SURVIVAL AND GROWTH OF *SALMONELLA* POONA ON AND IN TISSUES OF CANTALOUPES CO-INFECTED WITH PLANT PATHOGENIC MOLDS AND YEASTS

by

GLENNER MARIE RICHARDS

(Under the Direction of LARRY R. BEUCHAT)

ABSTRACT

Multistate outbreaks of salmonellosis associated with consumption of fresh cantaloupes from salad bars suggest that contamination occurred early in the farm to fork chain, rather than immediately before consumption. Factors that may influence the adherence, survival, and growth of *Salmonella enterica* serotype Poona on and in cantaloupes were investigated. The effects of temperature differentials between cantaloupes and *S*. Poona suspensions at 4°C and 30°C, on changes in fruit weight and populations of the pathogen recovered from rinds and stem scar tissues of Eastern and Western type cantaloupes were assessed. The weight of immersed cantaloupes increased by 0.13 – 0.43%, with Western cantaloupes showing greater increases. Initial temperature of the inoculum and cantaloupe affected weight increase by Eastern cantaloupes, but not Western type cantaloupes. The histology of cantaloupe rind and stem scar tissues augments attachment and penetration by contaminating *S*. Poona, potentially reducing effectiveness of sanitizer treatments. Proteolytic activity and changes in pH of cantaloupe rind caused by growth of the phytopathogens *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Geotrichum candidum*, and *Penicillium expansum* were studied. Survival and growth characteristics of *S*. Poona on the surface rind and in wounded tissue as affected by

co-infection with molds and storage at 4°C and 20°C for up to 21 days were determined. Populations of *S*. Poona on intact and wounded rind tissues at 4°C decreased by 1 – 2 logs, but increased by 3 – 6 logs at 20°C. Co-infection with molds did not affect populations of *S*. Poona recovered from cantaloupe rinds. The pathogen migrated from wounded tissues in the rind through pulp tissues to distances as great as 3 – 4 cm below the surface, with or without co-infection with phytopathogens. Migration and survival of *S*. Poona in cantaloupes were enhanced by co-inoculation with *C. cladosporioides* and, to a lesser extent, *P. expansum*. Ten yeasts were screened for antagonistic activity against *S*. Poona in cantaloupe juice and decay by *C. cladosporioides* and *G. candidum*, in wounds on the surface of cantaloupe rind. Some of the yeasts demonstrated their potential to restrict colonization of wounded tissues by phytopathogenic molds, particularly at low storage temperatures. Test yeasts did not markedly restrict growth of *S*. Poona in cantaloupe juice.

INDEX WORDS: Produce-related outbreaks, *Salmonella*, Cantaloupes, Metabiotic associations, Phytopathogenic molds, Biological control

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DEDICATION

This dissertation and all the work involved is dedication to:

My God whose faithfulness is new every morning.

Mom and Daddy Robinson, you sowed the seed for this dream. Daddy, Osmond Ransford Robinson (4/21/34 - 9/14/02), I am very sorry that I lost you before you could say, 'Cheers, to success'. Mom, Madge Yvonne Robinson, thanks for keeping the light aglow in the midst of our loss.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

The association of cancer and cardiovascular disease with consumption of saturated fat has led to significant diet modifications by many Americans. The meat-and-potato diet that accompanied the post war boom of the 1950s has been abandoned for a new American diet that emphasizes fruits, vegetables and grains, while de-emphasizing meats and foods with a high fat content. Public information campaigns such as Five-a-Day for Better Health by the National Cancer Institute promote increased consumption of fresh fruits and vegetables (Hedberg *et al.*, 1994; Sewell and Farber, 2001). Consumers have been demanding and purchasing more fruits and vegetables in recent years (Brackett, 1999).

Dietary changes have coincided with a dramatic increase in the variety of food items, including fruits and vegetables, available to the consumer. The increased demand for fresh produce has resulted in changes in the operation of food service establishments (Hedberg *et al.*, 1994). Restaurants have begun offering more raw produce in the form of salad bars and raw vegetarian main courses. While progress has been made in promoting heart-healthy diets in the past 40 years, these dietary changes have also altered the epidemiology of foodborne diseases in the United States (Hedberg *et al.*, 1994; Brackett, 1999).

Traditionally, foods implicated in outbreaks were undercooked meat, poultry or seafood, or unpasteurized milk and dairy products. This has changed and foods previously thought to be safe are now being recognized as being potentially hazardous (Tauxe, 1997). Fresh produce has a short shelf life and often has been consumed or discarded before the outbreak is recognized. Although still limited, an increasing proportion of reported outbreaks are being traced to fresh produce. Because fresh produce is widely distributed, most produce-related outbreaks are multistate events (Tauxe *et al.*, 1997).

Meeting the increased worldwide demand for fresh produce has required the implementation of intensive cultivation, harvesting and distribution measures. Local, national and international competition between producers may also result in cost-reduction measures in agricultural areas that have traditionally relied on low-paid workers. These measures could potentially increase the likelihood of produce becoming contaminated in the field or during packaging or distribution (Hedberg *et al.*, 1994). Fresh produce is now being mass-produced and distributed through large and complex networks. The size and complexity of these operations can greatly magnify the public health significance of contamination with foodborne pathogens. The presence of foodborne pathogens on fruits and vegetables results from exposure to human or animal waste, or to irrigation water or other water that has been contaminated by these sources. In the farm-to-table production, processing and distribution chain, there are various possible sources of pathogens and points of contamination of fruits and vegetables with disease-causing microorganisms. These sources include irrigation water, runoff water from livestock farms adjacent to fields and orchards, manure, wash water, handling by workers, and contact with contaminated surfaces, animal fertilizers applied in previous growing seasons, and feces of rodents and ruminants (Guo *et al*., 2002).

Mechanisms of produce-borne diseases share several common features. Contamination often occurs early in the production or handling process, rather than immediately before consumption. Widespread consumer demand and global food markets have resulted in the use of ingredients from many different locales and/or countries being combined in a single dish, making the specific source of contamination difficult to trace and the concomitant outbreaks hard to identify. All types of produce have the potential to harbor pathogens, but *Shigella* spp., *Salmonella* spp., enterotoxigenic and enterohemorrhagic *Escherichia coli, Campylobacter* spp.,

Listeria monocytogenes, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses, and parasites such as *Giardia lamblia, Cyclospora cayetanensis* and *Crytosporidium parvum* are of greatest public health concern (Beuchat, 2002). When compared with processed foods, fresh produce typically has fewer barriers, such as reduced water activities or preservatives, to inhibit microbial growth. A single and simple contravention can make the food unsafe, particularly with respect to pathogens such as *E. coli* O157:H7 and *Shigella* that have low infectious doses. Another notable influence on produce-related disease outbreaks is the introduction of new packaging technologies. In an attempt to extend the shelf life of fresh-cut fruits and vegetables, modified atmosphere packaging is used. Under these conditions, pathogens can potentially grow at a faster rate than food spoilage microorganisms, whereas under normal atmospheric conditions, pathogens may not be given an opportunity to multiply (Sewell and Farber, 2001). This can be potentially perilous because produce may appear safe while it is sustaining survival or growth of human pathogens.

Increased documentation of outbreaks associated with produce may be attributed to changes in the produce industry, social demographics, food consumption patterns, awareness of fresh fruits and vegetables as vehicles of infection (Beuchat and Ryu, 1997) and improved epidemiology and identification of foodborne pathogens (Sewell and Farber, 2001). Documentation of produce-related foodborne disease outbreaks has indicated that some produce (e.g., lettuce, watermelon, sprouts, cantaloupes, and tomatoes), enteric pathogens (*Salmonella, E. coli* O157:H7, and *Shigella*), and certain produce/pathogen combinations that are more frequently reported (Beuchat, 1996). The survival or growth of human pathogens on the surface of raw produce depends on the presence of free moisture, relative humidity and temperature (Brackett, 1987). Human pathogens contaminating produce during cultivation or at harvest are

likely to survive, and may increase in numbers during storage before consumption (Nguyen-the and Carlin, 2000).

Fruits and vegetables are perishable and maintain an active metabolism after harvesting (Arul, 1994; Wills *et al.*, 1998). Fungal infection, senescence and transpiration are the major factors that contribute to the early termination of the storage life of fresh produce. In fleshy fruits and vegetables, field infections may continue to develop after harvest. While in storage, new infections may be caused by the same or other pathogens (Agrios, 1997). Factors that accelerate senescence and favor microbial growth, such as physiological and mechanical injuries as well as exposure to high ambient temperature and humidity, are the major promoters of postharvest decay (Arul, 1994). Fleshy fruits and vegetables are generally kept at high relative humidity to avoid shrinkage, therefore providing conditions favorable for growth of pathogenic microorganisms, especially when wounds, cuts and bruises offer readily accessible nutrients. However, penetration through natural openings and directly through the cuticle and epidermis may also occur, especially when sound and infected produce are in contact. Once a fruit or vegetable becomes infected, development and spread of the infection increase as the storage temperature increases. At lower temperatures, pathogens and the diseases they cause develop more slowly or cease to develop at all (Agrios, 1997).

Preharvest infections require that the pathogen circumvent inherent or induced resistance of the host, or morphological barriers that inhibit the formation of an active disease lesion. Alternatively, the pathogen may enter a latent state on or in the host. The quiescent pathogen persists until the resistance of the host declines with advancing maturity or until a wound to the host tissue or some mechanism that weakens the host defenses activates the pathogen (Eckert and Ogawa, 1985). If physical injury is sustained, an active wound-healing process involving the

formation of corky cells to inhibit microbial invasion may ensue (Snowdon, 1990). Some of these factors change with time and, if the microorganism remains viable, invasion and complete colonization may eventually take place. Many molds are unable to penetrate intact skin of produce, but readily invade any break in the skin. The damage is often microscopic, but is sufficient for plant pathogens to gain access. In addition, tissue of produce at the point where it is severed from the mother plant can act as a route of entry.

Several intrinsic and extrinsic factors affect the rate of plant tissue decay. Environmental factors such as temperature, humidity and concentration of respiratory gases affect the rapidity and severity of tissue decay. Warm temperature and high humidity favor the development of postharvest decay and chilling injury generally predisposes tropical and subtropical produce to postharvest decay. In contrast, low temperature, low oxygen, high carbon dioxide and low humidity can restrict postharvest decay by either retarding the rate of ripening or senescence, depressing growth of the pathogen or both. Ripened fruits are more susceptible than immature fruits to microbial decay. Treatments that retard the rate of ripening will also slow the rate of growth of decay microorganisms.

Interactions between foodborne human pathogens and background microflora have been studied in meat and dairy products (Nguyen-the and Carlin, 1994; Francis *et al.*, 1999), but relationships on fruits and vegetables are ill defined (Beuchat, 2002). The antagonistic properties of lactic acid bacteria (Francis *et al.*, 1999) and pseudomonads toward pathogens on ready-to-use vegetables have been reviewed (Nguyen-the and Carlin, 1994). Metabiotic associations of molds and *Clostridium botulinum* on tomatoes (Draughon *et al.*, 1988) and molds and *Listeria monocytogenes* on fresh-cut apples (Conway *et al.*, 2000; Chardonnet *et al*., 2002) have been reported. Meager information has been published describing interactions between

Salmonella and *E. coli* O157:H7 and other microorganisms (Wells and Butterfield, 1997; Riordan *et al.*, 2000). *E. coli* O157:H7 is hypothetically more resistant than *Salmonella* to the acidic fermentation end-products of lactic acid bacterial populations (Francis *et al.*, 1999), but the behavior of pathogens on or in produce characterized by pH change caused by metabiotic activities of indigenous microflora has not been thoroughly investigated.

Interactions among microflora may have significant effects on the survival and growth of pathogens. These interactions need to be elucidated to ensure that traditional and novel mild preservation technologies can continue to be applied without compromising the safety of raw fruits and vegetables.

Indigenous microflora on produce

The initial microflora of fresh fruits and vegetables originates in the field and from harvesting and transportation equipment. Field sources include air, soil, insects, plant tissue exudates (ICMSF, 1998) and animals. Fresh produce that has not been pasteurized or sterilized has a microflora consisting of a specific association of microorganisms from the environment. The origin, development and succession of this association are governed by ecological factors that influence the physiological expression of microbial cells. Only microorganisms that possess the necessary physiological attributes to respond to intrinsic and extrinsic environmental pressures will survive (Goepfert, 1980). Eventually, given suitable environmental conditions, a particular microbial community will develop. If environmental factors, inclusive of the unique ecological factors associated with the specific produce permit, microflora will evolve to a specific spoilage association (Goepfert, 1980; Deak and Beuchat, 1996). The numbers and types of microorganisms present on freshly harvested fruit is dependent on environmental factors such

as the weather, the season, time of harvest within a season and type of fruit and its proximity to the ground, as well cultural practices inclusive of irrigation and preharvest treatment with chemicals such as fungicides.

As the flower bud develops into a flower and eventually matures into a fruit, surfaces are colonized by a succession of microorganisms. The location of these microorganisms is mostly limited to the surface of whole, sound fruits. The internal tissues are generally free from contamination. The cuticular protective cover on many fruits and vegetables and structural integrity due to cellulose and pectin, as well as the low pH by some produce, are important intrinsic factors that influence the microbial ecology. Only a few microbial species are capable of invading internal tissues of the fruit while it is attached to the plant**.**

Subsequent to harvest, fruits and vegetables are considered food products, although they continue to be living plant tissue. Being compartmentalized, plant tissues limit the diffusion of solutes and influence the survival and growth of microorganisms. Fruits and vegetables may contain chemical compounds that are inhibitory to microorganisms and may induce defense reactions similar to those found in growing plants against microbial invasion (Nguyen-the and Carlin, 2000). Only the microorganisms that possess the necessary physiological attributes to respond to intrinsic and extrinsic environmental pressures will survive (Goepfert, 1980).

Yeasts and molds are widely distributed in the environment and are present in the natural microflora of most leaves, flowers, tree exudates, and fruit surfaces. The population and relative proportion of species varies between commodities and is influenced by environmental, harvesting and storage conditions (Deak and Beuchat, 1996). Freshly harvested fruits often have a large yeast population. Mycological analysis has shown yeast population of $10^3 – 10^5$ cfu/g of sound, mature apples and $10^6 – 10^7$ cfu/g of grapes (Splittstoesser, 1996; Lund and Snowdon,

2000). On freshly harvested soft fruits (strawberries, raspberries and blackberries), the number of yeasts was $10^4 – 10^6$ cfu/g; mold population was up to 10^4 cfu/g and bacterial population $10^5 – 10^6$ cfu/g. With respect to shelf life and spoilage of most fruits, yeasts and molds play the most significant role. Various types of injury caused by weather, insects, birds, rodents and farm implements can be sustained before, during and after harvest (Snowdon, 1990). Breaking the skin of fruits during harvesting or handling operations can significantly modify the environment and allow the establishment of saprophytes (Mercier and Wilson, 1994).

Fruits that have been damaged are likely to have elevated yeast populations. Yeast cells introduced to exposed tissues are able to utilize sugars and other nutrients to support growth (Splittstoesser, 1996). Carbon dioxide and ethanol are the predominant metabolic by-products of many yeasts, but glycerol, acetaldehyde, pyruvic acid and α-ketoglutaric acid are also formed. Although yeasts may possess hydrolytic enzymes that degrade pectins, starch and certain proteins, enzymatic activity is usually much less than that exhibited by other aciduric microorganisms such as molds. Yeast populations on fruits have been extensively studied (Deak and Beuchat, 1996). With a few exceptions, yeasts are not capable of attacking plant tissues.

Molds represent the most important group of microorganisms within the microflora that cause decay of raw fruits. They are the main invaders causing fruits to rot, lose moisture and becoming mummified (Lund and Snowdon, 2000). Genera of molds (*Alternaria, Botrytis, Botrysphaeria, Colletotrichum, Diplodia, Monilinia, Penicillium, Phomopsis, Rhizopus* and *Sclerotinia)* and bacteria (*Erwinia* and *Pseudomonas)* are the main plant pathogens. A few molds, e.g., *Colletotrichum,* are able to penetrate the skin of healthy produce. Often the relationship between the host (fruit or vegetable) and the pathogen is reasonably specific. For example, *Penicillium digitatum* infects only citrus fruits and *P. expansum* primarily infects

apples and pears. Complete loss of the commodity occurs when one or a few pathogens invade and breakdown the tissues. This initial attack is rapidly followed by a broad spectrum of weak pathogens that magnify the damage caused by primary pathogens. Surface lesions caused by plant pathogenic microorganisms, without the internal tissues being affected, may cause deterioration of many commodities (Wills *et al.*, 1998).

Some molds cause latent infections that do not produce visible symptoms until postharvest. Defense mechanisms in the fruit are highly effective against nearly all fungi; however, some molds are highly specialized pathogens, attacking only one or two kinds of fruit. Others have a more general ability to invade fruit tissue (ICMSF, 1998). Only relatively few genera and species are able to invade a particular fruit type and cause extensive losses. Some species are plant pathogens that cause postharvest diseases of fruits while in transit or storage and result in extensive economic loss (Agrios, 1997). Mold-infected raw fruit may become soft after processing because pectinases were not inactivated by the thermal treatment. Some molds are xerophilic and are therefore potential spoilage agents of foods of low water activity such as dried fruits and fruit juice concentrate (Splittstoesser, 1996).

Control of Postharvest Diseases

Postharvest deterioration is a problem that persists from the time of harvest throughout the fruit and vegetable distribution chain, affecting the cost and availability of produce to the consumer and the ability of the producer to service distant markets (Arul, 1994). Control measures aimed at inhibiting deterioration of the produce until it is consumed generally begin in the field and continue through marketing. These include elimination of sources of infection, preharvest and/or postharvest spraying with fungicides for the control or elimination of causal

microorganisms and careful handling during harvest to minimize mechanical damage and subsequent microbial invasion.

The effectiveness of physical and chemical treatments used to control postharvest loss of fruits and vegetables depends on the ability of the treatment or agent to reach the pathogen. Physical treatments used to control postharvest deterioration include treatment with growth regulators that delay tissue senescence, low and high temperatures, low-oxygen atmosphere, modified relative humidity, ionizing radiation, good sanitation, and development of wound barriers (Eckert and Ogawa, 1985; Wills *et al*., 1998). Handling, storage, and transport at low temperature is the most important physical method to control postharvest deterioration because it delays ripening, physiological deterioration, and postharvest decay of fruits and vegetables (Smith, 1962). The remaining physical interventions are considered to be additional hurdles (Wills *et al*., 1998).

Chemical control

Chemical treatments to reduce postharvest decay of fruits and vegetables may be applied pre- or postharvest. The successful use of chemical treatment is dependent on the initial mold spore population, growth rate, depth of the infection within the host tissue, temperature, humidity, and depth to which the chemical can penetrate the host tissues. Efficacy of a chemical is dependent on its cost effectiveness, solubility if used as a solution, ability to inhibit growth of the pathogen and ability to kill the pathogen quickly. In addition, chemicals should not injure the product, leave toxic or unattractive residues, or impair the sensory qualities (Smith, 1962; Wills *et al*., 1998). Addition of chemicals to wash water was one of the earliest postharvest fungicide application methods, and is still extensively used at present. Chemicals may also be impregnated

into wraps or box-liners, or applied as fumigants, solutions and suspensions, or in wax (Wills *et al.*, 1998). Most of the chemicals used are fungistatic in action, inhibiting or reducing the rate of germination of spores or conidia and growth after germination, rather than causing death of the mold. A few sanitizers such as chlorine and sulfur dioxide are true fungicides. Sodium carbonate (Wills *et al*., 1998) and borax (sodium tetraborate) were the first chemicals to be extensively tested and accepted for use to reduce postharvest decay (Smith, 1962; Wills *et al*., 1998). Borax was predominantly used in the citrus industry to control *Penicillium digitatum* (green mold) and *P. italicum* (blue mold). Cantaloupe molds were also effectively reduced during transit and storage by washing for 0.5 to 2 min in warm water (37 – 42ºC) containing 2.5 – 5% borax (Smith, 1962).

Considered a more effective fungicide with broad-spectrum activity, sodium *o*-phenylphenate (SOPP) superseded sodium carbonate and borax in the 1950s. SOPP is not phytotoxic but is converted to a fungitoxic free phenol at appropriate sites. The *o*-phenylphenate anion (OPP) diffuses selectively into injury sites and is converted to the undissociated form, preventing infection at these sites during storage and marketing. SOPP can be applied as a dip or foam, or incorporated into wax applied before or after washing. The SOPP-OPP system was found to require pH control to prevent damage to the fruits. A residual tolerance level of 10 – 12 µg/g has been established.

Biphenyl, a compound with fungistatic activity, was subsequently introduced. Impregnated into fruit wraps or into paper sheets placed in the fruit containers, biphenyl sublimes into the atmosphere surrounding the fruits and prevents sporulation of *Penicillium* on the surface of infected fruits and transfer of infection to neighboring fruits. It is also used as a complement to SOPP on export fruits. The disadvantages associated with biphenyl include a

characteristic hydrocarbon odor, residues on the fruit surface sometimes exceeding that permissible limit of 70 – 100 μg/g, and development of resistant strains by *Penicillium* and *Diplodia* on fruits stored for up to 4 months. The use of biphenyl has been banned in some countries (Wills *et al*., 1998).

The benzimidazole group of chemicals, introduced in the late 1960s, includes thiabendazole (TBZ), benomyl, thiophanate methyl, and carbendazim. Benzimidazoles show systemic and residual activity, are not phytotoxic and have low mammalian toxicity. They also have a wide spectrum of antifungal activity at low concentrations, but are inactive against *Rhizopus, Alternaria, Geotrichum* and soft rot bacteria (Eckert and Ogawa, 1985; Wills *et al*., 1998). Major disadvantages of these fungicides are that they have limited solubility in water and resistant strains of *Penicillium* have developed as a result of their specific action that interferes with polymerization of the protein tubulin. The latter problem influenced the search for alternative treatments (Wills *et al*., 1998).

Sec-butylamine (2-AB) has limited use as an alternative fungicide, due to its relatively narrow spectrum of antifungal activity, and has been withdrawn from application. Guazantine offers control of benzimidazole-resistant molds, but multiple resistances have been reported. Triazoles, which include imazalil and prochloraz, are effective against a wide range of fungi, particularly benzimidazole-resistant strains. Their mechanism involves inhibition of demethylation during ergosterol biosynthesis. However, resistance has been reported (Eckert and Ogawa, 1985; Wills *et al*., 1998). The use of postharvest chemical treatments helps to extend the storage time of fruits and significantly affects the sucrose content (Caño *et al.*, 1987), but little is known about their effects on survival and growth of human pathogens in response to modifications in the behavior of indigenous microflora.

Biological control

Application of synthetic fungicides has been the primary method of controlling postharvest diseases. Interest in the development of alternatives has been generated in recent times due to concerns about carcinogenic risks and public safety (National Research Council, 1987), presence of chemical residues in the food chain, development of fungicide-resistant strains of postharvest pathogens, and deregistration of some of the more effective fungicides (Wilson and Wisniewski, 1989; Wilson *et al*., 1997; El-Ghaouth *et al*., 1998).

Essential oils and natural extracts from plants and microorganisms have been evaluated for safety and efficacy as fungicides (Wilson *et al*., 1997). Sugar analogs such as 2-deoxy-D-glucose have been known to effectively inhibit the growth of several yeasts and some filamentous fungi in the absence of metabolizable sugars (El-Ghaouth *et al*., 1997).

Native microflora on the surfaces of fresh produce have been assumed to be important in maintenance of quality and safety, by producing antimicrobial compounds, activating plant defense mechanisms, and suppressing human pathogens (Liao and Fett, 2001). The use of antimicrobial agents as alternatives to synthetic fungicides has shown significant potential for the control of postharvest disease of fruits and vegetables. Antagonistic yeasts and bacteria that display the capability to protect several types of fruits against postharvest pathogens have been isolated. Currently, two antagonistic microorganisms are commercially available as biocontrol agents. *Candida oleophila* Montrochus, a yeast, and *Pseudomonas syringae*, a bacterium are marketed under the trade names Aspire and Biosave-110, respectively (El-Neshway and Wilson, 1997; El-Ghaouth *et al*., 1998). Yeasts possess a number of attributes that make them useful biological control agents in the postharvest environment. They effectively colonize plant surfaces and produce extracellular compounds such as polysaccharides, which enhance their

survival while restricting colonization sites and flow of germination signals to pathogenic fungal propagules which may be present (Wilson *et al*., 1996). The mechanism by which antagonistic yeasts exert their biocontrol activity is unclear; however, nutrient competition (El-Ghaouth *et al*., 1998), site exclusion, direct parasitism and induced resistance have been suggested (Wilson *et al*., 1996). The biological activity of *Candida saitoana* and its interaction with plant pathogens *Botrytis cinerea* and *Penicillium expansum* in apple wounds were investigated (El-Ghaouth *et al*., 1998). *C. saitoana* proliferated in apple wounds, prevented the proliferation of the plant pathogens, stimulated several host defense reactions, and controlled decay of apple fruits. It was postulated that *C. saitoana* affects the ability of *B. cinerea* cells to degrade host tissue and establish a nutritional relationship (El-Ghaouth *et al*., 1998). The natural microflora was found to be inhibitory to *B. cinerea* at wound sites on Red Delicious apples while enhancing the biocontrol activity of *C. oleophila* (Mercier and Wilson, 1994).

The polypeptide antibiotic nisin was first used to prevent spoilage of Swiss cheese by *Clostridium butyricum.* Nisin has been used safely as a food preservative and has been approved for use in the United States since 1988 (Jay, 2000). Enhanced biocontrol activity of *C. oleophila* in the presence of nisin to control apple rot suggested that nisin stimulated the antagonist, inhibited the pathogen by preventing germination of spores, restricted growth of the germ tubes, and reduced the susceptibility of the wounds to infection (El-Neshway and Wilson, 1997).

Candida sake is commonly found in nature and is a component of the epiphytic community on mature fruits. This yeast has not been associated with warm-blooded animals. *C. sake* effectively controlled major postharvest diseases on apples when applied at 1.6×10^6 cfu/ml under commercial storage conditions (Viñas *et al*., 1998).

Biologically based control measures have not performed as consistently chemical applications and tend to have a limited range of application (El-Neshway and Wilson, 1997). Living organisms acting as 'biofungicides' have the disadvantage of not providing the direct and complete control offered by most synthetic fungicides (Wilson *et al*., 1996). Biological control of postharvest diseases is complex and involves a number of variables such as the rate and timing of application of biocontrol agents, the possible use of additives to enhance biocontrol activity, the physiological state of the host and environmental conditions used to store the produce (Mercier and Wilson, 1994).

There are limited reports describing the effect of biocontrol agents on human pathogens. Growth of *L. monocytogenes* on potato slices was significantly limited by the presence of fluorescent soft-rotting pseudomonads (Liao and Sapers, 1999). A saprophytic *P. syringae* strain (L-59-66) was effective in reducing the growth of human and decay pathogens on wounded apples (Janisiewicz *et al*., 1999). Growth of *Salmonella* Chester on pepper discs was not affected by the presence of native and uncharacterized flora. However, when co-inoculated with potential antagonistic yeast onto pepper discs, the population of *S.* Chester was reduced by approximately 1 log (Liao and Fett, 2001).

Salmonella and Salmonellosis

Salmonella spp. are facultatively anaerobic gram-negative rods within the taxonomic family Enterobacteriaceae (D'Aoust, 2001). Most members of the genus are motile by peritrichous flagella, but there are nonflagellated variants and nonmotile strains resulting from dysfunctional flagella (D'Aoust, 1997). Salmonella nomenclature has progressed through a succession of taxonomic schemes based on biochemical and serological characteristics and

principles of numerical taxonomy and DNA homology. The genus consists of more than 2,463 serovars that are classified within two species, *Salmonella* enterica and *Salmonella* bongori (D'Aoust, 1997).

Salmonellae grow optimally at 35°C to 37°C, catabolize a variety of carbohydrates into acids and gas, use citrate as a sole carbon source, produce hydrogen sulfide, and decarboxylate lysine and ornithine to cadaverine and putrescine, respectively. They are oxidase-negative and catalase-positive, with 50 to 53 mole % guanine plus cytosine (G+C) DNA content (D'Aoust, 2000). The biochemical identification of foodborne and clinical *Salmonella* isolates is generally coupled to serological confirmation involving agglutination of bacterial surface antigens with *Salmonella*-specific antibodies. These include O lipopolysaccharides (LPS) on the external surface of the bacterial outer membrane, H antigens associated with peritrichous flagella and the capsular (Vi) antigen, which occurs only in *S*. Typhi, *S*. Paratyphi C, and *S*. Dublin. All serovars are considered to be potentially pathogenic (Jay, 2000; D'Aoust, 2001).

Although the primary habitat of *Salmonella* is the intestinal tract of birds, reptiles, domestic and wild animals, humans, and insects, they may be found occasionally on other parts of the body. As intestinal forms, they are excreted in feces, which may be transmitted by insects and other living creatures to a large number of destinations, including water and food. When humans and other animals consume contaminated water and foods, salmonellae are again shed through fecal matter with a continuation of the cycle (Jay, 2000).

The genus *Salmonella* consists of resilient microorganisms that readily adapt to extreme environmental conditions. They have been shown to actively grow within a wide temperature range of 2°C to 47°C (Doyle and Cliver 1990; D'Aoust, 2000) and exhibit psychrotrophic properties as reflected in the ability to grow in foods stored at 2 to 4°C (Matches and Liston,

1968; D'Aoust, 1997). The physiological adaptability of *Salmonella* is demonstrated by its ability to proliferate at pH values ranging from 4.5 to 9.5, with an optimum pH for growth of 6.5 to 7.5. The minimum growth pH is dependent on the acid used to lower the pH (Chung and Goepfert, 1970; Doyle and Cliver, 1990; Asplund and Nurmi, 1991; D'Aoust, 1997; Jay, 2000). Water activity is an important factor determining the pH range for *Salmonella* growth. Most salmonellae can grow in foods in a water activity range of 0.945 - 0.999. As water activity shifts from the optimum for *Salmonella* growth, the permissive pH range narrows. Typical of other gram-negative bacteria, they are able to grow on a large number of culture media and produce visible colonies within 24 h at 37°C (Doyle and Cliver, 1990).

With respect to oxidation-reduction (OR) potential and nutrients, salmonellae are not fastidious. *Salmonella* can grow under aerobic as well anaerobic conditions, but growth can be inhibited by OR potential below –30mV (Doyle and Cliver, 1990). Generally, salmonellae are unable to ferment lactose, sucrose or salicin, but a few serovars are capable of lactose fermentation as an alternative. Glucose and certain other monosaccharides can be fermented to produce gas. *Salmonella* normally utilizes amino acids as nitrogen sources, but nitrate, nitrite and ammonia will serve as a sole source of nitrogen for *S*. Typhimurium (Jay, 2000).

Salmonellosis is the major bacterial foodborne disease in many countries (Asplund and Nurmi, 1991; D'Aoust, 2001) and has been increasing steadily as a public health problem over the last 50 years in the United States (Tauxe, 1991). Non-typhoidal salmonellae were estimated to cause approximately 1.5 million cases of infection, resulting in 15,000 hospitalizations and 500 deaths in the United States in 1999 (Mead *et al*., 1999). Foods of animal origin have historically been recognized as potential vehicles for *Salmonella*. According to the U.S. Department of Agriculture, the bacterium is acquired from meat, poultry, or eggs in 50 to 75% of

salmonellosis cases. Poultry is known to be the primary vehicle of transmission (FSIS, 1995; Leverentz *et al*., 2001). *Salmonella* as a leading cause of foodborne diseases, emanates from its ubiquity in the environment, its prominence in various sectors of the agriculture industry and from escalating movement of food and food ingredients in international trade (D'Aoust, 2001).

Major outbreaks of foodborne salmonellosis in the last two decades have involved a wide variety of foods and serovars (D'Aoust, 2001). Salmonellae remain well entrenched in the meat and poultry industries (D'Aoust, 2001); however, consumption of fresh fruits and vegetables, including seed sprouts (O'Mahony *et al*., 1990; Van Beneden *et al*, 1996; Mahon *et al*., 1997), unpasteurized apple cider (CDC, 1975), unpasteurized orange juice (Cook *et al*., 1998; CDC, 1999), raw tomatoes (Wood *et al*., 1991; Wessinger *et al*., 2000), watermelons (Gayler *et al*, 1955; Larson *et al*., 1979; Blostein, 1993), and cantaloupes (Ries *et al*., 1990; CDC, 1991) have also been linked to outbreaks of salmonellosis in recent years. Sources of *Salmonella* on produce include animal and human feces, and cross contamination from raw meat, poultry, or eggs (IFT/FDA, 2001).

The number of cells comprising the infectious dose ranges from 10 to 10^5 (IFT/FDA, 2001). The typical incubation period of non-typhoid salmonellosis is 18 to 72 h, and symptoms may include abdominal cramps, diarrhea with watery and possibly mucoid stools tinged with blood, chills, fever of short duration (< 48 h), nausea and vomiting that appear 8 to 72 h following exposure to the bacterium (D'Aoust, 2000). The clinical symptoms of uncomplicated enterocolitis usually subside within five days followed by a convalescent carrier state of one to several months during which the asymptomatic individual continues to excrete salmonellae. Non-typhoid salmonellosis can progress from an enterocolitic to a septicemic condition as a

result of the migration of salmonellae from the intestinal tract into deeper tissues through vascular and lymphatic conduits (D'Aoust, 2000).

Survival, growth, and removal of salmonellae on fruits and vegetables

The condition of produce tissue (healthy, intact tissue compared to damaged, diseased tissue) impacts attachment, infiltration and efficacy of sanitation practices. Microbial cells are known to lodge disproportionately in damaged and cut tissues compared to sound tissues (Weissinger *et al*., 2000). *E. coli* in damaged apples, for example, poses a greater risk for the contamination of cider than surface contamination of apples (Dingman, 2000). The epidermis of fruits and vegetables is covered with a multi-layered hydrophobic cuticle, which provides the primary barrier against invasion, insect and physical damage, and desiccation. Bacterial attachment is facilitated by the presence of stomata, lenticels, broken trichomes, or cracks in the cuticle. *E. coli* O157:H7 was observed to preferentially attach and penetrate the interior edge of cut surface of lettuce leaves as opposed to intact leaf surface (Seo and Frank, 1998). The rind of cantaloupes is complex, presenting a variety surfaces to which bacterium may bind. The ruptured cell surface presents a meshwork of raised tissue (the net) (Ukuku and Fett, 2002). Decontamination treatments are less effective in killing bacteria attached to or located within protective sites that occur naturally or as a result of injury.

Salmonella has been isolated from many different types of raw fruits and vegetables (Ercolani, 1976; Andrews *et al*., 1979; Roberts *et al.*, 1982; Garcia-Villanova Ruiz *et al.*, 1987). Several studies have been undertaken to assess the survival and growth of *Salmonella* in tissues of fruits and vegetables. *Salmonella* was associated more often with soft-rotted plant tissue (Wells and Butterfield, 1997) and injured tissue (Liao and Sapers, 2000; Liao and Cooke, 2001) than with their healthy and intact counterparts. Scanning electron microscopic observations

showed that retention of *S.* Chester on injured apple tissue, the stem, and calyx may be due in part to the physical entrapment of bacteria in the holes below the surface of the tissue (Liao and Sapers, 2000). When raw tomato fruits were immersed in a *S.* Montevideo suspension, more cells were retained in stem scar and core tissues than on tomato skin. Unlike bacteria inoculated onto the skin, *S*. Montevideo cells inoculated into wounds or onto slices multiplied over a 24-h period (Wei *et al.*, 1995).

The core tissue internalized higher numbers of *S.* Montevideo cells when tomatoes at 25°C were dipped into a suspension at 10°C compared to tomatoes dipped at 25 or 37°C. The population of viable *S*. Montevideo cells remained constant throughout subsequent storage for 18 days at 10°C, but storage at 20°C resulted in significant population increases. When tomatoes were stored at 5°C for 216 h, populations of *S*. Montevideo and aerobic mesophilic microorganisms remained constant. However, when stored at 20 and 30°C, significant increases in populations were observed (Zhuang *et al*., 1995). *Salmonella* is capable of rapid and prolific growth on cantaloupe, watermelon, and honeydew melons incubated at 23°C (Escartin *et al.*, 1989; Golden *et al*., 1993). However, when incubated at 5°C for 24-h period, populations of *Salmonella* on the melons remained unchanged (Golden *et al*., 1993).

At present, little is known about the mechanism by which bacteria become attached to surfaces of produce. Physical forces such as London-van der Waals attraction, electrostatic attraction between two surfaces and net gain entropy have been suggested to be involved. Bacterial age and culture conditions have also been identified as factors affecting attachment (Liao and Sapers, 2000).

The efficacy of washing treatments on detachment or inactivation of *Salmonella* on cantaloupe surfaces is dependent on the state and location of the cells on the outer surface of the

cantaloupes. *S.* Stanley was found to be able to survive on cantaloupe surfaces for at least 6 days, on root crops for 10 to 53 days, and on leafy vegetables for 1 to 40 days (Ukuku and Sapers, 2001). Firm attachment of bacteria to stem and calyx cavities, and injured tissue represent a major obstacle in developing effective methods for eliminating *Salmonella* from the surface of contaminated plant tissue.

Chlorine is widely used in the minimally processed fruit and vegetable industry. However, high concentrations may provoke irritation of eyes, skin or lungs of workers, be corrosive to metal components of machines, and perhaps leave residues on produce. Several serotypes of salmonellae inoculated onto shredded lettuce and diced tomatoes were able to grow and could not be eliminated with treatments of up to 200 ppm of chlorine (Weissinger *et al*., 2000). The effectiveness of chlorine on minimally processed spinach inoculated with *S*. Hadar was found to increase through a range of treatments up to 25 – 125 ppm for 2 – 8 min (Pirovani *et al.*, 2000). Dipping tomatoes inoculated with *S*. Montevideo in solutions containing 60 ppm and 110 ppm of free chlorine achieved significant population reductions. However, increased concentration up to 320 ppm chlorine did not significantly improve effectiveness in killing the pathogen (Zhuang *et al*., 1995; Beuchat *et al*., 1998). Chlorine was also reported to be less effective at killing cells that had been internalized to the core tissue than those that were on the surface tissue.

Trisodium phosphate (TSP) is known to be effective in killing *Salmonella* on poultry and red meats when applied to carcasses in the form of a chill or rinse water during processing. TSP was also observed to be highly effective in killing *S*. Montevideo on the surface of mature-green tomatoes, but less effective against internalized cells (Zhuang and Beuchat, 1996).

Salmonellosis associated with melons

Melon fruits are in contact with the ground during their development, enhancing their potential to be contaminated by pathogens. A field survey conducted by the Food and Drug Administration (FDA) during 1990 and 1991 revealed that 0.76 and 1.06%, respectively, of the melon rinds harbored several serovars of *Salmonella* (Madden 1992; Golden *et al*., 1993). Selected fresh produce, including cantaloupes imported into the United States was surveyed. Of 151 cantaloupes sampled, 7.3% were contaminated with *Salmonella* or *Shigella* (FDA, 2001a). The results of a similar survey of domestic fresh produce initiated in May 2000 indicated the presence of pathogens on 4.3% of 92 cantaloupes sampled (FDA, 2001b).

There have been numerous national and international incidents of salmonellosis associated with the consumption of fresh watermelons and cantaloupes. The association of salmonellosis with salad bars and fruit salads suggests introduction of *Salmonella* into the fruit from the rind by the slicing action, or the physical contact of cut pieces with contaminated rind. The netted texture of cantaloupe melon rind enables effective attachment of microorganisms and results in difficulty in sanitization. As few as 150 bacteria per cm^2 of the netted rind surface has been found to result in contamination of the interior pulp as a result of cutting whole cantaloupes (Suslow and Cantwell, 2001).

The potential of the contaminated tissue to support growth of *Salmonella* is exacerbated by display or storage at ambient temperature for an extended time (CDC, 1991; Golden *et al*., 1993). The high water activity and sugar content of tissue juices promote rapid proliferation of salmonellae. Cut cantaloupe is considered a potentially hazardous food in the FDA Food Code because it is capable of supporting the growth of pathogens due to low acidity (pH 5.2 to 6.7) and high water activity (0.97 to 0.99) (IFT/FDA, 2001).

An outbreak of salmonellosis involving *S*. Bareilly on watermelons occurred in 1950 in Minnesota, but was not reported in medical literature (Gaylor *et al*., 1955; Blostein, 1993). *S*. Miami was isolated from the internal flesh of watermelons that were responsible for 17 reported cases and one death in Massachusetts in 1954 (Gaylor *et al*., 1955). In 1979, an outbreak involving 18 cases of salmonellosis caused by *S*. Oranienberg linked to watermelons occurred in Illinois. *Salmonella* was not isolated from the melons but investigations revealed that damaged fruits were cut, covered with plastic film and displayed without refrigeration until sold (CDC, 1979). Cut cantaloupe from salad bars contaminated with *S*. Chester was associated with a large multistate outbreak estimated to involve more than 25,000 individuals and two deaths in 1989-1990 (Ries *et al*., 1990).

There were 26 primary cases and 13 secondary cases of salmonellosis associated with watermelon contaminated with *S*. Javiana in Michigan in 1991. It is suspected that the rind was contaminated and was not washed prior to cutting and serving at a picnic and school party over a two-day period (Blostein, 1993). In 1991, consumption of fruit salads containing sliced cantaloupes was epidemiologically linked to multistate outbreaks in the U.S. and Canada, involving more than 400 cases. *S*. Poona was isolated from cantaloupes originating in either Texas or Mexico (CDC, 1991). *S*. Saphra was isolated from cantaloupes sourced from Mexico and implicated in a 1997 outbreak involving 24 cases in California. Multiple purchase sites suggest that contamination occurred during production or harvest, and that retail storage at ambient temperature was a contributor (Mohle-Boetani *et al*., 1999).

In 1998, there were 22 cases of salmonellosis attributed to *S*. Oranienberg on cantaloupes consumed at various venues in Ontario, Canada (Deeks *et al*., 1998). Although the pathogen was not isolated from the fruit, it has been theorized that pathogen from the surface contaminated the

flesh during slicing and that the cut fruit was stored at room temperature for an extended period of time prior to consumption (IFT/FDA, 2001). *S*. Poona was associated with cantaloupes that were epidemiologically linked to multistate outbreaks in 2000 involving 43 cases (Ukuku and Sapers 2001; IFT/FDA, 2001), in 2001 with 50 cases (Walker, 2001), and in 2002 with 58 cases (CDC, 2002).

Cantaloupes

Cultivation and postharvest handling

Cantaloupes (*Cucumis melo* L. var. *reticulatus* Naud.), also commonly called muskmelons, are members of the taxonomic family Cucurbitaceae, which includes squash, pumpkins, cucumbers, watermelons, and gourds (Boyhan *et al*., 1999; Orzolek *et al*., 2001). The species *Cucumis melo* is subdivided into seven botanical variants: cantaloupensis, reticulatus, inodorous, flexuosus, conomon, chito and dudaim. Only the reticulatus and inodorous variants are of commercial significance in the United States. The inodorous variety includes honeydew melons (Boyhan *et al*., 1999).

Cantaloupes are annual plants that produce long running, non-climbing vines that are prostrate on the soil. Healthy plants have a canopy of large, soft, hairy leaves that are generally lobed and heart-shaped (Anonymous, 1999). Individual plants exhibit andromonoecious flowering, first producing groups of male flowers in leaf axils, and then producing single perfect flowers (Peet, 2001). Bee pollination is required to make fruit set possible, as pollen is too heavy and sticky to make wind pollination successful (Hemphill, 2002). Cantaloupes are round to oval fruits with sizes ranging from 5 to 8 inches in diameter and length, and weighing 3 to 7 pounds. They are characterized by the roughened appearance due to the network of corky surface tissue

(Anonymous, 1999; Orzolek *et al*., 2001; Hemphill, 2002) and the salmon-orange colored flesh with a musky aroma (Anonymous, 1999).

