Journal of Food Protection, Vol. 79, No. 8, 2016, Pages 1440–1445
doi:10.4315/0362-028X.JFP-16-075

Research Note

Internalization of *Listeria monocytogenes* in Whole Avocado

YI CHEN,* PETER EVANS, THOMAS S. HAMMACK, ERIC W. BROWN, AND DUMITRU MACARISIN*

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, 5100 Paint Branch Parkway, College Park, Maryland 20740, USA

MS 16-075: Received 16 February 2016/Accepted 4 April 2016

ABSTRACT

In recent years, tree fruits have emerged as a new concern for *Listeria monocytogenes* contamination. The objective of the current study was to evaluate the potential internalization of *L. monocytogenes* from the surface of avocados into the edible portions of the fruit during certain postharvest practices simulated in a laboratory setting. One set of intact avocados was spot inoculated with *L. monocytogenes* on the stem scar, and the second set was hydrocooled in water contaminated with *L. monocytogenes*. Under these experimental conditions, *L. monocytogenes* internalized into the avocado pulp through the stem or stem scar after both spot inoculation and hydrocooling. In avocados spot inoculated with 50, 130, 500, and 1,300 CFU per fruit, bacteria were detected in the edible portion adjacent to the stem scar within 15 days postinoculation during storage at 4°C. In avocados hydrocooled in water containing *L. monocytogenes* at 10^6 and 10^8 CFU/ml, bacteria reached the bottom end of the fruit, and the populations in the edible portion adjacent to the stem scar reached up to 5.90 to 7.19 log CFU/g within 10 to 15 days during storage at 4°C. Dye mixed with inoculum was useful for guiding subsequent sampling, but dye penetration patterns were not always consistent with bacterial penetration.

Key words: Avocado; Internalization; *Listeria monocytogenes*

Listeria monocytogenes is an intracellular foodborne pathogen that can survive and grow in a wide variety of reservoirs such as animals, the natural environment, food, and food processing environments *(22)*. The disease it causes, listeriosis, has a high case fatality rate, especially among immunocompromised and elderly individuals and pregnant women *(22)*. A variety of foods have been associated with listeriosis outbreaks: milk, cheese, fruits, vegetables, ready-to-eat deli meat, and hot dogs *(5, 8, 9, 28)*. In recent years, significant outbreaks and sporadic cases of listeriosis associated with fresh produce commodities have occurred, representing an emerging public health concern. In 2011, cantaloupes contaminated with *L. monocytogenes* caused a multistate outbreak in the United States resulting in 33 deaths, making it the worst listeriosis outbreak in history *(5, 28)*. In 2014, stone fruits, which do not usually support the growth of *L. monocytogenes*, were implicated in a multistate outbreak in the United States *(20)*. In 2015, a multistate outbreak in the United States that was associated with contaminated caramel apples caused 35 illnesses and three deaths *(6)*. Understanding the circumstances that lead to contamination of these foods with *L. monocytogenes* requires careful attention to how these foods are harvested, transported, and prepared.

Although cross-contamination from the fruit surface to the pulp during cutting by the consumer can pose a significant risk, internalization of bacterial pathogens during postharvest processing could also be an important contributing factor to foodborne diseases. Postharvest internalization of *Salmonella* in whole tomatoes was observed during the wet-dump stage, when a large amount of water was absorbed into the apoplastic free space of the fruit tissues. Apertures in tomato surfaces such as wounds, the stem scar, and lenticels located at the rim of the stem attachment area became infiltrated with water due to the temperature differential, dwell time, hydrostatic pressure, and the presence of residual surfactants *(1, 2, 10, 26, 30)*. Internalization of *Salmonella* through the tomato stem scar can also occur when the fruit is placed on a contaminated moist surface, even without immersion in water. Water infiltration into hydrocooled strawberries enabled passive internalization of phytopathogens suspended in water *(13)*. Peppers also take up water during hydrocooling *(10)*. Penteado et al. *(29)* found that water intake during hydrocooling of mangos resulted in *Salmonella* internalization into the fruit pulp. Similarly, *E. coli* O157:H7 was internalized in apples submerged in water containing this pathogen *(3)*. Eblen et al. *(12)* found that spot contamination of stem scars of oranges with a water suspension of *E. coli* O157:H7 and *Salmonella* led to internalization of these bacteria into the fruit.

