Chapter 19

Human Pathogens and the Health Threat of the Phyllosphere

M.T. Brandl
Produce Safety and Microbiology, USDA/ARS, WRRC, 800 Buchanan Str., Albany, CA 94710, USA
mbrandl@pw.usda.gov

Introduction

Human enteric illness has been associated traditionally with the consumption of contaminated meat products or water. However, foodborne illness linked to the contamination of fresh fruits and vegetables has been increasing dramatically since the 1970s (Sivapalasingam *et al.*, 2004). This increase may be explained in part by a noticeable shift in dietary habits in the USA between 1970-1979 and 1997. During these periods, the consumption of fresh fruits and fresh vegetables alone increased 34% and 26%, respectively (Anonymous, 2001). This trend is mainly attributable to the introduction of salad bars in restaurants and supermarkets, and of fresh-cut fruits and vegetables, pre-packaged salads and exotic and specialty produce in supermarkets (Anonymous, 2001). Along with this development, the intensification of agricultural production in industrialised countries has resulted in the disposal of increasing amounts of animal waste on agricultural fields and the frequent use of effluent or reclaimed water to irrigate crops. Application of contaminated irrigation water, or animal waste as raw or improperly composted manure, may introduce enteric pathogens in the field. Other causes for the increase in the incidence of enteric illness linked to fresh produce may include: (i) changes in processing and packaging methods, particularly the introduction of ready-to-eat fruits and vegetables on the market; (ii) the expansion of global trade, which provides fresh produce out of season in local markets; and (iii) the enhanced surveillance of foodborne illness by regulatory agencies.

The continued rise in the number of cases and outbreaks of foodborne illness associated with fresh produce is of great concern to the fresh produce industry and to public health agencies. This has fuelled a new wave of studies on the ecology of human pathogens on plants. Although still in its infancy, this field of research already has yielded new insights into human pathogen behaviour on plant surfaces. This chapter presents some of the information that is arising from this emerging field.

Epidemiology of Enteric Illness Linked to Produce

Incidence of enteric pathogens on produce

Large international epidemics originating from almonds (Isaacs *et al.*, 2005) and from sprouts grown from contaminated seeds (Mahon *et al.*, 1997) have occurred. In the USA, the number of outbreaks per year that are linked to produce doubled between the periods of 1973-1987 and 1988-1992 (Bean *et al.*, 1997; Mead *et al.*, 1999). Outbreaks have originated from lettuce (Ackers *et al.*, 1998; Hilborn *et al.*, 1999; Takkinen *et al.*, 2005), cilantro (Campbell *et al.*, 2001), parsley, tomato (Hedberg *et al.*, 1999; Cummings *et al.*, 2001; Anonymous, 2005), cantaloupe (Mohle-Boetani *et al.*, 1999; Anonymous, 2002a), and unpasteurised orange juice (Cook *et al.*, 1998; Anonymous, 1999a) and apple juice (Besser *et al.*, 1993), to name only a few. According to a report by the Center for Science in the Public Interest, produce was the second most important food vehicle of outbreaks in the USA between 1990 and 2002, and accounted for 20% of the total number of illness cases reported (Anonymous, 2002b). The food categories included in these data were fruits, vegetables and produce dishes. Table 19.1 lists the number of outbreaks by type of produce as reported to the Center for Disease Control and Prevention (CDC) in the USA from 1973 to 1997. A high proportion of the outbreaks were associated with salads, lettuce, juice, melon and sprouts. In other countries where surveillance of foodborne illness is extensive, a significant proportion of outbreaks has been attributed to fresh produce also. In England and Wales, salad, vegetables and fruit caused 6.4% and 10.1% of all outbreaks with a known food vehicle in the periods of 1993-1998 (Anonymous, 2000) and 1999-2000 (Anonymous, 2003), respectively.

Table 19.1. Produce items implicated in foodborne illness outbreaks in the USA, 1973-1997.

Items	No. of outbreaks
Multiple produce items	105
Salad	76
Mixed fruit	22
Mixed vegetables	7
Single produce items	85
Lettuce	25
Melon	13
Seed sprout	11
Apple and orange juice	11
Berry	9
Tomato	3
Green onion	3
Carrot	2
Apple	1
Pear	1
Pineapple	1
Basil	1
Celery	1
Cucumber	1
Fresh elderberry juice	1
Fresh-squeezed lemonade	1

Sivapalasingam *et al.* (2004). Reprinted with permission from the *Journal of Food Protection*. Copyright held by the International Association for Food Protection, Des Moines, Iowa, USA.

