and others undergoing the approval process, research is being focused on improving both the antagonist efficacy and the control system. One of the approaches to improving biocontrol agents is by selecting combinations of antagonists that will act better than each of the components separately.

A combination of the bacterium *Pseudomonas syringae* and the pink yeast *Sporobolomyces roseus* proved to have a marked advantage over each of the antagonists in controlling *Penicillium expansum* in apples, both in reducing the incidence of wound infections and in limiting rot diameter (Janisiewicz and Bors, 1995). After application of the mixtures, populations of *S. roseus* in the wounds were found to be consistently lower than those after individual applications, whereas populations of *P. syringae* were not affected by the presence of the other antagonist. When 35 nitrogen sources were tested for utilization by the antagonists, both *S. roseus* and *P. syringae* were found to utilize 14 sources, whereas *P. syringae* utilized an additional nine compounds. On the other hand, more carbon sources were utilized by *S. roseus* than by *P. syringae*. It was concluded that the populations of the antagonists in apple wounds form a stable community, dominated by *P. syringae*. This domination was attributed to the ability of *P. syringae* to use nitrogen sources, which form the limiting factor in the carbon-rich apple wounds. This is an example of the possibility of exploiting the differing nutritional features of the antagonists to achieve improved biological control at the wound site.

In a further study, Janisiewicz (1996) reported on the development of antagonist mixtures that were superior to individual antagonists in controlling blue mold (*P. expansum*) of apples; the development was based on ecological knowledge of the distributions of the antagonists in

time and space, and of their nutritional 'niche' differentiation. The microorganisms in these mixtures, which were isolated from exposed apple tissue before harvest, comprised mainly yeast populations. Following screening for their ability to control *P. expansum* on apple fruits, they were grouped into various nutritional clusters. Preference was given to antagonists colonizing the same fruits, followed by those colonizing different fruits but isolated at the same time. Nutritional differences between two antagonists in a mixture allowed populations of both antagonists to flourish in the same wound. The results indicated that combinations of antagonists that occupy different nutritional niches and coexist in the infection area are more effective in biological control treatments than the individual antagonists.

The advantage of antagonistic pairs over a single antagonist was described by Schisler et al. (1997) in the control of Fusarium dry rot (*Gibberella pulicaris,* anamorph *Fusarium sambucinum*) in stored potato tubers. The search for biological means to control this disease was stimulated by the development, over the years, of resistance to thiabendazole, the conventional chemical treatment. When the pathogen was challenged with pairs of antagonistic bacterial strains, it was found that successful pairs reduced Fusarium dry rot by an average of 70% versus controls, a level of control comparable with that obtained with 100 times the inoculum dose of a single antagonist strain. The successful coexistence of these pairs was partly attributed to the fact that compatible strains possessed differing carbon substrate utilization profiles. Similarly to the demonstration by Janisiewicz (1996) that combining antagonists on the basis of 'niche' differentiation enhanced the possibility of improved control of *P. expansum* on apples, diverse niches are also likely to be found in potato wounds. These could result in the exposure of *G. pulicaris* to higher total bacterial populations and wider ranges of nutrient competition when challenged by co-existing mixtures of microbial strains than when challenged by a single antagonist (Schisler et al., 1997). It does seem that determination of the substrate utilization profiles of strains of antagonist pairs, as well as evaluation of the colonization characteristics of successful and unsuccessful pairs of potato dry rot antagonists, would provide further clues as to the nature of the disease control success of some antagonistic pairs. However, the success of antagonistic pairs also may be attributable to the individual strains of the pair possessing complementary modes of action (Schisler et al., 1997).

E. COMBINED TREATMENTS TO IMPROVE DISEASE BIOCONTROL

Various additives have been shown to increase the effectiveness of some antagonistic microorganisms in controlling postharvest decay. The addition of $CaCl_2$ to antagonistic yeast suspensions was found to enhance their biocontrol activity and reduce the populations of yeast cells required to give effective control (McLaughlin et al., 1990; Droby et al., 1997). A combination of the yeast antagonist, *Pichia guilliermondii* (10^7 cells ml^{-1}) with $CaCl_2$ (136 mM) in dip application, significantly decreased the incidence of green mold caused by *Penicillium digitatum* in grapefruit wounds (Droby et al., 1997). Both spore germination and germ tube elongation of *P. digitatum* decreased with increasing $CaCl_2$ concentration. In addition, increased $CaCl_2$ concentration also resulted in the inhibition of pectolytic activity of a crude enzyme preparation of *P. digitatum*. Hence, the effects of calcium in reducing infection may be due to its effects on the host tissue, via enhanced cell-wall resistance to enzymatic degradation, or to direct effects on the pathogen by interfering with spore germination and inhibiting fungal pectolytic activity. However, the yeast also strongly maintained calcium homeostasis, and this ability probably allowed it to grow in a microenvironment which is inhibitory to the pathogen. Enhancement of biocontrol of the blue mold (*Penicillium expansum*) on apples has been achieved by adding nitrogenous compounds to suspensions of the bacterial antagonist *Pseudomonas syringae* applied to the fruits (Janisiewicz et al., 1992).

Other compounds capable of enhancing the activity of some antagonistic microorganisms are sugar analogs. The addition of 2-deoxy-D-glucose to the antagonists, *P. syringae* and *Sporobolomyces roseus* was found to enhance their antagonistic effect against *P. expansum* (Janisiewicz, 1994). This combination enabled the concentration of the antagonist required for the biocontrol of blue mold on apple fruits to be reduced tenfold. El Ghaouth et al. (1995) found that out of the various sugar analogs tested as potential fungicides against apple and peach pathogens, only 2-deoxy-D-glucose was effective in controlling decay in inoculated fruits. Recently this compound was found to be compatible with the antagonistic yeast, *Candida saitoana* (El Gaouth et al., 2000b). Although the growth of *C. saitoana in vitro* was reduced by 2-deoxy-D-glucose, the yeast grew normally in the presence of this compound when applied to wounds. A combination of the antagonistic yeast with a low dose of 0.2% of the sugar analog, applied to apple wounds prior to inoculation with *B. cinerea*

and *P. expansum*, or to lemon and orange wounds prior to inoculation with *P. digitatum*, significantly enhanced the biocontrol activity of *C. saitoana* against decay. The same combination was also effective against established infections when applied within 24 h after inoculation. In other words, the combination of the antagonistic yeast with the sugar analog had both protective and curative effects against the postharvest pathogens. Furthermore, its effectiveness on citrus fruits was found to be similar to that of the common fungicide, imazalil. It was also emphasized that the curative activity gained by the combined treatment represented an improvement over the currently available microbial biocontrol products that confer only a protective effect (El Ghaouth et al., 2000b).

Another combination aimed at improving the biocontrol of fruit decay by *C. saitoana* is that of the antagonistic yeast with glycolchitosan, a combination known as "a bioactive coating" (El Ghaouth et al., 2000a). The use of a bioactive coating became possible because the presence of glycolchitosan in apple wounds and on the fruit surface does not affect the natural increase of the yeast population. The combination of the antagonistic yeast with 0.2% glycolchitosan was more effective in controlling the natural infection caused by *B. cinerea* and *P. expansum* in various apple cultivars than either the yeast or the glycolchitosan alone. The bioactive coating was either similar or superior to thiabendazole in suppressing decay; it was also more effective than the antagonistic yeast alone in controlling natural infection of oranges and lemons (mainly by *P. digitatum*) and the control level was equivalent to that achieved with 2000 g ml^{-1} imazalil (Fig. 41).

Genetic manipulation of biocontrol fungi is another approach to improving the antagonist ability to control disease. To enhance their antagonistic potential against pathogenic fungi, Lalithakumari et al. (1996) selected two parent isolates of *Trichoderma* species for genetic manipulation: *Trichoderma harzianum,* which is an efficient biocontrol agent against plant pathogens, and *Trichoderma longibrachiatum*, which is tolerant to copper sulfate and carbendazim. The second isolate was selected as a co-parent because development of fungicide tolerance potential in a biocontrol agent is believed to be of great importance for integrated disease management. It was found that protoplast fusion of the two isolates, taken from young mycelia following cell-wall digestion, resulted in fusants that exhibited an enhanced antagonistic effect against several pathogens, along with tolerance to copper and carbendazim.

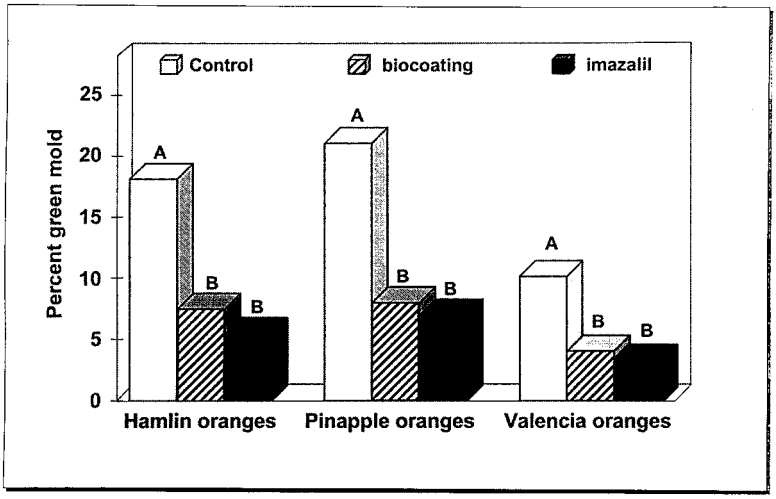


Fig. 41. Effect of the combination of *Candida saitoana* with 0.2% glycolchitosan on decay of orange cvs. Hamlin, Pineapple and Valencia after 21, 28 and 21 days at 18°C, respectively. Bars within each cultivar with the same letter are not significantly different according to Duncan's Multiple Range Test (p=0.05). (Reproduced from El Ghaouth et al., 2000a with permission of the American Phytopathological Society).

F. INTEGRATION INTO POSTHARVEST STRATEGIES

Several studies have highlighted the advantages of postharvest application of biological control agents over field or soil application. The major advantages are: (1) the convenience of bringing the antagonist in contact with the commodity, compared to its addition to the soil; (2) the possibility of acting under controlled conditions, created and maintained during storage and transportation (Wilson and Wisniewski, 1989); (3) the possibility of applying the biocontrol agents during the commercial process which the fruit and vegetables undergo in the packinghouse. For instance, it is possible to introduce *Bacillus subtilis* into the wax applied to peaches in the packing house to protect them from the brown rot caused by *Monilinia fructicola* (Pusey et al., 1988). The antagonistic yeast *Pichia guilliermondii* can be introduced into the wax mixture applied to citrus fruit, or applied as a separate step, in the packinghouse.

Compatibility between a microbial antagonist and a synthetic fungicide offers the option of using the antagonist in combination with reduced levels of the fungicide. Applying the yeast antagonist, *Pichia guilliermondii* to citrus fruit in combination with substantially reduced concentrations of thiabendazole (TBZ) reduced *Penicillium digitatum* decay to a level similar to that achieved by the currently recommended concentration of TBZ applied alone (Droby et al., 1993b). Thus, by adapting an integrated pest management system, we may expect not only to gain effective pest control but we can also maintain very low levels of chemical residues (Hofstein et al., 1994). The biological agent must, however, have low sensitivity to any of the supplemented chemical fungicides.

The intensive studies on biocontrol of postharvest diseases have led to the registration of two biological products for commercial postharvest applications to citrus fruits: Aspire, which is *Candida oleophila*, and BioSave™ 1000, which is *Pseudomonas syringae* (Brown, G.E. and Chambers, 1996; Brown, G.E. et al., 2000). In evaluating the efficacy of biological products for the control of citrus fruit pathogens, Brown and Chambers (1996) found that significant control of *P. digitatum* was obtained by each of the biological products but that the level of control, as well as consistency, were usually less pronounced than those obtained with standard rates of the fungicides, thiabendazole or imazalil. More recently Brown, G.E. et al. (2000) found that the major factor affecting the efficacy of the biological control is how quickly and how well the yeast colonizes injuries to the citrus fruit surface, including minor injuries involving only oil vesicles. This indication followed the finding that the peel oil was toxic to the *Candida* cells but not to spores of *P. digitatum*. However, combining Aspire with each of the chemicals used improved the results; sometimes combinations with a low rate of fungicide were sufficient to achieve effects similar to those obtained by the chemicals at standard rates.

GRAS (generally recognized as safe) compounds or natural products of plant origin, which have been suggested as alternatives to synthetic and conventional fungicides (see the Chapter on Chemical Control - Generally Recognized as Safe Compounds and Natural Chemical Compounds), could also be used in combination with biocontrol agents, complementing their activity. Pathogens treated with such antifungal substances might be weakened and become more vulnerable to the antagonist activity (Pusey, 1994).

The integration of postharvest biocontrol into modern production, storage and handling systems must begin before harvest. Several preharvest factors that affect fruit quality may have profound effects on the efficacy of postharvest biological control agents (Roberts, 1994). Preharvest calcium sprays during the growing season of apples and pears, as well as calcium application by immersion infiltration at postharvest, can increase fruit firmness, decrease the incidence of certain disorders and enhance resistance to postharvest infection (see the chapter on Means for Maintaining Host Resistance – Calcium Application). As was previously mentioned, calcium amendments and postharvest application of some antagonistic yeasts can be additive in reducing fruit decay and can significantly increase disease control compared with either treatment alone (McLaughlin et al., 1990; Droby et al., 1997). The advantages in increased firmness, enhanced resistance to postharvest decay and enhanced biocontrol efficacy under some circumstances reflect the multiple benefits of integrating postharvest biological control with cultural and production practices (Roberts, 1994).

Fruit maturity at harvest and at the application of antagonists is another factor affecting postharvest biological control. Late-picked, over-mature fruits are more susceptible to decay than are fruits picked at optimal storage maturity (Sommer, 1982). Working with apples and pears, and with different species of the antagonistic yeast *Cryptococcus*, Roberts (1990, 1994) found that fruit maturity markedly affected biocontrol efficacy: while excellent control was achieved on freshly harvested fruit, treatments of ripened fruit gave much lower levels of control. On the assumption that the infection process can be initiated at harvest, it would be advantageous to treat fruit with biocontrol agents as quickly as possible after harvest and to cool the fruit as rapidly as possible, to retard pathogen development. In fact, studies with *Mucor*-inoculated pears and antagonistic *Cryptococcus* species demonstrated maximal biocontrol effect when the yeasts were applied to the fruit soon after harvest (Roberts, 1990). The principle is to retard pathogen development while allowing the antagonistic microorganisms to colonize wound sites.

Postharvest factors, too, may have a major impact on the effectiveness of biological control. Temperature management, which is a critical factor in the maintenance of fruit quality and in pathogen development (see the chapter on Factors Affecting Disease Development), may also enhance

biological control of storage decay. Janisiewicz et al. (1991) demonstrated that as the storage temperature of apples and pears decreased, there was a reduction in the concentration of pyrrolnitrin (a metabolite of *Pseudomonas cepacia* and other *Pseudomonas* spp.) needed to protect the fruit from gray mold (*Botrytis cinerea*) and blue mold (*Penicillium expansum*).

An integrated strategy to control postharvest decay in pome and stone fruits has been advanced in recent years (Sugar et al., 1994; Spotts et al., 1998; Willett et al., 1992); it comprises several pre- and postharvest components (Sugar et al., 1994):

(1) Alteration of fruit nutrient status. This was found to influence the susceptibility of pome fruits to decay, and includes calcium applications, either as sprays during the growing season (Sugar et al., 1991) or by pressure infiltration into the fruit (Conway and Sams, 1983), which increase fruit calcium content and reduce the severity of decay. Lower fruit nitrogen content has also been associated with reduced disease severity. Management of pear trees for low fruit nitrogen (influenced by timing of fertilizer application) combined with calcium chloride sprays was found to reduce decay severity more than low nitrogen alone (Sugar et al., 1992).

(2) Maturity at harvest. The severity of several postharvest diseases of pears was found to increase as the fruit approached maturity or as harvest was delayed, within the range of harvested maturity (Spotts, 1985).

(3) Controlled atmosphere storage. Atmospheres with reduced O_2 and elevated CO_2 can reduce the severity of postharvest fungal decay in pome fruits by inhibiting fruit senescence (Chen et al., 1981), thereby maintaining host resistance to infection (Barkai-Golan, 1990).

(4) Application of low level of fungicide. All the components of the integrated program were found to be compatible with thiabendazole, which is used for postharvest decay control in pears (Roberts, 1991). This is relevant when the combined treatment is aimed at controlling the blue mold (*P. expansum*) in harvested pears. Thiabendazole is not effective against *Phialophora malorum*, the causal agent of side rot of pears (Willett et al., 1992).

Several yeast species, such as *Cryptococcus laurentii*, are capable of colonizing wounds of pear fruit under conditions of low temperature (0°C), ambient or reduced O_2, and ambient or elevated CO_2 (Roberts, 1991) and can, therefore, integrate into postharvest strategies. The

integration of early harvest, low fruit nitrogen, high fruit calcium, yeast or yeast + one-tenth of the label rate of thiabendazole, along with a controlled atmosphere (2% O_2, 0.6% CO_2), was found to reduce blue mold severity in inoculated fruit by 95, 61 and 98% in three successive years, after 2-3 months at 0°C. Side rot caused by *P. malorum* was completely controlled when the combined treatments included the antagonistic *Cryptococcus*, early harvest and high fruit calcium.

Control of brown rot of sweet cherry was achieved by postharvest application of a combination of the antagonistic yeast *C. laurentii* or *C. infirmo-miniatus,* with low doses of iprodione to the fruit (Chand-Goyal and Spotts, 1996b). However, following the ban on the application of iprodione less than 7 days before harvest, Spotts et al. (1998) showed that a combination of a single preharvest treatment with iprodione, in conjunction with postharvest biological control by *C. infirmo-miniatus*, gave significantly better control of the brown rot in stored sweet cherries than iprodione alone, although the yeast by itself had no suppressive effect on decay development. The effectiveness of such a combined treatment resulted from the fact that *M. fructicola* was sensitive to the residues of iprodione, whereas the antagonistic yeast was resistant to them.

In contrast to its lack of effect on *M. fructicola*, the yeast alone did suppress the development of *P. expansum*, which infected cherry fruits naturally. Combinations of preharvest iprodione and postharvest *Cryptococcus* applications resulted in the prevention of blue mold infection in storage (Spotts et al., 1998).

In addition, brown rot in sweet cherries, which was reduced by modified atmosphere packaging alone, could be further reduced as a result of synergism between the antagonistic yeast and the modified atmosphere. The O_2 and CO_2 percentages within the sealed package, after 42 days of storage at 0.5°C, were 11.4 and 5.1%, respectively; reduction of decay under these conditions was attributed to the accumulation of CO_2 in the atmosphere. Furthermore, when CO_2 dissolves in water, carbonic acid is produced and the pH is lowered. Such conditions may favor yeast growth. Thus, the *Cryptococcus*-MA synergism may result from a combination of suppression of *M. fructicola* and stimulation of *C. infirmo-miniatus* (Spotts et al., 1998). When these two postharvest treatments – biological control and modified atmosphere packaging – were combined with preharvest iprodione spray, the incidence of the brown rot was reduced from 41.5% in the control to only 0.4%.

A combined strategic approach was elaborated by Eckert (1991), who proposed to control wound-infecting pathogens by a series of treatments: (1) disinfection of the fruit surface and environment; (2) eradication or suppression of fungal spore germination at wound sites by a combination of fungicides; and (3) reduction of wound susceptibility to infection by the addition of biocontrol antagonists which act as protective agents. Since the biocontrol efficiency of the antagonist is enhanced by reduction of the spore concentration of the pathogen in the wound (see Fig. 39), a sanitation program that reduces pathogen contamination in water systems or on the equipment in the packing house may determine the level of success of a biological control program.

CHAPTER 12

NOVEL APPROACHES FOR ENHANCING HOST RESISTANCE

Synthetic fungicides are currently the primary means used for controlling postharvest diseases. The resistance developed by postharvest pathogens to some fungicides, and the withdrawal of a number of key fungicides in response to health concerns over pesticide contamination, have stimulated the search for alternative technologies for postharvest disease control. Among the various possible alternative means of control, much attention has been drawn to the wide range of natural substances with antimicrobial activity. Plant- and animal-derived fungicides may offer safe alternatives to synthetic fungicides (Wilson et al., 1994), and the potential use of these antimicrobial agents is discussed in the chapter, Chemical Control - Natural Chemical Compounds. Other alternatives to chemical treatments are the various physical means of suppressing postharvest decay development. These are discussed in the chapters, Means for Maintaining Host Resistance, and Physical Means. The use of antagonistic microorganisms, often isolated from plant surfaces, as biocontrol agents is another alternative and quite promising direction. The chapter on Biological Control is dedicated to this subject.

However, many of these treatments may also act as elicitors of enhanced resistance responses in harvested fruits and vegetables (Biles, 1991; Wilson et al., 1994). Fruits and vegetables may defend themselves against pathogen attack or further colonization of the tissue by inducing defense mechanisms in response to initial infection or to various chemical, physical or biological elicitors. These induced defenses are described as active mechanisms, which require host metabolism to function (Keen, 1992). Disease occurs when a potential pathogen not only circumvents the passive defenses, such as the structural barriers or the preformed antimicrobial compounds of the host, but also avoids elicitation of active defense responses in the tissues (Jackson and Taylor, 1996; Hutcheson, 1998). New approaches for controlling postharvest diseases have been based on activation of the natural defense responses induced by the host itself, by modulating them with suitable elicitors, as

well as on utilization of the resistance genes of a host via genetic crop modification or genetic engineering techniques.

A. INDUCED RESISTANCE

Inducible resistance in harvested tissues has recently joined the general concept that resistance in plants can be enhanced by modulating their natural defense mechanisms (Kuć, 1995). Activation of defense responses in harvested crops has been demonstrated in various host-pathogen interactions via application of physical, chemical or biological elicitors. This chapter will focus on the resistance-eliciting properties of these treatments and display their potential defense reactions.

1. PHYSICAL ELICITORS

Modified atmospheres, heat treatments, gamma radiation and ultraviolet (UV) light have been the main physical elicitors suggested as possible resistance inducers.

Modified Atmospheres

While conditions which stimulate ripening, such as ethylene application to various fruits, enhance decay incidence, conditions which inhibit ripening, such as low storage temperatures and controlled or modified atmospheres, contribute to decay suppression or retardation (see the chapter on Factors Affecting Disease Development). It has thus been found that storing avocado fruits in sealed plastic bags, along with the production of a CO_2-enriched atmosphere within the bag, delays fruit ripening. When ripening is delayed, the normal decomposition of a preformed antifungal diene compound in the fruit peel is retarded and, under these conditions, the appearance of anthracnose symptoms (*Colletotrichum gloeosporioides*) is delayed (Prusky et al., 1991). In a recent study, Ardi et al. (1998) showed that exposure of freshly harvested avocado fruits to CO_2 resulted in increased concentrations of the antioxidant epicatechin and of the antifungal diene compound in the peel. The normal decrease in the antifungal activity in the peel during ripening has been attributed to the activity of lipoxygenase, which oxidizes the antifungal compound to a non-active compound, while the activity of lipoxygenase is regulated by the epicatechin naturally present

in the fruit peel during ripening (Prusky et al., 1985b). Thus, by producing a high-CO_2 atmosphere, the increased concentrations of epicatechin during ripening may lead to increased antifungal activity in the host and, therefore, to maintaining the quiescent state of the fungus and reducing decay development.

