Journal of Food Protection, Vol. 68, No. 7, 2005, Pages 1352–1358

Effect of Relative Humidity on Preharvest Survival of Bacterial and Viral Pathogens on the Surface of Cantaloupe, Lettuce, and Bell Peppers

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MS 04-443: Received 29 September 2004/Accepted 1 March 2005

ABSTRACT

The purpose of this study was to compare the effects of humidity on the preharvest survival of microbial pathogens on cantaloupe, lettuce, and bell peppers. An additional goal was to evaluate *Clostridium perfringens* as an indicator of fecal contamination on produce. The microorganisms used in this study included *Escherichia coli, E. coli* O157:H7, *Shigella sonnei, Salmonella enterica* subsp. *enterica, Clostridium perfringens,* hepatitis A virus (HAV), feline calicivirus (FCV), and coliphage PRD1. The study took place in a controlled environment chamber that allowed for the control of temperature (18 to 26°C) and relative humidity. Survival rates under high (mean, 85.7 to 90.3%) and low (mean, 45.1 to 48.4%) relative humidity were compared. The surfaces of the edible portion of each plant were inoculated with the study microorganisms. Samples were collected throughout 2 weeks. More microorganisms survived significantly longer ($P < 0.05$) on cantaloupe than on lettuce and bell peppers. The type of produce on which each organism experienced the highest inactivation rate tended to change with relative humidity. The survival of microorganisms on produce surfaces was not uniformly affected by relative humidity. Of the studied microorganisms, HAV, PRD1, and *C. perfringens* were found to have the lowest inactivation rates, whereas FCV and *E. coli* ATCC 25922 tended to become inactivated most rapidly. *C. perfringens* generally survived longer than all other bacteria and FCV in all experiments. This trend suggests that *C. perfringens* may be an acceptable indicator of bacterial contamination and survival in various environments and on different types of crops.

It is currently estimated that the incidence of foodborne illness in the United States reaches 76 million cases per year. Of these, 325,000 led to hospitalizations, whereas 5,000 led to death. It has been estimated that viruses and bacteria cause 67 and 30% of these illnesses, respectively *(42)*. A growing trend in the consumption of fresh fruit and vegetables has brought with it an increase in foodborne outbreaks associated with such foods *(55)*.

Numerous fruits and vegetables have the potential to be contaminated with pathogenic microorganisms. Outbreaks of foodborne illness associated with cantaloupe have been caused by several serovars of *Salmonella enterica* during the past decade *(25, 43)*. In 2002, the U.S. Food and Drug Administration (FDA) issued an import alert on cantaloupes from Mexico after three multistate outbreaks of *S. enterica* serotype Poona occurred consecutively each spring from 2000 to 2002 *(16)*. Both *Shigella sonnei* and *Escherichia coli* O157:H7 outbreaks have been traced to lettuce *(32, 34)*. Hepatitis A virus (HAV) was determined to be the cause of a large foodborne outbreak in the United States associated with green onions in 2003 *(17)*. Iceberg lettuce *(48)*, raw blueberries *(12)*, and frozen strawberries *(20)* have also been implicated in HAV outbreaks. Norovirus outbreaks have been caused by the contamination of ready-to-eat foods, such as minimally processed and raw fruits and vegetables *(4, 18, 24, 37, 40, 44)*. Many of the foodborne pathogens associated with outbreaks that involved produce, such as HAV and *E. coli* O157:H7, have relatively low infectious doses *(41, 52)*.

Potential sources of preharvest contamination of fresh produce by pathogenic microorganisms may include feces, soil, wild and domestic animals, human handling, and inadequately composted manure *(8)*. It has also been shown that crops may become contaminated with enteric microorganisms through irrigation with wastewater *(47, 50)*. Such contamination of irrigation water has been found to occur due to human sewage pollution *(19, 20, 33, 39)*.