In the United States, cantaloupes are categorized on the basis of their fruit type. "Western" or "shipping" types have uniformly netted rinds lacking sutures and a firm, salmon-colored flesh. Traditionally, this type was grown in western states and shipped to distant states; however, in recent times they have been widely adapted and grown throughout the country. The "eastern" or "jumbo" cantaloupes are grown for local markets. They are characterized by fruits that are larger and less uniform in size than the western cantaloupes, with less uniform or no netting, deep sutures on the rind, and orange flesh (Boyhan *et al*., 1999; Hemphill, 2002).

Contingent on the cultivar and environment, fruits mature 35 to 55 days after full bloom and are harvested by maturity and not by size (Peet, 2001). The principal harvest indices are surface color and development of the abscission zone (Kasmire and Cantwell, 1992). The external color depends on variety and may still have a greenish cast at full slip (Hartz *et al*., 1996). Commercial maturity is ideally at the firm-ripe stage or "¾ to full slip" when a clear abscission from the stem occurs with light pressure, leaving no stem tissue attached to the fruit (Suslow *et al.*, 2000; Agblor and Waterer, 2001; Orzolek *et al*., 2001; Peet, 2001). Typically full slip is at 42 days after flowering, when cantaloupes have high sugar content and good flavor and aroma, but have a short storage life. For distant markets, less mature cantaloupes are harvested at "half-slip", but when the stem attachment area is smooth, rounded and lightly depressed. Harvesting at approximately 36 days after flowering has been suggested as a compromise for acceptable flavor and storage potential (Agblor and Waterer, 2001).

California, Arizona, Texas, Georgia and Indiana were the leading cantaloupe producing states in 1993-1997 (Rhodes, 2001). Most of the cantaloupes harvested in the U.S. are sold as

fresh produce in wholesale markets, cooperatives, local retailers, roadside stands, and pick-your own operations (Orzolek *et al*., 2001). Federal Grade Standards are based predominantly on external appearances and soluble solids. There are four U.S. fruit grades: Fancy and No.1 grades have a minimum of 11% and 10% soluble solids, respectively, while Commercial and No. 2. grades have a minimum of 9% soluble solids (Hurst, 1999; Suslow *et al*., 2000).

Cantaloupes are generally harvested manually and under cool early morning or late evening conditions (Agblor and Waterer, 2001; Peet, 2001). Examination by computerized tomography (CT) indicates that poorly handled fruits suffer mechanical injury that cause increased metabolic activity as measured by pectin esterase activity, fruit firmness, acidity and total soluble solids values (Halloran *et al*., 1999a). Dropping cantaloupes at a distance of more than 8 inches onto hard surfaces causes bruising and cracking (Hurst, 1999).

A flow diagram for postharvest handling systems for cantaloupes is shown in Figure 1.1. Pre-cooling to a typical endpoint of 10°C or preferably to 4°C by cold water, cold air or ice promptly after harvest is necessary to remove field heat, reduce respiration rate, and improve shelf life. Cooling in a room without the use of forced air is usually avoided as it takes 24 to 36 h to cool cantaloupes to 10°C. Forced air-cooling is the most common practice (Suslow *et al*., 2000), allowing cantaloupes to be cooled from 34°C to 16°C in 8 h (Agblor and Waterer, 2001). Hydrocooling is the most efficient method to rapidly cool to 10°C. Cooled melons are generally stored in a cold room and shipped in refrigerated trucks. Ice is blown in between rows of crates or waxed cartons and over packed containers for shipment. Although cantaloupes are susceptible to chilling injury, they are not injured by extended contact with ice (Hemphill, 2002). Typically 12 – 15 days of shelf life are attainable at storage temperatures of 2.2 – 5°C. Storage below this optimum range usually results in chilling injury.

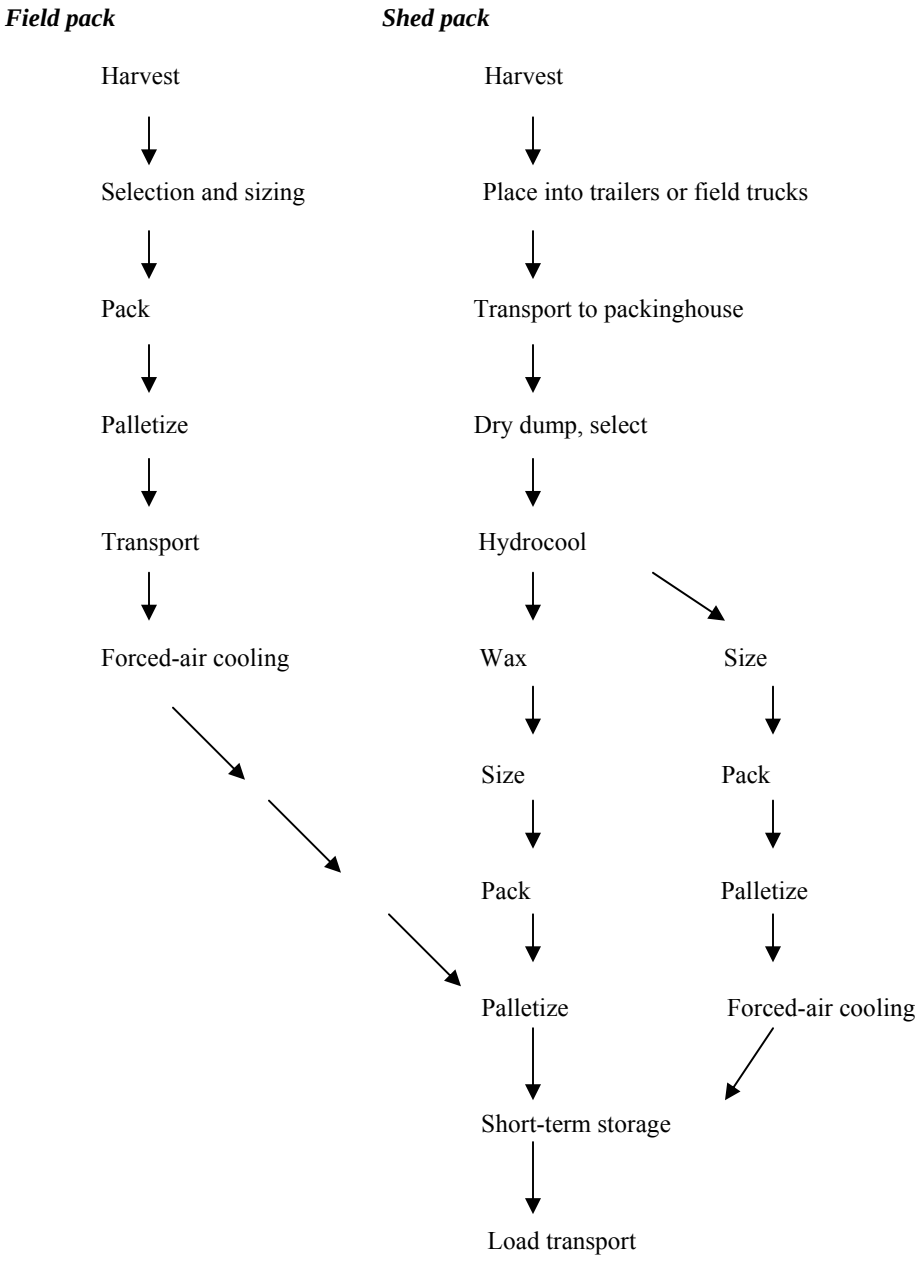

Figure 1.1 Postharvest handling system for cantaloupes (Adapted from Kasmire and Cantwell, 1992)

High relative humidity of 90 – 95% is essential to maximize postharvest quality and prevent desiccation through scuffed and damaged surface netting (Hurst, 1999; Suslow *et al*., 2000). Good quality is retained for approximately 14 – 21 days if cantaloupes are stored at 90 – 95% relative humidity and 8 – 12°C (Orzolek *et al*., 2001). Film wrapping is recommended to improve the shelf life of fruits that are intended for long distance shipping, because desiccation is a major cause of quality loss during shipping (Hemphill, 2002). Extended storage periods at higher humidity or the formation of condensate encourages the growth of molds on stem-scar tissue and rind surface (Suslow *et al*., 2000).

Consensus atmospheres of 3% O_2 and 10% CO_2 at 3°C with extended transit times of 14 – 21 days facilitates the benefit of delayed ripening, reduced respiration and associated sugar loss and inhibition of surface mold and decay (Kader, 1992; Suslow *et al*., 2000). Controlled atmosphere offers moderate benefits under most conditions; however, there is only limited use on the commercial scale.

Postharvest diseases

Cantaloupes are susceptible to postharvest fungal rots, especially under warm, wet conditions. Rots occur on the external surface of the fruit and gradually progress inwards in the flesh. Fungal pathogens of major concern are *Geotrichum*, *Alternaria*, *Penicillium, Cladosporium, Rhizopus*, and *Fusarium* (Suslow *et al*., 2000; Agblor and Waterer, 2001), and *Mucor* to a lesser extent (Suslow *et al*., 2000). *Alternaria* and *Cladosporium* rots occur frequently in cool storage. Dark brown or black lesions characterize *Alternaria* rot, and dark green or black lesions characterize *Cladosporium* rots. *Fusarium* and *Rhizopus* rots are problematic on fruit stored at room temperature. White and reddish hyphae on the netted surface

of fruits are symptoms of *Fusarium* rot. Softening and indentation of large areas of the flesh with little mycelial growth characterize *Rhizopus* rots (Agblor and Waterer, 2001).

Dipping in hot water 52 – 55°C for 0.5 to 1.0 min (Suslow *et al*., 2000) or for 2 min (Agblor and Waterer, 2001), or 59°C for 3 min (Hemphill, 2002) effectively reduces stem scar and surface molds, and controls fungal and bacterial rots. Hemphill further reported that addition of an undisclosed concentration of chlorine to hot water did not augment the benefits of hot water treatment. Warm wet melons are subject to microbial invasion, and shelf life is reduced by 3 – 4 days. It is recommended that the fruits be dried quickly, wrapped in plastic film, sealed in shipping boxes, and cooled to 4°C for up to 7 weeks of storage (Hemphill, 2002). Comparing fungicide application with hot water treatment, cantaloupe fruits (Galia F_1 cv.) treated with 3% benomyl for 90 s and then packed into perforated polyethylene bags for cold storage at 2°C and 85 to 90% relative humidity scored satisfactorily with respect to weight loss, titratable acidity, fruit firmness, pectin esterase, infection rate, and sensory quality. Hot water treated fruits had higher fungal infection rates and earlier fruit softening (Halloran *et al*., 1999b).

Chilling injury, the most common physiological disorder of cantaloupes, typically occurs after storage at less than 2°C for several days. Symptoms include pitting or sunken areas, failure to ripen, off-flavors and increased surface decay (Suslow *et al*., 2000; Agblor and Waterer, 2001). Sensitivity to chilling injury decreases as melon maturity and ripeness increases (Suslow *et al*., 2000).

Research Needs

The number of documented outbreaks of salmonellosis associated with consumption of cantaloupes has increased in recent years. There is a dearth of information about the ecology of fungal pathogens on cantaloupes in association with human pathogenic bacteria. The growth

habit, surface morphology, and postharvest conditions during processing, handling, and distribution of cantaloupes provide opportunities for exposure of the fruits to plant pathogenic molds and contamination of the rind and flesh by enteric pathogens such as *Salmonella*.

Research is needed to:

- Assess the effects of temperature differentials between cantaloupes and wash water on changes in fruit weight and populations of S*almonella* recovered from rinds and stem scar tissues of Eastern and Western type cantaloupes.
- Examine the association and interaction between plant pathogenic molds and *Salmonella* on rind and edible tissues of cantaloupe fruits.
- Determine the influence of postharvest processing, handling, and distribution conditions on the infiltration, survival, and growth of *Salmonella* on sound and injured tissues.
- Determine if the growth phytopathogenic molds in wounds on cantaloupe rinds facilitates migration of *S.* Poona into sub-surface mesocarp tissues.
- Examine yeasts for their potential to impair survival and growth of *S*. Poona in cell suspensions and in wounds on cantaloupe rind co-inoculated with phytopathogenic molds.

Information gathered from research will be useful to predict the behavior of *Salmonella* on damaged and sound tissues of cantaloupes and other produce, and to facilitate prudent decisions pertaining to good agricultural practices and proper postharvest handling practices to maximize microbiological safety of cantaloupes.

References

Agblor, S. and D. Waterer. June 2001. Muskmelons: Cantaloupe postharvest handling and storage. Agri-Food Innovation Fund (AFIF) Postharvest Specialist Program. University of Saskatchewan.

Agrios, G.N. 1997. Plant Pathology. Academic Press. San Diego, CA.

Andrews, W.H., C.R. Wilson, P.L. Poelma, A. Romero, and P.B. Mislivec. 1979. Bacteriological survey of sixty health foods. Appl. Environ. Microbiol. 37:559-566.

Arul J. 1994. Emerging technologies for the control of postharvest diseases of fresh fruits and vegetables, p. 1-13. *In* C.L. Wilson and M.E.Wisniewski (ed.), Biological control of postharvest disease. CRC Press. Boca Raton, FL.

Asplund, K. and E. Nurmi. 1991. The growth of salmonellae in tomatoes. Intl. J. Food Microbiol. 13:177-182.

Anonymous. 1999. Cantaloupe. Purdue University, Center for New Crops and Plant Produce. 18 February 1999, [Internet, WWW], ADDRESS: http://www.hort.purdue.edu/newcrop/Crops/Cantaloupe.html. Accessed 25 March 2002.

Beuchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59: 204-216.

Beuchat, L.R. and J-H. Ryu. 1997. Produce handling and processing practices. Emer. Infect. Dis. 3: 459-465.

Beuchat, L.R., B.V. Nail, B.B. Adler, and M.R.S. Clavero.1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. J. Food Prot. 61:1305-1311.

Beuchat, L.R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect. 4: 413-423.

Blostein, J. 1993. An outbreak of *Salmonella javiana* associated with consumption of watermelon. J. Environ. Health 56:29-31.

Boyhan, G.E., W.T. Kelley, and D.M. Granberry.1999. Culture- Cantaloupe and specialty melons. The University of Georgia, College of Agricultural and Environmental Sciences Cooperative Extension Service. Bulletin 1179. October 1999, [Internet, WWW], ADDRESS: http://www.ces.uga.edu/pubcd/b1179.htm Accessed 25 March 2002.

Brackett, R.E. 1987. Microbiological consequences of minimally processed fruits and vegetables. J. Food Qual.10:195-206.

Brackett, R.E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. Postharvest Biol. & Tech. 15:305-311.

Caño, M.P., J.L. De la Plaza, and L. Munoz-Delgado. 1987. Effect of several postharvest fungicide treatments on carbohydrate evolution of cold stored apples. Food Chem. 25:135-144.

Centers for Disease Control and Prevention (CDC). 1975. *Salmonella typhimurium* outbreak traced to a commercial apple cider – New Jersey. Morb. Mortal. Wkly. Rep. 24:87-88.

Centers for Disease Control and Prevention (CDC). 1991. Multistate outbreak of *Salmonella poona* infections – United States and Canada, 1991. Morb. Mortal. Wkly. Rep. 40:549-552.

Centers for Disease Control and Prevention (CDC). 1999. Outbreaks of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice – United States and Canada. Morb. Mortal. Wkly. Rep. 48:582-585.

Centers for Disease Control and Prevention (CDC). 2002. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupes from Mexico – United States and Canada, 2000 – 2002. Morbid. Mortal. Wkly. Rep. 51:1044-1047.

Chardonnet, C.O., E.E. Sams, W.S. Conway, F.A. Draughton, and J.R. Mount. 2002. Osmotic dehydration of apple slices with $CaCl_2$ and sucrose limits decay caused by *Penicillium expansum*, *Colletotrichum acutatum* and *Botrytis cinerea* and does not promote *Listeria monocytogenes* or total aerobic population growth. J. Food Prot. 65:172-177.

Chung, K.C. and J.M. Goepfert. 1970. Growth of *Salmonella* at low pH. J. Food Sci. 35:326-328.

Conway, W.S., B. Leverentz, R.A. Saftner, W.J. Janiewicz, C.E. Sams, and E. Leblanc. 2000. Survival and growth of *Listeria monocytogenes* on fresh cut apples and its interaction with *Glomerulla cingulata* and *Penicillium expansum*. Plant Dis. 84:177-181.

Cook, K.A., T.E. Dobbs, W.G. Hlady, J.G. Wells, T.J. Barrett, N.D. Puhr, G.A. Lancette, D.W. Bodager, B.L. Toth, C.A. Genese, A.K. Highsmith, K.E. Pilot, L. Finelli, and D.L. Swerdlow. 1998. Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. J. Am. Med. Assoc. 280:1504-1509.

D'Aoust, J-Y. 1997. *Salmonella* species, p. 129-158. *In* M.P. Doyle, L.R. Beuchat, and T.J. Montville (ed.), Food Microbiology: Fundamentals and Frontiers. ASM Press. Washington D.C.

D'Aoust, J-Y. 2000. *Salmonella,* p. 1233-1299. *In* B.M. Lund and G.W. Gould (ed.), The microbiological safety and quality of food. Aspen Publishers. Gaithersburg, MD.

D'Aoust, J-Y. 2001. Foodborne salmonellosis: current international concerns. Food Safety Magazine 7(2):10-17, 51.

Deak, T. and L.R. Beuchat. 1996. Handbook of food spoilage yeasts. CRC Press. Boca Raton, FL.

Deeks, S., A. Ellis, B. Ciebin, R. Khakhria, M, Naus, and J. Hockin. 1998. *Salmonella* Oranienberg, Ontario. Can. Comm. Dis. Rep. 24:177-179.

Dingman, D.W. 2000. Growth of *Escherichia coli* O157:H7 in bruised apple (*Malus domestica*) tissue as influenced by cultivar, date of harvest and source. Appl. Environ. Microbiol. 66:1077-1083.

Doyle, M.P. and D.O. Cliver. 1990. *Salmonella*, p.185-204. *In* D.O. Cliver (ed.), Foodborne diseases. Academic Press. Inc. San Diego, CA.

Draughon, F.A., S. Chen, and J.O. Mundt. 1988. Metabiotic association of *Fusarium*, *Alternaria* and *Rhizoctonia* with *Clostridium botulinum* in fresh tomatoes. J. Food Sci. 53:120-123.

Eckert, J.W. and J.M. Ogawa. 1985. The chemical control of post-harvest diseases: subtropical and tropical fruits. Ann. Rev. Phytopathology 23: 421-454.

El-Ghaouth, A., C.L., Wilson, and M. Wisniewski. 1997. Antifungal activity of 2-deoxy-D-glucose on *Botrytis cinerea, Penicillium expansum*, and *Rhizopus stolonifer*: Ultrastructural and cytochemical aspects. Phytopathol. 87:772-779.

El-Ghaouth, A., C.L., Wilson, and M. Wisniewski. 1998. Ultrastructural and cytochemical aspects of the biological control of *Botrytis cinerea* by *Candida saitoana* in apple fruit. Phytopathol. 88:282-291.

El-Neshway, S.M. and C.L. Wilson. 1997. Nisin enhancement of biocontrol of postharvest diseases of apple with *Candida oleophila*. Post. Biol. and Tech.10:9-14.

Ercolani, G.L. 1976. Bacteriological quality assessment of fresh marketed lettuce and fennel. Appl. Environ. Microbiol. 55:832-836.

Escartin, E.F., A. C. Ayala, and J.S. Lozano. 1989. Survival and growth of *Salmonella* and *Shigella* on sliced fresh fruit. J. Food Prot. 52:471-472.

Food and Drug Administration. 2001a. FDA Survey of imported fresh produce. FY 1999 Field assignment. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. Office of Plant and Dairy Foods and Beverages. January 30, 2001. [Internet, WWW], ADDRESS: http://www.cfsan.gov/~dms/prodsur6.html. Accessed 25 January 2002.

Food and Drug Administration. 2001b. FDA Survey of domestic fresh produce: interim results. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. June 30, 2001. [Internet, WWW], ADDRESS: http://vm.cfsan.fda.gov/~dms/prodsur8.html Accessed 25 January 2002.

Food Safety and Inspection Service (FSIS). 1995. Pathogen reduction: hazard analysis and critical control point (HACCP) systems; proposed rule. Fed. Regis. 60:6774-6889.

Francis, G.A., C. Thomas, and D. O'Bierne. 1999. The microbiological safety of minimally processed vegetables. Int. J. Food Sci. Tech. 34:1-22.

Garcia-Villanova Ruiz, B., R. Galvez Varga, and R. Garcia-Villanova Ruiz. 1987. Contamination of fresh vegetables during cultivation and marketing. Int. J. Food Microbiol. 4:285-291.

Gaylor, G.E., R.A. MacCready, J.P. Reardon, and B.F. Mc Kernan. 1955. An outbreak of salmonellosis traced to watermelon. Public Health Rep. 70:311-313.

Goepfert, J.M. 1980. Vegetables, fruit, nuts and their products, p. 606-642. *In* International Commission on Microbiological Specifications for Foods. Microbial ecology of foods, Food commodities. vol. 2. Academic Press. NY, NY.

Golden, D.A., E.J. Rhodehamel, and D.A. Kautter. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. J. Food Prot. 56:194-196.

Guo, X., J. Chen, R.E. Brackett, and L.R. Beuchat. 2002. Survival of *Salmonella* on tomatoes stored at high relative humidity, in soil, and on tomatoes in contact with soil. J. Food Prot. 65:274-279.

Halloran, N., M.U. Kasim, and R. Çagiran. 1999a. Determination of mechanical injury and effects of bruising on postharvest quality of cantaloupes. Acta Hort. (ISHS) 492:105-112. [Internet, WWW], ADDRESS: http://www.actahort.org/books/492/492_12.htm Accessed 26 March 2002

Halloran, N., M.U. Kasim, R. Çagiran, and A. Karakaya. 1999b. The effect of postharvest treatments on storage duration of cantaloupes. Acta Hort. (ISHS) 492:207-212. [Internet, WWW], ADDRESS http://www.actahort.org/books/492/492_26.htm Accessed 25 March 2002

Hartz, T.K., K.S. Mayberry, and J. Valencia. 1996. Cantaloupe production in California. Vegetable research and information center. Vegetable production series. University of California, Davis. Division of Agriculture and Natural Resources. Publication 7218.

Heard, G. 1999. Microbial safety of ready-to-eat salads and minimally processed vegetables and fruits. Food Australia 51:414-420.

Hedberg, C.W., K.L. MacDonald, and M.T. Osterholm. 1994. Changing epidemiology of foodborne disease: a Minnesota perspective. Clin. Infect. Dis.18:671-682.

Hemphill, D. 2002. Melons. Commercial Vegetable Production Guides. Oregon State University. [Internet, WWW], ADDRESS: http://www.orst.edu/Dept/NWREC/melon.html Accessed 25 March 2002.

Hurst, W.C. 1999. Good agricultural practices in the harvest, handling and packing of cantaloupes. Cantaloupe and specialty melons. The University of Georgia, College of Agricultural and Environmental Sciences Cooperative Extension Service. Bulletin 1179. October 1999, [Internet, WWW], ADDRESS: http://www.ces.uga.edu/pubcd/b1179.htm Accessed 25 March 2002.

Institute of Food Technologists/Food and Drug Administration of the United States Department of Health and Human Services (IFT/FDA). 2001. Analysis and evaluation of preventative control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. IFT/FDA Contract No. 223-98-2333. Task Order No. 3. Institute of Food Technologists. Chicago, IL.

International Commission on Microbiological Specifications for Foods (ICMSF). 1998. Microorganisms in foods. p. 216-251. Microbial ecology of commodities, vol. 6, Blackie Academic & Professional, London, UK.

Janisiewicz, W.J., W.S. Conway, and B. Leverentz. 1999. Biological control of postharvest decays of apple can prevent growth of *Escherichia coli* O157:H7 in apple wounds. J. Food Prot. 62:1372-1375.

Jay, J. 2000. Modern Food Microbiology. 6th ed. Aspen Publishers. Gaithersburg, MD.

Kader, A. A.1992. Modified atmosphere during transport and storage, p. 85-95. *In* A.A. Kader (ed.), Postharvest technology of horticultural crops. University of California, Division of Agriculture and Natural Resources. Publication 3311.

Kasmire, R.F. and M. Cantwell. 1992. Postharvest handling systems: Fruit vegetables, p. 261-266. *In* A.A. Kader (ed.), Postharvest technology of horticultural crops. University of California, Division of Agriculture and Natural Resources. Publication 3311.

Larson, A., J. Wallis, C. Lewandowski, D. Jenson, S. Potsic, M.K. Nickels, M. Lesko, C. Langkop, R.J. Martin, T. Endo, H.B. Ehrhard, and B.J. Francis. 1979. *Salmonella* Oranienberg gastroenteritis associated with consumption of precut watermelons- Illinois. Morb. Mortal. Wkly. Rep. 28:522-523.

Leverentz, B., W.S. Conway, Z. Alavidze, W.J. Janisiewicz, Y. Fuchs, M.J. Camp, E. Chighladze, and A. Sulakvelidze. 2001. Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: A model study. J. Food Prot. 64:1116-1121.

Liao, C-H. and G.M. Sapers. 1999. Influence of soft rot bacteria on growth of *Listeria monocytogenes* in potato tuber slices. J. Food Prot. 62:343-348.

Liao, C-H. and G.M. Sapers. 2000. Attachment and growth of *Salmonella* Chester on apple fruit and in vivo response of attached bacteria to sanitizer treatments. J. Food Prot. 63: 876-883.

Liao, C-H. and P.H. Cooke. 2001. Response to trisodium phosphate treatment of *Salmonella* Chester to fresh-cut green pepper slices. Can. J. Microbiol. 47:25-32.

Liao, C-H. and W.F. Fett. 2001. Analysis of native microflora and selection of strains antagonistic to human pathogens on fresh produce. J. Food Prot. 64:1110-1115.

Lund, B.M. and A.L. Snowdon. 2000. Fresh and processed fruits, p. 738-758. *In* B.L. Lund, T.C. Baird –Parker, and G.W. Gould (ed.), The microbiological safety and quality of food, vol. 1. Aspen Publishers, Gaithersburg, MD.

Madden, J.M. 1992. Microbial pathogens in fresh produce – the regulatory perspective. J. Food Prot. 55:821-823.

Mahon, B.E., A. Ponka, W. Hall, K. Komatsu, S. Dietrich, A. Siitonen, G. Cage, M. Lambert-Fair, P. Hayes, N. Bean, P. Griffin, and L. Slutsker. 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seed. J. Infect. Dis.175:876-882.

Matches, J.R. and J. Liston. 1968. Low temperature growth of *Salmonella*. J. Food Sci. 33:641-645.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P. M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.

Mercier, J. and C.L. Wilson. 1994. Colonization of apple wounds by naturally occurring microflora and introduced *Candida oleophila* and their effect on infection by *Botrytis cinerea* during storage. Biol. Contr. 4:138-144.

Mohle-Boetani, J.C., R. Reporter, S.B. Werner, S. Abbott, J. Farrar, S.H. Waterman, and D.J. Vugia. 1999. An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. J. Infect. Dis. 180:1361-1364.

National Research Council, Board of Agriculture. 1987. Regulating pesticides in food- The Delaney paradox, National Academy Press, Washington DC.

Nguyen-the, C. and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food. Sci. Nutri. 34:371-401.

Nguyen-the, C. and F. Carlin. 2000. Fresh and processed vegetables, p. 620-684. *In* B.L. Lund, T.C. Baird –Parker, and G.W. Gould (ed.), The microbiological safety and quality of Food, vol.1. Aspen Publishers, Gaithersburg, MD.

O'Mahony, P., J. Cowden, B. Smyth, D. Lynch, M. Hall, B. Rowe, E.L. Teare, R.E. Tettmar, A.M. Rampling, M. Coles, R.J. Gilbert, E. Kingcott, and C.L.R. Bartlet. 1990. An outbreak of *Salmonella* Saint-paul infection associated with bean sprouts in the UK. Epidemiol. Infect. 104:229-235.

Orzolek, M.D., G.L. Greaser, and J.K. Harper. 2001. Cantaloupes. Penn State Cooperative Extension. [Internet, WWW], ADDRESS: http://agalternatives.aers.psu.edu/crops/cantaloupe/ Accessed 25 March 2002.

Peet, M. 2001. Muskmelon and watermelon; Sustainable practices for vegetable production in the south. North Carolina State University. 4 October 2001, [Internet, WWW], ADDRESS: http://www.cals.ncsu.edu/sustainable/peet/profiles/c12melon.html Accessed 12 April 2002.

Pirovani, M.E., D.R. Güemes, J.H. Di Pentima, and M.A. Tessi. 2000. Survival of *Salmonella* Hadar after washing disinfection of minimally processed spinach. Lett. in Appl. Microbiol. 31:143-148.

Rhodes, D. 23 December 2001. US Cantaloupe and honeydew melon production statistics (1993-1997). Purdue University. School of Agriculture. Department of Horticulture and Landscape Architecture.

Ries, A.A., S. Zaza, C. Langkop, R.V. Tauxe, and P.A. Blake. 1990. A multistate outbreak of *Salmonella* Chester linked to imported cantaloupe. Abstract. 915, p. 238. *In* Programs and abstracts of 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

Riordan, D.C.R., G.M. Sapers, and B.A. Annous. 2000. The survival of *Escherichia coli* O157:H7 in the presence of *Penicillium expansum* and *Glomerulla cingulata* in wounds on apple surfaces. J. Food Prot. 63:1637-1642.

Roberts, D., G.N. Watson, and R.J. Gilbert. 1982. Contamination of food plants and plant products with bacteria of public health significance, p170-195. *In* M. Rhodes-Roberts and F.A. Skinner (ed.). Bacteria and plants. Soc. Appl. Bacteriol. Symp. Series No. 10. Academic Press, London.

Seo, K.H. and Frank, J.F. 1998. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning electron laser microscopy. J. Food Prot. 62:3-9.

Sewell, A.M. and J.M. Farber. 2001. Foodborne outbreaks in Canada linked to produce. J. Food Prot. 64:1863-1877.

Smith Jr., W.L. 1962. Chemical treatments to reduce postharvest spoilage of fruits and vegetables. The Botanical Review. 28:411-445.

Snowdon, A.L. 1990. A color atlas of post-harvest diseases and disorders of fruits and vegetables. Volume 1: General introduction and fruits. Wolfe Scientific, Barcelona, Spain.

Splittstoesser, D.F. 1996. Microbiology of fruit products, p. 261-292. *In* L.P. Somogyi, H.S. Ramaswamy, and Y.H. Hui (ed.), Processing fruits: Science and Technology. Biology, principles and applications. Technomic Publishing Co., Lancaster, PA.

Suslow, T.V., M. Cantwell, and J. Mitchell. 2000. Cantaloupes: Recommendations for maintaining postharvest quality. University of California, Davis. Postharvest Technology Research and Information Center. 26 June 2000, [Internet, WWW], ADDRESS: http://postharvest.ucdavis.edu/Produce/Produce facts/cantalope.html Accessed 25 March 2002.

Suslow, T. and Cantwell, M. 2001. Recent findings on fresh-cut cantaloupe and honeydew melon. Fresh cut. April 2001. Pp. 12, 20, 32-33.

Tauxe, R.V. 1991. *Salmonella*: A postmodern pathogen. J. Food Prot. 54:563-568.

Tauxe, R.V. 1997. Emerging foodborne diseases: An evolving public health challenge. Dairy Food Environ. Sanit. 17:788-795.

Tauxe, R., H. Kruse, C. Hedberg, M, Potter, J. Madden, and K. Wachsmuth. 1997. Microbial hazards and emerging issues associated with produce; a preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. J. Food Prot. 60:1400-1408.

Ukuku, D.O. and W. F. Fett. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. J. Food Prot. 65:1093-1099.

Ukuku, D.O. and G.M. Sapers. 2001. Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. J. Food Prot. 64:1286-1291.

Van Beneden, C.A., W.E. Keene, D.H. Werker, A.S. King, P.R. Cieslak, K. Hedberg, R.A. Strang, A. Bell, and D. Fleming. 1996. A health food fights back: an international outbreak of *Salmonella* Newport infections due to alfalfa sprouts, K47. Abstr. 36th, p. 259. Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.

Viñas, I., J. Usall, N. Teixidó, and V. Sanchis. 1998. Biological control of major postharvest pathogens on apple with *Candida sake*. Intl. J. Food Microbiol. 40: 9-16.

Walker, B. 2001. *Salmonella* outbreak prompts reminder to scrub cantaloupe before eating. State of Idaho. Department of Health and Welfare. 23 May 2001, [Internet, WWW], ADDRESS: http://www2.state.id.us/dhw/news/2001/01may23-cantaloupe.htm. Accessed 25 March 2002.

Wei, C.I., T.S. Huang, J.M. Kim, W.F. Lin, M.L. Tamlin, and J.A. Bartz. 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. J. Food Prot. 58:829-836.

Weissinger, W.R., W. Chantarapanont, and L.R. Beuchat. 2000. Survival and growth of *Salmonella* Baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. Intl. J. Food Microbiol. 62:123-131.

Wells, J.M. and J.E. Butterfield. 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Dis. 81:867-872.

Wills, R.B.H., D. Graham, B. McGlasson, and D. Joyce. 1998. Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals. CAB International. London, UK.

Wilson, C.L. and M.E. Wisniewski. 1989. Biological control of postharvest diseases of fruits and vegetables: An emerging technology. Annu. Rev. Phytopathol. 27: 425-441.

Wilson, C.L., M.E. Wisniewski, A. El-Ghaouth, S. Droby, and E. Chalutz. 1996. Commercialization of antagonistic yeasts for their biological control of postharvest diseases of fruits and vegetables. SIM News, September/October 1996. 46: 237-242.

Wilson, C.L., J.M. Solar, A. El-Ghaouth, and M.E. Wisniewski. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis. 81: 204-210.

Wood, R.C., C. Hedberg, and K.White. 1991. A multistate outbreak of *Salmonella javiana* infections associated with raw tomatoes. (Abstracts) *In* CDC Epidemic Intelligence Service, 40th Ann. Conf., Atlanta. U.S. Dept. of Health and Human Services, Public Health Science. p.69

Zhuang, R-Y., L.R. Beuchat, and F.J. Angulo. 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl. Environ. Microbiol. 61: 2127-2131.

Zhuang, R-Y. and L.R. Beuchat. 1996. Effectiveness of trisodium phosphate for killing *Salmonella* Montevideo on tomatoes. Lett. Appl. Microbiol. 22: 97-100.

CHAPTER 2

ATTACHMENT TO AND INFILTRATION OF CANTALOUPE RIND AND STEM SCAR TISSUES BY *SALMONELLA* POONA AS AFFECTED BY TEMPERATURE OF FRUIT AND INOCULUM[1]

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ABSTRACT

A negative temperature differential between fruits and water in which they are immersed has been shown to enhance infiltration of water and microorganisms into fruit tissues. The effect of temperature differentials on infiltration of *Salmonella* into cantaloupe rind has not been described. Using a full-factorial experimental design, we assessed the effects of temperature differentials between cantaloupes and suspensions of *Salmonella* Poona, each at 4°C and 30°C, on changes in fruit weight and populations of the pathogen recovered from rinds and stem scar tissues of Eastern and Western type cantaloupes. When the initial temperature of inoculum was 4°C, mean percent increase in Eastern cantaloupe weight caused by immersion for 5 min was significantly higher ($P \leq 0.05$) than when initial temperature of inoculum was 30°C, regardless of cantaloupe temperature. However, the initial temperature of inoculum and cantaloupe did not significantly affect weight gain by Western type cantaloupes. The mean weight gain by Western type cantaloupes was significantly greater ($P \leq 0.05$) than that of Eastern cantaloupes for all cantaloupe / inoculum temperature combinations. Populations of *S.* Poona recovered from stem scar tissues of Eastern and Western type cantaloupes were not significantly different ($P > 0.05$), regardless of cantaloupe / inoculum temperature combination. Attachment to or infiltration of Eastern but not Western type cantaloupe rind by *S.* Poona is enhanced when the cantaloupe is at 4°C compared to 30°C, regardless of the temperature of immersion suspension. The number of *S*. Poona cells recovered from rind tissue of Western cantaloupes at 30°C immersed in inoculum at 30°C was significantly less ($P \leq 0.05$) than that recovered from rinds of cantaloupes at 4°C or 30°C that were immersed in inoculum at 4°C. Results demonstrate that *S*. Poona in immersion water can adhere to and/or infiltrate rind and stem scar tissues of Eastern and Western type cantaloupes. However, the number of cells adhering to or infiltrating tissues is not dictated by

temperature differentials between cantaloupes and immersion suspensions but rather by structures unique to rind surface tissues.

INTRODUCTION

Salmonellae are considered to be a threat to public health worldwide and are a leading cause of foodborne disease in the United States. While the pathogen has been isolated from foods of animal and plant origin (Garcia-Villanova Ruiz *et al.*, 1987), animal products and by-products have been more frequently associated with transmission of salmonellosis (Bacon *et al.*, 2002). Most raw fruits and some raw vegetables have been historically considered to be among the safest foods because their acidic pH prevents the growth of most human pathogens and normal spoilage microflora usually have a competitive advantage (Brackett, 1987; Ukuku *et al.*, 2001). Produce safety has also been attributed in part to protection of subsurface tissues by physical and chemical barriers provided by the skin, rind, or cuticle that prevent or retard infiltration and propagation (Leverentz *et al.*, 2001). Human pathogens are highly sensitive to stresses such as periodic drying of plant surfaces, which has helped to limit their population size on plants (Suslow, 2002).

Coupled with changes in population demography, there have been changes in food consumption patterns. Many consumers in the United States are demanding more fresh and minimally processed produce. To satisfy the escalating consumer demand, there have been significant changes in methods used for growing, harvesting, processing, and distributing fresh and fresh-cut produce. The increased number of foodborne illness outbreaks associated with consumption of raw fruits and vegetables may be attributed, in part, to these changes (Beru and Salsbury, 2002; FDA, 1998).

Washing with unchlorinated or chlorinated water is a common postharvest practice used to clean and sanitize fruits and vegetables. However, water has the potential to be a source of contamination with human pathogens and a vehicle for spreading localized contaminants in field,

processing, transportation, and preparation environments (Suslow, 2002). In one study, contamination of cantaloupes and honeydew melons was traced to the primary wash tank or hydrocooler (Gagliardi *et al*., 2003). Use of water containing enteric bacteria, helminthes, protozoa, and viruses has been linked to contamination of produce (FDA, 1998). *Salmonella* has been isolated from water after washing raw fruits and vegetables (Ercolani, 1976; Wells and Butterfield, 1997). An outbreak of 250 cases of *Salmonella* Chester infection was linked to fresh and cut cantaloupes produced in Latin America (Brackett *et al.*, 1993). It was hypothesized that contaminated water was used to wash the fruits. In 1990 and 1993, two multistate outbreaks of salmonellosis involving at least 300 cases were linked to consumption of fresh tomatoes (CDC, 1993; Wood *et al*., 1991). Tomatoes involved in both outbreaks were traced to single packing facilities where wash water appeared to be the likely source of contamination.

Surveys conducted by the United States Food and Drug Administration revealed that rinds of 7.3% of imported cantaloupes and 4.3% of domestically grown cantaloupes were contaminated with *Salmonella* or *Shigella* (FDA, 2001a; 2001b). Numerous national and international outbreaks of salmonellosis have been epidemiologically linked to fresh cantaloupes (CDC, 1991; CDC, 2002; Deeks *et al*., 1998; IFT/FDA, 2001; Mohle-Boetani *et al*., 1999). *Salmonella* Poona was the predominant serotype responsible for these outbreaks. The association of these outbreaks with consumption of cantaloupes from salad bars and in fruit salads suggests that *Salmonella* is introduced into the fruit flesh from the rind during cutting or physical contact of cut pieces with the contaminated rind. Ukuku and Sapers (2001) showed that *Salmonella* Stanley on the surface of cantaloupes is transferred to internal tissues when cantaloupes are cut in preparation for consumption. As few as 150 *Salmonella* /cm^2 of netted

rind surface have been found to result in contamination of the interior pulp as a result of cutting whole cantaloupes (Suslow and Cantwell, 2001).

Within a few hours of harvesting, commercially grown Eastern type cantaloupe are commonly washed in large dump tanks containing chlorinated water at 45 - 50°C to remove soil and provide some level of sanitization, thereby reducing postharvest microbial spoilage. The temperature and probably the concentration of free chlorine in the water fluctuate during the course of the day. Factors such as air and water temperature, organic matter, and turbulence contribute to chlorine loss by chemical reaction and evaporation (Gagliardi *et al*., 2003). On average, fruits are immersed in water for 5 min before being brushed and partially dried using overhead fans at ambient temperature. Eastern cantaloupes are then cooled to 4 - 8°C with forced air before transporting to wholesale or retail markets. Postharvest handling of commercially grown Western (shipper type) cantaloupes is more diverse. Removal of field heat is mainly accomplished by forced-air cooling; however, hydrocooling and top icing are methods currently utilized in the industry to rapidly attain a temperature of 2 - 4°C. Some Western cantaloupes are coated with food grade vegetable-, petroleum-, beeswax- and / or shellac-based wax or resin immediately after the cooling process. To date, outbreaks of salmonellosis associated with cantaloupes in the United States have been associated with the Western type, but Eastern type cantaloupes may also serve as vehicles.

The extent of infiltration of water into fruits and vegetables is generally dependent on factors such as length of exposure, magnitude of temperature differential, immersion depth, agitation, viscosity of the external environment, and size and number of portals leading to internal airspaces (Bartz and Showalter, 1981). A negative temperature differential, i.e., the temperature of the fruit is higher than the temperature of the water in which it is immersed,

theoretically enhances infiltration of water and microorganisms it may contain. Infiltration of water and plant pathogens into tomatoes has also been shown to be influenced by time- and temperature-independent hydrostatic forces in addition to time-dependent temperature differential phenomena (Bartz, 1982; Bartz and Showalter, 1981). The effect of temperature differentials on infiltration of *Salmonella* into cantaloupe rind has not been described.

The objective of this study was to assess the effects of temperature differentials between cantaloupes and suspensions of *S*. Poona on changes in fruit weight and populations of the pathogen recovered from rinds and stem scar tissues of Eastern and Western type cantaloupes. The performance of two direct plating media for recovering *S*. Poona from inoculated cantaloupes was evaluated.

MATERIALS AND METHODS

Preparation of *S*. Poona and inoculum. Five strains of *Salmonella* Poona isolated from patients in cantaloupe-associated outbreaks of salmonellosis were used. Strains 00A3207, 01A3923, and 02A3275 were obtained from the State of California, Department of Health Services; strains 00A3279 and 01A242 were obtained from Dr. Trevor Suslow, University of California, Davis. Each strain was progressively made resistant to nalidixic acid by a series of nine transfers in tryptic soy broth (TSB; BBL/Difco, Sparks, MD) supplemented with increasing concentrations of nalidixic acid up to 50 µg /ml (TSBN). Cultures were incubated at 37°C for 24 h before transferring to TSB containing a higher concentration of nalidixic acid. Nalidixic acid-resistant cells were surface plated on tryptic soy agar (TSA; BBL/Difco) supplemented with 50 µg of nalidixic acid/ml (TSAN) and incubated at 37°C for 24 h. Stock cultures were stored on TSAN at 4°C.

Stock cultures were streaked on TSAN and incubated at 37°C for 24 h before transferring a single colony of each strain to 10 ml of TSBN and incubating at 37°C for 24 h. Two consecutive transfers of 24-h cultures were made via loop inocula into 10 ml of TSBN. The third transfer was made by depositing 2.0 ml of the 24-h TSBN culture in 198 ml of TSBN in a 500-ml Erlenmeyer flask. After incubation at 37°C for 24 h, cells were harvested by centrifugation at 5,500 x *g* for 15 min at 4°C in a Model J2-MI centrifuge (Beckman; Palo Alto, CA). Cells were washed three times in 200 ml of sterilized deionized water, then suspended in 200 ml of sterile deionized water. The population (CFU/ml) in each suspension was determined by serially diluting suspensions in sterile 0.1% (w/v) peptone water and surface plating samples (0.1 ml in duplicate) on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted. Random presumptive positive colonies of *Salmonella* were confirmed using triple sugar iron, lysine iron agar, and a *Salmonella* latex agglutination assay (Oxoid; Basingstoke, Hampshire, UK).