Avocado is one of the tree fruit commodities that have emerged as a new concern for *L. monocytogenes* contamination. *L. monocytogenes* has been isolated from avocado

* Authors for correspondence. Tel: 240-402-2783; Fax: 301-436-2332; E-mail: yi.chen@fda.hhs.gov (Y.C.), dumitru.macarisin@fda.hhs.gov (D.M.). These authors contributed equally to the conception and writing of this article.

pulp *(14)* and guacamole products *(15)*, and the contamination probably occurred in the production environment. However, the incidence of *L. monocytogenes* on fresh whole avocados has not been reported, possibly because previous surveillance work did not involve fresh whole avocados. Although postprocessing contamination can be a major contributor to the occurrence of *L. monocytogenes* in avocado pulp and guacamole products, research is needed to study the risk of *L. monocytogenes* contamination in fresh whole avocados.

Certain postharvest processing practices can affect the microbial contamination of fresh whole fruits and vegetables. After avocados are received at the packing facility, orchard litter can be removed using water alone or in combination with brushing. In some instances, high-pressure water blasting is necessary to remove insect pests or pollen deposits from the fruit rind *(21, 25)*. Washed fruits, occasionally still wet, are then packed in boxes with or without sheet inserts or liners and immediately transferred to cooling rooms. To reduce fruit water loss that usually causes patches on the skin, moisture is added to the cooling room by spraying water mist into the cooling airstream *(18)*. These procedures will increase the amount of free water on fruit surfaces, which can facilitate the survival and spread of *L. monocytogenes*.

To reduce avocado susceptibility to mechanical damage, physiological disorders, and phytopathogens, avocado fruits are commonly harvested in midafternoon when fruit turgidity is not high *(18)*. On the trees, avocado flesh temperatures well above 40°C have been recorded in direct sunlight even when ambient temperatures are 15 to 25°C *(32)*. Heat from fruit respiration also contributes to a considerable temperature increase in harvested avocados. Hence, rapid fruit precooling after harvest to near final storage temperatures is critical and particularly important for maintaining freshness during long-term storage *(23, 24, 33)*. The rate of cooling has a significant effect on the long-term storage potential, and vacuum cooling and hydrocooling are among the fastest cooling techniques. Avocados can be precooled to storage or transport temperatures in about 8 h by forced-air cooling *(31)* or in about 30 min by hydrocooling *(16)*. Hydrocooling was introduced in avocado packing houses in the 1970s *(4)* and produces rapid, inexpensive, and uniform cooling. Unlike other precooling techniques such as a cooling room, forced air, and vacuum cooling, hydrocooling causes no moisture loss, thereby improving the organoleptic properties of the fruit. Because of these advantages, hydrocooling has been adopted by some domestic and international avocado growers and packing houses, especially for the Hass variety *(23, 33)*, which accounts for about 90% of the world avocado market *(11)*. The ability of *L. monocytogenes* to internalize into avocado pulp during various postharvest practices, especially those that provide opportunities for pathogens in water to come in contact with the fruit, has not been explored. Avocado pulp supports the growth of *L. monocytogenes* *(19)*; therefore, any *L. monocytogenes* that internalizes into avocados could pose a significant health risk. The objective of the present study was to evaluate the impact of certain postharvest practices on the internalization of *L. monocytogenes* into the edible portions of fresh whole avocados.

MATERIALS AND METHODS

Avocado fruits. Bagged Hass avocados (Mission Avocados, Oxnard, CA) were obtained from a local supermarket, and physiologically mature but unripe fruits with no mechanical damage were selected for the study. Each avocado weighed 150 to 180 g.

Bacterial culture. *L. monocytogenes* serotype 1/2b strain AW001, previously isolated from fresh whole avocados in the United States in July 2014, was used for inoculation. The culture was grown at 37°C in brain heart infusion broth for 18 h and subsequently diluted in water to the desired levels.

