Survival and Growth of Salmonella in High-Moisture Pecan Nutmeats, In-Shell Pecans, Inedible Nut Components, and Orchard Soil

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ABSTRACT

Outbreaks of salmonellosis associated with almonds have raised interest in better understanding the behavior of Salmonella on other tree nuts. We undertook a study to determine the survival and growth characteristics of Salmonella on high-moisture (water activity of 0.96 to 0.99) pecan nutmeats, in-shell pecans, and inedible components (shuck, shell, and middle septum tissue) of in-shell pecans. Salmonella did not grow on high-moisture nutmeat halves, pieces, or granules stored at 4°C for up to 48 h. Growth did occur, however, at 21, 30, and 37°C. Increases of 1.77 to 5.87 log CFU/g of nutmeats occurred within 48 h at 37°C; the order in which nutmeats supported growth was granules > pieces > halves. Populations of Salmonella on and in high-moisture in-shell pecans (kernel water activity of 0.94) stored at 4, 21, 30, and 37°C for 8 days decreased by 0.52 to 1.19 log CFU/g. The pathogen grew on the surface of high-moisture (water activity of 0.99) pecan shucks and shells but died on middle septum tissue stored at 21, 30, and 37°C for up to 6 days. Salmonella died in water extracts of shucks and in pecan orchard soil saturated with water or shuck extract, but survived well for at least 18 weeks in dry soil. The ability of the pathogen to grow on high-moisture nutmeats and some of the inedible components of pecans emphasizes the importance of controlling or limiting the time pecans are exposed to water in preharvest and postharvest environments.

Documented outbreaks of salmonellosis associated with consumption of dry foods have been few compared with those associated with foods with water activity (aw) at levels allowing growth of salmonellae. There is, however, concern that foods with low water activity represent increasingly significant risks as vehicles of foodborne pathogens, particularly Salmonella (41). Notable examples implicating low-water-activity foods of plant origin in outbreaks of salmonellosis include chocolate (16), potato chips seasoned with paprika (26), toasted oat cereal (6), puffed cereal (10), a children’s snack (9), and halva (34). Nuts and nut products have also been associated with outbreaks of foodborne illness. Consumption of desiccated coconut (33, 39, 42, 50) and almonds (7, 22, 25) have been linked to salmonellosis, and cashew nuts have been reported to be a vehicle of Escherichia coli O157:H7, which caused infections (35). In-shell peanuts (24), a savory peanut snack (23, 43), and peanut butter (8, 11, 27, 32, 40) have been associated with outbreaks of salmonellosis. Salmonella has been isolated from almonds (7, 14, 18), pistachios (28), Brazil nuts (20, 29), cashew nuts (20), macadamia nuts (44), walnuts (20, 31, 38), and prepackaged mixed nuts (Brazil nuts, cashew nuts, almonds, peanuts, and walnuts) (29). With the exception of almonds, these tree nuts have not been implicated in outbreaks of salmonellosis.

Pecans have likewise not been associated with outbreaks of foodborne illness. However, exposure of pecans to pre- and postharvest environments imposes some level of risk of contamination with foodborne pathogens. Pecans that have fallen to the ground several days preceding harvest may absorb water from soil, potentially contaminated with foodborne pathogens originating from wild- and domestic-animal feces, inadequately composted manure, irrigation water, or run-off water from land inhabited by livestock. Postharvest exposure of pecans to water containing foodborne pathogens may also occur. Cleaning pecans, i.e., removal of leaves, sticks, stones, soil, and other debris from mechanically harvested nuts, can involve immersion in water. Wetting of in-shell pecans may occur during transport or handling or from leaks in roofs or walls of storage facilities. Pecans are conditioned (tempered) by immersing in cool and/or hot water, spraying with water, or steaming just preceding cracking and shelling. Water used to clean and condition pecans may or may not be chlorinated at a concentration needed to eliminate foodborne pathogens. Nutmeat pieces are separated from inedible components of the nuts after cracking and shelling by a chlorinated water vacuum flotation treatment, then dried to reduce the moisture content to a level that will prevent fungal growth and help to preserve sensory quality. The risk of contamination of pecans with Salmonella and other microorganisms exists at each step in the pre- and postharvest continuum.

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The presence of *E. coli* on pecans was reported as early as 1940 (36). Others have subsequently detected *E. coli* and infiltrated bacteria on pecan nutmeats (4, 12, 21) and in-shell pecans (30). In the latter study, the percent of samples positive for *E. coli* was six-fold higher when nuts were collected from orchards grazed with cattle as compared with samples collected from nongrazed orchards. The practice of grazing cattle and other ruminants in pecan orchards is highly discouraged, yet does exist to some extent in commercial operations as well as in small-scale operations from which nuts enter commercial trade, often through accumulators with minimal capability of providing information that would enable trace-back to the source. Preharvest contact of pecans with feces, water containing feces, or contaminated orchard soil could potentially result in infiltration by foodborne pathogenic microorganisms. *Salmonella* Enteritidis can survive in almond orchard soil for at least 180 days (15), persist for over 5 years (46), and migrate through almond hulls and shells (13). The addition of almond hull extract to almond orchard soil promotes growth of *Salmonella*, but its behavior in pecan orchard soil to which pecan shuck extract has been applied is not known.

*Salmonella* is able to grow at an *a*<sub>w</sub> as low as 0.93 (17), provided sufficient nutrients are available, and temperature and pH are not inhibitory. Uncontrolled or controlled wetting of pecans, either preharvest or postharvest, can result in water uptake and kernel *a*<sub>w</sub> values exceeding 0.93 (3). Given sufficient time, and in the absence of factors that would inhibit growth or cause death of *Salmonella*, populations of the pathogen on in-shell pecans, inedible nut components, or nutmeats could potentially increase to high numbers. Factors affecting survival and growth of *Salmonella* on high-moisture pecans have not been studied.