Equal volumes (400 ml) of suspensions of each strain were combined to generate 4 liters of a five-strain mixture of *S*. Poona containing approximately equal populations (9 $\log_{10}$ CFU/ml) of each strain. The mixture was divided into eight 500-ml portions, four of which were held at 4°C and the other four at 30°C for 3 h to attain desired inoculation temperatures. Immediately before being used to inoculate cantaloupes, each 500-ml suspension was combined with 11.5 liters of sterile deionized water of the same temperature (4°C or 30°C) to give a five-strain population of approximately 7 $\log_{10}$ CFU/ml.

Preparation and inoculation of cantaloupes. Full slip Eastern type cantaloupes (*Cucumis melo* L. var. *reticulatus* Naud; cultivar Athena) weighing 910 – 2680 g were purchased in August, 2002 from three vendors at the Georgia State Farmers' Market, Forest Park, GA.

These cantaloupes were used in three replicate experiments. Western (shipper type) cantaloupes (*Cucumis melo* L. var. *reticulatus* Naud; cultivar Ovassion) weighing 990 – 1500 g were purchased in January, 2003 from a single produce broker at the Georgia State Farmers' Market, Forest Park, GA. Eastern and Western cantaloupes were stored at 4°C for 4 – 6 days before using in experiments. For each study, sixteen Eastern and sixteen Western cantaloupes free of visible wounds, cuts, and bruises were randomly divided into four groups of eight. One group of each type was stored at 4°C and the other group was stored at 30°C for 24 h in forced air incubators. Inoculum (12 liters at 4°C or 30°C) was poured into polyethylene bags (61 cm x 91 cm; Fisher Scientific; Pittsburgh, PA) and placed in a 34-liter plastic container (32 cm long x 25 cm wide x 45 cm high; Rubbermaid; Wooster, OH). Four cantaloupes at 4°C or 30°C were randomly selected from groups of eight cantaloupes of each type, weighed, immersed in the suspension, and constantly agitated with gloved hands for 5 min to facilitate uniform exposure of the surface to the inoculum. A full-factorial experimental design using both types of cantaloupes and inocula, each at 4°C and 30°C, was followed.

After immersion in inocula for 5 min, the cantaloupes were removed and placed on elevated sterile mesh screens. The temperature of the suspensions was measured and approximately 2 min later, when inoculum on the surface of the cantaloupes had stopped dripping, cantaloupes were weighed. The surface of cantaloupes was dried by placing fruits in a laminar flow biosafety hood (class II, type A/B3) for 1 h at 22 ± 1°C before analyzing for populations of *S*. Poona.

Microbiological analysis. Samples of rind from two locations on each fruit were analyzed for populations of *S*. Poona. A piece of rind tissue (4.3 cm x 4.3 cm x ca. 0.5 cm deep) was excised using a sterile stainless steel knife. A sample of stem scar tissue (ca. 9 g) consisting

of the entire surface of the stem scar and a cone-shaped portion of subsurface tissue was removed using a sterile stainless steel spatula. Each sample was placed in a sterile stomacher 80 bag (10.1 cm x 15.2 cm) (Seward Medical Ltd.; London, UK) and pummeled with a small hammer to macerate the tissues. Forty milliliters of sterile lactose broth (LB; BBL/Difco) containing 50 μg of nalidixic acid/ml (LBN) was added and the mixture was pummeled for 1 min at normal speed in a Model 80 Stomacher (Seward Medical). The homogenate liquid was serially diluted in sterile 0.1% peptone water and surface plated (0.25 ml in quadruplicate and 0.1 ml in duplicate) on TSAN and bismuth sulfite agar (BSA; BBL/Difco) supplemented with 50 μg of nalidixic acid/ml (BSAN). Plates were incubated at 37°C for 24 h before presumptive *S*. Poona colonies were counted. Random presumptive-positive colonies were selected for confirmation using triple sugar iron, lysine iron agar, and a *Salmonella* latex agglutination assay (Oxoid).

Statistical analysis. Three replicate experiments were conducted. Data were analyzed to determine the effect of fruit and inoculum temperature differentials on the number of cells adhering to or infiltrating the surface and subsurface areas of the rind and stem scar tissue, as well as changes in weight of cantaloupes after immersion using general linear models on SAS software (Statistical Analysis Systems Institute; Cary, NC). Significant differences ($P \leq 0.05$) between mean values were determined using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Changes in temperature of inoculum and weight of cantaloupes. Listed in Table 2.1 are temperatures of cantaloupes and inocula before immersion of cantaloupes for 5 min and the temperature of inocula after immersion of cantaloupes. The initial temperature of inoculum and cantaloupe affected the mean percent increase in weight of Eastern type cantaloupes, but not the Western type. When the initial temperature of the inoculum was 4°C, the mean percent increase

in Eastern cantaloupe weight caused by immersion for 5 min was significantly higher ($P \leq 0.05$) than when the initial temperature of the inoculum was 30°C, regardless of the cantaloupe temperature. The significantly higher weight gain (0.24%) of Eastern cantaloupes at 30°C immersed in inoculum at 4°C (negative pressure differential), compared to weight gains of cantaloupes subjected to cantaloupe / inoculum temperature combinations of 4°C / 30°C or 30°C / 30°C, was expected. Based on the prediction that a negative temperature differential should result in uptake of more inoculum by cantaloupes compared to a positive temperature differential, the lack of significant difference in weight change by Western cantaloupes subjected to both differentials was not expected. Reasons for the lack of a significant difference between weight gain of Eastern cantaloupes at 30°C immersed in inoculum at 4°C versus cantaloupes at 4°C immersed in inoculum at 4°C are not clear. The temperature of the rind of cantaloupes after subjecting to the 4°C / 4°C treatment system would be lower than the temperature of the rind of cantaloupes after subjecting to the other three temperature differential systems. Exposure of the 4°C / 4°C system cantaloupes to an atmosphere with high relative humidity at 22 $\pm$ 1 °C after removal from the inoculum may have resulted in condensation of atmospheric moisture on the surface, thereby increasing the weight of the cantaloupes before they were weighed.

It is hypothesized that this aberrant weight increase may not have occurred during immersion of cantaloupes, but rather during the 2-min period between immersion and weight

TABLE 2.1. Changes in temperature of inoculum and weight of Eastern and Western type cantaloupes as affected by initial temperature of fruit and inoculum

Temperature (°C)					
	Inoculum				
		Final[a]		Weight Increase %[b]	
Cantaloupe	Initial	Eastern	Western	Eastern	Western
4	4	4.0 (0.0)	4.0 (0.0)	A 0.26 (0.02) B	A 0.43 (0.09) A
	30	28.3 (1.2)	27.0 (0.0)	B 0.18 (0.05) B	A 0.35 (0.13) A
30	4	7.7 (1.2)	8.0 (0.0)	A 0.24 (0.05) B	A 0.39 (0.08) A
	30	30.0 (0.0)	30.0 (0.0)	C 0.13 (0.02) B	A 0.40 (0.08) A

[a] Temperature of inoculum after immersing cantaloupes for 5 min. Number in parenthesis indicates standard deviation.

[b] Within cantaloupe type (Eastern or Western), mean values not preceded by the same letter are significantly different ($P \leq 0.05$). Mean values in each row that are not followed by the same letter are significantly different ($P \leq 0.05$). Number in parenthesis indicates standard deviation.

measurement. The stomata on leafy produce normally close after a small amount of water is lost, but rapid cooling of some tissues can cause the stomata to remain open (Wills *et al*., 1981). A rind surface meshwork of cantaloupe rind tissue, commonly referred to as the net, is an elaborate system of lenticels and cork cells comprising the complementary tissue of lenticels and the epiderm (Webster and Craig, 1976). Deviations from the norm in terms of uptake of water by cantaloupes as affected by temperature differential may be attributable in part to the complex lenticel structure of the rind net and stem scar tissues unique to cantaloupes. The surface of cantaloupe rind is replete with lenticels and cork cells that do not possess a mechanism for closing. A pervasive network of airspaces connecting the internal tissues of cantaloupe rind to the external atmosphere supplies air for normal respiratory processes. Gaseous exchange is facilitated by lenticular net development. When cantaloupes at a given temperature are immersed in water at a lower temperature, the decreased internal gas pressure theoretically causes an influx of water via portals such as lenticels and perhaps also the stem scar tissue. By extension, this behavior could contribute to retention of water by cantaloupes immersed in inoculum at 4°C.

The percentage weight gained by Western type cantaloupes as a result of immersion was significantly greater ($P \leq 0.05$) than that for Eastern cantaloupes for all cantaloupe / inoculum temperature combinations. The dissimilarity in weight gain response between Eastern and Western type cantaloupes is attributable to differences in fruit morphology and density of rind netting. Western type cantaloupes lack distinct sutures and the rinds are more densely and evenly netted than are rinds of Eastern cantaloupes. Eastern cantaloupes have more prominent crests and ridges, and rinds have a less uniform distribution of netting compared to rinds of Western cantaloupes. Areas on the rind of Eastern type cantaloupes that lack netting would be

expected to imbibe less water, but be more sensitive to the effects of temperature differentials owing to the lack of insulation assumed to be imparted by dense net tissue. Compared to Eastern type cantaloupes, the uniformity of exposed surface area and intensity of netting of Western cantaloupes could obscure the effects of temperature on water uptake. The dense netting of Western type cantaloupes is a manifestation of greater disruption of the cuticle, enabling retention of more water than netting of Eastern type cantaloupes.

Recovery of *S.* Poona from cantaloupes. Populations of *S.* Poona recovered from the rind tissue and stem scar tissue of cantaloupes were, respectively, 0.10 – 1.05 $\log_{10}$ CFU/cm^2 and 0.15 – 0.85 $\log_{10}$ CFU/g higher on TSAN than on BSAN, regardless of the fruit type or temperature of fruit or inoculum (Table 2). In only one of sixteen cantaloupe type / temperature / tissue type combinations (Western type, 4°C / 4°C, stem scar tissue) was the population recovered on TSAN significantly ($P \leq 0.05$) higher than on BSAN. The lower number of *S.* Poona recovered on BSAN is attributed to the inability of damaged or stressed cells to resuscitate when exposed to selective conditions imposed by this medium. Populations recovered on TSAN and BSAN from cantaloupes subjected to all other combinations of test parameters were not significantly different ($P > 0.05$). Tryptic soy agar is a nutrient-rich medium used for isolating and cultivating fastidious microorganisms whereas BSAN contains ingredients that are inhibitory to the growth of gram-positive bacteria and perhaps also injured *Salmonella*. Nalidixic acid was added to both media as a marker to eliminate or reduce the growth of gram negative microorganisms naturally occurring on the cantaloupe surface. Gram-negative bacteria are sensitive to nalidixic acid, having minimal inhibiting concentrations of < 10 μg/ml, unless adapted to grow in media containing higher concentrations.

TABLE 2.2. Populations of *Salmonella* Poona recovered from Eastern and Western cantaloupes as affected by initial temperature of fruit and inoculum

Temperature (°C)		Enumeration	Population[a]			
			Rind tissue (log_{10}CFU/cm^2)		Stem scar tissue (log_{10}CFU/g)	
Cantaloupe	Inoculum	medium	Eastern	Western	Eastern	Western
4	4	TSAN	A 5.00 A	A 5.25 A	A 5.83 A	A 5.56 A
		BSAN	A 4.90 A	A 5.01 A	A 5.19 A	A 5.32 B
4	30	TSAN	A 5.00 A	B 4.66 A	A 6.01 A	A 5.42 A
		BSAN	A 4.60 A	B 4.45 A	A 5.16 A	A 5.27 A
30	4	TSAN	B 4.56 A	A 5.32 A	A 6.00 A	A 6.78 A
		BSAN	C 3.51 A	A 5.01 A	A 5.58 A	A 6.32 A
30	30	TSAN	B 4.74 A	B 4.68 A	A 5.79 A	A 5.37 A
		BSAN	B 4.22 A	B 4.57 A	A 5.26 A	A 5.13 A

[a] Within enumeration medium, tissue type, and fruit type, values not preceded by the same letter are significantly different ($P \leq 0.05$). Within cantaloupe / inoculum temperature combination, tissue type, and fruit type, values not followed by the same letter are significantly different ($P \leq 0.05$).

Populations of *S.* Poona recovered from the rind of Eastern type cantaloupes after immersion in suspensions at 4°C or 30°C were significantly higher ($P \leq 0.05$) when cantaloupes were at 4°C compared to 30°C before immersion (Table 2.2). The population of *S.* Poona recovered on BSAN from rind of Eastern cantaloupes at 30°C was significantly higher ($P \leq 0.05$) when fruits were immersed in a warm (30°C) inoculum compared to populations recovered from fruits immersed in chilled (4°C) inoculum, although the low number of cells may be attributable in part to inability of injured cells to resuscitate and form colonies. Observations on populations recovered on both media were contrary to predictions based on observations on infiltration of microorganisms into tomatoes (Bartz, 1982; Bartz and Showalter, 1981) and apples (Buchanan *et al*., 1999; Burnett *et al*., 2000; Kenney and Beuchat, 2002) as affected by temperature differences between fruits and immersion suspensions. Results of these studies support the contention that uptake of a larger volume of water by warm fruits exposed to cold water, compared to cold fruits exposed to warm water, would allow more bacterial cells to adhere to or infiltrate fruits during hydrocooling. Comparative recovery of *S.* Poona from the rind and stem scar tissues of Eastern and Western cantaloupes exposed to positive and negative temperature differentials did not conform to these predictions. We recovered significantly higher ($P \leq 0.05$) numbers of *S.* Poona from the rind of chilled Eastern cantaloupes immersed in a warm suspension of the pathogen (positive temperature differential) than from warm cantaloupes immersed in a cool suspension (negative temperature differential). Our study indicates that adherence to or infiltration of Eastern but not Western type cantaloupe rind by *S.* Poona is enhanced when the cantaloupe is at 4°C compared to 30°C, regardless of the temperature of the immersion suspension. The higher number of cells adhering to or infiltrating cantaloupe rind at 4°C, compared to 30°C, apparently masks any effect temperature differential might have on

extent of adherence or infiltration. The number of *S*. Poona cells recovered from rind tissue of Western cantaloupes at 30°C immersed in inoculum at 30°C was significantly less ($P \leq 0.05$) than the number of cells recovered from rinds of cantaloupes at 4°C or 30°C that were immersed in inoculum at 4°C, indicating that immersion of warm fruits in warm inoculum diminishes adherence of *S*. Poona. As with Eastern cantaloupes, the effect of temperature differential between Western cantaloupes and inoculum did not conform to expectations based on observations made by others on infiltration of spoilage and pathogenic bacteria into tomatoes and apples.

The rind surface of Eastern and Western type cantaloupes is scabrous in texture due to disruption of the cuticular layer and presence of the net. The presence of these complex surface structures apparently negates predictions of infiltration based on the effects of temperature differentials on infiltration of bacterial populations into apples and tomatoes, which have a relatively even distribution of cuticle and smoother, much less porous surfaces. We hypothesize that the atypical comparative recovery of *S*. Poona from the rind and stem scar tissues of Eastern and Western type cantaloupes exposed to positive and negative temperature differentials is due largely to their unique surface tissue composition, structure, and respiratory activity.

The observation that a significantly higher ($P \leq 0.05$) population of *S*. Poona was recovered from Eastern cantaloupes at 4°C before immersion, compared to those at 30°C, concurs with observations on the attachment of *Escherichia coli* O157:H7 to lettuce leaves as affected by temperature (Takeuchi and Frank, 2000). They postulated that warm temperature promotes active respiration of lettuce tissues, and that the resultant gas movement produces a counterforce to resist penetration of *E. coli* O157:H7 cells. The attachment of *E. coli* O157:H7 to lettuce has also been attributed to changes in cell physiology and attachment mechanisms at

reduced temperature (Takeuchi *et al*., 2001). *S*. Poona may have exhibited a similar behavior on cantaloupe rind. Cooling of superficial air on the porous surface of rind during the drying process could have resulted in greater cell ingress of the tissue of warm Western cantaloupes that were immersed in the 4°C suspension compared to the warm cantaloupes that were immersed in 30°C suspension. If a reduction in temperature during drying enhances attachment or penetration of bacterial cells into damaged plant tissues, we postulate that a greater number of cells adhered to cooled Western cantaloupe rind tissues.

The outer surface of cantaloupes presents a variety of structures and surfaces to which bacteria may attach. As cantaloupe fruits mature, the hard epicarp undergoes progressive modification that involves the development of a network of corklike tissue originating from multi-layered hypodermal tissues (Webster and Craig, 1976). The surface becomes extensively cracked and deep fissures disrupt the cuticle and some of the subtending epidermal and hypodermal cells. The thickened and pitted cell walls have microscopic local thickenings and warts. Additionally, there are depressions and grooves with jointed conical hairs up to 375 μm in length on the net surface (Barber, 1909; Webster and Craig, 1976). It has been hypothesized that the presence of cuticle on fruits and other plant parts prevents or retards penetration by foodborne pathogens (Burnett and Beuchat, 2001; Kenney *et al*., 2001) and phytopathogenic microorganisms (Beattie, 2002; Morris *et al*., 2002). This function is attributed to the chemical nature of cutin, a highly polymerized hydrophobic material consisting of fatty acids. However, extensive disruption of the cuticle on cantaloupes would compromise such effectiveness (Webster and Craig, 1976). Damaged cuticle has been shown to enhance the ability of bacteria to adhere to plant tissues (Kenney *et al*., 2001). Microorganisms have been observed to concentrate in areas on fresh cut spinach where the cuticle and underlying tissues are damaged,

causing infection of the internal palisade parenchyma (Babic *et al*., 1996). When the topographical structures on apple skin was examined with scanning electron microscopy, attachment of *S*. Chester was primarily on surfaces of injured tissue and on stem and calyx parts, but rarely on the unbroken skin (Liao and Sapers, 2000). Preferential attachment of *S*. Chester was attributed to differences in topographical structures and physicochemical properties.

Populations of *S.* Poona recovered from the stem scar tissues of Eastern and Western type cantaloupes were not significantly different ($P > 0.05$), regardless of cantaloupe / inoculum temperature combination (Table 2.2). The stem scar tissue of tomatoes, on the other hand, is a primary site of infiltration of bacteria (Bartz, 1982; Bartz and Showalter, 1981; Zhuang and Beuchat, 1996). When raw tomato fruits were immersed in a suspension of *Salmonella* Montevideo, more cells were retained in the stem scar and core tissues than on tomato skin (Wei *et al*., 1995). Cantaloupe fruits mature on the vine with the peak of the respiratory climacteric coinciding with abscission at approximately 42 days after anthesis. The entire middle lamella surrounding cells at the stem / fruit tissue junction breaks down, and pectin dissolution and changes in polysaccharides result in a simultaneous separation of contiguous parenchyma cells within the abscission zone (Webster, 1975). Disjunct parenchyma cells soon collapse and several small cavities, which are separated from each other by intact parenchyma and vascular tissues, become evident. The anatomical and histochemical changes that occur in postharvest cantaloupes could affect attachment or infiltration of bacterial cells. Irregularities such as abrasions, crevices, and pits on the surface of cantaloupes enhance the ease of bacterial cell attachment and interfere with their removal (Ukuku *et al*., 2001). These processes may have occurred in Eastern and Western cantaloupes examined in our study, although the number of *S*.

Poona adhering to or infiltrating the stem scar surface and tissue was unaffected by differences in temperatures of cantaloupes and inocula.

Attachment of *E. coli* O157:H7 to the surface of lettuce (Takeuchi and Frank, 2000) and cantaloupes (Ukuku *et al.,* 2001) is influenced by the inoculum size. Attachment of bacteria to cantaloupe has also been attributed to surface charge and hydrophobicity of cells, as well as associated flagella, fimbriae, outer membrane proteins, and extracellular polysaccharides (Ukuku and Fett, 2002). The presence of waxes and suberin in cork cells within lenticels is thought to render the rind net impervious to water (Webster and Craig, 1976). However, the porous netted texture of cantaloupe rind promotes attachment and harborage of microorganisms, which results in difficulty in sanitization (Gagliardi *et al*., 2003).

This study demonstrates that *S*. Poona in immersion water can adhere to cantaloupe rind and stem scar tissues. The histology of the cantaloupe rind and stem scar tissues augments attachment and penetration, and may thereby reduce the effectiveness of sanitizer treatments. *Salmonella* and other foodborne pathogens attached to the external surfaces of cantaloupes are of great concern because they can be transferred to the edible pulp during cutting and removal of the rind. The abundance of nutrients and the near neutral pH of mature cantaloupe flesh would be expected to enhance the survival and growth of *Salmonella* and other foodborne pathogens, even at refrigeration temperature. It is important to develop and implement production, processing, and preparation practices that include vigilant monitoring of the microbiological quality of water that comes in contact with cantaloupes and prevent cross-contamination with pathogens from other sources at all points in the preharvest and postharvest system.

REFERENCES

Babic, I., S. Roy, A.E. Watada, and W.P. Wergin. 1996. Changes in microbial population on fresh cut spinach. Int. J. Food Microbiol. 31:107-119.

Bacon, R.T., J.N. Sofos, K.E. Belk, D.R. Hyatt, and G.C. Hyatt. 2002. Prevalence and antibiotic susceptibility of *Salmonella* isolated from beef animal hides and carcasses. J. Food Prot. 65:284-290.

Barber, K.G. 1909. Comparative histology of fruit and seeds of certain species of Cucurbitaceae. Bot. Gaz. 47:263-310.

Bartz, J.A. 1982. Infiltration of tomatoes immersed at different temperatures to different depths in suspensions of *Erwinia caratovora* subsp. c*aratovora*. Plant Dis. 66:302-305.

Bartz, J.A. and R.K. Showalter. 1981. Infiltration of tomatoes by aqueous suspensions. Phytopathology 71:515-518.

Beattie, G.A. 2002. Leaf surface waxes and the process of leaf colonization by microorganisms, p. 3-23. *In* S.E. Lindow, E.I. Hecht-Poinar, and V.J. Elliot (Ed.); Phyllosphere microbiology, APS Press, St. Paul, MN.

Beru, N. and P.A. Salsbury. 2002. FDA's produce safety activities. Food Safety Magazine. p.14, February/March 2002.

Brackett, R.E. 1987. Microbiological consequences of minimally processed fruits and vegetables. J. Food Qual.10:195-206.

Brackett, R.E., D.M. Smallwood, S.M. Fletcher, and D.L. Horton. 1993. Food safety: Critical points within the production and distribution system. *In* R.L. Shewfelt and S.E. Prussia (Ed.); Postharvest handling: A systems approach. Academic Press, San Diego, California.

Buchanan, R.L., S.G. Edelson, R.L. Miller, and G.M. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. J. Food Prot. 62:444-450.

Burnett, S.L. and L.R. Beuchat. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. J. Ind. Microbiol. Biotechnol. 27:104-110.

Burnett, S.L., J. Chen, and L.R. Beuchat. 2000. Attachment of *Escherichia coli* O157:H7 to the surface and internal structures of apples as demonstrated by confocal scanning laser microscopy. Appl. Environ. Microbiol. 66:4679-4687.

Centers for Disease Control and Prevention (CDC). 1991. Multistate outbreak of *Salmonella poona* infections – United States and Canada, 1991. Morbid. Mortal. Wkly. Rep. 40:549-552.

Centers for Disease Control and Prevention (CDC). 1993. Multistate outbreak of *Salmonella* serotype Montevideo infections. EPI-AID 93-79, 1993.

Centers for Disease Control and Prevention (CDC). 2002. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupes from Mexico – United States and Canada, 2000 – 2002. Morbid. Mortal. Wkly. Rep. 51:1044-1047.

Deeks, S., A. Ellis, B. Ciebin, R. Khakhria, M. Naus, and J. Hockin. 1998. *Salmonella* Oranienberg, Ontario. Can. Comm. Dis. Rep. 24:177-179.

Ercolani, G.L. 1976. Bacteriological quality assessment of fresh marketed lettuce and fennel. Appl. Environ. Microbiol. 55:832-836.

Food and Drug Administration (FDA). 1998. Guidance for industry. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. October 26, 1998. [Internet, WWW], ADDRESS: http://www.cfsan.gov/~dms/prodguid.html. Accessed 4 February 2002.

Food and Drug Administration (FDA). 2001a. FDA Survey of imported fresh produce. FY 1999 Field assignment. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. Office of Plant and Dairy Foods and Beverages. January 30, 2001. [Internet, WWW], ADDRESS: http://www.cfsan.gov/~dms/prodsur6.html. Accessed 25 January 2002.

Food and Drug Administration (FDA). 2001b. FDA Survey of domestic fresh produce: interim results. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. June 30, 2001. [Internet, WWW], ADDRESS: http://vm.cfsan.fda.gov/~dms/prodsur8.html Accessed 25 January 2002.

Gagliardi, J.V., P.D. Miller, G. Lester, and D. Ingram. 2003. On-farm and postharvest processing sources of bacterial contamination to melon rinds. J. Food Prot. 66:82-87.

Garcia-Villanova Ruiz, B., R. Galvez Vargas, and R. Garcia-Villanova. 1987. Contamination on fresh vegetables during cultivation and marketing. Intl. J. Food Microbiol. 4:285-291.

Institute of Food Technologists/Food and Drug Administration of the United States Department of Health and Human Services (IFT/FDA). 2001. Analysis and evaluation of preventative control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. IFT/FDA Contract No. 223-98-2333. Task Order No. 3. Institute of Food Technologists. Chicago, IL.

Kenney, S.J. and L.R. Beuchat. 2002. Survival of *Escherichia coli* O157:H7 and *Salmonella* Muenchen on apples as affected by application of commercial fruit waxes. Intl. J. Food Microbiol. 77:223-231.

Kenney, S.J., S.L. Burnett, and L.R. Beuchat. 2001. Location of *Escherichia coli* O157:H7 on and in apples as affected by bruising, washing, and rubbing. J. Food. Prot. 64:1328-1333.

Leverentz, B., W.S. Conway, Z. Alavidze, W.J. Janisiewicz, Y. Fuchs, M.J. Camp, E. Chighladze, and A. Sulakvelidze. 2001. Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: A model study. J. Food Prot. 64:1116-1121.

Liao, C.-H. and G. M. Sapers. 2000. Attachment and growth of *Salmonella* Chester on apple fruits and in vivo response of attached bacteria to sanitizer treatments. J. Food Prot. 63:876-883.

Mohle- Boetani, J.C. R. Reporter, S.B. Werner, S. Abbott, J. Farrar, S.H. Waterman, and D.J. Vugia. 1999. An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. J. Infect. Dis. 180:1361-1364.

Morris, C.F., M.H. Barnes, and R.J.C. McLean. 2002. Biofilms on leaf surfaces: Implications for the biology, ecology, and management of populations of epiphytic bacteria, p.139-155. *In* S.E. Lindow, E.I. Hecht-Poinar, and V.J. Elliot (Ed.); Phyllosphere microbiology. APS Press, St. Paul, MN.

Suslow, T.V. 2002. Production practices affecting the potential for persistent contamination of plants by microbial foodborne pathogen, p. 241-256. *In* S.E. Lindow, E.I. Hecht-Poinar, and V.J. Elliot (Ed.); Phyllosphere microbiology. APS Press, St. Paul, MN.

Suslow, T. and M. Cantwell. 2001. Recent findings on fresh-cut cantaloupe and honeydew melon. Fresh cut. April 2001. Pp. 12, 20, 32-33.

Takeuchi, K. and J.F. Frank. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. J. Food Prot. 63:434-440.

Takeuchi, K. A.N. Hassan, and J.F. Frank. 2001. Penetration of *Escherichia coli* O157:H7 into lettuce as influenced by modified atmosphere and temperature. J. Food Prot. 64:1820-1823.

Ukuku, D.O., V. Pilizota, and G.M. Sapers. 2001. Influence of washing treatment on native microflora and *Escherichia coli* population of inoculated cantaloupes. J. Food Safety. 21:31-37.

Ukuku, D.O. and G.M. Sapers. 2001. Effect of sanitizer treatments on S*almonella* Stanley

attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. J. Food Prot. 64:1286-1291.

Ukuku, D.O. and W. F. Fett. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. J. Food Prot. 65:1093-1099.

Webster, B.D. 1975. Anatomical histochemical modifications associated with abscission of *Cucumis* fruits. J. Amer. Soc. Hort. Sci. 100:180-184.

Webster, B.D. and M.E. Craig. 1976. Net morphogenesis and characteristics of the surface of muskmelon fruit. J. Amer. Soc. Hort. Sci. 101:412-415.

Wei, C.I., T.S., Huang, J.M. Kim, W.F. Lin, M.L. Tamlin, and J.A. Bartz. 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. J. Food Prot. 58:829-836.

Wells, J.M., and J.E. Butterfield. 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Dis. 81:867- 872.

Wills, R.B.H., T.H Lee, D. Graham, W.B. Mc Glasson and E.G. Hall. 1981. Postharvest. An introduction to the physiology and handling of fruit and vegetables. AVI publishing Company. New South Wales University Press, Australia.

Wood, R.C., C. Hedberg, and K. White. 1991. A multistate outbreak of *Salmonella* Javiana infections associated with raw tomatoes, p. 69. Abstr. Epidemic Intelligence Serv. 40th Annu. Conf. Centers for Disease Control and Prevention, Atlanta.

Zhuang, R.Y. and L.R. Beuchat. 1996. Effectiveness of trisodium phosphate for killing *Salmonella* Montevideo on tomatoes. Lett. Appl. Microbiol. 22:97-100.

CHAPTER 3

METABIOTIC ASSOCIATIONS OF MOLDS AND *SALMONELLA* POONA ON INTACT AND WOUNDED CANTALOUPE RIND[1]

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ABSTRACT

Salmonella Poona, a serotype rarely implicated in human infections, has recently caused several cantaloupe-associated outbreaks of salmonellosis in the United States and Canada. Metabiotic associations of molds and foodborne pathogens on produce have been reported. We tested proteolytic activity and measured changes in the pH of cantaloupe rind caused by growth of *Alternaria alternata, Cladosporium cladosporioides, Epicoccum nigrum, Geotrichum candidum*, and *Penicillium expansum,* molds commonly involved in postharvest decay, and determined survival and growth characteristics of *S*. Poona co-infected with each mold on the surface rind and in wounded rind tissue as affected by temperature. *C. cladosporioides, G. candidum*, and *P. expansum,* but not *A. alternata* and *E. nigrum*, showed proteolytic activity on agar media containing gelatin and/or casein, with concurrent increases in pH. Intact and mechanically wounded tissue of cantaloupe rinds were inoculated with a five-strain mixture of *S*. Poona and/or test mold. Five inoculation schemes were used: mold only, *S*. Poona only, mold and *S*. Poona simultaneously, mold then *S*. Poona 3 days later, and *S*. Poona then mold 3 days later. The pH of cantaloupe rinds inoculated with molds and stored at 20°C for 14 days was significantly higher ($P \leq 0.05$) than on day 0. However, only the pH of rinds inoculated with *C. cladosporioides* or *G. candidum* was significantly higher ($P \leq 0.05$) on day 21 than on day 0, when cantaloupes were stored at 4°C. An initial population of *S*. Poona increased from 3.3 $\log_{10}$ CFU/sample (ca. 7 cm^2) of cantaloupe rind to populations as high as 9.5 $\log_{10}$ CFU/sample during storage at 20°C for up 14 days, regardless of co-inoculation with molds. Populations of *S*. Poona decreased or remained constant at 4°C for up to 21 days. Results demonstrate that *S.* Poona can persist and grow on intact, wounded, and decaying cantaloupe rind at refrigerated and ambient temperatures and is not affected by the presence of test molds.

INTRODUCTION

Fresh produce, historically regarded as being microbiologically safe, is now recognized as a potential vehicle for foodborne pathogens. Numerous pathogenic microorganisms have been isolated from raw fruits and vegetables, and cantaloupes are among the produce that have been implicated in outbreaks of human infections (Beuchat, 1996; 2002). Cantaloupes provide variety, flavor, aesthetic appeal, and essential nutrients to diets. Minimally processed fresh-cut cantaloupe is widely accepted in the United States, largely because it offers convenience in preparation and serving. The per capita consumption of cantaloupes has increased in the United States in recent years. Domestic production and importation of cantaloupes have steadily increased to adequately meet the growing demand. However, postharvest decay limits shelf life in the fresh-fruit market (Sapers *et al*., 2001).

Several national and international outbreaks of salmonellosis have been epidemiologically linked to consumption of fresh cantaloupes (CDC, 1991; CDC, 2002; Deeks *et al*., 1998, IFT/FDA, 2001, Mohle-Boetani *et al*., 1999; Ries *et al.*, 1990). In October 2002, the United States Food and Drug Administration (FDA) issued an alert on importation of cantaloupes originating in Mexico. Outbreaks of salmonellosis were traced to cantaloupes originating in Mexico in 2000, 2001, and 2002 (FDA, 2002). *Salmonella* Poona, a relatively rare serotype, has caused four of seven cantaloupe-associated salmonellosis outbreaks. Typically, human infection with *S*. Poona is associated with exposure to reptiles. Three outbreaks of *S*. Poona infections were epidemiologically linked to cantaloupes grown on Mexican farms in the same region, suggesting the possibility of a unique natural reservoir of this serotype in the production, harvesting, or packing environment. It has been hypothesized that reptiles such as iguanas are attracted to feed on melons in the field or in packing sheds, thereby cross-

contaminating preharvest fruits or equipment and water used in washing and cooling processes (CDC, 2002).

Cantaloupe fruits may be in direct contact with the ground during their development on long, running, non-climbing vines that are prostrate on the soil. The growth habit of cantaloupes enhances the potential for fruits to be contaminated by pathogens that may be present in the soil. Postharvest handling may also bring cantaloupes in direct contact with various sources of foodborne pathogens. Fruits are particularly subject to contamination when rind surface integrity is compromised through disease, bruising, cutting, or peeling. Inadequate washing, storage, and handling augment the potential for growth of pathogens and cross contamination (Sewell and Faber, 2001). Field surveys conducted by the FDA during 1990 and 1991 revealed that 0.76% and 1.06%, respectively, of cantaloupe rinds harbored several serovars of *Salmonella* (Madden, 1992; Golden *et al*., 1993). More recently, 7.3% of 151 imported cantaloupes were found to harbor *Salmonella* or *Shigella* (FDA, 2001a). The results of a survey of domestic cantaloupes indicated the presence of these pathogens on 4.3% of 92 fruits (FDA, 2001b). Studies by Ukuku and Sapers (2001) indicate that outbreaks of salmonellosis may result from fresh-cut cantaloupes consumed 3 days after contamination, because cells attached to the cantaloupe rind for 3 or more days were difficult to kill using chlorine or hydrogen peroxide treatments.

The association of salmonellosis with cantaloupes on salad bars and fruit salads suggests an introduction of *Salmonella* into the edible flesh of fruits during slicing, or direct contact of the flesh with contaminated rind (Golden *et al.,* 1993). As few as 150 *Salmonella*/cm^2 of netted rind have been shown to contaminate the pulp as a result of cutting whole cantaloupes (Suslow and Cantwell, 2001). The potential for growth of *Salmonella* on tissue is exacerbated by displaying or storing cut cantaloupes at ambient temperature for an extended time (CDC, 1991; Golden *et*

al., 1993). Cut cantaloupe is considered a potentially hazardous food in the FDA Food Code because its low acidity (pH 5.2 to 6.7) and high water activity (0.97 to 0.99) enable the growth of pathogens (IFT/FDA, 2001).

By virtue of their differences in physical structure, intact plant tissues are resistant to infiltration and infection by most microorganisms. Plant diseases are the exception but occur only when several conditions are fulfilled (Snowdon, 1990). Extreme temperatures alter the physiology of raw fruits and vegetables, increasing their susceptibility to certain plant pathogens. Disease incidence and severity is dependent on the capability and population of microorganisms in the inoculum. Fungi are overwhelmingly responsible for postharvest diseases of fruits (Sommer *et al*., 1992). Cantaloupes are susceptible to postharvest fungal rots, especially under wet, warm conditions. Rots occur on the external surface of the fruit and gradually progress inward to the flesh. Phytopathogenic fungi of major concern in cantaloupes include species of *Alternaria*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Penicillium*, *Rhizopus*, and *Mucor* (Ceponis *et al*., 1986; Salunkhe and Desai, 1984; Suslow *et al*., 2000). These molds are also involved in spoilage of foods that are not necessarily of plant origin.

Infection of cantaloupes by plant pathogenic fungi and contamination with foodborne bacteria may occur before harvesting, at the time harvest, during handling, storage, transport, and marketing, or after purchase by the consumer. Metabiosis refers to circumstances in which growth of one microorganism creates favorable conditions for growth of another (Odlaug and Pflug, 1979). Metabiotic associations of molds and *Clostridium botulinum* on tomatoes (Draughon *et al.*, 1988) and *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fresh-cut apples (Chardonnet *et al.*, 2002; Conway *et al.*, 2000; Riordan *et al.*, 2000) have been reported. The behavior of foodborne pathogens such as *Salmonella* on or in cantaloupes as affected by

metabiotic activities of plant pathogens has not been investigated. During transportation, storage, and display at the retail level, physically sound and rotting fruits can come in direct contact with each other. Cross contamination with *Salmonella,* followed by growth of the pathogen, would increase the safety risk to the consumer. Thrifty consumers may be willing to purchase fruits with decaying areas, pare away these areas, and consume the presumably safe remainder.

The objective of this study was to examine the association between selected molds pathogenic to cantaloupes and *S.* Poona on the surface of intact rind and in wounds in the rind. Changes in pH caused by growth of molds were monitored, as were survival and growth of *S.* Poona in co-infected tissue as affected by temperature.

MATERIALS AND METHODS

Culture conditions for *S.* Poona and preparation of inoculum. Five strains of *S.* Poona isolated from patients in cantaloupe-associated outbreaks of salmonellosis were used. Strains 00A3207, 01A3923, and 02A3275 were obtained from the State of California, Department of Health Services, while strains 00A3279 and 01A242 were obtained from Dr. Trevor Suslow, University of California, Davis. Each strain was progressively made resistant to nalidixic acid by a series of nine transfers in tryptic soy broth (TSB; BBL/Difco, Sparks, MD) supplemented with increasing concentrations of nalidixic acid up to 50 μg/ml (TSBN). Cultures were incubated at 37°C for 24 h before transferring to TSB containing a higher concentration of nalidixic acid. The nalidixic acid-resistant cells were surface plated on tryptic soy agar (TSA; BBL/Difco) supplemented with 50 μg of nalidixic acid/ml (TSAN) and incubated at 37°C for 24 h. Stock cultures were stored on TSAN at 4°C.

Stock cultures were streaked on TSAN and incubated at 37°C for 24 h before transferring cells from a single colony of each strain to 10 ml of TSBN and incubating at 37°C for 24 h. Three consecutive 24-h transfers of suspensions were made via loop inocula into 10 ml of TSBN. After incubation at 37°C for 24 h, the cells were harvested by centrifugation at 2,500 x *g* for 10 min in a Centra CL2 centrifuge (International Equipment Company, Needham Heights, MA). Cells were washed two times in 10 ml of sterile deionized water, then resuspended in 10 ml of sterile 0.1% (w/v) peptone water. The population (CFU/ml) in each suspension was determined by serially diluting suspensions in sterile 0.1% peptone water and surface plating samples (0.1 ml in duplicate) on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted. Random presumptive-positive colonies of *Salmonella* were confirmed using triple sugar iron, lysine iron agar, and a *Salmonella* latex agglutination assay (Oxoid, Basingstoke, Hampshire, UK).

Equal volumes (5 ml) of suspensions of each strain were combined to give 25 ml of a five-strain mixture of *S*. Poona containing approximately equal populations (ca. 9 $\log_{10}$ CFU/ml) of each strain. The suspension was serially diluted to give an inoculum that contained ca. 5 $\log_{10}$ CFU of each strain/ml.

Procedures for growing molds and preparation of inocula. Strains of *A. alternata, C. cladosporioides, E. nigrum, G. candidum,* and *P. expansum* isolated from cantaloupes were obtained from Dr. Trevor Suslow, University of California, Davis. Molds were maintained on dichloran rose bengal chloramphenicol (DRBC) agar (BBL/Difco) and potato dextrose agar (PDA) (BBL/Difco) slants at 4°C, and subcultured every 14 days by streaking on fresh DRBC agar and PDA plates and incubating at 25°C for 5 days.

Cultures were streaked on DRBC agar and incubated at 25°C for 5 days. Suspensions of conidia or arthroconidia (*G. candidum*) were prepared by pipetting 10 ml of sterile deionized water containing 0.05% Tween 80 onto the surface of the 5-day-old cultures and gently massaging for 1 min with a sterile glass rod. Using a sterile Pasteur pipette, the conidia suspension was removed from the surface of the plate and deposited in a sterile test tube. The number of conidia (CFU/ml) in each suspension was determined by serially diluting samples in sterile deionized water and surface plating (0.1 ml in duplicate) on DRBC agar. Plates were incubated at 25°C for 5 days before colonies were counted. The number of conidia in each suspension was adjusted to ca. 4 – 5 $\log_{10}$ CFU/ml by diluting in sterile deionized water containing 0.05% Tween 80.

Determination of proteolytic activity of molds. *A. alternata, C. cladosporioides, E. nigrum, G. candidum*, and *P. expansum* were examined for extracellular proteolytic activity on gelatin agar (GA, pH 5.9) and standard methods casienate (SMC, pH 6.3) agar. GA contains (per liter of deionized water): 30 g of gelatin (BBL/Difco), 10 g of tryptone (BBL/Difco), 10 g of sodium chloride (Fisher Scientific, Fair Lawn, NJ), and 15 g of agar (BBL/Difco). The mixture was heated to dissolve ingredients, then autoclaved at 121°C for 15 min before cooling to 50°C and pouring 18 ml into petri plates (100 x 15 mm). SMC agar contains (per liter of deionized water): 23.4 g of standard methods agar (SMA; BBL/Difco), 10 g of sodium casienate (ACROS; Morris Plains, NJ), 4.41 g hydrated trisodium citrate (ICN Biomedicals Inc., Aurora, OH), and 4.38 g of hydrated calcium chloride (Fisher Scientific). Trisodium citrate was dissolved in 1 liter of deionized water and divided into two 500-ml portions. SMA and calcium chloride were added to one 500-ml portion of trisodium citrate and heated to dissolve. Sodium casienate was added to the second 500-ml portion of sodium citrate and blended in a Model

56000 blender (Hamilton Beach / Procter Silex, Washington, NC) for 2 min. The two 500-ml portions were combined in a 6-liter Erlenmeyer flask and sterilized at 121°C for 15 min. Twenty milliliters of sterile 1 M calcium chloride solution was added to the cooled (50°C) agar and thoroughly mixed before pouring (18 ml) into petri plates (100 x 15 mm).

Molds were inoculated into GA and SMC by stabbing two locations (approximately 2 cm apart) on each plate with a probe containing conidia picked from 5-day-old colonies grown on DRBC agar slants at 25°C. Plates were incubated upright at 20°C for 7 days or at 4°C for 21 days before examining for evidence of proteolytic activity. Proteolytic activity was manifested by the development of a halo (zone of gelatin liquefaction or casienolysis) surrounding colonies on GA and/or SMC agar. The halo on the transparent GA appeared as a cloudy area surrounding the colonies, while on white, opaque SMC agar haloes appeared as translucent areas. Diameters of colonies and haloes were measured with a dial caliper (Monostat, Zurich, Switzerland). The surface pH of GA and SMC agar at the edge of each colony and 2 cm away from the colony was measured using a surface pH probe.