Although some of the outbreaks linked epidemiologically to produce may have originated from cross-contamination by water or another food item during food preparation or processing, the data presented above raise great concerns about the safety of raw produce consumption. Several surveys conducted by individual research groups revealed the occurrence of contaminated fresh fruits and vegetables at the marketplace (Ercolani, 1976; Garcia-Villanova Ruiz *et al.*, 1987; Wells and Butterfield, 1997). Ercolani (1976) reported that 68% and 72% of lettuce and fennel samples, respectively, purchased from retail markets in Italy over a period of two years, harboured one or more of six *Salmonella* serotypes. A survey conducted in Spain between 1981 and 1983 showed that 7.4% of the samples from a variety of vegetables were contaminated with *Salmonella* (Garcia-Villanova Ruiz *et al.*, 1987). More recently, a large survey of produce by the US Food and Drug Administration in 2000-2001 revealed that 1.6% and 4.4% of the samples from domestic and import distribution markets, respectively, were contaminated with human pathogens (www.cfsan.fda.gov). In addition, an ongoing survey by the Microbial Data Program of the US Department of Agriculture, showed that in 2002, 0.62% of the samples were contaminated with *E. coli* strains harbouring virulence factors (www.ams.usda.gov/science/mpo/MDPSumm02.pdf). The types of produce sampled in this survey were: cantaloupe, celery, leaf lettuce, romaine lettuce and tomatoes. Overall, these studies provide evidence that outbreaks may not necessarily occur according to a cross-contamination scenario, and suggest that plant contamination with human pathogens can occur in a pre-harvest fashion in the field or during post-harvest transit before distribution in the marketplace.

Sources of Contamination

Unlike meat and fresh-cut produce, which are regularly tested in the packing plant, most produce is not inspected for the presence of foodborne pathogens. The short shelf life of fresh fruits and vegetables makes it difficult to identify the exact source of the contamination when an outbreak occurs, and epidemiological studies must be used to trace back to the contamination source. Although good bookkeeping at the farm and throughout the distribution chain may help to link a particular contaminated product to a specific farm or field, the very source of the inoculum usually remains unidentified. For example, in an outbreak of illness caused by *E. coli* O157:H7-contaminated lettuce, manure piled in the vicinity of the lettuce fields, roaming free-range chickens in the cow pasture as well as in the lettuce fields, bird activity in the processing shed, and contaminated wash water that was used to clean produce, were all potential sources of inoculum and risk factors identified at the farm implicated in the outbreak (Hilborn *et al.*, 1999).

In general, application of improperly composted manure, contaminated water used for irrigation or for application of pesticides, and animal activity, which leaves droppings on the ground, are considered as the primary causes of contamination in the field. Poor sanitation facilities for farm workers in the field may account for a high percentage of illness transmitted via human faeces, such as shigellosis. In addition, insects may be an important epidemiological factor. In particular, flies, which rely on faecal material and rotten fruit as a protein source to develop eggs (Lauzon, 2003), may act as vectors of faeces-borne pathogens. Both, the vinegar fly (*Drosophila melanogaster*) and the Mediterranean fruit fly (*Ceratitis capitata*) were shown to transmit *E. coli* to wounded and intact apples, respectively (Janisiewicz *et al.*, 1999; Sela *et al.*, 2005).

While airborne transmission is recognised as an important factor in the dispersal of pathogens among livestock (Dowd *et al.*, 2004), the role of bioaerosols as a source of bacterial contamination of produce has remained unexplored. Bioaerosols are a probable

source of inoculum in field crops since the presence of high quantities of animal faeces on the ground and of large manure piles at animal production facilities may lead to the transport of enteric pathogens via air currents and their deposition onto crops in the field.

Enteric Pathogens Linked to Foodborne Illness from Produce

A variety of bacterial enteric pathogens have been isolated from fresh fruits and vegetables and have caused produce-linked outbreaks. They include *S. enterica*, *Listeria monocytogenes*, *E. coli*, *Shigella* spp., *Campylobacter* spp., *Yersinia* spp. and *Staphylococcus aureus*.