We undertook a study with three objectives. The first objective was to determine survival and growth characteristics of *Salmonella* in high-moisture pecan nutmeats, in-shell pecans, and inedible components (shuck, shell, and middle septum) of in-shell pecans. The second objective was to determine if survival and growth characteristics of *Salmonella* are affected on exposure to shuck extract and pecan orchard soil containing the extract. The third objective was to determine survival characteristics of *Salmonella* in pecan orchard soil as affected by moisture content. The overall goal was to learn more about the behavioral characteristics of *Salmonella* in pecan nutmeats, in-shell pecans, inedible components of pecans, and pecan orchard soil on exposure to high-moisture conditions simulating those that may occur in pre- or postharvest pecans.

**MATERIALS AND METHODS**

**Source of pecans.** In-shell Desirable variety pecans (*Carya illinoinensis*), pecan shucks, a mixture of pecan shells and middle septum tissue, and nutmeats were obtained from commercial shellers in Georgia. Shucks had been removed from nuts before the nuts were cleaned. Shells and middle septum tissues were separated by hand in the laboratory. Three standard nutmeat grades (48) were used: mammoth halves (250 halves or fewer per pound [551 or fewer per kg]), medium pieces (maximum diameter, 6/16 in. [0.95 cm] and minimum diameter, 3/16 in. [0.48 cm]; i.e., will pass through a round opening 0.95 cm in diameter but not a round opening 0.48 cm in diameter), and granules (maximum diameter, 2/16 in. [0.32 cm] and minimum diameter, 1/16 in. [0.16 cm]). In-shell pecans, shucks, shells, middle septum tissues, and nutmeats were stored at 4°C until used in experiments.

**Source of soil.** Soil from commercial pecan orchards in four southern Georgia counties (Crisp, Lowndes, Mitchell, and Peach) was collected in early May 2009; predominant soil types in these counties are Tifton, Tifton and Pelham, Tifton and Wagram, and Faceville, respectively. Samples were taken from the top 2 cm of soil under the canopies of well-established and managed orchards, dried at 21°C, and stored at 4°C until used in experiments.

**Measurement of moisture content.** The moisture content of kernels removed from in-shell pecans in the laboratory, as well as nutmeats, shucks, shells, and middle septum tissue obtained from commercial shellers, was determined before and after various treatments. In-shell pecans were cracked with a mechanical cracker and nutmeats were separated from the shells, middle septum tissue, and packing material. Nutmeats were chopped in a One-Touch Chopper (model HC306, Black and Decker, Towson, MD). Shucks were cut into pieces with a knife and shells and middle septum tissues were crushed and broken to reduce the size of pieces to dimensions not exceeding 4 mm. The moisture contents of 5-g samples of halves, pieces, granules, shucks, shells, middle septum tissue, and orchard soil were determined with a moisture analyzer (model HB43-S, Mettler Toledo, Greifensee, Switzerland). Samples were dried at 130°C for 5 to 7 min (nutmeats) or 8 to 10 min (inedible materials), depending on the initial moisture content. Weight loss was attributed to removal of water during drying. The percent moisture in nutmeats and inedible materials was calculated.

**Measurement of *a*<sub>w</sub> values.** Portions of the nutmeats and inedible portions of pecans prepared for moisture analysis were used to determine *a*<sub>w</sub> values. Measurements of *a*<sub>w</sub> values were made with 3-g samples and an AquaLab model CX2 Water Activity Meter (Decagon Devices, Inc., Pullman, WA).

**Salmonellae used and preparation of inocula.** The inoculum contained a mixture of five *Salmonella* serotypes: Anatum, strain 6802, isolated from raw peanuts; Enteritidis, strain ATCC BAA-1045, from raw almonds; Oranienburg, strain 1839, from pecans; Sundsvall, strain 1659, from pecans; and Tennessee, strain K4643, clinical isolate from a patient in an outbreak of salmonellosis associated with consumption of peanut butter.

Salmonellae were grown at 37°C for 24 h in tryptic soy broth (TSB; Becton Dickinson, Sparks, MD) supplemented with nalidixic acid (50 µg/ml) (TSBN). One milliliter of culture of each serotype was surface spread on each of four large (150 by 15 mm) petri plates containing 60 ml of TSBN supplemented with agar (15 g/liter) (TSAN). Plates were incubated at 37°C for 24 to 26 h. Cells were grown on an agar medium rather than in broth because at least one of the serotypes (*Salmonella* Enteritidis) used in the study appears to have increased resistance to drying on almonds when cells for inoculum are grown on TSA rather than in TSB (45). To harvest cells, 5 ml of sterile 0.1% peptone water was deposited on the lawn that had developed on the surface of each plate, and cells were suspended in the peptone by gently rubbing the lawn with a sterile glass rod. Cell suspensions harvested from four plates of each serotype were pooled and analyzed for populations of *Salmonella* (see procedure described below). Equal
Survival and growth on pecan halves, pieces, and granules. The effect of temperature on survival and growth of Salmonella on high-moisture mammoth halves, medium pieces, and granules of Desirable variety pecans was determined. A five-serotype suspension of Salmonella was prepared as described above. The suspension was serially diluted by $10^{-4}$ in sterile deionized water; 16 ml of the diluted suspension was added to 1.6 liters of sterile deionized water to give a low-population suspension. Halves, pieces, and granules (800 g each) at 21°C were placed in bags (ca. 40 by 60 cm) fabricated from fiberglass insect screen (Phifer, Inc., Tuscaloosa, AL) in the laboratory and immersed, with gentle agitation, in the suspension (21°C) for 30 s. Nutmeats were removed from the suspension, drained, and held at 21°C for 5 min. The moisture content and $a_w$ values were measured. Nutmeats (25 g, wet weight) were placed in Stomacher 400 bags (Seward, West Sussex, UK), sealed, and held at 4, 21, 30 and 37°C for 0, 6, 24, and 48 h before analyzing for the presence (by enrichment) and populations of Salmonella.