Preparation and inoculation of cantaloupes. Full slip Eastern type cantaloupes (*Cucumis melo* L. var. *reticulatus* Naud, cultivar Athena) were purchased in July – August, 2002 from a grocery in Griffin, GA and stored at 4°C for 2 days. Prior to using in experiments, the cantaloupes were adjusted to 22 $\pm$ 1°C over a 16 – 20 h period. Twenty-four unwashed cantaloupes, free of visible wounds, cuts, and bruises, and supported by a 15-cm diameter plastic potting saucer (Ivex, Tustin, CA), were randomly divided into eight groups of three fruits each and placed in covered 27-liter polypropylene containers (59 cm long x 43 cm wide x 15 cm high; Sterilite, Townsend, MA) in which twenty-eight holes (ca. 6 mm diameter) were made to minimize the condensation of water on the surface of rinds and facilitate ventilation inside the

container. Each of the three fruits in the eight groups (treatments) represented a replicate. A permanent ink marker was used to make six 3-cm diameter circles (two parallel rows of three, ca. 6 cm apart) on the rind of each cantaloupe, designating sites for inoculation. The end (8 mm wide) of a sterile stainless steel spatula was used to create a wound 4 mm deep in the center of three of the six circles. This procedure resulted in six inoculation sites (three intact and three wounded) on each cantaloupe. Each pair of inoculation sites (one intact and one wounded) represented sampling locations for each of three sampling days (days 7, 14, and 21) on each replicate cantaloupe. The surface pH of the intact rind and the cut surface of wounded rind within the circles was determined with a surface probe using a Basic pH meter (Denver Instrument Company, Arvada, CO). Cantaloupes were inoculated with mold and/or *S.* Poona suspensions using a Precision Microliter Pipette (Rainin, Woburn, MA). Inoculum was deposited at 2 – 3 locations inside the 3-cm circles on the surface of intact rinds or directly in the cut rind tissue. Five inoculation schemes were used:

(i) Mold only: Cantaloupes were inoculated with 20 µl of conidia suspension at each of three intact and three wounded sites on six fruits on day 0. One group of three cantaloupes was stored at 4°C and the other group of three cantaloupes was stored at 20°C.

(ii) *S.* Poona only: Cantaloupes were inoculated with 20 µl of *S*. Poona suspension at each of three intact and three wounded sites on six fruits on day 0. One group of three cantaloupes was stored at 4°C and the other group was stored at 20°C.

(iii) Mold and *S*. Poona: Cantaloupes were inoculated with 10 µl of conidia suspension and 10 µl of *S*. Poona suspension at each of three intact and three wounded sites on six fruits on day 0. One group of three cantaloupes was stored at 4°C and the other group was stored at 20°C.

(iv) Mold, then *S*. Poona 3 days later: Cantaloupes were inoculated with 20 µl of mold conidia suspension on day 0, followed by 20 µl *S*. Poona suspension on day 3 at each of three intact and three wounded sites on three fruits. All three cantaloupes were stored at 20°C.

(v) *S*. Poona, then mold 3 days later: Cantaloupes were inoculated with 20 µl of *S*. Poona suspension on day 0, followed by 20 µl of mold conidia suspension on day 3 at three intact and three wounded sites on three fruits. All three cantaloupes were stored at 20°C.

The inoculum on the surface of cantaloupes was dried by placing the fruits in a laminar flow hood (class II, type A/B3) for 2 h at 22 $\pm$ 1°C before placing in polyethylene containers and incubating at 4°C or 20°C for up to 21 days.

Microbiological analyses. Intact and wounded rinds of cantaloupes were analyzed for the presence of test molds and the presence and number of *S*. Poona 7, 14, and 21 days after inoculation. For cantaloupes inoculated with mold and *S*. Poona on two different days (days 0 and 3), analysis was done 7, 14, and 21 days after the second inoculation day.

After the pH of the site inoculated with mold or mold and *S*. Poona was measured, samples were removed from the surface with a sterile inoculation loop, streaked on duplicate DRBC agar, and incubated at 25°C for 5 days. Isolates were examined to confirm the presence of test molds.

Samples of intact and wounded rind tissues (ca. 3 cm diameter, 4 mm deep) inoculated with *S*. Poona or *S*. Poona and mold were excised using a sterile spatula and placed in separate stomacher 80 bags (Seward Medical Ltd., London, UK). Forty milliliters of sterile lactose broth (LB; BBL/Difco) containing 50 µg of nalidixic acid/ml (LBN) was added. The sample was hand rubbed and macerated, then pummeled for 1 min at normal speed in a Model 80 Stomacher (Seward Medical). The liquid portion of the homogenate was serially diluted in sterile 0.1%

peptone water and surface plated (0.25 ml of undiluted liquid in quadruplicate and 0.1 ml of diluted liquid in duplicate) on TSAN. Plates were incubated at 37°C for 24 h before presumptive-positive *S*. Poona colonies were counted. Random presumptive-positive colonies were selected for confirmation as described above. Homogenates containing LBN and rind tissue were incubated at 37°C for 24 h. For samples that did not yield *S*. Poona colonies on TSAN, the LBN/rind mixture was examined for the presence of *S*. Poona. A 1-ml sample of the enriched mixture was inoculated into 10 ml of selenite cysteine (SC) broth (BBL/Difco), incubated at 37°C for 24 h, and surface plated on bismuth sulfite agar (BSA; BBL/Difco) supplemented with 50 μg of nalidixic acid/ml (BSAN). Plates were incubated at 37°C for 24 h before random presumptive-positive colonies were selected for confirmation as described above.

Statistical analysis. Three replicate experiments were conducted and three samples were analyzed at each sampling time. Mean values were analyzed to determine significant differences in pH of intact and wounded cantaloupe rind inoculated with mold and/or *S.* Poona. Significant differences in populations of *S.* Poona on intact and wounded cantaloupe rind as affected by co-infection with mold and storage temperature were also determined. General linear models on SAS software (Statistical Analysis Systems Institute, Cary, NC) were used. Significant differences ($P \leq 0.05$) between mean values were determined using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Proteolytic activity of molds. The five molds selected for this study were examined for their ability to hydrolyze gelatin and casein in GA and SMC agar, respectively. As evidenced by the formation of haloes around colonies, *C. cladosporioides* and *G. candidum* were proteolytic at 4 and 20°C (Table 3.1). *C. cladosporioides* showed proteolytic activity at 4°C on GA but not on

TABLE 3.1. Colony diameter, evidence of proteolytic activity, and changes in pH of media resulting from growth of molds.

		4°C				20°C			
		Diameter (mm)		pH[a]		Diameter (mm)		pH[a]	
Mold	Medium[b]	Colony	Halo	Colony	Medium	Colony	Halo	Colony	Medium
A. alternata	GA	28.0 (2.7)[c]	0	6.6 (0.3) A	6.1 (0.2) B	43.4 (5.7)	0	7.7 (0.4) A	7.0 (0.3) B
	SMC	28.7 (3.0)	0	7.0 (0.5) A	6.4 (0.2) B	52.5 (2.8)	0	8.4 (0.1) A	8.2 (0.1) B
C. cladosporioides	GA	7.6 (1.9)	0	6.2 (0.1) A	6.0 (0.1) B	16.9 (1.8)	29.3 (3.8)	8.0 (0.2) A	7.5 (0.2) B
	SMC	10.2 (1.1)	12.5 (1.3)	7.0 (0.3) A	6.3 (0.2) B	29.7 (1.8)	43.3 (3.3)	8.3 (0.1) A	8.2 (0.1) A
E. nigrum	GA	25.3 (4.5)	0	6.1 (0.2) A	6.0 (0.1) A	50.6 (3.0)	0	7.9 (0.1) A	6.9 (0.7) B
	SMC	39.5 (8.2)	0	6.6 (0.6) A	6.3 (0.2) A	55.5 (6.2)	0	8.5 (0.1) A	8.2 (0.3) A
G. candidum	GA	11.8 (0.7)	13.5 (0.7)	7.3 (0.1) A	6.3 (0.2) B	30.7 (4.7)	0	8.1 (0.2) A	8.0 (0.2) A
	SMC	15.1 (1.4)	18.0 (1.4)	7.7 (0.1) A	6.8 (0.2) B	48.3 (2.2)	52.1 (2.4)	8.5 (0.1) A	8.3 (0.1) B
P. expansum	GA	23.6 (1.4)	0	7.0 (0.1) A	6.2 (0.1) B	33.6 (4.2)	60.5 (3.1)	6.8 (0.1) A	6.3 (0.2) B
	SMC	33.4 (1.7)	0	7.2 (0.1) A	6.8 (0.2) B	39.3 (3.0)	43.0 (3.4)	8.0 (0.2) A	7.9 (0.2) A

[a] pH of media was measured at the edge of the colonies (colony) and 2 cm away from the edge (medium). The initial pH values of GA and SMC agar were 5.9 and 6.3, respectively; inoculated media were incubated for 21 days at 4°C or 7 days at 20°C. Within incubation temperature, mean values in the same row for pH at the edge of the colonies and 2 cm away from the colonies that are not followed by the same letter are significantly different ($P \leq 0.05$).

[b] GA = Gelatin Agar; SMC = Standard Method Casienate agar.

[c] Numbers in parentheses indicate standard deviation.

SMC agar. *G. candidum* showed proteolytic activity at 20°C on SMC agar but not on GA. *P. expansum* was proteolytic on both media at 20°C, but neither at 4°C. *A. alternata* and *E. nigrum* did not exhibit proteolytic activity, regardless of the medium or incubation temperature.

Growth of molds showing proteolytic activity caused a significant ($P \leq 0.05$) increase in pH of media at the edge of colonies compared to the pH of media 2 cm away from colonies (Table 3.1). Growth of *G. candidum* at 4°C caused the initial pH of GA and SMC agar at the edges of colonies to increase from 5.9 and 6.3, respectively, to 7.3 and 7.7. *A. alternata*, *C. cladosporioides*, and *P. expansum* also alkalinized GA and SMC agar at 4°C, but to a lesser extent. At 20°C, the pH of GA at the edge of *G. candidum* colonies increased to 8.1; the pH of the SMC agar at the edges of *E. nigrum* and *G. candidum* colonies increased to 8.5. When *A. alternata* was incubated at 20°C for 7 days, the pH of media at the edge of the colonies was significantly higher ($P \leq 0.05$) than the pH of 2 cm away from the edge. The pH at the edge of *C. cladosporioides* and *P. expansum* colonies was significantly higher ($P \leq 0.05$) on GA, but not on SMC agar, compared to the pH 2 cm away. Conversely, the pH at the edge of *G. candidum* colonies was significantly higher ($P \leq 0.05$) than the pH 2 cm away on SMC agar, but not on GA.

The lack of significant differences in pH at the two sites on GA or SMC agar inoculated with *C. cladosporioides, G. candidum*, and *P. expansum* and incubated at 20°C is attributed, in part, to extensive growth and colony development, causing the pH of media in the entire plates to increase. Deamination of proteins by molds results in the production of ammonia. Evolution of volatile alkalinizing compounds such as ammonia may have contributed to an increase in pH of media at some distance from the colonies. The *A. alternata* and *E. nigrum* species used in this study did not exhibit proteolytic activity, although growth at 20°C was accompanied by

alkalinization on GA and SMC agar. This indicates that biochemical activities other than gelatin liquefaction or casienolytic activity result in by-products that cause an upward shift in pH. The alkalinizing ability of molds is a species attribute (Mundt, 1978). Secretion of ammonia by *Colletotrichum* species and *A. alternata* is thought to enhance mold pathogenicity because it increases the pH to a more favorable level for growth (Eshel *et al*., 2002; Prusky *et al.*, 2001).

Changes in pH of cantaloupe rind as affected by inoculation with mold and temperature. The pH of the cantaloupe rind tissue inoculated with molds increased during storage at 4 or 20°C for up to 21 days (Table 3.2). Changes were larger in cantaloupes incubated at 20°C than at 4°C. At the time of inoculation (day 0), the pH values of intact rind surface and wounded rind tissue were 5.55 – 6.47 and 5.25 – 6.49, respectively. After storage of cantaloupes inoculated with molds for 21 days at 4°C, the pH of intact rind and wounded tissue was 5.84 – 6.81 and 5.38 – 7.42, respectively. The pH values of the surface of intact rind and wounded rind tissue of cantaloupes inoculated with mold and stored for 14 days at 20°C were 6.05 – 8.26 and 6.29 – 8.12, respectively. The pH of the intact rinds inoculated with mold but not *S*. Poona and incubated at 20°C did not change significantly or was significantly higher ($P \leq 0.05$) during 14 days of storage. The pH of intact rinds of cantaloupes inoculated only with *C. cladosporioides* or *G. candidum* and stored at 4°C for 21 days was significantly higher ($P \leq 0.05$) than on day 0. This is attributed to the alkalinizing ability of *C. cladosporioides* and *G. candidum* at 4°C, as shown in Table 3.1. The pH of intact cantaloupe rinds inoculated with *A. alternata*, *E. nigrum,* or *P. expansum* and stored at 4°C did not change significantly. This concurs with the lack of proteolytic activity on GA and SMC agar incubated at 4°C.

TABLE 3.2. pH of intact and wounded rind tissues of cantaloupes inoculated with mold and/or *Salmonella* Poona

Mold	Storage temp. (°C)	Inoculum	pH[a]							
			Intact rind surface				Wounded rind surface			
			Day 0	7	14	21	0	7	14	21
A. alternata	4	Mold only	A 6.17 A	A 6.39 A	A 5.72 A	A 6.19 A	B 5.90 A	A 6.27 A	A 6.20 A	A 6.99 A
		Salm.[b] only	A 6.47 A	A 6.42 A	A 5.95 A	A 6.21 A	A 6.12 A	A 6.10 A	A 5.69 B	A 5.60 B
		Salm. and mold (d 0)[c]	A 6.14 B	A 6.54 A	A 5.70 C	A 6.19 B	A 6.10 A	A 6.35 A	A 5.55 A	A 6.82 A
	20	Mold only	AB 6.24 AB	B 5.89 B	A 6.61 A	nd[d]	A 6.11 B	A 6.49 AB	A 7.00 A	nd
		Salm. only	B 6.07 A	AB 6.50 A	A 7.42 A	nd	A 5.56 B	A 6.60 A	A 7.34 A	nd
		Salm. and mold (d 0)	A 6.47 A	AB 6.21 A	A 6.81 A	nd	A 5.96 B	A 6.80 AB	A 7.46 A	nd
		Mold (d 0), Salm. (d 3)	AB 6.27 A	B 5.91 B	nd	nd	A 5.51 A	A 6.24 A	nd	nd
		Salm. (d 0), mold (d 3)	B 6.08 A	A 6.86 A	nd	nd	A 6.09 B	A 6.91 A	nd	nd
C. cladosporioides	4	Mold only	A 6.15 B	A 6.24 B	A 6.33 AB	A 6.46 A	B 5.79 C	A 6.34 B	A 6.26 BC	A 7.42 A
		Salm. only	A 6.47 A	A 6.42 A	A 5.94 A	A 6.21 A	A 6.12 A	A 6.10 A	A 5.69 B	C 5.60 B
		Salm. and mold (d 0)	A 6.28 A	A 6.21 A	A 5.86 A	A 6.81 A	A 6.08 B	A 6.07 B	A 5.63 B	B 6.81 A
	20	Mold only	A 6.19 B	A 6.10 B	A 8.26 A	nd	AB 5.68 C	A 6.33 B	A 7.67 A	nd
		Salm. only	B 6.07 A	A 6.50 A	A 7.42 A	nd	AB 5.56 B	A 6.60 A	A 7.34 A	nd
		Salm. and mold (d 0)	A 6.18 B	A 6.20 B	A 7.65 A	nd	B 5.25 B	A 6.97 A	A 7.28 A	nd
		Mold (d 0), Salm. (d 3)	A 6.20 A	A 6.03 B	nd	nd	A 5.87 A	A 6.67 A	nd	nd
		Salm. (d 0), mold (d 3)	A 6.28 A	A 6.14 A	nd	nd	A 6.13 A	A 7.26 A	nd	nd

Continued

TABLE 3.2, *continued*

Mold	Storage temp. (°C)	Inoculum	pH							
			Intact rind surface				Wounded rind surface			
			Day 0	7	14	21	0	7	14	21
E. nigrum	4	Mold only	A 6.46 A	A 5.84 A	A 6.17 A	A 6.76 A	A 6.17 A	A 5.86 A	A 6.44 A	A 6.65 A
		Salm. only	A 6.28 A	A 5.68 A	A 5.94 A	A 6.29 A	A 5.99 A	A 5.67 A	A 5.83 A	A 6.28 A
		Salm. and mold (d 0)	A 6.55 A	A 6.13 A	A 5.92 A	A 6.42 A	A 6.05 A	A 5.41 A	A 6.78 A	A 6.78 A
	20	Mold only	A 5.94 B	A 5.89 B	A 6.92 A	nd	B 5.93 B	A 7.08 A	A 7.34 A	nd
		Salm. only	A 6.16 A	A 5.96 A	A 6.24 A	nd	B 6.07 B	A 5.84 A	A 6.80 A	nd
		Salm. and mold (d 0)	A 6.30 A	A 6.04 A	A 6.61 A	nd	C 5.61 B	A 5.99 B	A 7.20 A	nd
		Mold (d 0), Salm. (d 3)	A 5.86 B	A 6.24 AB	A 6.82 A	nd	A 6.49 AB	A 6.17 B	A 7.35 A	nd
		Salm. (d 0), mold (d 3)	A 6.20 A	A 6.13 A	A 6.05 A	nd	B 6.10 A	A 6.26 A	A 6.29 A	nd
G. candidum	4	Mold only	B 5.77 B	A 6.65 A	AB 6.48 A	A 6.68 A	B 5.78 B	A 6.75 A	A 6.72 A	A 6.79 A
		Salm. only	A 6.47 A	A 6.42 A	B 5.94 A	A 6.21 A	A 6.12 A	B 6.10 A	B 5.69 B	B 5.60 B
		Salm. and mold (d 0)	A 6.37 B	A 6.78 A	A 6.83 A	A 6.62 A	A 6.48 B	A 6.96 B	A 6.96 A	A 6.58 B
	20	Mold only	A 6.24 B	A 6.63 B	A 7.99 A	nd	A 6.16 B	A 6.92 B	A 8.05 A	nd
		Salm. only	AB 6.07 A	A 6.50 A	A 7.42 A	nd	A 5.56 B	A 6.60 A	A 7.34 A	nd
		Salm. and mold (d 0)	C 5.55 C	A 6.59 B	A 7.62 A	nd	A 5.62 C	A 6.65 B	A 8.12 A	nd
		Mold (d 0), Salm. (d 3)	B 5.89 A	A 6.05 A	nd	nd	A 5.85 A	A 6.01 A	nd	nd
		Salm. (d 0), mold (d 3)	AB 5.96 A	A 6.19 A	nd	nd	A 5.66 A	A 6.61 A	nd	nd

Continued

TABLE 3.2, *continued*

Mold	Storage temp. (°C)	Inoculum	pH							
			Intact rind surface				Wounded rind surface			
			Day 0	7	14	21	0	7	14	21
P. expansum	4	Mold only	B 6.15 A	A 6.18 A	A 6.22 A	A 5.84 A	A 5.86 A	A 5.99 A	A 5.94 A	A 5.38 A
		Salm. only	A 6.47 A	A 6.42 A	A 5.95 A	A 6.21 A	A 6.12 A	A 6.10 A	A 5.69 A	A 5.60 B
		Salm. and mold (d 0)	C 5.76 A	A 5.99 A	A 5.99 A	A 5.67 A	A 5.67 A	A 6.16 A	A 5.89 A	A 5.71 A
	20	Mold only	B 6.06 B	A 6.54 B	A 7.30 A	nd	A 5.92 B	AB 7.00 A	A 7.20 A	nd
		Salm. only	B 6.07 A	A 6.50 A	A 7.42 A	nd	A 5.56 B	B 6.60 A	A 7.34 A	nd
		Salm. and mold (d 0)	B 6.09 B	A 6.42 B	A 7.88 A	nd	A 5.89 C	AB 6.96 B	A 7.57 A	nd
		Mold (d 0), Salm. (d 3)	C 5.85 A	A 7.17 A	nd	nd	A 5.75 B	A 7.41 A	nd	nd
		Salm. (d 0), mold (d 3)	A 6.54 A	A 5.96 A	nd	nd	A 5.81 A	B 6.46 A	nd	nd

[a] Within mold, incubation temperature, rind surface condition, and incubation time, values in a column that are not preceded by the same letter are significantly different ($P \leq 0.05$). Within rind surface condition, mean values in a row that are not followed by the same letter are significantly different ($P \leq 0.05$).

[b] *Salmonella* Poona.

[c] Values in parentheses indicate the day on which cantaloupes were inoculated.

[d] nd, not determined. Cantaloupes were severely decayed and were therefore not analyzed.

The pH of intact rinds of cantaloupes inoculated with *S*. Poona but not mold, then stored at 4°C or 20°C, did not change significantly after 21 and 14 days, respectively (Table 3.2). In some cases, simultaneously inoculating rind with *S.* Poona and a test mold resulted in increased pH of intact rind during the storage period. Inoculation of intact cantaloupe rinds with *S*. Poona and *G. candidum* simultaneously, followed by storage at 4°C or 20°C, resulted in a significant increase ($P \leq 0.05$) in rind pH. The pH of intact rinds of cantaloupes inoculated with *S*. Poona and *C. cladosporioides* or *P. expansum* on day 0 significantly increased ($P \leq 0.05$) during 14 days at 20°C, but not 21 days at 4°C. When the intact rinds were simultaneously inoculated with *S*. Poona and *A. alternata* or *E. nigrum*, the rind pH did not change when the cantaloupes were stored at 4°C or 20°C.

When intact rinds were inoculated with a mold 3 days before inoculating with *S*. Poona, followed by storage at 20°C, changes in pH were variable for different molds (Table 3.2). The pH of rind inoculated with *A. alternata* or *C. cladosporioides* decreased significantly ($P \leq 0.05$), while inoculation of rind with *E. nigrum* resulted in a significant increase ($P \leq 0.05$); the pH of intact rind inoculated with *G. candidum* or *P. expansum* did not change significantly. When intact rinds were inoculated with *S*. Poona 3 days before inoculating with molds, the pH did not change significantly within 7 or 14 days at 20°C.

Changes in pH of wounded cantaloupe rinds, as influenced by inoculation with *S*. Poona and/or mold and storage temperature, were similar to those observed for intact rinds (Table 3.2). The pH values of wounded cantaloupe rinds inoculated with *C. cladosporioides* or *G. candidum* and then stored at 4°C or 20°C for 21 and 14 days, respectively, were significantly higher ($P \leq 0.05$) than initial (day 0) values. *A. alternata, E. nigrum,* and *P. expansum* did not cause a

significant change in the pH of wounded rind tissue of cantaloupes stored at 4°C, but significantly increased the pH ($P \leq 0.05$) at 20°C.

The pH values of wounded rind tissue of cantaloupes inoculated with *S*. Poona only and then incubated for 21 days at 4°C were unchanged or significantly reduced ($P \leq 0.05$). However, the pH of wounded rind tissues of all cantaloupes inoculated with *S*. Poona only and stored at 20°C significantly increased ($P \leq 0.05$) after 14 days.

The effect of simultaneous inoculation of wounded tissues with *S*. Poona and a test mold on the pH of the tissue was assessed. Wounded cantaloupe rind tissues inoculated simultaneously with *S*. Poona and *C. cladosporioides*, but not other test molds, showed a significant increase ($P \leq 0.05$) in the pH after storage at 4°C for 21 days. However, wounded rind tissues of cantaloupes simultaneously inoculated with *S.* Poona and molds, followed by storage at 20°C, showed significant increases ($P \leq 0.05$) in pH after 14 days. When wounded rind tissues were inoculated with *E. nigrum* or *P. expansum* 3 days before inoculating with *S*. Poona and storing at 20°C, the pH increased significantly ($P \leq 0.05$). Only the wounded cantaloupe rinds inoculated with *A. alternata* 3 days after *S*. Poona was introduced into the wound showed a significant increase ($P \leq 0.05$) in pH at 20°C.

Salmonellae are physiologically adaptable microorganisms, able to proliferate at pH 4.5 - 9.5, with 6.5 - 7.5 being the optimum pH for growth (D'Aoust, 1997). In this study, although inoculated intact and wounded rinds of cantaloupes stored at 4°C did not show overt symptoms of decay, the pH of inoculated tissues increased. The rise in pH would likely favor survival and growth of *S*. Poona. Riordan *et al*. (2000), observed that enhanced growth of *E. coli* O157:H7 in wounds on apples at 22 ± 1°C co-inoculated with *Glomerella cingulata* was correlated with a rise in pH at the infected site, even though decay was not evident. It was postulated that the

increase in pH of apple tissue was due to enzymatic activity of *G. cingulata* that led to the production of alkaline end products or its ability to metabolize malic acid and lower acidity. *Aspergillus gracilis*, *Cladosporium, Fusarium, Penicillium,* and *Rhizoctonia* have been reported to raise the pH of tomato juice from levels that are normally inhibitory to levels that facilitate growth and toxin production by *Clostridium botulinum* (Draughon *et al*., 1988; Huhtanen *et al*., 1976; Odlaug and Pflug, 1979). The molds used in our study could have created microenvironments within or adjacent to wounds on cantaloupe rinds that would favor survival and growth *S.* Poona.

Recovery of molds from intact and wounded rinds. Populations of molds in inocula deposited on the surface of intact cantaloupe rind or into wounds on the rind are shown in Table 3.3. Molds were recovered from intact and wounded rinds stored at 4°C for up to 21 days or 20°C for up to 14 days after inoculation. Molds were detected in a larger number of wounded rinds than in intact rinds. This observation was most evident for rinds inoculated with *E. nigrum* and *P. expansum*. *E. nigrum*, *P. expansum,* and *G. candidum* are not capable of readily penetrating the cuticle and epidermis of the host but if they gain entry through the stem scar or a wound or a natural opening, they can rapidly decay mature produce (Ceponis, 1966; Eckert, 1980; Snowdon, 1990; Wiant, 1937). Most of the test molds isolated from wounded cantaloupe rind tissues were free of other molds, while isolates from the intact rinds consisted of test molds together with other molds naturally present on the cantaloupes. This suggests that the test molds competed more effectively with the natural mycoflora in wounded tissues than on intact tissues.

Recovery of *A. alternata* and *C. cladosporioides* from cantaloupes stored at 4°C was consistently more frequent than recovery from cantaloupes at 20°C.

TABLE 3.3. Presence of molds inoculated onto intact rind and into wounds of cantaloupe rind.

Mold	Storage temp. (°C)	Inoculum	Population[b]	Presence of mold on storage day[a]					
				Intact rind surface			Wounded rind surface		
				Day 7	14	21	7	14	21
A. alternata	4	Mold only	2.76	4	4	6	5	6	6
		Salm.[c] and mold (d 0)[d]	2.46	6	4	5	5	5	6
	20	Mold only	2.76	2	2	nd[e]	4	3	nd
		Salm. and mold (d 0)	2.46	1	0	nd	4	5	nd
		Mold (d 0), Salm. (d 3)	2.76	4	nd	nd	6	nd	nd
		Salm. (d 0), mold (d 3)	2.79	1	nd	nd	4	nd	nd
C. cladosporioides	4	Mold only	3.62	4	0	4	1	6	6
		Salm.[c] and mold (d 0)	3.32	2	4	4	1	6	5
	20	Mold only	3.62	2	3	nd	6	3	nd
		Salm. and mold (d 0)	3.32	1	2	nd	4	4	nd
		Mold (d 0), Salm. (d 3)	3.62	1	nd	nd	0	nd	nd
		Salm. (d 0), mold (d 3)	3.04	1	nd	nd	0	nd	nd
E. nigrum	4	Mold only	1.97	0	3	3	5	5	6
		Salm.[c] and mold (d 0)	1.67	0	0	1	3	4	4
	20	Mold only	1.97	0	1	nd	6	5	nd
		Salm. and mold (d 0)	1.67	0	0	nd	4	4	nd
		Mold (d 0), Salm. (d 3)	1.97	0	1	nd	6	6	nd
		Salm. (d 0), mold (d 3)	0.96	0	0	nd	0	3	nd

Continued

TABLE 3.3, *continued*

Mold	Storage temp. (°C)	Inoculum	Population	Presence of mold on storage day					
				Intact rind surface			Wounded rind surface		
				Day 7	14	21	7	14	21
G. candidum	4	Mold only	3.28	4	6	6	6	6	6
		Salm. and mold (d 0)	2.97	6	6	5	6	6	6
	20	Mold only	3.25	0	6	nd	6	6	nd
		Salm. and mold (d 0)	2.94	0	0	nd	6	6	nd
		Mold (d 0), Salm. (d 3)	2.94	2	nd	nd	6	nd	nd
		Salm. (d 0), mold (d 3)	3.53	4	nd	nd	6	nd	nd
P. expansum	4	Mold only	1.87	0	0	0	0	6	5
		Salm.[c] and mold (d 0)[d]	1.57	0	3	2	0	5	6
	20	Mold only	1.87	0	4	nd	6	4	nd
		Salm. and mold (d 0)	1.57	0	4	nd	6	6	nd
		Mold (d 0), Salm. (d 3)	1.87	1	nd	nd	4	nd	nd
		Salm. (d 0), mold (d 3)	2.21	0	nd	nd	0	nd	nd

[a] Number of samples out of six that were positive for mold 7, 14, and 21 days after inoculation.

[b] Population of test mold ($\log_{10}$ CFU per 10 µl or 20 µl of inoculum) on day 0. Cantaloupes were inoculated with 10 µl of conidia suspension and 10 µl of *S.* Poona suspension on day 0, or 20 µl of either conidia or *S.* Poona suspension on day 0 followed by 20 µl of either *S.* Poona or conidia suspension, respectively, on day 3.

[c] *Salmonella* Poona.

[d] Values in parentheses indicate the day on which cantaloupes were inoculated.

[e] nd, not determined. The cantaloupes were severely decayed and were therefore not analyzed.

Storage at low temperature retards decay by preserving resistance of the host to parasitism and retarding growth of the pathogen directly (Eckert, 1980). Low temperature, however, exacerbates decay of some fruits by *Alternaria* and *Cladosporium* (Morris and Wade, 1983; Snowdon, 1990).

Propagules of plant pathogenic molds are abundant on the surfaces of fruits and vegetables as they approach maturity in the field. Removal of cantaloupes from the vine creates stem scars through which molds, as well as foodborne pathogens, may enter the tissue and grow. Damage to the surface of cantaloupe rind during harvesting is inevitable, thus creating additional sites of entry for microorganisms. Sanitation is essential to reduce the population of microorganisms on the surface of cantaloupes, especially if they are washed with potentially contaminated water after harvest. The incidence of postharvest diseases that originate in wounds may be substantially reduced by changing handling practices which damage the fruit surface and by exposing crops to environmental conditions favoring the development of cellular barriers to the penetration of pathogens (Eckert, 1980).

Preharvest cantaloupes may be infected by penetration of certain molds through the intact cuticle or through wounds and natural openings on the surface of the fruit. Alternatively, many postharvest diseases can be initiated through mechanical injuries sustained during harvest (Eckert, 1980). Mature cantaloupes are susceptible to invasion by specific plant pathogens because they are high in moisture and nutrient content and are no longer protected by some of the intrinsic factors that confer resistance during development on the plant. Cantaloupes are easily injured as they approach maturity and are therefore more vulnerable to pathogens that can enter tissue through wounds. This situation is accelerated during transportation and storage

when cantaloupes are in close proximity, resulting in accelerated and uncontrolled ripening caused by release of ethylene by decaying fruits.

Recovery of *S.* Poona from intact and wounded cantaloupe rinds. Populations of *S.* Poona in 10 µl or 20 µl of inocula applied to intact rind of cantaloupes are shown in Table 3.4. The population of *S.* Poona 7 days after inoculation was significantly lower ($P \leq 0.05$) than the initial population, regardless of co-inoculation with mold or incubation temperature. Some cells would have succumbed to environmental stresses during the drying period prior to storage and throughout the 21-day storage period at 4°C. *S.* Poona was recovered from intact rinds of cantaloupes stored at 4°C for up to 21 days however, and grew on cantaloupes stored at 20°C. While the population of *S.* Poona recovered from cantaloupes stored at 20°C for 7 days was significantly lower ($P \leq 0.05$) than the population on day 0, with one exception (inoculation with *S.* Poona and *E. nigrum* on day 0), a significantly higher population ($P \leq 0.05$) was recovered from cantaloupes stored for 14 days. Survival and growth of *S.* Poona at 4 and 20°C on intact rinds were essentially unaffected by co-inoculation with mold, regardless of the order in which inocula were applied. The near neutral pH, nutrient availability, and high a_w on the surface of the rind favored growth of *S.* Poona at 20°C, even in the absence phytopathogenic molds.

With the exception of wounded rind co-inoculated with *G. candidum,* the population of *S.* Poona 21 days after inoculation and storage at 4°C was significantly lower ($P \leq 0.05$) than the population initially applied, regardless of co-inoculation with mold (Table 3.5). Only wounded tissue of rinds inoculated simultaneously with *S.* Poona and *E. nigrum* or *P. expansum* and stored at 4°C required enrichment to detect viable *S.* Poona. Regardless of the inoculation scheme used, the population of *S.* Poona recovered from wounds after storage for 7 and 14 days at 20°C was significantly higher ($P \leq 0.05$) than the population initially applied.

TABLE 3.4. Populations of *Salmonella* Poona recovered from inoculated intact rind of cantaloupes

Mold	Storage temp. (°C)	Inoculum	Population[a] Day 0	7	En[b]	14	En	21	En
A. alternata	4	Salm.[c] only	A 3.64 A	A < 1.60 B	0/3	A < 1.60 B	2/3	A < 1.60 B	2/3
		Salm. and mold (d 0)[d]	A 3.34 A	A < 1.60 B	1/3	A < 1.60 B	1/3	A < 1.60 B	0/3
	20	Salm. only	A 3.64 B	A 1.46 C	2/2	A 6.53 A	-	nd[e]	-
		Salm. and mold (d 0)	A 3.34 B	A 1.38 C	0/2	A 5.89 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 A	A 1.45 B	0/2	nd	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.64 A	A 0.69 B	1/0	nd	-	nd	-
C. cladosporioides	4	Salm. only	A 3.64 A	A < 1.60 B	0/3	A < 1.60 B	2/3	A < 1.60 B	2/3
		Salm. and mold (d 0)	A 3.34 A	A < 1.60 B	0/3	A < 1.60 B	0/3	A < 1.60 B	1/3
	20	Salm. only	A 3.64 B	A 1.46 C	2/2	A 6.53 A	-	nd	-
		Salm. and mold (d 0)	A 3.34 B	A 1.25 C	2/2	A 6.23 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 A	A 1.61 B	1/1	nd	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.64 A	A 1.83 B	-	nd	-	nd	-
E. nigrum	4	Salm. only	A 3.11 A	A < 1.60 B	1/3	A < 1.60 B	2/3	A < 1.60 B	2/3
		Salm. and mold (d 0)	A 2.81 A	A < 1.60 B	1/3	A < 1.60 B	1/3	A < 1.60 B	0/3
	20	Salm. only	A 3.11 B	A 1.25 C	0/2	A 7.78 A	-	nd	-
		Salm. and mold (d 0)	A 2.81 A	A < 1.60 B	3/3	B < 1.60 B	2/3	nd	-
		Mold (d 0), Salm. (d 3)	A 3.42 B	A < 1.60 C	3/3	AB 5.20 A	0/1	nd	-
		Salm. (d 0), mold (d 3)	A 3.11 A	A 0.88 B	2/2	AB 2.84 A	0/1	nd	-

Continued

TABLE 3.4, *continued*

Mold	Storage temp. (°C)	Inoculum	Population Day 0	7	En[b]	14	En	21	En
G. candidum	4	Salm. only	A 3.51 A	A < 1.60 B	0/3	A < 1.60 B	2/3	A < 1.60 B	0/3
		Salm. and mold (d 0)	A 3.21 A	A < 1.60 B	3/3	A < 1.60 B	1/3	A < 1.60 B	1/3
	20	Salm. only	A 3.64 B	A 1.46 C	2/2	A 6.53 A	-	nd	-
		Salm. and mold (d 0)	A 3.34 B	A 0.76 C	1/2	A 5.72 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 A	A 2.80 B	1/1	nd	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.64 A	A 3.70 A	-	nd	-	nd	-
P. expansum	4	Salm. only	A 3.64 A	A < 1.60 B	0/3	A < 1.60 B	2/3	A < 1.60 B	2/3
		Salm. and mold (d 0)	A 3.34 A	A < 1.60 B	2/3	A < 1.60 B	1/3	A < 1.60 B	0/3
	20	Salm. only	A 3.64 B	A 1.46 C	2/2	A 6.53 A	-	nd	-
		Salm. and mold (d 0)	A 3.34 B	B < 1.60 C	1/3	A 6.29 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 A	A 4.88 A	1/1	nd	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.64 A	B < 1.60 B	2/3	nd	-	nd	-

[a] Population of *S.* Poona on day 0 indicates $\log_{10}$ CFU per 10 µl or 20 µl of inoculum. Populations 7, 14, and 21days after inoculation with *S*. Poona indicate $\log_{10}$ CFU/rind sample analyzed. Within mold, incubation temperature, and incubation time, mean values in a column not preceded by the same letter are significantly different ($P \leq 0.05$). Mean values in a row not followed by the same letter are significantly different ($P \leq 0.05$).

[b] Number of samples positive for S. Poona out of the number analyzed by enrichment.

[c] *Salmonella* Poona.

[d] Values in parentheses indicate the day on which cantaloupes were inoculated.

[e] nd, not determined. Cantaloupes were severely decayed and therefore not analyzed.

TABLE 3.5. Populations of *Salmonella* Poona recovered from inoculated wounded rind of cantaloupes

Mold	Storage temp. (°C)	Inoculum	Population[a] Day 0	7	En[b]	14	En	21	En
A. alternata	4	Salm.[c] only	A 3.64 A	A 2.13 B	1/1	A 2.01 B	1/1	A 1.04 B	2/2
		Salm. and mold (d 0)[d]	A 3.34 A	A 3.45 A	-	A 3.24 A	-	A 1.84 B	1/1
	20	Salm. only	A 3.64 B	A 9.03 A	-	A 9.38 A	-	nd[e]	-
		Salm. and mold (d 0)	A 3.34 B	A 8.66 A	-	A 9.21 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 B	A 7.75 A	-	nd	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.64 B	A 8.27 A	-	nd	-	nd	-
C. cladosporioides	4	Salm. only	A 3.64 A	A 2.13 B	1/1	A 2.01 B	1/1	A 1.04 B	2/2
		Salm. and mold (d 0)	A 3.34 B	A 4.13 A	-	A 3.69 B	-	A 2.05 B	-
	20	Salm. only	A 3.64 B	AB 9.03 A	-	A 9.38 A	-	nd	-
		Salm. and mold (d 0)	A 3.34 B	B 8.57 A	-	A 9.32 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 B	B 8.92 A	-	nd	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.64 B	A 9.53 A	-	nd	-	nd	-
E. nigrum	4	Salm. only	A 3.11 A	A 2.44 B	-	A 1.76 B	1/1	A <1.60 B	3/3
		Salm. and mold (d 0)	A 2.81 A	A 3.41 A	-	A <1.60 B	3/3	A <1.60 B	2/3
	20	Salm. only	A 3.11 C	A 7.88 B	-	A 9.41 A	-	nd	-
		Salm. and mold (d 0)	A 2.81 C	A 4.72 B	0/1	A 9.44 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.42 C	A 7.80 B	-	A 9.20 A	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.11 B	A 8.87 A	-	A 9.48 A	-	nd	-

Continued

TABLE 3.5, *continued*

Mold	Storage temp. (°C)	Inoculum	Population Day 0	7	En	14	En	21	En
G. candidum	4	Salm. only	A 3.51 A	A 2.13 B	1/1	B 2.01 B	1/1	B 1.04 B	2/2
		Salm. and mold (d 0)	A 3.21 B	A 4.89 A	-	A 4.91 A	-	A 4.74 A	-
	20	Salm. only	A 3.64 B	A 9.03 A	-	A 9.38 A	-	nd	-
		Salm. and mold (d 0)	A 3.34 C	A 7.84 B	-	A 8.91 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 B	A 8.18 A	-	nd	-	nd	-
		Salm. (d 0), mold (d 3)	A 3.64 B	A 8.58 A	-	nd	-	nd	-
P. expansum	4	Salm. only	A 3.64 A	A 2.13 B	1/1	A 2.01 B	1/1	A 1.04 B	2/2
		Salm. and mold (d 0)	A 3.34 A	A <1.60 B	2/3	A <1.60 B	2/3	A <1.60 B	0/3
	20	Salm. only	A 3.64 B	A 9.03 A	-	A 9.38 A	-	nd	-
		Salm. and mold (d 0)	A 3.34 B	A 8.87 A	-	A 7.47 A	-	nd	-
		Mold (d 0), Salm. (d 3)	A 3.48 B	B 6.74 A	-	nd	-	nd	-
		Salm. (d 0), mold (d 3)	a 3.64 b	a 8.72 a	-	nd	-	nd	-

[a] Population of *S.* Poona on day 0 indicates $\log_{10}$ CFU per 10 µl or 20 µl of inoculum. Populations 7, 14, and 21 days after inoculation with *S.* Poona indicate $\log_{10}$ CFU/rind sample analyzed. Within mold, incubation temperature, and incubation time, mean values in a column not preceded by the same letter are significantly different ($P \leq 0.05$). Mean values in a row not followed by the same letter are significantly different ($P \leq 0.05$).

[b] Number of samples positive for S. Poona out of the number analyzed by enrichment.

[c] *Salmonella* Poona.

[d] Values in parentheses indicate the day on which cantaloupes were inoculated.

[e] nd, not determined. Cantaloupes were severely decayed and therefore not analyzed.

Larger populations of *S*. Poona were recovered from wounded rind than from intact rind (Tables 3.5 and 3.4, respectively). The number of *S*. Poona recovered from wounded rind tissues of cantaloupes stored at 4°C was 1 – 4 logs higher than that from intact tissues. The number of *S*. Poona recovered from wounded tissue of cantaloupes stored at 20°C was 2 – 6 logs higher than recovered from intact tissues. Nutrients were more available in wounds to facilitate resuscitation and support growth of injured *S*. Poona cells. The growth of *Salmonella* Stanley on the rind of cantaloupe cubes was also attributed to the availability of nutrients, especially when storage temperature was favorable (Ukuku and Sapers, 2001). Foodborne pathogens have been observed to consistently adhere preferentially to injured sites, cut edges, and blossom and stem scars compared to intact surfaces of fresh produce (Annous *et al*., 2001; Lukasik *et al*., 2001; Reina *et al*., 2002; Takeuchi and Frank, 2001). When soft rot and healthy tissues of potato, carrot and pepper tissues were inoculated with *Salmonella* Typhimurium, the population of viable cells multiplied 3- to 10-fold on soft rot tissues compared to healthy tissues (Wells and Butterfield, 1997).

Storage temperature affected the survival and growth of *S*. Poona on intact and wounded rinds of cantaloupes (Tables 3.4 and 3.5, respectively). Higher numbers of *S*. Poona were recovered from rinds of cantaloupes stored at 20°C compared to cantaloupes stored at 4°C. Del Rosario and Beuchat (1995) observed that the population of *E. coli* O157:H7 on inoculated cantaloupe and watermelon cubes and rinds remained unchanged or decreased, respectively, when stored at 5°C, but grew at 25°C. Similar observations were made when *S*. Stanley was inoculated on fresh-cut cantaloupe cubes (Ukuku and Sapers, 2001). *Salmonella* has been observed to grow on cut papaya, jimica, and watermelon held at 22 – 27°C (Escartin *et al.*, 1989) as well as cut cantaloupe and honeydew stored at 23°C (Golden *et al.*, 1993). In the present

study, intact rinds of cantaloupes co-inoculated with *S*. Poona and test molds, then stored at 4°C, required enrichment for detection of *S*. Poona. However, with the exception of wounded tissues inoculated with *S*. Poona and *E. nigrum* or *P. expansum* on day 0, viable cells were recoverable without enrichment of cantaloupes stored at 4°C (Table 3.5). Cold storage was reported to adversely affect the survival of *E. coli* O157:H7 on injured apple tissues co-inoculated with *G. cingulata* (Riordan *et al.*, 2000).

