

PRODUCE

SURVEY OF BIOSECURITY PRACTICES IN PRODUCE OPERATIONS IN THE SOUTHEAST (M. A. Harrison, K. Simmons, W. C. Hurst, and J. A. Harrison)

Fresh produce is potentially vulnerable to tampering because it is usually eaten raw or in a minimally processed state. It is grown, harvested and packed literally “in the open” and there is typically no kill step to destroy microbial pathogens prior to consumption. In a collaborative effort with researchers from Georgia, South Carolina and Florida, this study was undertaken to assess the current status of security at fresh produce facilities in these states. Security audit forms were prepared and used to survey growers, packers, and fresh-cut processing operations. A total of 25 farms, 25 packinghouses and 7 fresh-cut produce processing operations were surveyed. Practically all of the fresh-cut processors have a written security plan, conduct security training for their employees, and have restricted access to their facilities. However, only about half of the farm and/or packinghouse operations provide employee security training, and only one farm and one packinghouse surveyed have written security plans. About half (52%) of the packinghouses surveyed have perimeter fencing and only half have locks on the cooler doors. Documentation of any sort of security practice is lacking among both growers and packers. Survey data collected to date indicates that while fresh-cut processing facilities are dealing with current security challenges, farm and packing operations in the tri-state region are lagging behind. More training programs and assistance to increase awareness and to facilitate incorporation of feasible, preventative measures are needed by segments of the industry.

SURVEY OF YEASTS FOR ANTAGONISTIC ACTIVITY AGAINST *SALMONELLA* POONA IN CANTALOUPE JUICE AND WOUNDS IN RINDS CO-INFECTED WITH PHYTOPATHOGENIC MOLDS (G. M. Richards, J. W. Buck, and L. R. Beuchat)

Biological control is a process by which plant disease is minimized by application of a natural biological process and/or the product of a natural biological process, either preharvest or postharvest. 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. A yeast, *Candida oleophila* Montrocher, and a bacterium, *Pseudomonas syringae*, have been used to control postharvest diseases of pome and citrus fruits. Yeasts are the main group of microorganisms being investigated for biocontrol activity because they can colonize the surface of fruits and vegetables for long periods under reduced-moisture conditions, produce extracellular polysaccharides that enhance their survival, restrict colonization sites and flow of germination cues to fungal propagules, and use available nutrients to rapidly proliferate.

Metabiotic associations between phytopathogenic molds and foodborne bacteria on apples and tomatoes have been described. Some species of molds produce proteolytic enzymes that release alkaline by-products into the surrounding tissues that cause localized increase in pH. This may create a more favorable environment for enteric pathogens such as *Salmonella* to survive and grow. The objective of this study was to examine yeasts for their ability to impair survival and growth of *Salmonella* Poona in cantaloupe juice and in wounds on cantaloupe rind co-inoculated with phytopathogenic molds. Changes in the pH of cantaloupe juice and rind tissue, as well as the size of infected surface of and tissues surrounding wounds as affected by co-inoculation with yeasts, molds, and *S. Poona* were determined.

We examined ten yeasts for potential antagonistic activity against survival and growth of *S. Poona* in cantaloupe juice and decay by *Cladosporium cladosporioides* and *Geotrichum candidum* in wounds on

cantaloupe rind. Cantaloupe juice was inoculated using five schemes: *S. Poona* only (1.10 log₁₀ cfu/ml), high (3.93 – 5.21 log₁₀ cfu/ml) or low populations (1.79 – 3.26 log₁₀ cfu/ml) of yeasts only, and *S. Poona* combined with high or low populations of yeasts. High initial populations of *Debaryomyces hansenii*, *Pichia guilliermondii*, and *Pseudozyma* sp. were antagonistic to *S. Poona* in cantaloupe juice stored at 20°C for 48 h. Wounds in cantaloupe rinds were inoculated with yeast and mold or yeast, mold, and *S. Poona* and cantaloupes were stored at 4°C for 14 days or 20°C for 7 days. The pH of rind tissue inoculated with *C. cladosporioides* and yeasts increased significantly ($P \leq 0.05$) at 20°C. Wounds that were inoculated with *P. guilliermondii*, together with *C. cladosporioides* or *G. candidum*, did not show mold growth at 4 and 20°C. Populations of *S. Poona* (6.40, 7.26, and 7.98 log₁₀ cfu/sample) were lower in wounds co-inoculated with *G. candidum* and three of the test yeasts (*D. hansenii*, *P. guilliermondii*, and *Cryptococcus albidus*, respectively) compared to co-inoculation with *G. candidum* or the other seven yeasts. *Candida oleophila* and *Rhodotorula glutinis* showed the most promise in reducing the population of *S. Poona* in wounds in rinds of cantaloupes co-inoculated with *G. candidum* and stored at 4°C.