Survival and growth on and in in-shell pecans. Studies were done to determine the survival and growth characteristics of Salmonella on and in high-moisture in-shell Desirable variety pecans. A five-serotype suspension of Salmonella harvested from TSAN plates as described above was used as an inoculum. The suspension was serially diluted by $10^{-3}$ in sterile deionized water, and 20 ml as added to 4 liters of sterile deionized water to prepare a high-population inoculum; a low-population inoculum was prepared by serially diluting the five-serotype suspension by $10^{-7}$ and adding 20 ml of the diluted suspension to 4 liters of sterile deionized water. Populations of Salmonella in both suspensions were determined by diluting samples in sterile 0.1% peptone water and plating on TSAN; colonies formed on plates incubated at 37°C for 22 to 24 h were counted.

Undamaged in-shell pecans (2,000 g) at 21°C were immersed in high- or low-inoculum suspension (21°C) for 24 h. Pecans were drained and the weight was determined; the moisture content and $a_w$ values of nutmeats were also determined. Five-pecan samples (approximately 50 g per sample, dry weight) were analyzed for the presence and populations of Salmonella. These nuts were considered as day 0 samples when monitoring survival and growth of Salmonella as affected by storage time and temperature. Bags containing inoculated nuts (five per 1-qt [0.95-liter] Ziploc [S.C. Johnson & Son, Racine, WI] freezer bag) were sealed and placed in groups in l-gal (3.79-liter) Ziploc bags, according to the intended incubation temperature. The presence (by enrichment) and populations of Salmonella in nuts stored at 4, 21, 30, and 37°C for 0, 1, 2, 5, and 8 days were determined.

Survival and growth on pecan shucks, shells, and middle septum tissue. Survival and growth characteristics of Salmonella on the surface of high-moisture ($a_w$ of 0.99) pecan shucks, shells, and middle septum tissue as affected by temperature were determined. A five-serotype suspension was prepared as described above. An inoculum was prepared by serially diluting the suspension by $10^{-3}$ in sterile deionized water.

Six hundred grams of shucks, shells, and middle septum tissue of mature pecans (Fig. 1, stage 8) were separately soaked with occasional mixing in deionized water (4°C) for 24 h. The volume of water in which each type of inedible material was soaked was 20% more than the volume needed for 100% saturation. These amounts were determined in preliminary studies to be 920, 240, and 460 ml for shucks, shells, and middle septum tissue, respectively. Soaked materials were drained and moisture content and $a_w$ values were determined. To 800 g of water-saturated inedible pecan material, 40 ml of Salmonella inoculum was added. Inoculated materials were thoroughly mixed and 25-g samples were deposited in 1-qt (0.95-liter) Ziploc bags. Sealed bags were incubated at 4, 21, 30, and 37°C for 0, 1, 2, 3, and 6 days. Samples were analyzed for the presence and populations of Salmonella.

Preparation of shuck extract. Pecan shucks were collected from the ground in commercial orchards or at shell cleaner cleaning operations, dried at 21°C to approximately 4% moisture, and stored at 4°C until used. Shucks (3,000 g) were combined with 9,000 ml of deionized water (4°C) and steeped for 24 h at 4°C before homogenizing, along with unabsorbed water, in an Osterizer blender (Oster, 14 speed, Sunbeam Products, Inc., Boca Raton, FL). The homogenate (12 kg) was combined with 12 liters of deionized water, held at 4°C for 24 h, and filtered through eight layers of cheesecloth. The filtrate (shuck extract) was stored at −20°C until used in studies designed to determine its effect on survival or growth of Salmonella.

Survival in shuck extract. Shuck extract (pH 5.40) prepared as described above was diluted in sterile deionized water at ratios of 1:0 and 1:1 (extract-water [vol/vol]) to give concentrations of 100 and 50%, respectively; 0.05 M potassium phosphate buffer (pH 5.4) was used as a control (0% extract) medium. A five-serotype suspension of Salmonella was diluted by $10^{-4}$ and $10^{-8}$ to give medium- and low-population inocula, respectively. To buffer and undiluted or diluted extract (9 ml), 1 ml of inoculum was added and thoroughly mixed. Tubes were incubated at 21 and 37°C for 0, 24, and 48 h before analyzing for Salmonella.

Survival in soil saturated with shuck extract or water. The effect of survival on soil surface of Salmonella in pecan orchard soil saturated with shuck extract or water (control) was determined. A five-serotype suspension of Salmonella was prepared as described above. Soil (200 g) obtained from pecan orchards in four Georgia counties was placed in 1-pt (0.473-liter) glass jars, and 100 ml of shuck extract or sterile deionized water (control) was added. Preliminary studies had shown that a 2:1 ratio (soil–water or extract [wt/vol]) resulted in water-saturated soil, a condition mimicking that in orchard soil after substantial rainfall, which not uncommonly occurs during the time between nut dehiscence and mechanical harvesting of pecans in areas of the southeastern United States where pecans are commercially grown. Soil saturated with shuck extract or water was thoroughly mixed, followed by adding 2 ml of a diluted ($10^{-7}$ in sterile deionized water) five-serotype suspension of salmonellae and again thoroughly mixing. The pH of the hull extract and soil to which extract or water were added was determined. Lids with a 5-mm-diameter hole in the center were placed on the jars. Inoculated soil stored at 21 and 37°C for 0, 4, 11, 18, 25, and 49 days was analyzed for presence and populations of Salmonella.