The objective of this study was to evaluate the effect of relative humidity on the survival of enteric pathogens on the surface of fruits and vegetables under preharvest conditions. Cantaloupe, lettuce, and green peppers were chosen for this study because they represent produce with varying surface textures, amounts of foliage, and overall plant structure. Cantaloupe and lettuce were also selected because of their association with viral and bacterial outbreaks. *E. coli* O157:H7 ATCC 43894, *E. coli* ATCC 25922, *S. sonnei* ATCC 9290, *Salmonella* Typhimurium ATCC 43971 (also known as *Salmonella enterica* subsp. *enterica*), HAV, and coliphage PRD1 were chosen for this study because of their association with foodborne outbreaks and their use as sur-

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TABLE 1. *Daily environmental conditions in the controlled environment chamber during experiments*

Crop	Condition	Inoculation date	Ending date	Air temperature (°C)			Relative humidity (%)			Light intensity (W/m^2)	CO_2 level (ppm)
				Maximum	Minimum	Mean	Maximum	Minimum	Mean		
Cantaloupe	Dry	10/16/02	10/30/02	26.5	18.0	22.7	80.1	30.8	47.1	282.8	1,130.4
	Humid	09/25/02	10/09/02	30.6	18.0	22.7	99.6	39.2	90.3	214.9	1,368.8
Lettuce	Dry	01/21/03	02/04/03	26.0	21.2	24.8	68.6	28.6	45.1	348.9	826.9
	Humid	02/13/03	02/27/03	26.0	16.4	24.6	96.7	67.7	85.7	348.8	1,046.6
Bell pepper	Dry	05/30/03	06/13/03	31.2	21.6	24.8	75.6	37.0	48.4	316.2	968.0
	Humid	06/16/03	06/30/03	28.1	22.6	24.8	95.7	53.9	86.1	285.2	996.7

rogates for pathogens. Because human caliciviruses, such as Norwalk virus, cannot be grown under in vitro conditions, feline calicivirus (FCV) was selected as a surrogate. Previously, FCV was used as a surrogate by other researchers *(2, 10, 23)*.

An additional goal of this research was to evaluate *Clostridium perfringens* as an indicator of fecal contamination on produce. An acceptable fecal indicator organism is currently unavailable due to the possibility that plant materials may serve as a natural reservoir for traditional indicators such as coliforms *(36)*. In addition, hardier enteric pathogens, such as protozoan parasites and enteric viruses, experience longer extraenteral survival than some traditional indicators such as coliforms *(28)*.

C. perfringens is a sulfite-reducing anaerobic spore-forming bacterium that is exclusively fecal in origin *(28)*. The spores can survive in the environment for extended periods, are heat resistant, and are resistant to disinfection *(28)*. They have been found to survive longer on produce and in soil than *E. coli* and coliphage *(38)*. For these reasons, *C. perfringens* was proposed as an indicator of fecal contamination of produce. It was first proposed as an indicator of fecal contamination of water in 1899 *(11)*. *C. perfringens* has been found to survive much longer than both coliforms and fecal coliforms in water *(30)*.

MATERIALS AND METHODS

Preparation of microorganisms. Bacteria and viruses used in this experiment were obtained from the American Type Culture Collection (ATCC, Rockville, Md.) or from the University of Arizona Department of Soil, Water, and Environmental Science culture collection. Media were obtained from BBL, Becton Dickinson (Sparks, Md.) unless otherwise stated. *E. coli* O157:H7 ATCC 43894, *E. coli* ATCC 25922, *S. sonnei* ATCC 9290, and *S. enterica* subsp. *enterica* ATCC 43971 were grown for 18 to 24 h in tryptic soy broth at 37°C. *C. perfringens* ATCC 3624 was grown in cooked meat media for 18 to 24 h at 37°C. Duncan-Strong raffinose (Sigma Chemical Co., St. Louis, Mo.) was used to sporulate *C. perfringens* as previously described *(57)*. Coliphage PRD1 was propagated in *Salmonella* Typhimurium ATCC 19585 *(31)*. The HAV strain HM175 and FCV were propagated in the fetal rhesus kidney-derived (Frhk-4) cell line and Crandell's feline kidney (CRFK) cell line, respectively *(22, 29, 54)*.

Controlled environment chamber. A controlled environment chamber was designed, constructed, and tested for the present study. The experiment was performed at the Controlled Environmental Agricultural Center (CEAC) of the University of Arizona, Tucson, Ariz. The chamber allowed for the control of environmental conditions, such as temperature, relative humidity, light intensity, and carbon dioxide levels. Environmental conditions were selected to represent humidity and temperatures typical of various growing conditions in the United States and Central America. The dimensions of the chamber were 3.66 by 3.05 by 2.44 m. Before inoculation, appropriate time was allowed for the conditions to reach desired levels. Two experiments were conducted with each plant type to compare the effect of relative humidity on microbial survival for a total of six experiments. Triplicate samples of each plant type for each organism were collected. Because of the costs and magnitude of each environmental condition, it was not possible to replicate each environmental condition. Two dehumidifiers and two humidifiers were set up in the growth chamber to control relative humidity. High and low levels of relative humidity were used. Environmental conditions are described in Table 1. Environmental data were recorded in a data logger (Datalogger 21X, Campbell Scientific Inc., Logan, Utah) every 5 min.