A high-CO_2 atmosphere may also induce higher levels of resorcinols in mango fruits, resulting in the retardation of *Alternaria alternata* infection (Prusky and Keen, 1995). Similarly, application of subatmospheric pressure to mango fruits retards ripening and, in parallel, retards the decrease in the level of the preformed antifungal resorcinol compounds in the peel. This decrease occurs naturally during ripening. Under these conditions the renewed development of *A. alternata,* which is found in a quiescent state in the fruit, is delayed (Droby et al., 1986).

Heat Treatments

Heat treatment can affect many physiological processes of the harvested fruit and vegetable. Depending on fruit species, cultivar, and physiological age, on the one hand, and on the temperature and length of exposure to heating, on the other hand, this treatment may lead to retardation of the ripening process. In many cases, along with the retardation of fruit ripening and the maintenance of firmness during storage, heating may also induce enhanced resistance of the host to pathogens.

Various mechanisms by which heat treatment may induce host resistance have been suggested. Heat may function as a stress factor, inciting the accumulation of phytoalexins in the host tissue. A range of coumarin-derived antifungal compounds was detected in the peels of citrus fruits, following heat treatment (Ben Yehoshua et al., 1992; Kim et al., 1991). Kim et al. (1991) reported on the enhanced accumulation of the antifungal coumarin scoparone in heat-treated *Penicillium*-inoculated lemon fruits, and a correlation was drawn between the level of the antifungal activity in the host tissues and the development of disease resistance. Heat treatments may also result in the inducement of specific proteins of various molecular weights, known as 'heat shock proteins', in the plant tissue (Freeman et al., 1989; Vierling, 1991). A correlation was drawn between the accumulation of 'heat shock proteins' and the enhancement of thermotolerance of the fruit following heat treatments. The inducement of such proteins in fruits and their consequent enhanced thermotolerance may improve the efficacy of heat treatments in reducing

postharvest decay, through the possibility of using higher and more effective temperatures.

Gamma Radiation

Ionizing radiation can suppress decay development by directly affecting fungal spores and hyphae. It may also suppress decay indirectly, by delaying the ripening and senescence processes of harvested fruits and vegetables and contributing to the maintenance of their natural resistance to pathogen invasion or development. However, gamma radiation may also function as a stress factor, capable of inducing phytoalexin formation. Induction of the phytoalexins, scopoletin and scoparone was reported in citrus fruit following gamma irradiation at 1-4 kGy (Riov, 1971; Riov et al., 1971), and their antifungal activity has been demonstrated (Dubery and Schabort, 1987). However, the part that the induced phytoalexins play in the complex of radiation effects on the pathogen or the defense responses of the host to effective radiation doses has not yet been evaluated.

Low-dose Ultraviolet (UV) Light

UV illumination is another physical treatment that may induce resistance against pathogen infection (Wilson et al., 1994). UV-C (wavelength below 280 nm) light at low doses was found to induce resistance in a wide array of commodities, such as onions (Lu et al., 1987), carrots and peppers (Mercier et al., 1993), tomatoes (Liu et al., 1993), sweet potatoes (Stevens et al., 1990), peaches (Lu et al., 1991), various citrus fruits (Chalutz et al., 1992; Droby et al., 1993a; Porat et al., 1999; Rodov et al., 1992), and table grapes (Nigro et al., 1998).

The enhanced resistance that follows UV treatment may be mediated by the activation of various defense responses in the host tissue. The first such response is the accumulation of phytoalexins to inhibitory levels. In carrot slices, the UV-induced resistance of the tissue to *Botrytis cinerea* and *Sclerotinia sclerotiorum* infection coincided with the induction of the phytoalexin, 6-methoxymellein (Mercier et al., 1993). The induced resistance was expressed only after 1 week of storage, when the concentration of 6-methoxymellein in the tissue had reached inhibitory levels. The content of this compound in UV-treated slices that were held at 1 and 4°C remained elevated for up to 35 days after treatment.

Another phytoalexin, scoparone (6,7-dimethoxy-coumarin), has been linked to UV-induced resistance in citrus fruits (Rodov et al., 1992). Its accumulation in kumquat fruits reached its peak (530 $\mu g\ g^{-1}$) 11 days

after illumination (254 nm), but its concentration declined rapidly, returning to trace levels typical of non-illuminated fruit, 1 month after treatment. The accumulation of the phytoalexin was correlated with increased antifungal activity in the flavedo, and resulted in enhanced resistance of the fruit to infection by *Penicillium digitatum*. Such a defense against infection was achieved when illumination was applied before inoculation with *P. digitatum*, without direct contact of the pathogen with the light. Moreover, illumination of previously inoculated fruit failed to prevent decay development. The experiments with kumquats clarified that the fruit/pathogen interaction depends on the relative rates of fungal growth and of resistance development by the host. Inoculation of the fruit before UV treatment gives an advantage to the pathogen, while illumination applied 2 days before inoculation was sufficient to improve fruit resistance. Host resistance is thus dependent on the existence of a lag period between illumination and inoculation, during which the phytoalexin can be accumulated and suppress subsequent pathogen development (Rodov et al., 1992).

The enhancement by UV treatment of citrus fruit resistance to *P. digitatum* infection has also been explained in part by the induction of the key enzymes in the secondary metabolite pathway. It was thus found that in citrus fruits, the onset of UV-induced resistance coincided with the induction of phenylalanine ammonia lyase (PAL) (a key enzyme in the phenylpropanoid pathway) and peroxidase activities (Chalutz et al., 1992; Droby et al., 1993a). PAL activity in the peel of grapefruits increased within 24 hours after UV treatment and remained elevated for 72 hours, while peroxidase activity reached its maximum 72 hours after treatment. Both enzymes are considered to play a role in the induced resistance of plants against pathogens.

An additional explanation for the induced resistance of citrus fruits following UV treatment has recently been offered by Porat et al. (1999), who found that exposure of grapefruits to UV light (254 nm), followed by inoculation with *P. digitatum*, resulted in the accumulation of chitinase protein in the peel tissue. Whereas UV treatment or wounding alone had almost no effect on the levels of β-1,3-endoglucanase protein, the combination of the two treatments induced the accumulation of both chitinase and glucanase proteins. Since the two pathogenesis-related proteins are hydrolysers of fungal cell wall polymers and may inhibit fungal development, it was suggested that the resistance of citrus fruit to fungal development induced by UV treatment had been mediated by the

induced accumulation of chitinase and glucanase protein (Porat et al., 1999).

Although the potential of UV light as an inducer of defense reactions has been proven for various fruits and vegetables, this treatment by itself is unlikely to provide disease control comparable with that provided by synthetic fungicides. El Ghaouth (1994) suggests that in order to extend UV effects during a significant storage period, UV treatment should be combined with other alternatives such as a biological control by means of antagonistic microorganisms.

Studying the effects of UV-C illumination on the natural microbial epiphytic population on the surface of grape berries, Nigro et al. (1998) found a significant increase in the population of antagonistic yeasts and bacteria on berries treated with 0.25-0.5 kJ m^{-2} of UV-C energy. These results indicate that the UV-C light doses capable of inducing disease resistance have no negative impact on the epiphytic population and may even elevate the populations of some of the efficient antagonistic yeasts and bacteria which may serve as natural biological control agents on the fruit (see the chapter on Biological Control).

2. CHEMICAL ELICITORS

Among the chemical elicitors, we include those already well known, as well as newly introduced compounds that have been found to be associated with natural defense processes.

Ethylene as Inducer of Hypersensitive Response

Some disease-resistant plants restrict the spread of fungal, bacterial or viral pathogens to a small area around the point of initial penetration, where a necrotic lesion appears. This phenomenon, referred to as the hypersensitive reaction, may lead to acquired resistance to subsequent pathogen attack after the initial inoculation with the lesion-forming pathogens. The ability of ethylene to induce a hypersensitive response had already been demonstrated in the 1970s by G.E. Brown (1975, 1978): treatment of harvested Robinson tangerines with ethylene was found to cause a loss of chlorophyll and an increase in the content of carotenoids in the fruit. In parallel, the treatment stimulated the formation of infection hyphae by the appressoria of *Colletotrichum gloeosporioides* present on the fruit peel, and enhanced disease development. However, when the appressoria are removed from the peel before ethylene application, the ethylene-treated fruit, which becomes orange in color, develops resistance to invasion by subsequently applied fungal spores. In

this case, the penetration of epidermal cells by the hyphae of *Colletotrichum* results in the formation of several layers of necrotic cells around the hyphae. The necrotic cells of the host were found to be filled with phenolic compounds which restricted fungal development to the site of penetration and prevented further spread of the disease (Brown, G.E., 1978).

Chitosan and Defense Reactions

Chitosan, a deacetylated derivative of chitin, has demonstrated fungicidal activity against many fungi, including soil-borne and postharvest pathogens. Because of its polymeric nature, chitosan can also form gas-permeable films and create an internal modified atmosphere. It has, therefore, the potential for use as an edible antifungal coating material, regulating gas and moisture exchange by fruits and vegetables (Wilson et al., 1994). For discussion of these features of chitosan, see the chapter, Chemical Control – Natural Chemical Compounds. However, in addition to its direct antifungal capability and its polymeric nature, chitosan is also a potential elicitor of phytoalexins (Kendra et al., 1989) and of various low-molecular-weight, pathogenesis-related (PR) proteins, such as chitinases, chitosanases and β-1,3-glucanases.

By inducing these PR proteins, chitosan application may be able to promote defense responses in the tissues. These enzymes hydrolyze the main components of fungal cell walls (Boller, 1993; Bowles, 1990) and are considered to play a major role in constitutive and inducible resistance of plants against invading pathogens (El Ghaouth, 1994).

The inducement of such antifungal hydrolases by chitosan has been recorded in strawberries, bell peppers and tomato fruits, in which they have remained elevated for up to 14 days after treatment. In chitosan-treated bell peppers the production of lytic enzymes was followed by a substantial reduction of chitin content of the cell walls of invading fungal hyphae, as exhibited by a reduction of chitin labeling in the walls (El Ghaouth and Arul, 1992). Studies with bell peppers showed that chitosan treatment caused severe cytological damage to hyphae of invading *Botrytis cinerea* and interfered with the capacity of the fungus to secrete polygalacturonases. These activities were expressed in the reduced maceration of the host cell-wall components, pectin and cellulose, by the pathogen (El Ghaouth et al., 1997). The induction of lytic enzymes in harvested tissue by prestorage treatment with chitosan could, therefore, lead to the restriction of fungal colonization. The

persistence of defense enzymes in the tissue following elicitation by chitosan may also contribute to the delay in the reactivation of quiescent infections, which naturally takes place when tissue resistance declines. Because of these features, chitosan has been considered a promising means for enhancing disease resistance and has been offered as a possible alternative to synthetic fungicides (El Ghaouth, 1994).

Salicylic Acid and Defense Reactions

Plant infection by fungi, bacteria and viruses may lead to a hypersensitive response which prevents pathogen spread, by the development of necrotic lesions at the site of infection. In addition, the localized infection may induce enhanced resistance against further infection by a variety of pathogens. This phenomenon is known as systemic acquired resistance (Raskin, 1992; Buchel and Linthorst, 1999). Along with the development of a hypersensitive reaction and systemic acquired resistance to subsequent pathogen attack, the systemic synthesis of low-molecular-weight pathogenesis-related (PR) proteins has been recorded (Carr and Klessing, 1989). Among the various PR proteins discovered, we find the enzymes, β-1,3-glucanases and chitinases, which are associated with defense responses.

Although PR proteins are induced in pathological situations, they can also be induced by the application of certain chemicals that partly mimic the effects of pathogen infection, as well as by wounding or other stresses (Buchel and Linthorst, 1999). Salicylic acid is a natural compound capable of inducing such proteins even in the absence of pathogenic organisms.

Salicylic acid, which belongs to the diverse group of plant phenolics, has ubiquitous distribution in plants. Many plant scientists have used salicylic acid and aspirin (acetylsalicylic acid) interchangeably in their experiments. Aspirin undergoes spontaneous hydrolysis to salicylic acid, although it has not been identified as a natural plant product.

Increasing evidence suggests that endogenous salicylic acid plays an important role in the activation in plant tissues, of defense responses against pathogen attack (Kessman et al., 1994; Buchel and Linthorst, 1999). It is no wonder that recent development in the area of new antifungal compounds has focused on this compound and its functional analogs. Exogenic application of salicylic acid or aspirin has been found to induce PR proteins in leaves of some crops (Renault et al., 1996); no information, however, is available on the ability of such treatments to induce PR proteins in fruits, where they might be induced in reaction to wounding or other stresses. On the other hand, studies with pear cell

suspension culture indicated that salicylic acid may act as an inhibitor of ethylene biosynthesis, by blocking the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Leslie and Romani, 1986) and, therefore, may function as an inhibitor of fruit ripening. Studies with tomato fruits showed that salicylic acid may inhibit the increased expression of ACC gene, which is activated by fruit wounding (Li et al., 1992). By functioning as an inhibitor of ethylene production, salicylic acid would be able to enhance disease resistance against pathogens that do not attack non-ripening tissues.

Antioxidants

Antioxidants are another class of compounds that can reduce postharvest decay by modulating the natural fruit resistance. The antifungal diene, which is responsible for the inhibition of *Colletotrichum gloeosporioides* development in the young unripe avocado fruit, is apparently oxidized by lipoxygenase during fruit ripening, allowing the fungus to resume colonization of the fruit tissues. The activity of this enzyme in the peel of ripening fruit is regulated by the natural antioxidant epicatechin (Prusky and Keen, 1993). Laboratory studies showed that infiltration or dip treatment of avocado fruit with several antioxidants, such as α-tocopherol, butylated hydroxytoluene, butylated hydroxyanisole and *tert*-butyl hydroquinone, inhibited lipoxygenase activity, retarded the decrease of the antifungal diene and, consequently, inhibited the development of anthracnose (Prusky et al., 1985b). Following these findings Prusky et al. (1995) showed that a dip or spray of avocado fruit (cvs. Hass and Fuerte) with a commercial formulation of the antioxidant, butylated hydroxyanisole (1200 μg a.i. ml^{-1}) or the antioxidant combined with the fungicide prochloraz (250 μg a.i. ml^{-1}) consistently reduced the incidence of postharvest anthracnose caused by *C. gloeosporioides* in small and semi-commercial experiments. The effect of the antioxidant plus prochloraz lasted longer than that of the antioxidant alone, although prochloraz alone, even at higher concentrations, did not always reduce decay incidence. It was concluded that the antioxidant, which is a common food additive, prevented the conversion of quiescent infections into active ones, a process associated with fruit ripening and reduced resistance to infection.

3. BIOLOGICAL ELICITORS

The ability of various antagonistic microorganisms to act as alternatives to systemic fungicides and control postharvest diseases may be connected with their ability to produce and secrete antibiotic

substances, or their ability to injure the pathogen directly. In many cases, these microorganisms, which grow rapidly and colonize wounds, have been hypothesized to act by competing with postharvest wound pathogens, for space and nutrients (see the chapter on Biological Control). However, in several cases, antagonistic yeasts can also act as inducers of resistance in the host tissue.

The antagonistic yeast, *Pichia guilliermondii*, which is effective in controlling a wide variety of postharvest diseases in citrus fruits, apples and peaches, has been shown to induce enhanced levels of phenylalanine ammonia lyase (PAL) in citrus fruit peel (Wisniewski and Wilson, 1992), indicating the induction of a defensive response. *P. guilliermondii*, as well as an isolate of the yeast *Candida famata*, which is similarly effective against *Penicillium digitatum* in citrus fruits (Arras, 1996), contributes to enhanced host resistance by inducing the formation of the phytoalexin scoparone or of scoparone plus scopoletin, respectively, in the fruit peel (Rodov et al., 1992; Arras, 1996).

Studies with unripe avocado fruits indicated that their resistance to anthracnose, caused by *Colletotrichum gloeosporioides*, was related to the presence of an antifungal diene compound in the peel (Prusky and Keen, 1993). It has been suggested that fruit resistance to infection could be modulated, not only by delaying the normal decline of the antifungal diene by retarding fruit ripening, but also by increasing its synthesis. Challenge inoculation with the avocado pathogen, *C. gloeosporioides* (Prusky et al., 1990), or with a non-pathogenic mutant of *Colletotrichum magna* (Prusky et al., 1994) led to increased levels of the antifungal diene. These results suggest that the non-pathogenic *Colletotrichum* mutant is capable of enhancing the natural defense mechanism of avocado fruits, leading to the prolongation of the quiescent period of the pathogen and thus to disease inhibition.

Wild and Wilson (1996) have recently detected a host defense reaction in apples, which reduced decay development in fruits that had been challenged by *Penicillium expansum*, the typical blue mold fungus of apples. The application of the protein synthesis inhibitor, cycloheximide prevented the reaction and resulted in more than 700% increase in decay. Following cycloheximide application, inoculation of apples with the citrus pathogen, *P. digitatum* resulted in green mold rot development, although the fungus does not normally attack apple fruits. Once the citrus *Penicillium* became established in the apple, it progressed at a higher rate than that of the apple *Penicillium*. It was also found that if

rot caused by *P. digitatum* progressed through an apple and came into contact with a damaged region, it developed around the damage site leaving a halo of uninfected tissue. This area was suggested to be the location in which the host defense reaction occurred (Wild and Wilson, 1996).

In a recent study, Ippolito et al. (2000) found that the antagonistic yeast-like fungus, *Aureobasidium pullulans* multiplied rapidly in apple wounds, and reduced decay incidence in apples inoculated with *Botrytis cinerea* and *P. expansum*. However, they also found that the antagonist was capable of inducing the activities of β-1,3-glucanase, chitinase and peroxidase in the treated wounds on the fruits. This induction was exhibited in a transient increase in the enzymatic activity which started 24 h after the application of the antagonist, and reached a maximum 48 or 96 h after the treatment. The three enzymes are considered to be potentially important in host resistance mechanisms: chitinase and β-1,3-glucanase are hydrolases, capable of hydrolyzing fungal cells (Schlumbaum et al., 1986; Wilson et al., 1994), while peroxidase is involved in lignin formation and the production of structural barriers against pathogens (El Ghaouth et al., 1998; Chittoor et al., 1999). It was thus suggested that the induced activity of the glucano-hydrolases and peroxidase, together with the capacity to out-compete the pathogen for nutrients and space may be the basis of the biocontrol activity of *A. pullulans* (Ippolito et al., 2000).

B. GENETIC MODIFICATION OF PLANTS

1. DISEASE-RESISTANT TRANSGENIC PLANTS

Genetic transformation of plants for desired traits – such as improved yield, increased size or enhanced disease resistance – is not a new concept. Classical breeding has always provided new plant varieties which have desirable characteristics. However, the process can be too slow and inexact. Often pathogens can mutate too quickly for breeding of disease resistance varieties by classical methods. Progress in the development of bioengineering techniques provided the opportunity to modify plants, and enabled new transgenic plants with greater disease resistance to be developed more rapidly than via classical methods (Mount and Berman, 1994). A transgenic plant contains, within its genome, a foreign DNA that has been introduced artificially via genetic

engineering. The creation of such plants involves the introduction, from unrelated plant species, of genes for disease resistance (Boller, 1993). This technology not only allows for a wider genetic diversity but it may enable us to add multiple, diverse resistance genes to one plant variety. Furthermore, creating plant cultivars with inheritable genes for disease resistance is the most environmentally sound strategy for disease management.

Desirable target genes for creating useful transgenic plants are usually isolated from plant viruses, bacteria, fungi or other plants, depending on the traits desired. Widely used genes are those known to be inhibitory to fungal growth, and they are often induced following fungal invasion (Boller, 1993; Broekaert et al., 1995). These genes were reported to provide a quantitative improvement in resistance when introduced into a transgenic host in greenhouse studies (Jongedijk et al., 1995). However, the selection of beneficial target genes for pathogen resistance requires a thorough understanding of the pathogen, the host and the host-pathogen interactions. This knowledge can come from a multitude of genetic and physiological experiments.

2. SOURCES OF GENES FOR BIOENGINEERING PLANTS

Mount and Berman (1994) pointed out several natural compounds known to have antimicrobial activity against pathogenic fungi or bacteria, which could be possible sources for genes valuable for bioengineering of plants. The natural antibiotic compounds present an example of such sources. Transgenic plants with genes for antibiotic production may prevent initial infection of plant tissue by postharvest pathogens. Sources for novel antibiosis genes may come from plants themselves or from the many microorganisms being studied for use as biological control agents. If the antibiosis is due to a compound with isolatable genes, the genes might be suitable for bioengineering of plants. However, the widespread use of antibiotics is not recommended, mainly because of the possibility that the pathogen could rapidly develop resistance to the antibiotic compounds.

Chitinases and β-1,3-glucanases are other antifungal compounds which are effective against fungal cell-wall polymers and are believed to be involved in plant defense mechanisms against fungal infection. Once a hydrolase gene that is effective against the pathogen has been identified, a desired transgenic plant can be created through molecular manipulation. The insertion of a chitinase gene into tobacco and canola plants was shown to result in enhanced resistance to *Rhizoctonia solani*

but not to *Cercospora nicotianae*, suggesting that other factors besides chitinase, may be involved in disease resistance (Broglie et al., 1991). A later study describes enhanced resistance to fungal attack in transgenic plants by co-expression of chitinase and glucanase genes (Zhu et al., 1994). Other microbial enzymes normally induced in plants, such as peroxidase and other pathogen-defense-related compounds, might also be exploited for bioengineering (Bowles, 1990; Mohan and Kolattukudy, 1990).

The knowledge and understanding of postharvest disease etiology and physiology may be of much help in selecting new genes and developing new transgenic plants, resistant to postharvest diseases. For example, the knowledge that wounding leads to fungal penetration into the host and triggers postharvest disease development (Barkai-Golan and Kopeliovitch, 1981; Brown, G.E. and Barmore, 1983; Eckert and Ratnayake, 1994) may lead to genetic alteration of the plant to make it more physically resistant to wounding or to enhance the production of wound-healing compounds to shorten the time that the wounded tissue is vulnerable to pathogen attack (Mount and Berman, 1994). Similarly, understanding the role of the pathogen cell-wall-degrading enzymes in plant tissue deterioration may lead to the creation of new transgenic plants by which the pathogen polygalacturonase (PG) is suppressed. Following the characterization of PG-inhibiting proteins from pears by Stotz et al. (1993), it was suggested that the pear promoter might affect a fruit-specific expression of the gene, resulting in the inhibition of *Botrytis cinerea*. It was further suggested that the characterization of the protein inhibitor from pears should lead to the expression of PG-inhibiting proteins in transgenic plants and possibly to the inhibition of decay development. In fact, Powell et al. (1994) reported that transgenic tomato fruits expressing the gene of fungal PG-inhibiting glycoproteins of pears, were more resistant to *B. cinerea* than the control fruits.