EFFICACY OF GASEOUS CHLORINE DIOXIDE AS A SANITIZER FOR KILLING *SALMONELLA*, YEASTS, AND MOLDS ON BLUEBERRIES, STRAWBERRIES, AND RASPBERRIES (K. V. Sy, K. H. McWatters, and L. R. Beuchat)

Sanitizers such as gaseous chlorine dioxide (ClO₂) have been explored as alternatives to aqueous chemicals for sanitizing fruits and vegetables eaten raw. Gaseous ClO₂ has some advantages over chlorinated water in that it can break down phenolic compounds and remove phenolic tastes and odors from the water, does not react with ammonia, and has 2.5 times the oxidation capacity of chlorine. The bactericidal efficacy of gaseous ClO₂ is also not markedly affected by pH as well as it has greater sporicidal activity. Several studies have shown gaseous ClO₂ to be effective in killing enteric pathogens on several fruits and vegetables. However, the efficacy of gaseous ClO₂ gas in killing or removing *Salmonella*, yeasts, and molds on small fruits has not been reported. The objective of this study was to evaluate gaseous ClO₂ for its effectiveness in killing *Salmonella* inoculated onto the surface of blueberries, strawberries, and red raspberries. Inactivation of yeasts and molds naturally occurring on the fruits was also determined.

An inoculum (100 µl, 6.0 - 6.8 log₁₀ cfu/g of fruit) containing five serotypes of *Salmonella enterica* was deposited on the skin, calyx tissue, or stem scar tissue of blueberries, skin or stem scar tissue of strawberries, and skin of red raspberries, dried for 2 h at 22°C, then held for 20 h at 4°C and 2 h at 22°C before treatment. Sachets containing reactant chemicals were formulated to release gaseous ClO₂ at concentrations of 4.1, 6.2, and 8.0 mg/L of air within treatment times of 30, 60, and 120 min, respectively, at 23±1°C. Treatment with 8.0 mg of ClO₂/L significantly ($\alpha = 0.05$) reduced the population of *Salmonella* on blueberries by 2.4 – 3.7 log₁₀ cfu/g. Lethality was higher to cells in inoculum placed on the skin, compared to the stem scar tissue. Populations of *Salmonella* on strawberries treated with 8.0 mg of ClO₂/L were reduced by 3.8 – 4.4 log₁₀ cfu/g. A significant reduction of 1.5 log₁₀ cfu/g of raspberries was also achieved. Treatment with 4.1 – 8.0 mg of ClO₂/L caused reductions in populations of yeast and molds on blueberries, strawberries, and raspberries of 1.4 – 2.5, 1.4 – 4.2, and 2.6 – 3.0 log₁₀ cfu/g, respectively. Lethality of ClO₂ to *Salmonella*, yeasts, and molds was higher when fruits were treated at 75 – 90% relative humidity than at lower relative humidity. Treatment with 4.1 mg/L ClO₂ did not markedly affect the sensory quality of fruits stored for up to 10 days at 8°C. Results indicate that gaseous ClO₂ has promise as a sanitizer for small fruits.

***LISTERIA MONOCYTOGENES* SURVIVAL IN REFRIGERATOR DILL PICKLES** (J. Kim, E. M. D'Sa, M. A. Harrison, J. A. Harrison, and E. L. Andress)