A 400-W high-pressure sodium (PL780N, PL Lighting Systems, Beamsville, Ontario) light source for the controlled environment chamber was programmed with a 12-h photoperiod to simulate natural conditions. The wavelength of the light ranged from 346 to 1,003 nm, with an average value of 690 nm. Light intensity over the crop surfaces fluctuated close to 300 W/m^2.

Plant samples. Cantaloupe (*Cucumis melo* var. *reticulates*, Mission variety hybrid, Willhite Seed Inc., Poolville, Tex.), iceberg lettuce (*Lactuca sativa*, Beacon variety, Paragon Seed Inc., Salinas, Calif.), and bell peppers (*Capsicum* spp., California Wonder, Willhite Seed Inc.) were planted in a greenhouse near the CEAC and irrigated hydroponically. The plants were grown to maturity and transferred to the controlled environment chamber on the day they were to be inoculated. Once inside the controlled environment chamber, a hydroponic system was used for irrigation.

Plant inoculation. Inocula were applied to the surface of the cantaloupe and bell pepper fruit. Lettuce leaf surfaces were inoculated in the lettuce experiments. Before inoculation, square boundaries (3 by 3 cm) were placed on each plant to identify the inoculated portions of the plant. The boundaries were positioned in such a way as to receive the most direct light from the overhead lights. Permanent markers were used to draw the squares on lettuce and bell pepper. Due to the hydrophobicity of the cantaloupe, caulk (no. 12585, Ace Hardware, Oak Brook, Ill.) was used to create boundaries that would prevent the inoculum from moving out of the specified area. For uniformity, leaves were arranged in a way to prevent inoculated areas from being shaded.

Because of limited space on the cantaloupe surface, only four squares were placed on each plant. Each square was inoculated with two microorganisms. The microorganisms were paired as follows: (i) *C. perfringens* and *S. sonnei*, (ii) PRD1 and HAV, (iii)

E. coli ATCC 25922 and FCV, and (iv) *S. enterica* subsp. *enterica* and *E. coli* O157:H7. A micropipetter was used to spot inoculate the designated areas of the fruit with 50 μl of stock of each organism. Lettuce and bell peppers were inoculated in the same manner.

Light exposure control. A control experiment was conducted to determine the effect of light intensity on microbial survival. This experiment was performed in the controlled environment chamber with each of the previously described fresh produce experiments. Three light conditions were evaluated, including full light exposure, shaded exposure, and no direct exposure. A micropipetter was used to inoculate petri dishes with 100 μl of *E. coli* ATCC 25922. Petri dishes were placed on top of a table for full exposure to the light; for shaded exposure, dishes were placed underneath the shade created by plant leaves. For no direct exposure, the petri dishes were placed under the plastic containers in which the cantaloupe plants were contained or under a dark plastic cover in the case of lettuce and bell peppers.

Sample collection and recovery of microorganisms. Background samples were collected before each plant inoculation to determine if select microorganisms were already present on the produce. *E. coli* O157:H7, *E. coli* ATCC 25922, *S. sonnei*, and *S. enterica* subsp. *enterica* were chosen for this purpose. It was assumed that the absence of these microorganisms would indicate the absence of all other microorganisms used in the study.

Inoculated cantaloupe and bell pepper fruit, as well as lettuce leaves, were collected on days 0, 1, 3, 5, 7, 10, and 14 or until microbial numbers were below the detection limits for two consecutive sampling dates. Triplicate samples were collected in plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, Wis.) on each sampling date and stored on ice during transport to the laboratory. Light exposure control samples were collected in an identical manner.

The inoculated areas (3 by 3 cm) were removed from each plant sample using sterile paring knives. The approximate weight of the removed sections of the plant sample for cantaloupe, lettuce, and bell peppers were 15, 0.5, and 4 g, respectively. Each removed portion of the plant sample was placed in a plastic bag with 50 ml of elutant and shaken for 20 min on a horizontal shaker at approximately 200 strokes per minute (New Brunswick Scientific Company, New Brunswick, N.J.). Beef extract (3%) was used as an elutant for PRD1-HAV and *E. coli* ATCC 25922–FCV samples *(6)*, whereas 0.01 M phosphate-buffered saline (PBS) with a pH of 7.0 was used for *C. perfringens–S. sonnei* and *E. coli* O157:H7–*S. enterica* subsp. *enterica* samples *(58)*. After shaking, the 3% beef extract and PBS were removed and collected. Beef extract pH was adjusted to 7 to 8. For the *E. coli* ATCC 25922 light exposure control samples, petri dishes were placed in plastic bags with 0.01 M PBS and shaken for 20 min in the previously described manner.