Using *nor* (non-ripening) or *rin* (ripening inhibitor) tomato mutants that block many aspects of the ripening process of the fruit, including softening of the tissues and color development, Barkai-Golan et al. (1986) and Giovannoni et al. (1989) found that the tomato's own PG plays an important part in the total PG activity in the host during pathogenesis, and probably takes a major role in the degradation of cell wall pectic substances. Following these findings, it was further suggested that the suppression of fruit PG might be useful in increasing fruit shelf life (Giovannoni et al., 1989). Bioengineering this enzyme has been accomplished with tomatoes (Kramer et al., 1990; Smith et al., 1988) and

the resulting produce had a longer shelf life with no effect on fruit texture or color development.

C. MANIPULATION OF ETHYLENE BIOSYNTHESIS AND GENETIC RESISTANCE IN TOMATOES

The importance of ethylene ('the ripening hormone') in accelerating ripening and senescence in harvested fruits, and the considerable shortening of shelf life through its effects (see the chapter, Factors Affecting Disease Development – the Effects of Ethylene) led to the search for ways to suppress its influence in a reversible manner. Among the solutions offered to achieve this goal were the prevention of ethylene production by the plant tissue or the construction of a mutant plant whose fruits would not ripen until treated with ethylene (Theologis, 1992).

Elucidation of the pathway for ethylene synthesis in higher plants by Yang and Hoffman (1984) has been a major contribution, not only to the understanding of the biochemistry of this process, but also to the possibility of manipulating it to suppress or prevent ethylene production. An efficient way to prevent ethylene synthesis in tomato fruits was the inhibition of 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase), a key enzyme in the biosynthesis of ethylene, by an ACC-synthase antisense transgene. This function led to an almost complete inhibition of the ethylene precursor, ACC synthase, and to the production of mutant tomato plants with non-ripening fruits (Oeller et al., 1991). Using tomato plants in which synthesis of an ethylene-forming enzyme had been inhibited by an antisense gene, Picton et al. (1993) showed that the degree of inhibition of ripening was dependent upon the stage of development at which the fruits were detached from the plant: the effects were much more pronounced when fruits were detached from the vine before the onset of color change. Application of exogenous ethylene to such fruits only partially restored fruit ripening: it failed to increase lycopene accumulation to the level in the normal ripening fruits, and the ethylene-treated fruits demonstrated persistent resistance to over-ripening and shriveling.

Another approach to the manipulation of ethylene biosynthesis was reported by Klee et al. (1991), who introduced a bacterial gene encoding an ACC-metabolizing enzyme into tomato plants. As a result of this procedure, inhibition of the accumulation of ACC and, consequently, a

reduction in ethylene production and a marked inhibition of the ripening of the tomato fruit, were recorded.

Inhibition of fruit ripening by these approaches has not only had a direct effect of prolonging the physiological life of the harvested fruit, but also an indirect effect on decay development, which is strongly related to the stage of ripening. The relationship between the stage of maturity and the resistance of harvested fruit to postharvest diseases is probably associated with the declining ability of the mature tissue to produce defensive compounds and structural barriers. Furthermore, the pathogen is capable of producing and activating pectolytic enzymes only during fruit ripening or in the mature fruit, where it decomposes the pectic compounds of the fruit cell walls, thus causing tissue degradation.

Studies at the genetic level were also dedicated to direct enhancement of fruit disease resistance (Martin et al., 1993). This was achieved in a plant-pathogen system in which a single resistance gene in the plant responded specifically to a single avirulence gene in the pathogen, i.e., in a 'gene-for-gene' system. Such interactions have been described for various plant-pathogen pairs (Keen and Buzzel, 1991). Susceptibility to disease results in plant-pathogen systems in which either of two genes – the plant resistance gene or the pathogen avirulence gene – is absent from the interacting organisms.

Martin et al. (1993) used the relationship between tomato and the bacterium *Pseudomonas syringae* pv. *tomato*, the causative agent of bacterial speck, to isolate a plant resistance gene by map cloning. Since the avirulence gene from the bacterium was found to induce resistance specificity in tomato cultivars containing the plant resistance gene, it was concluded that the interaction between the tomato and the bacterium involves a 'gene-for-gene' system, and when tomatoes susceptible to *Pseudomonas* are transformed with the plant resistance gene they become resistant to the pathogen.

The tomato plant offers many advantages for the cloning of a resistance gene on the basis of their position on the genetic linkage map; since the tomato has been the subject of more than 50 years of plant breeding, many loci have been identified that confer resistance to various fungi, bacteria and viruses (Martin et al., 1993).

POSTHARVEST DISEASE SUMMARY
FOUR FRUIT GROUPS

The present chapter presents the major postharvest diseases of four specific groups of fruits: subtropical and tropical fruits; pome and stone fruits; soft fruits and berries and solanaceous fruit vegetables. Aspects addressed include the life cycles and modes of infection of the pathogens, factors affecting disease development, and approaches to disease prevention and suppression.

These descriptions should give us not only a general view on the various aspects of the postharvest pathology of different fruits, but may also enable us to compare among the diseases elicited by a given pathogen on a variety of hosts and among those elicited by different pathogens on a certain host. This chapter highlights two important features common to many postharvest diseases: (a) that wound pathogens play a dominant part in postharvest pathology, sometimes as major pathogens responsible for serious losses, and sometimes as minor pathogens, frequently associated with senescent or weakened tissue; (b) the importance of quiescent infections, characteristic of many postharvest pathogens at some stage between their arrival at the host or their initial penetration into the tissues, and the development of an active disease.

Since understanding the nature of the pathogens and their modes of infection forms the basis for the development and subsequent application of suitable control methods, notes on control measures appropriate to each group of comodities accompany the disease descriptions. Furthermore, since many postharvest studies in the last decade have focused on the development of new control measures, especially new alternatives to synthetic fungicidal compounds, these new options are included in the notes. However, we should always keep in mind that detailed chemical, physical and biological means for disease control, as well as means for enhancing or eliciting host resistance, are addressed in separate chapters, for which the present chapter is intended as a supplement. Comprehensive descriptions of postharvest diseases of fruits and vegetables have recently been given by Snowdon (1990, 1992) and by Beattie et al. (1989; 1995).

I. SUBTROPICAL AND TROPICAL FRUITS

This group of fruits comprises unrelated species, most of which are climacteric (pineapple being an exception). All the fruits of tropical and subtropical origin are characterized by their perishability: the harvested fruits have lost most of the intrinsic resistance characteristic of young and immature fruits, are rich in nutrients, have high moisture contents and are susceptible to attack by several pathogenic fungi (Eckert, 1990). The storage life of tropical fruits is relatively short – generally a few weeks under optimal conditions. The use of refrigeration to extend their storage life is limited since these fruits are sensitive to chilling injury and cannot be stored below their critical chilling range (Wang, 1990). Sensitivity to cold storage varies with the fruit species or cultivar, and also with the state of maturity. Because of their sensitivity to chilling injury, bananas should be stored at 13-16°C, lemons, grapefruits or mangoes at 10-15°C and papayas at 7-10°C. Under these conditions, pathogens such as *Colletotrichum gloeosporioides* or *C. musae* will continue to develop on mango or banana fruits, respectively, as will *Penicillium digitatum, P. italicum* and *Geotrichum candidum* on various sensitive cultivars of citrus fruits. In fact, postharvest decay losses of tropical and subtropical fruits have been estimated to be up to 50%, especially in developing countries where postharvest handling and storage are not optimal (Eckert, 1990). However, the demand for tropical fruits in the markets of non-producing countries continues to increase, as indicated by the expansion of trade among widely separated countries, although to reach some markets the fruits must undergo journeys long in terms of distance and time (Burdon, 1997).

CITRUS FRUITS

1. Wound Pathogens

Penicillium digitatum **Sacc.** (the green mold fungus) and ***P. italicum*** **Wehmer** (the blue mold fungus) are the main wound pathogens of citrus fruits, causing the most common and the most devastating postharvest diseases. They occur in all citrus growing countries, worldwide and may attack the fruits in packinghouses, in transit, in storage and in the market. The green mold, which is specific to citrus fruit, is more prevalent than the blue rot, and may account for most of the postharvest decay of citrus fruit in storage. The two fungi

may appear together in the same lot or even on the same fruit. Early studies by Fawcett and Berger (1927) have shown that the green mold fungus grows more rapidly at moderate temperatures and, therefore, it predominates during short-term transit and storage. *Penicillium italicum* may sometimes develop in storage as a hyper- parasite over the green mold decay.

Conidia of the Penicillia are present during the season in the atmosphere of citrus growing areas, particularly in citrus packinghouses and their surroundings, on the packinghouse equipment or on the hands of selectors and packers (Barkai-Golan, 1966). Disease is characteristically initiated through wounds and mechanical injuries sustained during harvesting, packing and handling (Kavanagh and Wood, 1967). Wounds may also result from piercing by the Mediterranean fruit fly and other fruit-piercing insects (Roth, 1967). Fruits are particularly susceptible to infection during wet or humid weather, and the postharvest temperature is another factor determining the green and blue mold fungi development. The optimal temperature range for both fungi is 20-27°C, within which the fruits may rot within a few days. Although fungal growth is reduced at lower temperatures, a very slow rate has still been recorded at 4.5-10°C, allowing the fungi to progress under these conditions when storage is extended or in overseas shipments. At 0-1°C the growth of the two Penicillia is arrested, but these temperatures result in chilling injury, expressed in pitting and internal physiological injury (Smoot et al., 1983).

At an early stage both fungi cause a soft rot of the peel. Following this stage, a white mycelium develops from the center of the affected soft area; it later starts sporulating from the center of the colony, which is the older part of the infected area. The sporulating part becomes olive green in the case of *P. digitatum* and blue in the case of *P. italicum*. The rot at this stage is characterized by three circles. The colored sporulating center (1) is surrounded by (2) a band of white mycelium which has not yet sporulated or has only just begun to form conidiophores, or conidiophores plus sterigmata (which will later bear the spores). This white band is surrounded in turn by (3) a definite band of water-soaked peel, which has not yet developed the white fungal mycelium.

Softening of the peel tissue is associated with the activity of pectolytic enzymes produced by the pathogen during pathogenesis. Under dry conditions the decayed fruit shrinks and becomes 'mummified'. The blue mold spreads directly from the decayed fruit into uninjured, healthy

fruits, causing 'nesting'. The green mold typically infects adjacent fruits that have been injured (Barmore and Brown, 1982). The dusting of sound fruit with *Penicillium* spores from decayed fruit, termed soilage, is often of greater economic importance in retail cartons than is the decayed fruit (Stange and Eckert, 1994).

Penicillium digitatum is capable of producing ethylene during its development (Biale, 1940; Ilag and Curtis, 1968). The production of ethylene, which is considered to be the ripening hormone, increases fruit respiration (Achilea et al., 1985a; Biale and Shepherd, 1941), hastens peel coloring and accelerates button senescence (hence leading to the initiation of stem-end rotting). By producing and releasing ethylene, *P. digitatum* reduces the storage life of healthy fruits in the same container or sometimes even in the same room.

***Geotrichum candidum* Link ex Pers.**, the sour rot fungus, has been reported from most citrus growing areas. The disease is usually of less importance than the green mold and blue mold decays or the stem-end rots. It is, however, particularly important after prolonged wet seasons when heavy losses have been recorded. Furthermore, the importance of sour rot may generally be underestimated because initial infections are easily overgrown by other molds (Smoot et al., 1983).

Sour rot is primarily a disease in storage and in transit, although infection may also occur on the tree. The spores (oidia), which are thin-walled cells derived from the fragmentation of fungal hyphae, are soil inhabitants being splashed during irrigation or rain to low hanging fruits or contaminating the fruit by soil contact. Infection may initiate preharvest at injuries caused by insects or via mechanical wounds sustained during harvesting and packinghouse handling (Laville, 1974). It is found most often on lemons, limes and grapefruits, which are often stored for extended periods, but they may infect other citrus fruits as well. Ripe fruit is more susceptible to the pathogen than green and immature fruit (Baudoin and Eckert, 1985).

Early symptoms are similar to those of the green and blue mold decay, being a water-soaked spot that enlarges along with or following the growth of the fungus within the fruit tissues. At a later stage, a thin water-soaked, off-white mycelium is developed on the affected area. When a total decomposition of the fruit tissues occurs, a spore-containing juice leaks from the rotten fruit and can infect healthy adjacent fruits.

2. Stem-End Pathogens

The principal stem-end rot pathogens of citrus fruits are:

(i) ***Diplodia natalensis*** P.E., frequently referred to as ***Botryodiplodia theobromae*** **Pat. [(perfect state:** ***Physalospora rhodina*** **Berk. & Curt.) Cooke];**

(ii) ***Phomopsis citri*** **Fawcett (perfect state:** ***Diaporthe citri*** **Wolf);**

(iii) ***Dothiorella gregaria*** **Sacc. (perfect state:** ***Botryosphaeria ribis*** **Grossenb. & Duggar).**

(iii) ***Alternaria citri*** **Ell. & Pierce**, which was renamed ***A. alternata*** **(Fr.) Keissler pv.** ***citri*** (Solel, 1991).

Stem-end rots caused by *D. natalensis* and *P. citri* are the major postharvest diseases in citrus fruits grown in humid subtropical areas with high rainfall during the growing season. These fungi, as well as *D. gregaria*, may assume the sexual state, namely perithecia, which give rise to ascospores. However, this form is not commonly found and the asexual spores, borne within pycnidia, are the only spores important for infection. The pycnidia are produced in dead wood and in living tissues of stems and leaves.

Infection may be initiated at any stage of fruit development, when wind and splashes of rain carry the pathogen spores to the surface of immature fruits on the tree. However, immature fruits are resistant to invasion, and the fungi remain quiescent in floral remnants under the sepals of the fruit and do not become active until the buttons become senescent and begin to separate from the fruit (Brown, G.E. and Wilson, 1968). *Diplodia* usually progresses rapidly down the central axis, frequently reaching the stylar-end; it grows along the tissues that divide the fruit into segments of the pulp, taking on the appearance of fingers that connect the rotted ends of the fruit (Smoot et al., 1983). Similar symptoms can arise from *Dothiorella* development. *Phomopsis* decay is characterized by some shriveling of the decayed tissue, and this helps to separate the decayed from the sound tissue. The fungus similarly progresses down the core but does not reach the stylar end until most of the rind has decayed. Infection by the three fungal species may, however, originate at injuries on the side or at the stylar end of the fruit.

Stem-end rots caused by *Diplodia, Phomopsis* and *Dothiorella* may occur in the same lots or even on the same fruit, and it is often difficult or impossible to distinguish among them. When *Diplodia* and *Phomopsis* develop on the same fruit, an important factor in determining which will predominate is the temperature (Smoot et al., 1983): the optimum

temperatures for growth are 30°C for *Diplodia* and 23°C for *Phomopsis* and *Dothiorella*. In general *Phomopsis* occurs throughout the harvest season, whereas *Diplodia* is more common during the ethylene-degreening season and in the late spring, when there are warmer weather and frequent rains. Under humid conditions a superficial, fine white mycelium may be developed on the *Phomopsis*-infected fruits. Surface growth is seldom seen on *Diplodia*-infected areas.

Alternaria citri is widely distributed in citrus-growing areas. Being dispersed by wind and air currents in the grove, the conidia can be found at the stem end, as well as on or underneath the 'button' of citrus fruits of any age. In Navel oranges, fungal conidia may be located at the stylar end (Singh and Khanna, 1966). However, infection may occur only if the fruit has been injured or physiologically weakened by unfavorable growing conditions. After harvest, infection may originate in peel injuries or more frequently through the stem end, resulting in stem-end rotting. Freshly harvested lemons, however, are resistant to *Alternaria* infection while immature. Infection through the button occurs only at the senescent stage, when the fungus is capable of progressing into the fruit, affecting the central core and to some extent the inner tissues of the rind. In this case, internal rot is frequently developed before the appearance of external disease symptoms (Brown, G.E. and McCornack, 1972).

In oranges, grapefruits and some hybrids, the internal tissue becomes black; hence the common name for *Alternaria* infection - the black rot (Singh and Khanna, 1966). In Navel oranges, infection usually originates at the stylar end. The ability of the fungus to penetrate the fruit through senescent buttons may explain the prolonged prevalence of *A. citri*. Similarly, citrus fruits that have been degreened by ethylene to accelerate color development are liable to be infected by *A. citri*. Ethylene treatment, by hastening senescence and thereby the death of the green button, predisposes the fruit to invasion by stem-end fungi. In contrast, the application of growth-regulating substances such as 2,4-D retards senescence of the fruit button and delays disease initiation (Eckert and Eaks, 1989).

3. *Phytophthora* spp.

***Phytophthora citrophthora* (Smith & Smith) Leon., *Phytophthora hibernalis* Carne, *Phytophthora nicotianae* (van Breda de Haan) var. *parasitica* (Dastur) Waterh.** and other *Phytophthora* species are the causal organisms of brown rot of citrus fruits.

Brown rot is a major fruit disease in all citrus growing areas and is

particularly prevalent after late-season rains, which play an important part in disease development. The fungi are common in soil and survive in their sexual state as thick-walled oospores. Fungi in the asexual state – sporangia and zoospores – are formed under moist conditions (Hough et al., 1980) and the mobile, swimming zoospores are splashed by rain or irrigation water onto low-hanging fruits in the tree. Rains and moisture can also carry the zoospores to higher fruits. Infection occurs only when the fruit remains wet for a relatively long period, during which the fungus may penetrate directly into the non-injured peel (Feld et al., 1979). If the fruit dries before the zoospores can germinate and penetrate into the peel, the infection process is arrested. Fruits infected on the tree will later drop onto the ground and, in fact, the appearance of infected fruits on the ground around the tree may indicate the presence of an active infection on the tree. At harvest, fruits which had unnoticed incipient infections on the tree are carried along with healthy fruits into the packinghouse. In such a case, the disease will develop during storage or transit.

The decayed area on citrus fruit is firm and leathery, characterized by brownish peel discoloration. Under humid conditions, a white delicate mycelium usually develops on the infected area. Another characterization of the brown rot is its distinctive aromatic or fermented odor (Smoot et al., 1983). During storage, the disease can spread by contact with other fruits (Klotz and DeWolfe, 1961). At a later stage, secondary soft rot bacteria may invade fruits infected by brown rot and turn the firm decay into a soft one.

4. *Colletotrichum gloeosporioides* (Penz.) Sacc. [Perfect state: *Glomerella cingulata* (stonem.) Spauld. & v. Schrenk]

Colletotrichum gloeosporioides, the causal fungus of anthracnose, is not included in the list of the major pathogens in citrus fruits. However, anthracnose may be a serious disease of tangerines, tangerine hybrids and other mandarin fruits harvested early in the fall, when long periods of ethylene exposure are required for promoting fruit color (Brown, G.E., 1975); it is usually only of minor importance on oranges, grapefruits and lemons.

The fungus exhibits both the sexual state (perithecia with ascospores) and the asexual state (acervuli with conidia). Acervuli with conidia are formed in dead branches in the grove. Conidia from this source are spread by winds and rains and may infect citrus fruit on the tree only when it has been weakened by drought, frost damage or unfavorable

cultural conditions. The infection may remain latent in the form of appressoria, in which case disease is manifested only during transport and storage (Fisher, 1970; Brown, G.E., 1975). Anthracnose may invade the fruits after harvest, in the packinghouse, in storage or in the market; it gains entrance to the fruit via mechanical injuries or dead buttons. Mature green fruits are sensitive to infection after prolonged exposure to ethylene during the degreening, since this treatment renders the intact peel easily penetrated by the pathogen. Affected fruits are characterized by the development of sunken lesions that, under humid conditions, form abundant salmon-pink masses of spores that later turn into brown-black masses.

The incidence of anthracnose can be reduced by washing, which removes the quiescent appressoria (Smoot and Melvin, 1971; Brown, G.E., 1975), before degreening with ethylene. This, however, cannot be done commercially because washing retards the degreening process. Several factors affect the severity of anthracnose: (1) orange-colored fruits are more resistant to the disease than pale or green-colored ones (Brown, G.E. and Barmore, 1976); (2) degreening with ethylene at concentrations higher than those required for optimal chlorophyll degradation (5 μl per liter of air) favors anthracnose development; (3) fungicide application prior to degreening reduces anthracnose development (Brown, G.E. and Barmore, 1976). Resistance to anthracnose was induced in mature green tangerines treated with ethylene after being washed to remove dormant appressoria (Brown, G.E. and Barmore, 1977). Application of ethephon (a chemical which produces ethylene on degradation) to Robinson tangerines 5-7 days before harvest significantly reduced the incidence of anthracnose (Barmore and Brown, G.E., 1978); control was attributed to the accumulation in the interior of the fruit of ethylene which induced physiological changes leading to the development of resistance.

5. *Trichoderma viride* Pers. ex S.F. Gray

This fungus, which is responsible for Trichoderma rot in storage, is frequently considered to be a pathogen of minor importance. It can, however, cause serious loss in some citrus-growing areas.

The fungus is known as a soil inhabitant and is capable of decomposing cellulose. Germinating conidia enter through mechanical injuries on the fruit, at the stem end or the stylar end (Gutter, 1961). This fungus is often overgrown by other organisms, particularly by *Penicillium digitatum* with which *T. viride* is often confused because of

the development of green or green-yellow masses of conidia on the infected area under humid conditions (Gutter, 1961). In the presence of *P. digitatum, Trichoderma* growth is stimulated, probably by the production and emanation of ethylene by *P. digitatum*; this enhances fruit senescence and consequently fruit susceptibility (Brown, G.E. and Lee, 1993). *T. viride* can sometimes be spread into healthy uninjured fruits during storage, causing 'nesting'.

Control Measures

The possibility of penetration of the wound pathogens into the fruit via injuries in the peel shows why the control of insects in the grove and careful handling throughout the harvesting process, to prevent skin breaks and bruises, are of primary importance in preventing the development of infections. The severity of postharvest wound infections is also directly related to the damage caused to the crop by rough handling after harvest (Sommer, 1982). Thus, careful handling should always be a continuous concern; the fact that preharvest fungicide sprays in the grove have not always been effective in reducing postharvest decay is probably because most injuries occur during harvesting or packinghouse handling.

Sanitation to minimize the presence of infective inoculum is also of great importance for disease prevention. It includes the removal of fallen or decayed fruit from the grove and from the packinghouse and its surroundings, sanitation of packinghouses by fumigation (with formaldehyde or other fumigants), sanitation of field boxes with disinfecting solutions or steam, and unloading and cleaning fruit arriving in the packinghouse in a separate area, to reduce contamination in the processing, packing and storage areas (Smoot et al., 1983).

In addition to sanitation procedures, strategies for control of postharvest diseases incited by wound pathogens include: (a) inactivation of germinating spores in fresh wounds; (b) protection of the peel from infection at a later time by depositing a fungitoxic residue in wounds or on the surface of the fruit; and (c) inhibition of *Penicillium* sporulation on the surface of decaying fruits and of subsequent contact spread in storage (Eckert, 1990). Non-selective, water-soluble salts of fungicides, such as sodium *ortho*-phenylphenate or sodium carbonate, applied generally prior to 1960, were fairly effective against wound pathogens if applied to the fruit soon after harvest (before the pathogen penetrated the tissue). The introduction of the systemic benzimidazole compounds offers highly effective fungicides for controlling wound infection by *Penicillium* spp.