The microbial ecology of fruits and vegetables can be influenced at every step from production through the point of consumption. Traditionally, attention has been focused on postharvest physiology and pathology of produce, recognizing that improper handling practices can cause decay and extensive economic loss. Reports on the presence of human bacterial pathogens on raw agricultural commodities have accentuated the need to consider fruits and vegetables as vehicles of microorganisms capable of causing disease (Beuchat, 2002). Research has shown that there are interactions between postharvest plant pathogens and human bacterial pathogens. While *P. expansum* in decayed tissue of sliced apples impeded the survival of *L. monocytogenes* by reducing the pH, *G. cingulata* was observed to stimulate growth at 10 and 20°C by increasing the pH of decaying tissue (Conway *et al*., 2000). A study of healthy and soft-rotted fruits and vegetables in retail markets revealed that the incidence of *Salmonella* on produce that had undergone bacterial soft rot was twice that of healthy samples (Wells and Butterfield, 1997). Various practices employed in production, processing, and transportation of fresh produce can influence microbial safety. Bacterial and fungal rots on fresh produce, generally regarded as signs of poor handling, storage, and sanitation, could be warnings for possible enteric bacterial contamination. Foodborne pathogens can persist on fresh produce through the point of consumption. Treatments such as washing with chlorinated water cannot be

relied on to eliminate pathogens from produce (Brackett *et al.*, 1993). It is unlikely that a consumer would intentionally eat the decayed portion of a cantaloupe. However, removal of bruised or moldy tissue, followed by cutting the remainder for consumption is not an uncommon practice. The development of alkalinized tissue as a result of mold growth could be a plausible explanation for retention of viability of *Salmonella* and associations of salmonellosis with raw fruits and fruit products. Cellulases and pectinases produced by phytopathogens cause softening and release of cell fluids from plant tissues. It is conceivable that release of cell sap and liquids could allow dislocation and relocation of foodborne pathogens from wounded and decaying tissues to otherwise wholesome tissue on the same fruit or adjacent fruits.

This study demonstrates that *S.* Poona can persist on intact, wounded, and decaying cantaloupe rind at refrigerated and ambient temperatures. Molds commonly involved in postharvest decay of cantaloupes increased the pH of intact and wounded rinds and enhanced the survival of *S*. Poona. Storage at 4°C, compared to 20°C, reduced the rate of fruit senescence and decay by phytopathogens, and enhanced the rate of death of *S*. Poona. Injury and high temperature abuse enhanced the growth of *S*. Poona on cantaloupe rind. Recovery of larger numbers of *S*. Poona from wounded tissues of cantaloupes, particularly fruits stored at 20°C, emphasizes the importance of developing agronomic and postharvest handling practices that prevent contamination, damage, and deterioration. The proteolytic activities of *C. cladosporioides* and *G. candidum*, in particular, portend the need for further investigation of potential metabiotic associations with *S*. Poona on cantaloupe rind and in cantaloupe cubes that may promote survival and growth of foodborne pathogens in cantaloupes and other fruits.

REFERENCES

Annous, B. A., G. Sapers, A. Mattrazzo, and D. Riordan. 2001. Efficacy of washing with a commercial flatbed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. J. Food Prot. 64:159-163.

Beuchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59:204-216.

Beuchat, L.R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect. 4:413-423.

Brackett, R.E., D.M. Smallwood, S.M. Fletcher, and D.L. Horton. 1993. Food Safety: Critical points within the production and distribution system. *In* R.L. Shewfelt and S. E. Prussia (Ed.), Postharvest Handling: a Systems Approach. Academic Press, San Diego, CA.

Centers for Disease Control and Prevention (CDC). 1991. Multistate outbreak of *Salmonella* Poona infections – United States and Canada, 1991. Morbid. Mortal. Wkly. Rep. 40:549-552.

Centers for Disease Control and Prevention (CDC). 2002. Multistate outbreaks of *Salmonella* Serotype Poona infections associated with eating cantaloupes from Mexico – United States and Canada, 2000 – 2002. Morbid. Mortal. Wkly. Rep. 51:1044-1047.

Ceponis, M.J. 1966. Occurrence of *Geotrichum candidum* in western melons on the New York market. Plant Dis. Rep. 50:222-224.

Ceponis, M.J., R.A. Cappellini, and G.W. Lightner. 1986. Disorders in muskmelon shipments to the New York market, 1972 –1984. Plant Dis. 70:605-607.

Chardonnet, C.O., E.E. Sams, W.S. Conway, F.A. Draughon, and J. R. Mount. 2002. Osmotic dehydration of apple slices with $CaCl_2$ and sucrose limits decay caused by *Penicillium expansum*, *Colletotrichum acutatum* and *Botrytis cinerea* and does not promote *Listeria monocytogenes* or total aerobic population growth. J. Food Prot. 65:172-177.

Conway, W.S., B. Leverentz, R.A. Saftner, W.J. Janisiewicz, C.E. Sams, and E. Leblanc. 2000. Survival and growth of *Listeria monocytogenes* on fresh cut apples and its interaction with *Glomerella cingulata* and *Penicillium expansum*. Plant Dis. 84:177-181.

D'Aoust, J-Y. 1997. *Salmonella* species, p. 129-158. *In* M.P. Doyle, L.R. Beuchat, and T.J. Montville (ed.), Food Microbiology: Fundamentals and Frontiers. ASM Press. Washington D.C.

Deeks, S., A. Ellis, B. Ciebin, R. Khakhria, M. Naus, and J. Hockin. 1998. *Salmonella* Oranienberg, Ontario. Can. Comm. Dis. Rep. 24:177-179.

Del Rosario, B.A. and L.R. Beuchat. 1995. Survival and growth of Enterohemorrhagic *Escherichia coli* O157:H7 in cantaloupe and watermelon. J. Food Prot. 58:105-107.

Draughon, F.A., S. Chen, and J.O. Mundt. 1988. Metabiotic association of *Fusarium*, *Alternaria*, and *Rhizoctonia* with *Clostridium botulinum* on fresh tomatoes. J. Food Sci. 53:120-123.

Eckert, J.W. 1980. Postharvest diseases of fresh fruits and vegetables – etiology and control. *In* N.F. Haard and D.K. Salunkhe (Ed.), Postharvest Biology and Handling of Fruits and Vegetables. AVI Publishing Company, Inc. Westport, CT.

Escartin, E.F., A.C. Ayala, and J.S. Lozano. 1989. Survival and growth of *Salmonella* and *Shigella* on sliced fresh fruit. J. Food Prot. 52:471-472.

Eshel, D., I Miyara, T. Ailing, A. Dinoor, and D. Prusky. 2002. pH regulates endoglucanase expression in virulence of *Alternaria alternata* in persimmon fruit. Mol. Plant Microbe Interact. 15:774-779.

Food and Drug Administration. 2001a. FDA Survey of imported fresh produce. FY 1999 Field Assignment. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. Office of Plant and Dairy Foods and Beverages. January 30, 2001. [Internet, WWW], ADDRESS: http://www.cfsan.gov/~dms/prodsur6.html. Accessed 25 January 2002.

Food and Drug Administration. 2001b. FDA Survey of domestic fresh produce: interim results. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. June 30, 2001. [Internet, WWW], ADDRESS: http://vm.cfsan.gov/~dms/prodsur8.html. Accessed 25 January 2002.

Food and Drug Administration. 2002. FDA issue import alert on cantaloupes from Mexico. US Food and Drug Administration. FDA Talk Paper. October 28, 2002. [Internet, WWW], ADDRESS: http://www.fda.gov/bbs/topics/ANSWERS/2002/ANS01167.html. Accessed 12 December 2002.

Golden, D.A., E.J. Rhodehamel, and D.A. Kautter. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. J. Food Prot. 56:194-196.

Huhtanen, C.N., J. Naghski, C.S. Custer, and R.W. Russell. 1976. Growth and toxin production by *Clostridium botulinum* in moldy tomato juice. Appl. Environ. Microbiol. 32:711-715.

Institute of Food Technologists/Food and Drug Administration of the United States Department of Health and Human Services (IFT/FDA). 2001. Analysis and evaluation of preventative control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. IFT/FDA Contract No. 223-98-2333. Task Order No. 3. Institute of Food Technologists. Chicago, IL.

Lukasik, J., M. Bradley, T. Scott, W-Y. Hsu, S. Farrah, and M. Tamplin. 2001. Elution, detection and quantification of Polio I, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157:H7 from seeded strawberries and tomatoes. J. Food Prot. 64:292-297.

Madden, J.M. 1992. Microbial pathogens in fresh produce – the regulatory perspective. J. Food Prot. 55:821-823.

Mohle-Boetani, J. C., R. Reporter, S.B. Werner, S. Abbott, J. Farrar, S.H. Waterman, and D.J. Vugia. 1999. An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. J. Infect. Dis. 180:1361-1364.

Morris, S.C. and N.L. Wade. 1983. Control of postharvest disease in cantaloupes by treatment with guazantine and benomyl. Plant Dis. 67:792-794.

Mundt, J.O. 1978. Effect of mold growth on the pH of tomato juice. J. Food Prot. 41:267-268.

Odlaug, T.E. and I.J. Pflug. 1979. *Clostridium botulinum* growth and toxin production in tomato juice containing *Aspergillus gracilis*. Appl. Environ. Microbiol. 37:496-504.

Prusky, D., J.L. McEvoy, B. Leverentz, and W.S. Conway. 2001. Local modulation of host pH by *Colletotrichum* species as a medium to increase virulence. Mol. Plant Microbe Interact. 14:1105-1113.

Reina, L.D., H.P. Fleming, and F. Breidt, Jr. 2002. Bacterial contamination of cucumber fruit through adhesion. J. Food Prot. 65:1881-1887.

Ries, A.A., S. Zaza, C. Langkop, R.V. Tauxe, and P.A. Blake. 1990. A multistate outbreak of *Salmonella* Chester linked to imported cantaloupe. Abstract. 915, p. 238. *In* Programs and abstracts of 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

Riordan, D.C.R., G.M. Sapers, and B.A. Annous. 2000. The survival of *Escherichia coli* O157:H7 in the presence of *Penicillium expansum* and *Glomerulla cingulata* in wounds on apple surfaces. J. Food Prot. 63:1637-1642.

Salunkhe, D.K. and B.B. Desai. 1984. Postharvest biotechnology of vegetables. Volume 2. CRC Press. Boca Raton, FL.

Sapers, G.M., R.L. Miller, V. Pilizota, and A.M. Mattrazzo. 2001. Antimicrobial treatments for minimally processed cantaloupe melon. J. Food Sci. 66:345-349.

Sewell A.M. and J.M. Faber. 2001. Foodborne outbreaks in Canada linked to produce. J. Food Prot. 64:1863-1877.

Snowdon, A.L. 1990. A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and

Vegetables. Volume 1: General Introduction and Fruits. Wolf Scientific Ltd., Barcelona, Spain.

Sommer, N.F., R.J. Fortlage, and D.C. Edwards. 1992. Postharvest diseases of selected commodities. *In* Adel A. Kader (Ed.), Postharvest Technology of Horticultural Crops. Publication 3311. University of California, Division of Agriculture and Natural Resources.

Suslow, T.V., M. Cantwell, and J. Mitchell. 2000. Cantaloupes: Recommendations for maintaining postharvest quality. University of California, Davis. Postharvest Technology Research and Information Center. June 26, 2000. [Internet, WWW], ADDRESS: http://postharvest.ucdavis.edu/Produce/Producefacts/cantaloupe.html Accessed 25 March 2002.

Suslow, T. and M. Cantwell. 2001. Recent findings on fresh-cut cantaloupe and honeydew melon. Fresh cut. April 2001. Pp.12, 20, 32-33.

Takeuchi, K. and J. Frank. 2001. Expression of red-shifted green fluorescent protein by *Escherichia coli* O157:H7 as a marker for the detection of cells on fresh product. J. Food Prot. 64:298-304.

Ukuku, D.O. and G.M. Sapers. 2001. Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. J. Food Prot. 64:1286-1291.

Wells J.M. and J.E. Butterfield. 1997. *Salmonella* contamination with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Dis. 81:867-872.

Wiant, J.S. 1937. Investigations of the market diseases of cantaloupes and honeydew and honey ball melons. United Sates Department of Agriculture. Technical Bulletin No. 573. September 1937.

CHAPTER 4

INFECTION OF CANTALOUPE RIND WITH *CLADOSPORIUM CLADOSPORIOIDES* AND *PENICILLIUM EXPANSUM*, AND ASSOCIATED MIGRATION OF *SALMONELLA* POONA INTO EDIBLE TISSUES[1]

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ABSTRACT

Although cantaloupe fruits are in contact with the ground during their development, enhancing their potential to be contaminated by pathogens, the internal tissues are expected to be free from microorganisms. Fungal infections of the rind tissue gradually progress inward toward the edible flesh. *Salmonella* Poona has been the predominant serotype responsible for national and international outbreaks associated with consumption of fresh cantaloupes. This study was undertaken to determine if the growth of two phytopathogens, *Cladosporium cladosporioides* and *Penicillium expansum,* in wounds on cantaloupe rinds facilitates migration of *S.* Poona into sub-surface mesocarp tissues. Wounded sites in cantaloupe rind were inoculated with *S*. Poona only, *S.* Poona and mold simultaneously or mold followed by *S*. Poona 3 days later. A cylindrical plug (ca. 3 cm diameter and 4 cm deep) was cut transversely into four segments (0 –1 cm, 1 – 2 cm, 2 – 3 cm, and 3 – 4 cm) representing distances from the rind surface. Regardless of the type of inoculum and the time of storage subsequent to inoculation, the pH of the flesh was significantly higher ($P \leq 0.05$) as the distance from the rind surface increased. Naturally-occurring and test microorganisms on the rind surface introduced into internal tissues during wounding as well as physiological changes in cantaloupe tissue contributed to these changes. The test molds were recovered from the inoculated rind as well as the underlying tissues throughout storage at 20°C for 10 days. *S*. Poona persisted and grew in the wounds in rinds on inoculated cantaloupe incubated. Recovery of *S*. Poona from tissues 3 – 4 cm below the inoculated wound supports the hypothesis that foodborne pathogens can migrate from the site of inoculation into adjacent mesocarp tissues. Results demonstrate that migration and survival of *S*. Poona in the internal tissues of cantaloupes were enhanced by co-inoculation with *C. cladosporioides* and to a lesser extent, *P. expansum*.

INTRODUCTION

Salmonellosis is the major bacterial foodborne disease in many countries (Asplund and Nurmi, 1991; D'Aoust, 2001) and has been increasing steadily as a public health problem over the last 50 years in the United States (CDC, 2000). Non-typhoidal salmonellae were estimated to cause approximately 1.5 million cases of infection, resulting in 15,000 hospitalizations and 500 deaths in the United States in 1999 (Mead *et al*., 1999). *Salmonella,* as a leading cause of foodborne disease, is attributable in part to its presence in a wide range of environmental sources, its prominence in various sectors of the agricultural and food industry, and from escalating movement of food and food ingredients in international trade (D'Aoust, 2001).

Foods of animal origin have historically been recognized as potential vehicles for *Salmonella*. However, consumption of fresh fruits and vegetables, including seed sprouts (O'Mahony *et al*., 1990; Van Beneden *et al.*, 1996; Mahon *et al*., 1997), unpasteurized apple cider (CDC, 1975), unpasteurized orange juice (Cook *et al*., 1998; CDC, 1999), raw tomatoes (Wood *et al.*, 1991; Wessinger *et al*., 2000), watermelons (Gayler *et al.*, 1955; Larson *et al*., 1979; Blostein, 1993), and cantaloupes (CDC, 1991; CDC, 2002; Deeks *et al*., 1998; IFT/FDA, 2001; Mohle-Boetani *et al.,* 1999; Ries *et al*., 1990) have also been linked to outbreaks of salmonellosis in recent years. Sources of origin of *Salmonella* detected on produce include animal and human wastes, irrigation water, runoff water from livestock farms adjacent to fields and orchards, manure, wash water, handling by workers, and contact with contaminated surfaces, animal fertilizers applied in previous growing seasons, and feces of rodents and ruminants (Guo *et al*., 2002). Cross contamination from raw meat, poultry, or eggs has also been identified as a potential mechanism for contamination of produce (IFT/FDA, 2001).

Cantaloupe fruits are in contact with the ground during their development, enhancing the potential for contamination by pathogens. The netted texture of cantaloupe melon rind enables effective attachment of microorganisms and results in difficulty in sanitization (Ukuku *et al*., 2001). A field survey conducted by the Food and Drug Administration (FDA) during 1990 and 1991 revealed that 0.76 and 1.06%, respectively, of the melon rinds harbor several serovars of *Salmonella* (Madden 1992; Golden *et al*., 1993). Selected fresh produce, including cantaloupes imported into the United States, was surveyed. Of 151 cantaloupes sampled, 7.3% were contaminated with *Salmonella* or *Shigella* (FDA, 2001a). The results of a similar survey of domestic fresh produce initiated in May 2000 showed the presence of *Salmonella* and *Shigella* on 4.3% of 92 cantaloupes sampled (FDA, 2001b). In a field study to assess the microbiological quality of 433 samples of leafy green vegetables and cantaloupes, *Salmonella* was isolated from 3.3% of the cantaloupes examined (Kleman *et al*., 2003). Contamination of the rinds of cantaloupes and honeydew melons has been traced to the primary wash tank or hydrocooler used to clean fruits after harvest (Gagliardi *et al*., 2003). *Salmonella* Poona has been the predominant serotype responsible for national and international outbreaks associated with consumption of fresh cantaloupes.

The internal tissues of produce are generally free of microorganisms. The cuticular protective cover on many fruits and vegetables and structural integrity afforded by cellulose and pectin, as well as the low pH by some produce, are important intrinsic factors that influence the microbial ecology. Being compartmentalized, plant tissues limit the diffusion of solutes that influence the survival and growth of microorganisms. Fruits and vegetables may contain chemical compounds that are inhibitory to microorganisms and may induce defense reactions against microbial invasion (Nguyen-the and Carlin, 2000). Only the microorganisms that

possess the necessary physiological attributes to respond to intrinsic and extrinsic environmental pressures will survive (Goepfert, 1980). The association of salmonellosis with salad bars and fruit salads suggests introduction of *Salmonella* into the fruit from the rind by the slicing action, or physical contact of cut pieces with contaminated rind (Suslow and Cantwell, 2001).

Cantaloupes are susceptible to postharvest fungal rots, especially under warm, wet conditions. Infection of the external surface of the fruit gradually progresses inwards toward the flesh. Fungal pathogens of major concern in cantaloupes are *Geotrichum*, *Alternaria*, *Penicillium, Cladosporium, Rhizopus*, and *Fusarium* (Suslow *et al*., 2000; Agblor and Waterer, 2001) and, to a lesser extent, *Mucor* (Suslow *et al*., 2000). Complete loss of the commodity occurs when one or a few fungal pathogens invade and begin to breakdown the tissues. This initial attack is often rapidly followed by a broad spectrum of weak pathogens that magnify the damage caused by primary pathogens.

Several studies have been undertaken to assess the survival and growth of *Salmonella* in tissues of fruits and vegetables. *Salmonella* was associated more often with soft-rotted (Wells and Butterfield, 1997) and injured plant tissues (Liao and Sapers, 2000; Liao and Cooke, 2001) than with their healthy, intact counterparts. The potential of the contaminated tissue to support growth of *Salmonella* is exacerbated by display or storage of cantaloupes at ambient temperature for an extended time (CDC, 1991; Golden *et al*., 1993). The high water activity and sugar content of tissue juices may promote rapid proliferation of salmonellae. Cut cantaloupe is considered a potentially hazardous food in the FDA Food Code because it is capable of supporting the growth of pathogens due to low acidity (pH 5.2 to 6.7) and high water activity (0.97 to 0.99) (IFT/FDA, 2001). The study reported here was undertaken to determine if the

growth of two molds, *Cladosporium cladosporioides* and *Penicillium expansum,* in wounds on cantaloupe rinds facilitates migration of *S.* Poona into sub-surface mesocarp tissues.

MATERIALS AND METHODS

Preparation of *S.* Poona and inoculum. Five strains of *S.* Poona isolated from patients in cantaloupe-associated outbreaks of salmonellosis were used. Strains 00A3207, 01A3923, and 02A3275 were obtained from the State of California, Department of Health Services, while strains 00A3279 and 01A242 were obtained from Dr. Trevor Suslow, University of California, Davis. Each strain was progressively made resistant to nalidixic acid by a series of nine transfers in tryptic soy broth (TSB; BBL/Difco, Sparks, MD) supplemented with increasing concentrations of nalidixic acid up to 50 μg/ml (TSBN). Cultures were incubated at 37°C for 24 h before transferring to TSB containing a higher concentration of nalidixic acid. The nalidixic acid-resistant cells were surface plated on tryptic soy agar (TSA; BBL/Difco) supplemented with 50 μg of nalidixic acid/ml (TSAN) and incubated at 37°C for 24 h. Stock cultures were stored on TSAN at 4°C.

Stock cultures were streaked on TSAN and incubated at 37°C for 24 h before transferring cells from a single colony of each strain to 10 ml of TSBN and incubating at 37°C for 24 h. Three consecutive 24-h transfers of suspensions were made via loop inocula into 10 ml of TSBN. After incubation at 37°C for 24 h, the cells were harvested by centrifugation at 2,500 x *g* for 10 min in a Centra CL2 centrifuge (International Equipment Company, Needham Heights, MA). Cells were washed two times in 10 ml of sterile deionized water, then resuspended in 10 ml of sterile 0.1% (w/v) peptone water. The population (CFU/ml) in each suspension was determined by serially diluting suspensions in sterile 0.1% peptone water and surface plating samples (0.1 ml in duplicate) on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted.

Random presumptive-positive colonies of *Salmonella* were confirmed using triple sugar iron, lysine iron agar, and a *Salmonella* latex agglutination assay (Oxoid, Basingstoke, Hampshire, UK).

Equal volumes (10 ml) of suspensions of each strain were combined to give 50 ml of a five-strain mixture of *S*. Poona containing approximately equal populations (9 $\log_{10}$ CFU/ml) of each strain. The suspension was serially diluted to give an inoculum that contained ca. 5.7 $\log_{10}$ CFU/ml.

Procedures for growing molds and preparation of inocula. Strains of *Cladosporium cladosporioides* and *Penicillium expansum* isolated from cantaloupes were obtained from Dr. Trevor Suslow, University of California, Davis. Molds were maintained on dichloran rose bengal chloramphenicol (DRBC) agar (BBL/Difco) and potato dextrose agar (PDA) (BBL/Difco) slants at 4°C, and subcultured every 14 days by streaking on fresh DRBC agar and PDA plates and incubating at 25°C for 5 days.

Each mold was streaked on DRBC agar and incubated at 25°C for 5 days. Suspensions of conidia were prepared by pipetting 10 ml of sterile deionized water containing 0.05% Tween 80 onto the surface of the 5-day-old cultures and gently massaging for 1 min with a sterile glass rod. Using a sterile Pasteur pipette, the conidial suspension was removed from the surface of the plate and deposited in a sterile test tube. Based on observations from preliminary work (data not shown), the number of conidia in each suspension was adjusted to ca. 4 – 5 $\log_{10}$ CFU/ml by diluting in sterile deionized water containing 0.05% Tween 80. The number of conidia (CFU/ml) in each suspension was determined by serially diluting samples in sterile deionized water and surface plating (0.1 ml in duplicate) on DRBC agar. Plates were incubated at 25°C for 5 days before colonies were counted.

Preparation and inoculation of cantaloupes. Full slip Western type cantaloupes (*Cucumis melo* L. var. *reticulatus* Naud) were purchased in September 2003 from a grocery in Griffin, GA and stored at 4°C for 2 days. Prior to using in experiments, the cantaloupes were adjusted to 22 ± 1°C over a 16 to 20-h period. Twenty-four unwashed cantaloupes, free of visible wounds, cuts, and bruises, and placed on a 15-cm diameter plastic potting saucer (Ivex, Tustin, CA), were randomly divided into six groups of four fruits each and placed in covered 27-liter polypropylene containers (59 cm long x 43 cm wide x 15 cm high; Sterilite, Townsend, MA) in which twenty-eight holes (ca. 6 mm diameter) were made to minimize the condensation of water on the surface of rinds and facilitate ventilation inside the container.

Each of the four fruits in the six groups (treatments) represented a sample for each day of analysis (days 3, 5, 7, and 10). A permanent ink marker was used to make four 3-cm diameter circles (ca. 4 cm apart) on the rind of each cantaloupe, designating sites for inoculation. The end (8 mm wide) of a sterile stainless steel spatula was used to create a wound 4 mm deep in the center of each circle. This procedure resulted in four inoculation sites on each cantaloupe, all of which were analyzed at each sampling time.

The pH of the cut tissue of wounded rind was determined with a surface pH probe (Basic pH meter, Denver Instrument Company, Arvada, CO). Two different lots of fruits were used. One group of four cantaloupes was left untreated and the wounded tissues on the cantaloupes in the other five groups were inoculated with *S.* Poona only or *S*. Poona and mold (*C. cladosporioides* or *P. expansum*) suspensions using a Precision Microliter Pipette (Rainin, Woburn, MA). Inoculum was deposited directly in the cut rind tissue. Three inoculation schemes were used:

(i) *S.* Poona only: Wounded rind tissues on cantaloupes were inoculated with 10 µl of *S.* Poona suspension containing ca. 5.9 $\log_{10}$ CFU/ml on day 0.

(ii) *S.* Poona and mold: Wounded rind tissues on cantaloupes were inoculated with 10 µl of *S.* Poona suspension containing ca. 5.9 $\log_{10}$ CFU/ml and 10 µl of mold suspension containing ca. 4 – 5 $\log_{10}$ CFU/ml on day 0.

(iii) Mold, then *S.* Poona 3 days later: Wounded rind tissues on cantaloupes were inoculated with 10 µl of mold suspension containing ca. 4 – 5 $\log_{10}$ CFU/ml, followed by 10 µl *S.* Poona suspension containing ca. 5.2 $\log_{10}$ CFU/ml, 3 days later.

Inoculated cantaloupes were kept in a laminar flow hood (class II, type A/B3) for 2 h at 22 $\pm$ 1°C, then incubated at 20°C for up to 10 days before analyzing for the presence of test molds and presence/population of *S.* Poona.

Microbiological analyses. Wounded inoculated rinds of cantaloupes were analyzed for the presence of *C. cladosporioides* or *P. expansum* and the presence and number of *S.* Poona 3, 5, 7, and 10 days after inoculation. For cantaloupes inoculated with mold on day 0 and *S.* Poona on day 3, analysis was done 3, 5, 7, and 10 days after inoculating with *S.* Poona.

At each inoculation site, a plug of tissue (ca. 3 cm diameter and 4 cm deep) was excised from the cantaloupe using a sterile stainless steel spatula. The cylindrical plug was placed on a sterile petri dish and cut transversely into four equal portions (ca. 3 cm diameter and 1 cm thick), representing segments four distances (0 – 1 cm, 1 – 2 cm, 2 – 3 cm, and 3 – 4 cm) from the site at which the wounded rind was inoculated with *S.* Poona only or *S.* Poona and a test mold. Each portion of the plug was placed into a separate sterile stomacher 80 bag (Seward Medical Ltd., London, UK). The samples were hand rubbed and juice (ca. 0.2 ml) from the

macerated tissue was removed to determine the pH using a Basic pH meter (Denver Instrument Company, Arvada, CO). A sterile inoculation loop was used to remove a sample from the stomacher bag and streak on duplicate DRBC agar plates. DRBC agar plates were incubated at 25°C for 5 days before the examining for the presence of test molds. Twenty milliliters of sterile lactose broth (BBL/Difco) supplemented with 50 μg of nalidixic acid/ml (LBN) were added to the bags of macerated tissue samples and the mixture was pummeled for 1 min at normal speed in a Model 80 Stomacher (Seward Medical). The liquid portion of the undiluted homogenate was surface plated (0.25 ml in quadruplicate and 0.1 ml in duplicate) on TSAN; samples (0.1 in duplicate) of homogenate diluted in 0.1% peptone were also surface plated on TSAN. Plates were incubated at 37°C for 24 h before presumptive-positive *S*. Poona colonies were counted. Random presumptive-positive colonies were selected for confirmation as described above. Homogenates containing LBN and rind tissue were incubated at 37°C for 24 h. For samples that did not yield *S*. Poona colonies on TSAN, the LBN/tissue mixture was examined for the presence of *S*. Poona. A 1-ml sample of the pre-enriched mixture was inoculated into 10 ml of selenite cysteine (SC) broth (BBL/Difco), incubated at 37°C for 24 h, and surface plated on bismuth sulfite agar (BSA; BBL/Difco) supplemented with 50 μg of nalidixic acid/ml (BSAN). Plates were incubated at 37°C for 24 h before random presumptive-positive colonies were selected for confirmation as described above.

Statistical analysis. The experiment was done two times. Samples from four cantaloupes were analyzed at each sampling time in both replicate trials. Mean values were analyzed to determine if significant differences exist in pH of tissue and in populations of *S.* Poona in each of the four segments of the plug at each storage time. Significant differences in pH and in *S*. Poona population in a given segment of the plug as affected by storage time were

also determined. General linear models on SAS software (Statistical Analysis Systems Institute, Cary, NC) were used. Significant differences ($P \leq 0.05$) between mean values were determined using Duncan's multiple range tests.

RESULTS AND DISCUSSION

pH of cantaloupe tissues as affected by inoculation with *S.* Poona or co-inoculation with S. Poona and mold. The pH of cantaloupe tissues at the time of inoculation is contingent on the distance of the tissue from the rind surface. Two different lots of fruits were used resulting in different initial pH for cantaloupes for cantaloupes used in control and uninoculated fruits. The pH of pulp tissues increased in each successive 1-cm segment from the site of inoculation inwardly to the mesocarp tissue adjacent to the seed cavity (Table 4.1). This gradient was unaffected by inoculation with *S*. Poona or co-inoculation with *S*. Poona and *C. cladosporioides* or *P. expansum*. Regardless of the type of inoculum and the time of storage subsequent to inoculation, the pH of the flesh further away from the rind surface (3 – 4 cm) was significantly higher ($P \leq 0.05$) than that of the tissue within 1 cm of the rind surface.

Ten days after wounding, the pH of the uninoculated (control) rind (0 – 1 cm) and subsurface tissues (1 – 3 cm) was significantly higher ($P \leq 0.05$) than respective tissues on the previous sampling days. However, the pH of tissues of control tissues on day 0 was not significantly different ($P > 0.05$) in the four segments of the plug. The increase in pH is attributed metabolic processes associated with tissues and to the growth of alkalinizing molds that naturally occurred on the rind surface and were introduced into the fleshy tissue during the wounding process. Prolific mold growth was observed in wounded rinds.

When wounds were inoculated with *S*. Poona only, the pH of the rind and the flesh tissues (up to 3 cm deep) also increased significantly ($P \leq 0.05$), peaking between days 5 and 7.

TABLE 4.1. pH of wounded rind and mesocarp tissues of cantaloupes inoculated with *Salmonella* Poona and molds

Inoculum	Distance [a] (cm)	pH[b] Day 0	3	5	6	7	8	10	13
Control	0 - 1	D 5.33 C	C 5.74 B	C 6.02 B		B 5.98 B		A 7.11 A	
(not inoculated)	1 - 2	C 5.81 B	C 5.91 B	C 6.00 B		B 6.00 B		A 7.10 A	
	2 - 3	B 6.27 B	B 6.11 B	B 6.18 B		B 6.15 B		A 6.65 A	
	3 - 4	A 6.47 B	A 6.54 B	A 6.56 B		A 6.56 B		A 6.84 A	
S. Poona only (d 0)[c]	0 - 1	C 6.07 C	D 6.19 BC	B 6.50 A		C 6.45 A		C 6.38 AB	
	1 - 2	B 6.55 B	C 6.38 B	B 6.53 B		B 6.84 A		BC 6.44 B	
	2 - 3	AB 6.69 B	B 6.59 B	B 6.67 B		B 6.85 A		B 6.57 B	
	3 - 4	A 7.00 AB	A 6.90 AB	A 7.06 A		A 7.05 A		A 6.75 B	
S. Poona and	0 - 1	C 6.07 B	B 6.25 AB	B 6.33 A		B 6.40 A		B 6.36 A	
C. cladosporioides (d 0)	1 - 2	B 6.55 AB	A 6.55 AB	B 6.33 B		A 6.82 A		B 6.45 B	
	2 - 3	AB 6.69 AB	A 6.64 AB	AB 6.42 B		A 6.84 A		AB 6.62 AB	
	3 - 4	A 7.00 A	A 6.68 A	A 6.67 A		A 6.98 A		A 6.89 A	
S. Poona and	0 - 1	C 6.07 A	B 6.14 A	B 6.17 A		B 5.33 B		B 4.64 B	
P. expansum (d 0)	1 - 2	B 6.55 A	A 6.46 A	A 6.52 A		AB 5.87 A		AB 5.19 B	
	2 - 3	AB 6.69 A	A 6.64 A	A 6.60 A		A 6.40 A		A 5.70 B	
	3 - 4	A 7.00 A	A 6.74 A	A 6.76 A		A 6.77 A		A 5.91 B	
C. cladosporioides (d 0)	0 - 1	C 6.07 B			C 6.56 A		A 6.53 A	B 5.73 B	C 5.65 B
and *S*. Poona (d 3)	1 - 2	B 6.55 AB			BC 6.61 A		A 6.45 A	A 6.03 A	B 6.18 A
	2 - 3	AB 6.69 AB			B 6.78 A		A 6.44 A	AB 6.44 A	A 6.50 A
	3 - 4	A 7.00 A			A 7.06 AB		A 6.68 C	A 7.26 A	A 6.76 BC
P. expansum (d 0)	0 - 1	C 6.07 A			C 5.10 A		C 4.74 AB	B 4.39 B	B 5.22 A
and *S*. Poona (d 3)	1 - 2	B 6.55 A			B 6.19 A		B 5.40 B	B 4.76 B	B 5.38 B
	2 - 3	AB 6.69 A			A 6.66 A		A 6.53 A	A 5.70 B	AB 5.86 B
	3 - 4	A 7.00 A			A 6.72 A		A 6.86 A	A 6.33 A	A 6.32 A

[a] Distance is measured from the site of inoculation on the wounded rind surface inwardly toward the edible tissue.

[b] Within inoculum and incubation time, mean values in a column that are not preceded by the same letter are significantly different ($P \leq 0.05$). Mean values in a row that are not followed by the same letter are significantly different ($P \leq 0.05$).

[c] Values in parentheses indicate the day on which cantaloupes were inoculated. For cantaloupes inoculated with mold day 0 and *S*. Poona on day 3, pH measurements were made 3, 5, 7, and 10 days after inoculation with *S.* Poona.

Significant increases in pH of rind tissue (0 – 1 cm) occurred within 5 days after inoculation. Simultaneous co-inoculation of wounds with *S*. Poona and *C. cladosporioides* caused a significant increase in the pH of the cantaloupe rind tissue (0 – 1 cm) within 5 days. The pH of flesh tissues (1 – 4 cm) was essentially unchanged during the 10-day storage period. The effect of simultaneous co-inoculation of wounds in rind tissue with *S*. Poona and *P. expansum* on the pH of the rind and flesh tissues was different from that of wounds caused by co-inoculation with *S*. Poona and *C. cladosporioides*. Wounded tissues inoculated with *S*. Poona and *P. expansum* showed a significant decrease in the pH of the rind tissue (0 – 1 cm) on days 7 and 10. The pH of the mesocarp (1 – 4 cm) below the rind surface remained unchanged for 7 days but decreased significantly between 7 and 10 days. Differences in changes in pH of tissues inoculated with the two molds are attributed in part to differences in their alkalinizing capabilities. The production of acids, such as lactic and pyruvic acids by *S*. Poona may also have contributed, to reductions in pH.

The pH of rind tissue (1 – 2 cm) inoculated with *C. cladosporioides* 3 days before inoculating with *S*. Poona decreased significantly between 5 and 7 days after inoculation with *S.* Poona. The pH of the flesh 1 – 3 cm below the surface did not change significantly ($P > 0.05$); however, at 3 – 4 cm, the pH fluctuated during the 10-day storage period. When the wounds were inoculated with *P. expansum* 3 days prior to inoculation with *S*. Poona, the pH of rind tissue (0 – 1 cm) decreased on day 7, then increased at day 10. The pH of mesocarp tissues (1 – 3 cm) trended downward during storage for 10 days but the pH 3 – 4 cm below the surface of the rind did not change significantly ($P > 0.05$). Fluctuations in pH of tissues in the four segments were indicative of changes in microbial ecology during storage. The rind surface of cantaloupes is inhabited by large numbers of yeast and mold species. Unlike molds, yeasts are generally

incapable of invading and infecting tissues and will act as secondary agents of spoilage (Splittstoesser, 1987). In addition to differences in alkalinizing capabilities of *C. cladosporioides* and *P. expansum* and growth of *S*. Poona in tissues, the decline in pH in the latter stages of the storage period may also reflect metabolic activities of yeasts.

Overall changes in pH of tissues at or near the site of inoculation occurred before more distant sites. This suggests that the inocula and naturally-occurring microorganisms were responsible for biochemical changes in tissues that began at the site of inoculation and progressed inwardly. Pseudomonads and lactic acid bacteria on fruits and vegetables capable of producing pectin-degrading enzymes may cause maceration and release of juices from cantaloupe tissues (Laminkanra *et al*., 2003). Wounding of plant tissues also induces elevated ethylene production and consequently increases the rate of tissue respiration. Starch breakdown is enhanced, stimulating the tricarboxylic acid cycle and the electron transport chain (Brecht, 1995). The escalated release of carbon dioxide and formation of carbonic acid causes the pH of the wounded tissues to decrease. There would also be elevated levels of sugars available for metabolizing by the microorganisms inoculated into the wounds and those that were naturally resident on the rind surface. The metabolic activities of the microorganisms in the wounds would elicit changes, such as increasing or decreasing the pH of the tissue environment, depending on constituents utilized and by products accumulated. Change in pH of uninoculated tissue reinforces the importance of preventing growth of molds that may influence the survival and growth of *Salmonella* and other foodborne pathogens.

Recovery of molds from cantaloupe tissues. Populations of *C. cladosporioides* and *P. expansum* deposited into wounds on the rind of cantaloupes are shown in Table 4.2. The molds were recovered from inoculated rind tissue as well as the underlying mesocarp closest to the seed

TABLE 4.2. Detection *of molds in rind and mesocarp tissues of cantaloupes after inoculation*.

Inoculum	Distance [a] (cm)	Population[c]	Presence of mold on storage day[b] 3	5	6	7	8	10	13
S. Poona and	0 - 1	1.85	16	14		16		16	
C. cladosporioides (d 0)[d]	1 - 2		7	11		13		3	
	2 - 3		4	10		11		2	
	3 - 4		2	8		13		2	
S. Poona and	0 - 1	2.73	14	16		16		16	
P. expansum (d 0)	1 - 2		7	8		8		10	
	2 - 3		5	3		2		7	
	3 - 4		2	2		1		7	
C. cladosporioides (d 0)	0 - 1	1.85			16		16	14	14
and *S*. Poona (d 3)	1 - 2				3		0	3	3
	2 - 3				1		0	4	3
	3 - 4				0		0	3	1
P. expansum (d 0)	0 - 1	2.73			16		14	16	16
and *S*. Poona (d 3)	1 - 2				5		15	16	8
	2 - 3				3		8	16	8
	3 - 4				1		6	16	8

[a] Distance is measured from the site of inoculation on the wounded rind surface inwardly toward the edible tissue.

[b] Number of samples out of sixteen that were positive for mold 3 – 13 days after inoculation with mold.

[c] Population of test mold ($\log_{10}$ CFU per 10 µl of inoculum) on day 0. Cantaloupes were inoculated with 10 µl of conidial suspension and 10 µl of S. Poona suspension on day 0, or 10 µl of conidial suspension on day 0, followed by 10 µl of *S*. Poona suspension on day 3.

[d] Values in parentheses indicate the day on which the cantaloupes were inoculated.

cavity (3 – 4 cm from the surface) after storage at 20°C for 10 days. Molds were detected in consistently higher numbers of samples closer to the rind surface, regardless of inoculation schedule or storage time after inoculation. As storage time increased, the detection of test molds in tissues further from the inoculation site tended to increase.

When wounds on the rind surface were co-inoculated with *S*. Poona and *C. cladosporioides* or *P. expansum*, the molds progressively penetrated the underlying mesocarp tissue. Overall, detection of molds in tissues further away from the rind increased through days 7 – 10. Some decreases in number of positive samples were noted at extended storage times.

It has been reported that while lesions on fruits and vegetables caused by pathogenic microorganisms may detract from the appearance, the internal tissues may remain unaffected (Wills *et al.*, 1998). However, results presented here show that *C. cladosporioides* and *P. expansum* inoculated into wounds on the rinds of cantaloupes were capable of penetrating through the mesocarp to depths as great as 4 cm. The effects of these molds on appearance of mesocarp were different. *C. cladosporioides* did not affect the appearance of mesocarp tissue, but tissues extending from the rind to the seed cavity were softened in texture. In some cases, penetration of the mesocarp tissues by *P. expansum* hyphae was visible, causing discoloration, reduction in the moisture content and hardening of the internal tissues.

Propagules of phytopathogens are abundant in the atmosphere and on the surface of fruits and vegetables as they approach maturity in the field. Mature fruits and vegetables are highly susceptible to invasion by many pathogenic fungi because they are high in moisture and nutrients and are no longer protected by some of the intrinsic factors that confer resistance during their development on the plant. Additionally, many tissues of fruits and vegetables become more easily injured as they approach full maturity and therefore are more vulnerable to pathogens

entering through wounds. Abundance of *C. cladosporioides* and *P. expansum* conidia in the tissue environment as well as and increasing susceptibility of mature cantaloupes during the 10-day storage period modeled the infection process during storage of harvested fruits. During storage of the cantaloupes, the fungal mycelia were advanced through the susceptible tissues.

Sanitation is essential to reduce pathogen populations available for infection, especially if the crop is treated with water after harvest (Eckert, 1980). Harvesting of produce often results in tissue injuries at the point of severance of stem and fruit, creating a point of entry for pathogens. The incidence of postharvest diseases is enhanced by the presence of wounds could be substantially reduced by modifying the handling practices that damage the fruit surface and by exposing produce to environmental conditions that favor the development of cellular barriers to the penetration of pathogens. The wounds created on the cantaloupe rinds disrupted the intrinsic barriers and increased the availability of nutrients and moisture in the tissue environment. The ability of *C. cladosporioides* and *P. expansum* to survive and advance to deeper tissues was enhanced by the fact that the rind was disrupted.

Recovery of *S.* Poona from wounded rinds and mesocarp tissues. Populations of *S.* Poona in 10 µl of inocula deposited into wounds in cantaloupe rinds are shown in Table 4.3. *S.* Poona persisted and grew in the wounds on cantaloupes incubated at 20°C. Except for wounds simultaneously or sequentially inoculated with *P. expansum,* the population of *S.* Poona increased significantly higher ($P \leq 0.05$) within 3 days.