Listeria monocytogenes can survive and grow in refrigerated foods with pH levels of approx. 4.0-5.0 and salt concentrations of 3-4%. Refrigerator dill pickles fit this description. Contamination of this

product with *L. monocytogenes* could cause serious problems since these items are not heated prior to consumption. This study determined whether *L. monocytogenes* survives and grows in refrigerator dill pickles at three salt levels (1.3, 3.8, and 7.6%). Cucumbers were inoculated with *L. monocytogenes*. Brine mixtures were poured over the cucumbers and they were held at room temperature for one week and then stored under refrigeration for up to 3 months. The pH and percent NaCl and total aerobic, psychrotrophic, lactic acid bacteria, and *Listeria* counts were measured following the addition of brine, at 2, 4, and 7 days, during storage at room temperature, and then later at weekly intervals during refrigerated storage. There was a rapid decrease in pickle pH after four days at room temperature (from 6.2-6.3 to 4.4-4.8) followed by a gradual decrease. The percent NaCl in the pickles increased only slightly while held at room temperature from 0 to 0.101, 0.234, and 0.448% in 1.3, 3.8, and 7.6% salt mixtures, respectively. The initial *Listeria* population was 6-7 log cfu/in² on the surface and 4-5 log₁₀ cfu/g internally. There was approximately 1 log increase during fermentation at room temperature followed by a population decline during refrigerated storage, with a greater decrease in the pickles with the highest NaCl content. Populations of total aerobes and lactic acid bacteria increased. Based on old recommendations consumption of refrigerator dill pickles could typically be anytime after 3 days of refrigerated storage. Since *L. monocytogenes* may still be viable well after this point, there is a food safety risk and no recommendations to prepare this product in the home should be distributed.

MICROFLORA ON GEORGIA-GROWN CANTALOUPE RELATED TO PACKAGING AND HANDLING PRACTICES (E. D. Akins, M. A. Harrison, and W. C. Hurst)

In recent years, there has been foodborne illness outbreaks associated with the consumption of cantaloupe. Contamination of cantaloupes with microorganisms could occur anywhere from the field to the packing line. Cantaloupes are handled and packed differently in various regions of the United States. Typically, California cantaloupes are field packaged while in Georgia they are brought to sheds, washed, and packed. The objective of this study was to determine the number of microbes that are on cantaloupes coming out of the field, after washing, and after packing.

Four Georgia growers with packing facilities, who use slightly different variations in product handling, were visited four times during the 2004 cantaloupe harvest season. For each visit, 20 cantaloupes were sampled from each of the following steps: after transport from the field, after washing, and after packing. The washing method varied among the facilities with 2 using chlorinated water, 1 using heated water, and 1 using a combination of heat and chlorinated water.

There was a slight, but significant decrease in microbial populations between the samples from the field and after washing in dump tanks at the two farms using chlorinated treatments. Exposing cantaloupes to water between 41-50°C (105-122°F) water for 5-10 minutes did not result in a significant change in microbial populations. Similarly, microbial populations on the cantaloupes after packing were approximately the same as that on the prewashed cantaloupes. Washing, chlorination and hot water treatments applied under actual field packing conditions in Georgia do not significantly affect the total aerobic populations on cantaloupes.

MIGRATION OF *CAENORHABDITIS ELEGANS* TO MANURE AND MANURE COMPOST AND POTENTIAL VECTORING OF *SALMONELLA* NEWPORT TO FRUITS AND VEGETABLES (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

It is not uncommon for animal manure and manure compost to be applied to cropland soil as fertilizers. The application of manure and manure compost to soil may attract nematodes that feed on bacteria. Free-living, microbivorous nematode populations have been reported to increase in soils to which cattle manure slurry has been applied. The extent to which various types of manure and manure composts are incorporated into the soil can influence populations of nematodes. Sand homogeneously amended with a humus-litter mixture has been reported to support higher populations of *Caenorhabditis*

elegans compared to sand containing isolated patches of the humus-litter mixture. It is hypothesized that free-living nematodes such as *C. elegans* and possibly other genera may ingest human pathogens occasionally found in the soil and transport them through the soil matrix. As a worm migrates through soil it may come in contact with external tissues of plants, either by attraction mechanisms or by random chance. A study was undertaken to determine if *C. elegans* is attracted to bovine manure, turkey manure, composted bovine manure, composted turkey manure, and manure-amended soil inoculated with *Salmonella* Newport. Survival and reproduction of *C. elegans* in the same matrices not inoculated with *S. Newport* were investigated. Movement of *C. elegans* to lettuce, strawberries, and carrots on an agar medium and the ability of the nematode to transport *S. Newport* in soil to the surface of produce were also studied.