Spot inoculation of *L. monocytogenes*. The stem scar areas were inoculated with 100 μl of five different levels of *L. monocytogenes* (four low levels of 50, 130, 500, and 1,300 CFU per fruit and one high level of 10^6 CFU per fruit), and the inoculated avocados were subsequently stored at 4°C for up to 15 days to simulate storage conditions following postharvest processing and packing *(23)*. For two of the inoculation levels, 50 and 500 CFU per fruit, avocados were inoculated with both *L. monocytogenes* and 1% acid blue 9 (Chem-Impex International, Wood Dale, IL). The dye does not interfere with growth and colony formation of *L. monocytogenes* (data not shown).

Hydrocooling in the *L. monocytogenes*–dye solution. To simulate field temperatures of avocados exposed to direct sunlight, fruits were heated to 40°C by incubation in an Innova 42R programmable incubator (New Brunswick Scientific, Enfield, CT) with a relative humidity at 92%. Fruit temperature was measured as described elsewhere *(32)*. Prewarmed avocados were then submerged for 20 min in 6°C water containing *L. monocytogenes* and 1% acid blue 9. *L. monocytogenes* was added to the hydrocooling solution to achieve final levels of 10^6 and 10^8 CFU/ml. The high level of inoculum was used to allow enumeration of *L. monocytogenes* populations internalized into avocado flesh. During the hydrocooling process, water temperature was monitored and maintained at 6°C with ANOVA 40 refrigerated circulators (ANOVA Industries, Houston, TX). The avocados were subsequently removed from the water, dried in a biosafety hood for 1 h, and stored at 4°C for up to 15 days.

Fruit sampling and detection and enumeration of *L. monocytogenes* in avocado mesocarp. Fruits were analyzed after spot inoculation and hydrocooling. Before analysis, avocados were submerged in 1.5 liters of 0.525% sodium hypochlorite solution for 5 min to sterilize the fruit surface, washed in sterile deionized water twice, and then dried. The rinsates from the second wash were centrifuged at 3,500 × *g*, and the resulting pellets were resuspended in Butterfield's phosphate buffer (BPB) and plated onto both agar *Listeria* Ottavani & Agosti (ALOA; AEB520080, bioMérieux, St Louis, MO) and RAPID'*L.mono* agar (3563694, Bio-Rad Laboratories, Hercules, CA) to verify that the fruit surfaces were free of *L. monocytogenes* after the hypochlorite treatment. To further verify the efficiency of the surface disinfection method, the total aerobic plate count was determined for pellets resuspended in BPB as described in the *Bacteriological Analytical Manual* (BAM) *(27)*. For qualitative analysis, pulp was enriched in buffered *Listeria* enrichment broth (BLEB) as described in the BAM *(17)*. For quantitative analysis, pulp was homogenized and serially diluted in BLEB, and 150 μl of the pulp-