S. enterica

S. enterica is the enteric pathogen most commonly recovered from produce and the most frequent bacterial causal agent in outbreaks associated with produce (De Roever, 1998; Castillo and Rodriguez-Garcia, 2004). In the USA, between 1973 and 1997, 48% of the outbreaks from produce that had a known bacterial etiological agent, were caused by *Salmonella* (Sivapalasingam *et al.*, 2004). Most alarming are the recent large outbreaks caused by multi-drug resistant (MDR) *S. enterica* Typhimurium DT104 strains that were associated with the consumption of lettuce in the UK (Horby *et al.*, 2003) and in Finland (Takkinen *et al.*, 2005). *S.* Typhimurium phage type DT104 is an emerging pathogen that is resistant to at least five antibiotics (Threlfall *et al.*, 1994). Multi-drug resistance of pathogens interferes with treatment of infections and puts patients at higher risk of death. Consequently, the emergence of MDR human pathogenic strains associated with fresh produce is of great concern to public health agencies.

L. monocytogenes

Although not implicated in outbreaks as frequently as *S. enterica*, *L. monocytogenes* is one of the deadliest foodborne pathogens associated with produce. The case-fatality rate of *L. monocytogenes* is 20% and is highest among pregnant women, neonates and immunocompromised adults (Mead *et al.*, 1999). Because of its lethality, the USA and the UK have established a "zero tolerance" level of *L. monocytogenes* per 25-g food sample, and consequently, the pathogen has caused the highest number of recalls by the food industry. These recalls include not only meat products, but also red peppers, sprouts and lettuce (Gorski, 2003). In 1981, cabbage processed as coleslaw was the first food and vegetable reported to be associated with an epidemic of listeriosis; the implicated cabbage was suspected to have been fertilised with sheep manure, which was the most probable source of contamination (Schlech *et al.*, 1983). In a recent study in Italy, Caggia *et al.* (2004) detected the presence of *L. monocytogenes* in green table olives, even after thermal treatment. This reflects the enhanced ability of this species to resist heat under low water activity conditions. *L. monocytogenes* is a versatile foodborne pathogen that has the ability to grow at a wide range of temperatures and at low pH (Montville and Matthews, 2005), characteristics that make it more difficult to control in food, including produce.

E. coli

While various pathogenic *E. coli* strains have caused produce-related outbreaks, *E. coli* O157:H7 is the causal agent of the most publicised outbreaks linked to fresh fruits and

vegetables. O157:H7 is a dangerous serotype of *E. coli* that causes haemolytic-uremic syndrome, which can lead to death, particularly in children, the elderly and those with compromised immune systems. In 1990-1999, 18 out of 55 produce-related outbreaks were caused by *E. coli* O157:H7 (http://www.cspinet.org/new/prodhark.html). Many of these outbreaks were traced back to the consumption of lettuce (Sivapalasingam *et al.*, 2004), a popular fresh vegetable that is difficult to wash thoroughly and is mostly consumed raw. While the Food and Drug Administration in the USA has approved the use of irradiation to decontaminate ground beef, a main food-vehicle of *E. coli* O157:H7 outbreaks, the decontamination of produce remains a significant challenge.

Shigella spp.

Shigella is a foodborne pathogen for which primates, including humans, are the only reservoir. It has a low infectious dose and therefore, can be transmitted easily through the faecal-oral route (Montville and Matthews, 2005). The major cause of contamination of foods with *Shigella* is poor personal hygiene of food handlers. Thus, fresh produce can be contaminated when sanitary facilities for farm workers are lacking or are inconveniently located in the field. Foodborne outbreaks of shigellosis have been associated with several types of raw produce, including green onions, iceberg lettuce and uncooked baby maize (Anonymous, 1999b). In particular, in 1998, seven outbreaks of *Shigella sonnei* within a 2-month period in the USA and Canada were attributed to fresh parsley (Anonymous, 1999b), and another outbreak of a strain with the same PFGE subtype that sickened 300 people in California was linked to cilantro shortly after (Tsang *et al.*, 1999). These outbreaks, along with enterotoxigenic *E. coli* infections linked to parsley (Naimi *et al.*, 2003), were traced back to the same farm, revealing that unsafe agricultural practices have widespread public health consequences.

Campylobacter spp.