Survival in dry soil. Soil (700 g) obtained from pecan orchards in four Georgia counties was dried at 30°C for 2 weeks in an incubator at a relative humidity of 37%. The moisture content and $a_w$ values of the soil were measured. To prepare an inoculum for the dry soil, 330 g of sand (pure, 40 to 100 mesh; Argos

References omitted.
Organics, Geel, Belgium) were combined with 85 ml of a five-serotype suspension of *Salmonella* prepared as described above. After setting for 10 min at 21°C, the suspension was drained. Sand was placed in a shallow pan and held for 22 to 24 h at 21°C before transferring to a filter paper on a screen elevated approximately 17 cm over a bench top in a biosafety cabinet. The inoculated sand was dried for 3 h at 21°C and thoroughly mixed. The moisture content, a<sub>w</sub>, and population of *Salmonella* in the soil were determined. Soil (540 g) was combined with 60 g of inoculated sand and thoroughly mixed. Portions (200 g) of the mixture were placed in 1-qt (0.95-liter) glass jars, sealed by applying a lid, and stored for 0, 1, 2, 4, 8, 12, and 18 weeks at 4, 21, and 37°C before analyzing for *Salmonella*.

**Detection and enumeration of Salmonella.** In studies designed to determine survival and growth characteristics of *Salmonella* on high-moisture pecan halves, pieces, and granules, samples (25 g) were placed in Stomacher 400 bags, 100 ml of lactose broth (Becton Dickinson) supplemented with nalidixic acid (50 μg/ml) (LBN) was added to each bag, and the mixture was pummeled for 1 min at normal speed. The homogenate was spiral plated (WASP2, Microbiology International, Frederick, MD) or surface plated (quadruplicate 0.25-ml samples and duplicate 0.1-ml samples) on TSAN and bismuth sulfite agar (BSA; Becton Dickinson) supplemented with nalidixic acid (50 μg/ml) (BSAN). Enrichment broths were incubated at 37°C for 24 h and 42°C for 24 h, respectively, before streaking on BSAN. Presumptive-positive colonies that formed on BSAN plates within 48 h at 37°C were randomly selected for confirmation. Cells from selected presumptive-positive colonies were subjected to confirmation tests by using BBL Enterotube II (Becton Dickinson) or API 20E (bioMérieux, Inc., Hazelwood, MO) assays, and a *Salmonella* latex agglutination test (Oxoid, Ltd., Basingstoke, UK). The detection limit for enumerating *Salmonella* by direct plating was 4 CFU/g of nutmeat. The detection limit by enrichment was 1 CFU per approximately 25 g of nutmeat.

The presence and populations of *Salmonella* on and in inoculated high-moisture in-shell pecans stored at 4, 21, 30, and 37°C for up to 8 days were determined. Each in-shell pecan in five-pecan samples (approximately 50 g per sample) in a Ziploc bag was crushed with a hammer, thereby exposing the kernels. The contents of each bag were transferred to a Stomacher 400 bag. Two hundred milliliters of LBN were added to each bag and the mixture was vigorously shaken by hand for 30 s. After 3 to 5 min without shaking, the mixture was again vigorously shaken for 30 s. The LBN wash was spiral plated as described above. Samples of LBN wash (0.1 ml, in duplicate) serially diluted in 0.1% peptone water were also surface plated on TSAN and BSAN. Bags containing the...
mixture of crushed pecans and LBN, as well as TSAN plates on which samples had been applied, were incubated at 37°C for 24 h; BSAN plates were incubated at 37°C for 48 h. For samples anticipated to contain low numbers of Salmonella, preenriched LBN homogenates were enriched in tetrationionate and Rappaport-Vassiliadis broths, followed by streaking on BSAN as described above. Procedures for counting presumptive Salmonella colonies and confirmation tests are also described above. The detection limit for enumerating Salmonella in high-moisture in-shell pecans by direct plating was 4 CFU/g. The detection limit by enrichment was 1 CFU/5 in-shell pecans (1 CFU per approximately 50 g of in-shell pecans).

Duplicate 25-g samples of uninoculated and inoculated high-water-activity pecan shucks, shells, and middle septum tissue held at 4, 21, 30, and 37°C for up to 6 days were analyzed for the presence and populations of Salmonella. To each sample in a Stomacher 400 bag, 100 ml of LBN was added. The mixture of inedible pecan material and LBN was shaken vigorously for 30 s, allowed to set without agitation for 5 min, and again shaken for 30 s. The LBN wash was analyzed for the presence and populations of Salmonella as described above. The detection limit for enumerating Salmonella by direct plating was 4 CFU/g of shucks, shells, or middle septum tissue. The detection limit by enrichment was 1 CFU/25 g.

Populations of Salmonella in 0, 50, and 100% shock extract incubated for 24 or 48 h at 21 and 37°C were determined by surface plating undiluted samples (0.25 ml in quadruplicate and 0.1 ml in duplicate) and samples (0.1 ml in duplicate) serially diluted in sterile 0.1% peptone water on TSAN and BSAN; undiluted samples were also spiral plated on TSAN and BSAN. TSAN plates were incubated at 37°C for 24 h and BSAN plates were incubated at 37°C for 48 h before presumptive Salmonella colonies were counted and confirmed as described above.