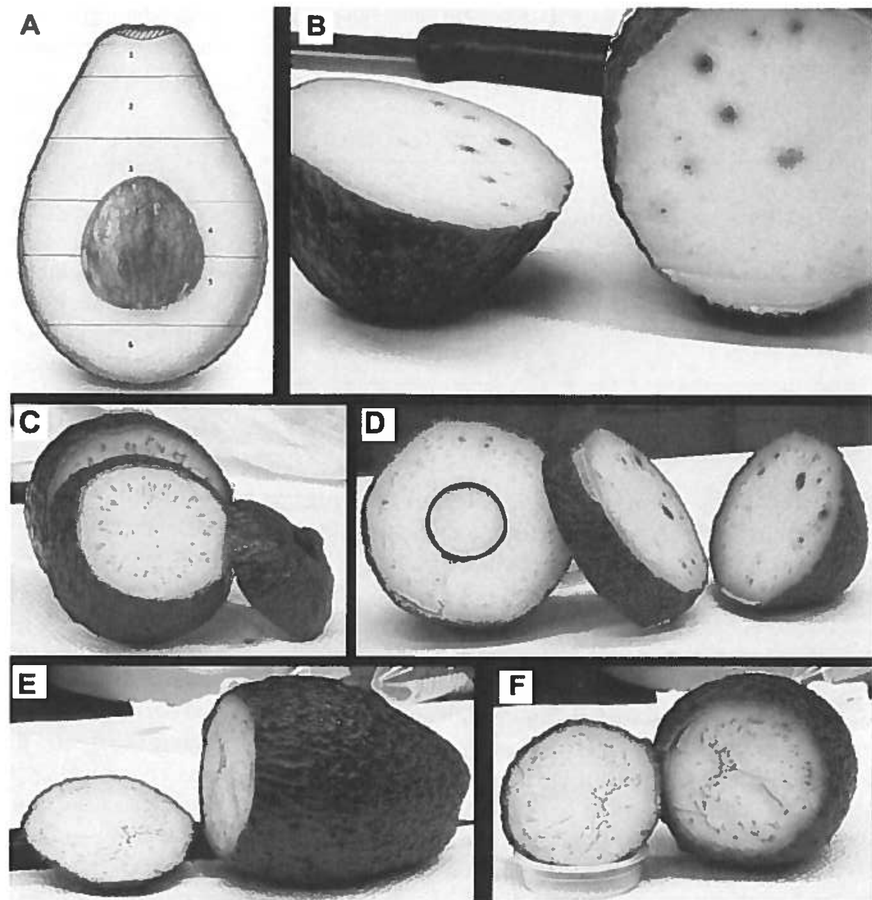

FIGURE 1. *(A) Six areas of avocado mesocarp evaluated in the analysis. (B) Dye permeation into mesocarp after spot inoculation of stem scars with* L. monocytogenes *(50 and 500 CFU per fruit) and dye. (C through F) Dye permeation into mesocarp after hydrocooling of avocados in water containing* L. monocytogenes *at 10^6 CFU/ml.*

broth mixture was spread plated onto four ALOA plates. This direct plating scheme had a lower limit of detection (LOD) of 16.6 CFU/g. The remainder of the pulp-broth mixture was incubated as described in the BAM *(17)* to determine whether any of the samples that were negative by direct plating still had detectable levels of the pathogen.

For spot inoculation at the four low levels (50, 130, 500, and 1,300 CFU per fruit), the pulp was analyzed on days 5 and 15. For spot inoculation at the high level (10^6 CFU per fruit), fruits were sampled on days 5 and 10. For hydrocooling at both levels of inoculum, fruits were sampled on days 5, 10, and 15. *L. monocytogenes* from areas 1, 2, 5, and 6 (Fig. 1A) was also enumerated for each fruit collected on days 10 and 15 after hydrocooling. Three biological replicates were performed for each inoculation level at each time point. The stem scar (nonedible) was removed using a sterile knife, and the avocado was cut transversally starting from the bottom end of the fruit. Edible pulp was collected from six areas at each time point (Fig. 1A).

Statistical analysis. For each time point (days 10 and 15) and each inoculation level, a one-way analysis of variance (ANOVA) was performed on the log CFU values to compare the levels of *L. monocytogenes* in different areas of the avocado after hydrocooling. For each area at each inoculation level, a *t* test was performed on the log CFU values to compare the levels of *L. monocytogenes* between day 10 and day 15 after hydrocooling. Some samples in areas 5 and 6 were positive for *L. monocytogenes* as confirmed after enrichment, but the levels were below the LOD (16.6 CFU/g) for direct plating. For statistical analysis, the level of *L. monocytogenes* in these samples was assumed to be half of the LOD (8.3 CFU/g).

RESULTS

Complete surface disinfection of the avocados that had been inoculated with *L. monocytogenes* by either spot inoculation or hydrocooling in bacterial suspension was critical to prevent pathogen transfer from the rind to the pulp during subsequent sampling. A 5-min wash in 0.525% sodium hypochlorite solution followed by thorough water rinsing provided complete disinfection of the fruit surface, and no *L. monocytogenes* or aerobic bacteria were recovered from the water rinsates. The *L. monocytogenes* strain used in the present study was isolated from a naturally contaminated avocado and was able to grow in avocado pulp but not on the skin, as demonstrated previously *(7)*.