In contrast to *Salmonella enterica* and pathogenic *E. coli*, which cause frequent foodborne outbreaks, *Campylobacter* infections appear to occur mainly as sporadic illnesses (Friedman *et al.*, 2000). While poultry products and contaminated water are considered to be major sources of *Campylobacter* illness, outbreaks of campylobacteriosis linked to contaminated fruits, vegetables or other produce-related products have been reported (Beuchat, 1996; Anonymous, 1999b; Jacobs-Reitsma, 2000). In a retrospective cohort study conducted in the UK, Evans *et al.* (2003) observed that salad vegetables were the second highest risk factor in relation to the number of individual cases of *Campylobacter* infection. Similarly, a review of the epidemiology of campylobacteriosis outbreaks and cases between 1990 and 1999 in the USA revealed that produce was associated with more cases of *Campylobacter* illness than any other food source during this period, and was second only to dairy products in the total number of outbreaks (Mandrell and Brandl, 2004). Although cross-contamination from other foods may help explain the unexpectedly high number of produce-related outbreaks compared to other food sources, it is noteworthy that several studies have reported the presence of *Campylobacter* species on produce sampled at the marketplace (Doyle and Schoeni, 1986; Park and Sanders, 1992; Kumar *et al.*, 2001), in ready-to-eat (Federighi *et al.*, 1999), and in modified atmosphere packaged (MAP) (Phillips, 1998) vegetables.

Other Pathogens

Y. enterocolitica, *Y. pseudotuberculosis* and *S. aureus* are involved in comparatively fewer outbreaks than the pathogens described above and cause mostly sporadic illness. However, yersiniosis is often misdiagnosed as acute appendicitis (Montville and Matthews, 2005) and outbreaks of this foodborne disease may have occurred from contaminated produce but remained unrecognised. For example, in an outbreak of *Y. pseudotuberculosis* from iceberg lettuce in Finland in 1998, five of 45 case patients underwent appendectomies (Nuorti *et al.*, 2004). The outbreak was nevertheless identified because of routine surveillance and of subtyping of clinical isolates on a broad geographical scale (Tauxe, 2004). As surveillance intensifies, and as fruits and vegetables become increasingly investigated as a likely vehicle of foodborne disease, other pathogens may join the list of potential etiological agents linked to outbreaks from produce.

Ecology of Pathogens on Produce

Most studies on the behaviour of human pathogens on plants have been performed on cut plants or plant tissue in order to simulate the contamination of produce during post-harvest handling and food processing. While the results of these studies do not necessarily reflect the dynamics of pre-harvest crop colonisation by enteric pathogens, they provide clues about the behaviour of pathogens on plants in a simple system with controlled variables. On the other hand, several studies have assessed the persistence of enteric pathogens on crops in the field over prolonged periods of time. The following section presents experimental evidence for the ability of enteric pathogens to attach, grow and survive on plant surfaces in pre- and post-harvest environments.

Attachment of Enteric Pathogens to Plant Tissue

Localisation

Studies of *E. coli* O157:H7 on store-bought lettuce indicate that the bacterial cells attach to leaves in a relatively short time period, and that not all cells are removed by vigorous washing (Seo and Frank, 1999; Takeuchi and Frank, 2000; Wachtel and Charkowski, 2002). Similar observations have been reported with other enteric pathogens and plant species (Reina *et al.*, 2002; Ukuku and Fett, 2002). In addition, enteric pathogens appear to attach preferentially to specific sites on plant tissue. After immersion of lettuce pieces in a suspension of *E. coli* O157:H7, the pathogen was observed predominantly on the cut edges of the leaves, whereas fewer cells attached to the intact cuticle region. Cells that attached to the undamaged leaf surface were located near stomates, on trichomes and on veins (Seo and Frank, 1999). Likewise, *S. enterica* was observed in the vein region of cilantro leaves after its inoculation onto potted plants (Brandl and Mandrell, 2002), and epiphytic bacteria are frequently located near trichomes and on the veins of leaves (Leben, 1988; Monier and Lindow, 2004). It remains unclear whether this common localisation of human pathogenic bacteria and epiphytic bacteria on specific sites on leaves is caused by the enhanced wettability of these sites or by the presence of plant factors that favour bacterial attachment.