C. elegans moved most rapidly to turkey manure and strawberries, with 35% and 60% of worms, respectively, associating with samples within 30 min. Survival and reproduction of *C. elegans* in test materials was not affected by the presence of *S. Newport*. Bovine manure and bovine manure compost inoculated with *S. enterica* serotype Newport (8.6 log₁₀ cfu/g) were separately placed in the bottom of a glass jar and covered with a layer of soil (5 cm) inoculated (50 worms/g) or not inoculated with *C. elegans*. A piece of lettuce, strawberry, or carrot was placed on top of the soil before jars were sealed and held at 20°C for up to 10 days. In the system using soil inoculated with *C. elegans*, *S. Newport* initially in bovine manure was detected on the surface of lettuce, strawberry, and carrot samples within 3, 1, and 1 days, respectively. The pathogen was detected on lettuce, strawberry, and carrot within 1, 7, and 1 days, respectively, when initially present in bovine manure compost. With one exception, the pathogen was not detected on the produce over the 10-day incubation period when *C. elegans* was not present in the soil. Results indicate that *C. elegans* has the potential for transporting pathogens in soil to the surface of preharvest fruits and vegetables in contact with soil.

INFECTION OF CANTALOUPE RIND WITH *CLADOSPORIUM CLADOSPORIODES* AND *PENICILLIUM EXPANSUM*, AND ASSOCIATED MIGRATION OF *SALMONELLA* POONA INTO EDIBLE TISSUES (G. M. Richards and L. R. Beuchat)

Cantaloupe fruits are often in contact with the ground during their development, enhancing the potential for contamination by microorganisms capable of causing human diseases. They are susceptible to postharvest fungal rots, especially under warm, wet conditions. Complete loss of the commodity occurs when one or a few fungal pathogens invade and begin to breakdown the tissues. We undertook a study to determine if the growth of two molds known to cause decay of cantaloupes, *Cladosporium cladosporioides* and *Penicillium expansum*, in wounds on rinds facilitate migration of *Salmonella* Poona into sub-surface mesocarp tissues.

Two phytopathogens, *Cladosporium cladosporioides* and *Penicillium expansum*, in wounds on cantaloupe rinds, were studied to assess their potential to facilitate 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) of inoculated tissue extending from the rind surface into edible tissues was removed and cut transversely into four segments (0 – 1, 1 – 2, 2 – 3, and 3 – 4 cm) representing distances from the rind surface. Regardless of the type of inoculum or the time of storage subsequent to inoculation, the pH of the tissues was significantly higher ($P \leq 0.05$) as the distance from the rind surface increased. Test microorganisms and naturally-occurring microorganisms on the rind surface which were introduced into internal tissues during wounding, as well as physiological changes in cantaloupe tissue, contributed to these changes. *C. cladosporioides* and *P. expansum* were recovered from the inoculated rind and underlying tissues throughout storage at 20°C for 10 days. *S. Poona* persisted and grew in wounds on rinds on inoculated cantaloupe incubated at 20°C. Recovery of *S. Poona* from tissues 3 – 4 cm below the inoculated wound supports the hypothesis that it can migrate from the site of inoculation into adjacent mesocarp tissues. Survival and migration of *S. Poona* into 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 enteric pathogens may still be present in remaining tissues.

METABIOTIC ASSOCIATIONS OF MOLDS AND *SALMONELLA* POONA ON INTACT AND WOUNDED CANTALOUPE RIND (G. M. Richards and L. R. Beuchat)

Several national and international outbreaks of salmonellosis have been epidemiologically linked to consumption of fresh cantaloupes. 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. Mesocarp tissues of fruits are particularly subject to contamination when rind surface integrity is compromised by disease, bruising, cutting, or peeling. Infection of cantaloupes by plant pathogenic fungi and contamination with foodborne pathogenic bacteria may occur before harvesting, at the time harvest, during handling, storage, transport, and marketing, or after purchase by the consumer. The behavior of foodborne pathogens such as *Salmonella* on or in cantaloupes as affected by metabiotic activities of plant pathogens has not been investigated. The objective of this study was to examine the association between selected molds pathogenic to cantaloupes and *Salmonella* 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.

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*. 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 were determined. *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, thus favoring survival and growth of salmonellae. Intact and mechanically wounded tissues 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. 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 log₁₀ cfu/sample (ca. 7 cm²) of cantaloupe rind to populations as high as 9.5 log₁₀ cfu/sample during storage at 20°C for up to 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 persistence and growth of *S. Poona* on intact, wounded, and decaying cantaloupe rind is not affected by the presence of molds.