Dye was mixed with the inoculum to assist in the subsequent sampling. Spot inoculation of the stem scar area led to the uptake and spread of the dye solution into the avocado mesocarp in areas 1, 2, and 3 (Fig. 1B); therefore, these three areas were analyzed for *L. monocytogenes.* Two of the three fruits inoculated at the 10^6 CFU per fruit level were positive for *L. monocytogenes* on day 5 in area 1, but no pulp areas were positive in avocados inoculated at lower levels (Table 1). On day 10, all three fruits inoculated at 10^6 CFU per fruit were positive in area 1, and on day 15 two of the three fruits inoculated with lower levels (50, 130, 500, and 1,300 CFU per fruit) were positive in area 1 (Table 1). Even though dye permeated the spot-inoculated fruits up to area 3, *L. monocytogenes* infiltrated only area 1 of the mesocarp.

TABLE 1. *Qualitative analysis of* L. monocytogenes *internalization into avocado fruits after spot inoculation at the stem scar*

Sample day	Inoculation level (CFU/fruit)	No. of positive fruits[a] Area 1	Areas 2 and 3
5	10^6	2	0
10	10^6	3	0
5	50, 130, 500, and 1,300	0	0
15	50 (with dye)	2	0
	130	2	0
	500 (with dye)	2	0
	1,300	2	0

[a] From three replicates. Areas are shown in Figure 1.

Hydrocooling caused the dye solution to permeate much more extensively into the avocado pulp than did spot inoculation. Dye solution permeated the entire fruit (Fig. 1C through 1F) via the stem scar but did not penetrate the fruit skin. All mesocarp areas (areas 1 to 6) were thus analyzed for *L. monocytogenes* internalization as described above. On day 5 after hydrocooling in water containing 10^6 CFU/ml *L. monocytogenes*, area 1 samples from all three replicate fruits were pathogen positive, and samples from all other areas were positive in at least one of the three replicates. On day 5 after hydrocooling in water containing 10^8 CFU/ml *L. monocytogenes*, samples from all areas were pathogen positive. Analysis of the avocado pulp on days 10 and 15 after hydrocooling revealed internalized *L. monocytogenes* in all areas of the mesocarp in all replicates. Enumeration of *L. monocytogenes* in various areas of the mesocarp revealed a gradient in bacterial populations. For each inoculation level at each time point, areas 1 and 2 (adjacent to the stem) were colonized at much higher levels than were areas 5 and 6 (bottom end of the fruit) (Table 2). No significant differences in internalization of *L. monocytogenes* or population growth were observed on days 10 and 15 for fruits hydrocooled in water containing 10^6 or 10^8 CFU/ml *L. monocytogenes*.

DISCUSSION

We hypothesized that water uptake by the fruit and internalization of bacteria occur via the stems or stem scars. To simulate contamination during the washing phase or during postpacking exposure to water condensate, avocados were spot inoculated in the stem scar areas with 100 μl of water containing various levels of *L. monocytogenes*. This study is the first to reveal that spot contamination of stem scar areas of whole avocados can lead to the internalization of *L. monocytogenes* into the edible portions of the fruit.

Experimental contamination of avocados during hydrocooling was conducted with two inoculum levels, 6 and 8 log CFU/ml, of *L. monocytogenes* in the hydrocooling solution. These high inoculum levels were chosen to evaluate the potential for bacterial internalization during hydrocooling and to quantitatively assess the populations of internalized *L. monocytogenes*. All six areas of the mesocarp were permeated by the dye solution (Fig. 1C through 1F) and were positive for *L. monocytogenes* (Table 2). This finding suggests that water influx during hydrocooling, possibly driven by the temperature differential, was strong enough to cause the infiltration of *L. monocytogenes* from the stem end to the bottom end of the avocado. Significantly higher ($P \leq 0.05$) levels of *L. monocytogenes* were recovered from the stem end (areas 1 and 2) than from the bottom end (areas 5 and 6), indicating that pathogen internalization took place through the stem scar and then spread into other areas of the fruit. Bacterial populations reached 6.16 to 7.19 log CFU/g at the stem end and 1.56 to 2.27 log CFU/g at the bottom end of the mesocarp on day 15 after hydrocooling. For fruits at both inoculation levels, in general the levels of *L. monocytogenes* in each area were

