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ORIGINAL ARTICLE

Survival and growth of *Salmonella* Enteritidis PT 30 in almond orchard soils

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Abstract

Aims: To evaluate factors potentially contributing to the long-term persistence of *Salmonella enterica* serovar Enteritidis phage type (PT) 30 in an almond orchard.

Methods and Results: Surface and subsurface soil temperatures, and air temperatures in a radiation shelter, were recorded during a 12-month period, and were used to identify relevant storage temperatures (20 or 35°C) for microcosms of two different soil types (clay and sandy loams) with moisture levels near saturation or near field capacity. *Salmonella* Enteritidis PT 30 was inoculated into the microcosms at 6 log CFU g^{-1} dry weight. Between 14 and 180 days of incubation, counts of *S.* Enteritidis PT 30 decreased rapidly at 35°C and were significantly different ($P < 0{\cdot}05$) from counts at 20°C, regardless of the soil type or moisture level. *Salmonella* was detected by enrichment of 10-g samples from all microcosms after 180 days of incubation at 20°C, but from none of the microcosms held at 35°C. To measure the potential for the growth of *S.* Enteritidis PT 30 in clay loam soil, an aqueous extract of almond hulls (containing 1·6% mono and disaccharides) or equivalent volume of water was added 7 days after inoculation. Significant ($P < 0{\cdot}05$) growth of *S.* Enteritidis PT 30 was observed within 8 or 24 h of adding hull extract, but not water, to soil.

Conclusions: Opportunities may exist for *S.* Enteritidis PT 30 to survive for an extended time in almond orchard soils and to grow in these soils where hull nutrients are released.

Significance and Impact of the Study: Temperature has a significant impact on the long-term survival of *S.* Enteritidis PT 30 in soil, and nutrients leached from almond hulls may result in *Salmonella* growth. These factors should be considered in the design of Good Agricultural Practices for almonds.

Introduction

Long-term survival of *Salmonella* in water, manure, soil and sediment has been well documented (Morse and Duncan 1974; Winfield and Groisman 2003). In studies where single *Salmonella* serovars have persisted in the environment for extended periods of time (Thompson *et al.* 1977; Twiddy *et al.* 1988; McLaren and Wray 1991; Mutalib *et al.* 1992; Sandvang *et al.* 2000; Baloda *et al.* 2001; Davies and Breslin 2003), those serovars isolated from environmental samples were also present in clinically diseased and asymptomatic farm or wild animals or insects, suggesting continual and perhaps recent reintroduction from the host species. In addition to regular re-inoculation, the establishment and multiplication of a pathogen in the environment may be another explanation for continuous isolation of a single strain from environmental samples. Although *Escherichia coli* are known to

proliferate in tropical ecosystems (Rivera *et al.* 1988), and *Salmonella* have been shown to multiply in polluted and unpolluted river water at temperatures as low as 10°C (Hendricks and Morrison 1967), the ability of enteric pathogens to exist as stable dividing populations in non-host environments (Cherry *et al.* 1972) remains to be further explored.

Traceback investigations of foodborne outbreaks are rarely successful in linking implicated food back to the environment in which it was grown (California Department of Health Services 2007). An exception was the traceback investigation associated with a 2000 to 2001 outbreak of salmonellosis from the consumption of raw almonds; in this case, the outbreak strain, *Salmonella enterica* serovar Enteritidis phage type (PT) 30, was detected in drag swabs of almond orchard floors over a large geographic area 1 year after the harvest of the outbreak almonds (Isaacs *et al.* 2005). *Salmonella* Enteritidis PT 30 was not isolated from animal or bird faeces (i.e. from rodents, migratory waterfowl or crows) collected from the farms and surrounding areas (California Department of Health Services 2002); the original source of this strain remains unknown. In a study of one outbreak-associated orchard from 2001 through 2006, the same strain was exclusively isolated at least once per year from surface drag swabs (Uesugi *et al.* 2007).

Among almond orchards, the soil type, moisture level and temperature vary and are dependent on location, planting density, tree size and irrigation management. The average almond orchard density is 225 trees per hectare, and in fertile loam soils, there can be as few as 135 trees per hectare (Hendricks 1996). Irrigation methods vary and may include surface (flood or furrow) sprinkler and micro-irrigation (drip and micro-sprinkler) (Prichard 1996).