At high dosage rates they have also provided a protective barrier on the fruit surface, that inhibits *Penicillium* sporulation on decaying fruits (Eckert and Ogawa, 1985). Imazalil, a systemic fungicide characterized by a different mode of action from that of the benzimidazole compounds, effectively controlled *P. digitatum* and *P. italicum* on citrus fruits, and its control extended to isolates resistant to the benzimidazole compounds. Both groups of fungicides, however, are ineffective against sour rot caused by *Geotrichum candidum* (Eckert and Ogawa, 1985). Following the evolution of *Penicillium* strains resistant to imazalil, as to other previously effective fungicides, new strategies and new chemicals are constantly being evaluated and developed. (See the chapter on Chemical Means – Postharvest Chemical Treatment).

Eckert and Eaks (1989) emphasized that since only *P. digitatum* biotypes resistant to the fungicides can sporulate on the treated fruit, fungicide application creates an ideal situation for the buildup of resistant types of *P. digitatum*, which would lead to poor decay control.

Curing citrus fruits (holding them at temperatures and humidities conducive to wound healing) has long been reported as a non-chemical method for controlling decay by wound pathogens (Hopkins and Loucks, 1948; Brown, G.E., 1973). Healing of wounds and control of postharvest decay has also been achieved by combining individual wrapping of citrus fruit in plastic film, which leads to the formation of a water-saturated atmosphere within the wrap, with curing at 36°C for 3 days (Ben-Yehsoshua et al., 1987). Stange and Eckert (1994) showed that dipping lemons in a surfactant solution prior to curing gave better control of the green mold than curing alone.

Much research has been conducted on the development of biological control means as alternatives to chemical treatments, for controlling postharvest decay (Wilson and Wisniewski, 1989; Brown, G.E. and Chambers, 1996). Chalutz and Wilson (1990) and Droby et al. (1992) found that yeast species of the genera *Debaryomyces* and *Pichia* are capable of inhibiting wound pathogens because of their rapid development in wounds in citrus fruit peel. Arras (1996) found that *Candida famata* was one of the most active yeasts against *P. digitatum* in wounded fruits, while Smilanick and Denis-Arrue (1992) and Smilanick et al., (1996) demonstrated that strains of the bacterium *Pseudomonas syringae* could reduce the incidence of postharvest rot by occupying the wounds. Isolates of the common yeast-like fungus, *Aureobasidium pullulans* were reported by Schena et al. (1999) to control *P. digitatum* at high concentrations on grapefruit.

Recently, two biological products have been registered for commercial postharvest applications to citrus fruits – **Aspire,** which is *Candida oleophila*, and **BioSave**™ 1000, which is *P. syringae* (Brown, G.E. and Chambers, 1996). Evaluating the efficacy of these biological products against citrus fruit pathogens, G.E. Brown and Chambers (1996) found that although significant control of *P. digitatum* was obtained with each, the level of control and its consistency were usually less pronounced than those obtained with standard rates of the usual chemicals (thiabendazole or imazalil). Combining each of these chemicals with Aspire improved the results and sometimes combinations of Aspire, with a low rate of fungicide, were sufficient to achieve effects similar to those obtained by the chemicals at standard rates. The biological products were not effective against stem-end rots caused by *Diplodia natalensis* or *Phomopsis citri* (Brown, G.E. and Chambers, 1996).

Several approaches have been used to control stem-end rots of citrus fruits arising from latent infections. Since infections at the stem end are related to the ability of the pathogens to invade the fruit through the senescent button tissues, these fruits have been controlled for over four decades with 2,4-D in order to retard senescence of the button, which usually harbors a quiescent infection of one of the stem-end pathogens. Maintaining the button green and young delays fungal penetration into the fruit (Eckert and Eaks, 1989).

Debuttoning of fruit at harvest was found to remove the source that is responsible for initial infection. Similarly, pruning out dead limbs and twigs to reduce the level of inoculum has also been found effective, but it is not economically practical (Smoot et al., 1983). The control of stem-end rots of citrus fruits was made possible by the introduction of the systemic fungicides (thiabendazole, benomyl, imazalil and prochloraz). Apparently, these systemic compounds penetrate the tissues and provide action against the pathogen at sites not accessible to the traditional water-soluble fungicides (Brown, G.E., 1981; Eckert, 1990).

Since brown rot is initiated in the grove, its control should start with preharvest disease control. Fungicidal sprays of fixed copper compounds applied to the lower fruits of the tree before the onset of the rainy season were found beneficial in suppressing disease development (Timmer and Fucik, 1975). Spraying the ground beneath the trees was found effective in inhibiting spore formation on the soil surface (Solel, 1983). Preventive measures also include the removal of fallen fruit from the grove, routine disinfection of the picking boxes and ensuring that they are not left on the soil during rainy or foggy periods (Smoot et al., 1983).

The benzimidazole compounds and imazalil applied to control the wound pathogens of citrus fruit are not effective against *Phytophthora*. On the other hand, metalaxyl is uniquely effective in eradicating incipient infections of *Phytophthora* and has no effect on the development of other postharvest diseases (Eckert and Ogawa, 1985). This compound delayed the development of brown rot on citrus fruit (Cohen, 1981), suppressed the growth of *Phytophthora* on the fruit and prevented contact spread of the disease on inoculated grapefruit at 11°C. Later studies (Ferrin and Kabashima, 1991) described the development of metalaxyl-resistant isolates of *P. parasitica* as indicated by the increased metalaxyl concentration needed for the suppression of mycelium growth and of sporangia and chlamidospore formation. Fosetyl aluminum applied after harvest was found to provide both protective and curative action against *P. parasitica* infection (Gaulliard and Pelossier, 1983).

Hot water dip treatments for 3 min at 46-49°C are effective in eradicating *Phytophthora* infections when applied shortly after infection (within a few days), while the fungus is still confined to the external layers of the fruit peel where heat will penetrate rapidly (Klotz and DeWolfe, 1961).

Disease control is dependent on careful handling and shipping of strong, top-quality fruits and the avoidance of prolonged storage. Harvesting of fruit at prime maturity can avoid the need for prolonged degreening of the immature fruit with ethylene, which generally increases fruit susceptibility to anthracnose. Preharvest ethephon sprays, which promote fruit ripening with relatively small color changes from green to yellow or orange, may allow the crop to be picked at prime maturity and may also lead to triggering the resistance mechanisms of the fruit (Barmore and Brown, G.E., 1978).

Suppression of citrus stem-end rots greatly depends on proper refrigeration during storage and transportation, and temperatures between 4 and 7°C are required to control these rots in transit. However, for various citrus fruits, such as lemons, limes, grapefruits, pomelos and certain hybrids, which are sensitive to low temperatures and develop chilling injuries, higher temperatures should be maintained. Since the minimum growth temperature of *Colletotrichum gloeosporioides* is 9°C (Sommer, 1985), temperatures below 10°C would delay the appearance of anthracnose symptoms. Storage temperatures below 10°C also result in a very slow growth of the wound pathogen *G. candidum*, which has a high optimal growth temperature of 25-30°C

(Laville, 1974), and suppress growth of the fungus *Trichoderma*, whose minimum growth temperature is about 5°C (Gutter, 1963).

BANANA

The most important diseases of bananas are: crown rot, caused by a complex of pathogens; anthracnose, incited by *Colletotrichum musae*; and fruit rot, caused by *Botryodiplodia theobromae.*

1. Crown Rot Fungi

Several fungi may be involved in banana crown rot, which is the principle cause of postharvest disease losses of bananas in international trade. They include *C. musae,* (Berk. & Curt.) v. Arx, *Fusarium* spp. [mainly *F. pallidoroseum* (Cooke) Sacc.], *Verticillium theobromae* (Turc.) Mason & Hughes, *B. theobromae* Pat., *Acremonium* spp., *Cephalosporium* spp. and *Ceratocystis paradoxa* (Dade) Moreau.

Crown rot pathogens commonly grow saprophytically and sporulate on senescent flower parts and leaf debris in the plantation. The spores are spread to the developing hands by rain splash and wind (Meredith, 1971). Harvested bananas carry the spores of pathogenic fungi into the tank of water, in which they are floated to permit latex to flow from the cut tissue of the crown (the portion of the node that has been severed from the stem). The tank (or the 'dehanding' tank) is believed to be the major site of crown rot inoculation, and the newly exposed tissue is vulnerable to infection (Eckert, 1990; Shillingford, 1977).

2. *Colletotrichum musae* (Berk. & Curt.) v. Arx

This fungus is one of the components of the crown rot complex, and the principle cause of banana anthracnose: one of the major diseases of bananas in all producing countries. The fungus exists on debris in its asexual state, as conidia located in a special acervulus. Spores are liberated by rain splash or irrigation water and dispersed by air currents onto young green fruits. Following germination under moist conditions, the conidia produce appressoria, which remain quiescent on the fruit skin. When the fruit ripens and its susceptibility to invasion increases, the fungus becomes active and infects the fruit. The presence of an antifungal compound in the unripe banana fruit has been related to the latency of *C. musae* in this fruit (Mulvena et al., 1969).

The infection of uninjured, immature fruit before harvest results in the formation of numerous, small dark circular spots which enlarge and tend to coalesce (Simmonds, 1963). However, *Colletotrichum* may cause a "non-latent" anthracnose, which is initiated at abrasions and scars sustained during harvesting and handling (Slabaugh and Grove, 1982). In this case, large lesions are produced (Shillingford and Sinclair, 1978). Both types of lesion eventually carry salmon-pink spore masses.

3. *Botryodiplodia theobromae* Pat.

In addition to being a component of the crown rot complex pathogens, *B. theobromae* is also responsible for banana finger rot. The abundance of conidia produced on decaying vegetation in banana plantations form the source of primary infection. Conidia are disseminated by air currents and rain to dying flower parts and are later capable of infecting the fruit when it ripens. Rotting usually begins at the tip of a finger but may occur at any wound on the fruit surface. The rot progresses rapidly at high temperatures, its optimum being about 30°C. Under these conditions, within a few days the rot becomes soft and dark, and typical black pycnidia appear, which give rise to conidia (Williamson and Tandon, 1966). Thus, in hot and humid weather, the harvested fruits can be expected to develop finger rot during transport and ripening. The disease may be serious in fruits held at a high temperature for more than 14 days in transit (Strover, 1972).

Control Measures

Control of postharvest diseases of banana fruits depends on several steps. The fruit should be harvested at the correct stage of maturity and handled carefully to avoid injury. Fallen leaves and flower bracts should be removed in the plantation and the packing station to reduce the numbers of infecting spores. The washing water in the tank, in which the banana 'hands' float to permit latex to flow from the cut crown, should be changed frequently to minimize the inoculum level. Along with reducing the inoculum level, wounds should be protected against fungal infection (Shillingford and Sinclair, 1978; Slabaugh and Grove, 1982). Application of a fungicide soon after dehanding is important, to protect wounds and to prevent infection of the cut crown surface (Eckert and Ogawa, 1985). Postharvest treatment with a systemic fungicide was found to be more effective in controlling decay than preharvest sprays (Ram and Vir, 1983). Cooling is important to suppress decay development, and it should be initiated as soon as possible after cutting the fruit.

Studying the potential for biocontrol of *Lasiodiplodia theobromae* in banana fruits, Mortuza and Ilag (1999) found that *Trichoderma viride* is capable of reducing rotting by up to 65% in inoculated banana fruits. The time of application of the antagonist and the inoculum levels of both the pathogen and the antagonist are important factors in the biocontrol activity. The best activity was exhibited at high concentrations of the antagonist and low concentrations of the pathogen, and when the antagonist was applied 4 hours prior to the pathogen.

MANGO

The most serious postharvest diseases of mango are anthracnose, caused by *Colletotrichum gloeosporioides*; and stem-end rot, caused by a complex of fungi, of which *Botryodiplodia theobromae* is the most common.

1. *Colletotrichum gloeosporioides* (Penz.) Sacc. [perfect state: *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk].

This pathogen causes anthracnose, which is responsible for severe losses of fruit in all mango-growing countries. It is the same fungus that causes anthracnose in avocado, citrus and papaya fruits.

C. gloeosporioides may exhibit both the asexual state (acervuli with conidia) and the sexual state (perithecia and ascospores). The asexual conidia are produced in wet seasons on dead twigs and leaves, from which they are washed down to the fruit. Perithecia have also been found on dead twigs and leaves but they probably do not play an important role in anthracnose infection (Fitzell and Peak, 1984). Disease is initiated by conidia in unripe mango fruits in the plantation, the fruit being susceptible to infection from blossoming and during its developmental stages on the tree. Germinating conidia give rise to appressoria, but at this stage or after the production of fine subcuticular hyphae, the infection remains quiescent. Fungal growth is renewed only when the fruit ripens (Daquioag and Quimio, 1979). The resistance of unripe mango fruits has been attributed to the presence of preformed antifungal compounds in the peel, which act on the subcutaneous hyphae from germinating appressoria (Prusky and Keen, 1993). A fruit with quiescent infection, without any visible symptoms, is liable to be stored and will start to rot later, during storage or marketing.

2. Stem-End Pathogens

Stem-end rot is a very serious postharvest disease of mango, responsible for heavy losses during transit and storage. The fungi involved in stem-end rots are mainly: *Botryodiplodia theobromae* Pat. [perfect state: *Physalospora rhodina* (Berk. & Curt.) Cooke], *Phomopsis* spp. and *Dothiorella* spp. In their asexual state – pycnidia with conidia – they persist in the orchard on dead wood. The sexual state (perithecia and ascospores) is also occasionally exhibited (Alvarez and Lopez, 1971). The conidia are washed down by rainwater, thus contaminating the fruit. Stem-end rots arise by invasion of the cut stem of the fruit at harvest or shortly after harvest (Pathak and Srivastava, 1969), but may originate at injuries in other locations on the fruit. The rot develops rapidly at room temperature, causing a soft-watery decay, which involves the whole fruit within several days, and is characterized by the development of minute black pycnidia over the lesions.

3. *Alternaria alternata* (Fr.) Keissler

This fungus usually develops from quiescent infections on the surface of mango fruits, where it creates typical black spots. Germinating conidia can penetrate the immature fruit via lenticels but infection remains quiescent until the onset of ripening after harvest (Prusky et al., 1983). The resistance of young mango fruits to fungal development has been related to the presence in the peel of fungitoxic concentrations of antifungal resorcinol compounds (Droby et al., 1986).

Control Measures

The major strategies for control of postharvest diseases of mangoes include regular sprays in the plantation, to reduce quiescent infections of *C. gloeosporioides*. Anthracnose can be controlled by immersing mature-green fruits in hot water at 55°C for 5 min after harvest, but this treatment does not satisfactorily control Diplodia stem-end rot (Spalding and Reeder, 1978). The heat treatment may slightly injure the fruit and accelerate the change in peel color from green to yellow. The addition of various fungicides to the hot water enabled the concentration of the fungicide and the level of the heat treatment to be reduced compared with those required when either was applied alone, resulting in improved control of anthracnose and stem-end rot (Spalding and Reeder, 1986b) and, in turn, a greater amount of fruit acceptable for marketing.

The efficiency of quarantine heat treatments (developed for fruit fly disinfestation in mangoes) as a means for postharvest disease control,

was evaluated by Coates and Johnson (1993). High-humidity (> 95% RH) hot air treatment alone (to a core temperature of 46.5°C for 10 min) reduced the incidence of anthracnose in mangoes stored for 14 days at 13°C prior to ripening at 22°C. A treatment consisting of high-humidity hot air combined with either a heated fungicide (benomyl) or an unheated superior fungicide (prochloraz) gave complete control of anthracnose under these storage conditions. The hot air treatment alone gave no control of stem-end rot caused by *Dothiorella* and *Lasiodiplodia* in mangoes stored at 13°C prior to ripening. A supplementary hot fungicide treatment was required for acceptable control of this disease in cool-stored mangoes. A low level of gamma radiation (1000 Gy), which is aimed at eradication of fruit fly infestations of mango fruits, improved the efficacy of hot water against both anthracnose and stem-end rots (Spalding and Reeder, 1986b). Effective control of postharvest diseases of mangoes during short-term storage at 20°C was provided by hot fungicide treatment followed by low-dose irradiation. Satisfactory disease control during long-term controlled atmosphere storage (5% O_2 and 1.5-2% CO_2 at 13°C) was achieved when mangoes were treated with heated and unheated fungicides combined with low-dose irradiation (Johnson et al., 1990). Irradiation in excess of 600 Gy caused surface damage in Kensington Pride mangoes, while cultivars Kent and Zill show more tolerance to irradiation.

A postharvest unheated fungicide application may substantially reduce the incidence of Alternaria black spot on mangoes (Prusky et al., 1983).

Moreno and Paningbatan (1995) reported on the biocontrol of Diplodia stem-end rot of mango, by the antagonistic fungus, *Trichoderma viride*. The antagonistic activity was enhanced at higher concentrations of the antagonist and at lower concentrations of the pathogen.

PAPAYA

Similarly to other tropical and subtropical fruits, the major postharvest diseases of papaya are anthracnose, caused by *Colletotrichum gloeosporioides*, and stem-end rots, caused by *Botryodiplodia theobromae* or *Phomopsis* spp. Other fungi may be associated with stem-end rots of papayas, such as *Phoma caricae-papayae* and *Phytophthora palmivora*. Various surface rots, caused by species of *Rhizopus, Fusarium, Alternaria, Stemphylium* and other pathogens, may infect papaya fruits and reduce their postharvest life.

1. *Colletotrichum gloeosporioides* (Penz.) Sacc. [perfect state: *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk]

Anthracnose, caused by this pathogen, is generally initiated by the asexual state of *C. gloeosporioides* (acervuli with conidia), although the sexual state (perithecia and ascospores) has been recorded. Conidia are produced on dying leaves or petioles and are spread by rain and air currents onto the fruit. Under moist conditions, the conidia germinate within a few hours to form appressoria and infection hyphae, which penetrate the cuticle and form quiescent infections in the immature fruit (Dickman and Alvarez, 1983). Renewed growth and characteristic disease symptoms, including fungal colonization and the development of an abundance of salmon-pink conidia, appear when the fruit ripens after harvest.

C. gloeosporioides is also responsible for multiple small lesions of the 'chocolate spot' type (Dickman and Alvarez, 1983), which similarly arise from quiescent infections but only occasionally develop into anthracnose lesions.

2. Stem-End Pathogens

Botryodiplodia theobromae Pat. and *Phomopsis* spp. are characteristic stem-end pathogens of tropical and subtropical fruits.

Infection takes place by conidia (produced within pycnidia), which are liberated and dispersed during wet conditions in the orchard. Germinating spores penetrate via newly cut stem ends or through crevices between the peduncle and the fruit flesh (Chau and Alvarez, 1979), but infection may also be initiated at any injury incurred by the fruit shortly before harvest, and which has not yet healed.

Phoma caricae-papayae (Tarr) Punith. (perfect state: *Mycosphaerella caricae* H.& P. Sydow) can infect uninjured developing fruit during wet weather (Simmonds, 1965). Infection may be initiated anywhere on the fruit surface, by asexual spores (conidia) or by sexual spores (ascospores produced within perithecia) (Chau and Alvarez, 1979). Infection may, however, occur via the cut stem during harvest resulting in the formation of stem-end rot (Srivastava and Tandon, 1971).

3. *Phytophthora palmivora* (Butler) Butler

Phytophthora infection is initiated during the rainy season by the asexual state of the fungus (sporangia with zoospores), although sexual spores (oospores) have also been recorded. Following dispersal of the lemon-shaped sporangia, the zoospores, which are liberated during wet

weather, are capable of infecting uninjured papaya fruits. Infected fruits fall to the ground and serve as a source of new infections in developing fruits (Srivastava and Tandon, 1971). Invasion may, however, occur at the cut stem and result in stem-end rot development (Alvarez and Nishijima, 1987).

4. *Rhizopus stolonifer* (Ehrenb. Ex Fr.) Lind

Rhizopus rot, caused by *Rhizopus stolonifer*, is a destructive rot of papaya. Spores of the fungus are ubiquitous and infection originates only via injuries inflicted during harvesting and handling. Severe problems occur particularly during extended rainy periods (Nishijima et al., 1990).

The fungus infects mature fruits, causing a soft watery rot. Under warm humid conditions, a loose white mycelium bearing black sporangia (giving rise to sporangiospores) is being developed. Infected fruits, which rapidly collapse, cause contact infection of neighboring fruits during shipment and storage (Quimio et al., 1975). Nishijima et al. (1990) found that the optimum temperature for disease development was about 25°C and that no disease developed at 5 or 35°C; they also showed that the fungus is consistently capable of infecting the fruit through lesions caused by *Colletotrichum gloeosporioides, Phomopsis* sp. or *Cercospora* sp.

Control Measures

Postharvest diseases of papaya are controlled by frequent fungicide sprays in the plantation followed by postharvest hot water and fungicide treatments (Eckert, 1990). The frequent fungicide sprays in the plantation control quiescent infections of *Colletotrichum* and incipient infections of *Phytophthora*, and reduce the inoculum levels of *Phoma (Mycosphaerella), Botryodiplodia* and other pathogens that invade the fruit through wounds (Alvarez et al., 1977; Bolkan et al., 1976). The traditional treatment of hot water at 48°C for 20 min was used in Hawaii for many years to control anthracnose, stem-end rots and incipient *Phytophthora* infections (Akamine, 1976; Aragaki et al., 1981). However, this treatment delayed color development and caused a slight heat injury, accompanied by an increase in *Stemphylium* infection (Glazener and Couey, 1984). A combination of shorter, hotter water sprays (54°C for 3 min) followed by a fungicide treatment, was recommended for papayas destined for long distance shipment (Couey and Alvarez, 1984).

In order to satisfy the quarantine requirements for eradication of fruit flies in papaya shipments to fly-free zones, the double hot water dip (30 min at 42°C followed by 20 min at 49°C) was adopted. This treatment,

coupled with regular field spraying programs, also provides control of postharvest diseases of papaya (Alvarez and Nishijima, 1987). However, in the light of the sensitivity of various papaya cultivars to heat injury, a single hot dip (49°C for 15 min) was suggested as optimal for disease control with minimum effects on fruit quality (Nishijima, 1995).

A hot water dip at 49°C for 20 min is effective in reducing early *Rhizopus* infections, mycelium being more sensitive to heating than fungal spores. Decay was further reduced and almost eliminated when a hot water treatment was coupled with fungicide field sprays. The main control measures against *Rhizopus* soft rot are, however, sanitation procedures. These include the removal of decaying fruit from areas around the packinghouse, and regular disinfestation of floors, packing lines and other packinghouse equipment and of bins used for transferring fruit from the field to the packer (Nishijima et al., 1990). Holding the fruit at 7-10°C will retard decay development during storage.

AVOCADO

The most significant postharvest diseases of avocado fruits are: anthracnose, caused by *Colletotrichum gloeosporioides*; fruit rot, caused by *Dothiorella gregaria*; and stem-end rots, caused by *Botryodiplodia theobromae*, *Dothiorella gregaria*, *Alternaria* spp. and *Phomopsis* spp. (Darvis, 1982; Muirhead et al., 1982). Other, less significant postharvest pathogens include *Fusarium* spp., *Pestalotiopsis versicolor*, *Rhizopus stolonifer*, *Pseudocercospora purpurea*, *Trichothecium roseum*, *Penicillium* spp., and the bacteria *Erwinia carotovora* and *Pseudomonas syringae*.