TABLE 2. *Qualitative and quantitative analysis of* L. monocytogenes *internalization into areas 1, 2, 5, and 6 of avocado pulp on days 5, 10, and 15 after fruits were hydrocooled in water containing* L. monocytogenes[a]

Sample day	Inoculation level (CFU/ml)	Area 1	Area 2	Area 5	Area 6
No. of positive fruits[b]					
5	10^6	3	2	1	1
	10^8	3	3	3	3
Mean ± SD *L. monocytogenes* (log CFU/g)[c]					
10	10^6	5.90 ± 0.65 A	3.43 ± 1.79 AB	0.92 ± 0 B[d]	0.92 ± 0 B
	10^8	5.51 ± 0.86 A	4.41 ± 2.08 AB	2.34 ± 0.79 BC	0.92 ± 0 C
15	10^6	7.19 ± 0.41 A	2.04 ± 1.32 B	2.17 ± 1.10 B	1.56 ± 1.11 B
	10^8	6.16 ± 0.20 A	5.14 ± 1.36 B	5.57 ± 4.54 B	2.27 ± 1.39 B

[a] Areas are shown in Figure 1.

[b] From three replicates.

[c] *L. monocytogenes* was enumerated in samples from areas 1, 2, 5, and 6 of fruits on days 10 and 15. A one-way ANOVA was performed to compare levels of *L. monocytogenes* between different areas for each time point at each inoculation level. Means with the same letters are not significantly different ($P > 0.05$). A one-way ANOVA was also performed to compare levels of *L. monocytogenes* in each area between day 10 and day 15 and between the two inoculation levels. No significant differences were found; thus, the results are not shown.

[d] For three replicates, samples from this area were positive for *L. monocytogenes* as indicated by subsequent growth in enrichment culture, but results were below the limit of detection (LOD) of direct plating (16.6 CFU/g or 1.22 log CFU/g) and therefore were assumed to be half of the LOD (8.3 CFU/g or 0.92 log CFU/g).

slightly higher at day 15 than at day 10, even though the difference was not significantly different probably because of the limited number of replicates and high individual fruit variability. Iturriaga et al. *(19)* found that *L. monocytogenes* could grow in avocado pulp under refrigeration conditions, with an approximately 1-log increase from day 10 to day 15 in avocado pulp stored at 4 to 7°C. This finding is consistent with our findings.

In the present study, the purpose of using the dye was to track the water uptake and distribution in the avocado mesocarp and to assist with subsequent sampling for microbiological analysis. The dye permeation and bacterial penetration into the mesocarp did not always occur to the same extent, which was not surprising because dye particles were much smaller than the *L. monocytogenes* cells. In spot-inoculated fruits, dye traveled further into the pulp than did *L. monocytogenes* cells (Tables 1 and 2 and Fig. 1); however, during hydrocooling, *L. monocytogenes* travelled as far as did the dye (Table 2 and Fig. 1). All areas that were positive for *L. monocytogenes* also contained dye, which indicates the usefulness of this dye for future studies on bacterial internalization into vegetal matrices.

Herein, we demonstrated for the first time the potential of *L. monocytogenes* to internalize into the mesocarp of avocados following experimental contamination by spot inoculation in stem scar area and by hydrocooling in contaminated water. Water uptake and *L. monocytogenes* infiltration took place through the stem scar and then spread within the mesocarp. As a result, the stem end of the pulp was more heavily colonized by *L. monocytogenes* than was the bottom end. These results also suggest that *L. monocytogenes* is not likely to penetrate the avocado skin. Because the results of the present study were obtained in a laboratory setting, a follow-up study of *L. monocytogenes* internalization in a pilot processing plant would allow more accurate determination of the risk of contamination associated with various postharvest practices used for avocado processing. Enhanced sanitation and control of water quality used for washing and precooling of avocados may prevent avocado colonization by *L. monocytogenes* under certain postharvest conditions.