Almonds are harvested after kernels have reached maturation and the hulls have begun to split and dry (Reil *et al.* 1996; Almond Board of California 2004). The nuts are shaken from the trees and left to dry on the orchard floor for 1 to 2 weeks. Almonds (kernels in hulls and shells) are then gathered from the orchard floor by mechanical sweeping, which effectively, although unintentionally, mixes the almonds with the top layers of soil. Orchard soil that may be contaminated thereby becomes a possible means for introduction of *Salmonella* onto almonds. Uesugi and Harris (2006) demonstrated that almond hull and shell slurries provide an excellent medium for the growth of *S.* Enteritidis PT 30. Almonds in their hulls may come into contact with moisture in the orchard in a number of ways, including: when almonds drop prematurely before preharvest irrigation is terminated; if rainfall occurs during harvest; and when almonds remain in the orchard following harvest – under these conditions, there is the potential for the growth of *S.* Enteritidis PT 30.

A better understanding of the factors that influence the persistence of *S.* Enteritidis PT 30 in almond orchards is important for developing almond-specific Good Agricultural Practices [U.S. Food and Drug Administration (FDA) 1998]. The objectives of this study were: (i) to determine surface and subsurface soil temperatures in an outbreak-associated almond orchard; (ii) to evaluate the impact of temperature, moisture level and soil type on the survival of *S.* Enteritidis PT 30 in almond orchard soils; and (iii) to determine the potential for the growth of *S.* Enteritidis PT 30 in almond orchard soils upon addition of almond hull extracts.

Materials and Methods

Almond orchard

The study site was a single representative 61-ha almond orchard on one of the three farms in California's western Fresno County linked to a 2000 to 2001 outbreak of salmonellosis (Isaacs *et al.* 2005; Uesugi *et al.* 2007). In this orchard, the tree density was 375 trees per hectare, and the orchard was irrigated by micro-sprinkling near the tree line. This management strategy resulted in a well-developed canopy that effectively shaded much of the orchard in the summer and early fall. Consistently, wet soil was typically observed at the tree line, and very dry soil was observed in the rows between the trees.

Soil- and air-temperature measurements

Subsurface soil temperatures were measured under the tree canopy at a depth of 8·5 cm, and air temperatures were measured in a radiation shelter under the tree canopy at a height approx. 30 cm above the soil surface. These temperatures were monitored from April 2004 through March 2005. Thermistors were used for temperature measurement, and the data were recorded at 1-h intervals using a DL2E datalogger (Dynamax, Houston, TX, USA). Surface-soil temperatures under the tree canopy and in the middle of the drive row were measured at midday from June to September 2004 and were also monitored approximately every 2 h on 4 August 2004, with a Raytek Raynger MX2 handheld infrared thermometer (Raytek Corporation, Santa Cruz, CA, USA).

Two types of soils were used in this study. A Cereni clay loam, consisting of 27–35% clay, was collected from four locations within the outbreak-associated almond orchard. The moisture content of this soil at field capacity was 21%. Soil pH in water paste, cation exchange capacity (CEC) and organic matter content were 7·6, 2·2 meq

per 100 g and 3·1%, respectively, as analysed by A&L Western Agricultural Laboratories (Modesto, CA, USA). A Milham sandy loam was collected from eight locations within a single 64-ha almond orchard located in another major almond-producing area (not associated with the outbreak) in western Kern County, California. All soil samples were stored at 4°C for up to four months and screened through a sieve (3·18-mm mesh) prior to use.

The soil moisture levels were calculated following standard procedures (U.S. Department of Agriculture 1996). A high moisture level, which was close to the saturation point (10% above field capacity), and a moderate moisture level, which was close to field capacity, were selected for this experiment. The amount of additional moisture required for the soils to reach these conditions was calculated and this amount of distilled water was added to the soil samples following inoculation.