In experiments focused on determining the behavior of Salmonella in pecan orchard soil saturated with shock extract or water, inoculated soil slurries incubated at 21 and 37°C for up to 7 weeks were thoroughly mixed at each sampling time before removing a sample (25 g) for analysis. Inoculated dry soil (25 g) stored at 4, 21, and 37°C for up to 18 weeks was also analyzed for Salmonella. Each sample was combined with 225 ml of LBN in a Stomacher bag and vigorously shaken by hand for 1 min. The populations of Salmonella in the soil and LBN mixture were determined by plating undiluted samples and samples serially diluted in 0.1% peptone water on TSAN and BSAN, as described above. Enrichment and confirmation procedures were also as described above. Data are presented on a wet weight basis for water- and extract-saturated soil and on a dry weight basis for dry soil (0.78 to 1.54% moisture, a_w of 0.33 to 0.36). The detection limit by direct plating was 4 CFU/g (ml) of extract- or water-saturated soil slurry on dry soil. The detection limit by enrichment was 1 CFU/25 g (ml) of slurry or dry soil.

Statistical analysis. All experiments were replicated at least three times. Values from duplicate or triplicate samples representing each test parameter combination in each replicate trial were analyzed with a general linear model on SAS software (version 8.0, SAS Institute Inc., Cary, NC). The least significant difference test was used to determine significant differences (α = 0.05) in mean values.

RESULTS AND DISCUSSION

Higher numbers of Salmonella on inoculated in-shell pecans, pecan nutmeats, inedible pecan materials, shock extract, and soil slurry were more often recovered on TSAN than on BSAN. In some instances, counts obtained on TSAN were significantly higher (α = 0.05). Only counts obtained from samples plated on TSAN are reported.

Survival and growth on nutmeats. As high as 42% of some varieties of in-shell pecans are reported to have cracked shells after cleaning (37). Weight gains by undamaged in-shell pecans, pecans with cracked shells, and pecans with pieces of shell missing can be up to 20, 42, and 53%, respectively, after immersion in water for 24 h (3). The a_w values of kernels in these pecans is in the range of 0.95 to 0.98. Recognizing that the a_w values of nutmeats in in-shell pecans and nutmeat halves after exposure to cleaning, conditioning, cracking, and shelling operations and of pieces and granules after flotation treatment may be high enough to support growth of Salmonella, we did a study to determine survival and growth characteristics of the pathogen in high-moisture mammoth halves, medium pieces, and granules; moisture contents were 12.3, 14.5, and 20.3%, respectively, and a_w values were 0.96, 0.97, and 0.99.

Survival and growth curves of Salmonella on high-water-activity nutmeat halves, pieces, and granules incubated at 4, 21, 30, and 37°C for up to 48 h are shown in Figure 2. Initial populations of Salmonella were 0.10 to 0.54 log CFU/g. The pathogen did not grow in nutmeats held at 4°C but remained viable for at least 48 h. Growth did, however, occur at 21, 30, and 37°C. Significant (α = 0.05) increases in Salmonella occurred within 6 h in halves and pieces stored at 30 and 37°C and in granules stored at 21, 30, and 37°C. Lag times were shorter, and maximum populations reached within 48 h were higher in nutmeats stored at 30 and 37°C than in nutmeats stored at 21°C. Salmonella grew most rapidly and to the highest population (6.41 log CFU/g) in pecan granules stored at 37°C. Maximum population was reached in medium pieces (5.26 log CFU/g) held at 37°C, whereas the maximum population on halves (2.31 log CFU/g) occurred at 30°C during the 48-h incubation period. Differences in lag times and maximum populations reached in the three types of nutmeats are attributed in part to the higher moisture content and a_w values in granules as compared with pieces, and in pieces as compared with halves. Of the three types of nutmeats, granules had the largest percentage of internal tissue exposed to water during the 30-s inoculation procedure, thereby resulting in the greatest amount of water being absorbed and creating a more favorable a_w value for growth of Salmonella. The amount of internal tissue (surface area per gram of nutmeats) accessible to Salmonella is highest for granules, followed by medium pieces and mammoth halves, in that order. A larger percentage of accessible internal nutmeat tissue would likely provide larger amounts of readily available nutrients for growth. Thus, the order of suitability of the three types of pecan nutmeats for supporting growth of Salmonella at a given temperature was likely influenced not only by a_w values, but also by the amount of internal tissue nutrients accessible to the pathogen.
Survival and growth on and in in-shell pecans. The rapid uptake of water by in-shell pecans, whether damaged (3), raises interest in learning more about the behavioral characteristics of *Salmonella*, which may become internalized on exposure of the pecans to contaminated water in pre- or postharvest environments. Survival and growth of the pathogen in high-moisture in-shell pecans (kernel moisture, 11.1%; kernel *a*$_w$, 0.94) stored at 4, 21, 30, and 37°C for up to 8 days was studied. An initial population of 6.49 log CFU/g decreased by 0.52 to 1.19 CFU/g of nuts during the 8-day storage period (Fig. 3). Within 5 days, the population on nuts held at 4°C was significantly lower (*p* < 0.05) than that on nuts held at 21, 30, or 37°C. In nuts initially containing a lower number of *Salmonella* (3.51 log CFU/g), populations on nuts stored for 8 days at 21, 30, or 37°C did not change significantly. A significant reduction (1.61 log CFU/g) occurred within 2 days in nuts stored at 4°C. Lack of growth of *Salmonella* at 21, 30, and 37°C may have been due in part to a kernel *a*$_w$ value (0.94) near the minimum for growth. Intact kernel testae limited nutrient availability, thereby also inhibiting growth. High numbers of the pathogen did survive, however, on in-shell pecans containing a high amount of moisture and at an *a*$_w$ value in the range that may occur in preharvest nuts for periods that may exceed 8 days or in postharvest environments in which nuts may be unintentionally exposed to water for several days or perhaps weeks in poorly maintained storage facilities or inadequately cleaned shell operations.