Recovery of *S.* Poona from tissues at depths up to 3 – 4 cm below the inoculated wound supports the hypothesis that foodborne pathogens can migrate from the site of inoculation into adjacent mesocarp tissues. In wounds in the rind tissue were inoculated with only *S.* Poona, the

TABLE 4.3. *Population of* Salmonella *Poona in wounded rind and mesocarp tissues of inoculated cantaloupes*

	Distance[a]	Population[b]								
Inoculum	(cm)	Day 0	3	En[c]	5	En	7	En	10	En
S. Poona only (d 0)[d]	0 - 1	3.90 C	A 5.58 B		A 6.28 AB		A 6.75 A		A 5.36 B	
	1 - 2		B <1.30 B	0/8	B 0.21 B	1/7	B 2.30 A	0/2	B <1.30 B	0/8
	2 - 3		B <1.30 B	0/8	B <1.30 B	1/8	B 1.70 A	2/4	B <1.30 B	0/8
	3 - 4		B <1.30 B	0/8	B <1.30 B	0/8	B 1.09 A	2/5	B <1.30 B	0/8
S. Poona and	0 - 1	3.90 B	A 5.36 A		A 5.74 A		A 5.70 A		A 5.98 A	
C. cladosporioides (d 0)	1 - 2		B <1.30 A	1/8	B <1.30 A	2/8	B <1.30 A	5/8	B <1.30 A	3/8
	2 - 3		B <1.30 A	2/8	B <1.30 A	1/8	B <1.30 A	2/8	B <1.30 A	1/8
	3 - 4		B <1.30 A	2/8	B <1.30 A	0/8	B <1.30 A	2/8	B <1.30 A	3/8
S. Poona and	0 - 1	3.90 ABC	A 5.97 A		A 4.94 AB		A 2.61 B	0/4	A 2.71 B	1/4
P.expansum (d 0)	1 - 2		B 0.47 B	3/7	B <1.30 A	3/8	B <1.30 A	0/8	B 0.75 A	1/3
	2 - 3		B <1.30 A	0/8	B <1.30 A	3/8	B <1.30 A	2/8	B 0.52 A	4/5
	3 - 4		B <1.30 A	1/8	B <1.30 A	2/8	B <1.30 A	0/8	B 0.24 A	4/6
C. cladosporioides (d 0)	0 - 1	3.20 C	A 3.77 B	1/1	A 6.33 A		A 5.11 B	0/1	A 4.74 AB	1/1
and S. Poona (d 3)	1 - 2		B <1.30 A	0/8	B 1.16 A	0/6	B 0.96 A	2/6	B <1.30 A	2/8
	2 - 3		B <1.30 A	5/8	B <1.30 A	2/8	B <1.30 A	3/8	B <1.30 A	2/8
	3 - 4		B <1.30 A	4/8	B <1.30 A	1/8	B <1.30 A	4/8	B <1.30 A	1/8
P. expansum (d 0)	0 - 1	3.20 A	A 0.66 B	0/6	A 0.60 B	0/6	A 0.30 B	1/7	A 1.11 B	0/5
and *S*. Poona (d 3)	1 - 2		A <1.30 A	0/8	A <1.30 A	0/8	A <1.30 A	1/8	B <1.30 A	0/8
	2 - 3		A <1.30 A	0/8	A <1.30 A	0/8	A <1.30 A	0/8	B <1.30 A	0/8
	3 - 4		A <1.30 A	0/8	A <1.30 A	0/8	A <1.30 A	0/8	B <1.30 A	0/8

[a] Distance is measured from the site of inoculation on the wounded rind surface inwardly toward the edible tissue.

[b] Population of *S*. Poona on day 0 indicates $\log_{10}$ CFU per 10 µl of inoculum. Populations 3, 5, 7, and 10 days after inoculation with *S*. Poona indicate $\log_{10}$ CFU/tissue sample analyzed. The minimum detection limit is 1.30 $\log_{10}$ CFU/sample. Within inoculum and incubation time, mean values in a column that are not preceded by the same letter are significantly different ($P \leq 0.05$). Mean values in a row that are not followed by the same letter are significantly different ($P \leq 0.05$).

[c] Number of samples positive for *S*. Poona out of the number analyzed by enrichment.

[d] Values in parentheses indicate the day on which cantaloupes were inoculated. For cantaloupes inoculated with mold on day 0 and *S*. Poona on day 3, analyses for the presence and number of *S*. Poona were made 3, 5, 7, and 10 days after inoculation with *S*. Poona.

pathogen was recovered from the mesocarp tissues 2 – 3 cm below the rind surface 5 days after inoculation. Within 7 days, *S*. Poona was recovered from tissues at a depth of 3 – 4 cm from the rind surface. At 10 days, *S*. Poona was not detected in tissue 1 – 4 cm below the inoculation site. The reason for the absence of the pathogen is not evident. In wounds simultaneously inoculated with *S*. Poona and *C. cladosporioides* or *P. expansum* or sequentially inoculated with *C. cladosporioides* and *S*. Poona, the pathogen was recovered from the tissues 3 – 4 cm below the rind after 3 days of storage and persisted for up to 10 days. This suggests that *C. cladosporioides* and *P. expansum* enhanced the migration of *S*. Poona away from inoculated tissue and promoted its survival in subsurface tissues. Increased recovery of *S*. Poona from deeper tissues could have been due to penetration by fungal hyphae or perhaps fluids as a result of maceration of the tissues. Inoculation of wounds with *P. expansum* 3 days before inoculating with *S*. Poona did not appear to facilitate the movement of *S*. Poona into sub-surface tissues.

C. cladosporioides appeared to promote survival and growth of *S*. Poona in cantaloupe tissues more than *P. expansum*. This may be attributable in part to proteolytic activity by *C. cladosporioides*, which would contribute to an increase the pH of the tissue, making it more favorable for *S*. Poona. Hardening of the mesocarp tissues inoculated by *P. expansum* would reduce the potential of *S*. Poona to move away from the site of inoculation. Although the pH of wounds in rind tissue inoculated with *P. expansum* decreased to as low as 4.39 (sd. $\pm$ 0.2), *S*. Poona continued to survive in these tissues. The optimum pH for growth of *Salmonella* is 6.5 – 7.5, but physiological adaptability of the pathogen has been demonstrated by its ability to grow at pH values ranging from 4.5 to 9.5 (D'Aoust, 1997; D'Aoust, 2000; Jay, 2000). *Salmonella* Montevideo has been reported to grow in chopped tomatoes (pH 4.1) (Zhuang *et al*., 1995) and *Salmonella* Baildon grew in diced tomatoes (pH 4.4) (Weissinger *et al*., 2000)

Microbial growth is among the factors that accelerate senescence of cantaloupes, causing economic loss by producers and consumers. To reduce these losses, visually sound portions of edible tissue at distances away from the site of infection are salvaged for consumption. This is a potentially hazardous practice, as *S*. Poona and probably other salmonellae may migrate away from the site of infection and contaminate apparently healthy tissues. The migration of *S*. Poona in tissues of cantaloupes was more evident when co-inoculated with *C. cladosporioides* than with *P. expansum*. This may be attributable to more extensive breakdown of tissues by *C. cladosporioides* versus hardening of some tissues by *P. expansum. S.* Poona was not recovered from tissues compacted as a result of growth of *P. expansum*.

Phytopathogens produce extracellular enzymes such as cellulases and pectinases to decompose plant tissues that are not digestible by other organisms. Fruit decay pathogens produce pectolytic enzymes that degrade cell wall pectin and macerate tissues (Bruton *et al*., 1998). Release of fluids, softening of the tissues, and the creation of channels and cavities by fungal mycelia would promote passive translocation of *S*. Poona from the site of inoculation in the cantaloupe rind to tissues within the mesocarp. *Salmonella* is not fastidious with respect to oxidation-reduction (OR) potential (Doyle and Cliver, 1990) and can therefore survive in cantaloupe tissues with low OR potential. *S.* Montevideo has been reported to survive in internal tissues of tomatoes stored at 20 and 30°C (Zhuang *et al*. 1995). Although the mode of entry of *S*. Poona into internal cantaloupe tissues in the study presented here was different, viable *S*. Poona was shown to be present for up to 10 days at a distance of 3 – 4 cm from the rind surface.

The effectiveness of treatments to detach or inactivate *Salmonella* on cantaloupe surfaces is dependent on the location of the cells. Migration of *S*. Poona into sub-surface tissues would reduce the efficacy of decontamination practices that are currently being used. Solutions

containing chlorine at concentrations of 60, 120, and 320 µg/ml, for example, were less effective at killing *S*. Montevideo that had been internalized in the core tissue compared to those that were on the surface of tomatoes (Zhuang *et al*., 1995). Aqueous chlorine at 100 µg/ml failed to kill *S*. Montevideo inoculated into wounded sites on tomatoes (Wei *et al*., 1995). The efficacy of chlorine in killing microorganisms on the surface of raw produce is notably reduced by contact with organic matter (Beuchat *et al*., 1998). Therefore it is conceivable that application of chlorine by dipping or spraying would be ineffective against *Salmonella* in sub-surface tissues. Trisodium phosphate (TSP) was observed to be effective in killing *S*. Montevideo on the surface of mature-green tomatoes, but less effective against internalized cells (Zhuang and Beuchat, 1996). Similarly, *Salmonella* Chester in injured tissue of pepper discs were not killed by treatments of 3% to 12% TSP (Liao and Cooke, 2000). When apples were treated with hydrogen peroxide for the purpose of killing or removing *S*. Chester, populations remaining in the stem and calyx cavities were greater than on the skin (Liao and Sapers, 2000). Entrapment of bacteria below the surface of the fruits and vegetables limits the accessibility of sanitizers to the bacterial contaminants (Beuchat, 1999).

Salmonellosis associated with consumption of cantaloupes may be due to introduction of *Salmonella* on the rind into the pulp tissue by physical contact or during cutting (Suslow and Cantwell, 2001; Ukuku and Sapers, 2001). It is hypothesized that the stem scar tissues and wounds on the rind would trap bacteria. In a previous study, *S*. Poona was shown to infiltrate the intact rind and stem scar tissues during immersion in inocula. These cells could subsequently migrate to deeper tissues. The study reported here shows that contamination of the flesh can also result from migration of *S*. Poona from the wounded rind through the pulp tissues to distances as far as 3 – 4 cm, with or without assistance from phytopathogens. Migration and survival of *S*.

Poona in the internal tissues of cantaloupes were enhanced by co-inoculation with *C. cladosporioides* and to a lesser extent, *P. expansum*. Consumption of cantaloupes from which diseased tissue has been removed is not advisable because *S*. Poona and perhaps other pathogens may still be present in remaining tissues.

REFERENCES

Agblor, S. and D. Waterer. June 2001. Muskmelons: Cantaloupe postharvest handling and storage. Agri-Food Innovation Fund (AFIF) Postharvest Specialist Program. University of Saskatchewan.

Asplund, K. and E. Nurmi. 1991. The growth of salmonellae in tomatoes. Intl. J. Food Microbiol. 13:177-182.

Beuchat, L.R. 1999. Surface decontamination of fruits and vegetables eaten raw: a review. World Health Organization. WHO/FSF/FOS/98.2.

Beuchat, L.R., B.V. Nail, B.B. Adler, and M.R.S. Clavero. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. J. Food Prot. 61:1305-1311.

Blostein, J. 1993. An outbreak of *Salmonella javiana* associated with consumption of watermelon. J. Environ. Health 56:29-31.

Brecht, J.K. 1995. Physiology of lightly processed fruits and vegetables. Hort Sci. 30:18-22.

Bruton, B.D. W.S. Conway, K.C. Gross, J.X. Zhang, C.L. Biles, and C.E. Sams. 1998. Polygalacturonases of a latent and wound postharvest fungal pathogen of muskmelon fruit. Postharv. Biol. Technol. 13:205-214.

Centers for Disease Control and Prevention (CDC). 1975. *Salmonella typhimurium* outbreak traced to a commercial apple cider – New Jersey. Morb. Mortal. Wkly. Rep. 24:87-88.

Centers for Disease Control and Prevention (CDC). 1991. Multistate outbreak of *Salmonella poona* infections – United States and Canada, 1991. Morb. Mortal. Wkly. Rep. 40:549-552.

Centers for Disease Control and Prevention (CDC). 1999. Outbreaks of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice – United States and Canada. Morb. Mortal. Wkly. Rep. 48:582-585.

Centers for Disease Control and Prevention (CDC). 2000. Surveillance of foodborne disease outbreaks – United States 1993 – 1997. Morb. Mortal. Wkly. Rep. Surveillance Summaries 49 (SS01):1-51.

Centers for Disease Control and Prevention (CDC). 2002. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupes from Mexico – United States and Canada, 2000 – 2002. Morbid. Mortal. Wkly. Rep. 51:1044-1047.

Cook, K.A., T.E. Dobbs, W.G. Hlady, J.G. Wells, T.J. Barrett, N.D. Puhr, G.A. Lancette, D.W. Bodager, B.L. Toth, C.A. Genese, A.K. Highsmith, K.E. Pilot, L. Finelli, and D.L. Swerdlow. 1998. Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. J. Am. Med Assoc. 280:1504-1509.

D'Aoust, J-Y. 1997. *Salmonella* species, p. 129-158. *In* M.P. Doyle, L.R. Beuchat, and T.J. Montville (ed.), Food microbiology: Fundamentals and frontiers. ASM Press. Washington D.C.

D'Aoust, J-Y. 2000. *Salmonella,* p. 1233-1299. *In* B.M. Lund and G.W. Gould (ed.), The microbiological safety and quality of food. Aspen Publishers. Gaithersburg, MD.

D'Aoust, J-Y. 2001. Foodborne salmonellosis: current international concerns. Food Safety Magazine 7(2):10-17, 51.

Deeks, S., A. Ellis, B. Ciebin, R. Khakhria, M, Naus, and J. Hockin. 1998. *Salmonella* Oranienberg, Ontario. Can. Comm. Dis. Rep. 24:177-179.

Doyle, M.P. and D.O. Cliver. 1990. *Salmonella*, p.185-204. *In* D.O. Cliver (ed.), Foodborne diseases. Academic Press. Inc.

Eckert, J.W. 1980. Postharvest diseases of fresh fruits and vegetables – Etiology and control, p. 81-117. *In* N.F. Haard and D.K. Salunkhe (Ed.) Postharvest biology and handling of fruits and vegetables. The AVI Publishing Company, Inc., Westport, Connecticut.

Food and Drug Administration. 2001a. FDA Survey of imported fresh produce. FY 1999 Field assignment. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. Office of Plant and Dairy Foods and Beverages. January 30, 2001, [Internet, WWW], ADDRESS: http://www.cfsan.gov/~dms/prodsur6.html Accessed 25 January 2002.

Food and Drug Administration. 2001b. FDA Survey of domestic fresh produce: interim results. US Food and Drug Administration. Center for Food Safety and Applied Nutrition. June 30, 2001, [Internet, WWW], ADDRESS: http://vm.cfsan.fda.gov/~dms/prodsur8.html Accessed 25 January 2002.

Gagliardi, J.V., P.D. Miller, G. Lester, and D. Ingram. 2003. On-farm and postharvest processing sources of bacterial contamination to melon rinds. J. Food Prot. 66:82-87.

Gayler, G.E., R.A. MacCready, J.P. Reardon, and B.F. Mc Kernan. 1955. An outbreak of salmonellosis traced to watermelon. Public Health Rep. 70:311-313.

Goepfert, J.M. 1980. Vegetables, fruit, nuts and their products. p. 606-642. *In* International Commission on Microbiological Specifications for Foods. Microbial ecology of foods Volume 2. Food commodities.

Golden, D.A., E.J. Rhodehamel, and D.A. Kautter. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. J. Food Prot. 56:194-196.

Guo, X., J. Chen, R.E. Brackett, and L.R. Beuchat. 2002. Survival of *Salmonella* on tomatoes stored at high relative humidity, in soil, and on tomatoes in contact with soil. J. Food Prot. 65:274-279.

Institute of Food Technologists/Food and Drug Administration of the United States Department of Health and Human Services (IFT/FDA). 2001. Analysis and evaluation of preventative control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. IFT/FDA Contract No. 223-98-2333. Task Order No. 3. Institute of Food Technologists. Chicago, IL.

Jay, J. 2000. Modern food microbiology. 6th ed. Aspen Publishers. Gaithersburg, MD.

Kleman, L., L. Jaykus, D. Moll, C. Moe, C. Martinez, and J. Anciso. 2003. A field study of the microbiological quality of fresh produce. Abstr. T33, p. 170. 90th Annual Meeting Intl. Assc. Food Prot. New Orleans, LA.

Laminkanra, O., B. Juavaez, M.A. Watson, and O.A. Richard. 2003. Effect of cutting and storage on sensory traits of cantaloupe melon cultivars with extended postharvest shelf life. J. Sci. Food Agric. 83:702-708.

Larson, A., J. Wallis, C. Lewandowski, D. Jenson, S. Potsic, M.K. Nickels, M. Lesko, C. Langkop, R.J. Martin, T. Endo, H.B. Ehrhard, and B.J. Francis. 1979. *Salmonella* Oranienberg gastroenteritis associated with consumption of precut watermelons- Illinois. Morb. Mortal. Wkly. Rep. 28:522-523.

Liao, C-H. and G.M. Sapers. 2000. Attachment and growth of *Salmonella* Chester on apple fruit and *in vivo* response of attached bacteria to sanitizer treatments. J. Food Prot. 63: 876-883.

Liao, C-H. and P.H. Cooke. 2001. Response to trisodium phosphate treatment of *Salmonella* Chester to fresh-cut green pepper slices. Can. J. Microbiol. 47:25-32.

Madden, J.M. 1992. Microbial pathogens in fresh produce – the regulatory perspective. J. Food Prot. 55:821-823.

Mahon, B.E., A. Ponka, W. Hall, K. Komatsu, S. Dietrich, A. Siitonen, G. Cage, M. Lambert-Fair, P. Hayes, N. Bean, P. Griffin, and L. Slutsker. 1997. An international outbreak of S*almonella* infections caused by alfalfa sprouts grown from contaminated seed. J. Infect. Dis. 175:876-882.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P. M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.

Mohle-Boetani, J.C., R. Reporter, S.B. Werner, S. Abbott, J. Farrar, S.H. Waterman, and D.J. Vugia. 1999. An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. J. Infect. Dis. 180:1361-1364.

Nguyen-the, C. and F. Carlin. 2000. Fresh and processed vegetables, p. 620-684. *In* B.L. Lund, T.C. Baird –Parker, and G.W. Gould (ed.), The microbiological safety and quality of food, vol. 1. Aspen Publishers, Gaithersburg, MD.

O'Mahony, P., J. Cowden, B. Smyth, D. Lynch, M. Hall, B. Rowe, E.L. Teare, R.E. Tettmar, A.M. Rampling, M. Coles, R.J. Gilbert, E. Kingcott, and C.L.R. Bartlet. 1990. An outbreak of *Salmonella* Saint-Paul infection associated with bean sprouts in the UK. Epidemiol. Infect. 104:229-235.

Ries, A.A., S. Zaza, C. Langkop, R.V. Tauxe, and P.A. Blake. 1990. A multistate outbreak of *Salmonella* Chester linked to imported cantaloupe, Abstr. 915. Abstr. 30th, p. 238. Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.

Splittstoesser, D.F. 1987. Fruits and fruit products, p. 101-128. *In* L.R. Beuchat (Ed.). Food and Beverage Mycology. AVI New York, NY.

Suslow, T.V., M. Cantwell, and J. Mitchell. 2000. Cantaloupes: Recommendations for maintaining postharvest quality. University of California, Davis. Postharvest Technology Research and Information Center. 26 June 2000, [Internet, WWW], ADDRESS: http://postharvest.ucdavis.edu/Produce/Produce facts/cantalope.html Accessed 25 March 2002.

Suslow, T. and Cantwell, M. 2001. Recent findings on fresh-cut cantaloupe and honeydew melon. Fresh cut. April 2001. Pp. 12, 20, 32-33.

Tauxe, R.V. 1991. *Salmonella*: A postmodern pathogen. J. Food Prot. 54:563-568.

Ukuku, D.O., V. Pilizota, and G.M. Sapers. 2001. Influence of washing treatment on native microflora and *Escherichia coli* population of inoculated cantaloupes. J. Food Safety 21:31-47.

Ukuku, D.O. and G.M. Sapers. 2001. Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. J. Food Prot. 64:1286-1291.

Van Beneden, C.A., W.E. Keene, D.H. Werker, A.S. King, P.R. Cieslak, K. Hedberg, R.A. Strang, A. Bell, and D. Fleming. 1996. A health food fights back: an international outbreak of *Salmonella* Newport infections due to alfalfa sprouts, K47. Abstr. 36th, p. 259. Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.

Wei, C.I., T.S. Huang, J.M. Kim, W.F. Lin, M.L. Tamlin, and J.A. Bartz. 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. J. Food Prot. 58:829-836.

Weissinger, W.R., W. Chantarapanont, and L.R. Beuchat. 2000. Survival and growth of *Salmonella* Baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. Intl. J. Food Microbiol. 62:123-131.

Wells, J.M. and J.E. Butterfield. 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Dis. 81:867-872.

Wills, R.B.H., D. Graham, B. McGlasson, and D. Joyce. 1998. Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals. CAB International. London, U.K.

Wood, R.C., C. Hedberg, and K.White. 1991. A multistate outbreak of *Salmonella javiana* infections associated with raw tomatoes. (Abstracts) p.69. *In* CDC Epidemic Intelligence Service, 40th Ann. Conf., Atlanta. U.S. Dept. of Health and Human Services, Public Health Science.

Zhuang, R-Y., L.R. Beuchat, and F.J. Angulo. 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl. Env. Microbiol. 61: 2127-2131.

Zhuang, R-Y. and L.R. Beuchat. 1996. Effectiveness of trisodium phosphate for killing *Salmonella* Montevideo on tomatoes. Lett. Appl. Microbiol. 22: 97-100

CHAPTER 5

EXAMINATION OF YEASTS FOR ANTAGONISTIC ACTIVITY AGAINST *SALMONELLA* POONA IN CANTALOUPE SUSPENSION AND IN WOUNDS IN RINDS CO-INFECTED WITH PHYTOPATHOGENIC MOLDS[1]

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ABSTRACT

The application of yeasts as biocontrol agents to prevent fungal decay of fruits and vegetables has been described. However, little is known about the potential antagonistic effects of yeasts against human bacterial pathogens that may contaminate produce. Ten yeasts were screened for antagonistic activity against survival and growth of *Salmonella* Poona at 20°C in cantaloupe juice and decay by two phytopathogens, *Cladosporium cladosporioides* and *Geotrichum candidum*, in wounds on the surface of cantaloupe rind stored at 4 and 20°C. Cantaloupe juice was inoculated using five inoculation schemes: *S*. Poona only, high (3.93 – 5.21 $\log_{10}$ CFU/ml) or low populations (1.79 – 3.26 $\log_{10}$ CFU/ml) of yeasts only, and *S*. Poona combined with high or low populations of yeasts. The populations of *S*. Poona and yeasts in juice, as well as pH and °brix, were determined 6, 24, and 48 h after inoculation. High populations of *Debaryomyces hansenii, Pichia guilliermondii,* and *Pseudozyma* sp. were antagonistic to *S*. Poona in cantaloupe juice stored at 20°C after 48 h ($P \leq 0.05$). With or without yeasts, growth of *S*. Poona caused the pH of the cantaloupe juice to decrease ($P \leq 0.05$). Wounds in cantaloupe rinds were inoculated with yeast and mold or yeast, mold, and *S*. Poona and incubated at 4°C for 14 days or 20°C for 7 days. The pH of the wounded rind tissue inoculated with *C. cladosporioides* and yeasts increased significantly ($P \leq 0.05$) at 20°C. Results indicate that at 4°C, *Candida oleophila, Candida sake, D. hansenii, P. guilliermondii, Pseudozyma* sp., and *Rhodotorula glutinis* co-inoculated with *G. candidum* retarded the increase in pH and prevented changes of the magnitude that occurred in tissue inoculated with *G. candidum* alone. Wounds that were inoculated with *P. guilliermondii* together with *C. cladosporioides* or *G. candidum* did not show visible mold growth. At 20°C, populations of *S*. Poona (6.40, 7.26, and 7.98 $\log_{10}$ CFU/sample) were lower in wounds co-inoculated with *G. candidum* and three of the

test yeasts (*D. hansenii*, *P. guilliermondii*, and *Cr. albidus*, respectively) compared to co-inoculation with *G. candidum* (9.16 $\log_{10}$ CFU/sample) and the other yeasts (> 8.49 $\log_{10}$ CFU/sample). While *C. oleophila* and *R. glutinis* showed most promise for reducing the population of *S.* Poona in the wounds in cantaloupes co-inoculated with *G. candidum* and stored at 4°C, *Cr. albidus*, *D. hansenii*, and *P. guilliermondii* were most effective in controlling growth at 20°C.

INTRODUCTION

Fresh and minimally processed produce are highly perishable. Postharvest deterioration is a problem that persists from the point of harvest through the marketing chain, ultimately resulting in increased cost and decreased availability of produce to consumers and delivery to distant markets. Early termination of storage life of fresh produce is attributed to fungal infection, senescence, and transpiration (Arul, 1994). Subsequent to harvest, physiological senescence and microbial deterioration are accelerated. These processes are inextricably bound because the ability of plant tissues to resist microbial invasion declines through senescence. Worldwide postharvest losses of crops have been estimated to be in the range of 5 – 50% of the harvested crop (Eckert and Ogawa, 1985; Salunkhe and Desai, 1984; Wilson and Wisniewski, 1989). Storage diseases, especially those caused by fungal pathogens infecting surface wounds, are responsible for substantial postharvest losses of most fruits and vegetables (Droby *et al*., 1996; Snowdon, 1990). Developments in the produce industry, such as mechanical harvesting and bulk handling, can aggravate postharvest diseases because of increased wounding and the creation of microenvironments that promote disease development (Arul, 1994).

Since the 1960s, synthetic fungicides have been the primary method of controlling postharvest diseases (Eckert and Ogawa, 1985). In recent times, chemical control of plant pests and diseases has come under scrutiny as there are indications that some pesticides and fungicides pose potential oncogenic risks when applied to processed foods (National Research Council, 1987). Furthermore, some postharvest pathogens have developed resistance to fungicides that have been registered for the control of postharvest diseases (El-Ghaouth, 1997; Wilson and Wisniewski, 1989). Increasing awareness of the potential negative side-effects of fungicides on the ecosystem, growing interest in pesticide-free agricultural products, and consumers' demand

for food free of microbial toxins, pathogens, and chemical residues have resulted in an escalation in research aimed at identifying alternative methods to improve the quality and shelf-life of fresh produce.

Biological control refers to the process of curbing incidence and preventing spread of plant diseases by applying a natural biological process and/or the product of a natural biological process, either preharvest or postharvest (Wilson and Wisniewski, 1989; Wilson, 1997). Among the possible approaches are the use of antagonistic microorganisms, inherent or induced resistance, and natural plant- or animal-derived products (Wilson and Wisniewski, 1994). Ideal antagonists need to meet several criteria to be successfully developed for commercial use on harvested crops. These characteristics include: genetic stability, efficacy at low concentration, not fastidious in nutrient requirements, the ability to survive adverse environmental conditions such as low temperature and controlled atmosphere storage, effectiveness against a wide range of pathogens on a variety of fruits and vegetables, amenable to production on an inexpensive medium, easy to dispense, the microbe does not produce metabolites that are deleterious to human health, resistance to pesticides, compatibility with commercial processing procedures, and not pathogenic to plant tissues (Droby *et al*., 1996).

Biological control does not equal or surpass chemical control in the field, with respect to efficiency or consistency (Wilson and Wisniewski, 1994). Inconsistent results of biological control methods applied in the field have been attributed to the inherent variability in living systems and vagaries of the environment. Prospects for biological control are better for postharvest diseases than field infections because the postharvest environment is easier to control, more conducive to the application of biological control agents, and more similar to conditions in the laboratory than in the field (Wilson and Wisniewski, 1994). Effective

biological control of fungal pathogens by introduction of an antagonist has been reported on apple, apricot, cherry, citrus, grape, nectarine, peach, pear, pepper, persimmon, plum, potato, strawberry, and tomato (Janisiewicz and Korsten, 2002; Wilson and Wisniewski, 1989). A yeast, *Candida oleophila* Montrocher, and a bacterium, *Pseudomonas syringae,* were registered by the United States Environmental Protection Agency in 1995 for control of postharvest diseases of pome and citrus fruits, and have been made commercially available under the trade names Aspire™ and Biosave 110™, respectively. Yeasts are the main group of microorganisms being investigated for use as artificially introduced antagonists because they can colonize the surface of fruits and vegetables for long periods under reduced-moisture conditions, produce extracellular polysaccharides that enhance their survival and restrict colonization sites and flow of germination cues to fungal propagules, use available nutrients to rapidly proliferate, and are minimally affected by pesticides (Droby *et al*., 1996). There are indications that the mechanisms by which antagonistic yeasts exert their protectant effect involve competition for nutrients, site exclusion, direct parasitism, and perhaps induced resistance (Droby *et al*., 1989; Droby and Chalutz, 1994; El-Ghaouth, 1997; El-Ghaouth *et al*., 1998; Janisiewicz *et al*., 2000; Walker *et al*., 2001; Wisniewski *et al*., 1991).

Although outbreaks of produce-associated foodborne illnesses continue to be fewer in number compared to outbreaks linked to consumption of meat and meat products, microbial contamination of fresh produce has been of increasing concern (Buck *et al*., 2003). The percentage of outbreaks of foodborne illnesses that have been associated with consumption of fresh and minimally processed fruits and vegetables or their products has been increasing. The number of documented produce-related outbreaks doubled between 1973-1987 and 1988-1992. Outbreaks with identified etiology were caused predominantly by bacteria, primarily *Salmonella*

(Buck *et al*., 2003). Results of experiments evaluating antimicrobial-producing lactic acid bacteria such as *Lactobacillus acidovorus*, *L. casei*, *L. plantarum*, *Lactococcus lactis,* and *Pediococcus* spp. to control *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus* on meat, cheese, and vegetables are promising (Lewus *et al*., 1991; Muriana, 1996; Stevens *et al*., 1991). Applications have positive and negative implications, as compounds produced by the antagonists may initially kill foodborne pathogens, but pathogens may eventually develop resistance to these compounds. The possibility of developing resistance to a biocontrol agent whose mechanism of resistance is based on competitive exclusion is less likely to occur (Janisiewicz *et al*., 1999a).

Metabiotic associations between phytopathogenic molds and foodborne bacteria on apples and tomatoes have been described (Conway *et al*., 2000; Draughon *et al*., 1988; Huhtanen *et al*., 1976; Mundt, 1978; Odlaug and Pflug, 1979; Wade and Beuchat, 2003). Some molds produce proteolytic enzymes that release alkaline substances into the surrounding tissues and cause the pH to increase. This may create a more favorable environment for enteric pathogens such as *Salmonella* to survive and grow. In our previous studies, *Salmonella* Poona was shown to persist on intact rind at refrigerated temperature and grow on intact, wounded, and decaying cantaloupe rind at refrigerated and ambient temperatures. Injury and abusive storage temperature enhance survival and growth of phytopathogenic molds and *S*. Poona on cantaloupe rinds. Disruption of tissue and cell integrity in fresh cut and wounded fruits results in an increased availability of nutrients and water to support contaminating microorganisms. Modification of the pH and enhanced nutrient and moisture availability are factors that support *S*. Poona on wounded cantaloupe rind tissues, especially at ambient temperature.

While the success of antagonistic yeasts in controlling fungal spoilage and decay of produce has been demonstrated, much less is known about the antagonistic effect of yeasts against bacteria. Bilinski *et al.* (1985) surveyed nearly 400 yeast strains belonging to 31 genera for antibacterial activity. *Kluyveromyces thermotolerans* and *Hanseniospora uvarum* inhibited the growth of *Lactobacillus plantarum* and *Bacillus cereus*, but not gram-negative bacteria. Yeasts isolated from the phylloplane produced antibacterial compounds that inhibited the growth of *Pseudomonas fluorescens* and *S. aureus* (McCormack *et al*., 1994). In light of concerted efforts to develop biological control protocols to reduce the effects of postharvest fungal and bacterial pathogens, studies to determine the response of human pathogens to potential biocontrol microorganisms have been done. *Saccharomyces boulardii* was shown to be capable of inhibiting multiplication of *Salmonella* and *Shigella* (Rodrigues *et al*., 1996). Decline in the population of enteropathogenic *Escherichia coli* O157:H7 in apple cider was attributed to interaction of the pathogen with naturally occurring yeasts (Janisiewicz *et al*., 1999a). *P. syringae* L-59-66, the antagonist used to control postharvest decay of pome fruits, was reported as preventing growth of *E. coli* O157:H7 on wounded apple tissue (Janisiewicz *et al*., 1999b). The objective of this study was to examine yeasts for their potential to impair survival and growth of *S*. Poona in cell suspensions and in wounds on cantaloupe rind co-inoculated with phytopathogenic molds. Changes in the pH of cell suspensions and rind tissue and surface diameter of inoculated wounds as affected by co-inoculation with yeasts, molds, and *S*. Poona were determined.

MATERIALS AND METHODS

***S*. Poona inoculum.** Five strains of *S.* Poona isolated from patients in cantaloupe-associated outbreaks of salmonellosis were used. Strains 00A3207, 01A3923, and 02A3275

were obtained from the State of California, Department of Health Services, and strains 00A3279 and 01A242 were obtained from Dr. Trevor Suslow, University of California, Davis. Each strain was progressively made resistant to nalidixic acid by a series of nine transfers in tryptic soy broth (TSB; BBL/Difco) supplemented with increasing concentrations of nalidixic acid up to 50 μg/ml (TSBN). Cultures were incubated at 37°C for 24 h before transferring to TSB containing a higher concentration of nalidixic acid. The nalidixic acid-resistant cells were surface plated on tryptic soy agar (TSA; BBL/Difco) supplemented with 50 μg of nalidixic acid/ml (TSAN) and incubated at 37°C for 24 h. Stock cultures were stored on TSAN at 4°C.

Preparation of *S*. Poona inoculum for *in vitro* study. Stock cultures of *S*. Poona were streaked on TSAN and incubated at 37°C for 24 h before transferring cells from a single colony of each strain to 10 ml of TSBN and incubating at 37°C for 24 h. Three consecutive 24-h transfers of suspensions were made via loop inocula into TBSN. After incubation at 37°C for 24 h, the cultures were serially diluted in sterile deionized water and finally cantaloupe juice (see preparation below) to give inocula that contained 2.10 $\log_{10}$ CFU/ml. The population of *S*. Poona was determined by surface plating serially diluted suspensions in cantaloupe juice (0.25 ml in quadruplicate and 0.1 ml in duplicate) on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted.

Preparation of *S*. Poona inoculum for wound inoculation. *S*. Poona cells were grown as described above. Subsequent to the three consecutive 24-h transfers of cultures and incubation in TSBN at 37°C for 24 h, cells were harvested by centrifugation at 2,500 x *g* for 10 min in a Centra CL2 centrifuge (International Equipment Company, Needham Heights, MA). Cells were washed two times in 10 ml of sterile deionized water, then suspended in 10 ml of sterile 0.1% (w/v) peptone water. The population (CFU/ml) in each suspension was determined

by serially diluting suspensions in sterile 0.1% peptone water and surface plating samples (0.1 ml in duplicate) on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted. Random presumptive-positive colonies of *Salmonella* were confirmed using triple sugar iron, lysine iron agar, and a *Salmonella* latex agglutination assay (Oxoid, Basingstoke, Hampshire, U.K.).

Equal volumes (10 ml) of suspensions of each strain were combined to give 50 ml of a five-strain mixture of *S*. Poona containing approximately equal populations (ca. 9 $\log_{10}$ CFU/ml) of each strain. The suspension was serially diluted to give an inoculum that contained ca. 6.5 log10 CFU/ml.

Preparation of cantaloupe juice for *in vitro* study. Eastern type cantaloupes (*Cucumis melo* L. var. *reticulatus* Naud.) were purchased in June – July, 2003 from a grocery in Griffin, GA and stored at 4°C for 2 days before adjusting them to 22 ± 1°C over a 16 to 20-h period. The rind was removed from four unwashed cantaloupes using a stainless steel knife. The seeds were pared from the mesocarp and discarded. The mesocarp was diced and juice was extracted in a commercial juice extractor (The Juiceman Jr., Trillium Health Products, J.M. Marketing Inc. Seattle WA). The extracted juice, pulp, and fiber were filtered through a 5½-inch-diameter strainer with 1-mm diameter holes (Inter-American Products, Cincinnati, OH). The filtrate was filtered two additional times through four-ply graphics arts cheesecloth (Vertec, Graphic Arts Products, Walpole, MA) to remove fibrous tissue and pulp. To allow sedimentation and removal of large particles, 100-ml portions of the juice were centrifuged at 15,300 x *g* for 15 min at 4°C in a Model J2-MI centrifuge (Beckman, Palo Alto, CA). The liquid portions were collected and centrifuged twice. Using a vacuum, the juice was filtered through four sheets of 5.5-cm diameter Whatman #2 Qualitative filter paper (Whatman Limited, England). To sterilize the cantaloupe

juice, the filtrate was successively vacuum filtered using microfilters with pore diameters of 0.65 µm (Millipore Filter Corporation, Bedford, MA), 0.45 µm (Millipore, Ireland) and 0.20 µm (Nalgene Filtration Products, Nalge Nunc International, Rochester, NY). The pH and °brix of the sterile cantaloupe juice were determined using a Basic pH meter (Denver Instrument Company, Arvada, CO) and an ABBE-3L refractometer (Spectronic Instruments Inc., Rochester, NY), respectively. Nine-milliliter aliquots of the cantaloupe juice were dispensed into sterile test tubes (16 x 125 mm) and stored at 4°C for approximately 16 h. Immediately before inoculation, the temperature of the sterile cantaloupe juice was adjusted to 22 ± 1°C.

Preparation of cantaloupes for wound inoculation. Western (shipper type) cantaloupes were purchased in March – April, 2003 from a fresh produce broker at the Georgia State Farmers' Market, Forest Park, GA. The cantaloupes were stored at 4°C for 2 days, then adjusted to 22 ± 1°C over a 16-to 20-h period before inoculating with test organisms. Forty-eight unwashed cantaloupes, free of visible wounds, cuts, and bruises, and supported by a 9-cm diameter plastic petri plate, were placed in 27-liter polypropylene containers (59 cm long x 43 cm wide x 15 cm high; Sterilite, Townsend, MA) in which twenty-eight holes (ca. 6 mm diameter) were made to facilitate ventilation after a cover was applied. A permanent ink marker was used to make six 3-cm diameter circles on the rind of each cantaloupe, designating sites for inoculation. Circles did not encompass the stem end or blossom end. The end (8 mm wide) of a sterile stainless steel spatula was used to create a wound 4 mm deep in the center of each circle. This procedure resulted in six wounded sites for inoculation on each cantaloupe. Two cantaloupes were used for each treatment and each cantaloupe represented a replicate. The rinds of four other cantaloupes were not wounded. These cantaloupes were designated as intact, un-

inoculated controls. The pH of the cut surface of the wounded rind tissue and intact rind surface was determined using a Basic pH meter (Denver Instrument Company, Arvada, CO).

Culture conditions for yeasts and preparation of inocula. Yeasts isolated from various plants were obtained from Dr. James Buck, Department of Plant Pathology, University of Georgia, Griffin, GA (Table 5.1). Some strains of these yeasts have been shown to inhibit the growth of phytopathogens (Chand-Goyal and Spotts, 1997; Lima *et al*., 1998; Mercier and Wilson, 1994; Viñas *et al*., 1998; Wilson *et al*., 1996; Wisniewski *et al*., 1991). The yeasts were streaked on potato dextrose agar (PDA) (BBL/Difco, Sparks, MD) and incubated at 25°C for 48 h. Cells of a single colony of each yeast were transferred to a 250-ml Erlenmeyer flask containing 50 ml of tryptone glucose yeast extract broth (TGY, pH 6.0) and incubated on a rotary shaker at 25 $\pm$ 1°C for 48 h. TGY broth contains (per liter of deionized water): 100 g of glucose, 5.0 g of tryptone, and 5.0 g of yeast extract.

To prepare yeast inocula for the *in vitro* cantaloupe juice study, cultures in TGY broth were serially diluted in sterile deionized water and finally cantaloupe juice to give inocula that contained 3.97 – 5.86 $\log_{10}$ CFU/ml for high population inocula and 1.87 – 3.26 $\log_{10}$ CFU/ml for low population inocula. The populations of yeasts were determined by surface plating serially diluted suspensions in cantaloupe juice (0.25 ml in quadruplicate and 0.1 ml in duplicate) on dichloran rose bengal chloramphenicol (DRBC) agar (BBL/Difco). Plates were incubated at 25°C for 5 days before colonies were counted.

For the wound inoculation study, cells in 10 ml of 48-h cultures of yeasts were centrifuged at 7,000 x *g* for 10 min at 4°C in a Model J1-MI centrifuge (Beckman, Palo Alto, CA) and washed twice in sterile deionized water. Cells in pellets were resuspended in 10 ml sterile deionized water and the population (CFU/ml) in each suspension was determined by

serially diluting in sterile deionized water and surface plating samples (0.1 ml in duplicate) on PDA. Plates were incubated at 25°C for 48 h before colonies were counted. Cell counts ranged from ca. 7 to 9 $\log_{10}$ CFU/ml of suspension.

Table 5.1. Yeast isolates screened for antagonistic activity against molds and *Salmonella* Poona on cantaloupes

Yeast	Source
Ascomycetes	
Candida oleophila	C.L. Wilson[1]
Candida sake	C.L. Wilson
Debaryomyces hansenii	C.L. Wilson
Pichia guilliermondii	C.L. Wilson
Basidiomycetes	
Aureobasidium pullulans	Geranium leaf
Cryptococcus albidus	NRRL Y - 1400
Cryptococcus laurentii	NRRL Y - 2536
Pseudozyma sp.	Creeping bentgrass
Rhodotorula glutinis	Geranium leaf
Rhodotorula mucilaginosa	NRRL Y - 2510

[1] Appalachian Fruit Research Center, ARS/USDA.

Procedures for growing molds and preparation of inocula. Cultures of *Cladosporium cladosporioides* and *Geotrichum candidum* isolated from cantaloupes were obtained from Dr. Trevor Suslow, University of California, Davis. The isolates were maintained on DRBC agar and PDA. Molds were subcultured by streaking on DRBC agar and incubating at 25°C for 5 days.

Suspensions of conidia or arthroconidia were harvested by pipetting 10 ml of sterile deionized water containing 0.05% Tween 80 onto the surface of the 5-day-old cultures and gently rubbing for 1 min with a sterile glass rod. Using a sterile Pasteur pipette, conidial suspensions were removed from the surface of plates and deposited in a sterile test tube. The number of conidia of *C. cladosporioides* and arthroconidia and vegetative cells of *G. candidum* (CFU/ml) in suspensions was determined by serially diluting samples in sterile deionized water containing 0.05% Tween 80 and surface plating (0.1 ml in duplicate) on DRBC agar. Plates were incubated at 25°C for 5 days before colonies were counted. The number of colony forming units (CFUs) in each suspension was adjusted to ca. 4.64 $\log_{10}$ CFU/ml for *C. cladosporioides* and 5.61 $\log_{10}$ CFU/ml for *G. candidum* by diluting in sterile deionized water containing 0.05% Tween 80.

Effect of yeasts on *S.* Poona in cantaloupe juice (*in vitro* study). Test tubes containing 9 ml of cantaloupe juice were inoculated with 1 ml of a low-population inoculum of *S.* Poona and/or 1 ml of high or low population of yeast inoculum, all made in cantaloupe juice.

Five inoculation schemes were used to inoculate cantaloupe juice:

(i) Low population of *S.* Poona only: Tubes containing 9 ml of cantaloupe juice were inoculated with 1 ml of *S.* Poona suspension containing ca. 2.1 $\log_{10}$ CFU/ml.

(ii) High population of yeast only: Tubes containing 9 ml of cantaloupe juice were inoculated with 1 ml of yeast suspension containing ca. 5.9 $\log_{10}$ CFU/ml.

(iii) Low population of yeast only: Tubes containing 9 ml of cantaloupe juice were inoculated with 1 ml of yeast suspension containing ca. 3.5 $\log_{10}$ CFU/ml.

(iv) High population of yeast and low population of *S*. Poona: Tubes containing 9 ml of cantaloupe juice were inoculated with 1 ml of yeast suspension containing ca. 5.9 $\log_{10}$ CFU/ml and 1 ml of *S*. Poona suspension containing ca. 2.1 $\log_{10}$ CFU/ml.

(v) Low population of yeast and low population of *S*. Poona: Tubes containing 9 ml of cantaloupe juice were inoculated with 1 ml of yeast suspension containing ca. 3.5 $\log_{10}$ CFU/ml and 1 ml of *S*. Poona suspension containing ca. 2.1 $\log_{10}$ CFU/ml.

Immediately after inoculation, the pH and °brix of cantaloupe juice suspensions were determined. A tube containing uninoculated cantaloupe juice was used as the control in all treatments. Inoculated and uninoculated cantaloupe juice was incubated at 20°C.

Measurement of pH and °brix. The pH and °brix of the cantaloupe juice were determined. A sterile 1-ml serological pipette was used to withdraw 0.2 ml of the suspension. Three drops of suspension were dispensed on the sterile surface of a plastic petri dish for pH determination, while two drops were placed on the measurement prism of the ABBE-3L refractometer (Spectronic Instruments, Rochester, NY) for °brix determination.