Inoculum preparation and incubation of microcosms

Unless otherwise indicated, all media were obtained from Difco, Becton Dickinson (Sparks, MD, USA). A variant of *S.* Enteritidis PT 30 (ATCC BAA-1045; Uesugi and Harris 2006), capable of growing in the presence of 50 μg ml^{-1} of nalidixic acid (Sigma, St Louis, MO, USA), was used in all experiments. This strain was originally isolated from the recalled 2000 to 2001 outbreak-associated almonds (Isaacs *et al.* 2005). Nalidixic acid resistance was used to aid in the identification of *Salmonella* from the background microflora, and did not alter the growth characteristics of the strain (data not shown). Prior to each experiment, the inoculum was prepared as described in Danyluk *et al.* (2005) with the addition of nalidixic acid (50 μg ml^{-1}) to all media. Inoculum concentrations were determined by serial dilution in Butterfield's phosphate buffer (BPB) and plating the inoculum onto tryptic soy agar (tryptic soy broth and 1·5% granulated agar) supplemented with nalidixic acid (TSAN; 50 μg ml^{-1}) and bismuth sulfite agar supplemented with nalidixic acid (BSAN; 50 μg ml^{-1}). When necessary, serial dilutions of the inoculum in BPB were made prior to soil inoculation to achieve lower inoculum levels.

Soil (500 ± 0·5 g) was weighed into a polyethylene bag (Bitran, 30·5 by 30·5 cm; Com-Pac Int., Carbondale, IL, USA), and 5 ml of the inoculum was added. This inoculum volume was previously determined (by visual inspection of soil consistency) to be the maximum that could be added without causing the soil to clump. After the addition of the inoculum, the bag was closed and rubbed by hand for 1 min, and the contents were poured out of the bag and spread over two sheets of filter paper (46 by 57 cm; Fisherbrand Qualitative P8; Fisher Scientific, Pittsburgh, PA, USA) that had been folded in half and placed on a metal drying rack inside a large plastic tub. The inoculated soil was allowed to dry for 18 ± 2 h at ambient (23 ± 3°C) temperature, and the samples were then pooled into one polyethylene bag (Bitran, 40·6 by 40·6 cm) and mixed thoroughly by inverting the bag by hand for 1 min. Uninoculated soil samples used as controls were wetted with BPB and then allowed to dry under identical conditions.

The pooled soil was divided into polyethylene bags (Bitran, 30·5 by 30·5 cm), and sterile distilled water was added to achieve the desired moisture levels. Moistened inoculated soil (100 g) was placed in sterile 120-ml polypropylene specimen containers (Fisher Scientific) with screw caps. Controls were set up with 100 g of moistened uninoculated soil in the 120-ml containers. The containers were incubated at 20 ± 2°C or 35 ± 2°C, with lids slightly ajar, and the soils were sampled at days 0, 3, 7, 14, 28, 56, 90, 120, 150 and 180.

Almond extracts and microcosm growth

The whole Carmel variety almonds in their hulls and shells were obtained from the Almond Board of California. The almonds were held in sealed polyethylene bags inside sealed plastic tubs at ambient temperature (23 ± 3°C) for up to 12 months. Hulls and shells were later removed by hand from some almonds for extract preparation. Fifty grams of whole almonds (hull, shell and kernel), almond hulls only or almond shells only, were added to 5 l of sterile distilled water to form a slurry in a 5-l Erlenmeyer flask. Each flask was then placed on a shaker (Labline, Barnstead International, Dubuque, IA, USA) and rotated at 150 rev min^{-1} for 24 ± 2 h at 4 ± 2°C. Following 24 h of shaking, the slurry was filtered through eight layers of sterile cheesecloth, and the resulting extract was stored in 50-ml aliquots at −20 ± 2°C. Prior to use, the extract was placed on the bench top for several hours to warm to ambient temperature.

Soluble sugars (including sucrose, glucose and fructose) represent 26·5% of the dry weight of an almond hull (Saura-Calixto *et al.* 1983, 1984). Sucrose, D-glucose and D-fructose levels in whole almond extract, hull extract and shell extract were determined using the R-Biopharm Enzymatic BioAnalysis kit (Roche, Darmstadt, Germany) following the manufacturer's instructions.