Although similarities in the pattern of attachment of human pathogens and epiphytic bacteria to plants appear to exist, clear differences also have been observed. Takeuchi *et al.* (2000) evaluated strains of *E. coli* O157:H7, *Pseudomonas fluorescens*, *S. enterica* and *L. monocytogenes* for their attachment to the intact and cut region of lettuce leaf pieces. *P.*

fluorescens attached preferentially and in the greatest number to the intact cuticle of the leaf, *E. coli* O157:H7 and *L. monocytogenes* attached better to the cut edges, and *S. enterica* bound equally to both sites, suggesting that the plant epiphyte may be better adapted to the phyllosphere than the human pathogens tested in the study. Additional evidence of such differential behaviour among bacterial species in adhesion to plant tissue has been presented. Barak *et al.* (2002) showed that four non-pathogenic *E. coli* strains isolated from field-grown cabbage attached to alfalfa sprouts (cotyledons, hypocotyls and roots) better than *E. coli* O157:H7, and that *S. enterica* attached in 10- to 1000-fold greater numbers than *E. coli* O157:H7 strains. However, in a separate study, strains of *E. coli* O157:H7 implicated in produce outbreaks attached to lettuce roots at ca. 10-fold higher levels than did two of five non-pathogenic *E. coli* strains (Wachtel *et al.*, 2002). Such differences in attachment have been observed also with strains of *L. monocytogenes*. While minimal differences between attachment of seven strains of *L. monocytogenes* were detected on cut radish tissue (Gorski *et al.*, 2003), 100- to 1000-fold differences were reported in the attachment and colonisation of different *L. monocytogenes* strains to alfalfa sprouts (Gorski *et al.*, 2004). These differences in the strength of attachment at the bacterial species and strain level, as well as with various plant species, suggest that specific factors may be involved in the attachment of enteric pathogens to plant tissue.

Attachment Factors

It is likely that some of the plant attachment mechanisms in enteric pathogens are similar to those described in plant-associated bacteria. These attachment factors may include lectins, polysaccharides, flagella, fimbriae, cellulose fibrils and nonfibrillar adhesins. At present, few fundamental studies have been initiated to identify plant attachment factors in human pathogens and their corresponding moiety in plants. Gorski *et al.* (2003) screened a mutant library of *L. monocytogenes* for attachment to radish slices at 30°C and identified three mutants that were reduced in attachment by at least 10-fold compared to the parental strain. Two mutations were in genes of unknown function within an operon encoding flagellar biosynthesis, but only one of these caused a lack of motility. The third mutant carried an insertion in a gene necessary for the transport of arabitol. The motility mutant was impaired in attachment also at 10 and 20°C, whereas the arabitol transport mutant was decreased in attachment also at 10°C. Interestingly, no difference in attachment between the mutants and the parental strain was observed when the assay was performed at 37°C. The effect of temperature in this system suggests that *L. monocytogenes* may express different plant attachment factors under different environmental conditions.

S. enterica serovar Thompson was observed in the cilantro phyllosphere by immunoelectron microscopy after its inoculation and incubation on cilantro plants (Brandl and Monier, 2006). The high resolution of the electron microscope combined with antibodies specific to the flagella of serovar Thompson allowed observation of the flagella of the pathogen cells located on the leaf cuticle. The flagella appeared to anchor the bacterial cells to the leaf surface, suggesting that they may serve for attachment to plants. Flagella have been described to have a role in the adhesion of *Azospirillum brasilense* to wheat roots based on experiments with a flagellar mutant as well as with purified flagella (Croes *et al.*, 1993). Besides flagella, other adhesins such as pili and fimbriae also are involved in the attachment of several bacterial species to plants surfaces (Korhonen *et al.*, 1986; Vesper and Bauer, 1986; Vesper, 1987; Romantschuk *et al.*, 2002). Recently, several *S. enterica* mutants were identified that have reduced adhesion to alfalfa sprouts (Barak *et al.*, 2005). In addition to mutants that had insertions in previously uncharacterised genes, one mutant was inactivated in curli production. Curli are thin aggregative fimbriae

produced by *E. coli* (Olsen *et al.*, 1989) and *S. enterica* (Romling *et al.*, 1998), which mediate binding to and invasion of animal cells (Sukupolvi *et al.*, 1997; Uhlich *et al.*, 2002). It is possible therefore, that curli fimbriae have a role in the attachment of *S. enterica* to both animal and plant tissue.