ACKNOWLEDGMENT

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Food and Drug Administration.

REFERENCES

1. Bartz, J. A. 1982. Infiltration of tomatoes immersed at different temperatures to different depths in suspensions of *Erwinia carotovora* subsp. *carotovora*. *Plant Dis.* 66:302–305.
2. Bartz, J. A. 1999. Washing fresh fruits and vegetables: lessons from treatment of tomatoes and potatoes with water. *Dairy Food Environ. Sanit.* 19:853–864.
3. 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.
4. Campbell, C. 1994. Handling of Florida-grown and imported tropical fruits and vegetables. *HortScience* 9:975–978.
5. Centers for Disease Control and Prevention. 2011. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August–September 2011. *Morb. Mortal. Wkly. Rep.* 60:1357–1358.
6. Centers for Disease Control and Prevention. 2015. Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. apples (final update). Available at: http://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/. Accessed 28 March 2016.
7. Chen, Y. Unpublished data.
8. Chen, Y., W. Zhang, and S. J. Knabel. 2005. Multi-virulence-locus sequence typing clarifies epidemiology of recent listeriosis outbreaks in the United States. *J. Clin. Microbiol.* 43:5291–5294.
9. Chen, Y., W. Zhang, and S. J. Knabel. 2007. Multi-virulence-locus sequence typing identifies single nucleotide polymorphisms which differentiate epidemic clones and outbreak strains of *Listeria monocytogenes*. *J. Clin. Microbiol.* 45:835–846.
10. Corey, K. A., and Z. Y. Tan. 1990. Induction of changes in internal gas pressure of bulky plant organs by temperature gradients. *J. Am. Soc. Hortic. Sci.* 115:308–312.
11. Crane, J. H., G. Douhan, B. A. Faber, M. L. Arpaia, G. S. Bender, C. F. Balerdi, and A. F. Barrientos-Priego. 2013. Cultivars and rootstocks, p. 200–233. *In* B. Schaffer, B. N. Wolstenholme, and A. W. Whiley (ed.), The avocado: botany, production and uses, 2nd ed. CABI, Cambridge, MA.
12. Eblen, B. S., M. O. Walderhaug, S. Edelson-Mammel, S. J. Chirtel, A. De Jesus, R. I. Merker, R. L. Buchanan, and A. J. Miller. 2004. Potential for internalization, growth, and survival of *Salmonella* and *Escherichia coli* O157:H7 in oranges. *J. Food Prot.* 67:1578–1584.
13. Ferreira, M. D., J. A. Bartz, S. A. Sargent, and J. K. Brecht. 1996. An assessment of the decay hazard associated with hydrocooling strawberries. *Plant Dis.* 80:1117–1122.
14. Food Safety News Team. 2011. *Listeria* concern prompts avocado recall. Available at: http://www.foodsafetynews.com/2011/09/listeria-concern-prompts-avocado-recall. Accessed 28 March 2016.
15. Food Safety News Team. 2011. *Listeria* test leads to avocado dip recall. Available at: http://www.foodsafetynews.com/2011/08/listeria-test-causes-avocado-dip-recall. Accessed 28 March 2016.
16. Gaffney, J., and C. Baird. 1975. Susceptibility of West Indian avocados to chilling injury as related to rapid cooling with low temperature air or water. *Proc. Fla. State Hortic. Soc.* 88:490–496.
17. Hitchins, A. D., K. Jinneman, and Y. Chen. 2016. Detection and enumeration of *Listeria monocytogenes* in foods, chap.10. *In* Bacteriological analytical manual. U.S. Food and Drug Administration. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm. Accessed 28 March 2016.
18. Hofman, P. J., J. Bower, and A. Woolf. 2013. Harvesting, packing, postharvest technology, transport and processing, p. 489–540. *In* B. Schaffer, B. N. Wolstenholme, and A. W. Whiley (ed.), The avocado: botany, production and uses, 2nd ed. CABI, Cambridge, MA.
19. Iturriaga, M. H., S. M. Arvizu-Medrano, and E. F. Escartin. 2002. Behavior of *Listeria monocytogenes* in avocado pulp and processed guacamole. *J. Food Prot.* 65:1745–1749.
20. Jackson, B. R., M. Salter, C. Tarr, A. Conrad, E. Harvey, L. Steinbock, A. Saupe, A. Sorenson, L. Katz, S. Stroika, K. A. Jackson, H. Carleton, Z. Kucerova, D. Melka, E. Strain, M. Parish, and R. K. Mody. 2015. Notes from the field: listeriosis associated with stone fruit—United States, 2014. *Morb. Mortal. Wkly. Rep.* 64:282–283.
21. Jamieson, L. E., D. C. Whiting, A. B. Woolf, A. White, and R. M. McDonald. 2000. Water-blasting avocados to remove leafroller eggs, p. 371–374. *In* Proceedings of the 53rd New Zealand Plant Protection Conference, Christchurch, 7 to 10 August 2000.
22. Kathariou, S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *J. Food Prot.* 65:1811–1829.
23. Kok, R. D., J. P. Bower, and I. Bertling. 2010. Low temperature shipping and cold chain management of 'Hass' avocados: investigation into reducing shipping costs. *S. Afr. Avocado Growers Assoc. Yearb.* 33:33–37.
24. Lütge, A., J. P. Bower, and I. Bertling. 2010. An investigation into ultra-low temperature shipping and cold chain management of 'Fuerte' avocados—an opportunity to reduce shipping costs. *S. Afr. Avocado Growers Assoc. Yearb.* 33:39–43.