Microbial analysis. *E. coli* ATCC 25922 was assayed using the Colilert quanti-tray system (IDEXX, Westbrook, Mass.). *E. coli* O157:H7 and *S. enterica* subsp. *enterica* were assayed by the spread plate method using Hektoen agar, whereas *S. sonnei* was assayed using XLD agar. *C. perfringens* was assayed using m-CP media (Acumedia, Baltimore, Md.) as previously described *(5)*. All bacterial samples were incubated on their respective media for 18 to 24 h at 37°C. PRD1 coliphage was assayed using the plaque-forming unit method with the bacterial host *Salmonella* Typhimurium ATCC 19585 on tryptic soy agar incubated for 18 to 24 h at 37°C *(31)*.

Both HAV and FCV were assayed using the Frhk-4 and CRFK cell lines, respectively *(22, 29, 54)*. Briefly, cell lines were grown into confluent monolayers in plastic tissue culture flasks in Eagle's minimum essential medium (MEM) with 10% fetal bovine serum. The growth media was decanted and Tris-buffered saline (Sigma Chemical Co., St. Louis, Mo.) was used to rinse the monolayer. Cell monolayers were then inoculated with samples, covered with MEM (2% fetal bovine serum), and incubated at 37°C in 5% CO_2. For the CRFK cell line, MEM was supplemented with 200 μl/liter of sodium pyruvate. Both viruses were quantified using the Reed-Muench $TCID_{50}$ method *(46)*.

Statistical analysis. Microbial inactivation rates were determined using the equation $(N_t/N_0) = 10^{-k_d t}$, where N_t is the density of surviving microorganisms (number per square centimeter) at time t, N_0 is the initial density of microorganisms (number per square centimeter), t is time (days), and k_d is the inactivation rate (1/day). Inactivation rates and their standard deviations were calculated using Minitab Statistical Software release 13.32 (Minitab Inc., State College, Pa.). Analysis of variance of inactivation rates using standard deviation was conducted using Microsoft Excel Version 9 (Microsoft Corporation, Redmond, Wash.). Values below the detection limit were considered to be at the detection limit. Days in which all triplicate samples were below the detection limit were not included in the analysis. Differences between inactivation rates were considered statistically significant if $P < 0.05$.

RESULTS

Bacterial and viral survival on produce surfaces. Microbial inactivation rates are reported in Table 2. Inactivation rates were used to compare the survival of microorganisms on the various produce surfaces and levels of relative humidity. The surface of cantaloupe favored longer survival for most microorganisms (7 of 8 organisms) than lettuce or bell pepper. This trend reached significance ($P < 0.05$) for PRD1, HAV, FCV, and *E. coli* ATCC 25922. Only one organism, *S. enterica* subsp. *enterica*, experienced its lowest inactivation rate on lettuce, which was statistically significant ($P < 0.05$). *E. coli* O157:H7 had a significantly higher inactivation rate ($P < 4.50 \times 10^{-5}$) on lettuce compared with cantaloupe and bell pepper, regardless of relative humidity.

In dry conditions, most organisms (7 of 8 organisms) experienced their lowest inactivation rates on the surface of cantaloupe, of which values were statistically significant ($P < 0.05$) for PRD1, HAV, FCV, and *C. perfringens.* No clear trend of microbial survival was observed in humid conditions. When the two relative humidity conditions were compared, it was observed that most of the microorganisms survived longer on the surfaces of lettuce (5 of 8 organisms) and bell peppers (6 of 8 organisms) in dry conditions, of which the survival of PRD1, HAV, and *E. coli* ATCC 25922 on lettuce and the survival of *E. coli* ATCC 25922 on bell peppers were statistically significant ($P < 0.05$). *S. enterica* subsp. *enterica* survived significantly longer in humid conditions on lettuce ($P = 0.019$). *C. perfringens* had a significantly higher inactivation rate ($P = 0.005$) in humid conditions on green peppers. Overall, no trend exists for the effect of relative humidity on microbial survival on the surface of cantaloupe. On cantaloupe, *E. coli* O157:H7 and *S. enterica* subsp. *enterica* experienced a significantly