To determine the volume of hull extract or sterile distilled water to use in the growth studies, water was added to soil just to the point where pooling of liquid was visible after mixing. The muddy consistency of this saturated soil resembled that observed in the almond orchards during rain events. After 7 days of holding inoculated Cereni clay loam soil (approximately 10^1, 10^3 and 10^5 CFU g^{-1} dry weight) at ambient temperature (23 ± 3°C), 100 ml

of hull extract was added to 500 g of soil in polyethylene bags (30·5 by 30·5 cm). The hull extract and soil mixture was then rubbed by hand for 60 s. To serve as a control, 100 ml of sterile distilled water was added to 500 g of inoculated soil as described for the hull extract. In addition, 100 ml of hull extract was added to 500 g of uninoculated soil. Soil containers were held for 28 days at ambient temperature, with lids slightly ajar, and sampled at days 0, 7 (prior to addition of hull extract or water), 7 (8 h after addition of hull extract), 8, 10, 14 and 28.

Recovery, enumeration and enrichment of inoculated cells

Two 10 ± 0·3 g subsamples from each soil microcosm were added to 90 ml BPB in 145-ml polypropylene flip-top vials (Hardy Diagnostics, Santa Maria, CA, USA). Samples were shaken vigorously 50 times in a 30-cm arc, allowed to stand for 5 min, and then shaken an additional five times before serial dilution in 9-ml BPB. Samples (0·1 ml) were spread-plated onto TSAN and BSAN. In addition to plating 0·1 ml of the lowest dilution (10^{-1}), four spread plates of 0·25 ml of the lowest dilution were prepared to improve the detection limit. Colonies on plates were counted by hand after 24 ± 2 h (TSAN) or 48 ± 2 h (BSAN) of incubation at 35 ± 2°C. Results were reported as the log of the number of survivors per gram dry weight of soil.

When counts fell below the limit of detection, enrichment for *Salmonella* was conducted following the FDA Bacteriological Analytical Manual (BAM) method (Andrews and Hammack 2003), using 10-g soil samples. Enrichment isolates producing reactions that were typical of *Salmonella* were confirmed with specific *Salmonella* O antisera group D1 factors 1, 9 and 12 (Difco, Becton Dickinson) from triple sugar iron (Difco, Becton Dickinson) slants. Background soil populations were determined by plating serial dilutions (described earlier) onto half-strength TSA and incubating for 5 days at 28°C.

Statistics

A multivariate analysis of variance was performed with the Statistica 6 software package (StatSoft, Inc., Tulsa, OK, USA). Differences between the mean values were considered significant at $P < 0{\cdot}05$. The rate of reduction of *Salmonella* cells during incubation was determined after performing a linear regression on the survival curves and calculating the reduction per day.

Results

Air and soil temperatures in the almond orchard

Air temperatures measured under the tree canopy from April 2004 to March 2005 ranged from below 0°C to approximately 33°C (Fig. 1a). Subsurface soil temperatures under the tree canopy at a depth of 8·5 cm varied less than the corresponding air temperatures and ranged from 3 to 26°C over the same period (Fig. 1b). The mid-day surface-soil temperatures measured from June to September 2004 ranged from 20 to 35°C under the tree