1. *Colletotrichum gloeosporioides* (Penz.) Sacc. (Perfect state: *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk)

This fungus, which is responsible for anthracnose, may exhibit both the asexual state (acervuli and conidia) and the sexual state (perithecia and ascospores), but the asexual conidia play the main role in fungal infection. Initial infection of avocado peel takes place in the plantation during the growing season but, since avocados do not ripen on the tree, the infection remains quiescent in the immature fruits (Binyamini and Schiffmann-Nadel, 1972). Quiescence is exhibited by appressoria that are formed by germinating conidia and are located on the fruit surface, or by thin infection hyphae that penetrate under the cuticle or the external layers of the epidermis (Muirhead, 1981b; Prusky et al., 1990). Renewed

fungal growth and complete penetration of the fruit peel occur only after the onset of ripening (Prusky and Keen, 1993). Infected avocados may thus be harvested without any visible symptoms and the disease develops later during storage or marketing. Typical symptoms of anthracnose, which appear only when the fruit begins to soften, include circular dark spots and, later, sunken lesions which, under humid conditions, give rise to masses of salmon-pink conidia.

2. *Dothiorella gregaria* Sacc. (perfect state: *Botryosphaeria ribis* Grossenb. & Duggar)

Dothiorella may exhibit both the asexual state (pycnidia with conidia) and the sexual state (perithecia and ascospores) on dead twigs and leaves. Under wet conditions spores may infect the fruits on the tree, via their stomata and lenticels, and the infection then remains quiescent. Progressive lesions generally develop when the fruit begins to soften after harvest (Labuschagne and Rowell, 1983). The same fungus, alone or in association with other fungi, is also responsible for stem-end rot (Muirhead et al., 1982).

3. Stem-End Fungi

The stem-end fungi are found on dead branches and bark of avocado trees. *Botryodiplodia theobromae* Pat. [perfect state: *Physalospora rhodina* (Berk. & Curt.) Cooke], is typically a wound pathogen and avocado infections frequently take place during harvest. The decay originates at the stem end and proceeds, rather uniformly, towards the blossom end. It may infect the pedicel during fruit development but, since avocados do not ripen on the tree, the infection remains quiescent until after harvest, when the fruit ripens. *Dothiorella gregaria* and *Phomopsis* spp., however, are capable of causing latent infections in developing fruits (Peterson, 1978). Generally, stem-end rots caused by *Alternaria alternata* are less common. However, after prolonged commercial use of the systemic fungicide, thiabendazole to treat avocados, *Alternaria alternata*, which is insensitive to this fungicide, has become a major cause of stem-end rot in stored fruits (Zauberman et al., 1975).

Control Measures

Anthracnose development may be reduced by preventing injuries to the fruit, in the orchard or during harvesting and handling (Smoot et al., 1983). The disease is particularly important during prolonged storage,

since the fruit may mature and ripen during this period. Both orchard spraying and postharvest fungicidal dips have been used to control postharvest avocado diseases (Darvis, 1982; Muirhead et al., 1982).

A reduction of the incidence of postharvest anthracnose of avocado was recorded after dipping or spraying with an antioxidant compound, which led to the maintenance of host resistance. A combination of the antioxidant with fungicide (prochloraz) application reduced decay consistently and for a longer period, while fungicidal treatments alone did not always result in disease reduction (Prusky et al., 1995).

Looking for a non-chemical method to control postharvest diseases of avocados in South Africa, Korsten et al. (1995) evaluated the inhibitory capacity of bacteria isolated from avocado leaf and fruit surfaces, against fruit pathogens. *Bacillus subtilis*, when incorporated into the commercial wax and applied to the fruit in the packinghouse, was found to be the most effective antagonist against the *Dothiorella/Colletotrichum* fruit rot complex and against stem-end rot.

Under commercial conditions, decay development can be delayed by storing the fruit at 5-6°C for short-term shipping or storage. However, several cultivars are sensitive to such low temperatures and should be stored at higher temperatures. A controlled atmosphere of 2% O_2 and 10% CO_2 at 7°C can extend the storage period of avocados (Spalding and Reeder, 1975). When the fruit must be held for longer periods, a combination of low temperatures and fungicide treatments is needed (Muirhead et al., 1982).

PINEAPPLE

The main postharvest diseases of pineapple are black rot, caused by *Thielaviopsis paradoxa*, and fruitlet core rot, caused by several wound pathogens.

1. *Thielaviopsis paradoxa* (de Seynes) Höhnel [perfect state: *Ceratocystis paradoxa* (Dade) Moreau]

This fungus is the cause of black rot, which is the most significant postharvest disease of pineapple (Rohrbach and Phillips, 1990), and which is sometimes referred to as soft rot or stem-end rot, depending on the mode of infection. The fungus survives in plant debris in the soil in the form of thick-walled chlamydospores. Infective asexual conidia are splashed onto the fruit by rain. The sexual state (perithecia with

ascospores) has also been recorded occasionally (Liu and Rodriguez, 1973). Infection may originate in the field via growth cracks or via insect punctures, but fungi generally penetrate into the fruit through the cut stem end when the fruit is removed from the stalk or through injuries inflicted during harvesting and handling (Rohrbach and Phillips, 1990). The disease develops rapidly at tropical temperatures. The fungal optimal growth temperature is approximately 26°C and it becomes inactive below 10°C. Moist conditions at harvest are important for decay development. High percentages of decay in a shipment usually indicate that the fruit was harvested during or shortly after a prolonged rainy period (Smoot et al., 1983).

2. Fruitlet Core Pathogens

Several fungi, such as species of *Cladosporium, Penicillium* and *Trichoderma*, can invade wounds during harvest but frequently develop as surface molds (Eckert, 1990). Some fungi, including species of *Fusarium* and *Penicillium*, and bacteria, including species of *Erwinia, Pseudomonas* and *Acetobacter*, either singly or in combination, may cause "fruitlet core rot" (Rohrbach and Taniguchi, 1984). While *Penicillium funiculosum* enters the developing floret through the unopened bud, *Fusarium moniliforme* var. *subglutinans* and the pathogenic bacteria infect the developing fruit via the open flower (Rohrbach and Phillips, 1990). Fungal spores or bacterial cells are deposited in the floral cavities by water splash or by insects that both carry the pathogens and damage the tissue prior to infection (Bolkan et al., 1979; Mourichon, 1983). Infection results in brown soft rot of the axis of individual fruitlets and can rarely be detected from the outside of the fruit. A longitudinal section reveals the extension of the affected area towards the heart of the fruit (Snowdon, 1990). The decay may develop in ripe or mature fruit, in the field or during transit and marketing.

Control Measures

Since fungal infection is associated with the presence of wounds, careful handling is needed at all stages to prevent injury, and packing has to be designed to prevent the sharp 'crown' leaves from piercing adjacent fruits (Snowdon, 1990). Damaged and wet fruits and those with an excessive number of growth cracks should be excluded from fresh fruit shipments (Smoot et al., 1983). Measures to reduce fruitlet core rot incited by the various pathogens start in the field, with the control of insects that spread the disease (Rohrbach and Phillips, 1990).

For several decades black rot was controlled by disinfection of the cut area of the fruit with sodium *ortho*-phenylphenate, sodium salicylanilide or benzoic acid within 2 h after cutting (Eckert and Ogawa, 1985). Several studies indicated that better control of black rot could be achieved by postharvest fungicide applications (Cho et al., 1977; Eckert, 1990; Hernandez Hernandez, 1990), which should be applied within 6 to 12 h after harvest. Thiabendazole and benomyl may also be added to a wax formulation applied to harvested fruits to control internal browning and water loss, although they are less effective when mixed with wax than when applied in water (Cho et al., 1977).

Refrigeration at 7-8°C arrests fungal development, even in the presence of deep wounds, open for fungal penetration (Hernandez Hernandez, 1990). This temperature is suitable for ripe fruit during storage or transportation, but it may cause mature green fruits to fail to ripen normally and to develop good flavor, therefore higher storage temperatures (10-13°C) are required for such fruits.

PERSIMMON

Several fungi are involved in postharvest decay of persimmon fruits. The most important are species of *Alternaria, Botrytis, Colletotrichum, Phoma, Cladosporium, Penicillium, Mucor* and *Rhizopus.*

Alternaria alternata (Fr.) Keissler

This pathogen causes black spot disease or Alternaria rot. Direct penetration of developing fruits can occur during the entire growth period of the fruit. The infection remains quiescent and regains activity only after harvest, when the fruits ripen (Prusky et al., 1981). Symptoms generally appear after 10 weeks of storage at -1°C and, at a later stage, the disease may involve the entire fruit surface. In addition, fungal penetration frequently occurs via small cracks formed on the fruit shoulder beneath the calyx as the fruit approaches harvest maturity. In the high humidity environment of the rainy season, preharvest disease symptoms appear beneath the calyx of riper fruits in the orchard (Prusky et al., 1981; Perez et al., 1995). When the season is dry, no decay is recorded at harvest.

Control Measures

Protective fungicide treatments in the orchard are ineffective in reducing decay development beneath the calyx (Perez et al., 1995). A preharvest

spray with gibberelic acid (GA_3), applied in the orchard 10-14 days before harvest, delays fruit softening and extends the storage life of the fruit (Ben-Arie et al., 1986). Following three GA_3 sprays (20 $\mu g\ ml^{-1}$) during the development of the fruit in the orchard, the calyx was found to remain erect until harvest and a smaller area was covered with the black spot disease after 3 months of storage at 0°C. GA_3, at concentrations of up to 200 $\mu g\ ml^{-1}$, had no direct effect on fungal growth *in vitro* or on inoculated fruit, and the effect of GA_3 on disease development was attributed to the enhancement of fruit resistance to infection (Perez et al., 1995).

GUAVA

The fact that the guava fruit peel is easily broken leads to postharvest infections by many wound pathogens (Eckert, 1990). These include: *Colletotrichum gloeosporioides, Botryodiplodia theobromae, Ceratocystis paradoxa* and species of *Phomopsis, Phoma, Fusarium, Pestalotia, Penicillium, Aspergillus, Rhizopus, Mucor* and *Erwinia* (Adisa, 1985b; Arya et al., 1981; Brown, B.I. et al., 1984; Singh and Bhargava, 1977; Ramaswamy et al., 1984; Wills et al., 1982).

1. *Colletotrichum gloeosporioides* (Penz.) Sacc. [perfect state: *Glomerella cingulata* (Stonem.) Spauld & v. Schrenk]

Anthracnose, caused by this pathogen, is the most significant disease of guavas (Adisa, 1985). The fungus, which characteristically also infects other tropical and subtropical fruits, attacks most cultivars of guava, although several cultivars have been found to offer some resistance (Tandon and Singh, 1970).

2. *Botryodiplodia theobromae* Pat.

Botryodiplodia rot, caused by this pathogen, is another disease common to various tropical and subtropical fruits. It may invade guavas in the orchard, causing a dry stem-end rot of developing fruits. Decay of ripe fruits results from infection through the stem end or via wounds and causes a soft breakdown of the tissue (Adisa, 1985; Srivastava and Tandon, 1969).

3. *Phomopsis* spp.

Infection by these pathogens may also be initiated either in the orchard or after harvest (Srivastava and Tandon, 1969); it usually occurs

at the stem end, or at the distal end in the region of the persistent calyx. Affected areas of the skin turn soft and wrinkled along with the appearance of numerous pycnidia with the asexual spores.

Control Measures

Sanitation activities in the orchard, careful harvesting and handling and preventive preharvest fungicide sprays may reduce postharvest diseases of guava incited by wound pathogens (Srivastava and Tandon, 1969; Ito et al., 1979; Ramaswamy et al., 1984). Good control has been achieved by postharvest fungicide dips of guava inoculated with the wound pathogens *Pestalotia, Phoma, Gloeosporium* and *Aspergillus* (Singh and Bhargava, 1977; Arya et al., 1981). Wills et al. (1982) found that under natural infection conditions, a heated fungicide was more effective than some single fungicides at ambient temperatures. Refrigeration at 5°C enables prolongation of the postharvest life of guavas by up to 3 weeks while lower temperatures may cause chilling injury (Brown, B.I. and Wills, 1983).

LITCHI

Litchis may be infected by many postharvest pathogens during storage and transport (Prasad and Bilgrami, 1973; Scott et al., 1982). Pathogens characteristic of tropical and subtropical fruits, such as *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*, do not spare this one. Wound pathogens, such as *Rhizopus* spp. or *Geotrichum candidum*, for which wounding is a prerequisite for infection, may penetrate the fruit shell via cracks resulting from sun scorch or via punctures produced by fruit-piercing insects (Scott et al., 1982). Such injuries not only provide an avenue for fungal penetration into the fruit but may also allow the exudation of juices that stimulate mold development (Prasad and Bilgrami, 1973). However, the main postharvest problem with litchis is associated with physiological changes exhibited in darkening and loss of the natural red color of the fruit. This phenomenon involves the formation of polyphenols in response to desiccation and injury (Akamine, 1976).

Control Measures

Disease control should begin in the orchard, with preventive measures against fruit insects, including 'bagging' of developing fruits (Prasad and Belgrami, 1973). In order to reduce postharvest decay, the fruits can also

be treated with a wax formulation containing fungicide (Prasad and Bilgrami, 1973) or dipped in a fungicide solution, which may be heated or unheated (Scott et al., 1982; Brown, B.I. et al., 1984). The physiological changes in the natural fruit color can be controlled by packing the fruit in a plastic film that maintains a high relative humidity around it and reduces moisture loss (Akamine, 1976), or by altering the pH of the fruit pericarp (Fuchs et al., 1993).

II. POME AND STONE FRUITS

POME FRUITS

Eckert and Ogawa (1988) divided the major postharvest pathogens of pome fruits into two groups: (a) those that cause quiescent infections of lenticels, including *Gloeosporium album, G. perennans* and *Nectria galligena*; and (b) those that preferably enter through wounds after harvest, including *Penicillium expansum, Botrytis cinerea, Monilinia* spp., *Mucor* spp., *Rhizopus* spp., *Alternaria alternata, Stemphylium botryosum* and *Cladosporium herbarum*. The rots in the lenticels are initiated in the orchard in the late summer, in areas with late summer rainfall and are a major problem in apples grown in the United Kingdom and Northern Europe (Edney, 1983). In drier apple production areas, the main problems are caused by wound pathogens that invade the fruit after harvest through injuries sustained during harvesting and handling and via puncture wounds, bruised lenticels, etc. In fact, the wound pathogens are of major importance in all apple and pear production areas. Other important pathogens of pome fruits are species of *Phytophthora* that may become a serious problem during rainy seasons for fruit from orchards with heavy soils (Edney, 1978), and *Colletotrichum gloeosporioides* (Syn. *Gloeosporium fructigenum*), the bitter rot fungus, which is capable of direct penetration of the intact skin (Brook, 1977), while harvested fruits are infected via injuries. *Botryosphaeria* spp., the black and white rot fungi, are of importance in several areas of the USA (Snowdon, 1990).

Pathogens of minor importance that may occasionally be found on harvested apples and pears include *Stemphylium botryosum, Cladosporium herbarum, Trichothecium roseum,* and species of

Phomopsis, Nigrospora, Fusarium, Epicoccum, Aspergillus, Trichoderma and others (Snowdon, 1990).

A. *Penicillium expansum* Link

The blue mold rot, caused by *P. expansum*, is the commonest and one of the most destructive rots of harvested apples and pears. Other *Penicillium* species, such as *P. cyclopium, P. crustosum* and *P. verrucosum*, may occasionally cause the blue mold rot (Barkai-Golan, 1974; Hall and Scott, 1989a).

Germinating conidia invade the fruit mainly through wounds or bruises incited during harvesting and handling. Penetration can also take place via the lenticels, under favorable conditions (Baker and Heald, 1934), or at the site of infection by other pathogens, such as species of *Gloeosporium, Phytophthora* and *Mucor* (Snowdon, 1990). The susceptibility of lenticels to penetration is enhanced in over-mature fruit, during prolonged storage, or by bruising or puncturing (Hall and Scott, 1989a). The fungus produces pale brown to brown soft-watery spots that enlarge rapidly under shelf life conditions. Under humid conditions, conidia-bearing conidiophores, grouped to form coremia, are formed on the surface of the lesion. As the conidia mature, they become blue-green and form masses which give the decay its typical color. Since decay development is favored by high humidity, the blue mold is more of a problem on fruits stored or shipped in plastic film liners (Hall and Scott, 1989a). Decay can progress, albeit slowly, during cold storage; rapid development begins when the fruits are transferred to warmer conditions. The fungus can spread during the long months of storage, by contact between infected and sound fruit, forming 'nests' of decay.

Several strains of *P. expansum* can produce the mycotoxin patulin while they develop in apples and pears. The mycotoxin may be highly toxic to animal tissue and may also display carcinogenic and mutagenic properties (Stott and Bullerman, 1975). The ability to produce patulin and the amounts of patulin produced depend on fungal strain, fruit cultivar, storage temperature and storage atmosphere (Lovett et al., 1975; Paster et al., 1995). The amounts of patulin produced by different strains of the fungus in Golden Delicious apples were found to range from 2 to 100 $\mu g\ g^{-1}$ (Sommer et al., 1974). However, patulin production by a given strain has also been found to differ at different temperatures (Paster et al., 1995). Studying patulin production in apples stored under controlled atmosphere of 1% CO_2, 3% O_2 at 1°C, Lovett et al. (1975) reported that only one of the two *Penicillium* strains tested produced

mycotoxin, and the amounts produced were considerably reduced, as compared with production in air. Sommer et al. (1974) found that two strains of *P. expansum* produced patulin in Golden Delicious apples stored in a controlled atmosphere containing 7.5% CO_2 and 2% O_2 at 23°C, although at markedly lower levels than in air. Working with other *P. expansum* strains, Paster et al. (1995) found that patulin production was inhibited in Starking apples stored in an atmosphere of 3% CO_2, 2% O_2 at 25°C, although fungal growth was still 70% of that of the control. Under 3% CO_2, 10% O_2, patulin production was only slightly reduced.

B. *Botrytis cinerea* Pers. [perfect state: *Botryotinia fuckeliana* (de Bary) Whetzel]

The gray mold rot caused by this fungus can cause heavy losses of pome fruits, particularly pears. The fungus survives as sclerotia in the soil and on plant debris, and under favorable conditions asexual spores (conidia) are formed. Under wet conditions the conidia may infect dying blossoms (Combrink et al., 1983) and remain quiescent in the flower parts (DeKock and Holz, 1991). Fruit infection occurs later, after renewal of the pathogen growth during storage and marketing. *B. cinerea* can infect the fruit through wounds incurred during harvesting and handling, or directly via skin breaks (Spotts and Peters, 1982a). In fact, the resistance of various apple cultivars to infection by *B. cinerea* and other wound pathogens has recently been related to the force required to break the fruit skin (Spotts et al., 1999).

Lesions are dry and firm at first, but become soft as the rot advances. Under humid conditions, abundant gray-brown conidia are produced. Sclerotia may also be formed on well advanced lesions. The fungus spreads very readily during storage by contact between infected and sound fruit, forming 'nests' of decaying fruit. This type of infection markedly increases the infection rate in storage (Edney, 1978). In addition, *B. cinerea* may penetrate apple fruits via the sinus between the calyx and the core cavity, in apple cultivars characterized by open sinuses, resulting in core rot (Spotts et al., 1988).

C. *Monilinia* spp.

Monilinia rot may be incited by three species of *Monilinia*: *Monilinia fructigena* (Aderh. & Ruhl.) Honey, which is widespread in Europe, Asia and South America, but is uncommon in North America; *M. fructicola* (Wint.) Honey, which occurs in North and South America, South Africa, Japan, Australia and New Zealand; and *Monilinia laxa* (Aderh. & Ruhl.)

Honey, which occurs occasionally in Europe, Asia and some regions of North America (Snowdon, 1990).

The three species are wound pathogens capable of invading apple and pear fruits before and after harvest (Edney, 1983). Fruits infected early in the growing season develop rot on the tree, exhibit firm lesions and eventually turn into shriveled 'mummies', which may remain attached to the tree or fall to the ground. Infections initiated in the field continue to develop in storage, where they spread by contact between infected and sound fruits (Cole and Wood, 1961). The fungus survives the winter and, when environmental conditions become favorable, produces asexual spores (conidia). The conidia are dispersed throughout the orchard and infect the fruits via wounds. The fungi may exhibit the sexual state (apothecia with ascospores) and, on liberation, the ascospores may infect the flower parts, thus spreading the disease into developing fruits.

D. *Gloeosporium album* Osterw. [perfect state: *Pezicula alba* Guthrie] and *Gloeosporium perennans* Zeller & Childs [perfect state: *Pezicula malicorticis* (Jackson) Nannf.]

These two lenticel pathogens cause *Gloeosporium* rot. Infection is spread by the asexual spores (conidia produced in acervuli), which are dispersed by rain and contaminate the fruit during the growing season (Edney, 1983). Following germination on the fruit surface, the fungus enters the developing fruits via lenticels (Bompeix, 1978) and remains quiescent until after harvest (Noble and Drysdale, 1983). The sexual state (apothecia with ascospores) has been recorded but is not regarded as an important source of inoculum. *G. perennans* produces circular rots, frequently with a yellow center, that have led to the common names 'target spot' or 'bull's eye' rot. However, identification of the species is mainly by microscopic examination.

E. *Cylindrocarpon mali* (Allesch.) Wollenw. [perfect state: *Nectria galligena* Bresad.]

This lenticel pathogen is responsible for Cylindrocarpon or Nectria fruit rot. The fungus exhibits both the sexual (perithecia with ascospores) and the asexual (conidia produced in sporodochia) states, and both forms may be found in cankers on the tree. Conidia are dispersed by rain splash while ascospores are frequently carried by air currents to greater distances (Swinburne, 1983). Infection may occur through the calyx-end or the stem-end and result in the fruit decaying

on the tree. However, the fungus can penetrate via the lenticels and remain quiescent until after harvest. The quiescent stage has been related to the synthesis of the phytoalexin, benzoic acid in the tissues; after harvest, with the loss of the phytotoxic activity of the phytoalexin, the pathogen resumes active growth and decay is developed (Swinburne, 1983). The resistance of apple tissues to decay may, however, change because of the development of *N. galligena* mutants resistant to benzoic acid (Seng et al., 1985).

F. *Colletotrichum gloeosporioides* (Penz.) Sacc. [perfect state: *Glomerella cingulata* (Stonem.) Splaud. & v. Schrenk] and *Colletotrichum acutatum* J.H. Simmonds

These two species are involved in bitter rot of apples, which may cause heavy losses in warm, wet growing areas (Sutton, 1990).