TABLE 2. *Inactivation rates for microorganisms on produce surfaces at 18.0 to 30.6°C*[a]

	Cantaloupe				Lettuce				Bell pepper			
	Dry[b]		Humid[c]		Dry		Humid		Dry		Humid	
Organism	k_d (1/day)	R^2	k_d (1/day)	R^2	k_d (1/day)	R^2	k_d (1/day)	R^2	k_d (1/day)	R^2	k_d (1/day)	R^2
PRD1	0.03 ± 0.01	0.50	0.55 ± 0.09	0.76	0.09 ± 0.02	0.66	0.25 ± 0.08	0.39	0.14 ± 0.03	0.67	0.08 ± 0.02	0.47
Hepatitis A virus	0.01 ± 0.03	0.50	0.06 ± 0.04	0.20	0.12 ± 0.03	0.57	0.29 ± 0.05	0.71	0.11 ± 0.04	0.31	0.18 ± 0.02	0.76
Feline calicivirus	0.28 ± 0.05	0.71	1.11 ± 0.33	0.70	1.13 ± 0.18	0.87	1.06 ± 0.28	0.74	0.63 ± 0.19	0.58	0.80 ± 0.16	0.76
Escherichia coli O157:H7	0.27 ± 0.05	0.64	0.07 ± 0.05	0.14	4.51 ± 0.38	0.98	4.90 ± 0.12	1.00	0.32 ± 0.16	0.27	0.33 ± 0.24	0.20
Escherichia coli ATCC 25922	0.37 ± 0.14	0.36	0.20 ± 0.06	1.00	1.09 ± 0.46	0.48	5.00 ± 2.13	0.73	0.79 ± 0.22	0.60	5.08 ± 1.11	0.91
Shigella sonnei	0.22 ± 0.03	0.71	0.22 ± 0.10	0.53	0.43 ± 0.35	0.20	2.46 ± 1.57	0.55	1.16 ± 1.45	0.24	1.48 ± 1.27	0.41
Salmonella enterica	0.24 ± 0.04	0.69	0.13 ± 0.03	0.63	0.35 ± 0.15	0.30	0.06 ± 0.03	0.17	0.20 ± 0.10	0.21	0.78 ± 0.51	0.31
Clostridium perfringens	0.04 ± 0.02	0.22	0.13 ± 0.06	0.30	0.14 ± 0.02	0.72	0.10 ± 0.04	0.23	0.14 ± 0.03	0.74	0.08 ± 0.01	0.66

[a] Values are mean ± standard deviation.
[b] Mean relative humidity ranged from 45.1 to 48.4%.
[c] Mean relative humidity ranged from 85.7 to 90.3%.

lower inactivation rate ($P = 0.007$) in humid conditions, whereas PRD1 and FCV survived significantly longer in dry conditions. Of the evaluated microorganisms, PRD1, HAV, and *C. perfringens* tended to survive the longest. Although not statistically significant in all instances, *C. perfringens* had lower inactivation rates than nearly all other bacteria in all experiments. PRD1 experienced a similar trend, having lower inactivation rates than most non–spore-forming bacteria, except on cantaloupe in humid conditions. Both *C. perfringens* and PRD1 had significantly lower rates of inactivation ($P < 0.05$) than *E. coli* ATCC 25922, except on cantaloupe in humid conditions, as well as significantly lower inactivation rates ($P < 0.05$) than FCV in all experiments. HAV had significantly lower inactivation rates than *E. coli* ATCC 25922 and FCV in all experiments. PRD1, HAV, and *C. perfringens* had significantly lower rates of inactivation than all other organisms on cantaloupe in dry conditions, as well as *E. coli* O157:H7 on lettuce in dry and humid conditions. In humid conditions, *C. perfringens* survived significantly longer than HAV on lettuce and bell peppers ($P < 0.008$) and PRD1 on both lettuce and cantaloupe ($P < 0.043$).

Overall, *E. coli* ATCC 25922 and FCV tended to inactivate most rapidly. In humid conditions, all microorganisms survived significantly longer than FCV on cantaloupe. PRD1 had a significantly higher rate of inactivation than all other organisms on cantaloupe in humid conditions, except for FCV. In humid conditions, *E. coli* ATCC 25922 experienced the highest inactivation rate of all other organisms on green peppers, which was statistically significant. *E. coli* O157:H7 experienced the most rapid inactivation rate on lettuce in dry conditions. This rate reached statistical significance.