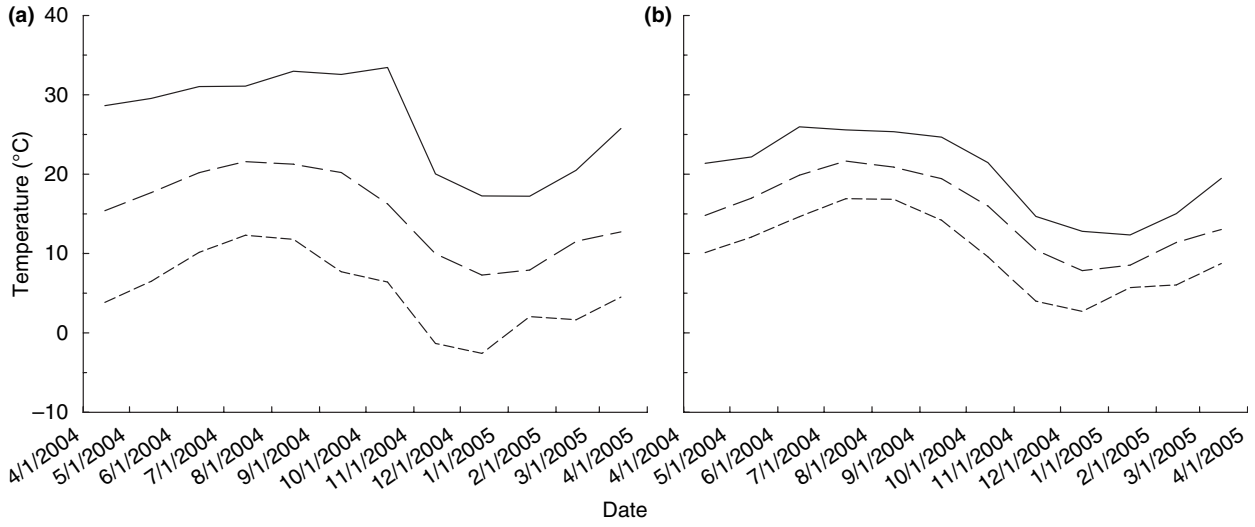

Figure 1 Monthly high, average and low air temperatures at 30 cm above soil surface under the tree canopy (a), and monthly high (—), average (– – –) and low (- - -) soil temperatures at a depth of 8·5 cm under the tree canopy (b), measured from April 2004 through March 2005 in an almond orchard associated with a 2000–2001 salmonellosis outbreak.

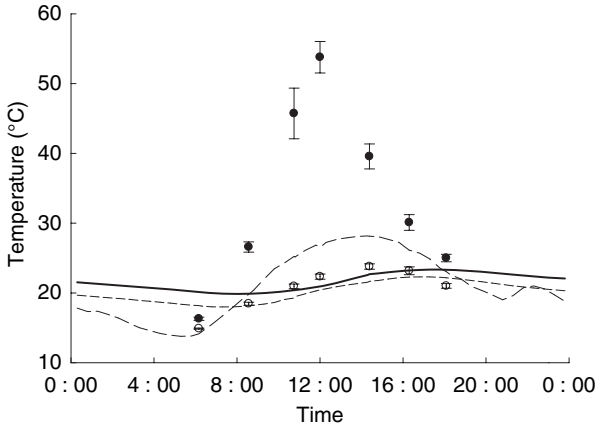

Figure 2 Summer-day (4 August 2005) variation in air and soil temperatures in almond orchard associated with a 2000–2001 salmonellosis outbreak: air under tree canopy 30 cm above soil surface (– – –); soil at a depth of 8·5 cm under tree canopy (- - -); soil at a depth of 8·5 cm under drive row (—); surface soil temperature under tree row (○) and surface soil temperature in the centre of drive row (●). Y axis error bars represent standard deviations.

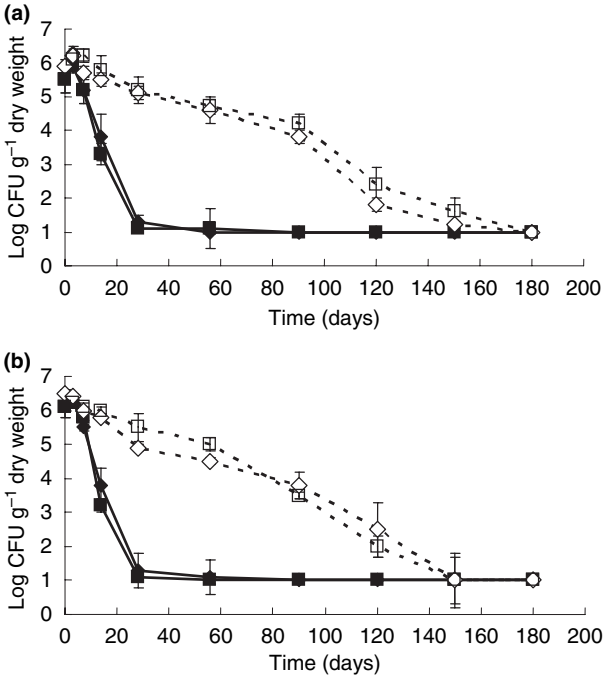

Figure 3 Survival of *Salmonella* Enteritidis phage type (PT) 30 in Cereni clay loam (a) and Milham sandy loam (b) soils at: moderate moisture and 35 ± 2°C (■); high moisture and 35 ± 2°C (♦); moderate moisture and 20 ± 2°C (□); and high moisture and 20 ± 2°C (◇). Limit of detection is approximately 1 log CFU g^{-1} dry weight. Values are the average of duplicate samples from each of three experiments (n = 6). Y axis error bars represent standard deviations.