Some strains of *C. gloeosporioides* are capable of exhibiing both the sexual state (perithecia with ascospores) and the asexual state (acervuli with conidia), while others produce only asexual spores. Both types of spores can take part in disease initiation (Sutton and Shane, 1983). Infection takes place in the orchard during the growing season, in warm, wet weather, the optimal temperature for growth being about 26°C. Spores are washed down onto the fruit by rain, and infection can take place by direct penetration of germinating spores into the intact fruit (Brook, 1977). At a later stage, completely decayed fruits turn into 'mummies', a source of immense quantities of conidia which serve as inoculum for the following year (Brook, 1977). Bitter rot is also found in storage following fungal penetration through wounds sustained during harvesting, handling and marketing.

Bitter rot symptoms appear as circular lesions with the fungal reproductive structures (acervuli, perithecia or both) often in concentric rings on the fruit lesion (Shi et al., 1996). Isolates of the two *Colletotrichum* species exhibit a considerable variation in colony color, but they generally produce acervuli with typical orange to salmon-pink conidial masses (Jones et al., 1996). Apple isolates of *C. gloeosporioides* differ from apple isolates of *C. acutatum* in the morphology of their conidia and by a faster rate of colony growth (Shi et al., 1996). The frequency of occurrence of the various pathogens on apples varies among orchards and may be influenced by many variables including environmental factors, sampling date, host cultivar and management practices.

G. *Phytophthora cactorum* (Lebert & Cohn) Schroet. and *Phytophthora syringae* (Kleb.) Kleb.

These two species, which are the causal pathogens of Phytophthora rot, are soil-borne. Infection is closely related to rainfall which splashes infected soil onto fruits close to the ground (Edney, 1978). The asexual spores (zoospores), which are the main agents of infection, come into contact with low-hanging fruits and generally infect them via the lenticels. This process requires free water or wetness (Edney, 1978). Fallen apple and pear leaves have also been found to bear the sexual state of *Phytophthora* (oospores) and are considered to be an important source of inoculum in the orchard (Harris, 1979). After a resting period, and under wet conditions, the oospores germinate to form sporangia that release new zoospores. Fruit harvested in the early stages of disease development undergo rotting later, in storage. Decay is spread during storage by contact of infected fruit with sound fruit, forming 'nests' of decayed fruit (Edney, 1978).

H. *Botryosphaeria* spp.

Two species are responsible for apple and pear rots (Combrink et al., 1984; Brown, E.A. and Britton, 1986): *Botryosphaeria obtusa* (Schw.) Shoem (imperfect state: *Sphaeropsis* sp.) and *Botryosphaeria ribis* Grossenb. & Duggar (imperfect state: *Dothiorella* sp.).

B. obtusa [syn. *Physalospora obtusa* (Schw.) Cooke], the cause of the black rot, and *B. ribis*, the cause of the white rot, may both produce the asexual state (pycnidia with conidia) and the sexual state (perithecia with ascospores). The conidia are water-borne while the ascospores may be dispersed to greater distances by air currents (Snowdon, 1990). Both stages are capable of producing the disease. The fungi may penetrate into the fruit via lenticels, bruises, or cracks, while in the orchard, in transit or in storage. Young fruits are resistant to rotting (Sitterly and Shay, 1960), becoming susceptible on maturation. Thus, fruit harvested shortly after infection will rot later in storage.

Symptoms may vary with the temperature but both fungi can be controlled during storage at about 0°C.

I. *Mucor piriformis* Fischer

Mucor rot can develop on various cultivars of pears and apples. The fungus in the sexual state, as zoospores, can survive hot dry periods (Michaelides and Spotts, 1986), while the asexual state (sporangia with sporangiospores) is of prime importance in the fungal infection. The fungus may penetrate the fruit through injuries of the skin (Combrink

and Fourie, 1984) or via the stem end (Lopatecki and Peters, 1972). In some apple cultivars, penetration occurs through the open calyx tube (Sharples and Hims, 1986; Spotts et al., 1988) resulting in core rot. Although the susceptibility of apple cultivars to *M. piriformis* may differ among different locations or different years, Granny Smith and Braeburn have generally been found to be the most susceptible cultivars and Royal Gala the most resistant one to Mucor infection (Spotts et al., 1999). Fruits with unseen initial infection may be introduced into the storeroom where fungal development proceeds even at 0°C (Lopatecki and Peters, 1972). According to the site of fungal penetration, lesions may appear on the fruit surface (Combrink and Fourie, 1984), at the stem end (Lopatecki and Peters, 1972), or in the core region (Spotts et al., 1988).

The rot is soft and watery, and under humid conditions is characterized by the appearance of minute black heads, which are the sporangia of the fungus. It differs from Rhizopus watery rot in its lack of a sour odor (Combrink and Fourie, 1984).

J. *Rhizopus* spp.

Rhizopus stolonifer (Ehrenb. ex Fr.) Lind and, to a lesser extent, *Rhizopus oryzae* Went & Prinsen Geerligs, are the cause of Rhizopus rot of pome fruits. However, while Rhizopus rot is a serious postharvest disease of stone fruits, it is not considered a major disease of pome fruits.

Infection is initiated by the asexual spores (sporangiospores), which are common components of the air spora (Barkai-Golan et al., 1977b). The rot is soft and watery and, at its progressive stage, releases juices having a sour odor. The sexual state (zygospores) has rarely been reported. The fungus is heterothallic and requires the presence of two physiologically different and compatible mycelia for sexual reproduction (Alexopoulos, 1961). The development of both species of *Rhizopus* is inhibited at temperatures below 5°C and the rot is, therefore, prevented at the storage temperatures recommended for apples.

K. *Alternaria alternata* (Fr.) Keissler

This fungus is the main cause of Alternaria rot in pome fruits. Infection is initiated by conidia that are produced in abundance on leaf debris and other plant material in the orchard. The conidia, which are very important components of the air spora (Barkai-Golan et al., 1977b), are disseminated by wind and rain, and are generally present on the fruit surface at harvest time. The fungus is a weak pathogen that frequently colonizes damaged or senescent fruit. Several different symptoms can be produced following

Alternaria infection, including small, black corky lesions, shallow dark-colored rot, or black decay of the stem (Snowdon, 1990). In addition, the fungus can penetrate the fruit via open calyces causing core rot of apples (Ceponis et al., 1969; Combrink et al., 1985). It has also been reported as a secondary infection of bitter pit, one of the most important physiological disorders of apples (Ben-Arie, 1975).

Some metabolites of *A. alternata* strains may be toxic to animals and humans. Stinson et al. (1981) reported that the main toxins produced by the fungus during its growth in apples were alternariol and alternariol monomethyl ether, with maximum concentrations of 5.8 and 0.23 mg per 100 g tissue, respectively. Higher concentrations of both toxins were produced by another strain of *A. alternata*, which was held at 25°C for several weeks (Ozcelik et al., 1990).

L. *Stemphylium botryosum* Wallr. [perfect state: *Pleospora herbarum* (Pers.) Rabenh.] and *Cladosporium herbarum* (Pers.) Link

These two species are weak pathogens that tend to attack weakened or senescent tissue. *C. herbarum* is mainly of interest because of its association with scald and other types of physiological disorders in various apple cultivars (Edney, 1983), while *S. botryosum* may attack lesions resulting from sunscald (Snowdon, 1990). Cladosporium rot has been found to accompany side rot caused by *Phialophora malorum* on pears (Sugar and Powers, 1986). For both pathogens the susceptibility of the fruit to decay increases during extended storage periods.

M. *Trichothecium roseum* Link.

Trichothecium is a weak pathogen that invades pome fruit via wounds or lesions incited by other pathogens. It frequently follows the scab fungus (*Venturia inaequalis*) or the black rot fungus (*Botryosphaeria obtusa*). It is also one of the fungi involved in the development of core rot in apple fruits (Raina et al., 1971). The fungus is common under non-refrigerated conditions since its growth is greatly inhibited below 10°C. The decay is characterized by the production of pink conidia and by the bitter taste of the infected fruit.

Cultivar Resistance

Since decay pathogens are dependent on the presence of a wound to initiate infection, resistance of the epidermis (the skin) to breakage may be an important factor in the resistance of apple cultivars to decay.

Studying the force required to break the epidermis of several cultivars, as a criterion for resistance to wound pathogens, Spotts et al. (1999) found that the epidermis of Golden Delicious and Jonagold was more easily broken than that of other cultivars, while the epidermal tissues of Fuji and Granny Smith were the most resistant to puncture.

The resistance of apples of various cultivars to core rot infection, initiated by the penetration of *B. cinerea, P. expansum, Mucor piriformis* and other pathogens, via the sinus between the calyx and the core cavity (Combrink et al., 1985; Spotts et al., 1988), was evaluated according to the presence of open sinuses (Spotts et al., 1999). The highest percentage of fruits with open sinuses (with a mean of 38%) was recorded for Fuji fruits; Granny Smith and Braeburn had the fewest fruits with open sinuses, averaging 1.0 and 0%, respectively. Various biochemical and physiological factors may influence the resistance of the cortex tissue of apple fruits to decay pathogens. These include the presence of glycoprotein inhibitors of pectolytic enzymes of the pathogen (Brown, A.E., 1984) and the accumulation of the phytoalexinic compound, benzoic acid (Seng et al., 1985).

Control Measures

The choice of treatment for controlling postharvest diseases of pome fruits depends, to a large extent, on the nature of the pathogens involved, the source of infection and the time of infection (Edney, 1983; Eckert and Ogawa, 1988). The lenticel rots are caused largely by pathogens present in the orchard and this enables us to treat these infections by preharvest orchard sprays. In areas suffering from heavy losses as a result of lenticel rots, orchards are sprayed with fungicides in the late summer to suppress the production of inoculum of *Gloeosporium* spp. and *Nectria galligena* and to protect the fruit lenticels from quiescent infections (Corke and Sneh, 1979; Edney, 1983). Corke and Sneh (1979) found that the efficiency of preharvest systemic fungicides in reducing decay by *Gloeosporium perennans* was related to their ability to suppress fungal sporulation. After harvest, the fruits are treated with a systemic fungicide to suppress the development of quiescent infections of *Gloeosporium* in the lenticels. The control of the lenticel rotting is considered to be one of the greatest contributions made by systemic benzimidazole compounds following their introduction as postharvest fungicides in the late 1960s. It is their ability to reach the lenticels, which are located within the fruit peel, that enables these fungicides to control rotting (Leroux et al., 1975). However, the penetration ability of

these fungicides contributed to their persistence in the fruit throughout the storage period exerting continuous pressure for the selection of resistant strains of *Penicillium expansum* and *Botrytis cinerea*, which would not be controlled by postharvest treatments (Eckert and Ogawa, 1988).

Various fungicides with differing modes of action are applied after harvest, alone or in combination, to control postharvest infections initiated by wound pathogens, such as *P. expansum, B. cinerea, Alternaria alternata, Cladosporium herbarum* and *Aspergillus* spp. (see the chapter on Chemical Means). Protection against *Phytophthora* spp., which can invade apple fruits before harvest, should be provided by orchard sprays. A postharvest treatment has to contend with infections aged several days, and should also be able to prevent the contact spread of the pathogen during storage (Edney, 1978; Edney and Chambers, 1981). *Mucor piriformis*, which can also spread during storage, cannot be controlled by most of the fungicides; the common method for controlling this pathogen is the addition of chlorine or sodium *ortho*-phenylphenate to the washing water in the packinghouse, to reduce the level of pathogenic fungal spores brought into the water with the fruit (Spotts and Peters, 1982b).

Calcium treatments applied primarily to control physiological disorders in apples have also resulted in reduced fungal infection. Preharvest calcium sprays were found to reduce the incidence of storage lenticel rot caused by *Gloeosporium* spp. (Sharples and Johnson, 1977) and bitter rot caused by *Colletotrichum gloeosporioides* and *C. acutatum* (Biggs, 1999). A reduction in the rate of *P. expansum* rot was found after postharvest calcium application (Conway and Sams, 1983; Conway et al., 1987; 1994a). Although the reduction in disease development following calcium treatments has generally been attributed to the suppression of physiological diseases and the improved keeping quality of the fruit, direct effects of calcium on spore germination and colony growth have been recorded for several pathogens, such as *P. expansum, B. cinerea* (Conway et al., 1994a) and *Colletotrichum* spp. (Biggs, 1999). (See the chapter on Means for Maintaining Host Resistance – Calcium Application).

Several studies have reported on the ability of antagonistic microorganisms to control postharvest diseases of apples. Control of both *B. cinerea* and *P. expansum*, the two major pathogens of apple fruit, was achieved with several epiphytic bacteria isolated from apple leaves (Sobiczewski et al., 1996). The yeast, *Candida oleophila* was effective in

controlling *B. cinerea*, when applied to fresh wounds on apples (Mercier and Wilson, 1995), while *Candida sake* was effective against *P. expansum* when applied preharvest (Teixido et al., 1998). Preharvest application of *Bacillus subtilis*, the yeast *Rhodotorula* and the yeast-like fungus *Aureobasidium* suppressed postharvest decay of apples by *P. expansum, B. cinerea* and *Pezicula malicorticis* (Leibinger et al., 1997). Postharvest control of *P. expansum* rot in pear fruits, was achieved with antagonistic yeasts, used either as field sprays or as postharvest applications (Chand-Goyal and Spotts, 1996a). (See the chapter on Biological Control).

Refrigeration is the most commonly used method for suppressing decay after harvest. Apples are generally held at temperatures above 0°C, the optimal storage temperature varying with the sensitivity of the cultivar to chilling injury. Pear cultivars are able to benefit from storage at -1°C. Chilling the fruit in the packinghouse by refrigerated forced air or by hydrocooling slows ripening and reduces pathogen growth. The addition of chlorine to the cold water disinfects the fruit surface and prevents the buildup of pathogen propagules in the water (Eckert and Ogawa, 1988). Rapid removal of the field heat by hydrocooling to about 4.5°C markedly reduces *Monilinia* growth and arrests *Rhizopus* and *Colletotrichum* development, although the fungi remain alive and resume growth when the fruit is returned to higher temperatures.

Further extension of the storage life of apples can be achieved by controlled atmosphere storage, frequently accompanied by the removal of ethylene. This involves a careful regulation of the temperature, the humidity and the levels of oxygen and carbon dioxide in the atmosphere (see the chapter on Means for Maintaining Host Resistance – Modified and Controlled Atmospheres).

STONE FRUITS

Stone fruits are highly perishable and decay is the major problem in handling them, both for the fresh market and for processing (Eckert and Ogawa, 1988). The susceptibility of stone fruits diminishes in the order: cherries, nectarines, peaches, plums and apricots. The main postharvest pathogens of stone fruits are: *Monilinia* spp., *Botrytis cinerea, Rhizopus stolonifer, Penicillium expansum, Mucor piriformis, Colletotrichum gloeosporioides, Alternaria alternata* and *Cladosporium herbarum*. The relative importance of these pathogenic species differs in different fruits:

Penicillium, Alternaria and *Cladosporium* are more common in plums and cherries, while *Rhizopus* causes heavy losses mainly in peaches, nectarines and apricots.

A. *Monilinia* spp.

Three species of *Monilinia* are involved in brown rot of stone fruits: *M. fructicola* (Wint.) Honey, *M. fructigena* (Aderh. & Ruhl.) Honey and *M. laxa* (Aderh. & Ruhl.) Honey.

Brown rot is of major importance in stone fruits in many countries and, if the weather is wet and cool in the spring, it can attain epidemic levels in peaches, nectarines and apricots (Holz et al., 1998). There are, however, differences in the geographical distributions of the three *Monilinia* species. While *M. laxa* is a serious problem in apricots in the United States, it may attack all stone fruits to some extent in Chile and South Africa (Eckert and Ogawa, 1988). *Monilinia fructicola* is a most important pathogen in the Americas, South Africa, Japan, Australia and New Zealand, while *M. fructigena* is a common pathogen in some stone fruits in Europe and Asia, but not in the United States (Snowdon, 1990).

The fungi survive the winter as mycelium in rotted or mummified fruits in the orchard. In wet weather, abundant conidia are produced and are spread in the orchard by rain splash and wind (Tate and Corbin, 1978) or by insects. In addition to the asexual conidia, *M. fructicola* frequently produces stromata (sclerotial mat or resistant fungal tissue), that give rise to the sexual state - apothecia with ascospores (Biggs and Northover, 1985; Willetts and Hadara, 1984). In other species of *Monilinia* apothecial production is rare (Willetts and Hadara, 1984). Examining the conditions under which *M. fructicola*-infected fruit develop stromata and produce apothecia, Holz et al. (1998) found that apothecia were formed only from infected fruits that had been subjected to a drying and cold temperature incubation, during which a stromatization process takes place. In the orchard, apothecia are formed during the bloom, from mummies that have survived the winter. They commonly appear on orchard floors with natural vegetation or cover crops, which may create a habitat that reduces desiccation. Apothecial production has not been observed in infected fruits which are attached to trees, but only in those lying on moist soils (Hong et al., 1996; Willetts and Hadara, 1984). Liberated ascospores may serve as a major source of primary inoculum for initiating infection of blossoms (Hong et al., 1996).

The optimal temperature for daily discharge of ascospores from the apothecia is 15°C, although high discharge can occur at any temperature

within the range of 10-25°C. Ascospore germination and germ-tube elongation rise with the increasing temperatures, from 7 to 15°C (Hong and Michailides, 1998). Reduction in ascospore discharge at higher temperatures (up to 25°C) is due to the faster disintegration of apothecia. However, high temperatures can also expedite drying of the apothecia in orchards when air humidity is low. Such information may help in the development of warning systems and in scheduling fungicide application against brown rot infection.

Following invasion of apricot flower parts, the fungus may progress into young fruits, where infection remains quiescent until the fruit ripens (Wade and Cruickshank, 1992). Quiescent visible infections of *M. fructicola* have been described on plums (Northover and Cerkauskas, 1994), whereas symptomless latent infections were reported on cherries (Adaskaveg et al., 2000). Additional infection may occur while the fruits are maturing via stomata, via hair sockets in peaches (Hall, 1971) or directly through the skin. However, the most common mode of invasion is through preharvest wounds caused by insects, hail or other adverse weather, or via injuries sustained during harvest and postharvest handling.

Decay is characteristically firm (Byrde et al., 1973). In advanced stages, spore masses, which are often arranged in concentric rings, cover the surface of the affected area. Within a few days at room temperature, the entire fruit may be decayed. Brown rot can spread rapidly by contact infection, forming 'nesting' during storage and marketing.

B. *Botrytis cinerea* Pers. [perfect state: *Botryotinia fuckeliana* (de Bary) Whetzel]

This fungus is one of the major pathogens of stone fruits in all producing areas. It survives as sclerotia in the soil or on dead plant material. During cool weather the fungus sporulates and inoculum for infection may be readily available. Infection may be initiated throughout the growing season under natural orchard conditions. Fourie and Holz (1994) indicated that *B. cinerea* does not penetrate young nectarine and plum fruits via floral parts to establish latent infections as in pears and apples (DeKock and Holz, 1991; Tronsmo and Raa, 1977), strawberries and raspberries (Powelson, 1960; Dashwood and Fox, 1988), grapes (McClellan and Hewitt, 1973) and cucumbers (Elad, 1988); in nectarines and plums the pathogen is likely to be introduced by field infection of developing fruit. Conidia germinate in water on the surfaces of both green and mature fruits, and the germ

tubes may give rise to appressoria when they have reached lengths of 10-15 μm. Thin infection hyphae (or infection pegs) formed from the inner appressorium wall enter the substomatal cavity of the fruit via stomata. In the green fruit no further fungal growth occurs, probably because of resistance reactions characteristic of young tissue, such as the presence of preformed phenolic compounds or callose at the site of infection (Fourie and Holz, 1995). When nectarines are invaded by *B. cinerea* at an advanced stage of fruit maturity (near the picking-ripe stage), the majority of infection hyphae do penetrate the cuticle. In plum fruits at this stage of maturity, only a small number of successful penetrations have been recorded. In both nectarines and plums, however, susceptibility to infection increases with fruit maturity. Enhanced infection in mature nectarine fruits can also result from the appearance of cuticular micro-cracks on the fruit surface. These can provide alternative penetration routes for the pathogen (Fourie and Holz, 1995). The fruit can also be invaded via injuries sustained during harvesting and handling.

The gray mold rot is soft and, under humid conditions, produces abundant surface mycelium. Under dryer conditions sporulation is prolific and a mass of gray conidia are formed, which constitute a ready source for initiation of new infections.

C. *Rhizopus* spp.

Rhizopus stolonifer (Ehrenb. ex. Fr.) Lind and *Rhizopus oryzae* Went & Prinsen Geerligs cause Rhizopus rot, one of the most serious postharvest diseases of stone fruits (Hall and Scott, 1989b). It inflicts heavy losses, especially in peaches, nectarines and cherries. *Rhizopus* spp. exist on dead material and their asexual spores (sporangiospores) are disseminated in the air where they form important components of the air spora and are responsible for disease initiation. The fungi generally infect the fruit via injuries sustained during harvesting and handling. Infected areas are water-soaked and covered with a profuse white mycelium which gives rise to globular sporangia with new sporangiospores. The fruit becomes very soft and, at the progressive stage of the disease, releases juices having a sour odor. During storage a rotted fruit infects sound fruit by contact (Hall and Scott, 1989b), causing extensive 'nesting'.

The fungi tolerate high temperatures, having optima of 25°C for *R. stolonifer* and 35°C for *R. oryzae*, and infection is favored by a warm, moist environment (Pierson, 1966).

D. *Penicillium expansum* Link.

This fungus is a common postharvest pathogen of stone fruits in all producing areas, but can attack only injured or over-mature fruit. Fruits that have been heat treated to reduce incipient infections of brown rot are also sensitive to *Penicillium* infection (Smith, W.L. and Anderson, 1975). During storage, conidiophores bearing blue-green conidia are produced in coremia, sometimes arranged in concentric circles around the point of infection. A white edge remains around the rotten area as it develops (Hall and Scott, 1989b). The infected tissue is moist and characterized by a musty odor. Since the growth of *Penicillium* is greatly reduced at low temperatures, rapid pre-cooling and cold storage markedly suppress its development.

E. *Colletotrichum gloeosporioides* (Penz.) Sacc, [perfect state: *Glomerella cingulata* (Stonem.) Spauld & v. Schrenk]

This fungus is the causal agent of anthracnose. Some strains can exhibit the sexual state (perithecia with ascospores), but infection is generally initiated by the asexual spores (conidia produced in acervuli). The fungus penetrates the fruit mainly via wounds caused by twig abrasion while on the tree (Rittenburg and Hendrix, 1983), and disease symptoms may appear in the orchard. If infection occurs shortly before harvest and the disease cannot yet be detected in the packinghouse, it will develop later in storage. During harvesting and handling operations, infections may take place through wounds and can be spread from diseased fruit to sound fruit by contact.

Lesions are firm and sometimes tend to coalesce and cover the fruit (Ramsey et al., 1951). However, almost no progress takes place below 5°C. Under humid conditions, a mycelium-bearing salmon-pink mass of spores, ready to initiate new infections, is developed.

F. *Mucor piriformis* Fischer

This fungus is generally considered a disease of minor importance in stone fruits. However, following its isolation from several stone fruits, mainly peaches and nectarines, it has been regarded as a threat since, in contrast to *Rhizopus*, this pathogen can develop in cold storage even at 0°C (Smith, W.L. et al., 1979), and is not controlled by available fungicide treatments (Eckert and Ogawa, 1988).