In an attempt to identify plant factors that affect the attachment of *S. enterica* to cilantro leaves, polymers were purified from leaf surface extracts and tested individually for attachment by *S. enterica*. One of the few compounds to which *S. enterica* attachment was significantly superior to the control, was identified by mass spectrometry as stigmasterol (Mandrell *et al.*, 2006), a sterol detected also in other leaf extracts (Esmelindro *et al.*, 2004). It is unclear whether such particular components of the cuticle layer of leaves interact specifically with *S. enterica* cells upon their arrival in the phyllosphere, or if they mediate bacterial adhesion through electrostatic and hydrophobic bonds. Hassan and Frank (2003) have probed the nature of the forces that drive the adhesion of *E. coli* O157:H7 to the leaf surface of lettuce pieces. Their results indicated that highly hydrophobic surfactants were the most effective in removing *E. coli* O157:H7 cells from the intact portion of the leaf cuticle, and thus, that hydrophobic interactions are involved in the attachment of this pathogen to leaves. In a study on the attachment of several foodborne pathogens to the outer surface of whole cantaloupe melons, Ukuku and Fett (2002) demonstrated a significant linear correlation between the ability of the various bacterial species to attach to the cantaloupe surface and their relative cell hydrophobicity and surface charge. Therefore, it is likely that passive mechanisms of attachment that are dictated by the chemical nature of bacterial cell surfaces and plant surfaces, including electrostatic and hydrophobic interactions, are involved in the adhesion of human pathogens to plants, in addition to more specific factors (e.g. adhesins). This model has been proposed previously for the attachment of plant pathogenic bacteria and fungi to plants (Romantschuk *et al.*, 1996).

Fitness of Enteric Pathogens on Plants

Growth

It is the ability of immigrant foodborne pathogenic bacteria, not only to attach, but also to grow and/or survive in the plant environment that determines their fate in that habitat and their potential to cause foodborne illness. Few studies have investigated the ability of enteric pathogens to multiply on plant surfaces. Brandl and Mandrell (2002) developed a model with cilantro and a clinical isolate of *S. enterica* serovar Thompson that was linked to an outbreak from cilantro in California (Campbell *et al.*, 2001), to assess the role of various biotic and abiotic factors in the fitness of this pathogen in the phyllosphere. Bacterial population dynamics revealed that *S. enterica* reached lower population sizes than *Pseudomonas chlororaphis* and *Pantoea agglomerans*, two common epiphytic bacterial colonisers, after their inoculation onto the leaves of cilantro plants incubated under humid conditions in the laboratory. Despite this lower apparent fitness, microcolonies of GFP-labelled *S. enterica* cells, located mainly in the vein area of the leaf, were observed as soon as 2 days after inoculation. Larger colonies and high density aggregates were visualised on the veins of old leaves after 9 days of incubation under conditions conducive to the presence of free water on the leaves (Plate 4, see colour plate section). Leaf surface areas between the veins also hosted cells of the pathogen, but at much lower density, following a distribution pattern that has been observed previously with epiphytic bacterial species (Leben, 1988; Monier and Lindow, 2004).

S. enterica grows optimally at 37°C. When inoculated cilantro plants were incubated under high humidity at 30 or 37°C, *S. enterica* achieved higher growth rates and population sizes in the cilantro phyllosphere than at 24°C (Fig. 19.1) (Brandl and Mandrell, 2002). This suggested that under growth-conducive conditions, in this case warm temperature and high water availability, *S. enterica* is capable of multiplying rapidly in the phyllosphere and thus, reaching significant population sizes. It is noteworthy that the ratio of *S. enterica* to resident bacteria population sizes decreased after an initial increase during the first day following inoculation. A probable scenario explaining this change in the competitiveness of *S. enterica* over time is that the high growth rate of the pathogen at warm temperatures allows it to utilise simple nutrient sources that are present on the leaf surface efficiently. However, once that nutrients of this type are depleted, the pathogen may be less adapted to derive much energy from the remaining nutrients and is outcompeted by the resident microbiota. Evidence from studies by O'Brien and Lindow (1989) also supports the hypothesis that *S. enterica* can colonise the phyllosphere under conditions of high water availability on the plant surface. In their studies, performed in a growth chamber at 24°C, *S.* Typhimurium and *E. coli* colonised the wet leaves of maize and bean plants to population levels similar to those of three strains of *P. syringae*. This level of fitness on plants has not been observed with all enteric pathogens. For example, *C. jejuni*, a foodborne pathogen with a microaerophilic and thermophilic lifestyle, was unable to grow and survived poorly in the phyllosphere and rhizosphere of several plant species (Brandl *et al.*, 2004), indicating a lack of microsites on leaves and roots that are suitable for colonisation by fastidious human pathogens.