canopy and from 35 to 55°C in the centre of the drive row (data not shown). Soil- and air-temperature data collected on 4 August 2004, a representative summer day, are shown in Fig. 2. The soil temperatures at a depth of 8·5 cm were about 20°C under the tree canopy and an average of 1–1·5°C warmer in the drive row (Fig. 2); little change in temperature was observed over 24 h. However, the surface-soil temperatures under the canopy and in the drive row ranged from 15 to 24°C and 16 to 54°C, respectively, over the 24-h period. Based on these data, 20 and 35°C were selected as representative orchard-soil temperatures for subsequent soil inoculation studies.

Survival of *Salmonella* in soil

Counts of *S.* Enteritidis PT 30 on TSAN and BSAN were not significantly different for any test parameter ($P > 0{\cdot}05$); therefore, only data from TSAN are shown. Following inoculation, the initial levels of *Salmonella* ranged from 5·5 log CFU g^{-1} dry weight in the Cereni clay loam at near saturation moisture to 6·6 log CFU g^{-1} dry weight in the Milham sandy loam at near field capacity.

Temperature was identified as the most important factor influencing the long-term survival of *Salmonella* in almond orchard soils. *Salmonella* Enteritidis PT 30 survived for a longer time in soils at the cooler (20 ± 2°C) temperature (Fig. 3). Counts of *S.* Enteritidis PT 30 decreased rapidly at 35 ± 2°C and were significantly different ($P < 0{\cdot}05$) from counts at 20 ± 2°C between 14 to 150 days of incubation. No significant differences in counts ($P > 0{\cdot}05$) were found between the Cereni clay loam and the Milham sandy loam soils held under the same conditions, or between the soils at near saturation and near field capacity moisture levels at each temperature (Fig. 3).

At 35 ± 2°C, the densities of *S.* Enteritidis PT 30 approached the limit of detection (approximately 1 log CFU g^{-1} dry weight) at day 28; therefore, the rate of reduction was calculated using data points from day 0 to day 28. The rates of reduction at near saturation and near field capacity moisture levels were 0·17 (R^2 = 0·97) and 0·17 (R^2 = 0·96) log CFU g^{-1} dry weight per day, respectively, for Cereni clay loam, and 0·20 (R^2 = 0·99) and 0·20 (R^2 = 0·95) log CFU g^{-1} dry weight per day, respectively, for Milham sandy loam. Thus, soil moisture had no influence, and the soil type had only a minor impact on the survival of *S.* Enteritidis PT 30 in our study.

From 90 days of incubation at 35 ± 2°C onwards, *S.* Enteritidis PT 30 was only detected in soils by enrichment of 10-g samples (Table 1). *Salmonella* Enteritidis PT 30 could not be detected by enrichment of six 10-g samples of Cereni clay loam or Milham sandy loam at near saturation (high moisture) from 120 or 150 days of incubation onwards, respectively. At 180 days of incubation at 35 ± 2°C, *S.* Enteritidis PT 30 was not detected in any of the 24 enriched 10-g samples.

Table 1 Enrichments positive for *Salmonella* Enteritidis phage type (PT) 30, following long-term incubation

Soil	Temperature (°C)	Moisture	Number of positive samples out of six samples enriched at day			
			90	120	150	180
Cereni	35 ± 2	High	4	0	0	0
		Moderate	6	4	4	0
	20 ± 2	High	NA*	NA	NA	5
		Moderate	NA	NA	NA	6
Milham	35 ± 2	High	4	2	0	0
		Moderate	4	2	2	0
	20 ± 2	High	NA	NA	NA	5
		Moderate	NA	NA	NA	5

* NA, not applicable.

In soil samples incubated at 20 ± 2°C, *Salmonella* declined at a slower rate (Fig. 3). At 20 ± 2°C, the average rates of reduction at near saturation and near field capacity moisture levels were 0·031 (R^2 = 0·92) and 0·028 (R^2 = 0·87) CFU g^{-1} dry weight per day, respectively, for Cereni clay loam, and 0·031 (R^2 = 0·98) and 0·033 (R^2 = 0·98) CFU g^{-1} dry weight per day, respectively, for Milham sandy loam.