Infection takes place when the asexual spores (sporangiospores within sporangia) penetrate wounded fruits and cause a soft, watery rot. During

storage the fungus produces a coarse, erect, white mycelium that gives rise to globular black sporangia with new sporangiospores.

G. *Alternaria alternata* (Fr.) Keissler

Alternaria rot is common mainly in plums and cherries. The fungus survives on dead material in the orchard and its spores are disseminated in the air. Infection is usually associated with injury that occurs during harvesting and handling. In cherries, fungal penetration can take place through cracking or splitting of the skin while the fruit is still on the tree (Snowdon, 1990). In apricots, the fungus can penetrate the sound fruit via stomata (Larsen et al., 1980).

Lesions are firm, slightly sunken, and bear a dense mat of olive-green or dark conidia.

H. *Cladosporium herbarum* (Pers.) Link

Cladosporium survives on dead plant material in the soil and produces an abundance of conidia, which are disseminated in the air, and constitute the most common component of the air spora (Gregory, 1973; Barkai-Golan et al., 1977b). *Cladosporium herbarum* is a weak pathogen infecting fruit that has been damaged by rain or rough handling. Plums that have been shaken from the tree and collected from the ground exhibited a particularly high incidence of infection (Michaelides et al., 1987).

Lesions are limited in area and covered with a velvety mat of dark green spores. Thanks to its low minimal growth temperature of -4°C (Sommer, 1985), *Cladosporium* can grow and infect the fruit even at cold storage temperatures.

Control Measures

Postharvest disease control of stone fruit pathogens consists of an integrated combination of pre- and postharvest fungicide applications, sanitation practices, cold storage, modified atmosphere storage and biological control techniques. Chilling the fruit, which is the most commonly used method, both slows ripening and inhibits the growth of most decay pathogens (Eckert and Ogawa, 1988).

To control infection by wound pathogens, such as *B. cinerea, P. expansum, A. alternata, R. stolonifer* and *M. piriformis*, control measures should include the prevention of mechanical damage during harvesting and handling, and application of sanitation practices in the orchard and in the packinghouse, to minimize the level of infective

spores. To control brown rot (*Monilinia* spp.) sanitation should include the removal of blighted flowering shoots and mummified fruits from the orchard (Holz et al., 1998) along with careful harvesting and handling to avoid injuries that would enable easy penetration of the pathogen into the fruit. Fungicidal sprays are generally necessary to protect the flowers from infection and to control brown rot in the orchard (Zehr, 1982). Postharvest fungicide treatments with dips, sprays or wax formulations containing a systemic fungicide have provided good control of *M. fructicola*. However, with the appearance of resistant strains of the pathogen, the use of unrelated chemicals has been recommended (Michailides et al., 1987). Combinations of different fungicides have been effective in controlling both brown rot and Rhizopus rot (Wade and Gipps, 1973). The effectiveness of fungicides against brown rot may be improved by combining them with a hot water treatment. Such a combination has enabled the concentration of the fungicide to be reduced without impairing the effectiveness of the treatment (Jones and Burton, 1973).

Hot water alone (52°C, 2.5 min) has been found to control incipient infections of *M. fructicola* and *R. stolonifer* in nectarines and peaches, but the control was accompanied by physiological injuries to the fruit. Combining hot water treatment with a fungicide enabled decay control to be achieved at a lower temperature, and no injury was observed during storage (Smith, W.L. and Anderson, 1975). However, Phillips and Austin (1982) found that some changes in peaches had already developed after treatment with hot water above 45°C. When stone fruits are immersed in a hot water fungicide bath, the time of immersion and the temperature should be carefully controlled to avoid fruit injury. Heat injury of the fruit may also lead to increased sensitivity to *P. expansum* infection (Smith, W.L. and Anderson, 1975).

Several calcium salts, particularly calcium propionate and calcium silicate, were found to reduce both the incidence and the severity of brown rot in wound-inoculated peaches (Biggs et al., 1997). The salts directly reduced *M. fructicola* growth and inhibited its pectolytic activity. Minimal growth and maximal inhibition of enzymatic activity occurred after calcium propionate application.

Several studies have been dedicated to the control of postharvest diseases of stone fruits by biological means. Whereas the first biocontrol studies focused on the use of antagonistic bacteria, later studies used mainly yeast species and epiphytic fungi. The control of brown rot and Rhizopus rot in peaches was achieved with the bacteria, *Bacillus subtilis*

and *Enterobacter cloacae* (Pusey and Wilson, 1984; Wilson et al., 1987b). *Bacillus subtilis* has also controlled both brown rot and Alternaria rot in sweet cherries (Utkhede and Sholberg, 1986). Two species of *Pseudomonas* were used to control brown rot in peach and nectarine fruits (Smilanick et al., 1993), while reduction of brown rot in sweet cherries was achieved with two epiphytic fungi, *Aureobasidium* and *Epicoccum* (Wittig et al., 1997). The combination of preharvest fungicide treatment with postharvest yeast application gave a better control of brown rot in sweet cherries than the fungicide alone, although the yeast alone did not affect disease development. Combining the double treatment with modified atmosphere packaging of sweet cherries resulted in a further increase in decay control and almost eliminated brown rot during storage (Spotts et al., 1998). The advantage of a combined biological and chemical control over the single treatments has been reported also for *P. expansum* in harvested nectarines (Lurie et al., 1995). (See the chapter on Biological Control).

III. SOFT FRUITS AND BERRIES

The title applied to this group of fruits by Snowdon (1990) indicates the complex of fruits involved. They include strawberries and raspberries, which are not true berries, and blueberries, cranberries and gooseberries, which are berries that grow on bushes. Snowdon (1990) added to these grapes and kiwifruits, which are berries that grow on vines. The fruits in this group are not related botanically but are all native to the temperate zones. They are all very delicate and should be treated with great care. Their storage life is greatly shortened by both physiological and pathological deterioration. However, decay development is the primary cause of loss in soft fruits, and any treatment capable of controlling or delaying decay and thus prolonging the storage life by a few days, is appreciated.

The major postharvest pathogen of fruits in this group is *Botrytis cinerea*, the causal agent of gray mold. Species of *Mucor* and *Rhizopus*, the causal organisms of "leak" disease, are major pathogens of strawberries and raspberries. Other fungi responsible for postharvest rots include *Colletotrichum* spp., which cause anthracnose in strawberries, raspberries and blueberries, and *Phytophthora* spp., which initiate leather rot in strawberries (Snowdon, 1990). *Alternaria alternata*, which is of minor importance in strawberries and raspberries,

may be one of the main pathogens of blueberries and gooseberries (Dennis et al., 1976). Similarly, *Phomopsis* spp., which are generally of minor importance in strawberries, may be of greater importance in harvested blueberries (Milholland and Daykin, 1983). Other fungi responsible for postharvest rotting include species of *Cladosporium, Penicillium* and *Pestalotia* (Snowdon, 1990). The incidence of the less common or minor pathogens may vary from year to year, from season to season and from place to place, suggesting that climate factors may affect the amount of inoculum available or the infection resistance of the fruit (Dennis, 1983a).

STRAWBERRIES AND RASPBERRIES

A. *Botrytis cinerea* Pers. [perfect state: *Botryotinia fuckeliana* (de Bary) Whetzel]

The gray mold fungus is a most important pathogen of soft fruits, worldwide. It survives on organic debris in the field, frequently as sclerotia, and under favorable conditions it sporulates, releasing quantities of spores that serve as a potential source of inoculum for infection of flowers (Braun and Sutton, 1987). Temperature and duration of humidity have the greatest effects on inoculum production: the optimal temperature for sporulation is between 17 and 18°C, but under sustained humidity the temperature range for profuse sporulation extends to 15-22°C (Sosa-Alvarez et al., 1995). Under alternating wet and dry periods, the total duration of high humidity and the length of wet periods determine the amount of inoculum produced. No sporulation occurs at 30°C.

During the flowering and fruiting season fungal spores are common in the atmosphere (Jarvis, 1962), and at harvest time the fruit is already contaminated with *Botrytis* spores. Conidia are deposited on flowers by air or water (Braun and Sutton, 1987) and primary infection of strawberries takes place through senescent floral parts where the conidia remain quiescent in the base of the receptacle. Disease is manifested as stem-end rot only when the fruit ripens (Powelson, 1960), so that the fruits may be harvested in apparently sound condition only to decay during transit and marketing (Aharoni and Barkai-Golan, 1987).

In raspberry fruits the fungus invades stigmas and styles, resulting in infection of individual fruitlets (Williamson and McNicol, 1986). Mummified fruits and receptacles entirely covered with fungal spores are often found on raspberry plants. After harvest, infections of strawberries

and raspberries can arise, either from germination of spores on the surface of the fruit or from renewed growth of the quiescent infection that had been initiated in the field. Davis and Dennis (1979) found that the majority of strawberry infections occurring during storage appear on the surface of the fruit and only a small proportion are initiated from quiescent infections at the stem-end. Sporulation on diseased flowers and fruits becomes an important source of secondary inocula in annual production systems where flowers are continuously produced over several months (Legard et al., 2000).

The decay is brown and firm, and its surface becomes covered with an abundance of conidiophores bearing gray-brown conidia. Under humid conditions, a white to gray mycelium with only a few spores characteristically grows over the lesions (Dennis, 1983a). Sclerotia are rarely found, and their development is dependent on *Botrytis* strains or isolates and environmental conditions. Having a minimal growth temperature of about -2°C, the gray mold is capable of development even at the low temperatures used for storage (Dennis and Cohen, 1976). The decay can spread readily by contact between rotten and healthy fruits, causing 'nesting' during storage (Sommer et al., 1973). Rapid reduction of the temperature from the field level to 1°C, and low-temperature storage can only delay fungal development.

B. *Rhizopus* spp. and *Mucor* spp.

These fungi are responsible for the "leak" disease, a destructive soft watery disease of soft fruits. The *Mucor* species involved are *M. piriformis* Fischer and, occasionally, *M. hiemalis* Wehmer. The *Rhizopus* species are *R. stolonifer* (Ehrenb. ex Fr.) Lind, a heterothallic species (requiring the presence of two physiologically compatible mycelia for sexual reproduction), and in some areas *R. sexualis* (Smith) Callen, a homothallic species (having a self-fertile mycelium).

Mucor and *Rhizopus* are soil inhabitants but, unlike *Botrytis*, they do not sporulate on plant debris in the field. Sporulation usually occurs only on infected ripe fruits. The soil-borne inocula of the two fungi cause limited early infection in the plantation. After being established in ripe fruits the inoculum level increases and the latter part of the season is characterized by enhanced contamination and higher rates of infection by these fungi (Dennis, 1978). Infection is initated by the asexual spores (sporangiospores). Germination of sexual spores (zygospores) of *R. stolonifer* has been observed only rarely (Alexopoulos, 1961), while those of

R. sexualis have never been observed to germinate (Snowdon, 1990). Direct infection can occur if ripe fruits touch the ground or become contaminated by splashing rain (Harris and Dennis, 1980). Infection progresses rapidly, especially in injured fruits.

Infections of *Mucor* and *Rhizopus* result in a water-soaked appearance. In *Mucor*, infections in the invaded area rapidly become covered with visible black sporangia containing the asexual spores (sporangiospores). These spores are the inocula for further infection, both in the field and after harvest. Under warm and dry conditions, *Rhizopus* spp. can initiate a similar infection. At their progressed stage of development, both *Mucor* and *Rhizopus* are capable of breaking down the fruit tissue and so causing juice leaks. During storage and marketing further rotting is caused by contact infection between infected and healthy fruits and through contamination with spore-laden juices.

At 5°C or below, *Rhizopus* rots can be controlled. Harris and Dennis (1980) showed that asexual spores of *R. sexualis* were inactivated at 0°C and did not recover their viability when the fungus was moved to a higher temperature. *Mucor piriformis* can, however, infect the fruit and develop even at 0°C.

BLUEBERRIES AND GOOSEBERRIES

The most important causes of postharvest decay of **blueberries** are *Botrytis cinerea* Pers., *Alternaria alternata* (Fr.) Keissler and, in some instances, *Alternaria tenuissima* (Kunze:Fr.) Wiltshire, *Colletotrichum* spp. and *Phomopsis vaccinii* Shear. In many cases the stem scar is the predominant site of infection for various pathogens of blueberries (Ceponis and Cappellini, 1983; Milholland and Daykin, 1983). *Mucor piriformis* Fischer, the cause for "leak" disease in strawberries and raspberries, has been reported by Dennis and Mountford (1975) as an important pathogen of blueberries as well. Species of *Monilinia*, which cause the brown rot of stone fruits, are important field pathogens of blueberries (Barta, 1987). *Monilinia* invades the berries through the flowers, giving rise to diseased fruits that either fall to the ground and turn into 'mummies' or are harvested along with healthy berries. This is why *Monilinia* species are also included among the postharvest pathogens of blueberries.

The major postharvest pathogens of **gooseberries** are *B. cinerea, M. piriformis* and *A. alternata. Botrytis* invades the fruit in the field via

the senescent calyx. Infection of the fruit by *Mucor* frequently occurs following contamination by soil splash after heavy rain (Dennis, 1983a). Infection by *Alternaria* (and sometimes also by *Stemphylium*) via the calyx end similarly occurs in the field prior to harvest, and may result in premature fall of infected berries from the bushes. The fungus initially colonizes the seeds. However, when the fruits are stored at ambient temperatures, the fungus can progress and begin to infect the pericarp.

Control Measures

Chemical control of postharvest diseases of soft fruits has mainly focused on the suppression of *B. cinerea*, the most important pathogen of these fruits. Since infection originates in the field, fungicidal sprays during the flowering period are the first step in controlling the disease. Preharvest fungicidal application of benzimidazole successfully controlled decay in the early 1970s (Jordan, 1973). Following the emergence of *B. cinerea* strains resistant to these chemicals, they have been replaced by fungicides of the dicarboximide group, and preharvest spraying of strawberries with iprodione during the flowering period provided good control of the gray mold disease during storage (Aharoni and Barkai-Golan, 1987). However, these fungicides, similarly to the benzimidazoles, are not effective against Phycomycetes fungi, such as *Rhizopus* spp. and *Mucor* spp. (Eckert and Ogawa, 1988), and *B. cinerea* strains resistant to the dicarboximides have also developed (Hunter et al., 1987). Postharvest fungicidal applications are not practical for ripe berries because of their sensitivity to wetting.

A rapid reduction of the temperature from the field level to below 5°C is important for retarding decay development in strawberries during shipment (Harris and Harvey, 1973). This is currently achieved by forced-air cooling, although hydrocooling, which removes field heat from the berries more rapidly than air cooling, cleans them and does not cause moisture loss. The latter has not been recommended because wetting may lead to excessive decay (Mitchell, 1992). In a recent study of the decay hazards associated with hydrocooling, Ferreira et al. (1996) concluded that hydrocooling, with the addition of proper chlorination, has promise as a method for rapid cooling and cleaning of strawberries as well as for reducing the level of *B. cinerea* inoculum on the fruit surface. These investigators considered that if cooled, good-quality berries were subsequently stored at the low temperatures recommended (0 to 1°C), the risk associated with hydrocooling would be minimal.

Elevating the CO_2 content of the atmosphere to 20-30% is a known procedure for suppressing decay in strawberries during transit. A level of 20% CO_2 both reduces decay in strawberries and retards their softening, without injuring the berries or impairing their flavor (Harris and Harvey, 1973). Reduced decay can also be achieved by wrapping strawberries in polyvinyl chloride (PVC) film which leads to the accumulation of a high level of CO_2 within the package (Aharoni and Barkai-Golan, 1987). Low O_2 concentrations can reduce both *B. cinerea* and *R. stolonifer* decay in stored strawberries, but excessively low O_2 results in the development of persistent off flavors in the fruit as a result of the accumulation of alcohols and aldehydes in the tissue (Sommer, 1982). Storing blueberries in 15-20% CO_2 at 2°C for 7-14 days reduced the decay caused mainly by *B. cinerea, Alternaria* spp. and *Colletotrichum* spp. and prolonged the shelf life of the fruit (Ceponis and Cappellini, 1985).

Several investigators have studied the reduction of postharvest diseases of strawberries by antagonistic organisms. Preharvest spraying of strawberry flowers with antagonistic non-pathogenic isolates of *Trichoderma* species reduced the incidence of gray mold in storage (Tronsmo and Dennis, 1977). Storage diseases of both *B. cinerea* and *R. stolonifer* were reduced after application of the yeast-like fungus, *Aureobasidium pullulans* to strawberries grown under plastic tunnels (Lima et al., 1997). The antagonistic effect was more pronounced when the fungus was applied at the flowering stage, and under these conditions the antagonistic fungus was more effective than the fungicidal treatment (vinclozolin). Moline et al. (1999) found that of the many bacteria isolated from the surface of non-treated strawberries, the most effective ones were species of *Pseudomonas* and *Chryseobacterium*, which were capable of reducing the incidence of gray mold under field conditions.

GRAPES

The major postharvest problems of grapes are desiccation, bruising and decay (Capellini et al., 1986), decay being directly related to bruising. The main decay pathogens of cold-stored grapes at -1°C are *Botrytis cinerea, Cladosporium herbarum, Alternaria* spp., *Rhizopus stolonifer, Aspergillus niger* and *Penicillium* spp. (Hewitt, 1974; Nelson, 1985).

A. *Botrytis cinerea* Pers. [perfect state: *Botryotinia fuckeliana* (de Bary) Whetzel]

The gray mold rot caused by *B. cinerea* is the most important disease of grapes, and can cause very heavy losses in all grape-producing countries, especially after wet seasons (Pearson and Goheen, 1988).

The fungus persists in the soil and can be found in infected vines and on decaying plant material. The pathogen exhibits both the sexual state (apothecia with ascospores) and the conidia, which are the asexual state (Snowdon, 1990). Both sporulation and infection take place under wet conditions. When late-harvested grapes have been exposed in the field to high humidity, dew and rainfall, a blossom infection may occur; the fungus develops a quiescent infection in the young developing fruits that cannot be eradicated by the SO_2 treatment (Eckert and Ogawa, 1988). The fungus resumes activity when the fruit matures (Marais, 1985). Thus, grapes which were apparently sound when harvested and packed may develop rotting only at a later stage (Nelson, 1956). Secondary infection spreads by contact during storage, resulting in the formation of 'nests' of decay among the grapes.

Decay is manifested by a brown discoloration, which later becomes covered with an abundance of gray-brown spores (Marais, 1985). Under humid conditions, a sporeless mycelium is developed.

B. *Cladosporium herbarum* (Pers.) Link

This species is the cause of Cladosporium rot in stored grapes. It is an important cause of spoilage because of its ability to develop in grapes in cold storage.

The pathogen has frequently been reported as the most important airborne fungus (Gregory, 1973). Primary infections occur before harvest. The fungus is capable of penetration through the intact fruit or at the blossom end of the berry and infection is favored by wet seasons (Hewitt, 1974). The disease is characterized by circular black spots or lesions which, after removal to shelf-life conditions, become covered with olive-green conidia-bearing mycelium. The decay is shallow and does not extend to the seeds. The affected tissue is attached to the skin and can be removed with it (Harvey and Pentzer, 1960).

C. *Alternaria alternata* (Fr.) Keissler

This fungus develops on dying flowers that then become a source of airborne spores. Infection can occur on any part of the fruit but is often initiated at the stem end. Disease initiation and development are favored

by wet weather (Hewitt, 1974). The lesion is dark brown and firm and the margins of the infected area are less distinct than those of Cladosporium rot (Harvey and Pentzer, 1960).

D. *Aspergillus niger* v. Tieghem

This pathogen can be a serious problem when grapes are marketed at ambient temperatures (Mandal and Dasgupta, 1983). The fungus survives on plant debris in the soil at high temperatures (25-30°C) and airborne spores infect berries via injuries caused by insect punctures, splits and stem-end fractures. Infection occurs only in mature berries; young berries are resistant to infection even when wounded (Abdelal et al., 1980). Infection can also take place through injuries incurred during handling. Since the fungus does not grow at temperatures below 5°C, cold storage is effective in suppressing disease development (Matthee et al., 1975).

E. *Penicillium* spp.

Several species of *Penicillium*, including *P. citrinum* Thom, *P. cyclopium* West. and *P. expansum* Link, can infect harvested grapes causing the blue mold rot (Barkai-Golan, 1974). Although infection can sometimes be initiated before harvest, the disease is usually associated with wounds and cracks in the skin at harvest (Harvey and Pentzer, 1960). Infection is induced in injured berries by conidia, which are dispersed by air currents, winds and insects. Decay continues to develop, although at a very slow rate, during the prolonged cold storage of grapes at 0°C (Harvey and Pentzer, 1960), and it may spread through the bunch.

F. *Rhizopus stolonifer* (Ehrenb. Ex Fr.) Lind and *R. oryzae* Went & Prinsen Geerligs

Rhizopus rot, incited by both of these species, can become a problem for grapes that are marketed at ambient temperatures (Mandal and Dasgupta, 1983).

The fungi exist and sporulate in the soil and on plant debris. Asexual spores (sporangiospores) are spread by air currents and primary infection is via injuries (Barbetti, 1980). *R. oryzae* is also capable of penetrating the intact skin of mature berries in the presence of exuded grape juice. Infection continues to spread during storage, when rotted berries infect adjacent healthy berries by contact. The infected tissue rapidly becomes covered with sporangia (white when young, later turning black), which are borne on sporangiophores that arise in clusters on the white mycelium.

Both species have high optimal growth temperatures (30-35°C for *R. oryzae* and 20-25°C for *R. stolonifer*) and their development can thus be suppressed, similarly to that of *A. niger*, by refrigeration. Growth is resumed, however, on transfer of the fruits to shelf-life conditions.

Control Measures

Minimizing the amount of debris in the vineyard by sanitation measures, and injury prevention by gentle handling of the berries are important for reducing postharvest diseases initiated by wound pathogens. Thinning of bunches may also result in reduced cracking and splitting (Barbetti, 1980).

Cold storage after rapid removal of the field heat is the basic means for decay suppression in grapes, while keeping them in a cool room throughout marketing will suppress decay at the end of the marketing chain (Beattie and Dahlenburg, 1989). The classic fungicidal means for decay control is periodical fumigation of the fruit with sulfur dioxide gas (Eckert and Ogawa, 1988), but this treatment may result in damage to the berries and in deposition of sulfite residues on their surface (Marois et al., 1986). Looking for alternatives to SO_2, Forney et al. (1991) found that vapor-phase hydrogen peroxide suppressed germination of *B. cinerea* conidia on grapes and reduced the incidence of decay in non-inoculated fruits, without affecting their color or soluble solids contents. Fumigation of grapes with low concentrations of acetic acid was suggested by Sholberg et al. (1996) as a suitable alternative to SO_2 for controlling decay in cold storage. At 0.27% (vol/vol), acetic acid-treated grapes did not show any phytoxicity and there were no differences between SO_2 and acetic acid treatments regarding fruit color and composition. In addition to table grapes, wine grapes could also benefit from fumigation with acetic acid. Acetaldehyde vapors have also been considered as a possible treatment for controlling postharvest decay in grapes (Avissar and Pessis, 1991). However, acetic acid was found to be much more inhibitory to *B. cinerea* spores than acetaldehyde, and the latter has been reported to have some adverse effects on fruit quality, such as damage to the berries and an off-flavor in Thompson Seedless grapes (Pesis and Frenkel, 1989).