25. Mandemaker, A. J. 2003. Effect of postharvest water blasting on fruit pesticide residues. *N. Z. Avocado Growers Assoc. Annu. Res. Rep.* 3:66–70.
26. Marvasi, M., G. J. Hochmuth, M. C. Giurcanu, A. S. George, J. T. Noel, J. Bartz, and M. Teplitski. 2013. Factors that affect proliferation of *Salmonella* in tomatoes post-harvest: the roles of seasonal effects, irrigation regime, crop and pathogen genotype. *PLoS One* 8:e80871.
27. Maturin, L., and J. T. Peeler. 2001. Aerobic plate count, chap. 3. *In* Bacteriological analytical manual. U.S. Food and Drug Administration. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm. Assessed 28 March 2016.
28. McCollum, J. T., A. B. Cronquist, B. J. Silk, K. A. Jackson, K. A. O'Connor, S. Cosgrove, J. P. Gossack, S. S. Parachini, N. S. Jain, P. Ettestad, M. Ibraheem, V. Cantu, M. Joshi, T. DuVernoy, N. W. Fogg, Jr., J. R. Gorny, K. M. Mogen, C. Spires, P. Teitell, L. A. Joseph, C. L. Tarr, M. Imanishi, K. P. Neil, R. V. Tauxe, and B. E. Mahon. 2013. Multistate outbreak of listeriosis associated with cantaloupe. *N. Engl. J. Med.* 369:944–953.
29. Penteado, A. L., B. S. Eblen, and A. J. Miller. 2004. Evidence of *Salmonella* internalization into fresh mangos during simulated postharvest insect disinfestation procedures. *J. Food Prot.* 67:181–184.
30. Vigneault, C., J. A. Bartz, and S. A. Sargent. 2000. Postharvest decay risk associated with hydrocooling tomatoes. *Plant Dis.* 84:1314–1318.
31. Watkins, J. B., and S. L. Ledger. 1990. Forced air cooling, 2nd ed. Queensland Department of Primary Industries, Brisbane, Queensland, Australia.
32. Woolf, A. B., J. H. Bowen, and I. B. Ferguson. 1999. Preharvest exposure to the sun influences postharvest responses of 'Hass' avocado fruit. *Postharvest Biol. Technol.* 15:143–153.
33. Yeasrley, C., N. Lallu, J. Burdon, M. Punter, and S. Osman. 2002. Effect of prepacking holding temperatures on shelf life quality of Hass avocados. *N. Z. Avocado Growers Assoc. Annu. Res. Rep.* 2:1–12.