Almond extracts

Sucrose, D-glucose and D-fructose levels were determined in aqueous extracts of whole almonds (hull, shell and kernel), almond hulls alone or almond shells alone (Table 2). Sugar concentrations in shell extracts were significantly lower ($P < 0·05$) than in both whole almond and hull extracts. However, no significant difference in the sugar content ($P > 0·05$) was found between whole almond and almond hull extracts; hull extract was used in subsequent growth studies.

Growth of *Salmonella* Enteritidis PT 30 in almond orchard soil

Following inoculation, the soil was held at room temperature for 7 days, prior to the addition of hull extract or sterile distilled water (control), to allow *S.* Enteritidis PT 30 populations to stabilize in the soil. The total aerobic counts of soil microcosms were monitored on half-strength TSA. The levels of culturable aerobic microorganisms on half-strength TSA (initially approximately 6 log CFU g^{-1}) increased within the first 24 h after adding hull extract to approximately 7 log CFU g^{-1} and stabilized at that level (data not shown).

Table 2 Sugar contents of extracts prepared from whole almonds, almond hulls or almond shells

Extract	Sucrose (g l^{-1})*	D-Glucose (g l^{-1})	d-Fructose (g l^{-1})
Whole almond	12·3 ± 0·3†	0·4 ± 0·2	2·3 ± 0·5
Hull	13·4 ± 0·6	0·8 ± 0·3	2·0 ± 0·4
Shell	3·3 ± 0·2	0·2 ± 0·2	1·4 ± 0·2

*Sugar values reported as g l^{-1} based on enzymatic determination.
†n = 3.

Increases in *S.* Enteritidis PT 30 levels of 0·5, 0·9 and 0·8 log CFU g^{-1} dry weight were observed within 24 h after adding sterile water to the soils inoculated with *S.* Enteritidis PT 30 at approximately 1, 3 and 5 log CFU g^{-1} dry weight, respectively (Fig. 4). These increases were not significantly different ($P > 0·05$) from those observed in the inoculated control without addition of liquid, but demonstrate that slight growth occurred even in the absence of adding a rich growth medium. *Salmonella* was not detected in uninoculated controls supplemented with hull extract.

Significant ($P < 0·05$) growth of *Salmonella* occurred within 8 h of adding hull extract to soil inoculated at 5 log CFU g^{-1} dry weight and 24 h after adding hull extract to soils inoculated at 1 and 3 log CFU g^{-1} dry weight. Within the first 24 h, increases of 0·5, 2·0 and 1·9 log CFU g^{-1} dry weight were observed for soils with initial inoculum levels of 1, 3 and 5 log CFU g^{-1} dry weight, respectively. Maximum increases of *S.* Enteritidis PT 30 of 0·7, 2·7 and 2·4 log CFU g^{-1} dry weight for inocula of 1, 3 and 5 log CFU g^{-1} dry weight, respectively, were observed in all cases 7 days after addition of hull extract. Twenty-one days after the addition of hull extract, *Salmonella* levels had decreased slightly from the maximum levels reached; however, this decrease was not significant ($P > 0·05$).

Discussion

Similar studies of foodborne pathogens in sand support the importance of temperature to survival, with greater survival at cooler temperatures (Parker and Mee 1982; DeRoin *et al.* 2003). These studies also found a relative lack of influence of moisture level. Soil type has been identified as an important variable in pathogen survival in other studies (Roper and Marshall 1978; Natvig *et al.* 2002), with clay soils supporting greater survival of enteric pathogens than sandy soils.

Large daily temperature shifts were observed in the surface soil located in the centre of the drive row, but temperatures beneath the soil surface were remarkably stable. Temperature fluctuations at the surface may not provide an environment favourable for extended *Salmonella* survival. However, production practices that disturb the upper soil layers and generate large volumes of