In a previous study (2), we observed that *Salmonella* survived on the shell surface of dry in-shell pecans stored at −18, −7, and 5°C for 32 weeks. We have also observed that *Salmonella* survives for up to 78 weeks on and in in-shell pecans inoculated with essentially the same immersion procedure used in the present study, dried to 3.2 to 3.6% kernel moisture, and stored at −20 to 37°C (3). In the present study, high-moisture (11.1%) nuts stored for more than 8 days were not analyzed for *Salmonella*. Kernels in preharvest in-shell pecans may contain 11.1% or more moisture for more than 8 days before delivery to cracking-shelling operations. While the results of our study show that *Salmonella* can survive in high-moisture pecans, we also observed mold growth on nuts during the latter days of the 8-day storage period, particularly on nuts stored at 30 and 37°C. These nuts may or may not be segregated from sound nuts preceding conditioning, cracking, and shelling and, aside from concerns about *Salmonella*, introduce potential safety hazards associated with mycotoxins.

Others have described the behavior of *Salmonella* on in-shell tree nuts. Blessington et al. (5) reported that reductions of the pathogen on immersion-inoculated, dried in-shell walnuts stored at 23°C for up to 12 weeks were substantial. *Salmonella* *Enteritidis* PT30 is known to survive at high populations on dry almond kernels stored at −20 and 4°C for 18 months (45). The behavior of *Salmonella* on...
high-moisture in-shell walnuts and almonds has not been described.

Survival and growth on shucks, shells, and middle septum tissue. Salmonella Enteritidis has been reported to grow in slurries of almond hulls and shells (47) and in almond orchard soil containing an aqueous extract of almond hulls (15). Populations of Salmonella Enteritidis in walnut wash water, hulls, and hull slurry, in contrast, have been observed to decline by about 3 log CFU/ml (g) over a 4-week period at 23°C (5). We did a study to determine the survival and growth characteristics of Salmonella on high-water-activity pecan shucks, shells, and middle septum tissue. Results are shown in Figure 4. The moisture contents (wt/wt) of shucks, shells, and septum tissue were 60.4, 31.6, and 49.2%, respectively; surface pH values were 6.08, 5.61, and 4.95. The a_w values of the three inedible nut materials ranged from 0.993 to 0.999. Salmonella grew on the surface of shucks stored at 21, 30, and 37°C and on shells stored at 30 and 37°C. Significant increases (α = 0.05) of 2.77 log CFU/g of shells and 2.43 log CFU/g of shucks occurred within 1 day at 37°C. The greatest increase (3.51 log CFU/g) occurred on shucks held at 37°C for 6 days. In contrast, significant decreases in Salmonella were observed within 1 day on middle septum tissue held at 21, 30, and 37°C. After storage for 6 days at 4, 21, 30, and 37°C, populations decreased by 0.50, 1.96, 2.92, and 3.36 log CFU/g of tissue, respectively.

Uesugi and Harris (47) described the behavior of Salmonella Enteritidis in almond hull and shell slurries. The pathogen grew rapidly in both slurries held at 24°C for 4 days. Growth occurred in hull slurry but not in shell slurry held at 15°C. The more favorable nutrient source provided by almond hulls, as compared with shells, was thought to be a major factor contributing to higher populations of Salmonella Enteritidis being reached in hull slurries. Higher amounts of sugars, protein, and oil in pecan shucks, as compared with shells, may also have resulted in conditions more favorable for growth of Salmonella.

The ability of Salmonella to survive or grow on the surface of inedible high-moisture pecan components may also be attributable to other factors. In our study, aside from assumed differences in amount and type of nutrients available for growth of Salmonella on these components, the pH of shucks (6.08) would be more favorable for survival and growth, as compared with the pH of shells (5.61) or middle septum tissue (4.95). Low nutrient availability and pH of septum tissue, however, may not be entirely responsible for the rapid inactivation of Salmonella. Pecans contain high amounts of phenolic compounds (1, 19). In an earlier study (2), we observed that middle septum tissue (packing tissue), at a concentration of 0.2% in TSB (pH 7.0) supplemented with yeast extract (0.5%), was lethal to Salmonella Senftenberg. Tissue was bacteriostatic at a concentration of 0.1% and delayed growth at 0.05%. Lethal and inhibitory activities were attributed to polyphenolic compounds that are present at high concentrations in middle septum tissues and in the testae of pecan kernels. Results from the present study confirm earlier observations that components in middle septum tissue in pecans are lethal to Salmonella.

Observations on the ability of almond hull slurry and high-moisture pecan shucks to support the growth of Salmonella are contrary to those reported for walnut hull pieces and slurry (5). Salmonella Enteritidis was observed to decrease slowly but significantly (α = 0.05) on high-moisture walnut hull pieces and in slurry stored at 23°C for 4 weeks. An initial population of 9 to 10 log CFU/g of hull slurry decreased to levels detectable by enrichment after storing slurries for 9 months. Lack of nutrients, acidic pH, or other factors, alone or in combination, may have caused death of the pathogen.