Effect of yeasts on growth of molds in wounds. Cantaloupes were adjusted to 22 ± 1°C before wounds were inoculated with yeast suspension. Using a Precision Microliter Pipette (Rainin, Woburn, MA), 10 µl of yeast suspension was deposited into each wound in cantaloupe rinds. The cantaloupes were kept at 22 ± 1°C for 2 h, followed by inoculation of wounds with 10 µl of mold suspension. Cantaloupes were kept at 22 ± 1°C for an additional 2 h before incubating at 4°C for 21 days or 20°C for 7 days and analyzing for presence and populations of test yeasts and molds.

The pH of inoculated tissues and using a dial caliper (Monostat, Zurich, Switzerland), width of decayed area developing at wounded inoculation sites were measured initially and 7 and 14 days after inoculation and storage at 20°C and 4°C, respectively.

Test for antagonistic activity of yeasts against *S*. Poona in wounds. Wounds in cantaloupe rinds were inoculated with yeast and mold or only mold as described above; 10 µl of *S*. Poona suspension containing ca. 6.49 $\log_{10}$ CFU/ml was then deposited in each wound. The cantaloupes were kept at 22 ± 1°C for 2 h before incubating at 4°C for 14 days or 20°C for 7 days.

Microbiological analyses of juice. Inoculated and uninoculated cantaloupe juice was analyzed at 6, 24, and 48 h after inoculation for populations of *S*. Poona and test yeast. *S*. Poona populations were determined by surface plating juice (0.25 ml in quadruplicate and 0.1 ml in duplicate of undiluted juice and 0.1 ml in duplicate of suspensions serially diluted in 0.1% peptone water) on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted. Random colonies were selected for confirmation as described above.

Yeast populations in inoculated juice were determined by surface plating undiluted juice (0.25 ml in quadruplicate and 0.1 ml in duplicate) and juice serially diluted in 0.1% peptone water (0.1 ml in duplicate) on DRBC agar. Plates were incubated at 25°C for 5 days before colonies were counted.

Microbiological analyses of wound tissue. The pH of cut tissue in wounded, uninoculated and inoculated sites on cantaloupes stored at 20°C for 7 days or 4°C for 14 days was measured before removing samples for microbiological analysis. To test for the presence of test yeast and mold inoculated into wounds, a sterile inoculation loop wetted in sterile deionized water was used to remove samples from the surface of wound tissue. Samples were streaked on

duplicate DRBC agar plates and incubated at 25°C for 5 days. Isolates were examined to confirm the presence of test yeasts and/or molds.

Samples of wounded rind tissues (ca. 3 cm diameter, 4 mm deep) inoculated with yeast, mold, and *S*. Poona or mold and *S*. Poona were excised using a sterile spatula and placed in separate stomacher 80 bags (Seward Medical Ltd., London, UK). Forty milliliters of sterile lactose broth (LB; BBL/Difco) containing 50 μg of nalidixic acid/ml (LBN) was combined with the tissue. The sample was hand rubbed and macerated, then pummeled for 1 min at normal speed in a Model 80 Stomacher (Seward Medical Ltd.). The liquid portion of the homogenate was serially diluted in sterile 0.1% peptone water and the undiluted homogenate (0.25 ml in quadruplicate and 0.1 ml in duplicate) and diluted homogenate (0.1 ml in duplicate) were surface plated on TSAN. Plates were incubated at 37°C for 24 h before presumptive-positive *S*. Poona colonies were counted. Random colonies were selected for confirmation as described above.

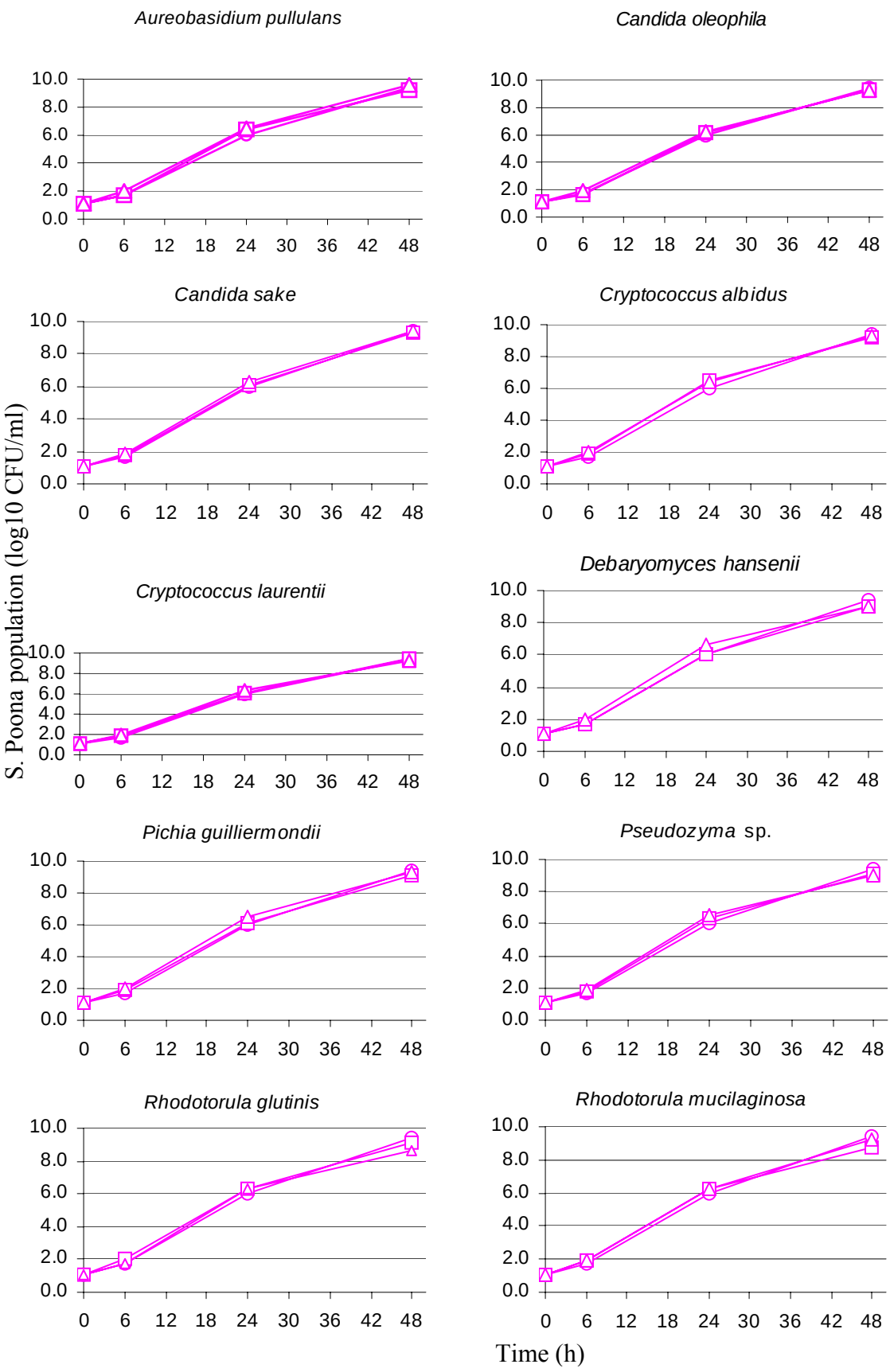
Statistical analysis. Experiments for the *in vitro* study were replicated three times. Mean values were analyzed to determine significant differences in populations of *S.* Poona in cantaloupe juice inoculated with *S*. Poona only and co-inoculated with *S*. Poona and high or low populations of yeasts. General linear models on SAS software (Statistical Analysis Systems Institute, Cary, NC) were used. Significant differences ($P \leq 0.05$) between mean values were determined using Duncan's multiple range tests. Experiments on intact and wounded cantaloupes were done two times.

RESULTS AND DISCUSSION

Growth of *S.* Poona and potentially antagonistic yeasts in cantaloupe juice. The initial population of *S*. Poona inoculated into sterile cantaloupe juice was 1.10 $\log_{10}$ CFU/ml (Fig. 5.1). With the exception of three test yeasts (*D. hansenii*, *P. guilliermondii*, and *Pseudozyma* sp.)

Figure 5.1. Populations of *S*. Poona in cantaloupe juice. Nine milliliters of cantaloupe juice were inoculated with 1 ml *S*. Poona suspension (1.10 $\log_{10}$ CFU/ml) or co-inoculated with 1 ml of *S*. Poona suspension and 1 ml of a suspension containing a high population (3.97 – 5.86 $\log_{10}$CFU/ml) or a low population (1.87 – 3.26 $\log_{10}$ CFU/ml) of yeast. The inoculated cantaloupe juice was incubated at 20°C for up to 48 h before populations of *S*. Poona were determined.

Key: *S*. Poona only (○); *S*. Poona co-inoculated with high yeast population (□); *S*. Poona co-inoculated with low yeast population (Δ).


Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotorula mucilaginosa
S. Poona population (log10 CFU/ml)
Time (h)
0.0
2.0
4.0
6.0
8.0
10.0
0
6
12
18
24
30
36
42
48

the presence and population of potentially antagonistic yeasts did not affect survival and growth of *S.* Poona. Cantaloupe juice inoculated with only *S*. Poona contained populations of 1.70, 6.00, and 9.41 $\log_{10}$ CFU/ml after incubation for 6, 24, and 48 h, respectively, at 20°C. Cantaloupe juice inoculated with *S*. Poona and a high population of yeast (3.97 – 5.86 $\log_{10}$CFU/ml) contained *S*. Poona populations of ca. 1.83, 6.22, and 9.17 $\log_{10}$ CFU/ml 6, 24, and 48 h, respectively, after inoculation. *S*. Poona co-inoculated with a low population of yeast (1.87 – 3.26 $\log_{10}$ CFU/ml), were ca. 1.94, 6.40, and 9.21 $\log_{10}$ CFU/ml after 6, 24, and 48 h, respectively.

Comparing *S*. Poona population in cantaloupe juice inoculated with *S*. Poona only with that in juice co-inoculated with low populations of yeasts, there were no significant differences in *S*. Poona populations ($P > 0.05$) at any sampling time. However, when co-inoculated with high populations of *D. hansenii, P. guilliermondii,* or *Pseudozyma* sp., the *S*. Poona population was significantly less ($P \leq 0.05$) at 48 h than in juice inoculated with *S*. Poona only. These results indicate that while low populations of yeast did not affect the growth of *S*. Poona (Table 5.2), high populations of *D. hansenii, P. guilliermondii,* and *Pseudozyma* sp. are potentially antagonistic to *S*. Poona in cantaloupe juice stored at 20°C statistically, but not practically feasible (Table 5.3).

Yeasts inoculated into cantaloupe juice at high or low populations, or co-inoculated with *S*. Poona were enumerated 6, 24, and 48 h after inoculation (Fig. 5.2). Yeast populations changed by (-) 2.72 and 2.48 $\log_{10}$ CFU/ml and (-) 1.28 and 4.37 $\log_{10}$ CFU/ml, respectively, in juice inoculated with high and low populations. Comparing the growth in juice inoculated with the two populations, it is apparent that the low populations grew at a more rapid rate. This observation is attributable to a comparatively greater availability, on a per cell basis,

Table 5.2. Populations of *Salmonella* Poona in cantaloupe juice co-inoculated with low populations of yeast

Microorganism	Population (log_{10} CFU/ml)[a]			
	Time (h) 0	6	24	48
S. Poona only (control)	1.10	a 1.70	a 6.00	a 9.41
S. Poona and yeast				
A. pullulans	1.10	a 1.99	a 6.47	a 9.59
C. oleophila	1.10	a 1.93	a 6.24	a 9.27
C. sake	1.10	a 1.89	a 6.27	a 9.43
Cr. albidus	1.10	a 2.05	a 6.42	a 9.32
Cr. laurentii	1.10	a 1.95	a 6.35	a 9.25
D. hansenii	1.10	a 1.97	a 6.63	a 9.02
P. guilliermondii	1.10	a 2.02	a 6.54	a 9.35
Pseudozyma sp.	1.10	a 1.92	a 6.58	a 9.01
R. glutinis	1.10	a 1.73	a 6.28	a 8.67
R. mucilaginosa	1.10	a 1.92	a 6.25	a 9.20

[a] Mean values within the same column that are not preceded by the same letter are significantly different ($P \leq 0.05$).

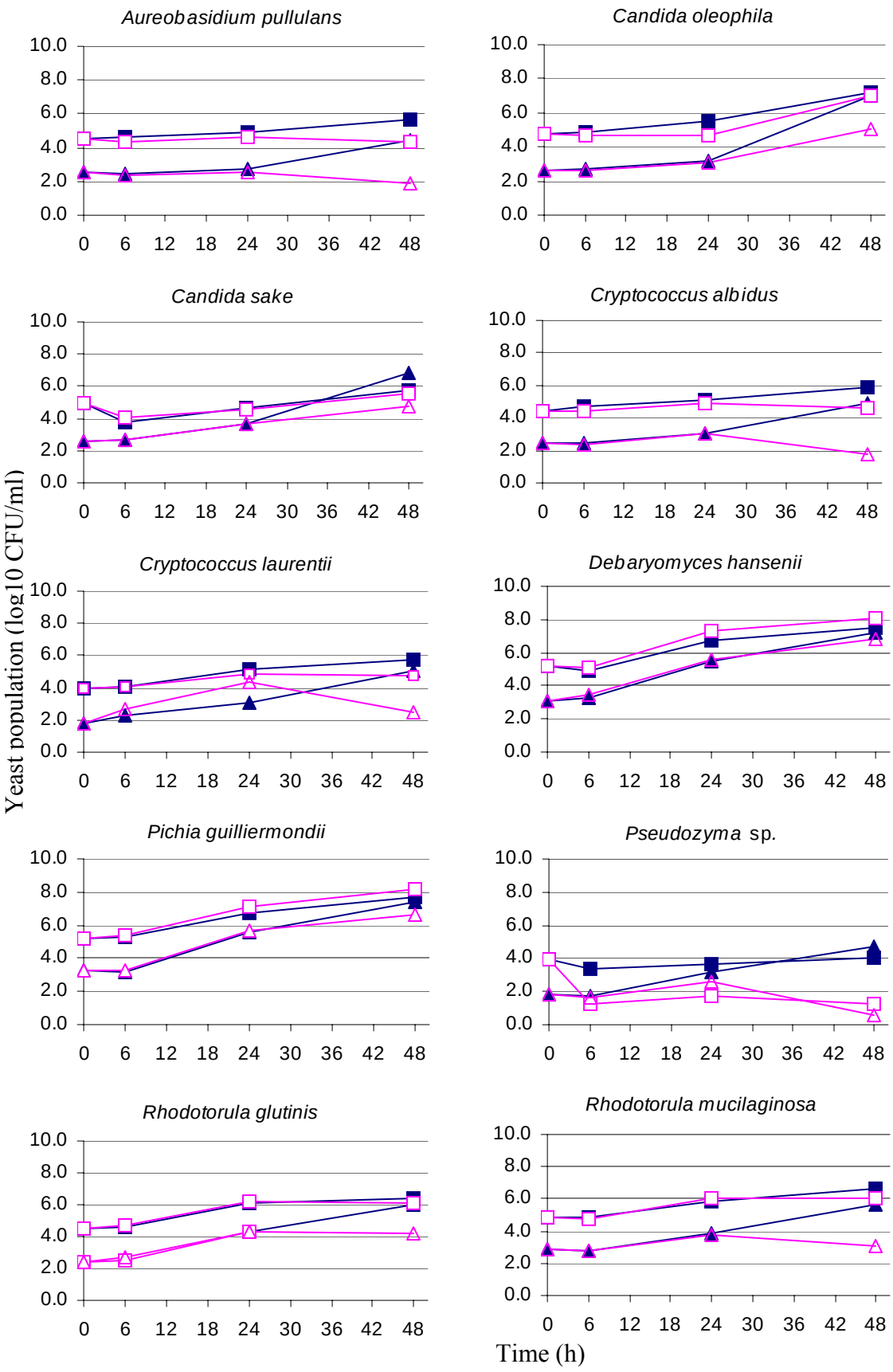
Table 5.3. Populations of *Salmonella* Poona in cantaloupe juice co-inoculated with high populations of yeast

Microorganism	Population ($\log_{10}$ CFU/ml)[a]			
	Time (h) 0	6	24	48
S. Poona only (control)	1.10	a 1.70	a 6.00	a 9.41
S. Poona and yeast				
A. pullulans	1.10	a 1.69	a 6.41	a 9.21
C. oleophila	1.10	a 1.62	a 6.17	a 9.29
C. sake	1.10	a 1.79	a 6.12	a 9.30
Cr. albidus	1.10	a 1.92	a 6.46	a 9.25
Cr. laurentii	1.10	a 1.88	a 6.06	a 9.46
D. hansenii	1.10	a 1.73	a 6.02	b 9.02
P. guilliermondii	1.10	a 1.91	a 6.09	b 9.15
Pseudozyma sp.	1.10	a 1.77	a 6.31	b 9.09
R. glutinis	1.10	a 2.06	a 6.30	a 9.12
R. mucilaginosa	1.10	a 1.92	a 6.28	a 8.79

[a] Mean values within the same column that are not preceded by the same letter are significantly different ($P \leq 0.05$).

Figure 5.2. Populations of yeasts in cantaloupe juice. Nine milliliters of cantaloupe juice were inoculated with 1 ml of a suspension containing a high population (3.97 – 5.86 $\log_{10}$ CFU/ml) of yeast, 1 ml of a suspension containing a low population (1.87 – 3.26 $\log_{10}$ CFU/ml) of yeast, or 1 ml of *S*. Poona (1.10 $\log_{10}$ CFU/ml) suspension and 1 ml of a suspension containing a high population of yeast, or 1 ml of *S*. Poona suspension and 1 ml of suspension containing low population of yeast. The inoculated cantaloupe juice was incubated at 20°C for up to 48 h before populations of yeast were determined.

Key: High yeast population only (■); low yeast population only (▲); high yeast population co-inoculated with *S*. Poona (□); low yeast population co-inoculated with *S*. Poona (Δ).


Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotorula mucilaginosa
Yeast population (log10 CFU/ml)
Time (h)
0.0
2.0
4.0
6.0
8.0
10.0
0
6
12
18
24
30
36
42
48

of nutrient sources in juice inoculated with a low population of yeasts. There is greater competition for nutrients in juice inoculated high populations. Additionally, a higher initial yeast population may result in a more rapid production and accumulation of metabolic by products which may restrict the growth of cells.

While the populations of yeasts continued to increase between 24 and 48 h in juice inoculated with high or low populations of yeast only, the populations of *A. pullulans*, *Cry. albidus*, *Cry. laurentii*, *Pseudozyma* sp., *R. glutinis*, and *R. mucilaginosa* began to decrease after 24 h in juice co-inoculated with *S*. Poona (Fig. 5.2). At 6 and 24 h after inoculation, the populations of yeast recovered from the juice were not significantly different from the populations in juice co-inoculated with *S*. Poona ($P > 0.05$). With the exception of juice co-inoculated with low populations of *Cry. laurentii, D. hansenii*, and *P. guilliermondii*, the populations of yeast co-inoculated with *S*. Poona began to decrease at 48 h ($P \leq 0.05$).

With the exception of *D. hansenii, P. guilliermondii,* and *Pseudozyma* sp. at high initial populations, overall, the presence and population of antagonistic yeasts did not appear to influence the survival and growth of *S*. Poona in cantaloupe juice. Within 6, 24, and 48 h of inoculation, the population of *S*. Poona increased by 0.72, 5.11, and 8.16 $\log_{10}$ CFU/ml, respectively, in juice co-inoculated with yeasts. Results from our study indicate that, overall, potentially antagonistic yeasts strains examined in this study do not compete effectively with *S*. Poona in cantaloupe juice and therefore would not be effective in controlling the growth of *S*. Poona on cantaloupes.

Changes in pH and total soluble solids content of cantaloupe juice as affected by inoculation with *S.* Poona and yeasts. At the time of inoculation, the pH of cantaloupe juice

was 6.29 – 7.12, depending on the lot selected for examination (Fig. 5.3). The pH of uninoculated juice increased from 6.78 to 7.04 over the 48-h storage period at 20°C. When cantaloupe juice was inoculated with only *S*. Poona, there was a slight increase in pH during the first 24 h, but a very rapid decrease to 5.44 between 24 and 48 h. Although the pH of cantaloupe juice inoculated with *C. oleophila, C. sake, D. hansenii*, and *P. guilliermondii* trended downward after 24 h, all the yeasts considered, the pH of the juice 48 h after inoculation with high or low populations of yeasts was 6.28 – 7.45 and 6.43 – 7.51, respectively, indicating that the level of inoculum did not have marked effect on the juice pH. However, when the cantaloupe juice was co-inoculated with high or low populations of yeasts along with *S.* Poona, the pH decreased to 5.13 – 5.92 and 5.10 – 5.89, respectively, over the 48 h storage period. Growth of *S*. Poona caused the pH of the environment to decrease.

Inoculation of cantaloupe juice with *S*. Poona, with or without yeasts, resulted in a sharp decrease in juice pH. This may be attributable to metabolism of amino acids, resulting in excretion of weak acids to maintain internal pH and consequently reduce the pH of the external environment. Eighteen amino acids have been identified in cantaloupe fruits and the total amino acid content decreased by 40% after storage at 20°C (Lamikanra *et al*., 2000). Some of these amino acids were used to support bacterial growth, formation of other compounds, and catabolic degradation. While utilization of acids by the yeasts would have caused the pH of the juice to increase, excretion of acids by *S.* Poona would maintain the initial pH of the juice.

The initial total soluble solids content (TSSC, measured as °brix) in uninoculated cantaloupe juice (4.6 – 8.7, mean = 7.6) decreased slightly to 7.4 (Fig. 5.4). When the juice was inoculated with *S*. Poona, the TSSC decreased to ca. 7.1 over the 48-h storage period at 20°C.

Figure 5.3. Effect of *S*. Poona and yeasts on pH of cantaloupe juice. Nine milliliters of cantaloupe juice were inoculated with 1 ml of *S*. Poona suspension (1.10 $\log_{10}$ CFU/ml), 1 ml of a suspension containing a high population (4.00 – 5.86 $\log_{10}$ CFU/ml) or a low population (1.87 – 3.26 $\log_{10}$ CFU/ml) of yeast, or 1 ml of *S*. Poona suspension and 1 ml of yeast suspension containing a high population a low population of yeast. Suspensions were incubated at 20°C for 6, 24, or 48 h before the pH was measured.

Key: pH of uninoculated juice (●); juice inoculated with *S*. Poona only (○); high yeast population only (■); high yeast population and *S*. Poona (□); low yeast population only (▲); low yeast population and *S*. Poona (Δ).

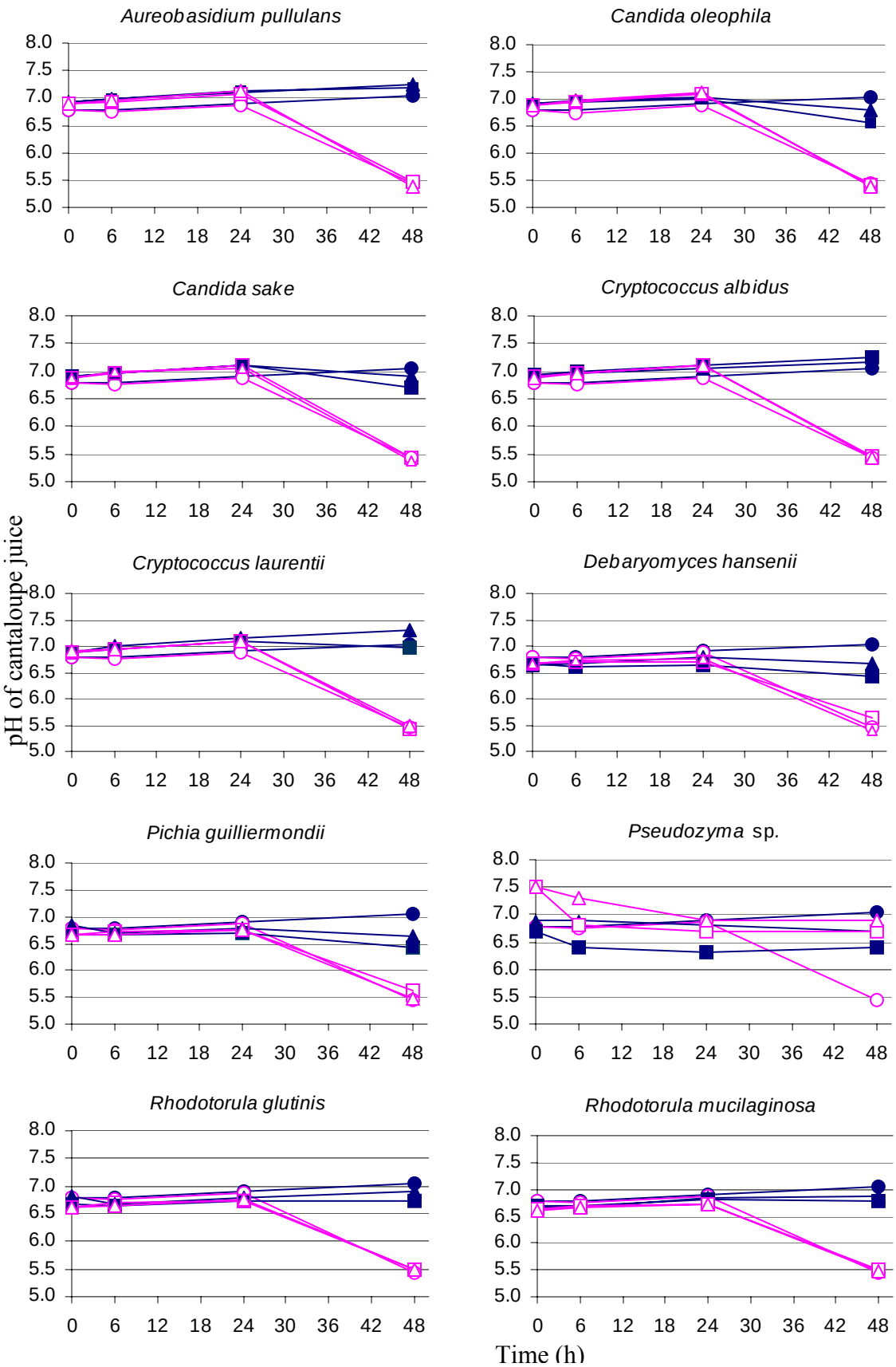
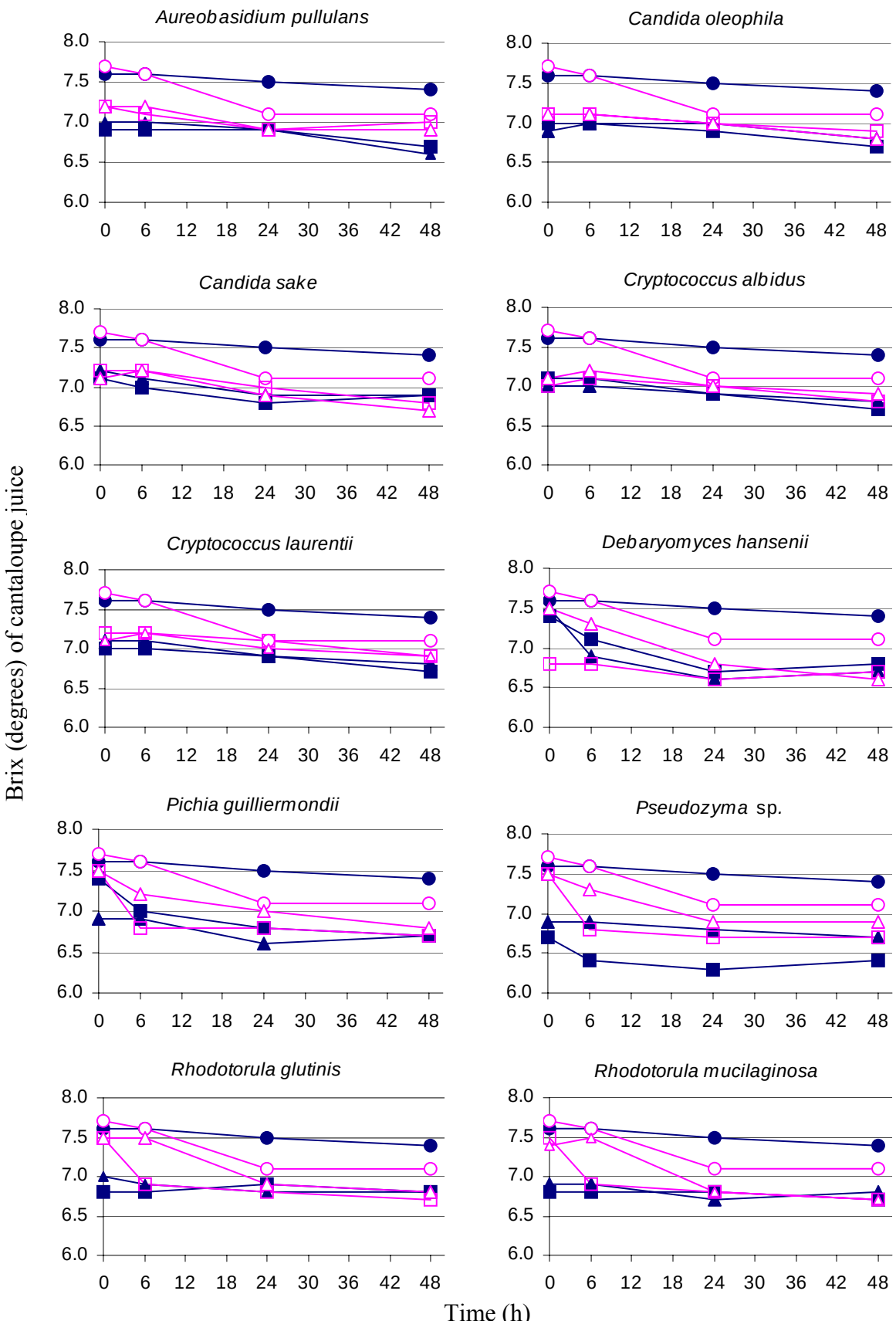

Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotorula mucilaginosa
pH of cantaloupe juice
Time (h)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
0
6
12
18
24
30
36
42
48

Figure 5.4. Effect of *S*. Poona and yeasts on total soluble solids content (TSSC) of cantaloupe juice. Nine milliliters of cantaloupe juice were inoculated with 1 ml of *S*. Poona suspension (1.10 $\log_{10}$ CFU/ml), 1 ml of a suspension containing a high population (1.30 – 5.86 $\log_{10}$ CFU/ml) or a low population (1.87 – 3.26 $\log_{10}$ CFU/ml) of yeast, or 1 ml of *S*. Poona suspension and 1 ml of yeast suspension containing a high population or a low population of yeast. Suspensions were incubated at 20°C for 6, 24, and 48 h before TSSC was measured.

Key: TSSC of uninoculated juice (●); juice inoculated with *S*. Poona only (○); high yeast population only (■); high yeast population and *S*. Poona (□); low yeast population only (▲); or low yeast population and *S*. Poona (Δ).


Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotorula mucilaginosa
Brix (degrees) of cantaloupe juice
Time (h)
8.0
7.5
7.0
6.5
6.0
0
6
12
18
24
30
36
42
48

The TSSC of juice inoculated with only high or low populations of yeasts decreased from 4.6 – 8.7 (mean = 7.0) to 4.6 – 8.2 (mean = 6.7) and from 4.6 – 9.0 (mean = 7.0) to 4.6 – 8.2 (mean = 6.8), respectively. When high or low populations of yeasts were co-inoculated into cantaloupe juice with *S*. Poona, the TSSC of juices decreased from 4.8 – 8.9 (mean = 7.2) to 4.6 – 8.4 (mean = 6.8) and 4.6 – 8.8 (mean = 7.3) to 4.6 – 8.2 (mean = 6.8), respectively. Regardless of type of inoculum, the decline in TSSC during incubation at 20°C for 48 h was not significant ($P > 0.05$). Negligible changes in the TSSC of cantaloupe juice inoculated with yeasts suggest that the yeasts were preferentially metabolizing acids instead of sugars. Changes in pH and TSSC of cantaloupe juice were observed to occur only between 24 and 48 h. The content of malic acid, the major acid in cantaloupes, has been reported to decrease in fruits at held at 20°C (Lamikanra *et al*., 2000).

Change in rind pH. There was inherent variability in pH of cantaloupe tissue. This is attributed to different stages of maturity of cantaloupes. To enable comparisons to be made, change in the pH of rinds instead of absolute pH values was used as the parameter to assess the effects of yeasts, molds, and *S*. Poona on pH of the surface rinds and the cut tissue of wounds in cantaloupe rinds.

There was a greater increase in the mean pH of the surface of uninoculated rinds of cantaloupes stored at 4°C for 21 days compared to storage at 20°C for 7 days (Figs. 5.5 and 5.6), respectively. The pH of intact surface rind of the melons stored at 4°C and 20°C increased by 0.04 – 0.48 units and decreased by 0.46 – 0.29 units, respectively, for cantaloupes used as controls in the studies involving *C. cladosporioides* (Fig. 5.5). In cantaloupes inoculated with *G. candidum,* the pH of intact rind of the melons stored at 4°C

Figure 5.5. Effect of yeasts and *C. cladosporioides* on changes in pH of intact and wounded tissue in cantaloupe rinds. Wounds in cantaloupe rinds were co-inoculated with 10 µl of *C. cladosporioides* suspension (2.64 $\log_{10}$ CFU) and 10 µl of yeast suspension (5.98 – 7.32 $\log_{10}$ CFU). The initial pH of tissue stored at 4°C and 20°C was 5.23 – 6.10 and 5.31 – 6.64, respectively. Change in the pH of the cantaloupe rind tissue [difference between initial pH on day 0 and pH after storage at 4°C for 21 days (shaded bars) and 20°C for 7 days (solid bars)] was monitored.

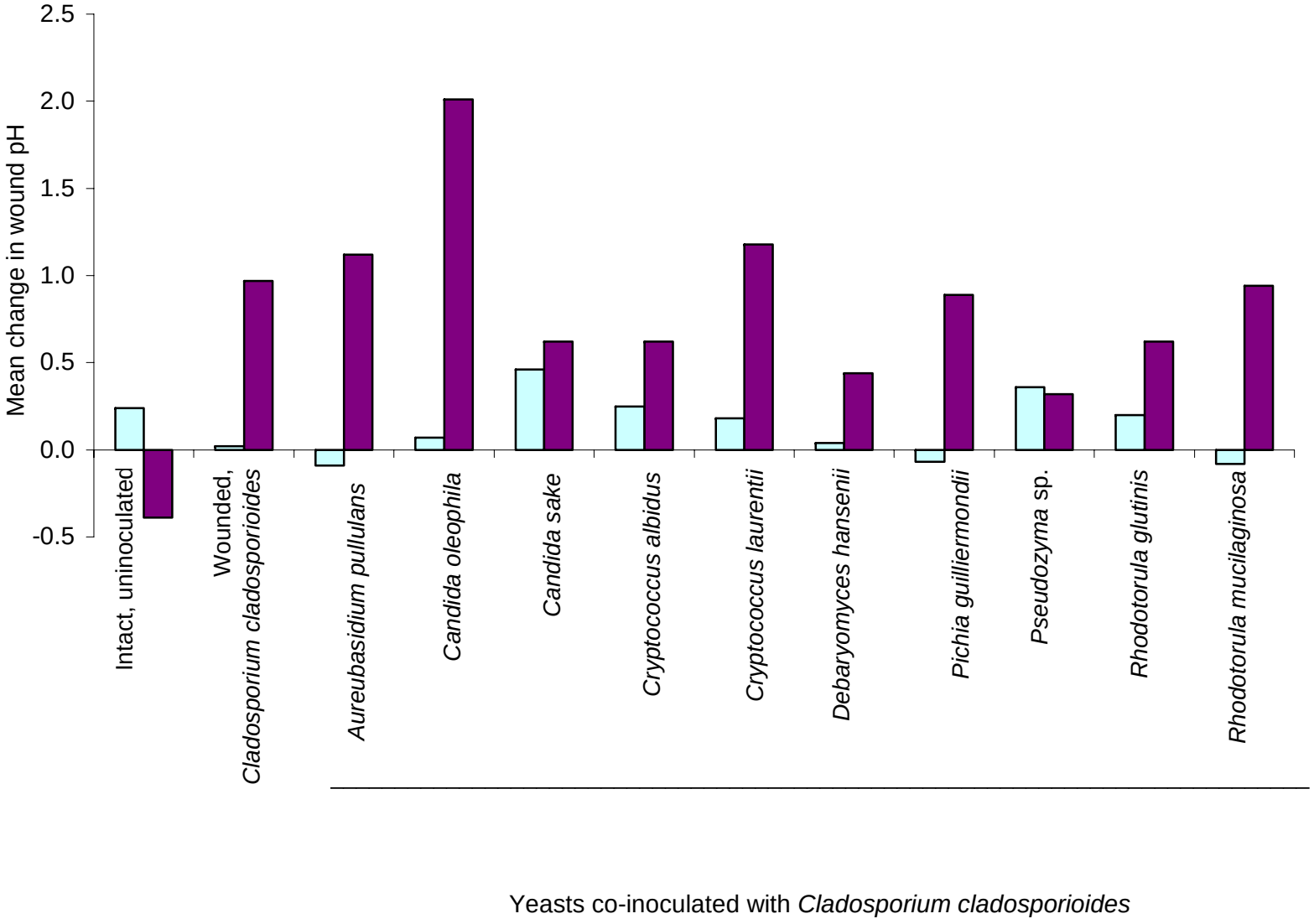
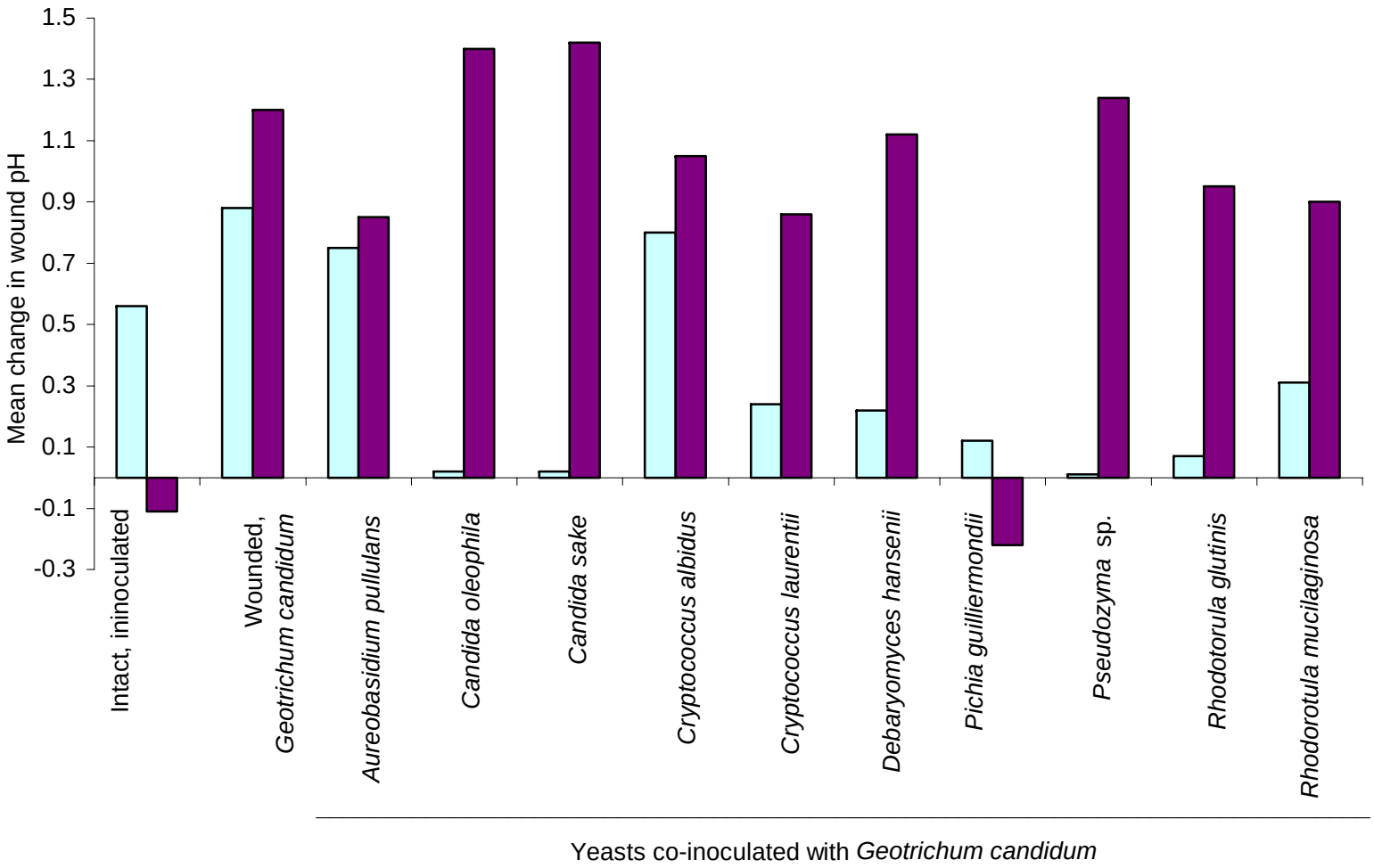

2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Mean change in wound pH
Intact, uninoculated
Wounded, Cladosporium cladosporioides
Aureubasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotorula mucilaginosa
Yeasts co-inoculated with Cladosporium cladosporioides

Figure 5.6. Effect of yeasts and *G. candidum* on changes in pH of intact and wounded tissue in cantaloupe rinds. Wounds in cantaloupe rinds were co-inoculated with 10 µl of *G. candidum* suspension (3.51 $\log_{10}$ CFU) and 10 µl of yeast suspension (5.98 – 7.32 $\log_{10}$ CFU). The initial pH of tissue stored at 4°C and 20°C was 5.16 – 6.11 and 5.32 – 6.4, respectively. Change in the pH of the cantaloupe rind tissue [difference between initial pH on day 0 and pH after storage at 4°C for 21 days (shaded bars) and 20°C for 7 days (solid bars)] was monitored.


1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
Mean change in wound pH
Intact, ininoculated
Wounded, Geotrichum candidum
Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodorotula mucilaginosa
Yeasts co-inoculated with Geotrichum candidum

increased by 0.12 – 1.23 units; melons stored at 20°C decreased by 0.23 or increased by 0.13 units (Fig. 5.6). The differences in pH between these two groups of control melons are attributed to differences in the level of maturity of the fruits used in the two studies.

The pH of the uninoculated, intact rind surface of cantaloupes in which wounds were inoculated with *C. cladosporioides* or with yeast and *C. cladosporioides* significantly increased ($P \leq 0.05$) when cantaloupes were stored for 21 days at 4°C and decreased when at 20°C for 7 days (Fig. 5.5). In wounds on cantaloupe rinds inoculated with *C. cladosporioides* only or *C. cladosporioides* and yeasts, the effect of storage temperature on change in pH showed the opposite trend, i.e. the increase in pH was greater in cantaloupes stored at 20°C than at 4°C.

Inoculation of wounds with *C. cladosporioides* only, followed by storage at 4°C for 21 days, did not cause a significant change in pH ($P > 0.05$); at 20°C, however, the pH of wound tissue increased significantly by 0.97 within 7 days ($P \leq 0.05$). When the wounds were co-inoculated with *C. cladosporioides* and yeasts and stored at 20°C, the pH of wounded rind tissue increased significantly ($P \leq 0.05$). The magnitude of increase in pH of wounded tissues was lowest when *C. cladosporioides* was co-inoculated with *C. oleophila*. Increases in the pH wounded rind of cantaloupes stored at 4°C after inoculation with *C. cladosporioides* and yeasts were not significant ($P > 0.05$), except with *C. sake* and *Pseudozyma* sp. Wounds co-inoculated with *A. pullulans, P. guilliermondii*, or *R. mucilaginosa,* and *C. cladosporioides* exhibited decreased pH when the cantaloupes were stored at 4°C; however, these decreases were not significant ($P > 0.05$).

Similarly, in wounded cantaloupe rinds inoculated with *G. candidum* only or *G. candidum* and yeasts, with the exception of wounded tissue inoculated with *G. candidum* and *P. guilliermondii*, the increase in pH was higher in cantaloupes stored at 20°C than at 4°C (Fig.