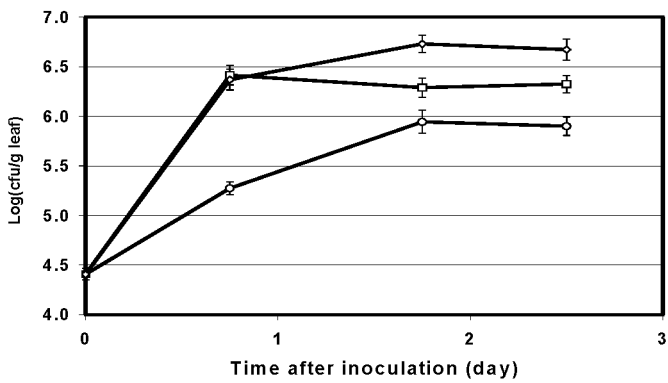

Fig. 19.1. Population dynamics of *S. enterica* in the cilantro phyllosphere after its inoculation onto plants and incubation under high humidity at 24°C (○), 30°C (□) and 37°C (◊).

Despite its epiphytic growth under optimal conditions, *S. enterica* did not multiply in the cilantro phyllosphere under dry conditions (Brandl and Mandrell, 2002). This is unlike *P. syringae*, a common coloniser of plant surfaces, which is able to grow on bean leaves in the laboratory even under conditions of low water availability (Wilson and Lindow, 1992). Similar differences between *P. syringae* strains, and *S. enterica* and *E. coli* on dry plant surfaces were reported by O'Brien and Lindow (1989). It is noteworthy, however, that after an initial phase of overall decline, which has been observed also with epiphytic bacteria, the population size of *S. enterica* stabilised on the dry leaf surface of cilantro plants maintained at low relative humidity (Fig. 19.2). Most importantly, the human pathogen recovered well to reach high population levels under subsequent wet conditions on the leaves. This implies

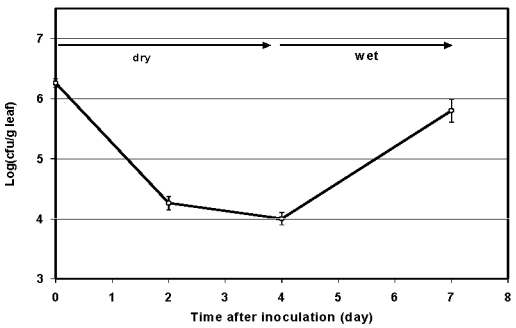

Fig. 19. 2. Population sizes of *S. enterica* on cilantro leaves after its inoculation onto plants incubated under dry conditions at 50% RH, and then under conditions allowing for the presence of water on the plant surface.

that even small populations of the pathogen that survived desiccation stress on crops in the field or during storage may increase to infectious dose levels during growth-conducive conditions before the produce is consumed. It has been reported that *S. enterica* forms large heterogeneous aggregates on cilantro leaves (Brandl and Mandrell, 2002), and that up to 54% of the pathogen population on leaves reside in large aggregates (Brandl *et al.*, 2005). The presence of such aggregates may have important implications for the survival of *S. enterica* under stressful conditions on plants, as was demonstrated for *P. syringae* in the bean phyllosphere (Monier and Lindow, 2003).

Survival

The frequent detection of enteric pathogens on produce, even before it is distributed to retailers, suggests that pre-harvest contamination occurs and that the pathogens have the ability to survive on crops after their arrival in that habitat. Consequently, there is a great interest in determining the survival of enteric pathogens on plants in order to better assess the risks associated with contamination of produce in the field. The survival of enteric pathogens on plants has been documented in several soil microcosm-based studies in the laboratory. Wachtel *et al.* (2002) investigated the association of *E. coli* O157:H7 with lettuce seedlings after their growth from seeds in soil that was irrigated with an aqueous suspension of the pathogen. *E. coli* O157:H7 cells were detected on the hypocotyls and cotyledons, as well as on the roots of the seedlings, where they formed small colonies.

Evidence of growth of E. coli O157:H7 on lettuce was demonstrated also when plants were spray-irrigated with water containing low concentrations of the pathogen; following this growth period, the population of E. coli O157:H7 on the lettuce leaves declined, but the pathogen was still detectable 30 days after inoculation at plant maturity (Solomon, 2003). Similar results were obtained when spinach seeds inoculated with *E. coli* were planted in soil microcosms, and the upper part of the plants tested for the presence of *E. coli* at harvest (Warriner *et al.*, 2003). In growth chamber experiments using soil cores and simulated seasonal environmental conditions, *S.* Typhimurium survived on the leaves of arugula plants until harvest when inoculated manure was applied to the soil in the "summer" and the crop harvested in the "autumn", but was undetectable at harvest when the manure was applied in the "spring" (average daily temperature <10°C) and crops harvested in the "summer" (Natvig *et al.*, 2002). This suggested a critical role for warm temperatures in the population sizes that *S. enterica* can achieve on leaf surfaces, as reported by Brandl and Mandrell (2002).