Survival in pecan shuck extract. On drying of mature pecans on the tree, the four shucks surrounding the nut open and the nut falls to the ground (Fig. 1, stage 8). Shucks may remain on the tree for several weeks but, like the nuts, they too eventually fall to the ground. Reports on the growth of
**TABLE 1. Number of Salmonella recovered from water and pecan shuck extract (50 and 100% solution) as affected by incubation temperature and time**

<table>
<thead>
<tr>
<th>Initial population (log CFU/ml)</th>
<th>Extract (%)</th>
<th>Temp (°C)</th>
<th>Incubation time (h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.75</td>
<td>0</td>
<td>21</td>
<td>A 1.75 A</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>21</td>
<td>A 1.75 A</td>
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<tr>
<td>50</td>
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<td>A 1.75 A</td>
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<td>100</td>
<td>21</td>
<td>37</td>
<td>A 1.75 A</td>
</tr>
<tr>
<td>5.69</td>
<td>0</td>
<td>21</td>
<td>A 5.69 A</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>21</td>
<td>A 5.69 A</td>
</tr>
<tr>
<td>50</td>
<td>21</td>
<td>37</td>
<td>A 5.69 A</td>
</tr>
<tr>
<td>100</td>
<td>21</td>
<td>37</td>
<td>A 5.69 A</td>
</tr>
</tbody>
</table>

* Values in the same row that are not followed by the same letter are significantly different (α = 0.05). Within the same initial population and incubation temperature, values in the same column that are not preceded by the same letter are significantly different.

*Salmonella* Enteritidis in almond hull slurry (47) and growth-promoting effects of hull extract on *Salmonella* in almond orchard soils (15), coupled with our observation on the growth of *Salmonella* on the surface of high-moisture pecan shucks, raised interest in determining if pecan shuck extract will support growth. Shown in Table 1 are populations of *Salmonella* recovered from 0% (0.05 M potassium phosphate buffer, pH 5.4) and 50 and 100% water extract (pH 5.4) of pecan shucks. Unlike the suitability of high-moisture shucks to support growth of *Salmonella* (Fig. 3), suspending cells in shuck extract resulted in death of the pathogen. Inactivation was more rapid in 50% shuck extract than in buffer and, in turn, in 100% extract as compared with 50% extract. In contrast, populations of *Salmonella* in buffer did not decrease significantly during the 48-h incubation period. Temperature (21 and 37°C) did not significantly affect the lethality of extract at a given concentration. These observations are contrary to those from experiments showing that *Salmonella* is able to grow on the surface of high-moisture shucks. A low concentration of water-soluble nutrients and the acidic pH, perhaps together with high concentrations of polyphenolic compounds and other antimicrobials in the extract, may have caused death of *Salmonella*.

**Survival in soil.** Pecans that have fallen to the ground may be in contact with water-saturated soil for several days before they are mechanically or hand harvested. Shucks that have separated from the nuts, as well as those still attached, are subject to leaching on exposure to rainwater or water from other sources that have accumulated in low areas on the orchard floor. The desperate behavior of *Salmonella* on high-moisture shucks versus shuck extract raised interest in investigating survival characteristics of the pathogen in water- and shuck extract–saturated pecan orchard soil. Shown in Table 2 are populations of *Salmonella* recovered from soil saturated with either water (control) or a water extract of shucks and held at 21 and 37°C for up to 49 days. The acidic pH (5.40) of the extract reduced the pH of the soil and extract mixture to 5.30 to 5.87, depending on the origin of the soil, compared with the pH (5.36 to 6.62) of water-saturated soils. The decrease in pH of soils from four counties on saturating with shuck extract varied, with soil from Crisp County being least affected and soil from Mitchell County being most affected. This corresponds with relative differences in initial pH of soils and may indicate differences in buffer capacity owing to the type and amount of organic matter in soils obtained from orchards in the four counties.

Regardless of origin of the soil, presence of shuck extract, or temperature, *Salmonella* did not grow in water- or extract-saturated soil during the 49-day storage period. On the contrary, the pathogen slowly died to reach undetectable levels (<1 CFU/25 g) within 4 to 49 days. Initial populations (1.22 to 1.43 log CFU/g of soil) decreased to populations less than 1 CFU/25 g in all test soils by the end of the 49-day storage period. Overall trends in the rate of inactivation of *Salmonella* indicate that death may have been more rapid at 37°C than at 21°C. This is in agreement with studies showing that *Salmonella* Enteritidis decreased more rapidly in high-moisture almond orchard soil stored at 35°C than in soil stored at 20°C (15).

*Salmonella* does not appear to persist longer in water-saturated pecan orchard soil as compared with shuck extract–saturated soil (Table 2). This is in agreement with observations on inactivation of *Salmonella* suspended in shuck extract (Table 1). Death of *Salmonella* in extract-saturated soils did not appear to be affected by their lower pH as compared with that of water-saturated soils. The origin of the soil did not have a marked effect on the rate of inactivation of *Salmonella*. *Salmonella* Enteritidis has been reported to slowly decline in walnut hull wash water (initial population was 9 to 10 log CFU/ml) held at 23°C for 4 weeks (5). The pathogen was not detected by enrichment of inoculated wash water held for 9 months. These observations on death of *Salmonella* in water that may contain lethal components leached from walnut hulls are in agreement with our observations that inactivation of the
pathogen is most likely caused by antimicrobials present in pecan shuck extract and soil saturated with the extract. In contrast, lack of evidence for enhanced growth because of adding pecan shuck extract to soil is contrary to observations on the growth-promoting effects of almond hull extract on Salmonella on addition of the extract to orchard soil (15). The latter study reported significant growth of Salmonella Enteritidis in soil containing almond hull extract as compared with soil containing water within 8 h at 23 ± 3°C. The pH of the almond orchard soil to which hull extract or water was added was 7.6. This pH is considerably higher than that of water-saturated pecan orchard soils (pH 5.36 to 6.62) used in our study. Differences in methods used to prepare extracts from almond hulls and pecan shucks in the two studies, as well as procedures for inoculation and analysis of soils make it difficult to compare results and draw valid conclusions concerning differences and similarities in the behavior of Salmonella on exposure to almond hull extract versus pecan shuck extract.