***E. coli* ATCC 25922 survival in light exposure control.** In light exposure control experiments, with the exception of the cantaloupe experiment under humid conditions, samples with full exposure experienced a 99.9% reduction in less than 1 day on the plastic surface of the petri dishes, whereas those with shaded and no exposure reached a 99.9% reduction in less than 3 days. In the cantaloupe experiment under humid conditions, samples with full exposure to light achieved a 3-log reduction in less than 5 days. In the same experiment, samples with no exposure to direct light and shaded samples were inactivated (99.9%) after 10 days. Overall, no difference was observed between samples with no direct exposure to light and those that were shaded.

DISCUSSION

The light exposure control experiments revealed that, under the conditions studied, *E. coli* ATCC 25922 with no direct exposure to light and those that were shaded survived 2 to 5 days longer than those with full exposure to the light. Although there are differences in the surface characteristics of plastic and plant tissue, these results suggest that the degree of light exposure influences the survival of microorganisms in the studied conditions. UV light has previously been found to effectively reduce enteric pathogen populations on surfaces *(27, 59)*, including lettuce leaves *(60)*. Further studies are necessary to determine if shade from plant material such as leaves might afford significantly longer survival to microorganisms on plant surfaces.

The surface of cantaloupe favored the longest survival of all of the evaluated microorganisms except *S. enterica* subsp. *enterica*, which was most persistent on lettuce. Previous research suggests that the rougher or more irregular the surface of produce, the longer viruses are able to survive *(6)*. The surface texture and structure of vegetables also plays an important role in the attachment and survival of bacteria *(35)*. Cantaloupes have irregular lenticles, known as netting, on their rind, thus providing a variety of surfaces where bacteria can attach *(56)*. It has also been suggested that when open, the lenticles might provide additional sites for microorganisms to colonize and might provide protection from disinfection *(26)*. The complexity of cuticular waxes of vegetables surfaces also plays a role in microbial entrapment *(1)*. The more complex the wax structure, the greater the chance of entrapment *(3)*. However, with the exception of *E. coli* O157:H7 and HAV, the type of produce on which each organism experienced the highest inactivation rate changed with relative humidity. This suggests that the ability of the evaluated microorganisms to survive on the surface of cantaloupe, lettuce, and bell peppers is influenced by relative humidity.

Studies have shown that at 20°C HAV experiences the longest survival at low relative humidity *(9, 51)*. In contrast, high relative humidity has been found to afford *Salmonella* longer survival on the surface of tomato plants *(45)*. Although this study found significantly greater survival of HAV in dry conditions on lettuce and *S. enterica* subsp. *enterica* in humid conditions on lettuce and cantaloupe, the overall survival of microorganisms on the surfaces of different types of produce and under different relative humidity is variable.

Some pathogenic microorganisms, such as HAV, are stable in the environment *(53)*. Viruses have previously been shown to survive on the surface of vegetables for more than 2 months under suitable conditions *(7)*. Based on the calculated inactivation rates from this study, 99.9% reduction of HAV could take as long as 822 days in preharvest conditions. The occurrence of microorganisms such as *Salmonella* and *E. coli* on preharvest cantaloupe and in sources of irrigation water has been documented *(13)*. Because of the potential survival of pathogenic microorganisms on produce surfaces, measures should be taken to lessen the exposure of produce to fecal contamination as harvest time approaches. Even if the produce surfaces are not consumed, as with cantaloupe, risk still exists due to the contamination of the edible flesh during slicing *(14, 15)*. Other potential risks include the transfer of pathogens onto the hands of a harvester or consumer *(49)* and kitchen contamination during preparation.

C. perfringens tended to survive longer on the surfaces of fresh produce than other bacteria in both dry and humid conditions, especially fecal coliforms. In some instances, *C. perfringens* survived significantly ($P < 0.05$) longer than hardier pathogens, such as HAV. *C. perfringens* has not been isolated in soils in Arizona by the method used in this

study *(21, 38)*. These characteristics suggest that *C. perfringens* may be an acceptable indicator of bacterial contamination and survival in various environments and on different types of crops.

ACKNOWLEDGMENTS

This work was supported by a Center for Food Safety and Applied Nutrition grant from the FDA (grant FD-U-002109-01). The authors thank Pat Gundy and the Environmental Virology Laboratory at the University of Arizona, Tucson, for their assistance with viral assays.

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