5.6). The pH of wounded rind tissue inoculated with only *G. candidum* increased significantly ($P \leq 0.05$) by 0.88 and 1.20 units at 4°C and 20°C, respectively. In cantaloupes stored at 4°C, and in some cases at 20°C, the pH of wounds inoculated with *G. candidum* and yeasts did not increase as much as the pH of wounds inoculated with *G. candidum* only. Results indicate that at 4°C, *C. oleophila, C. sake, D. hansenii, P. guilliermondii, Pseudozyma* sp., and *R. glutinis* retarded the increase in pH and prevented changes of the magnitude that occurred in tissue inoculated with *G. candidum* alone. Except in wounds co-inoculated with *P. guilliermondii*, the pH of the wounded rind tissue co-inoculated with *G. candidum* and yeasts was significantly higher ($P \leq 0.05$) after storage at 20°C for 7 days. *P. guilliermondii* limited the increase in pH of wound tissue at 4°C but caused a decrease in the pH of wounded tissue of cantaloupes stored at 20°C.

Injury of rind and flesh of cantaloupes, either by cutting or infection with molds, causes the integrity of cells to be disrupted and increases the availability of nutrients and water to phytopathogens and other microorganisms that may occur naturally on the surface. Release of products as a result of proteolysis and fermentation of tissue constituents, as well as utilization of acids, may contribute to changes in the pH. Consequently, wounding eventually causes changes the physiology, texture, and flavor of the fruit (Lamikanra *et al*., 2003). Oxalic, citric, malic, and succinic acids, together with glucose, fructose, and sucrose are present in edible tissues of freshly cut cantaloupe. In our previous study and that of Lamikanra *et al*. (2000), the pH of cut tissue of cantaloupes stored at 20°C increased over a 5-day period, but the pH of cut tissue stored at 4°C did not change substantially. The pH of wounded tissues increased more than that of the intact surface tissue. This is attributed to an increased availability of nutrients on the cut surface, which enhanced metabolic activities and consequently the production of by-products or

utilization of acids by yeasts and/or molds inoculated into the wounds. Some of the test yeasts, as well as *G. candidum* and *C. cladosporioides,* are capable of producing extracellular proteolytic enzymes that would catalyze biochemical reactions that produce alkaline substances that increase the pH of the environment (Deak and Beuchat, 1996). Yeasts and bacteria are able to take up nutrients more rapidly and in greater quantities than are the germ tubes of mycelial fungi (Droby *et al*., 1989). Reduction in the amounts of exogenous nutrients restricts conidial germination and hyphal development, which also gives yeasts a competitive advantage. Fermentative yeasts could contribute to controlling increases in pH of tissues by producing acids. *C. oleophila, C. sake*, and *P. guilliermondii* are fermentative, while the other test yeasts are non-fermentative (Kurtzman and Fell, 1999). Among the test yeasts, *P. guilliermondii* was most effective in limiting the increase in pH of wounds in the rinds of cantaloupes co-inoculated with *G. candidum*.

Change in diameter of wounds. The diameter of wounds inoculated with *C. cladosporioides* only or co-inoculated with *C. cladosporioides* and yeasts increased to 1.5 – 3.2 mm (mean = 2.3 mm) when the cantaloupes were stored at 4°C for 21 days and 3.0 – 5.0 mm (mean = 3.4 mm) when they were stored at 20°C for 7 days (Fig. 5.7). When wounds in cantaloupe rinds were inoculated with *G. candidum* only or co-inoculated with *G. candidum* and yeasts, the approximate diameter of the infected surface area around the wounds increased from 1 mm (day 0) to 1.5 – 3.7 mm (mean = 2.5 mm) after 21 days at 4°C and 2.0 – 6.0 mm (mean = 3.7 mm) after 7 days at 20°C (Fig. 5.8).

The size of wounds inoculated with only *C. cladosporioides* or *G. candidum* increased to 2.5 and 3.0 mm, respectively, when cantaloupes were stored at 4°C and 20°C. Storage at 4°C

Figure 5.7. Effect of yeasts and *C. cladosporioides* on the diameter of infected area around inoculated wounds in cantaloupe rinds. Wounds (1 mm in diameter) in cantaloupe rinds were co-inoculated with 10 µl of *C. cladosporioides* suspension (2.64 $\log_{10}$ CFU) and 10 µl of yeast suspension (5.98 – 7.32 $\log_{10}$ CFU). Cantaloupes were stored at 4°C for 21 days (shaded bars) and 20°C for 7 days (solid bars).

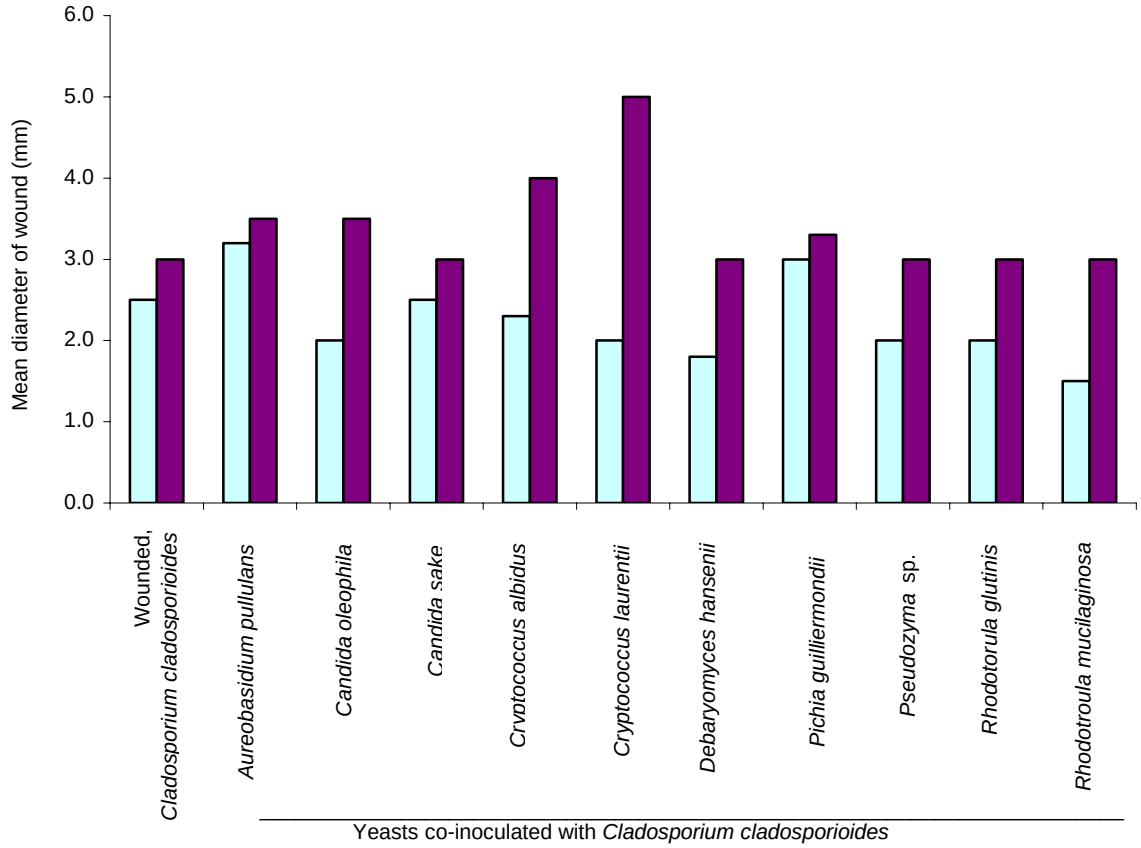
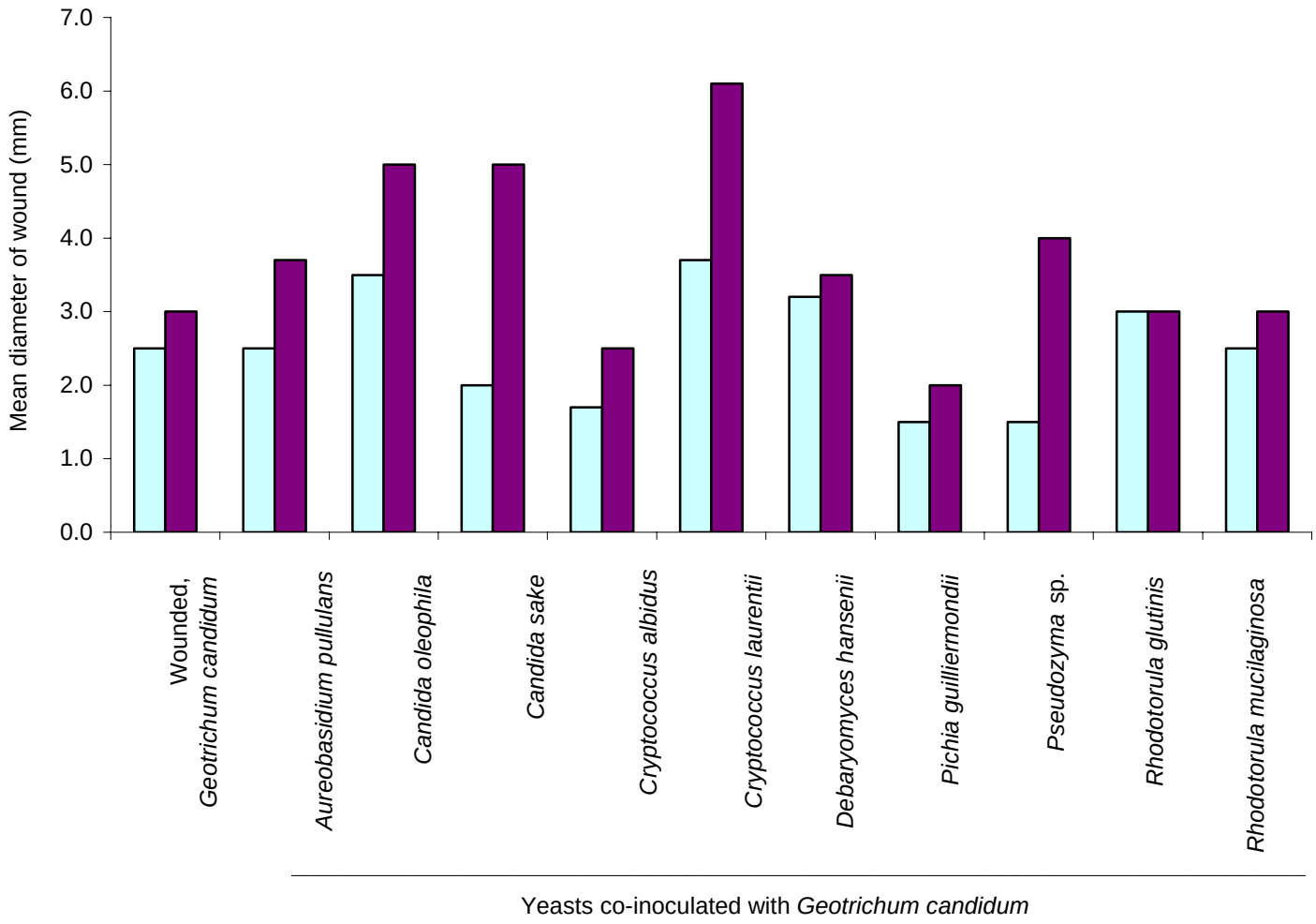

Mean diameter of wound (mm)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Wounded, Cladosporium cladosporioides
Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotroula mucilaginosa
Yeasts co-inoculated with Cladosporium cladosporioides

Figure 5.8. Effect of yeasts and *G. candidum* on the diameter of infected area around inoculated wounds in cantaloupe rinds. Wounds (1 mm in diameter) in cantaloupe rinds were co-inoculated with 10 µl of *G. candidum* suspension (3.51 $\log_{10}$ CFU) and 10 µl of yeast suspension (5.98 – 7.32 $\log_{10}$ CFU). Cantaloupes were stored at 4°C for 21 days (shaded bars) and 20°C for 7 days (solid bars).


Mean diameter of wound (mm)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Wounded, Geotrichum candidum
Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotorula mucilaginosa
Yeasts co-inoculated with Geotrichum candidum

helped to minimize visible growth of the inoculated molds. Co-inoculation of wounds with yeasts and *C. cladosporioides* or *G. candidum* did not inhibit decay at 20°C. However, *D. hansenii* and *R. mucilaginosa* were the most effective among test yeasts in limiting visible surface growth of *C. cladosporioides* (Fig. 5.7). *Cr. albidus, P. guilliermondii*, or *Pseudozyma* sp. retarded the expansion of visible decay by *G. candidum* to a mean surface diameter of < 2 mm after 21 days at 4°C (Fig. 5.8).

Increased wound size can also be attributed, in part, to water loss, particularly during extended storage. When wounds are created in the rinds, cells and tissues are ruptured, resulting in the release of nutrients and water. Moisture loss by evaporation from the tissues causes shrinkage of ruptured cells on and near the surface of wounds, thus expanding the wound size. Plant tissues are in equilibrium with an atmosphere at a relative humidity of 99 to 99.5%. Any reduction in water vapor pressure in an atmosphere below 99 – 99.5% would result in water loss. In intact tissues, water in intercellular spaces is not directly exposed to the external atmosphere. However, cutting or peeling a fruit or vegetable exposes interior tissues and drastically increases the rate of water evaporation (Brecht, 1995). The higher relative humidity (70 – 80% RH) in storage containers at 4°C compared to that in containers at 20°C (45%) in our study may have contributed to some of the changes in wound size during storage.

Mycelia of *G. candidum* were not visually apparent in wounded rind of cantaloupes stored at 4°C, regardless of co-inoculation with yeasts, but mycelial growth was obvious when fruits were stored at 20°C. Introduction of *C. cladosporioides* and *G. candidum* into wounds facilitated deterioration of the tissues as a result of production of cellulases and pectinases, especially at abusive storage temperature (20°C), causing a release of fluids from tissues. Rotting plant tissues are more likely to offer harborage to foodborne pathogens. In a survey of

more than 400 fruits and vegetables, the incidence of *Salmonella* in healthy tissues was reported to be approximately 10%, whereas 20% of the samples with bacterial rot were confirmed positive for the organism (Wells and Butterfield, 1997). Inoculation of soft rot and healthy tissues of potato, carrot, and pepper with *Salmonella* Typhimurium resulted in a 3- to 10-fold increase in population, compared to healthy tissues.

P. guilliermondii appeared to offer some control of visual mold deterioration of cantaloupe tissues at 4 and 20°C. Wounds that were inoculated with *P. guilliermondii*, together with *C. cladosporioides* or *G. candidum,* did not show obvious mold growth.

Populations and presence of yeasts and *G. candidum* in co-inoculated wounds. Populations and presence of yeasts and *G. candidum* inoculated into wounds in cantaloupe rind are listed in Table 5.4. Test yeasts were recovered from cantaloupes stored at 4°C for 7 and 14 days or 20°C for 7 days. Yeasts were more dominant than *G. candidum* in cantaloupes stored at 4°C. *G. candidum* as well as test yeasts were detected in a higher number of wounds on cantaloupes stored at 20°C. Results indicate that the yeasts competed with *G. candidum* for space and nutrients and their potential as antagonists against *G. candidum* was enhanced at low temperature.

Change in pH and recovery of *S.* Poona from wounded cantaloupe rinds. In wounds co-inoculated with *G. candidum,* yeasts, and *S.* Poona (Fig. 5.9), changes in pH were similar to those occurring in wounds inoculated with *G. candidum* and yeasts (Fig. 5.6). Infection of apples and tomatoes with molds has been reported to cause the pH of tissues to increase and promote the growth of *Clostridium botulinum* (Draughon *et al*., 1988), *Escherichia coli* O157:H7 (Riordan *et al*., 2000), and *Listeria monocytogenes* (Conway *et al*., 2000). It was anticipated that some of the test yeasts would produce ethanol or acids that would minimize increases in pH

Table 5.4. Presence of yeasts and *G. candidum* inoculated into wounds in cantaloupe rinds

Microorganism	Population[b]	Storage Temp. (°C)	Presence of yeast and *G. candidum*[a]			
			Yeast		*G. candidum*	
			Day 7	14	7	14
G. candidum	3.26	4	-[c]	-	4	4
		20	-	-	nd[d]	nd
A. pullulans	5.62	4	4	4	4	3
		20	4	nd	4	nd
C. oleophila	6.48	4	4	4	2	2
		20	4	nd	4	nd
C. sake	6.36	4	4	4	4	2
		20	4	nd	4	nd
Cr. albidus	5.97	4	4	4	3	4
		20	2	nd	4	nd
Cr. laurentii	5.58	4	4	4	3	2
		20	4	nd	4	nd
D. hansenii	6.36	4	4	4	1	3
		20	4	nd	4	nd
P. guilliermondii	6.51	4	4	4	2	3
		20	2	nd	4	nd
Pseudozyma sp.	5.48	4	4	0	1	4
		20	3	nd	4	nd
R. glutinis	5.26	4	4	4	3	2
		20	4	nd	4	nd
R. mucilaginosa	6.23	4	4	4	3	3
		20	1	nd	4	nd

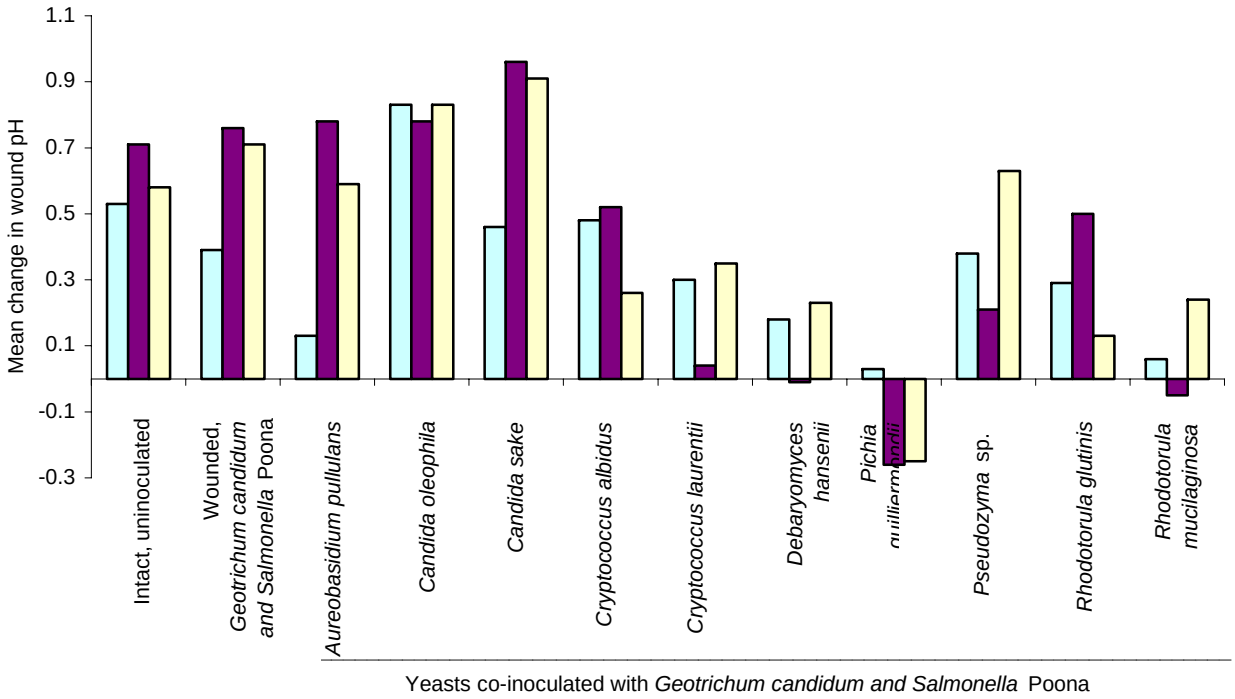
[a] Number of positive samples out of four samples analyzed.

[b] Populations of *G. candidum* and yeasts ($\log_{10}$ CFU per 10 µl of inoculum) on day 0. Wounds were co-inoculated with 10 µl of *G. candidum* suspension (3.26 $\log_{10}$ CFU), 10 µl of yeast suspension (5.26 – 6.51 $\log_{10}$ CFU), and 10 µl of *S.* Poona suspension (4.49 $\log_{10}$ CFU).

[c] Not inoculated with yeasts.

[d] Not determined; cantaloupes were discarded after 7 days.

Figure 5.9. Effect of yeasts, *G. candidum*, and *S*. Poona on change in pH of tissue in wounds in cantaloupe rinds. Wounds in cantaloupe rinds were co-inoculated with 10 µl of *G. candidum* suspension (3.26 $\log_{10}$ CFU), 10 µl of yeast suspension (5.26 - 6.51 $\log_{10}$ CFU), and 10 µl of *S*. Poona suspension (4.49 $\log_{10}$ CFU). The initial pH values of tissue stored at 4°C and 20°C were 5.54 – 6.64 and 5.75 – 6.49, respectively. Change in the pH of the cantaloupe wound issue [difference between initial pH on day 0 and pH after storage at 4°C for 7days (shaded bars), 4°C for 14 days (solid bars) and 20°C for 7 days (open bars)] was monitored.


1.1
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
Mean change in wound pH
Intact, uninoculated
Wounded, Geotrichum candidum and Salmonella Poona
Aureobasidium pullulans
Candida oleophila
Candida sake
Cryptococcus albidus
Cryptococcus laurentii
Debaryomyces hansenii
Pichia guilliermondii
Pseudozyma sp.
Rhodotorula glutinis
Rhodotorula mucilaginosa
Yeasts co-inoculated with Geotrichum candidum and Salmonella Poona

caused by metabolic activities of *C. cladosporioides* and *G. candidum*, thereby inhibiting survival and growth of *S*. Poona. However, overall, this phenomenon was not evident.

The population of *S*. Poona in 10 µl of inoculum deposited into wounds in the rind of cantaloupes was 4.49 $\log_{10}$ CFU/ml (Table 5.5). *S*. Poona survived but the population did not change significantly ($P > 0.05$) in cantaloupes co-inoculated with *G. candidum* or *G. candidum* and yeast, followed by storage at 4°C for 7 or 14 days. Exceptions were cantaloupes inoculated with *C. oleophila* or *R. glutinis* in which progressive declines in the populations of *S*. Poona occurred during the 14-day storage period ($P \leq 0.05$).

S. Poona grew on the cut tissue in wounded rinds of cantaloupes stored at 20°C, regardless of co-inoculation with *G. candidum* or *G. candidum* and yeasts. The population of *S*. Poona increased to 9.16 $\log_{10}$ CFU/sample (ca. 7 cm^2 of rind surface with contiguous tissue ca. 0.5 cm thick) in wounds co-inoculated with *G. candidum* only. In the presence of *G. candidum* and yeasts, the population of *S*. Poona increased to as high as 9.60 $\log_{10}$ CFU/sample when the cantaloupes were stored at 20°C for 7 days. The population of *S*. Poona increased by an average of 4.17 $\log_{10}$ CFU/ sample. Growth of *S*. Poona was lowest in wounds co-inoculated with *D. hansenii* (6.40 $\log_{10}$ CFU/sample), *P. guilliermondii* (7.26 $\log_{10}$ CFU/sample), and *Cr. albidus* (7.98 $\log_{10}$ CFU/sample). Co-inoculation of *S*. Poona with *G. candidum* and *D. hansenii* or *P. guilliermondii* resulted in increases of only 1.91 and 2.77 $\log_{10}$ CFU/sample, respectively, indicating inhibitory activity against *S*. Poona. While *C. oleophila* and *R. glutinis* appear to be most promising for reducing the population of *S*. Poona in the wounds in cantaloupes stored at 4°C, *Cr. albidus, D. hansenii*, and *P. gulliermondii* were more effective in controlling growth at 20°C.

Table 5.5. Populations of *S.* Poona recovered from wounds in cantaloupe rinds co-inoculated with *S.* Poona, *G. candidum,* and yeasts.

	Population[a]			
	4°C			20°C
Microorganism[b]	Day 0	7	14	7
G. candidum (control)	4.49	4.54	4.53	9.16
G. candidum plus yeast				
A. pullulans	4.49	4.47	4.36	9.53
C. oleophila	4.49	4.31	3.64	8.97
C. sake	4.49	4.48	4.47	8.49
Cr. albidus	4.49	4.42	4.18	7.98
Cr. laurentii	4.49	4.59	4.39	9.35
D. hansenii	4.49	4.56	4.37	6.40
P. guilliermondii	4.49	3.92	4.61	7.26
Pseudozyma sp.	4.49	4.87	4.41	9.09
R. glutinis	4.49	3.83	3.88	9.60
R. mucilaginosa	4.49	4.49	4.55	9.39

[a] Population of *S.* Poona on day 0 indicates $\log_{10}$ CFU per 10 µl of inoculum. Populations 7 and 14 days after inoculation with *S.* Poona indicate $\log_{10}$ CFU/rind sample analyzed.

[b] Wounds in cantaloupe rinds were inoculated with 10 µl of *G. candidum* suspension (3.26 $\log_{10}$ CFU) and 10 µl of *S.* Poona suspension (4.49 $\log_{10}$ CFU) (control) or 10 µl of *G. candidum* suspension, 10 µl of *S.* Poona suspension, and 10 µl of yeast suspension (5.26 – 6.51 $\log_{10}$ CFU). See Table 2 for populations of each yeast in 10 µl of inoculum.

The non-cellulosic neutral sugar composition of cell walls of mature cantaloupes is ca. 11.3 mg/100 mg of wall tissues (Gross and Sams, 1984). These sugars include rhaminose, fructose, arabinose, xylose, mannose, galactose, and glucose. All test yeasts utilize glucose, fructose, and mannose, but there are large differences among species with respect to utilization of other sugars and amino acids (Deak and Beuchat, 1996). Generally, *Salmonella* are able to ferment glucose and other monosaccharides and utilize a wide range of compounds as sources of nitrogen. Yeasts can potentially be antagonistic by competing with bacteria for these nutrients in fruit tissues. In studies conducted by Nunes *et al*. (2002), wounds in apples and pears were co-inoculated with *C. sake* and the bacterium *Pantoea agglomerans* to assess their abilities to enhance their biocontrol capabilities. *C. sake* dominated the wounds because of its higher capacity to utilize nitrogen sources. The low nitrogen and vitamin contents in tissue fluids in wound sites after colonization of *C. sake* caused a decline in the population of *P. agglomerans*.

Wounded apple tissue has been observed to be an excellent substrate for the growth of *E. coli*, supporting exponential growth (Dingman, 2000; Janisiewicz *et al*., 1999b). Decreased survival of *E. coli* O157:H7 in unpasteurized cider compared to sterile apple juice was attributed to interaction with natural populations of yeasts (Janisiewicz *et al*. 1999b). The authors recommended that explorative work be undertaken to identify yeasts that prevent potential colonization of human pathogens.

Strains of the yeasts used in this study have been assessed by others to determine their potential as antagonists and ability to prevent colonization of phytopathogens in wounds in fruits. *A. pullulans*, *Cr. laurentii,* and *R. glutinis* were shown to produce antifungal and antibacterial compounds that were inhibitory to *P. fluorescens* and *S. aureus* (McCormack *et al*., 1994). These yeasts have also been shown to inhibit several fungal pathogens in wounds on apples,

pears, strawberry, kiwi, grapes, oranges, mandarins, and grapefruits (Lima *et al*., 1999). Conidial germination and mycelial growth by *Penicillium expansum* were reduced by rhodotorulic acid and siderophore produced by *R. glutinis* (Calvente *et al*., 1999). *C. oleophila* has been used to develop commercial formulations for application to pome and citrus fruits (Droby *et al*., 1998; Mercier and Wilson, 1994; Wilson *et al*., 1996) and *C. sake* is reported to be an effective antagonist for the control of major postharvest pathogens on pome fruits at small and semi-commercial scale trials in packinghouses (Abadias *et al*., 2001; Viñas *et al*., 1998). Successful biocontrol activity of *Pseudozyma flocculosa* has been attributed to its ability to produce antifungal fatty acids that affect the mycelial growth and conidial germination of fungi (Avis and Bélanger, 2001).

Rapid colonization, competition for nutrients, and secretion of cell wall-degrading enzymes have been suggested as the mechanisms by which *D. hansenii* and *P. guilliermondii* (previously called *D. hansenii*) achieve biological control of phytopathogens (Droby *et al.*, 1989; Petersson and Schnürer, 1995). The ability of *P. guilliermondii* to attach firmly to the hyphae of *Botrytis cinerea* and produce high levels of β- (1-3) glucanase activity were found to be additional characteristics that improved its effectiveness as a biological control agent (Wisniewski *et al*., 1991). *P. guilliermondii* has been reported to elicit the formation of phytoalexins (scoparone and scopoletin) in citrus fruit wounds at concentrations that are fungitoxic (Lima *et al.,* 1999). These control methods would be acceptable for use in the biological control of phytopathogens and foodborne pathogens because they do not involve secretion of potentially harmful antibiotics.

Some of the yeasts tested by others **(**Droby *et al.*, 1989; Droby *et al*., 1998; Lima *et al*., 1999; Mercier and Wilson, 1994; McCormack *et al*., 1994; Petersson and Schnürer, 1995;

Wilson *et al*., 1996; Wisniewski *et al*., 1991) were demonstrated in this study to have potential to restrict colonization and metabolic activities of plant pathogenic molds in wounds in cantaloupe rinds, particularly at low storage temperatures. Results of our study indicate that *C. oleophila* and *R. glutinis* at 4°C and *Cr. albidus, D. hansenii*, and *P. gulliermondii* at 20°C could also be efficacious as antagonists to *S*. Poona in the wounds co-infected with phytopathogens. The populations of *S*. Poona in cantaloupe juice co-inoculated with *D. hansenii*, *P. guilliermondii*, and *Pseudozyma* sp. at high populations was significantly less than that of the control. However, the overall results of the *in vitro* study suggest that the yeasts evaluated were unable to vigorously compete and efficiently restrict the growth of *S*. Poona in cantaloupe juice. The success of biological control depends on the ability of the antagonist to rapidly become established in the environment, out-compete the pathogen for resources, and create a medium that is unsuitable for the pathogen.

REFERENCES

Abadias, M., N. Teixidó, J. Usall, A. Benabarre, and I. Viñas. 2001. Viability, efficacy, and storage stability of freeze-dried biocontrol agent *Candida sake* using different protective and rehydration media. J. Food Prot. 64:856-861.

Arul, J. 1994. Emerging technologies for the control of postharvest diseases of fresh fruits and vegetables. *In* C.L. Wilson and M.E. Wisniewski (Ed.), Biological Control of Postharvest Diseases. Theory and Practice. CRC Press, Boca Raton, FL.

Avis, T.J. and R.R. Bélanger. 2001. Specificity and mode of action of the antifungal fatty acid cis-9-heptadecenoic acid produced by Pseudozyma flocculosa. Appl. Environ. Microbiol. 67:956-960.

Bilinski, C.A., G. Innamorato, and G.G. Stewart. 1985. Identification and characterization of antimicrobial activity in two yeast genera. Appl. Environ. Microbiol. 50:1330-1332.

Brecht, J.K. 1995. Physiology of lightly processed fruits and vegetables. HortSci. 30:18-22.

Buck, J. W., R. R. Walcott, and L.R. Beuchat. 2003. Recent trends in microbiological safety of fruits and vegetables. Plant Health Progress. http://www.apsnet.org. Accessed 29 January 2003.

Calvente, V., D. Benuzzi, and M.I.S. de Tosetti. 1999. Antagonistic action of siderophores from *Rhodotorula glutinis* upon the postharvest pathogen *Penicillium expansum*. Int. Biodeter. Biodegrad. 43:167-172.

Chand-Goyal, T. and R.A. Spotts. 1997. Biological control of postharvest diseases of apples and pear under semi-commercial and commercial conditions using three saprophytic yeasts. Biol. Control 10:199-206.

Conway, W.S., B. Leverentz, R.A. Saftner, W.J. Janisiewicz, C.E. Sams, and E. Leblanc. 2000. Survival and growth of *Listeria monocytogenes* on fresh cut apples and its interaction with *Glomerella cingulata* and *Penicillium expansum*. Plant Dis. 84:177-181.

Deak, T. and L.R. Beuchat. 1996. Handbook of Food Spoilage Yeasts. CRC Press, Boca Raton, FL.

Dingman, D. W. 2000. Growth of *Escherichia coli* O157:H7 in bruised apple (*Malus domestica*) tissue as influenced by cultivar, date of harvest, and source. Appl. Environ. Microbiol. 66:1077-1083.

Draughon, F.A., S. Chen, and J.O. Mundt. 1988. Metabiotic association of *Fusarium, Alternaria*, and *Rhizoctonia* with *Clostridium botulinum* on fresh tomatoes. J. Food Sci. 53:120-123.

Droby, S., E. Chalutz, C. L. Wilson, and M.E. Wisniewski. 1989. Characterization of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruit. Can. J. Microbiol. 35: 794-800.

Droby S. and E. Chalutz., 1994. Mode of action of biocontrol agents of postharvest diseases. *In* C.L. Wilson and M.E. Wisniewski (Ed.), Biological Control of Postharvest Diseases. Theory and practice. CRC Press, Boca Raton, FL.

Droby, S., E. Chalutz, M.E. Wisniewski, and C.L. Wilson. 1996. Host response to introduction of antagonistic yeasts used for control of postharvest decay. *In* C.E. Morris, P.C. Nicot, and C. Nguyen-the (Ed.), Aerial Plant Surface Microbiology. Plenum Press, New York, NY.

Droby, S., L. Cohen, A. Daus, B. Weiss, B. Horev, E. Chalutz, H. Katz, M. Keren-Tzur, and A. Shachnai. 1998. Commercial testing of Aspire: A yeast preparation for the biological control of postharvest decay of citrus. Biol. Control 12:91-101.

Eckert, J.W. and J.M. Ogawa. 1985. The chemical control of postharvest diseases: subtropical and tropical fruits. Ann. Rev. Phytopathol. 23:421-454.

El-Ghaouth, A. 1997. Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases. J. Industrial Microbiol. Biotechnol. 19:160-162.

El-Ghaouth, C.L. Wilson, and M.E. Wisniewski. 1998. Ultrastructural and cytological aspects of the biological control of *Botrytis cinerea* by *Candida saitoana* in apple fruit. Phytopathology 88: 282-291.

Gross, K.C. and C.E. Sams. 1984. Changes in cell wall neutral sugar composition during fruit ripening: A species survey. Phytochemistry 23: 2457-2461.

Huhtanen, C.N., J. Naghski, C.S. Custer, and R.W. Russell. 1976. Growth and toxin production by *Clostridium botulinum* in moldy tomato juice. Appl. Environ. Microbiol. 32:711-715.

Janisiewicz, W.J., W.S. Conway, and B. Leverentz. 1999a. Biological control of postharvest decays of apple can prevent growth of *Escherichia coli* O157:H7 in apple wounds. J. Food Prot. 62:1372-1375.

Janisiewicz, W.J. W.S. Conway, M.W. Brown, G.M. Sapers, P. Fratamico, and R.L. Buchanan. 1999b. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. Appl. Environ. Microbiol. 65:1-5.

Janisiewicz, W.J., T.J. Tworkoski, and C. Sharer. 2000. Characterizing the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. Phytopathology 90:1196-1200.

Janisiewicz, W.J. and L. Korsten. 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40:411-441.

Kurtzman, C.P. and J.W. Fell. 1999. The Yeasts, A Taxonomic Study. Elsevier, Amsterdam, The Netherlands.

Lamikanra, O., J.C. Chen, D. Banks, and P.A. Hunter. 2000. Biochemical and microbial changes during the storage of minimally processed cantaloupe. J. Agric. Food Chem. 48:5955-5961.

Lamikanra, O., B. Juaraez, M.A. Watson, and O.A. Richard. 2003. Effect of cutting and storage on sensory traits of cantaloupe melon cultivars with extended postharvest shelf life. J. Sci. Food Agric. 83:702-708.

Leverentz, B., W.S. Conway, Z. Alavidze, W.J. Janisiewicz, Y. Fuchs, M.J. Camp, E. Chighladze, and A. Sulakvedidze. 2001. Examination of bacteriophage as a biocontrol method of *Salmonella* on fresh-cut Fruit: A model study. J. Food Prot. 64:1116-1121.

Lewus, C.B., A. Kaiser, and T.J. Montville. 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. 57:1683-1688.

Lima, G., S. Arru, F. De Curtis, and G. Arras. 1999. Influence of antagonist, host fruit and pathogen on biological control of postharvest fungal diseases by yeasts. J. Indus. Microbiol. Biotechnol. 23:223-229.

McCormack, P.J., H.G. Wildman, and P. Jeffries. 1994. Production of antibacterial compounds by phylloplane-inhabiting yeasts and yeastlike fungi. Appl. Environ. Microbiol. 60:927-931.

Mercier, J. and C.L. Wilson. 1994. Colonization of apple wounds by naturally occurring microflora and introduced *Candida oleophila* and their effect on infection by *Botrytis cinerea* during storage. Biolog. Control 4:138-144.

Mundt, J.O. 1978. Effect of mold growth on the pH of tomato juice. J. Food Prot. 41:267-268.

Muriana, P.M. 1996. Bacteriocins for control of *Listeria spp*. in food. J. Food Prot. 56:944-948.

National Research Council, Board of Agriculture. 1987. Regulating pesticides in food – The Delany Paradox, National Academy Press, Washington, DC.

Nunes, C., J. Usall, N. Teixidó, R. Torres, and I. Viñas. 2002. Control of *Penicillium expansum* and *Botrytis cinerea* on apples and pears with the combination of *Candida sake* and *Pantoea agglomerans*. J. Food Prot. 65:178-184.

Odlaug, T.E. and I.J. Pflug. 1979. *Clostridium botulinum* growth and toxin production in tomato juice containing *Aspergillus gracilis*. Appl. Environ. Microbiol. 37:496-504.

Petersson, S. and J. Schnürer. 1995. Biocontrol of mold growth in high-moisture wheat stored under airtight conditions by *Pichia anomala*, *Pichia guilliermondii*, and *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 61:1027-1032.

Riordan, D.C.R., G.M. Sapers, and B.A. Annous. 2000. The survival and growth of *Escherichia coli* O157:H7 in the presence of *Penicillium expansum* and *Glomerella cingulata* in wounds on apple surfaces. J. Food Prot. 63:1637-1642.

Rodrigues, A.C.P., R.M. Nardi, E.H. Bambirra, E.C. Vieira, and J.R. Nicoli. 1996. Effect of *Saccharomyces boulardii* against experimental oral infection with *Salmonella* Typhimurium and *Shigella flexneri* in conventional and gnotobiotic mice. J. Appl. Bacteriol. 81:251-256.

Salunkhe, D.K. and B.B. Desai. 1984. Postharvest Biotechnology of Fruits. Vol. 1. CRC. Boca Raton, FL.

Snowdon, A. L. 1990. A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables. Volume 1: General Introduction and Fruits. CRC Press, Boca Raton, FL.

Stevens, K.A., B.W. Sheldon, N.A. Klapes, and T.R. Klaenhammer. 1991. Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. Appl. Environ. Microbiol. 57:3613-3615.

Viñas, I. J. Usall, N. Teixidó, and V. Sanchis. 1998. Biological control of major postharvest pathogens on apple with *Candida sake*. Intl. J. Food Microbiol. 40:9-16.

Wade, W.N. and L.R. Beuchat. 2003. Proteolytic fungi isolated from decayed and damaged raw tomatoes and implications associated with changes in pericarp pH favorable for survival and growth of foodborne pathogens. J. Food Prot. 66:911-917.

Walker, R., C.M.J. Innes, and E.J. Allan. 2001. The potential biocontrol agent *Pseudomonas antimicrobica* inhibits germination of conidia and outgrowth of *Botrytis cinerea*. Lett. Appl. Microbiol. 32:346-348.

Wells, J.M. and J.E. Butterfield. 1997. *Salmonella* contamination with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Dis. 81:867-872.

Wilson, C.L. and M.E. Wisniewski. 1989. Biological control of postharvest diseases of fruits and vegetables: An emerging technology. Annu. Rev. Phytopathol. 27:425-441.

Wilson, C. L. and M.E. Wisniewski. 1994. Biological control of postharvest diseases. CRC Press, Boca Raton, FL.

Wilson, C.L., M.E. Wisniewski, A. El Ghaouth, S. Droby, and E. Chalutz. 1996. Commercialization of antagonistic yeasts for the biological control of postharvest diseases of fruits and vegetables. J. Industrial Microbiol. 46:237-242.

Wilson, C.L., J.M. Solar, A. El-Ghaouth, and M.E. Wisniewski. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis. 81: 204-210.

Wisniewski, M., C. Biles, S. Droby, R. McLaughlin, C. Wilson, and E. Chalutz. 1991. Mode of action of the postharvest biocontrol yeast, *Pichia guilliermondii*. I. Characterization of attachment to *Botrytis cinerea*. Phys. Mol. Plant Pathol. 39:245-258.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Studies were undertaken to determine the survival and growth characteristics of *Salmonella* Poona on and in cantaloupes as affected by conditions to which the fruits may be exposed preceding cutting for preparation to consume. Specific objectives of the studies documented in this dissertation were:

1. To determine if temperature differentials between cantaloupes and suspensions of *S.* Poona influences infiltration of the pathogen into rind and stem scar tissues of Eastern and Western type cantaloupes.
2. To determine survival and growth characteristics of *S*. Poona on the surface of intact rind and in wounded rind tissue as affected by storage temperature and co-infection with phytopathogenic molds.
3. To determine if *S*. Poona migrates from tissues in the cantaloupe rinds to edible internal tissues.
4. To examine yeasts for their potential to impair survival and growth of *S*. Poona in cell suspensions and in wounds in the cantaloupe rind co-inoculated with phytopathogenic molds.

This research demonstrated that *S*. Poona in wash water adheres to rind and stem scar tissues of cantaloupes. The initial temperature of the inoculum and cantaloupe affected the mean percent weight increase in weight by Eastern type cantaloupes, but not the Western type. Adherence to or infiltration of Eastern but not Western type cantaloupe rind by *S.* Poona is enhanced when the cantaloupe is at 4°C compared to 30°C. The histology of cantaloupe rind and stem scar tissues augments attachment and penetration of *S*. Poona, and may therefore reduce the effectiveness of sanitizer treatments.

S. Poona persists on intact, wounded, and decaying cantaloupe rind at refrigerated and ambient temperatures. Phytopathogenic molds can increase the pH of cantaloupe tissue by

proteolytic activity and perhaps other alkalinizing activities. However, recovery of *S.* Poona after incubation at 4°C and 30°C was not affected by co-infection with molds. Abusive storage temperatures enhance survival and growth of *S*. Poona on wounded cantaloupe tissues. These findings underscore the importance of proper postharvest handling and storage to prevent contamination, physical damage, and deterioration.

S. Poona is capable of migrating from wounded tissues in the rind through the pulp tissues to depths up to 4 cm in edible tissues, with or without co-inoculation with phytopathogens. Migration and survival of *S*. Poona in cantaloupes were enhanced by co-inoculation with *Cladosporium cladosporioides* and, to a lesser extent, *Penicillium expansum*.

Some of the yeasts examined in this study for biocontrol activity exhibited potential to restrict colonization and metabolic activities of plant pathogenic molds in cantaloupe rinds, particularly at low storage temperatures. *Candida oleophila* and *Rhodotorula glutinis* at 4°C and *Cryptococcus albidus*, *Debaryomyces hansenii*, and *Pichia guilliermondii* at 20°C could also be efficacious as antagonists to *S*. Poona in wounds co-infected with phytopathogenic molds. While the populations of *S*. Poona in cantaloupe juice co-inoculated with *D. hansenii, P. guilliermondii*, and *Pseudozyma* sp. at high populations were significantly less that of the control, the overall results of the in vitro study suggest that the yeasts evaluated were unable to vigorously compete and efficiently restrict the growth of *S*. Poona in cantaloupe juice.

Biological control activities by the yeasts investigated do not prevent survival and growth of *S.* Poona in cantaloupe tissues. Adherence of *S*. Poona to rind and stem scar tissues, migration of the pathogen from subsurface edible tissues, and recovery of larger populations from wounded tissues, particularly from cantaloupes stored at abusive temperatures, underscore the importance

of proper postharvest handling and storage to prevent contamination, physical damage, and deterioration.