In contrast to the behavior of Salmonella in water- and shuck extract–saturated soil, the pathogen survived well in dry soil (0.78 to 1.54% moisture) for at least 18 weeks. Survival was not significantly affected (α = 0.05) by the origin of the soil. Shown in Figure 5 are survival curves as affected by storage temperature. Data represent means of counts from test soils obtained in four counties. The initial number of Salmonella in soil (6.77 log CFU/g) stored at 4°C for 18 weeks did not decrease significantly. However, significant decreases occurred within 1 week and between 1 and 8 weeks in soil stored at 21°C; significant decreases occurred within 1 week in soil stored at 37°C but not between 1 and 12 weeks. Significant reductions occurred between 8 and 12 weeks and between 12 and 18 weeks in soil stored at 21°C; significant reductions occurred between 12 and 18 week in soil stored at 37°C. At each storage time, the number of Salmonella recovered from soil stored at 21°C was significantly lower (α = 0.05) than was the number recovered from soil stored at 4°C and, in turn, the number recovered from soil stored at 37°C was significantly lower than was the number recovered from soil stored at 21°C. Reductions in initial populations in soil stored at 4, 21, and 37°C for 18 weeks were 0.35, 2.61, and 3.42 log CFU/g, respectively. These studies indicate that viability of Salmonella in pecan orchard soil is markedly affected by moisture content. In situations where soil remains dry for an extended period, the pathogen can remain viable for at least 18 weeks (126 days), even at elevated temperature.

### TABLE 2. Survival of Salmonella in soil as affected by origin of soil, presence of pecan shuck extract, and temperature

<table>
<thead>
<tr>
<th>Origin of soil (Georgia county)</th>
<th>Temp (°C)</th>
<th>Liquid phase</th>
<th>pH of saturated soil</th>
<th>Storage time (days):</th>
<th>0</th>
<th>4</th>
<th>11</th>
<th>18</th>
<th>25</th>
<th>49</th>
</tr>
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<td>Crisp</td>
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<td>Water</td>
<td>5.36</td>
<td>1.37</td>
<td>1.00 (1/1)</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(2/3)</td>
<td>(0/3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Extract</td>
<td>5.29</td>
<td>1.37</td>
<td>1.00 (0/2)</td>
<td>(0/3)</td>
<td>(1.00 (0/1)</td>
<td>(1/3)</td>
<td>(0/3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Water</td>
<td>5.36</td>
<td>1.37</td>
<td>1.30 (2/2)</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(0/3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Extract</td>
<td>5.29</td>
<td>1.37</td>
<td>1.30 (2/2)</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(0/3)</td>
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<tr>
<td>Lowndes</td>
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<td>Water</td>
<td>6.41</td>
<td>1.43</td>
<td>2.45 (1/1)</td>
<td>1.48 (2/2)</td>
<td>1.63</td>
<td>1.30 (1/1)</td>
<td>(0/3)</td>
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<td>37</td>
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<td>6.41</td>
<td>1.43</td>
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<td></td>
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<td>5.75</td>
<td>1.43</td>
<td>1.70</td>
<td>(0/3)</td>
<td>(0/3)</td>
<td>(0/3)</td>
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<tr>
<td>Mitchell</td>
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<td>Water</td>
<td>6.62</td>
<td>1.43</td>
<td>1.70</td>
<td>(0/3)</td>
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<td>(0/3)</td>
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<tr>
<td></td>
<td>21</td>
<td>Extract</td>
<td>5.87</td>
<td>1.43</td>
<td>2.00 (2/2)</td>
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<td></td>
<td>37</td>
<td>Water</td>
<td>6.62</td>
<td>1.43</td>
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<td>(0/3)</td>
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<td>Extract</td>
<td>5.87</td>
<td>1.43</td>
<td>2.00 (2/2)</td>
<td>(3/3)</td>
<td>(3/3)</td>
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<tr>
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<td></td>
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<td>(3/3)</td>
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<td>(3/3)</td>
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<tr>
<td></td>
<td>37</td>
<td>Extract</td>
<td>5.30</td>
<td>1.43</td>
<td>2.00 (2/2)</td>
<td>(3/3)</td>
<td>(3/3)</td>
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<tr>
<td></td>
<td>37</td>
<td>Extract</td>
<td>5.30</td>
<td>1.43</td>
<td>2.00 (2/2)</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(0/3)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers of Salmonella (log CFU/g of soil, wet weight basis).

Values are shown only for the samples (one, two, or three samples) from three replicate trials not giving counts by direct plating. Limit of detection by direct plating was 4 CFU/g of soil; limit of detection by enrichment was 1 CFU/25 g of soil.

**FIGURE 5. Survival of Salmonella in dry soil (0.78 to 1.54% moisture, aw of 0.33 to 0.36) stored at 4, 21, and 37°C for up to 18 weeks. Data points represent mean counts from inoculated soils obtained from four counties.**
In summary, results show that *Salmonella* can grow on the surface of high-moisture (a_w of 0.96 to 0.99) pecan nutmeats, in-shell pecans, shucks, and shells, but is inactivated on the middle septum tissue and in water extract of shucks. These observations emphasize the importance of controlling or limiting the time potentially contaminated in-shell pecans and nutmeats are exposed to water, both in pre- and postharvest environments. *Salmonella* does not survive in orchard soil saturated with water or shuck extract, but survives well in dry soil. Studies to determine inactivation characteristics of *Salmonella* as affected by seasonal variations in soil moisture content and temperature need to be done before recommendations can be made concerning the minimum time needed between application of manure or compost that may contain the pathogen and the time pecans are harvested.

**ACKNOWLEDGMENTS**

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