Contamination of the entire plant, including the seeds, was reported in a study where *Arabidopsis thaliana* was grown in soil irrigated with *S. enterica* or *E. coli* O157:H7 (Cooley *et al.*, 2003). In this model system, movement of *S. enterica* up the plant to the seeds was eliminated when nonmotile mutants were used. Seed contamination has a direct impact on the microbial safety of sprouts, which are among the top food vehicles of outbreaks associated with produce (Sivapalasingam *et al.*, 2004). Due to factors inherent to their mode of production, such as warm temperature, ample water and high levels of nutrients exuded during seed germination, sprouts offer an ideal environment for the amplification of human pathogens. Furthermore, as is recognised already for plant pathogens, the presence of human pathogens on seeds has important implications for the contamination cycle on the farm, since the planting of contaminated seeds may spread or reintroduce the pathogen into the field.

Field studies have demonstrated that enteric pathogens are able to survive for extended periods of time on vegetables and in soil inoculated by applying contaminated water to the crops or manure to the soil in the field. Ercolani (1979) provided the first evidence that enteric pathogens can persist on crops under agricultural conditions. After inoculation of young lettuce plants, *S.* Typhi was recovered from the plants at harvest, whether the crop was grown in the field during the winter or summer season. More than 20 years later, with a heightened concern regarding the microbial safety of produce, similar field investigations were conducted with an avirulent mutant of *S.* Typhimurium (Islam *et al.*, 2004b) and a nontoxigenic mutant of *E. coli* O157:H7 (Islam *et al.*, 2004a). Irrigation with contaminated water or application of manure to the soil, at the seedling stage, resulted in the persistence of both enteric species for at least 3 and 6 months in the lettuce and parsley phyllosphere, respectively. It is significant that the pathogens survived in the soil at higher rates when the crops were in the ground than after harvest. This observation suggests that the aerial part of leafy plants, such as lettuce and parsley, may contribute to the persistence of enteric pathogens in the field, either by providing a continuous source of inoculum or by creating physicochemical conditions that are conducive to the survival of the pathogens in soil or on roots. The effect of roots on the enhanced survival of *E. coli* O157:H7 in soil microcosms has been proposed (Gagliardi and Karns, 2002), and may be part of a plethora of factors that affect the fitness of enteric pathogens in the agricultural environment.

Conclusion

The prevailing dogma about the fitness of human pathogens in the plant environment has been that enteric pathogens lack the ability to grow and survive on plants, and thus should be of little threat to the microbial safety of fresh produce. Emerging outbreaks are challenging this concept by providing evidence that foodborne illness can occur from contaminated produce. This is supported by recent studies that demonstrated that human pathogens have the ability to colonise and/or survive on plant surfaces. Although not evolutionarily as well adapted to colonise plants as plant-associated or plant pathogenic bacteria, enteric pathogens such as *S. enterica* and *E. coli* appear to be fit enough on plants to grow or persist in that environment. It is still unclear, however, whether this fitness is sufficient to explain their common association with enteric illness from the consumption of fresh fruits and vegetables, or if post-harvest conditions that promote the growth of these human pathogens are required to achieve the infectious doses necessary to cause disease in their host.

It is clear that the prevention and control of crop contamination with enteric pathogens in the pre- and post-harvest environments will require an increased understanding of their ecology in those habitats. At present, there is a great lack of fundamental knowledge of the bacterial and plant determinants that enable foodborne pathogens to multiply and survive on plant surfaces. Genomic information about most common enteric pathogens is now available and should be valuable for the investigation of the molecular events that drive the fitness of these pathogens on plants, and their interaction with the resident plant microbiota. Such knowledge may help identify critical control points for intervention strategies or treatments for the decontamination of produce. A better understanding of the biology of enteric pathogens in the plant environment also will provide insight into their minimum infectious dose when present in the plant-food matrix, and help assess the risks associated with the consumption of contaminated produce. Present epidemiological data suggests that enteric pathogens should not be discounted as important transient members of plant microbial communities.

Acknowledgements

Work in my laboratory is funded by the US Department of Agriculture, Agriculture Research Service CRIS projects 201-5325-210-44 and 201-5325-210-45.

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