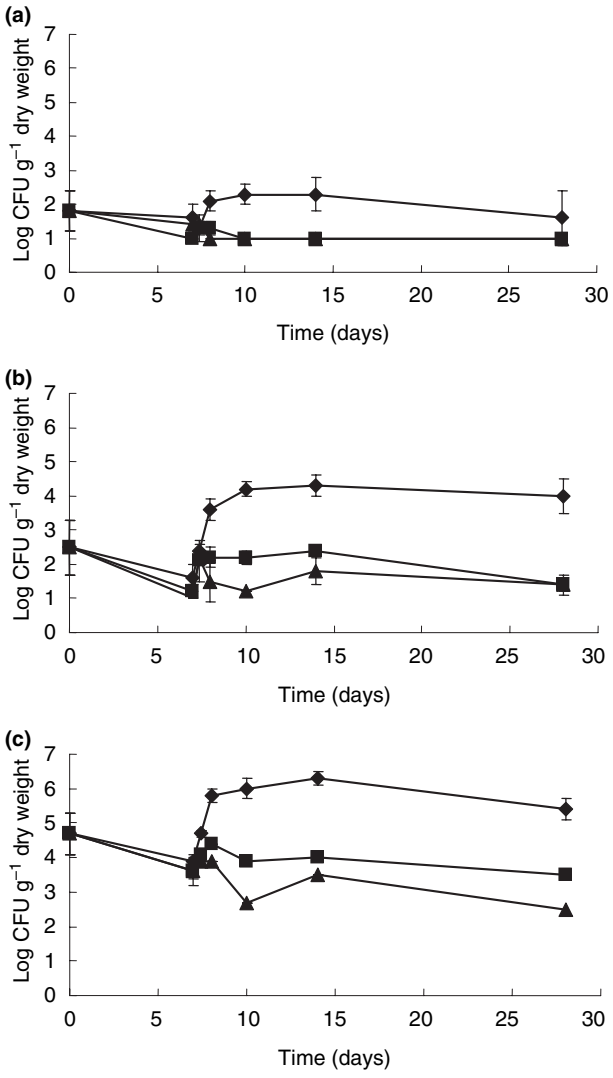

Figure 4 Growth of *Salmonella* Enteritidis phage type (PT) 30 in Cereni clay loam soil inoculated with approximately 10^1 (a), 10^3 (b) and 10^5 (c) CFU g^{-1} dry weight: growth in soil without added hull extract or sterile water (▲), and with hull extract (◆) or sterile distilled water (■) added on day 7. Values are the average of duplicate samples from each of three experiments (n = 6). *Y* axis error bars represent standard deviations.

dust, such as sweeping the almonds from the orchard floor during harvest, have the potential to mix the surface and subsurface layers of soil and spread existing organisms throughout the environment (Uesugi and Harris 2006). The impact of widely fluctuating temperatures on the survival of *Salmonella* in soil remains to be determined.

Uesugi and Harris (2006) demonstrated that almond hull slurries provide an excellent medium for the growth of *S.* Enteritidis PT 30. At 24°C, *S.* Enteritidis PT 30 populations increased significantly in a hull slurry within the first 6 h, and reached a maximum of 7·8 log CFU g^{-1} at 24 h. The slurry was a thick mixture of hulls and water, and the authors concluded that the high sugar levels of this slurry supported substantial growth of *S.* Enteritidis PT 30. The hull extract used in the present study, prepared at the same ratio previously used (Uesugi and Harris 2006), contained a level of sugars (15 g l^{-1}) that is comparable with or higher than that of many commercially available microbiological growth media.

Several events can lead to the wetting of almond hulls in the field and thus provide conditions supportive of *Salmonella* growth. Almonds may drop prematurely from trees and become wetted by irrigation, rain may fall during the harvest when nuts are on the ground, or almonds left on the trees after harvest may eventually drop to the ground where they could lie during the winter rainy season. Significant rainfall occurred during the harvest season in the orchards associated with the 2000 to 2001 outbreak. Further investigations into the rain event associated with that outbreak indicated that although the early rain may have played a role, it could not have been the only factor because the outbreak began prior to the distribution of almonds that had been rained upon (Uesugi and Harris 2006).

Salmonella Enteritidis PT 30 is capable of extended survival (at least 180 days) in typical almond orchard soils even in the absence of growth substrates. In addition, opportunities may exist for *S.* Enteritidis PT 30 to grow in soils where almond hull nutrients are released, thereby providing a mechanism for prolonged persistence of *Salmonella* in the almond orchard environment in the absence of an identified animal vector. Future studies are warranted to determine if the behaviour of other *Salmonella* serovars and enteric pathogens is similar to our observations of *Salmonella* Enteritidis PT 30 in soil.

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