KIWIFRUIT

Several fungi are responsible for storage decay of kiwifruit. They include *Botrytis cinerea, Penicillium* spp., *Phomopsis actinidiae,*

Alternaria alternata and species of *Colletotrichum, Botryosphaeria* and *Phoma* (Hawthorne et al., 1982; Opgenorth, 1983; Pennycook, 1985; Sommer et al., 1994).

A. *Botrytis cinerea* Pers. [perfect state: *Botryotinia fuckeliana* (de Bary) Whetzel]

The gray mold rot caused by *B. cinerea* is the most serious disease of kiwifruit in storage (Sommer et al., 1994). *Botrytis* conidia are capable of surviving on the surface of kiwifruit, where they remain viable and infectious throughout the growing season (Walter et al., 1999). Under moist conditions invasion may take place via senescent floral parts at the blossom end, after which the fungus remains quiescent for some time in storage before resuming activity (Opgenorth, 1983; Sommer et al., 1983). *Botrytis* may also penetrate the fruit through the cut stem or through wounds in the skin (Sommer et al., 1983). Conidia on the leaves and those accumulated on the hairy surface of kiwifruit are the major sources of inoculum for infection of the picking wound at harvest or after it (Pennycook and Manning, 1992).

Infection results in soft rot at the site of penetration, and it continues to develop, although very slowly, at 0°C. During prolonged storage the fungus can spread by contact from decayed to sound fruit, causing a typical 'nesting' of gray mold rot. In addition to the direct losses caused by *B. cinerea*, infected fruit produce ethylene in cold storage, which accelerates softening of healthy fruits (Brook, 1991).

B. Other Pathogens

Species of *Phoma, Colletotrichum* and *Botryosphaeria* are capable of colonizing dying flower parts and entering the young fruits on the tree. However, they remain quiescent at the site of penetration until the fruit ripens after harvest. Hence, the name 'ripe rot' frequently given to these rots (Snowdon, 1992). *Phomopsis actinidiae* (perfect state: *Diaporthe actinidiae* Sommer & Beraha) may initiate stem-end rots when the fruit ripens (Hawthorne et al., 1982). Although the fungal development is suppressed in cold storage, growth is resumed when the fruit is transferred to shelf-life conditions. Decay can also be caused by several species of *Penicillium,* which penetrate the fruit via injuries. *Alternaria* species may grow on the fruit surface but are not considered primary pathogens (Eckert and Ogawa, 1988).

Control Measures

Cold storage suppresses decay development, although *B. cinerea* grows slowly even at 0°C. In order to prevent softening of kiwifruit during storage, the environment must be kept free of ethylene, which enhances the ripening process (see the chapter on Factors Affecting Disease Development – Effects of Ethylene). Care must be taken, therefore, to exclude damaged, diseased or over-mature fruits that produce ethylene, and to avoid storage together with ethylene-producing commodities, such as apples and pears (Snowdon, 1992). Storage life can be further extended by the use of controlled atmosphere (Arpaia et al., 1987) or modified atmosphere with ethylene removal (Ben-Arie and Sonego, 1985).

Reduction of postharvest decay can also be achieved by curing kiwifruit after harvest. Looking for the optimum conditions for curing, Bautista-Baños et al. (1997) found that holding the fruit at 10-20°C in relative humidity higher than 92% for no more than 3 days, resulted in the lowest subsequent disease incidence, without lowering fruit quality during cold storage.

Postharvest decay, which often results from latent infection initiated in senescent floral parts or stem-end scars (receptacles) in the vineyard, can be reduced by preharvest fungicidal sprays (such as iprodine or vinclozolin) beginning at the end of the blossom period (Beever et al., 1984). Michailides and Morgan (1996) found a direct relationship between the incidence of *B. cinerea* in the fruit sepals and receptacles and the incidence of gray mold after several months of storage; they therefore suggested that the predicted incidence of decay in storage based on the rate of latent infections in the vineyard could be used to determine when preharvest fungicidal sprays are needed and justified.

IV. SOLANACEOUS FRUIT VEGETABLES

'Fruit vegetables' of the family Solanaceae include tomatoes, peppers and eggplants. The main pathogens of these fruits are *Botrytis cinerea, Alternaria alternata, Erwinia carotovora, Rhizopus stolonifer, Mucor* spp., *Geotrichum candidum, Fusarium* spp., *Phytophthora* spp. and *Rhizoctonia solani.* Of these, *B. cinerea, A. alternata* and *E. carotovora* are the most important pathogens of fruits from any region. In general, *B. cinerea* is the major cause of loss in temperate countries, while in hotter areas the common causes of decay are *R. stolonifer, G. candidum*

and *E. carotovora* (McColloch et al., 1982), which are characterized by high optimal growth temperatures. Other pathogens may be of local or occasional importance. Various fungi, such as *Trichothecium roseum* and species of *Phomopsis, Colletotrichum, Phoma, Pythium, Sclerotinia, Stemphylium, Cladosporium, Penicillium* or *Aspergillus*, may also be involved in fruit decay but are considered of minor importance.

A. *Botrytis cinerea* Pers. [perfect state: *Botryotinia fuckeliana* (de Bary) Whetzel]

This fungus is generally regarded as the major pathogen in greenhouse-grown tomatoes and peppers, while *Alternaria* is of greater importance in field-grown crops (Dennis, 1983b). *B. cinerea* can persist in the soil or on plant debris as sclerotia and, under cool, moist conditions, it produces an abundance of asexual spores (conidia) which are dispersed by air currents. The pathogen can penetrate young fruits through senescent flower parts (Lavy-Meir et al., 1988), through growth cracks and wounds or via the stem end, before or during harvesting. However, if the fruits are weakened by exposure to chilling in the field or during storage, they become susceptible to direct penetration, and lesions may develop anywhere on their surface (McColloch et al., 1982). In addition to penetrating chill-injured fruit, the fungus can directly penetrate the cuticle of immature fruit but, in this case, further growth is arrested and 'ghost spots' are formed on the growing fruit (Verhoeff, 1974).

The infected tissue is soft and water-soaked. Under humid conditions, an abundance of gray-brown conidia, sometimes accompanied by black sclerotia, are formed and serve as inoculum for new infections. A considerable proportion of the rots caused by *B. cinerea* spread during storage by contact between infected and sound fruits.

B. *Alternaria alternata* (Fr.) Keissler

This is a very common pathogen of solanaceous fruits (Barkai-Golan, 1981), which can limit their commercial life after harvest. The fungus survives on plant debris and its conidia are very important components of the air spora.

Alternaria is a weak pathogen that requires injured or weakened tissue for penetration and development. When free water forms on the surface of ripening fruit from rain, dew or overhead irrigation, spores germinate in response to water-soluble nutrients on the fruit surface (Pearson and Hall, 1975). The fungus can enter via growth cracks, insect injuries or mechanical damage, but infection is often initiated at the

calyx scar (Dennis 1983b; Fallik et al., 1994b). Incipient infections, which may appear at the margin of the stem scar, remain quiescent unless the fruits are subjected to weakening conditions, including chilling injury, sunscald or over-maturing. The enhancing effects of chilling and of high-temperature treatments on the susceptibility of tomato fruits to *Alternaria* injection has been exhibited at both the mature-green and mature development stages (Barkai-Golan and Kopeliovitch, 1989). Prolonged storage can also increase fruit susceptibility. Although the optimal growth temperature of the fungus is 28°C, it may continue growing during storage, thanks to the relatively high temperatures recommended for these cold-sensitive fruits.

A. alternata infections result in firm lesions, slightly sunken and covered in dense, olive-green to black masses of conidia (McColloch et al., 1982). Pearson and Hall (1975) described incipient infections of *A. alternata* on green fruits, which failed to resume activity when the fruits ripened. This phenomenon resembles the 'ghost spotting' described by Verhoeff (1974) in green tomatoes infected by *B. cinerea.*

The development of *A. alternata* in tomatoes may be associated with the production of several non-specific toxic metabolites in the infected fruits. Stinson et al. (1981) found that the main toxin in infected tomato fruits was tenuazonic acid; others, such as alternariol, alternariol monomethyl ether and altenuen were found in much smaller amounts. However, working with another strain of *A. alternata*, Ozcelik et al. (1990) reported that the major toxin in infected tomatoes was alternariol, followed by alternariol methyl ether. Fungal growth, along with toxin production, occurred at temperatures between 4 and 25°C.

C. *Erwinia carotovora* ssp. *carotovora* (Jones) Dye and *Erwinia carotovora* ssp. *atroseptica* (van Hall) Dye

These soft rot bacteria are among the most common postharvest pathogens of tomatoes and peppers, and are found in the soil and plant debris wherever they are grown. These pathogens are spread by splashing of contaminated soil, by wind, by insects and by postharvest washing (Bartz, 1982). They are wound pathogens, requiring moisture to initiate infection, and entering the fruit under warm, wet conditions, through breaks in the skin, the cut stem or any injury incurred during picking and packing (Volcani and Barkai-Golan, 1961; Sherman et al., 1982). Growth and multiplication of the bacteria are favored by wet conditions and temperatures of 24-30°C. Under these conditions, the entire fruit may rot in a few days.

The infected tissue becomes soft and water-soaked and, later, the skin may split liberating an infecting liquid. Decay spreads rapidly from fruit to fruit, forming nests of decay, especially under shelf-life conditions at temperatures above 20°C. Keeping the fruits under refrigeration during transport and storage suppresses bacterial growth.

D. *Rhizopus stolonifer* (Ehrenb. Ex Fr.) Lind

This species is the cause of Rhizopus rot, which can occur wherever tomatoes, peppers and eggplants are grown. In tropical countries another species, ***R. oryzae* Went & Prinsen Geerligs**, is common (Snowdon, 1992).

R. stolonifer is widely distributed in the soil and in the atmosphere and, although it may produce sexual spores (zygospores), it commonly exists in the asexual state as sporangia and sporangiospores. Spore germination occurs in a warm, moist environment and penetration takes place via injuries or damaged tissues (Barkai-Golan and Kopeliovitch, 1981). The infected area of tomatoes and peppers appears water-soaked through the distended skin, which often ruptures and releases an abundance of liquid. Sporulation occurs on the surface of infected fruits and the disease is spread to adjacent fruit, resulting in extensive 'nesting' during storage (Barkai-Golan and Kopeliovitch, 1981). However, the disease develops slowly in fruits stored at the recommended temperatures.

E. *Geotrichum candidum* Link ex Pers.

This fungus is responsible for the sour rot in stored tomatoes. It is a soil-borne pathogen whose spores are transmitted mainly by insects. Flies are known to transfer spores from rotten fruits to healthy fruits and also to create wounds that enable fungal penetration (Butler, 1961). The fungus is a wound pathogen, infecting both mature-green and ripe fruits via the stem scar or any wound on the fruit surface. If fruits are infected shortly before harvest, symptoms will not be visible at harvest and the decay can develop during storage or marketing.

G. candidum has a high optimum growth temperature (about 30°C) and can develop under warm, moist conditions. The infected tissues in ripe fruits are soft and watery whereas those in mature-green fruits are characteristically water soaked. In both cases decay is accompanied by a sour odor. At an advanced stage, the infected skin splits and a white-to-creamy mycelium begins to develop on the exposed tissue and forms conidia (oidia or arthrospores) by fragmentation of the hyphae (Butler, 1961).

F. *Fusarium* spp.

Several *Fusarium* spp. may cause Fusarium rot of tomatoes, peppers and eggplants. The most frequently recorded are *F. equiseti* (Corda) Sacc., *F. avenaceum* (Corda ex Fr.) Sacc., *F. moniliforme* Sheldon, *F. solani* (Mart.) Sacc. and *F. oxysporum* Schlecht (Snowdon, 1992).

The *Fusarium* spp. are common inhabitants of the soil. The conidia are dispersed by wind or water, and fruits arriving into storage may already be contaminated with them. The fungi are weak pathogens that infect only wounded fruits or those that have been weakened by chilling temperatures. Fruits stored at too low temperatures for a long time are particularly susceptible to infection.

The decayed area is usually water-soaked and becomes covered by a mycelium which may be white, yellow or pinkish, according to the *Fusarium* involved. The rot extends into the center of the fruit and the infected tissue appears pale brown (Dennis, 1983b).

G. *Phytophthora infestans* (Mont.) de Bary

This fungus induces late blight of potatoes and tomatoes and may cause serious losses in many areas during wet seasons. Infection is initiated by the asexual state (sporangia and zoospores), although *P. infestans* can also exhibit the sexual state (oospores) (Hermansen et al., 2000).

Disease development depends on weather conditions prior to harvesting. The optimal temperature for infection is 15-18°C, and wet conditions are essential for infection capability of the zoospores (McColloch et al., 1982). Infection of leaves, stems and fruits may be by germinating sporangia or may follow the release of zoospores from the sporangium (Alexopoulos, 1961). The zoospores, which gain mobility from their flagella, swim in the film of rainwater on the host. Infection frequently takes place at the edge of the stem-scar although, under optimal conditions, direct penetration of the fruit skin can also occur (Eggert, 1970). Tomatoes harvested from infected fields may be in the initial stages of infection, when lesions are not yet visible. Such fruits are packed and decay can occur during transit or at the market.

The infected area is brown, or rusty-tan, hard and with irregular margins. There is little spreading from diseased to healthy fruit in transit, in the ripening room or in storage.

H. *Phytophthora* spp.

Several species of *Phytophthora* induce Phytophthora rot of tomatoes, peppers and eggplants, the most common species being *P. nicotianae* var.

parasitica (Dastur) Waterh. and *P. capsici* Leonian (Snowdon, 1992).

These species are soil inhabitants and can survive in seeds as thick-walled oospores (the sexual state) or chlamidospores. Infection takes place in the field in warm, wet conditions, which favor the production of thin-walled sporangia, the asexual state (Bhardwaj et al., 1985) giving rise to numerous zoospores, which are swimming spores that must have free water to survive and cause fruit infection. They are capable of penetrating both unripe and ripe uninjured fruit. Infection is generally confined to fruits growing low down on the plant, near to or in contact with the soil (Springer and Johnston, 1982). When disease development is interrupted by harvest, growth continues afterwards and may be spread to healthy fruits by contact during storage.

In peppers, infection frequently originates at the stem end, whereas in eggplants lesions often appear near the distal end. Phytophthora rot of tomatoes is usually called 'buckeye rot' and is characterized by the formation of concentric, brown rings around the point of disease initiation. In humid conditions, an off-white mycelium, giving rise to numerous sporangia, is developed on the surface of the infected area (McColloch et al., 1982). The occurrence of the disease depends upon weather conditions, regardless of geographical location.

I. *Rhizoctonia solani* Kühn [perfect state: *Thanatephorus cucumeris* (Frank) Donk]

This fungus causes soil rot or Rhizoctonia rot, which is of importance mainly in tomatoes (Baker, K.F., 1970). *Rhizoctonia* is a soil-inhabiting fungus, which produces both the sexual state (a hymenium giving rise to basidiospores) and the sterile state (sclerotia). Infection occurs under wet conditions by splashing of contaminated soil onto the low-hanging fruits (Baker, K.F., 1970), but fruits in contact with the ground are generally infected by penetration of the fungus from the soil through injuries (Murphy et al., 1984). The disease develops on both green and ripe fruits and is common in tomatoes intended for processing, since they are generally grown without stakes and many of them remain in contact with the soil until fully ripe. The rate of infection depends upon weather conditions (McColloch et al., 1982): the optimal growth temperature is 26-27°C and there is little development below 10°C. Tomatoes that were infected in the field but showed no evidence of decay, or were overlooked during grading and packing, may decay during transit.

Typical spots on ripe fruits are firm and reddish brown, while the infected flesh is soft or even watery. At an advanced stage of

development, a white or gray mycelium may cover the lesion, and the fungus can spread from diseased to healthy fruit by contact (Strashnov et al., 1985).

J. Other Pathogens

Phomopsis spp. (perfect state: *Diaporthe* spp.)

These fungi are of minor importance in tomatoes and peppers, but infection of eggplants by *Phomopsis vexans* (Sacc. & Sydow) Harter may result in destructive decay during transit or storage. In fact, Phomopsis rot is included among the common diseases of eggplants. The fungus may exhibit the sexual state (perithecia and ascospores) (Gratz, 1942) although infection is usually induced by the asexual conidia which are borne within pycnidia. The fungus reaches the host in rainwater, germinates and penetrates directly through the healthy tissue of leaves, stems and fruits (Divinagracia, 1969). Fruits infected in the field just before harvest may seem healthy when packed but will decay later in storage. The optimal temperature for fungal growth is 26-30°C; there is little growth at or below 7°C (McColloch et al., 1982).

The lesions are brown, tough and characterized by the development of minute black bodies: the conidia-bearing pycnidia.

Colletotrichum spp. (perfect state: *Glomerella* spp.)

Several *Colletotrichum* spp., such as *C. coccodes* (Wallr.) Hughes, *C. capsici* (Sydow) Butler and Bisby, and *C. gloeosporioides* (Penz.) Sacc., may cause anthracnose in solanaceous fruits (Adikaram et al., 1982; Batson and Roy, 1982).

In addition to the asexual state (acervuli with conidia), some of the species exhibit the sexual state (perithecia with ascospores). The fungus may survive from season to season on plant debris (McColloch et al., 1982). Infection occurs during warm, wet weather, when conidia are splashed onto immature fruits by wind-driven rain, and germinate to form appressoria that adhere to the wax or the cuticle of the fruit. The infection hyphae that form from the appressoria pierce the cuticle and the fungus remains quiescent until the fruit ripens. Quiescent infection of peppers is associated with the production of phytoalexins in the young infected tissue (Adikaram et al., 1982). Anthracnose is primarily a disease of ripe fruits and, therefore, becomes a problem mainly in tomatoes and peppers grown for canning and left to ripen on the plant. Manandhar et al. (1995) reported on the ability of *C. gloeosporioides* to cause anthracnose on pepper fruits of all ages; disease incidence was correlated with cuticle and exocarp thickness.

With progress of the disease, the lesions become dark, characterized by salmon-pink masses of conidia in the center. In some cases black sclerotia are also formed.

***Cladosporium herbarum* (Pers.) Link**

Fungal conidia are generally found in the soil and are very common in the atmosphere. However, Cladosporium rot of tomatoes and peppers is of importance only during long transit or extended storage. The fungus is a weak pathogen, infecting fruits via mechanical damage, or following sunscald or chilling injury (Barkai-Golan, 1981). Lesions are circular, firm and with definite borders; at an advanced stage and under humid conditions, they become covered with a velvety olive-green mycelium bearing new conidia.

***Stemphylium botryosum* Wallr. [perfect state: *Pleospora herbarum* (Pers.) Rabenh.]**

This fungus may exhibit both the asexual state (conidiophores bearing conidia) and the sexual state (perithecia with ascospores). Infection occurs under moist conditions, being initiated at growth cracks, the edge of the stem scar or at any injury on the fruit surface. Lesions are similar to those formed by *A. alternata*, but *Stemphylium* is much less common. When the fungus exhibits the sexual state, the development of perithecia, which appear as minute black fruit bodies, may serve to distinguish between the two fungi (McColloch et al., 1982).

Control Measures

To reduce infection by wound pathogens such as species of *Alternaria, Goetrichum, Fusarium, Cladosporium, Stemphylium*, as well as *B. cinerea* that may also gain entry through wounds, care should be taken to prevent injury to the fruit during harvesting. Fruits showing growth cracks, sunscald or chilling injury in the field, which predispose the fruit to pathogen penetration, should be rejected at the packing stage.

To reduce the source of inoculum in the field, plant debris on which the fungi can proliferate, should be removed. Good sanitation practices around the packinghouses and regular disinfection of picking containers, are also advisable. Since infection by soft rot bacteria depends on wet conditions (Volcani and Barkai-Golan, 1961), the fruit should be picked while dry.

Fungicidal treatments to control Botrytis rot on staked tomatoes start with sprays during flowering, to protect the flowers and the young fruits

from preharvest infection (Eckert and Ogawa, 1988). Preharvest sprays with chlorothalonil are also effective in controlling *A. alternata* during storage (Davis et al., 1997). Sprays with two fungicides (metalaxyl and mancozeb) combined effectively control *P. infestans* in the field, reduce the inoculum level and minimize infections that lead to decay development in the markets (Eckert and Ogawa, 1988).

Use of chlorinated water at 38-43°C to wash tomatoes in the packinghouse prevents the buildup of inoculum in the water. The water should be properly chlorinated and frequently changed (Bartz, 1982) and the fruit has to be dried before being packed. After removal of the surface water, the fruit may be treated with postharvest fungicides. Postharvest fungicidal dips (using imazalil) were found to be effective against *A. alternata* in tomatoes and peppers (Spalding, 1980) and against *B. cinerea* in tomatoes (Manji and Ogawa, 1985). Most of the chemical compounds used are ineffective against *G. candidum* (Eckert and Ogawa, 1988).

Rotting is often retarded in storage since the pathogens grow very slowly at or below 10°C. However, the correct storage temperature should be carefully maintained, to prevent chilling injury (Kader, 1986), which enhances susceptibility to infection (Barkai-Golan and Kopeliovitch, 1981, 1989). While ripe tomatoes can be held for a few days at 7-10°C, mature green fruits, which are more susceptible to low temperature injury, should usually be held at or above 13°C. Relatively high storage temperatures are also recommended for the chill-sensitive eggplants (8-12°C) and peppers (7-10°C).

Reduction in the incidence of *B. cinerea, R. stolonifer* and *A. alternata* could also be achieved by biological means (Chalutz et al., 1991): application of a known antagonistic yeast (*Pichia guilliermondii*) to wounds on the surface of harvested ripe tomato fruits, prior to inoculation with the pathogens, reduced decay by 90%. The yeast had no effect on the pathogens in culture and its inhibitory effect on pathogens on the fruit was attributed to competition for nutrients. The efficacy of the antagonist in reducing decay was affected by the concentrations of both the yeast cells and the fungal spore suspension used for inoculation.

Fruits of the non-ripening tomato mutants, *nor* (non-ripening) and *rin* (ripening inhibitor), which are devoid of typical carotenoids of tomato and fail to soften (Tigchelaar et al., 1978), have been found to be less susceptible to postharvest diseases than normal tomato fruits (Barkai-Golan and Kopeliovitch, 1981; Lavy-Meir et al., 1989). Increased resistance toward *B. cinerea* has also been exhibited by the hybrid fruit

of the *nor* mutant (Barkai-Golan and Kopeliovitch, 1989; Lavy-Meir et al., 1989), which is characterized by color development and retarded softening (Tigchelaar et al., 1978). This resistance was, however, partially broken when the fruit was exposed to chilling temperature or hot water treatment, which favor fungal penetration (Barkai-Golan and Kopeliovitch, 1989).

Some cultivars of solanaceous fruits are available, which are resistant to certain postharvest pathogens. They include tomato and pepper cultivars resistant to various *Phytophthora* spp. causing fruit rot, tomato cultivars resistant to *P. infestans* and eggplant cultivars resistant to *Phomopsis* (Snowdon, 1992).

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